General Concepts in Integrated Pest and Disease Management

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Nectarine powdery mildew showing white mycelium growth on the green fruits (by Peter Sholberg, Pacific Agri-Food Research Centre/Centre de recherches agroalimentaires du Pacifique, Summerland, BC, Canada).

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PREFACE

The proposal for this series originated during a short term visit of Professor Mukerji to the Plant Protection Institute of CNR at Bari, Italy, in November 2005. Both editors agreed on the need to produce a volume focusing on recent advances and achievements which changed the practice of crop protection in the last decade. The *opera* rapidly evolved towards a long term editorial endeavour, yielding a multi-disciplinary series of five volumes.

In view of environmental and health concerns, a determined effort is currently made in almost any agroecosystem in the world, to reduce and rationalize the use of chemicals (pesticides, fungicides, nematocides etc.) and to manage pests/pathogens more effectively. This consciousness is not only related to the need of nourishing a still growing world population, but also derives from the impact of side effects of farming, like soil, water and environmental contamination, calling for a responsible conservation of renewable resources. There are increasing expectations at the producers and consumers levels, concerning low inputs agriculture and residues-free food. Disciplines like IPM/IDM (integrated pest management / integrated disease management) are now central to the science and technology of crop protection. In the classical version of IPM/IDM, a pesticide/fungicide is applied only when the pathogen population reaches a level that would lead to economic losses in the crop. In other words, classical IPM/IDM concentrates on reducing the numbers of noxious organisms through the application of agrochemicals. However, IPM/IDM actually means "A disease management system that, in the context of the associated environment and the population dynamics of the pest/pathogen species, utilises all suitable techniques and methods in a manner as compatible as possible and maintains the pest/pathogen population at levels below those causing economic injury". IPM/IDM in the broad sense has been defined as "the optimization of pest/pathogen control in an economically and ecologically sound manner, accomplished by the coordinated use of multiple tactics to assure stable crop production and to maintain pathogen pest damage below the economic injury level, while minimizing hazards to humans, animals, plants and the environment".

Plant health depends on the interaction of a plethora of microorganisms, including pathogens and pests, which give rise to a complex system based on multiple food webs and organisms interactions, including the physical and chemical environment in which plants grow. Thus IPM/IDM moves beyond a one-plant one-pathogen/one-pest control view of disease control towards an integrated view of plant health as a result of complex interactions. Moreover, the basic concern of IPM/IDM is with designing and implementing pest/disease management practices that meet the goals of farmers, consumers and governments in reducing pest/disease losses while at the same time safeguarding against the longer term risks of environmental pollution, hazard to human health and reduced agricultural sustainability.

Due to the large amounts of data available in IPM/IDM, the volume is not a comprehensive manual, because of the wide range of topics and the numerous, sometimes specific aspects, characterizing this discipline. However, our effort in compiling the contributions of the first volume of the series attempted to collect

concepts and achievements which will probably produce popular practices and tools, available in the next decades for crop protection. A growing number of discoveries, applications and technologies are available today for farming, gradually re-shaping worldwide pest and disease management and control. During the last decades, dramatic changes deriving from the digital and molecular revolutions were experienced in the way farmers may monitor and control pests and diseases, and some of them are sought and described in this first volume.

A first section covers modeling, management and environment related issues, ranging from advances in modeling and monitoring, to potentials of remote sensing technologies. The section also includes a review of resurgence and replacement causing pest outbreaks, a chapter describing the role of plant disease epidemiology in developing successful integrated management programs, a chapter describing the effects of climate changes on plant protection and two applied reviews, treating carrot and post-harvest diseases management. In a second section we grouped emerging technologies including the application of information technology or remote sensing and of *Bacillus thuringiensis* or mycorrhizae in IPM. In a third section, molecular issues in IPM/IDM are grouped, with chapters treating the management of insect borne viruses through transmission interference as an alternative to pesticides, the novel microbial compounds suitable for pest/disease control or the use of molecular diagnostic tools in IPM/IDM.

The volume is a compilation of the thoughts from a wide array of experts in the areas of plant protection, microbiology, plant pathology, ecology, agricultural biotechnology, food safety and quality, covering a wide range of problems and solutions proposed. The chapters are contributed by leading experts with several research years' expertise, investigating and applying advanced tools in their work, and offer several illustrations and graphs, helping the reader in his/her study.

A. Ciancio
K. G. Mukerji

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Section 1

Modeling, Management and Epidemiology

R. D. MAGAREY AND T. B. SUTTON

HOW TO CREATE AND DEPLOY INFECTION MODELS FOR PLANT PATHOGENS

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Abstract. This chapter is designed as a practical guide on how to create and deploy infection models for plant disease forecasting. Although, infection models have been widely and successfully used in plant pathology for many years, there is a general lack of standards for model development. In part, this is because most disease forecast models tend to be either complex or specialized. The first part of this guide is an overview of the biological considerations for infection, including temperature, moisture and splash dispersal requirements. The second part is a review of the strengths and weaknesses of new and commonly used infections models. Since weather conditions and infection risk alone does not determine disease severity, the guide provides some practical suggestions for integrating host, pest and cultural factors into a disease forecast in the third part of the chapter. The fourth part covers the best methods for collecting or obtaining the weather inputs used in infection models. The fifth section covers techniques for model validation both from a biological and commercial perspective. The final section briefly covers techniques for information delivery focusing on the internet.

1. INTRODUCTION

Plant pathologists, research scientists or agronomists tasked with constructing plant disease forecast models might realistically hope to go to a publication or an on-line source and find an encyclopedia-like model building reference. In an ideal world, these models would be generic such that they would be suitable for use on a many different diseases. It would be easy to 'plug and play' models into a disease forecasting system since the model inputs and outputs would be standardized. In addition, each model would contain a number of biologically based parameters and a reference table would give these parameter values or their ranges for economically important pathogens. Finally, if the encyclopedic site was on-line, it would be possible to upload a weather data file and test the model on-line.

Entomologists have an on-line resource available at the UC-Davis IPM web site (Anonymous, 2006) that meets some but not all of these ideal specifications. Approximately 90 degree day models are available at this web site. Each model has almost the same parameters: lower (and in some cases upper) developmental thresholds and the degree day requirements for each life stage. Another on-line resource has a library of these developmental requirements for over 500 insects (Nietschke *et al*., unpublished data). The consequence of these databases and other resources is that an entomologist can easily make prediction models for these pests with one simple model and inputs of daily average temperature.

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Plant pathologists are in a much less favorable position. In contrast to entomology, the UC Davis IPM web site has forecast models available for only 12 diseases. Although many more than 12 plant diseases have been successfully modeled, the complexities of the model design and the lack of standardization make such an encyclopedic task difficult if not impossible. More problematic than the lack of available models is the lack of standardization among models. Often there may be many different models for important diseases adding to the confusion. On the UC Davis site, two diseases have ten or more models each, some of them are quite different from the others. A quick perusal of the model database reveals a lack of standardization on almost every facet of model construction including model description, time steps, inputs, methods of calculating risk and outputs.

This of course does not mean that entomology is a more advanced science. Although some entomologists might wish to advocate such a position, there are many more fundamental reasons why it is harder to construct an encyclopedia resource for plant disease forecast models. The most important reason is that the insect models discussed above are simply predicting pest phenology based on temperature accumulation, while many plant disease models are predicting risk. Even when the model simply estimates the risk of infection it may integrate many complex biological processes such as sporulation, germination, spore dispersal and pathogen and host phenology, as will been seen later in the chapter. These biological complexities make the creation of a generic risk model difficult.

While biological complexity might be the principal reason, there are other contributing factors. Many plant pathologists work on one or two commodities and usually one or two diseases on each commodity. This tends to lead towards specialization in that many models created by scientists may be complex and highly customized. While this individual approach may help the scientists who create the models publish original research, it tends to work against standardization. There are of course some examples of models which have been successfully used generically. For example the FAST system for *Alternaria* like diseases on tomato has been adapted for apple, pear and potato (Madden *et al*., 1978; Montesinos & Vilardell, 1992; Shuman & Christ, 2005).

An additional factor limiting the ability of scientists to use models generically, is that many models do not have biologically based parameters which limits the ability to adapt a model to another pathogen. Since there is no standardization of model parameters, there is also no incentive for scientists to compile databases of these parameter values, a classic catch-22 situation. A final problem is that many models are simply based on statistical relationships between average or summary weather variables and observed disease incidence for a specific crop and location. It is unclear if these types of models would provide useful results when used in a different climate or pathosystem.

Another problem relates to the lack of standardization of environmental inputs. Some models were developed before automated weather stations were available to provide hourly weather data and instead use simple daily weather data. Leaf wetness has been historically difficult to measure (Magarey *et al*., 2005a), so some disease

models have used average relative humidity (RH) or hours above a specific RH threshold. In addition there might be differences about the canopy location or the protocol for collecting these weather inputs.

Given all these issues, it is tempting to wonder if an effort to standardize and catalog plant disease forecast or infection models is even practical. However, some of the negative points discussed above are possibly exceeded by many of the positive points about plant disease forecast models including: *i)* international experience with the use, application and development of disease forecast models for well over 50 years (Campbell & Madden, 1990); *ii)* many plant diseases are highly weather driven making them perfect candidates for forecasting (Waggoner, 1960); and *iii)* a good repository of published data to create infection models albeit not in a standardized format.

In this chapter, some of the practical issues for creating and using simple infection models for plant pathogens are examined. Infection models are a small subset of disease forecast models, however they are quite important because most plant disease are caused by fungi and most fungi with the exception of powdery mildews and some 'wound' pathogens' have some sort of environmental requirements (Huber & Gillespie, 1992; Waggoner, 1960). While many plant pathogenic processes are temperature driven, infection also requires moisture and moisture is limiting in most terrestrial environments (Magarey *et al*., 2005a). Infection is the process by which a plant pathogen initiates disease in a plant. In this paper, we use a very broad definition of infection, which may also include requirements for dispersal, spore germination and sporulation.

In our approach to infection modeling, we lean towards the fundamental approach rather than an empirical one (Madden & Ellis, 1988). In the fundamental approach, infection models are created from experiments in the laboratory and controlled environmental chambers and describe the infection response in relation to environmental parameters. An alternative is the empirical approach where qualitative rules or quantitative models are created based on statistical relationships often between summarized environmental inputs and disease observations in the field, usually from four of more years of data (Madden & Ellis, 1988). The empirical approach has the advantage that data from controlled or laboratory tests are usually not required. They may also have the advantage of being simple and easy to develop, especially those that are qualitative. However, the empirical approach may not lead itself well to generic and standardized approach since it likely to be a unique relationship for each pathosystem. Also the empirical relationship may not 'hold up' outside of the specific circumstance in which it is developed. Thirdly, with modern electronic weather data there is no longer a need for models to be developed from summary environmental variables. Although the empirical approach continues to be important in plant pathology, models developed using this approach are outside of the scope of this chapter.

In the first section of this chapter, we review the biological requirements for infection. This includes temperature, moisture and splash dispersal requirements of plant pathogens, factors usually incorporated into the infection model itself. The

second is a review of the strengths and weaknesses of new and commonly used infection models. Since weather conditions and infection risk alone does not determine disease severity, the guide provides some practical suggestions for integrating host, pest and cultural factors into a risk estimation. The fourth section deals with the best methods to collect or obtain the weather inputs used in infection models. The fifth section covers techniques for model validation and validation and in the final section techniques for information delivery are briefly discussed.

2. BIOLOGICAL REQUIREMENTS FOR INFECTION

Pathogens vary in their temperature and moisture requirements for infection (Table 1). An organism's temperature requirements for infection can be summarized by the cardinal temperatures, T_{min}, T_{opt} and T_{max}. Moisture requirements may be for free surface moisture or high humidity. In general, there is little practical difference between these two variables since high humidities measured at a standard weather station environment may constitute wetness in a canopy. Moisture duration requirements can be summarized by W_{min}, the minimum wetness duration requirement for infection (Magarey *et al*., 2005c).

Plant pathogens can have quite different temperature-moisture responses for infection (Fig. 1), for example web blotch of peanut caused by *Didymella arachidicola* has a high T_{min} and W_{min}, while cucurbit downy mildew caused by *Pseudoperonospora cubensis* has a relatively low T_{max} and W_{min}. Finally, there are bacteria such as *Erwinia amylovora* or xerophytic pathogens such as powdery mildews which may have little or no moisture requirement beyond that of rain for splash dispersal (Miller *et al*., 2003; Steiner, 1990).

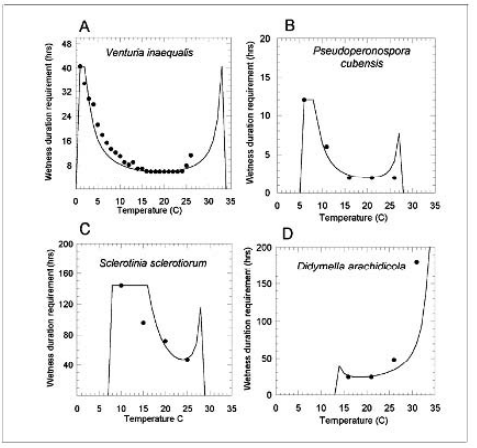

Figure 1. Comparison of temperature-moisture response for infection for four fungal pathogens: A) Venturia inaequalis *(causal agent of apple scab); B)* Pseudoperonospora cubensis *(cucurbit downy mildew); C)* Sclerotinia sclerotiorum *(white mold of beans); and D)* Didymella arachidicola *(peanut web blotch) (Magarey et al., 2005c).*

Table 1.Example of infection parameters for selected plant pathogens.

Pathogen	T_{min} [r]	T_{max} [s]	T_{opt} [t]	W_{min} [u]	W_{max} [v]	*References*
Didymella arachidicola	13.3	35	18.5	24	210	Subrahmanyam & Smith, 1989
Pseudoperonospora cubensis	1	28	20	2	12	Cohen, 1977
Sclerotinia sclerotiorum	1	30	25	48	144	Weiss *et al*., 1980
Venturia inaequalis	1	35	20	6	40.5	Stensvand *et al*., 1997
Sphaerotheca macularis f. sp. *fragariae*	5	24	30	0	NA*	Miller *et al*., 2003

*NA = not applicable.

Generally the temperature and moisture requirements for infection are determined in controlled environment studies where plants or plant parts are incubated in moist environments at various temperatures (Madden & Ellis, 1988; Rotem, 1988). Presently, there are probably about 100-200 pathogens where this infection response has been described (Magarey *et al*., 2005c). In the case where these data are not available and experiments can not be conducted, the moisture and temperature requirements for infection must be estimated from scientific reports such as germination requirements, growth in culture or field observations. Useful sources of information include the CABI Crop Protection Compendia and the APS Plant Disease Compendia. Literature searches in abstract databases such as CAB abstracts, AGRICOLA and BIOSIS are also helpful sources of information. A dated but extensive review of temperature requirements may be helpful if no other data are available (Togashi, 1949).

Some pathogens also require continuous moisture for infection while others can endure dry periods without disruption to the infection process. For example, two species of *Puccinia* are sensitive to dry interruptions of 1-2 hours, whereas *Venturia inaequalis* and *Cersospora carotae* are relatively insensitive and can survive for more than 24 hours (Magarey *et al*., 2005c). It should be noted that many published studies of interruption to wetness may not be representative of real world conditions where spores may be quickly desiccated and should be treated with caution. Interruptions to wetness can be handled by terminating the infection process or by reducing the severity of infection.

Infection potential may also be related to other parts of the disease cycle (Magarey *et al*., 1991; Xia *et al*., 2007). Temperature and moisture or high humidity may also be required for sporulation (Colhoun, 1973). For example grape downy mildew has a high relative humidity requirement for the formation of sporangia during secondary infection (Magarey *et al*., 1991).

Another important moisture requirement is for splash dispersal. Many pathogens have relatively heavy spores that are not easily liberated and dispersed by wind or rain splash may be required to liberate spores from a fruiting structure (Fitt & McCartney, 1986). For this requirement, 2 mm of rain has been used in the case of ascospores of grape powdery mildew to allow for the splash transport of ascospores from mature bark to new growth (Gadoury & Pearson, 1990). Only 0.25 mm of rain is required to splash *Erwinia amylovora* bacteria from overwintering cankers to the stigma, where it causes infection (Steiner, 1990). Rain 10 mm or more has been used as a splash requirement for grape downy mildew, because puddling is required to liberate sporangia from the soil, which must then be splashed up into the grape canopy (Magarey *et al*., 1991). The choice of a differences between these figures (0.25, 2 and 10 mm) may represent the difference in how far the spores must be splashed from their overwintering location.

Another requirement is light or dark. *Plasmopara viticola*, causal agent of grape downy mildew, requires darkness for formation of sporangia (Magarey *et al*., 1991) and apple scab ascospores are not released during darkness (Stensvand, *et al*., 1998). *Puccinia graminis* has a requirement for light to complete the infection process (Pfender, 2003).

3. INFECTION MODELS

After having determined the environmental requirements for infection it is necessary to have some sort of model to process the weather data into infection potential. The easiest way to create a model of infection potential is to use a simple rule using daily weather data. Commonly these combine minimum temperature and rain for example, the 10 C and 2.5 mm rule for grape powdery mildew ascosporic infection (Gadoury & Pearson, 1990) and the 10:10:24 rule for grape downy mildew infection (Magarey *et al*., 2002). There are also other examples of simple decision aids such as charts and graphs that use combinations of daily average temperature and hours of wetness per day (Seem & Russo, 1984). However usually for most pathogens, hourly weather data are required to capture the infection response and these call for a more complex model. The model is essentially a biological clock that tracks the accumulation of favorable conditions usually hour by hour. There may be initiation conditions to start the clock for example rain splash, daylight or darkness. The counter of the clock may be reset to zero by dryness or when relative humidity or temperature falls below a certain threshold or when spores have been liberated and no more are available.

There are a variety of modeling approaches which are summarized below (Table 2). The modeling approaches have their strengths and weaknesses and model selection depends upon a number of factors. These include the quantity of data

available for model development and also whether the developer is creating a suite of models or an individual model. A common approach to modeling is what we call a matrix. An example of matrix approach is the Wallin potato late blight model (Krause & Massie, 1975). In this matrix, rows represent the temperature requirement expressed as average temperature during the wetness period and columns represent moisture requirement expressed as hours above 90% RH. Lower temperatures and longer moisture periods yield higher disease severity combinations. Bailey took this concept one step further by creating an interactive generic matrix based upon combinations of temperature and relative humidity and the number of hours required to achieve infection at each combination (Bailey, 1999).

Where the infection response has been observed at multiple temperature and wetness combinations it is possible to create an infection model using regression equations, such as those based on polynomials, logistic equations, and complex three-dimensional response surfaces (Magarey *et al*., 2001; Pfender, 2003). These

Table 2. Comparison of different infection modeling approaches.

Approach	*Strengths*	*Weaknesses*
Matrix (Krause & Massie, 1975; Mills, 1944; Windels, *et al*., 1998)	Easy: converts moisture/temperature combinations into severity values or risk category. Tried and true approach.	Data to populate matrix may not be readily available.
Regression:		
– polynomial (Evans *et al*., 1992)	Used widely in plant pathology (Pfender, 2003; Magarey *et al*., 2005c).	Parameters not biologically based.
– logistic (Bulger *et al*., 1987)	Model already available for many economically important plant pathogens.	Requires data set for model development.
Three–dimensional response surface (Duthie, 1997)	Describes infection response in detail.	Parameters not biologically based. Complex, requires long processing time and extensive data set for model creation.
Degree wet hours (Pfender, 2003)	Simple, based on degree hours which is widely used in entomology. Requires only T_{min} and $T_{max.}$	Recently developed, assumes thermal response is linear.
Temperature-moisture response function (Magarey *et al*., 2005c)	Simple, based on crop modeling functions, requires only T_{min}, T_{opt} and $T_{max.}$	Recently developed.

models are now widely used in plant pathology, and so infection models are available for many economically important plant pathogens. The problem with many of these modeling approaches is that they are not generic and the model parameters are not biologically based, thus they do not serve as a good template to develop a suite of disease forecast models using the same general equation. If there are many observations (>60) of the temperature-moisture response it is also possible to create a 3-D response surface (Duthie, 1997). The three dimensional response surfaces may capture the infection response in the most detail but may be too complex and processing intensive for many operational disease forecasting applications.

A novel approach is the concept of degree hour wetness duration (Pfender, 2003). The beauty of the degree hour wetness duration concept is its simplicity and the fact that it aligns infection models closely with those used for insect phenology modeling. The weakness of the degree hour approach is that not all pathogens may respond in a linear fashion between T_{min} and T_{max}. Taking this one step further is our concept of the temperature-moisture response function (TMRF) (Magarey *et al*., 2005c). This is a modification of temperature-response function which is commonly used for crop modeling (Yan & Hunt, 1999). The models inputs are the cardinal temperatures for growth and the minimum wetness duration requirement. There are several advantages of TMRF including the fact that it only needs inputs of cardinal temperatures to model the infection response, thus the TMRF is ideally suited to creating simple infection models for exotic plant pathogens. Another reason for using the TMRF approach is that it aligns infection models with those used for crop modeling, thus potentially making it easier for infection models to be incorporated into more complex decision support systems.

The TMRF model calculates predicted infection severity values for a given wetness duration and temperature:

$$I = W f_{(T)} / W_{min} \geq W / W_{max} \quad (1)$$

where, W = wetness duration h, $f_{(T)}$ = temperature response function (Yin, *et al*., 1995), and $W_{min,\ max}$ = the minimum and maximum value of the wetness duration requirement.

For pathogens that require high relative humidity rather than free moisture the wetness requirement may also be defined as the number of hours above a relative humidity threshold. The critical disease threshold for the TMRF was defined as 20 % disease incidence or 5 % disease severity on an infected plant part at non-limiting inoculum concentration, but it could be a custom defined value. The parameter W_{max} provides an upper boundary on the value of W since temperature is not always a rate limiting factor. The model uses the temperature response function of Yin *et al*. (Yan & Hunt, 1999; Yin *et al*., 1995) which is a simplified and improved version of the rice clock model (Gao *et al*., 1992). The function uses a pathogen's cardinal temperatures, to estimate the shape parameter and the temperature response,

$$f_{(T)} = \left(\frac{T_{max} - T}{T_{max} - T_{opt}} \right) \left(\frac{T - T_{min}}{T_{opt} - T_{min}} \right)^{(T_{opt} - T_{min}) / (T_{max} - T_{opt})} \quad (2)$$

if $T_{min} \leq T \leq T_{max}$ and 0 otherwise, where T = mean temperature (°C) during wetness period, T_{min} = minimum temperature for infection, T_{max} = maximum temperature for infection, T_{opt} = optimum temperature for infection.

The advantages of the Yin function compared to other growth functions include the fact that the function has only three parameters (T_{min}, T_{opt}, and T_{max}) and each parameter has a clear biological meaning (Yan & Hunt, 1999). In developing the model, other crop growth functions were also examined and the Wang and Engel (Wang & Engel, 1998) and Yin (Yin *et al*., 1995) formulations had almost identical computational results.

Having made the choice of which type of infection model, the next practical consideration is the time step. Most model applications will use an hourly time step, since this is a standard for collection of meteorological data. With both hourly and daily data, it is necessary to know how many dry hours may interrupt a wet period without terminating the infection process. The additivity of two interrupted wet periods is determined by D_{50}, the critical dry-period interruption value. Consider the case of two wet periods W^1 and W^2 separated by a dry period D. The sum of the surface wetting periods W_{sum} is given as

$$\begin{aligned} W_{sum} &= W^1 + W^2 \text{ if } D < D_{50} \\ W_{sum} &= W^1, W^2 \text{ if } D > D_{50} \end{aligned} \quad (3)$$

The parameter D_{50} is defined as the duration of a dry period that will result in a 50% reduction in disease compared with a continuous wetness period. Some models use a relative humidity threshold of 90 or 95% for linking wet periods (Eisensmith & Jones, 1981). The value of D_{50} is sensitive to the time the dry period occurs and may vary from less than 2 hours to more than 24 h (Magarey *et al*., 2005c). As mentioned earlier some pathogens require rain splash so some infection models will require precipitation above a specific threshold to initiate the accumulation of infection values.

Meteorological inputs are fed into an infection model and the output is the hourly infection severity value. A daily severity value or a risk index is an arbitrary value which defines the predicted disease favorability for each day and is usually accumulated over time (Krause & Massie, 1975). Growers do not want to see hourly model output but would rather see a summarized daily output, usually the total of the hourly severity values for the day. When infection periods last several days it is important to report the maximum value for the event. For some diseases it might be more meaningful to report the accumulation of the infection severity values over a

week. In the next section, we will discuss how to convert a daily infection severity into a risk value that integrates a combination of host, pathogen and cultural factors.

4. DISEASE FORECAST

One of the most important considerations for implementing infection models is that moisture and temperature alone do not determine disease risk rather it is a combination of host, pathogen and cultural factors. In this section, we highlight a few selected factors that have potential to be integrated quantitatively with an infection event to create a disease forecast. In an article of this size it is not possible to do justice to any of these factors. Rather it is our intention to make readers aware that these are some of the factors that should be considered when deploying infection models. After introducing these factors, we suggest simple methods to incorporate these factors into risk models either qualitatively or quantitatively.

These factors vary in importance. For many crops, phenological susceptibility is critical factor. Populer identified two main types of susceptibility associated with the age of plant part (Populer, 1978). Type 1 where susceptibility rapidly increases and then rapidly declines during the growth period, after which it remains low. This would be typical of herbaceous stems on perennials which become lignified a short time after growth ceases and become resistant. Ontogenetic resistance of grape clusters to *Uncinula necator* the causal agent of grape powdery mildew (Gadoury *et al*., 2003) is a good example. In susceptibility Type II, resistance remains high until advancing maturity when the plant part becomes increasingly susceptible. An example is brown rot of stone fruit caused by *Monilinia fructicola*. Fruit become increasingly susceptible from pit hardening until harvest and especially susceptible during the 2-3 weeks while fruit ripens (Biggs & Northover, 1988; Luo & Michailides, 2001).

Phenological susceptibility has several advantages that make it amenable to modeling. Firstly phenology can often be calculated from a planting or bud burst date using a crop phenology model with inputs of day degrees. Crop phenological developmental requirements have been published for numerous models field and horticultural crops (Anonymous, 2006; Miller *et al*., 2001; Seem & Szkolnik, 1978). Second, the period of phenological susceptibility is usually known for most diseases. A good illustration of this is a figure showing overlapping risk windows for 12 pests graphed against apple growth stages in New York apple orchards (Gadoury *et al*., 1989). Phenological susceptibility has until recently rarely been quantified. One practical method is to inoculate plant parts at different phenological stages and then score disease severity (Gadoury *et al*., 2001). Although this technique works well it requires skilled technicians and is labor intensive. An educated guess might be made by observing disease severity in the field and then back dating observations by several weeks or more to take into account the incubation and development period for the pathogen. Target size is also related to crop phenology but may be a factor that highly susceptible and immature plant parts may have a small leaf, flower or fruit surface area.

Target size also may change microclimate when plants grow larger and denser, decreasing air circulation and increasing water holding capacity, both of which increase wetness duration. In addition to phenological susceptibility, it is often important to account for genotypic resistance. One approach is to multiply the accumulated risk values by a coefficient. For example (Matyac & Bailey, 1988) multiplied the risk index values for a peanut leaf spot infection model by 0.85 and 0.7 to represent two different levels of genotypic resistance.

The pest factors inoculum density would be valuable to include in a disease model. For most pathogens it may be difficult to quantify the level of inoculum in a reproducible fashion. In some cases primary inoculum may be estimated from a known relationship with weather variables for example: *i)* low temperature and survival of primary inoculum e.g. wheat leaf rust (Eversmeyer & Kramer, 1998); and *ii)* temperature dependent development and pest phenology e.g. apple scab ascospore maturation and release (Gadoury & MacHardy, 1982).

In other cases protocols have developed which allow for a quantification or semi-quantification of primary or secondary inoculum including *i)* visual quantification of survival or infective structures (Gadoury & MacHardy, 1986); *ii)* spore traps counts (Berger, 1973; Bugiani *et al*., 1996; Jedryczka *et al*., 2004); and *iii)* estimation of inoculum levels from an atmospheric transport model e.g. tobacco blue mold spores (Davis & Main, 1984).

Another important factor is the rate of pest reproduction or the rate of epidemic development. Some plant diseases are monocyclic and have a single cycle of disease development. Others are polycyclic and may have many generations of development. The rate at which the pathogen multiplies will in part be dependent upon the latent period, the time for the pathogen to produce another generation of propagules. Latent periods may be anywhere between 3-21 days or occasionally even longer.

When using infection models, it is important to understand that for most diseases new lesions may appear anywhere from several days to several weeks after an infection event. One of the most important cultural factors is fungicide management. There are models that can calculate spray cover based upon rainfall and time elapsed from the last spray (Smith & MacHardy, 1984; Stewart *et al*., 1998). Remaining spray cover percentage is calculated from elapsed time in days and rainfall using the following equations, which are processed sequentially:

$$Y_{(d)} = Y_{(d-l)} - a \quad (4)$$

$$Y_{(d)} = Y_{(d)} - (0.01\ Y_{(d)}\ w\ r_{(d)}) \quad (5)$$

where y = % cover, *d* = day, *a* = % cover decay rate/day, w = wash-off rate (% cover/mm rain/day) and r = rainfall (mm).

Many of the host, pest and cultural factors are qualitative and can be used to define, initiate or terminate the susceptible period. Some other factors can be

quantified and might be used to mathematically calculate risk. We suggest a simple risk index approach for quantifying the predicted daily disease severity from a raw infection severity value. The Disease Severity Index (DSI) is based on a relative risk index first developed for apple scab based on target size, target susceptibility and incoulum dose (Falk *et al.*, 1995). Falk *et al*. developed this index after realizing that while wetness duration may change risk by a factor of 6, the above relative risk index may change by a factor of 100. Generalizing this approach, we can define the DSI as a relative index of daily disease risk with a value between 0 and 1. It is the multiplicative product of a number of sub-indices also rated between 0 and 1. The first step in creating a risk index is to define what factors are important for quantifying the predicted disease severity. The daily relative risk for each factor is the predicted value divided by a commonly observed maximum value. The DSI is calculated from:

$$DSI = (a\,I\ b\,P\ c\,T\ d\,S) / e \qquad (6)$$

where DSI = Disease severity index, P = relative phenological susceptibility, T = relative target size, S = relative spray coverage, and a, b, c, d, e = weighted constants with a product of 1. The factors P T and S could be easily replaced by other pest, host or cultural factors but there are several important considerations. Each factor should be: a) quantitative; b) have a known maximum value; c) easily estimated, observed or measured; and d) reproducible. We suggested these four variables as potential risk indices since infection risk (I) can be estimated from an infection model, target size (T) and phenological susceptibility (P) could be calculated from a biofix date, a day degree model and a look-up table of susceptibility and target area, and spray relative cover (S) can be calculated as shown above. It may be tempting to include an observed inoculum level but these measurements may not be easily reproducible from one observer to another or may be difficult to quantify.

Since it may be difficult to interpret what infection severity values mean to managers, an alternative to the relative risk index is a dependency network or decision tree. A dependency network uses logical 'and/or' statements to link a host, pest and cultural factors to a particular risk level or management action (Travis & Latin, 1991). These pest and host factors may include past disease history, crop end use, variety, phenological stage and even a growers attitude to risk. The dependency network diagram can be easily created by a plant pathologist or agronomist experienced with the disease and later transferred into code by a computer programmer to form the basis of a decision support system. The dependency network diagrams formed the knowledge base for expert (decision support) systems built for grape pests (McDonald, 1997; Saunders *et al*., 1991).

Although infection models are usually run from hourly input, daily summary of model output is appropriate since it is unlikely growers will make management decisions on a temporal scale less than a day. Disease forecast models using numerical output units to quantifying risk or favorability often refer to them as daily severity values (Gleason *et al*., 1995; Krause & Massie, 1975), but others have used

terms such as daily infection values (Windels *et al*., 1998), or environmental favorability index (Fidanza *et al*., 1996). Numerical output is often accumulated or it may be summarized for a given temporal period longer than a day often using a moving average *(*Gleason *et al*., 1995). Accumulation or averaging of numerical output may be most appropriate when: *i)* a specific infection event lasts over several days; *ii)* infection events are non-discrete and overlap; *iii)* the rate of disease progress is relatively low or the influence of individual infection periods is small; *iv)* the period of crop susceptibility is long; and *v)* the model is being used to predict spray interval. Some infection models may use output units that are categorical or simply predict an infection date. An example of categorical output is the classic Mills table values of nil, light, medium or severe (Mills, 1944). Categorical or date based outputs are most appropriate for high value crops where individual infection periods are of relatively high consequence during the period of susceptibility.

One of the most important considerations for the predicted disease severity value is there should be clearly defined management consequences. In the validation section, we discuss techniques for commercial validation, the process of associating model output with a management recommendation, including the development of action thresholds. The action threshold may differ with many factors including crop end use or variety. The action thresholds may enable a farmer to correctly use the model output to: *i)* initiate the onset of a spray program; *ii)* determine the frequency of fungicide sprays or the length of a spray interval; *iii)* time individual fungicide applications (especially post-infection fungicides) or *iv)* initiate scouting. This approach can also be likened to the inactive, watch and warning systems borrowed from the field of meteorology (Magarey *et al*., 2002).

5. WEATHER INPUTS

In terms of weather inputs, a model developer has essentially three choices to make: *i)* the best choice of input variables; *ii)* locating the best sources of weather data; and *iii)* corrections for canopy microclimate.

5.1. Choice of Input Variables

Infection models generally run from inputs of temperature, free moisture (leaf wetness or high relative humidity) and precipitation. Historically, weather inputs were difficult to collect so often models ran from daily variables. However since the development of automated weather stations hourly weather data has become more widely used. Some systems have chosen to use smaller time intervals (e.g. 10, 12 or 15 minutes) but this should not be necessary provided that moisture duration is not underestimated. Temperature may vary with position in the canopy as will be discussed later in the section on microclimate. Some pathogens may be in the soil consequently, soil temperature may be a better predictor than air temperature. Although soil temperature is not commonly measured it can easily be derived or calculated from surface temperature and soil properties (Novak, 2005).

For moisture there has been a variety of different choices including relative humidity. Model developers have tended to use a variety of different relative humidity measures including hours above 90% RH, hours above 95% RH, and average relative humidity. However, relative humidity is not an absolute measure of the water content of the air, as it is dependent upon the air temperature. Consequently, it may not be a good predictor of a fungus' biological response to moisture. For this reasons, some researchers have chosen to use saturation vapor pressure deficit (Magarey *et al*., 1991). Some plant pathogens require only high atmospheric moisture but most require leaf wetness hence relative humidity has really been used as a surrogate variable. For these reasons leaf wetness may be a better choice for a moisture variable for most plant pathogens.

There have also been issues with standards for leaf wetness measurement. There have been a wide variety of sensors employed for its measurement and lack of general agreement about its definition (Magarey *et al*., 2005a). In recent years, simulation of leaf wetness has emerged as an alternative to measurement (Magarey *et al*., 2005a). Most simulation models calculate surface wetness from air temperature, relative humidity, net radiation and wind speed. It is only recently that simulated site-specific weather data has really made surface wetness simulation a really viable alternative. The main advantage of the simulation approach is that an on-site weather station is not required (Magarey *et al*., 2001). In addition, there are many protocol considerations with the use of sensors that can be avoided by the use of a simulation model. These include the type of sensor and placement in the canopy and maintenance issues.

5.2. Source of Weather Data

The second consideration for weather inputs is locating the best available sources. For many years ago there was little choice other than to obtain weather data from a nearby city or airport. The development of low cost reliable weather stations has given plant pathologists a much better access to data. As time went by more stations were deployed and some organizations established weather station networks to further enhance data availability. In recent years small low cost weather sensors have also become more widely available and improved in quality and capabilities. Now an even more advanced technology is emerging: simulated site-specific weather data (Magarey *et al*., 2001). This information is derived using spatial interpolation procedures and atmospheric modeling utilizing multiple data sources such as ground observations, radar and satellite images (Chokmani *et al.*, 2005; Hansen *et al*., 2000; Kim *et al.*, 2006; Workneh *et al*, 2005). The key advantage of this technology is that it removes the need for a farm weather station, apart form selected units for ground truthing. Another key advantage is that the information can be made site-specific rather than using data from the nearest station. We expect this to be especially the case as radar is increasingly used to estimate rainfall at spatial resolutions of 1 km^2 (Workneh *et al*., 2005). The other advantage is that the simulated site-specific data can also be forecast allowing management decisions to be made prior to an infection event. As these

technologies mature and merge we expect the quality and resolution of weather data available to farmers to continually improve.

5.3 Canopy Microclimate

The third consideration for weather variable is corrections for canopy microclimate. In a plant canopy, relative humidity often decreases, while wind speed increases with height in a plant canopy (Oke, 1978). The effect is most pronounced in dense field crop canopies that restrict air circulation. For example in peanut, we have found that RH stays almost constantly above 90% once the canopy rows close together (Fig. 2).

The effect may be less pronounced in some horticultural crops where air can circulate both above and beneath the canopy. So for some crop canopies standard weather data which is measured over a turf environment may not give a good estimate of canopy weather variables. This is not a problem when the weather station is deployed in the canopy, but it is a concern for those using either a weather station deployed over turf, such as a weather station from a government or commercial network. It is also an issue for those using simulated site-specific inputs since these inputs must be 'downscaled' or corrected for the canopy. This downscaling will be especially important for the estimation of derived variables such as soil temperature and leaf wetness and also relative humidity which is highly sensitive to microclimate. In these cases there is only really one solution and that is to observe weather conditions in the plant canopy. This is not so nearly a daunting

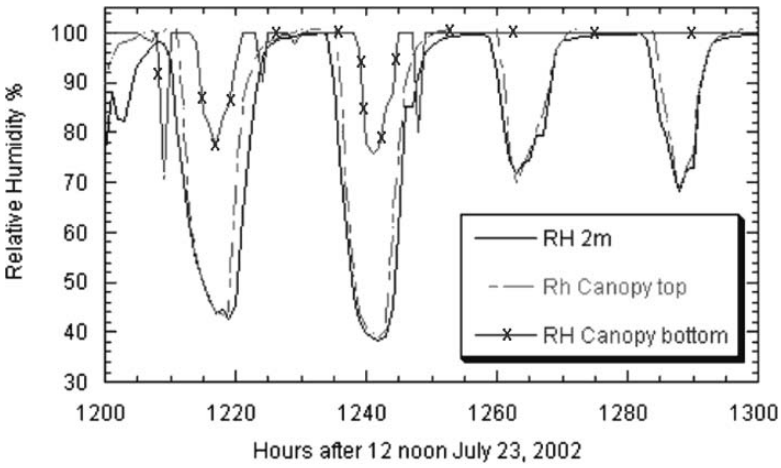

Figure 2. Comparison of relative humidity sensors above, at the top and at the bottom of a peanut canopy after row closure.

problem as even 10 years ago thanks to the proliferation of small low cost sensors that can be easily and quickly deployed in the canopy. Once the microclimate data are collected it is relatively easily to correct the data by a statistical relationship between standard weather station and microclimate weather data. There are also more sophisticated meteorological methods to derive canopy microclimate data, but these are beyond the scope of this chapter (Seem *et al*., 2000).

A consequence of the crop canopy profiles is that in field crops surface wetness duration after rain may be much longer in the bottom of the canopy than at the top (Huber & Gillespie, 1992; Magarey *et al*., 2005a). In contrast to this dew formation (dew falls) usually begins at the top of the canopy and may not saturate the entire canopy. Consequently the top of the canopy may have longer surface wetness durations after dew (Baxter *et al.*, 2005; Magarey *et al.*, 2005b). Exceptions to this may be in semi-arid climates where the soil is more important than the atmosphere for dew formation. It is really a good idea to visually observe the drying of the canopy after both rain and dew to understand the canopy surface wetness profile.

6. MODEL VALIDATION

Model validation might be either biological or commercial. Biological validation is the process of making sure that the model correctly predicts disease progress or risk under field conditions. Commercial validation should also test how well model output can be used to predict specific management options. This includes estimating action thresholds in model output units. This may be an important difference since a model output may be well correlated with disease progress but it might not be clear how to use the model output for management. Unfortunately, most published studies of infection models do not usually consider validation under field conditions. This is not only because these studies may take several years to complete but also because scientists who develop these models may be not be responsible for their use or application in the field.

There are many ways to validate an infection model and these include: *i)* exposure of trap plants; *ii)* historical comparisons; *iii)* management comparison with a routine spray program, and *iv)* expert opinion.

Probably the most popular method for biological model validation is using potted (trap) plants or harvested plant parts (e.g. fruits) exposed to natural or near natural field conditions (Aldwinckle *et al.*, 1980; Arauz & Sutton, 1989; Eisensmith & Jones, 1981; Grove *et al.*, 1985; MacHardy & Gadoury, 1989; Wilson *et al.*, 1990). In this approach, tagged plants are inoculated, placed near a known or harvested inoculum source or placed in an inoculum rich environment. The tagged plants are usually exposed to natural wetness events but artificial wetness such as misting or bagging may also used (Shaw *et al*., 1990). After each infection event or after certain time period, the plant or plant parts are removed and placed in a greenhouse or in a laboratory. Other studies may leave the infected plant in the field but this would probably only work in circumstances where natural inoculum and/or moisture sources were scarce. The advantage of this technique is that it provides a mixture of field and controlled laboratory validation. But this may be also a

disadvantage in that the methods and observations may be very similar to those with which the model was developed. Another disadvantage of this technique is that it is labor intensive.

Historical comparisons are another method that is useful for both biological and commercial validation. In this approach, infection model output is compared to disease observations or management recommendations over several years. Historical comparisons of model output with disease observations may be based upon correct prediction of discrete infection events (Jones, 1992), comparison with disease progress (Sutton *et al.*, 1986), comparison with disease outbreaks (Fidanza *et al.*, 1996) or comparison with seasonal summaries (Grunwald *et al.*, 2000; Spotts, 1977).

Disease observations may be recorded in various types of variety or fungicide trials. Disease observations may be end of season or may be frequently repeated such as those that are used in disease progress studies. Although the later may be the most rigorous for scientific comparisons there are several caveats. One is that disease progress measurements are very time consuming and usually require an unsprayed plot. The second limitation is that in most agricultural systems (especially high value crops) disease incidence and severity is kept to very low levels by fungicide application or other management practices. Consequently comparisons of model output to disease progress may be less than satisfying especially for commercial validation. Another limitation is that infection models do not predict disease progress, they predict periods of disease risk. Consequently, there must be some way to compare the observations and the predictions.

Validations may also be made with less scientific rigor by using other data sources. Most local agricultural consultants or extension agents have a good local knowledge of which seasons in recent history were severe and which ones were not. Some offices maintain records of crop loss or archive weekly advisories with risk ratings or fungicide applications per season. Although comparisons made using these types of data sources may not be the best for a biological validation, they may actually be the most useful for a commercial validation, since they answer the basic question — does the grower need to treat or take action? In the past historical comparisons were often frustrated because the weather data were usually not available for years and sites with disease observations or vice versa. Thanks to simulated historical site-specific weather information covered above, it is now relatively easy to create model output for specific location(s) and year(s). This output can be quickly summarized in a spreadsheet and compared with historical observations of disease or with historical management recommendations.

A sub-set of the historical comparisons method and one that is useful for commercial validation is comparison with multiple spray timings. The FAST model on pears was validated by creating 20 exposure periods each of four weeks duration during which time fungicide were not applied (Montesinos & Vilardell, 1992). Disease incidence and model output was then compared for each exposure period. Exposure periods might also be created on a smaller scale by inoculating tagged plants parts and then bagging them during the next spray application. A non-volatile

fungicide should be used. This technique was used for studies of ontogenetic resistance but could also be adapted for disease forecast model validation (Gadoury *et al*., 2003). These techniques are labor intensive, so rather than having to set-up a specific field trial for validation, it would be easier if it was possible to validate an infection model retrospectively using data collected for other studies. For some diseases with discrete infection events, it is possible to informally validate a model retrospectively by case studies where untreated infection events results in crop loss. A good example of this is grape downy mildew in south eastern Australia where infection events are rare and sporadic, making such comparisons relatively easy. In one case, a failed spray program was used to demonstrate retrospectively how to use a infection model to time sprays to determine if an infection period was missed and if a post-infection fungicide spray was needed (Magarey *et al*., 1991).

With advancing computing power it might be possible in the future to expand this technique using fungicide trial data. Often in fungicide trials, combinations of spray timings (for example early, mid or late season) are tested and disease intensity data are collected once or multiple times.

While a calendar schedule would include all of these sprays each year, a disease forecast model may enable some of these sprays to be skipped depending on weather conditions. Thus, it would seem possible that a comprehensive commercial validation could be made qualitatively or quantitatively by comparing different treatment schedules and disease outcomes with recommended sprays based on an infection model. The premise is that for each site and year combination it would be possible to determine which sprays in the program were the most important for preventing crop loss or maintaining low disease intensity. A disease model would 'fail' its validation if it: *i)* failed to recommended sprays that in the field trials maintained a low disease intensity or prevented crop loss; or *ii)* consistently recommended sprays that when missed in field trials did not result in increased disease intensity and/or crop loss.

Another option for commercial validation is to compare a calendar based program with a spray program based on an infection model. Often this type of validation will compare multiple action thresholds, so the best threshold can be selected. Usually, disease incidence or severity and the number of sprays is compared for calendar spray programs with the programs recommended by the infection model (Broome, *et al*., 1995; Cu & Phipps, 1993; Grunwald *et al*., 2000; Madden *et al*., 1978; Montesinos & Vilardell, 1992; Shtienberg & Elad, 1997; Vincelli & Lorbeer, 1989). It may also be important to compare amount, type, frequency of fungicide application, disease intensity at one or more times and yield. For the infection model to be useful it must either be more efficient or more effective than the calendar based program (Madden & Ellis, 1988). Another method for commercial validation is expert opinion (Stewart *et al*., 1998). In this case management recommendations from a model are compared to those made by a panel of experts for a real life data set of weather observations. There are some caveats with this process including the need to ensure there are enough 'challenging' decisions in each test validation data set.

7. INFORMATION DELIVERY

The final consideration for infection models is delivery of information to the end user (Xia *et al*., 2007). Unquestionably the internet is becoming the predominant method for communication. There are number of ways to convey infection model information over the internet. Text-based summaries remain one of the common methods for summarizing infection model output. One of the most practical methods is a table in which rows represent days and columns represent summarized weather variables and model output.

Graphs can also be used to summarize the same type of information. One of the most exciting methods is map-based tools that not only include disease forecasts but also include capabilities for real time survey and diagnostic data sharing and tools for extension specialists to make management recommendations or provide guidelines for growers.

The potential for this effort is illustrated by the development of the Legume Pest Information Platform for Extension and Education (L-PIPE) (Isard *et al.*, 2006). The L-PIPE was initially created in response to the incursion of soybean rust and was a collaborative effort with 30 US states. The purpose of the tool is to provide the public with a web based platform for extension and risk management for soybean rust (USDA-APHIS, 2005). The tool includes a map so users can zoom in and zoom out and a calendar so users can move forward or backward in time. The PIPE also includes additional menu selections for management guidelines, educational material and training opportunities.

The PIPE also has on-line tools for data collection via PDA, on-line forms or uploadable spreadsheets. Importantly, the L-PIPE is able to integrate data collection from diverse sources that included federal and state government, university, and industry.

There are many other issues related to the implementation and delivery of disease forecast models. A recent paper used the analogy with a water supply system to explain why some decision support systems fail, while others are never implemented and why some never meet the needs of those users whom the system was supposed to serve (Magarey *et al*., 2002).

In this chapter we have attempted to address the most practical considerations for the creation of infection models. It is our hope that it will serve as useful and practical guide for all aspects of infection model development from model design to implementation. We hope that some of the ideas and concepts suggested in this paper could be made available on-line in the form of a model development web site that could build upon the existing effort by UC Davis. Such a web site could contain a library of model types, parameter values for economically important plant pathogens, test data sets with validation data and publications related to disease forecasting.

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A REVIEW OF RESURGENCE AND REPLACEMENT CAUSING PEST OUTBREAKS IN IPM

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Abstract. Insect and mite pest resurgence occurs when an insecticide or acaricide treatment destroys the pest population and kills, repels, irritates or otherwise deters the natural enemies of the pest. The residual activity of the insecticide then expires and the pest population is able to increase more rapidly and to a higher abundance when natural enemies are absent or in low abundance. Replacement of a primary pest with a secondary pest occurs when an insecticide or acaricide treatment controls the primary pest and also destroys natural enemies of an injurious insect or mite that was regulated below an economic injury level by the natural enemies, thus, elevating the secondary pest to primary pest status. Disruption of natural controls is not always the cause of resurgence or replacement events. A dose-response phenomenon called hormesis can occur in pest populations exposed to sublethal doses of pesticides. This can cause an increase in fecundity (physiological hormoligosis) or oviposition behaviour (behavioural hormoligosis) of the pest leading to a significant increase in its abundance. Selective insecticides and acaricides coupled with natural enemies and host plant resistance have become the alternative methods more commonly used by growers that encounter these problems. The purpose of this chapter is to review pesticide-induced resurgence and replacement in modern cropping systems and methods for measuring and resolving these problems.

1. INTRODUCTION

Primary pest resurgence and replacement of a primary pest by a secondary one are two important consequences of chemical insect and mite control in agricultural systems. Resurgence and replacement were recognized as problems of use of synthetic organic pesticides on crops documented in over 50 cases soon after the broad scale application of these compounds in agriculture (Ripper, 1956). These problems have persisted up to the present day (Luck *et al*., 1977; Perkins, 1982; Pedigo & Rice, 2006). These complicated phenomena are not always solely caused by the removal of natural enemies: pesticide treatments also cause changes in the pest's behaviour, dispersal, development and fecundity indicating that resurgence and replacement may not be solely caused by destruction of the natural enemies of the pest (Hueck, 1953; Gerson & Cohen, 1989; Croft, 1990; Hardin *et al*., 1995).

The consequences of a resurgence or replacement event include: an increase in injury to the crop and potential losses in crop production (Dutcher *et al*., 1984; Braun *et al*., 1989); disruption of biological control programmes (Dutcher, 1983); an increase in management costs for additional chemical controls to prevent further injury (Horton *et al*., 2005) and, in perennial crops, an increase in the pest abundance that carries over to the next growing season (Dutcher, 1983; 2007).

A. Ciancio & K. G. Mukerji (eds.), General Concepts in Integrated Pest and Disease Management, 27–43.

Short term solutions range from application of the insecticide at a different time (Heyerdahl & Dutcher, 1985; Johnson *et al.,* 1976) or application rate (Sandhu *et al*., 1989) to switching to a pesticide that is safer to the natural enemies (Villanueva-Jimenez *et al.*, 1998) or switching to a pesticide with a different mode of action (Kerns & Stewart, 1999). In the long term, the pesticide causing the problem may be completely replaced by selective insecticides and acaricides (Grafton-Cardwell *et al*., 2005), new biological control agents (Hajek, 2004; Koch, 2003), conservation methods for natural enemies (Settle *et al*., 1996; Barbosa, 1997) and resistant crop varieties (Thomas & Waage, 1996). Selective pesticides, coupled with detailed on-site monitoring of the abundance of pest and beneficial species, research information on toxicity (Ruberson & Knutson, 2006) and sublethal effects of the pesticides to organism associated with the crop, have become important tools for growers that encounter these problems.

Research continues to demonstrate ways to integrate various pest control methods (Thomas, 1999). Growers now commonly integrate the new control techniques into their operations. A recent review of primary pest resurgence and replacement of the primary pest by a secondary pest has not been reported (Norris *et al*., 2002) and the purpose of this chapter is to review the types of pest resurgence found in modern cropping systems and methods for measuring and resolving these problems.

2. PRIMARY PEST RESURGENCE

Primary pest resurgence occurs when the target insect or mite population responds to an insecticide/acaricide treatment by increasing to a level at least as high (Hajek, 2004) or higher (Hardin *et al*., 1995) than in an untreated control or higher than the population level observed before the treatment (Pedigo & Rice, 2006). The resurgence may occur after the first application or after several applications of the insecticide/acaricide. Pest population outbreaks can be caused by many factors (Barbosa & Schultz, 1987) but pest resurgence occurs after a treatment of the crop with a chemical targeted at the pest population that is intended and expected to control the targeted pest.

The successful control of one primary pest can lead to an outbreak of a second primary pest when the two pest species feed on the same plant part. These outbreaks are not unexpected or unintended consequences of a pesticide treatment and are not part of this review. It is important for growers to consider the factors that can exacerbate the pest resurgence problem such as susceptibility of the variety to the pest, impact of fertilizer applications on pest reproduction and development, or pesticide treatments targeted to other pests.

The textbook example is the resurgence of California red scale populations in citrus after attempts to control it with DDT (Hajek, 2004). Recent examples of problems with primary pest resurgence are found in many cropping systems in the USA. Cyclamen mite is difficult to control with pesticides on biennial and perennial strawberry because they inhabit and increase in abundance in the protected areas of the developing leaves and flower buds. A key mortality factor, the predator, *Typhlodromus reticulatus* (Oudemans), typically runs across the

treated plant surface in search of prey and is more susceptible to acaricides. Many strawberries are now produced as an annual crop from transplants that are treated in hot water before planting and cyclamen mite is controlled (Zalom *et al*., 2005; Denmark, 2000). Petroleum oils applied on avocado in California to control mites can disrupt natural enemies leading to resurgence of Persea mite (USDA-ARS/CSREES, 2003a). Early season applications of pyrethroid and organophosphate insecticides, on cotton in Texas, destroy beneficial arthropods leading to resurgence of bollworms. Delaying early season bollworm sprays and switching from pyrethroids and organophosphates to biorational and microbial insecticides for bollworm control are not associated with bollworm resurgence. Dicrotophos initially controls aphids in Texas cotton but the aphid resurgence follows the application unless the dicrotophos is mixed with amitraz or profenophos (Stevensson & Matocha, 2005). Two-spotted mite and Banks grass mite rebound in field sweet corn after miticide treatments kill the phytophagous and predatory mites and not the phytophagous mite eggs (USDA-ARS/CSREES, 2003b; 2003c).

Broad spectrum insecticides are used in high-value fruit and nut orchards, vineyards, vegetable and tobacco farms, cotton, rice and wheat fields, and in turf and ornamental plant production when multiple pests attack the saleable parts of the plants. The organophosphate, carbamate and pyrethroid insecticides offer a rapid control technique in these situations, killing the pests on contact before they can injure the crop. The biorational insecticides often have to be ingested by the pests before the pests are killed and the crop is injured before the pests die. There is some trepidation among growers that cessation of the use of preventive sprays of broad spectrum insecticides and switching to more specific insecticides/miticides would lead to the resurgence of more problems. Lima beans with pod feeding damage cannot be sold at a premium price. Biorational insecticides that have to be ingested will not protect all the pods from injury. On New Jersey cranberry farms, the application of broad spectrum insecticides for control of multiple pests also controls the blunt-nosed leafhopper, a secondary pest and vector of a phytoplasma that causes false blossom disease. It is believed that the cessation of use of broad spectrum insecticides would lead to a resurgence of the leafhoppers and subsequently the disease. False blossom disease had a devastating effect on the industry in New Jersery between 1920 and 1960 reducing the productive area from 11,000 to 3,000 acres (USDA-ARS/CSREES, 2002c).

3. SECONDARY PEST RESURGENCE

Replacement of a primary pest with a secondary pest or a secondary pest outbreak occurs when a non-target, but injurious, pest population increases in a crop after it is treated with a pesticide to control a primary pest population (Hardin *et al*., 1995; Hajek, 2004). The increase is an unintended and unexpected consequence of the pesticide treatment. For example, pesticide sprays to control the codling moth, apple maggot and plum curculio on apple lead to resurgence of populations of white apple leafhopper, spotted tentiform leafminer, and European red mite

(Howitt, 1993). Season long sulfur sprays for control of powdery mildew on grapes often lead to resurgence of the spider mite, *Tetranychus pacificus* McGregor (Albers, 2002).

In Texas cotton fields, spider mites, aphids and whiteflies resurgence as a result of pyrethroid applications. Dicofol destroys predatory mite populations but not acarophagous insects and spider mite resurgence usually does not follow dicofol applications. Propargite kills spider mites more slowly than dicofol at temperatures above 29°C and mites will lay eggs before they die leading to resurgence in the hot summer months. An expensive solution to spider mite resurgence in cotton is the application of abamectin (Stevensson & Matocha, 2005).

In New Jersey peach orchards, resurgence of green peach aphid, a vector of Plum pox virus, is a result of the destruction of aphidophagous insects by pyrethroid insecticide sprays. Pyrethroid sprays prevent insect injury to the fruit (USDA-ARS/CSREES, 2002a; Horton *et al*., 2005).

In peanut fields of the southeastern USA, resurgence of the two-spotted spider mite is induced by the application of foliar sprays of insecticides and fungicides to control primary pests (USDA-ARS/CSREES, 2002b). Carbaryl applications for leafhopper control on carrots leads to aphid outbreaks (USDA-ARS/CSREES, 2000). The information gleaned from the USDA-ARS/CSREES pest management strategic planning documents cited in these last two sections are assemblages of actual research results and opinions of groups of experts in each cropping system. These documents serve to indicate that resurgence and replacement are current and important problems in agricultural systems in the USA.

4. DESTRUCTION OF NATURAL ENEMIES

The impact of pesticides on populations of beneficial insects or mites is an extremely important factor in the resurgence and replacement of pest populations. Though insecticides have been shown to cause increases in dispersal and fecundity in pestiferous aphids and mites and shorten the life span of mites (Hurej & Dutcher, 1994a; Gerson & Cohen, 1989), the destruction of natural enemies associated with the pest populations is most often assumed to be the cause of pest resurgence and secondary pest outbreaks. This brings us to the question: *Are natural enemies important regulating factors in a pest population?* If so, removal of natural enemies should result in an increase in abundance in the pest population greater than the abundance when the natural enemies were present. In natural populations of phytophagous insects, natural enemies may not always cause mortality that is proportionate to the prey population density and certain populations may not be regulated by natural enemies even when they are present (Dempster, 1983).

In agricultural systems phytophagous insect populations, the importance of natural enemies becomes apparent when they are destroyed by pesticides. For example, when leafminers on vegetables are treated with insecticides a moderate number of leafminers survive, and a low number of parasitoids survive resulting in an outbreak of the leafminer population in the next generation (Saito, 2004).

Simulation models indicate that simple density-independent mortality caused by natural enemies in agricultural systems is additive to mortality caused by other control tactics (Thomas, 1999). However, two or more species of natural enemies regulating the same pest population may have positive and negative interactions. Multiple species complexes of parasitic Hymenoptera have a strong regulatory effect on populations of leafmining lepidopterans in apple (Pottinger & LeRoux, 1971) and pecans (Dutcher & Heyerdahl, 1988). Conversely, ladybeetles feed on healthy aphids and parasitized aphids and reduce the impact of parasitism on aphid populations (Ferguson & Stiling, 1996). Generalist predators are not always synchronized with the pest populations and biological pest suppression is highly variable (Carroll & Hoyt, 1984; Dutcher, 1993). When generalist predators that control outbreaks are combined with specialist predators that control endemic populations, the probability of successful biocontrol is high. The introduction of the specific aphid parasite, *Trioxys pallidus* Haliday, greatly improved biological control of aphids in filberts and walnuts beyond the level of control provided by indigenous generalist predators (Messing & AliNiazee, 1988; Van den Bosch *et al*., 1979).

In other situations, specific natural enemies will achieve pest control. European red mite is controlled below economic injury levels by predatory mites in apple orchards (Cuthbertson *et al*., 2003; Hardman *et al*., 1985; Thistlewood, 1991). Parasites are important natural enemies and suppress diamondback moth populations in collards (Mitchell *et al*., 1997). The combined mortality imposed by multiple natural enemies of different types may control pests, e.g. predators, parasitic hymenopterans and viruses regulate populations of alfalfa loopers (Berry, 1998). Multicoloured Asian ladybeetle, on the other hand, is one species that controls many pests in several crops, e.g. aphids in pecan, apples, soybeans, scales in pine plantations, and multiple pests in corn and citrus (Koch, 2003). Temperature regulates insect and mite growth, development, reproduction and behaviour and the importance of insect and mite natural enemies changes with the ambient temperature as it affects the activity of the natural enemies and their abilities to attack prey (Skirvin & Fenlon, 2003).

5. HORMOLIGOSIS

Hormoligosis or hormesis is a phenomenon that occurs in the measurement of the dose-response to a series of concentrations of a chemical treatment. A low dose elicits a stimulatory response and a high dose elicits an inhibitory response (Calabrese & Baldwin, 2003). In these cases, the dose-response to the chemical treatment is an ∩-shaped curve and not linear or log-linear. That is, starting at a response of zero and a dose of zero (control level) and moving to higher doses the response is initially stimulatory and then inhibitory.

This type of relationship has been known to occur for over 75 years generally in biological systems and occurs in many dose-response experiments including the response of insect and mite reproduction to pesticides (Calabrese *et al*., 1999). DDT increases the egg production of European red mite (Hueck *et al*., 1952) and

granary weevil (Kuenen, 1958). Azinphosmethyl stimulates the reproduction of green peach aphid (Lowery & Sears, 1986a; 1986b). Other pesticides affect the reproductive rates of citrus thrips (Morse & Zareh, 1991) and western corn rootworm (Ball & Su, 1979). The reproductive rate of the brown planthopper increases when it is exposed to low doses of either deltamethrin or methyl parathion (Chelliah *et al*., 1980; Chelliah & Heinrichs, 1980). The fecundity of the two spotted spider mite increases after exposure to sublethal residues of carbaryl, DDT (Dittrich *et al*., 1974) or imidacloprid (James & Price, 2002). Green leafhoppers exposed to sublethal doses of imidacloprid had a lower reproductive rate than untreated green leafhoppers and hormoligosis did not occur (Widiatra *et al*., 2001). Whiteflies on cotton preferred to oviposit on plants treated with fenvalerate more than on plants treated with acephate and least on untreated plants. This was called behavioural hormoligosis (Abdullah *et al*., 2006).

Hormoligosis may occur anytime the pest is exposed to a sublethal dose. Pesticides, applied at lethal doses, are reduced to sublethal doses with time and exposure to climatic conditions in the field. The detection of hormoligosis requires detailed bioassays that measure the dose-responses of the insects and mites to a range of concentrations of the pesticide. The lowest dose and increments between doses in the bioassays need to be selected so that the low-dose stimulatory response can be detected and the higher doses should be selected to elicit inhibitory responses including a lethal response at the highest dose.

6. DETECTING AND MEASURING PEST RESURGENCE

Problems occur almost immediately when reviewing the literature to find actual documented examples of primary pest resurgence in field experiments or on farms. Many reports of pest outbreaks have insufficient pre-treatment and post-treatment data to ascribe the outbreak to resurgence (Hardin *et al*., 1995). The pest in the treated plot may increase to the same abundance as the untreated control but not be significantly higher than the population in the untreated control (Bagwell, 2005). Key pests are usually monitored intensively in crops and resurgence does not always progress to the point of an outbreak. When scouting information indicates that an outbreak is imminent, the crop is treated with an alternative chemical control tactic to prevent further injury to the crop and before the field can be examined for causes of the outbreak. Resurgence of polyphagous key pests that migrate between crops may not be detected if the resurgents leave the crop before the damage is detected. Often the phenomena will occur on farms with similar pesticide spray programs and commonalities in the management methods are assumed to be possible causes of the pest outbreak. Pest resurgence and replacement phenomena in cropping systems are difficult to recreate in an experimental setting, in order to elucidate the causes in sufficient detail to determine the relative importance of events leading to an outbreak.

Resurgence and replacement are often detected in insecticide efficacy trials. Efficacy (Abbott, 1925) and the resurgence index (Henderson & Tilton, 1955) are two well-known and practical formulae that are used to determine the overall effect

of an insecticide application. Abbott's formula for insecticide efficacy (E_A) measures the mortality caused by the insecticide in the pest population with a correction for natural mortality in the untreated pest population:

$$E_A = 100 \times (N_c - N_t) \div N_c$$

where. N_c = live individuals in the control after the treatment;
N_t = live individuals in the treatment after the treatment.

Henderson-Tilton's resurgence index calculates the change (E_{HT}) in pest population after the insecticide application:

$$E_{HT} = 100 \times [1 - (N_{ta} \div N_{ca} \times N_{cb} \div N_{tb})]$$

where, N_{cb} = live individuals in the control before treatment;
N_{tb} = live individuals in the treatment before treatment;
N_{ca} = live individuals in the control after treatment;
N_{ta} = live individuals in the treatment after treatment

The products of these formulae are percentages and simply describe the relative amount of change in insect abundances after a treatment and are not mathematical models describing the mechanisms causing population change. If pest abundance is greater in the treated than non-treated plants, each formula results in a negative value indicating that resurgence has occurred after the treatment. Abbott's formula will detect resurgence in pesticide trials where post-treatment estimates of pest abundance are recorded. Henderson – Tilton resurgence index can be calculated if pre-treatment and post-treatment estimates of pest abundance are recorded. Each method requires that pest abundance is monitored in an untreated control plot. A better approximation of the magnitude of the resurgence is determined by a resurgence ratio where the pest population is monitored for a period of time (30 days in Trumper & Holt, 1998) after the insecticide application and insect density is graphed over time in two plots - one treated and the other untreated. The ratio of the areas beneath the two curves (pest abundance area in treated over pest abundance area in untreated) indicates the intensity of the resurgence response (Trumper & Holt, 1998).

When the abundances of natural enemies are determined in the field studies, the information is of high practical value to growers. Among methods for evaluation of the importance of natural enemies (Luck *et al*., 1988), the insecticide-check method is commonly used (Lim *et al*., 1986) to assess natural enemies in crops. In the insecticide-check method, pesticides are used to selectively control natural enemies and not the pests to set up an array of situations in the field. The importance of parasitoids in regulating diamondback moth has been assessed by this method (Lim *et al*., 1986; Ooi, 1992; Muckenfuss *et al*., 1992). One advantage is the direct measurement of yield and quality of the crop under the different regimes created by the selective pesticides. The comparison to a standard and untreated control allows for measurement of the economic importance of

resurgence. For example, resurgence of tetranychid mites in cassava, following elimination of predators with permethrin sprays, resulted in economically significant reductions in yield (Braun *et al*., 1989). Direct observation of natural enemies in the field is also effective in evaluating the importance as biological control agents (Rosenheim *et al*., 1999). However, the abundance of the natural enemy and its dispersion over the field may differ considerably from the abundance and dispersion of the pest population and a separate sampling method is required to make a precise and accurate estimate of these parameters.

Detailed direct observations following the treatment are the best method to determine the cause or mechanism leading to resurgence or secondary pest outbreak. Causes of resurgence are ecological (destruction of natural enemies and altering insect behaviour) (Ripper, 1956; Margolies & Kennedy, 1988; Hurej & Dutcher, 1994a; 1994b), physiological (increasing fecundity) (Luckey, 1968; Widiarta *et al*., 2001) or both (stimulating oviposition behaviour) (Abdullah *et al*., 2006).

The highest level of confidence in making a change in spray recommendations is achieved when field results are coupled with laboratory or greenhouse bioassays of the relative toxicities of the insecticides used on the crop to pest and its associated natural enemies. These studies (Kerns & Stewart, 1999; Widiarta *et al*., 2001; Rebek & Sadof, 2003; Abdullah *et al*., 2006) require more time than is often available for the application of a short-term solution of the problem and are used to develop long term alternatives to the chemical control causing the offence in the first place.

The causes of pest population outbreaks after pesticide applications are difficult to determine without information on the pest and natural enemy and host plant populations before the occurrence of the pesticide treatment and resurgence event (Hardin *et al*., 1995). It is important to determine whether ecological or physiological mechanisms (or both) are causing the problem before changing the control practices. Pests can resurgence to higher population levels than is possible from simple release from natural enemies – e.g. the population increase response of pecan aphids to carbaryl treatments is higher than the intrinsic rate of increased measured in clip cages on untreated leaves (cf. Dutcher, 1983 and Kaakeh & Dutcher, 1992) - and presumably more than one factor is causing the resurgence.

7. PROBLEMS AND SOLUTIONS

Successful integrated control techniques for spider mites, leafminers and aphids are now in place in many cropping systems in the U.S. The problems associated with resurgence and replacement are assumed to primarily be caused by pesticide toxicity and sublethal effects of pesticide residues to various species in the cropping systems. Current solutions integrate proactive avoidance of pesticides that induce resurgence and replacement and development and implementation of alternative controls.

Spider mite population outbreaks often occur after the application of pyrethroid insecticides on crop plants. Pyrethroids act as toxicants, repellents, irritants and

antifeedants to the spider mites and associated phytoseiid mites (Penman & Chapman, 1988; Gerson & Cohen, 1989; Holland *et al*., 1994; Margolies & Kennedy, 1988). These actions alter the main factors controlling the population dynamics of the mites. Changes in the dispersal of the mites on the plant, the reproductive rate, and life span of the mites lead to mite abundance that is much higher than on untreated plants. Mites respond differently to plant surfaces treated with pyrethroid insecticides than to untreated surfaces. They select untreated surfaces for oviposition and egg production is inhibited on pyrethroid-treated surfaces. Spider mite behaviour on azinphosmethyl and carbaryl-treated leaf surfaces was similar to untreated surfaces (Penman *et al*., 1981). Pyrethroids increase the dispersal of mites from the plant. Mites run-off and spin-down from pyrethroid-treated surfaces and remain on untreated surfaces (Holland *et al*., 1994).

The pyrethroids repel spider mites as well as phytoseiid mites increasing dispersal and evening the distribution of mites on the plant. Spider mites on pyrethroid treated plants produce less webbing and more eggs than on untreated plants. Spider mite populations on pyrethroid treated plants may have a different sex ratio than on untreated plants if the pyrethroid is more toxic to one sex over the other (Gerson & Cohen, 1989).

Any single change or combination of changes may lead to spider mite resurgence. Simulation of a pyrethroid-induced spider mite outbreak on cotton indicated that changes in fecundity had the least affect; duration of the development time had an intermediate affect; and increased survival had the most significant affect on the induction of the mite outbreak (Trichilo & Wilson, 1993).

Integrated control programmes for phytophagous mites have solved many instances of mite resurgence. Selective acaricides are combined with the release of predatory mites to achieve sustained control in orchards and greenhouses. Long term solutions of replacing pesticides with sustainable biological controls are improving in effectiveness, especially for the release of predatory mites to control phytophagous mites. Currently, commercial insectaries produce very high quality predatory mites for release at a reasonable price. Predatory mites that can survive treatment with insecticide sprays for fruit and nut pests and overwintering in the orchard have been effectively integrated into orchard pest management. Predatory mite release is less costly when the predators become established in the orchard and do not have to be released each season (Dutcher, 2007). Apple growers that release predatory mites also conserve the predatory mites by spraying a safe insecticide for fruit pest control in an integrated pest control system. Hand dispersal of predatory mites evenly across the orchard in the fall by fastening foliage from trees with high mite density to new sites is also effective (Breth & Nyrop, 1998). Integration of biocontrol of twospotted spider mites with releasing predatory mites and chemical control of thrips with spinosad on ivy geranium in greenhouses is possible since spinosad controls thrips and does not disrupt predatory mites (Holt *et al*., 2006).

Leafmining insects rarely reach primary pest status where they are indigenous and are not exposed to pesticides. Hymenopterous parasitoids are typically key mortality factors common to many insect populations with leafminer larvae and removal of parasitoids by sprays for control of fruit feeding pests leads to

replacement (Pottinger & LeRoux, 1971; Dutcher & Heyerdahl, 1988). On grapefruit plants in the nursery it is possible to prevent primary pest resurgence of citrus leafminer, *Phyllocnistis citrella* Stainton, by the integration of biological control with the parasitoid, *Ageniaspis citricola* Logvinovskaya, and chemical control with azadirachtin or diflubenzuron and not abamectin. Abamectin at 0.1 times the lowest recommended field rate did not cause a resurgence of the citrus leafminer but did cause a significant reduction in the parasitism rate of leafminers by *A. citricola* (Villanueva-Jiménez *et al*., 1998). Pea leafminer, *Liriomyza huidobrenis* (Blanchard), a primary pest of potato in South America is difficult to control with adulticides due to insecticide resistance. The insecticide, oxamyl, is effective against the larvae in the mines and is relatively safe to parasitoids. Leafmining flies are difficult to control with older broad spectrum insecticides and the triazine insect growth regulator, cyromazine, is an effective treatment for control of larvae (Weintraub & Horowitz, 1995).

Aphids are among the more difficult insects to control and are often considered as superpests for their ability to reproduce rapidly through alternation of sexual and asexual reproduction. Furthermore, aphids are often vectors of plant diseases. Aphids are also able to detoxify pesticides and have a strong propensity to develop resistance to insecticides. Natural enemies are important mortality factors and resurgence of aphids is common after spray applications for primary pests (Dutcher, 1983). In the short term, resurgence of aphids has been solved by additional treatment of the crop with a neonicotinoid insecticide, such as, imidacloprid. Imidacloprid is currently the most widely used insecticide worldwide primarily due to its high efficacy and long residual activity against aphids, and to some extent other Sternorrhyncha - leaf hoppers and spittlebugs. Long term solutions to these pests outbreaks depend on whether or not the aphids, leafhoppers or spittlebugs are vectors of plant diseases. Non-vectors can be suppressed or even controlled below economically injurious population levels with conservation of natural enemies (Barbosa, 1997; Pickett & Bugg, 1998; Dufour, 2001). Growers have a lower tolerance for disease vectors and crop rotation, new pesticides and host plant resistance are used for control (Rocha-Peña *et al*., 1995).

Integration of two or more insect/mite pest control techniques as an alternative to control with a single technique has been successful (Pedigo & Rice, 2006) by combining: host plant resistance and natural enemies (Thomas & Waage, 1996); insecticide/miticide treatments followed release of natural enemies pest population monitoring and treatment of emerging problems with selective insecticidal/miticidal materials (Holt *et al*., 2006); and, monitoring pest and beneficial insect and mite populations and timing the treatment with a nonselective insecticidal material when pests and not beneficials are active (Heyerdahl & Dutcher, 1985).

Mango requires annual repeated treatments with pesticides for fruit flies, tree borers, seed weevils and leafhoppers as key pests. The crop is under attack by a these pests over the main portion of the growing season and pesticides are applied over the entire growing season to prevent injury to the crop. Pesticide treatments for fruit flies cause resurgence of scale insects. Growers use integrated pest management based on sampling and economic thresholds, use biopesticides and fruit fly resistant cultivars to prevent scale outbreaks (Peña *et al*., 1998).

Irrigated rice fields in Java and Indonesia have a high level of natural biological control. Experimental chemical exclusion of generalist predators in the early season lead to pest resurgence in the late season. Experimental increase in organic matter caused an increase in alternate prey for generalist predators and subsequently and increase in predator abundance. Tropical rice growers in the region are taught to conserve generalist predators by limiting pesticide sprays and increasing organic matter content of the fields (Settle *et al*., 1996).

In pecan orchards, pecan scab, pecan weevil and stink bugs are primary pests that are controlled with preventive applications of broad spectrum pesticides. Fungicide sprays for pecan scab control also destroy entomopathogenic fungal pathogens of pecan aphids (Pickering *et al*., 1990). Pyrethroids and carbaryl used for control of late season pecan weevil and kernel-feeding hemipterans destroy aphidophagous insects (Dutcher, 1983) and repel or kill predatory mites. The resulting secondary outbreaks of aphids and mites are controlled with additional aphidicides and miticides. The costs of pecan pest control with multiple sprays, tank mixtures and soil applied system insecticides can outweigh the value of the nut crop (Hudson & Pettis, 2005). Pecan producers now have alternative controls for primary pests that are not as harmful to natural enemies and resurgence is not common. Pecan producers have replaced chlorpyrifos with tebufenazide, diflubenzuron, or spinosad sprays for control of pecan nut casebearer and hickory shuckworm. Pecan weevil and stink bugs are controlled with broad spectrum insecticides, carbaryl and pyrethroids. Spray timing is improved with trapping of adults for weevil and stinkbugs and trap cropping for stinkbug. Resurgences of aphids and mites, following these sprays are controlled with additional chemical control - soil-applications of aldicarb or imidacloprid or foliage treatments with aphidicides and miticides. Additional biological control tactics are integrated into pecan orchard management for additional long-term suppression of aphids and mites, including: intercrops and food sprays to enhance aphidophaga (Dutcher, 2004); the introduction of predators and parasites for control of aphids; release of predatory mites (Dutcher, 2007); climatic monitoring to reduce the frequency of fungicide sprays and increase the survival of aphid pathogens (Pickering *et al*., 1990); and trap crops for control of stinkbugs.

Application of insecticides and acaricides with selective toxicity is one method to control pests while conserving beneficial insects and mites. Implementation requires laboratory bioassays to determine the relative toxicity of a pesticide to the predatory and parasitic insects and mites where beneficial insects are exposed to the pesticide. Assessment of beneficial insect and mite populations in field efficacy trials is also an important source of information of selectivity of pesticides. Integrated pest management of diamondback moth on collards in Virginia is possible with alternative insecticides that are less toxic to beneficial insects (Alonso, 2005). This author found that natural mortality of the diamondback moth was 98-99%. Certain broad spectrum insecticides were toxic to parasitoids at 1% of the field application rate. Methoxyfenozide, however, was less toxic than the broad spectrum and effectively controlled diamondback moth.

Foliage-feeding spider mites commonly resurgence to high abundance after pyrethroids and organophosphate sprays are applied to orchards for fruit pest

control. Toxicities of broad spectrum pyrethroids and organophosphates to phytophagous and predatory mites differs between the two types of mites. Cypermethrin and fenvalerate were more toxicity to predatory mites and less toxic to phytophagous mites than azinphosmethyl (Wong & Chapman, 1979). Azinphosmethyl has been selected over pyrethroids for fruit pest control in apples to conserve predatory mites (Penman *et al*., 1981). Fortunately, newer chemical controls with physiological selectivity in favour of natural enemies that do not cause these problems (Ruberson & Knutson, 2006), continue to replace the older chemicals and resurgence and replacement are no longer problems without, at least, short term solutions.

The toxicity of pesticides to pests and beneficial insects and mites differs between locations, environmental conditions and depends on the amount of exposure the insects and mites have had to the pesticide, how long the test organisms have been in artificial culture and many other extraneous factors. Even current ratings of pesticide safety or efficacy to insects and mites have to be used with caution. Growers with limited arsenals of new pesticides can selectively control the primary pest and not disrupt natural enemies with broad spectrum pesticides through ecological selectivity - timing of the treatment of the main crop before natural enemies are abundant; controlling the pests with pesticides in a trap crop planted outside of the main crop; preserving natural enemies on untreated crops planted outside of the main crop and further conserve natural enemies by habitat management (Barbosa, 1998; Pickett & Bugg, 1998) and biointensive integrated pest management (Dufour, 2001).

8. CONCLUSIONS

Pest management strategies for control insects and mites in cropping systems have undergone significant changes in the uses of new chemical pesticides and the integration of chemical and biological controls. Growers are adopting these new insect and mite pest management strategies to conserve biological control agents, reduce pesticide spray costs, achieve more effective control of key pests and reduce hazardous environmental effects of certain chemical pesticides. These new strategies also reduce primary and secondary pest resurgence.

Solutions to the resurgence and replacement problems following insecticide/acaricide applications to crop plants require two dynamic components – new technology and new information. Growers, in the U. S., currently access new information generated by state agricultural experiment stations, the pesticide industry and the U.S.D.A through cooperative extension programs including online publications, articles in grower-oriented publications, telephone call-in “hotlines”, production meetings, on-site visitation by scientists and specialists, diagnostic services, and field days.

Resurgence and replacement are common topics at the meetings and field days and proportionally more biorational insecticides (Grafton-Caldwell *et al*., 2005) are used today than five years ago. Growers require better technology for: monitoring pest and beneficial insect and mite populations, adjuvants to increase the retention

of a lethal dose of pesticide on the host plant; and prediction tools to improve timing of conventional insecticide treatments and successfully deploy biological control strategies (host plant resistance, natural enemies and microbial pesticides) and biorational insecticides.

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THE ROLE OF PLANT DISEASE EPIDEMIOLOGY IN DEVELOPING SUCCESSFUL INTEGRATED DISEASE MANAGEMENT PROGRAMS

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Abstract. The role of initial inoculum (y_o), rate (r) of pathogen or disease development (infection), and period of time (t) that the pathogen and host populations interact during the cropping period is revisited in modeling plant disease epidemics. The importance of quantitative informations and the relationship between initial inoculum and the rate of disease development represent key elements for identification of the most useful disease models to be used. For effective Integrated Disease Management of monocyclic or polycyclic epidemics, temporal population growth models of plant disease epidemics (monomolecular, exponential, logistic and Gompertz population models) are presented. Sanitation and disease management principles (exclusion, avoidance, eradication, protection, resistance and therapy) are described. Finally, the integration of IPM practices, at the disease components level, with Lieberg's Law of the Minimum is discussed.

1. INTRODUCTION

The primary objective in integrated disease management is to keep disease intensity below an economic injury threshold (Nutter, 2001; Zadoks, 1985) and thereby prevent reductions in crop yield and quality that negatively impact the producers' chance of making a profit and sustaining their farm enterprise (Mills & Nutter, 1991; Nutter & Guan, 2001). Disease management involves the integration of tactics to achieve one or more strategic goals. These goals are: *i*) eliminate or reduce initial inoculum, *ii*) reduce the rate of infection and/or *iii*) reduce the time that pathogen populations and host populations interact, to reduce disease intensity.

1.1. Importance of Quantitative Informations on y_o, r, and t

Quantitative knowledge concerning three key epidemiological parameters is paramount to develop cost–effective integrated disease management programs (Nutter *et al*., 1991). These key parameters are: *i*) the level of initial inoculum (y_o), assessed as either the initial pathogen population or initial disease population, *ii*) the rate (r) of pathogen or disease development (infection), and *iii*) the period of time (t) that pathogen and host populations interact during the cropping period, which is often measured in days for most annual crops and in years for perennial crops, (e.g. orchards, vineyards, ornamentals, etc.). Of these

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three parameters, quantitative information concerning the rate of disease development "r" is of great strategic importance.

Quantitative information concerning r provides a critical roadmap towards the ultimate goal of developing an integrated disease management program that will economically reduce disease risk, maintain the health and sustainability of the agroecosystem, and minimize crop losses (Madden & Nutter, 1995; Nutter, 1999; Nutter & Guan, 2001; Savary *et al.*, 2006). Moreover, the rate of disease development "r" can also be taken as a measure of disease risk (R).

If development (infection) "r" is very fast for a particular pathosystem, then the primary strategy would be to employ disease management principles that reduce "r". Disease risk (R) must be sufficiently lowered to a strategic threshold, below which the integration of other disease management principles – such as those that reduce initial inoculum (y_o) and/or reduce the period of time for disease development (t) – will provide an epidemiological benefit that further reduces disease risk. However, if the rate of disease development (r) is low, as in many pathosystems involving soil-borne pathogens, then the best strategy to reduce disease risk would be to employ disease management principles that reduce initial inoculum (y_o). Reductions in y_o delays the time to reach disease onset (y = 0.05) or any other specified level of y (e.g. an economic injury threshold).

1.2. The Relationship Between Initial Inoculum (y_o) and the Rate of Disease Development (r)

For producers to cost-effectively reduce disease risk, the epidemiological affects of specific disease management principles on reducing "y_o" and/or "r" must be known. A useful tool to help set strategic priorities regarding the interactive effects of reducing initial inoculum and the rate of disease development on plant disease epidemics is the sanitation ratio (Van der Plank, 1963). This simple calculation provides an estimate of how r and y_0 interact to delay the time it takes to reach any specified level of disease intensity (y) (e.g., disease onset, the time to reach 50% disease intensity, the time to reach an economic injury threshold, etc.).

The sanitation ratio is calculated by estimating the % reduction in initial inoculum resulting from one or more sanitation practices that reduce y_0, along with an estimate of the rate of disease development (r). The equation is:

$$\text{SR (delay in time)} \quad = \ln\left(\frac{y_o}{y_{ws}}\right) \cdot \frac{1}{r}$$

where the sanitation ratio (SR) is the estimated delay in time (t) required to reach a specified level of disease intensity, y_o is the level of initial inoculum present without sanitation (i. e. 100%), y_{ws} is the percentage of initial inoculum remaining after one or more sanitation practices performed (e. g., y_{ws} = 100% - 95% reduction in y_o due to crop rotation = 5%), and r is the estimated rate of disease development (change in transformed y vs. time).

For example, if r is estimated to be 0.2 ln units/day and a biological control agent is estimated (or known) to reduce initial inoculum by 90%, then the estimated delay in time (shift of disease progress curve to the right in time) resulting from the use of the biological control agent would be:

$$SR = \ln\left(\frac{100\%}{100\% - 90\%}\right) \cdot \frac{1}{0.2 \text{ ln}/\, day} = 12 \; days$$

Thus, in this example, the disease progress curve would be shifted 12 days to the right (12 days delay in the epidemic) (Fig. 1). If r in this example was reduced from 0.2 to 0.1 ln units/day by also using a resistant host cultivar, the delay in time would be 23 days. If r could be further reduced from 0.1 ln units/day to 0.02 ln units/day, the delay in the epidemic would be 115 days, which is usually sufficient for many annual crops to produce acceptable yields.

Conversely, when r is high, disease management principles that reduce initial inoculum (y_o) may not sufficiently delay the epidemic onset in order to reduce the disease risk and prevent crop losses. The relationship between r and reducing y_o by sanitation is shown in Table 1.

Table 1. Effect of the interaction between the rate of disease development (r) and the percentage reduction in initial inoculum (y_o) on delaying the insurgence of a plant disease epidemics.

	Percentage reduction in inoculum at source					
r [a]	20	50	80	90	95	99
0.02	11	35	80	115	150	230
0.1	2	7	16	23	30	46
0.2	1	4	8	12	15	23
0.4	0.5	2	4	6	7.5	12
0.6	0.3	1	2	3	5	6

[a] Rate of disease development.

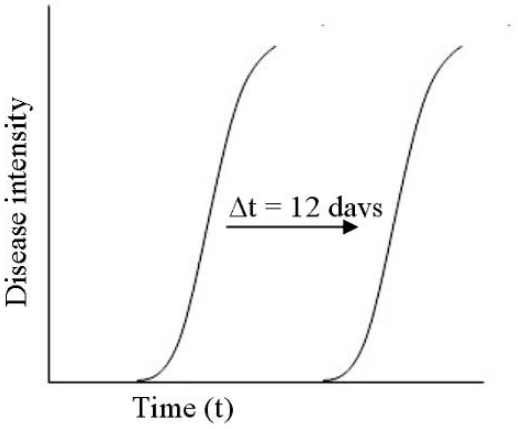

Figure 1. Theoretical delay in time of a plant disease epidemic as affected by sanitation practices reducing the initial inoculum (y_o) by 90%, if the rate of disease development is 0.2 in units/ day. The delay in time (t) in this example corresponds to 12 days.

The important concept gleaned from this table is that the lower the rate of disease development r, the more effective sanitation practices that reduce y_o become in delaying the epidemic. Conversely, the higher the rate of disease development (r), the less effective sanitation is in delaying the epidemic. Thus, disease management principles that reduce r must first be employed to sufficiently reduce this parameter to a rate below which disease management principles reducing y_o will provide a beneficial return on investment, in terms of keeping disease risk below the economic threshold (Nutter & Mills, 1990; Zadoks, 1985). Zadoks and Schein (1979) stated that, with regards to polycyclic pathosystems in which the pathogen has a high r and a short latent period, sanitation practices are generally not worthwhile.

1.3. Reducing y_o, r, and/or t for Effective Integrated Disease Management

Plant disease epidemics generally fit one of two main processes regarding the production of new dispersal units: the epidemics are monocyclic or polycyclic. Dispersal units can be defined as any recognizable device used for spread and survival of the pathogen, e.g. conidia, ascospores, basidiospores, mycelium, sclerotia, virus-infested (or infected) insect vectors, etc. (Zadoks & Schein, 1979).

Monocyclic pathosystems are so named because dispersal units (inoculum) are produced just once per growing season, and cannot become infection units (potential inoculum) until the next time a susceptible crop is grown and the environment is favorable for infection. However, when pathogen infection results in the production of new dispersal units that can become infective during

the same growing season (i.e., resulting in two or more disease cycles per cropping season), such epidemics are defined as polycyclic.

1.4. Selecting the Best Model to Estimate y_o, r, and t

The selection of a temporal model that best describes the temporal progress of plant disease epidemics involves consideration of both subjective and objective criteria (Madden, 1980; Nutter, 1997). The temporal population growth models commonly used to analyze plant disease epidemics are the monomolecular, exponential, logistic, and Gompertz population models (Zadoks & Schein, 1979; Nutter, 1997; Nutter & Parker, 1997). Expressions for the absolute rate of change in y with t (dy/dt) and the integrated and linearized forms of these models are given in Table 2. To select the most appropriate population growth model for disease progress datasets, several steps are involved (Campbell & Madden, 1990). One of the first steps is to graph y (usually disease or pathogen incidence or severity) versus time t. The shape of the disease/pathogen progress curve is very helpful in identifying the best model.

The next step is to graph the estimated dy/dt versus t. The shape of these curves will also help to identify the model that will best fit disease (and pathogen) progress. If the appropriate model is chosen, the plot of the transformed y versus time (t) should approximate a straight line.

After viewing these graphs, objective criteria for the acceptance or rejection of a model are provided by the estimated regression parameters and statistics, i. e. the r^2, cv, SE, and standard deviations of parameter estimates (Campbell & Madden, 1990; Nutter & Parker, 1997). Finally, the inspection of residual plots reveals if the model is satisfactory.

Table 2. Four population growth models used to describe temporal disease progress.

Model [a]	*Integrated expression* [i]	*Absolute rate equation*	*Linearized equation*
Monomolecular	$y = 1-(1-y_o)\exp(-r_M t)$	$dy/dt = r_M(1-y)$	$Ln[1/(1-y)] = \ln[1/(1-y_o)] + r_M t$
Exponential	$y = (y_o)\exp(r_E t)$	$dy/dt = r_E y$	$\ln(y) = \ln(y_o) + r_E t$
Logistic	$y = 1/\{1+[(1-y_o)/y_o)]\exp(-r_L t\}$	$dy/dt = r_L y(1-y)$	$Ln[y/(1-y)] = \ln[y/(1-y_o)] + r_L t$
Gompertz	$y = \exp\{\ln(y_o)\exp(-r_G t)\}$	$dy/dt = r_G[-\ln(y)]$	$-\ln[-\ln(y)] = \ln[-\ln(y_o)] + r_o t$

[a] y: disease intensity; t: time; r: rate parameter (i: code for models; r_M = rate for the monomolecular model; r_E = rate for the exponential model; r_L = rate for the logistic model; r_G = rate for the Gompertz model); y_o: constant of integration, corresponding to *y* at t = 0.

1.4.1. The Monomolecular Model

Plant disease pathosystems having a single cycle during the growing season are often best described by the monomolecular model (Table 2). The absolute rate of equation takes the form:

$$\frac{dy}{dt} = r_m\,(1-y)$$

The monomolecular model assumes that dy/dt is greatest at the beginning of the epidemic and that it slows in direct relation to the remaining amount of disease-free (or pathogen-free) plant tissue (1- y). For simplicity, it is assumed here that y is 1 (100%). The expression 1-y accounts for the constraints to further disease increase caused by the lack of healthy plants. At low levels of disease (y), the expression is $dy/dt \approx r_m$, and therefore dy/dt is not directly dependent on y and is similar to the linear model.

This model is also called the negative exponential model (Campbell & Madden, 1990) or the 'simple interest' model (Van der Plank, 1963). The monomolecular population growth model is applied to epidemics for which there is no spread from plant to-plant, i. e. there is no secondary spread within a growing season. This model can also be used to quantify temporal changes in cumulative virus incidence over time. For example, Jones (1979) annually recorded the incidence of aphid-borne viruses in raspberries based on visual symptoms beginning at planting (May 1971) through 1978. The disease progress curve for incidence of raspberry virus over the 8 year period is shown in Fig. 2A. The rate curve dy/dt decreases with time for this model (not shown) indicating that it provides a good fit to this epidemic. This is further indicated by the regression line and equation in Fig. 2B, with time explaining 96.9% of the variation in transformed virus incidence.

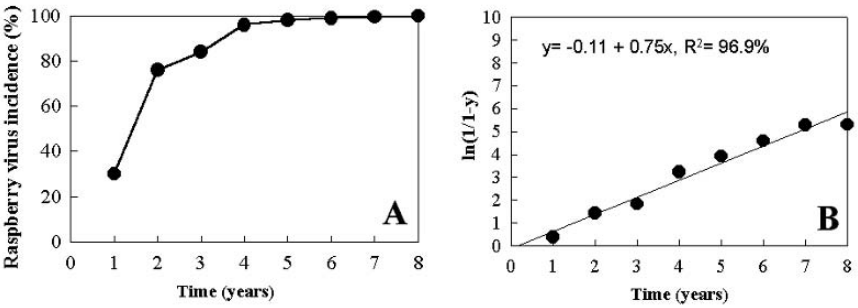

Figure 2. Disease progress curve for incidence of aphid-borne viruses in raspberry (A). Linear regression and equation using the monomolecular model (B).

1.4.2. The Exponential Model

The exponential model (Table 2) (also known as the logarithmic, geometric, or Malthusian model) is the simpler of the two 'compound interest' models of Van der Plank (1963). It is appropriate for polycyclic pathogens when they spread from plant-to-plant over time. That is, newly diseased (infected) individuals lead to more diseased (infected) individuals, within the same growing season. The exponential model dates back at least to Malthus who, in 1798, used it to predict future increase in the human population. The absolute rate equation takes the form:

$$\frac{dy}{dt} = r_E y$$

This model assumes that dy/dt increases throughout the epidemic and that the absolute rate of increase is directly proportional to the present level of disease intensity (y) as well as the rate of infection r_E. Therefore, this model implies that the carrying capacity of the host crop does not limit the absolute rate of disease increase. Disease (or pathogen) progress curves are typically J-shaped and lack an inflection point. A plot of the rate curve, dy/dt vs. time, also has no inflection point and dy/dt increases with time.

The exponential model has been used to model changes in disease prevalence on a geographic scale (Ward *et al.*, 1999) and can be applied to describe the very early phases of most polycyclic (compound interest) epidemics. This is because, when y is very small, there is little effect on the population of healthy plants or host tissue (1 - y) remaining to be used as a potential food source by the pathogen population. Figure 3A shows a pathogen progress curve for the cumulative number of US counties found to have Asian soybean rust (*Phakopsora pachyrhizi*) in commercial soybean over time in 2006. The exponential model was the most

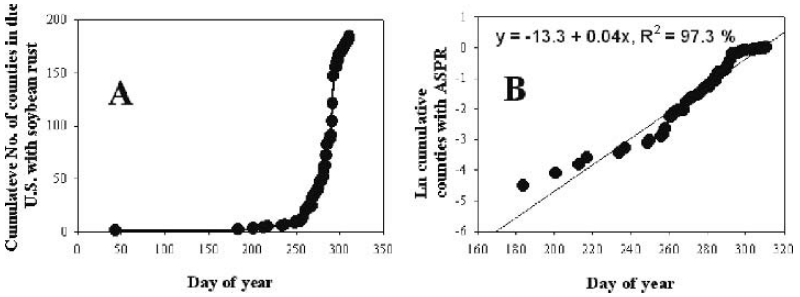

Figure 3. Cumulative number of counties in the U.S. with Asian soybean rust in 2006(A), and the linear regression line and equation using the exponential model (B).

appropriate to obtain a linear relationship between ln (y) and t, with time explaining 97.3% of the variation in ln (y) (Fig. 3B).

1.4.3. The Logistic Model

The logistic model (Table 2) was proposed by Verhulst in 1838 to represent human population growth and is probably the model most frequently used to describe plant disease epidemics that are polycyclic. It is the second type of compound interest model proposed by Van der Plank (1963) and may be appropriate for most plant disease epidemics, where there is plant-to-plant spread within the crop. Disease (and pathogen) progress curves typically have a characteristic sigmoid (S-shaped) form, with an inflection point at the time when disease intensity reaches a proportion of 0.5 (50%). The absolute rate equation takes the form:

$$\frac{dy}{dt} = r_l y(1-y)$$

The absolute rate curve (not shown) is symmetrical, with the highest rate occurring when y = 0.5 (50%). A biological interpretation is that, early in the epidemic, dy/dt accelerates as the level of disease (or pathogen) intensity (y) approaches 0.5 because an increasing number of newly diseased plants become infectious and contribute to additional diseased plants during the same growing season. At later stages of an epidemic when y > 0.5, the diminishing healthy plant tissue (green leaf area), represented by 1-y limits the rate of disease/pathogen increase. Thus, the absolute rate (dy/dt) of disease increase is proportional to the proportion of non-infected plant tissue late in the epidemic (1-y), the level of disease (or pathogen) intensity early in the epidemic (y), and the logistic rate of infection (r_l).

A typical S-shaped disease progress curve is demonstrated in Fig. 4A for the disease caused by *Bean pod mottle virus* (BPMV) in soybean. Soybean plants were planted on 5 May 2006 (day of year 125) and plants were tested for the presence of BPMV by ELISA. The first diseased plants were detected on 30 May (day of year 150) and BPMV disease incidence reached 99% by 17 August (day of year 229). The logistic model provided the best fit to this dataset, resulting in a linear relationship between logit BPMV incidence and day of year (Fig. 4B).

The slope (r_l) of the regression line for logit y versus t was 0.11 logits/day for both epidemics (insecticide versus the control treatment), which indicates that two insecticide applications (V1 and R2 growth stages) did not provide an epidemiological benefit in delaying the BPMV epidemic in 2006. The rate of infection was 0.11 logits per day which indicates that BPMV incidence was doubling every 6.3 days. It should be noted that r_l is an overall measure of the host, pathogen and environment interaction, including vector population dynamics. Using this model, the independent variable (time) explained 93-99% of the

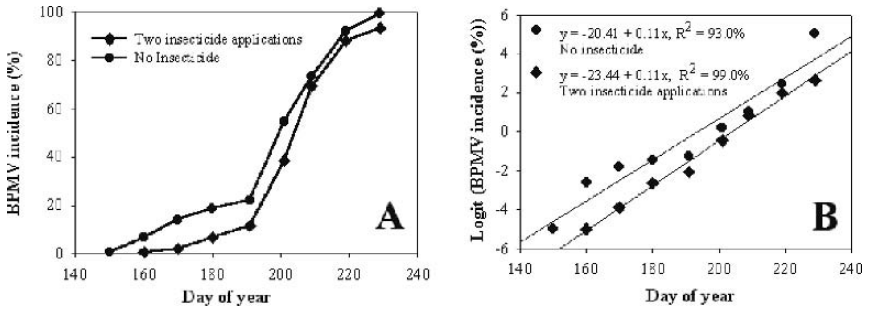

Figure 4. Pathogen progress curves of Bean pod mottle virus (A, incidence) in soybean variety NB 3001 at the Iowa State University Farm in 2006. The linearized model and logistic equation (B) show the relationship between day of year and logit BPMV incidence.

variation in logit disease incidence (y) indicating that the logistic model provided a good fit to the data.

1.4.4. The Gompertz Model

The Gompertz model (Table 2) is borrowed from an animal growth study and was originally proposed in 1825 (Madden, 1980). As with the logistic model, the progress curve has an inflection point, but it is located when y = 0.37 (1/e), and a large portion of the area under the rate curve is located to the right of the inflection point. The absolute rate equation takes the form:

$$\frac{dy}{dt} = r_g \; y \; [-\ln(y)]$$

The absolute rate curve reaches a maximum more quickly and then declines more gradually than the logistic model. This model would be appropriate for polycyclic (compound interest) diseases as an alternative to the logistic model. The 'correction factor' for decreasing healthy host plants (or healthy leaf area index) used in the logistic model (1-y) is replaced by -ln(y) in the Gompertz model.

An example of a disease progress curve that best fits by this model comes from Steinlage *et al.* (2002) for the *Soybean mosaic virus* (SMV) pathosystem. The pathogen progress curve (Fig. 5A) and the absolute rate curve (not shown) were both skewed.

Because of the logarithmic function in the equation for dy/dt, the appropriateness of this model implies that equal proportions of the pathogen's ability to increase are reduced with an increase in time. This may possibly be due to temporal dynamics of the aphid vector population and/or a trend towards increased host resistance as the crop matures. Figure 5B shows

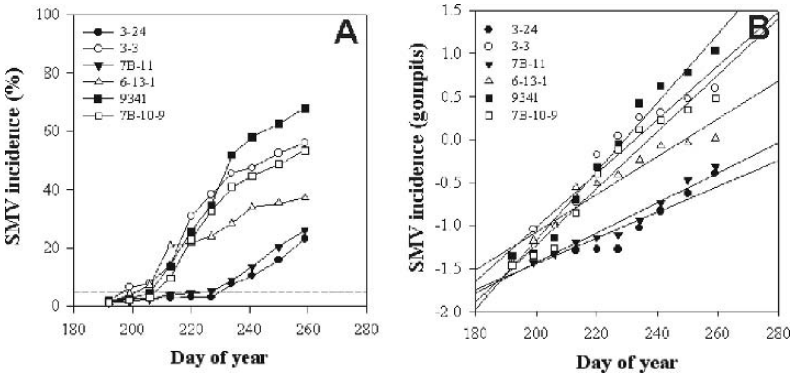

Figure 5. Pathogen progress of soybean mosaic virus (SMV) incidence in transgenic soybean lines and non-transgenic cultivar 9341 at the Iowa State University Farm at Ames, Iowa in 2000 (A). The linearized regression lines (B) using the Gompertz model, Δ gompits (pathogen incidence) vs. Δ time, which explained 87 to 97% of the variation in gompit SMV incidence (adapted from Steinlage et al., 2002).

that the Gompertz model provided an excellent fit to the transformed data, with r^2 values ranging from 87 to 97%.

2. SANITATION

Sanitation is the process that eliminates, reduces, or avoids initial inoculum (y_o) from which plant disease epidemics start (Zadoks & Schein, 1979; Van der Plank, 1963). For pathosystems involving plant pathogens with moderate-to-high infection rates (r), the strategic goal would be to first use integrated tactics that reduce the rate of infection (r), and then delay epidemic onset in time by reducing y_o. This can be accomplished by excluding, eliminating, reducing, and/or avoiding initial inoculum (y_o).

Epidemic onset is a threshold value of disease intensity (y) that is often operationally defined as the level of disease intensity (measured as either disease incidence or severity) when y = 0.05 (i. e. 5% incidence or severity).

Sanitation practices usually fall under one of three management principles: exclusion, avoidance, or eradication (Fig. 6). Again, for sanitation to be effective, the rate of an epidemic must be low, or slowed to a rate below which sanitation practices will effectively delay disease onset.

2.1. Disease Management Principle I: Exclusion (y_o)

Exclusion can be considered as the first line of defense in integrated disease management. The concept is quite simple – keep the pathogen out of crop production areas so that initial inoculum (y_o) is kept at zero. When y_o is kept at zero in polycyclic pathosystems, the absolute rate dy/dt will also be zero (no

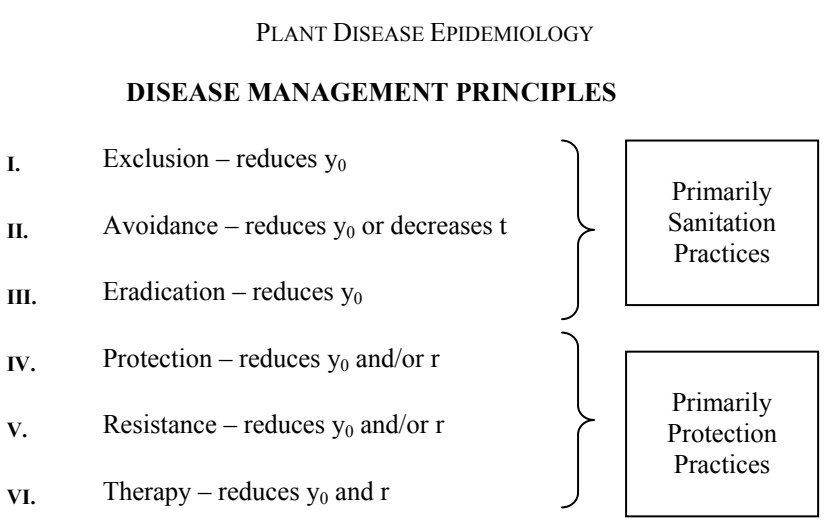

Figure 6. The "Principles of Disease Management" and their epidemiological effects on plant disease epidemics, where y_o represents initial inoculum, r represents the rate of pathogen development (infection), and t represents the time that host and pathogen populations interact to affect final disease intensity (y_{final}).

epidemic). Various disease management tactics can be used to keep the risk of pathogen introduction at zero.

2.1.1. Quarantine (y_o)

The prevention of both the introduction and interstate movement of disease and pests is an essential responsibility of regulatory agencies. Because of the potential threat to crops posed by the accidental or by the deliberate introduction of plant pathogens by bioterrorists (Fegan *et al.*, 2004; Nutter & Madden, 2005), exclusion of pathogen inoculum (y_o) is often attempted by establishing phytosanitary restrictions (known as quarantines) that prohibit the importation of agricultural commodities known to serve as routes of entry for new disease threats, (thus keeping initial inoculum equal to zero and no epidemic will develop).

Quarantine may totally prohibit the importation of specific agricultural products (or planting containers) for specific pathogen threats, or may allow the importation of agricultural products that are inspected prior to entry. Quarantine may be imposed for entire countries, regions, or states and provinces (Fry, 1982; Palti, 1981; Sill, 1982).

2.1.2. Seed/Plant Certification Programs (y_o)

Seed and plant certification programs involve the sampling and testing of plant materials (seed, tubers, bulbs, corms, root stocks, seedling transplants, shoots, budwood, etc.) before planting materials are seeded or transplanted into

production fields or orchards. Due to the large plant populations and volumes of seed that are imported/exported, not every individual in a host population can be sampled and inspected or tested for the presence of plant pathogens. Therefore, sampling is required to select a small proportion of the host population that will be inspected and/or tested.

Because sampling is used, not all individuals are inspected and/or tested and certification programs cannot scientifically (or legally) claim that the entire plant or seed population is absolutely pathogen-free (or disease-free) (i. e. y_o may not be equal to zero because sampling and detection thresholds limit the ability to detect extremely low levels of initial inoculum). It can only be stated, however, that specific pathogens were either not detected in a sample, or the inspected plant material was below an established threshold, based upon the testing procedures currently certified for use. Thus, even if a pathogen was actually present in a seed lot or plant sample that was certified "pathogen not detected", the level of initial inoculum (y_o) in the sample would be extremely low, and in most cases would be of little epidemiological consequence with regards to disease risk and crop loss. The exception would be those pathosystems in which r is extremely high.

2.2. Disease Management Principle II: Avoidance (t)

If a plant disease threat cannot be kept out of a crop production area by exclusion, then it makes sense to attempt to avoid disease risk in the form of y_o that is absent or very low, or the selection of planting sites in which the environment limits the infection efficiency of initial inoculum (y_o). This disease management principle is known as Avoidance, defined as the use of management tactics that avoid high disease risk planting sites, or exposure to environmental conditions that increase disease risk.

2.2.1. Avoidance of Disease Risk in Space (t)

One of the tactics most often utilized by farmers involves the selection of low disease risk planting sites, since it is clear to most farmers that disease risk is much higher in certain areas of the farm than in others. The avoidance of planting in cool, wet sites at the farm scale where pathogens that cause damping-off (*Pythium* and *Phytophthora* spp.) are more prevalent (i. e. the plants are at higher disease risk) compared to planting in warm, well-drained soils, is just one example of avoidance tactics. Conversely, root rot of bean caused by *Fusarium solani* f. sp. *phaseoli* is favored by dry soils and avoidance of these conditions will lower disease risk.

Another good example of avoidance involves topography and/or the presence of vegetation that inhibits air movement that results in longer dew periods (leaf wetness) and longer durations of high relative humidity that favor the development of many fungal pathogens. Pythium blight of turfgrass, caused by *Pythium aphanidermatum* is a good example (Nutter *et al*., 1983). If the consecutive number of hours of relative humidity $\geq$ 90% exceeds 16 hours

during hot summer temperatures (maximum t ≥ 30 °C, followed by night-time minimum temperatures ≥ 20 °C), then Pythium blight infection foci will be visible on turfgrass the following morning. The areas at highest disease risk within golf courses are the low-lying areas where air movement on greens, tees, and fairways is impeded by vegetation (trees) and/or topography (Nutter *et al*., 1983). The removal of some trees to facilitate air movement and their replacement with smaller shrubs or turfgrass can greatly lessen the risk for Pythium blight, thus avoiding this disease. The venting of warm, humid air from greenhouses at night is another example of avoidance, since this practice makes the greenhouse environment less conducive for many fungal pathogens.

Disease risk can vary at many geographic scales, i.e., site-specific (field scale), county-wide scales, regional scales, etc. An example of this avoidance deployed at the county or regional scale involves Stewart's disease of corn. In this pathosystem, disease risk is largely dependent on the overwintering survival of the insect vector population (the corn flea beetle) that serves as an overwintering survival refuge for the causal agent *Pantoea (Erwinia) stewartii,* and as the primary vector to transmit this bacterium to corn seedlings early in the growing season (Esker *et al*., 2006a).

Based upon mean monthly temperatures for December, January and February, plus knowledge concerning the presence/absence of Stewart's disease in a county the previous growing season, the predicted risk for Stewart's disease the ensuing season can be predicted and mapped using geographic information systems (GIS) (Nutter *et al*., 2002). The predicted risk for Stewart's disease in seed corn before the 2003 growing season in Iowa and Illinois is displayed in Fig. 7.

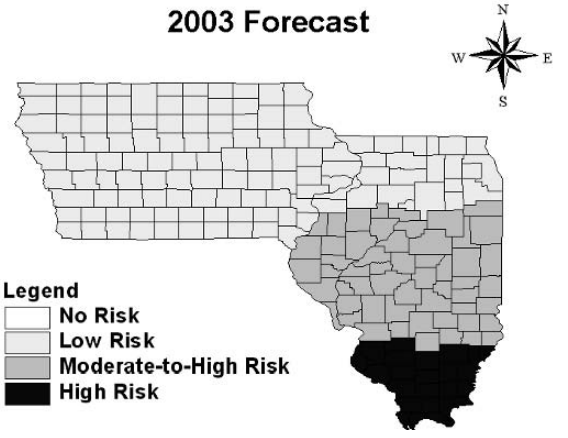

Figure 7. Map of the predicted pre-plant risk for Stewart's disease of corn in Iowa and Illinois in 2003. This disease risk map was generated by coupling the Iowa State Model for predicting the risk of Stewart's disease with a geographic information system (ArcGIS) (from Nutter et al., 2002).

Before planting seed corn in 2003, seed corn companies could use this map to make decisions concerning which seed corn producers to contract, to plant corn inbreds in Iowa and northern Illinois. By selecting counties within low disease risk, producers can "avoid" planting sites in counties with higher disease risk (based upon this Stewart's disease forecasting model).

There are other benefits that can be derived by mapping disease risk. For example, Fig. 7 also informs agricultural scientists where to best conduct field experiments in 2003 to evaluate and compare the efficacy of disease management tactics aimed at improving the management of the corn flea beetle (vector) population and/or Stewart's disease. For example, field trials to evaluate and compare the efficacy of insecticide seed treatments, or the relative levels of resistance/susceptibility of corn genotypes to *P. stewartii*, would best be located where the risk for Stewart's disease was predicted to be highest (red areas on the map). Thus, for the 2003 growing season, field experiments conducted in the southern third of Illinois would likely have provided the "toughest" test to quantify the efficacy of management tactics aimed at Stewart's disease. Such "disease risk maps" provide valuable information concerning the spatial distribution of predicted disease risk with regards to choice of planting sites (Coelho Netto & Nutter, 2005; Nutter *et al*., 2002).

2.2.2. Avoidance of Disease Risk in Time (t)

Avoidance of pathogen populations in time, such as choice of planting date, can be a highly effective management strategy for some pathosystems. The theory behind this strategy is to limit (avoid) the period of time (t) that pathogen and host populations, thereby limiting pathogen-induced crop loss (Nutter & Guan, 2001; Savary *et al*., 2006). By planting early in some pathosystems, the crop will have more time to develop before pathogen inoculum is active or present. One example involves legumes at risk of infection from aphids that have acquired *Cucumber mosaic virus* (CMV) from alternative hosts and can transmit CMV to legumes. Planting early, when conditions are dry, allows legume crops (such as lupin, chickpea, peas, etc.) to be well-established in their growth cycles before seasonal rains arrive that greatly increase the risk of CMV-infection, due to higher aphid populations that result from increased rainfall.

In some pathosystems, delayed planting may substantially decrease disease risk by avoiding early season sources of pathogen inoculum. A good example of this strategy again comes from the Stewart's disease of corn pathosystem (Esker *et al*., 2007). Adult corn flea beetles (*Chaetocnema pulicaria)* that survive Iowa winters and have already acquired the bacterium that causes Stewart's disease (by feeding on corn infected with *P. stewartii* the previous growing season), emerge from grassy areas adjacent to corn fields in April and begin to feed on corn seedlings as they emerge. However, by the end of May, most of the overwintering adult beetles have laid their eggs and died. By delaying planting, feeding by *P. stewartii*-infested beetles can be avoided, the bacterium is not

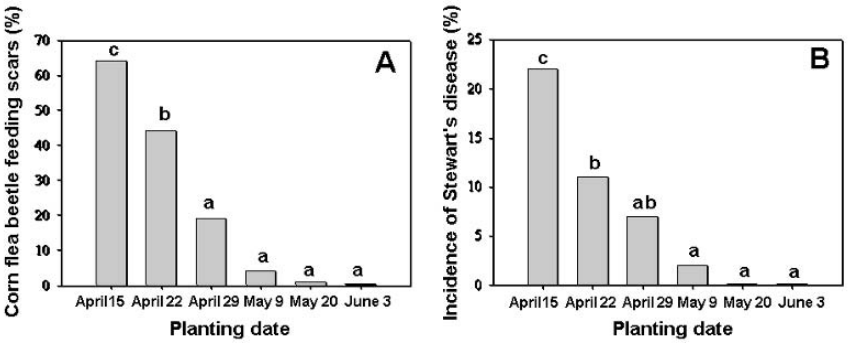

Figure 8. Effect of planting date on (A) the incidence of corn flea beetle feeding scars assessed at corn growth stage V5 (fifth leaf stage), and (B) incidence of Stewart's disease at growth stage V5. Means followed by same letter are not significantly different at the 0.05 level (adapted from Esker et al., 2007).

passed from adults to eggs, there will be a 2-3 week window for crop development before the first summer generation of adult corn flea beetles begin to emerge and feed on infected corn to acquire the pathogen (Esker & Nutter, 2003). The effect of delayed planting on the incidence of corn flea beetle feeding scars and on the incidence of Stewart's disease of corn is shown in Fig. 8. For each day that planting was delayed in 2002, Stewart's disease incidence was significantly decreased.

2.3. Disease Management Principle III: Eradication (y_o)

Eradication can be defined as any practice that reduces initial inoculum (y_o) at the source. Therefore, practices that involve the burial, burning, or removal of crop debris at the source (field) are examples of eradication. The removal of alternative weed hosts or alternate hosts that serve as potential sources of initial inoculum (at the field site) also fall under the principle of eradication.

Other practices that reduce initial inoculum at the source include crop rotation, the introduction of biological control agents, soil fumigation, steam sterilization or pasteurization, solarization, green manure crops, trap crops, and the incorporation of crop residues from other crop species (Fry, 1982; Lipps, 1985; Palti, 1981; Sharvell, 1979). All these practices reduce (to differing degrees) the amount of initial inoculum in the soil.

Initial inoculum (y_o) also can be reduced by a number of physical, chemical and biological practices. However, the use and success of specific eradication tactics is dependent upon the biology of the pathosystem (the rate of disease development, in particular), and the efficiency and cost of the eradication practice.

2.3.1. Eradication Through Crop Rotation (y_o)

Crop rotation is the practice of growing a prescribed sequence of different crops in the same field, to discourage the build-up of pathogen populations over time. Crop rotation is an extremely effective eradication tactic because most pathogens can only infect and cause disease within single plant families, and many plant pathogens cannot survive in the infested crop residue if one or more subsequent cropping cycles are seeded to non-host crops. Crop rotation can provide substantial disease management benefits by reducing initial inoculum (y_o) at the source (in the field).

In general, plant pathogens do not compete well against saprophytic organisms in the soil for food bases or space (Cook, 1977). Plant pathogens use plant (crop) residues both as a refuge and as a food base (Cook, 1977). Bruehl (1975) refers to this as the "possession principle". Pathogens that take possession of above ground host plant parts (stems, leaves, fruits, etc.) have much less competition with microbiota while the crop is developing, but as crops mature and senesce, infected crop tissue above ground becomes infested crop residue that resides on, or below, the soil surface. The key for plant pathogens to maintain possession of a residue substrate until the next susceptible crop is present, is a slow continued metabolism. However, a physical environment unfavorable for metabolism of a plant pathogen in possession of a crop residue may not be unfavorable for potential colonist residing outside infested crop residue. As crop residue degrades due to colonizing saprobes, pathogen survival quickly declines.

Certain soil amendments, fertilizers, green manure crops and trap crops can further increase the benefits of crop rotation by increasing biological activity of the soil microbiota (Cook, 1977; Palti, 1981). Thus crop rotation can provide excellent control, provided that infested crop residues are colonized and decayed before a susceptible host crop is reintroduced to the same field.

For plant pathogen that can successfully compete with saprophytic organisms and can also produce long-term survival structures (dispersal units), e. g. sclerotia, microsclerotia, dormant sexual spores, nematodes cysts, etc., as well as having a broad host ranges, rotation may be of little benefit. Trap plants can be highly effective in soil pathosystems in reducing y_o unless crop rotations are very long.

A variation on crop rotation schemes is to leave fields fallow. This is an ancient practice, which favors competing soil microbes in the absence of a susceptible host. Fallow periods as short as six weeks can have beneficial eradication benefits (Palti, 1981).

2.3.2. Removal of Alternate and Alternative Hosts (y_0)

The epidemiological importance of alternate and alternative hosts of plant pathogens is that they can serve as an overseasoning refuge (bridge from one crop growing season to the next time a susceptible crop is grown), as well as

providing a local source of initial inoculum for the next susceptible crop. It is important to differentiate between alternate versus alternative hosts.

Alternate hosts. Alternate hosts are plant hosts which the pathogen requires to complete its cycle. The eradication of alternate host plants is very effective in reducing local sources of initial inoculum (y_0). This is important in the stem rust of wheat/barberry pathosystem. Urediniospores (asexual spores) of the wheat stem rust pathogen (*Puccinia graminis* f. sp. *tritici*) produced on susceptible wheat plants cannot survive winter environments (overseason) in much of the US. Barberry (*Berberis vulgaris*) is the alternate host of *P. graminis* that enables the stem rust fungus to survive locally, by providing a host for basidiospores produced from overwintering telia. Barberry, in turn, serves as the source of local inoculum in the form of aeciospres to infect wheat, which directly leads to the production of urediniospores and regional stem rust epidemics (Peterson *et al.*, 2005). However, the eradication of barberry does not break the disease cycle because long distance dissemination of urediniospores from the Southern-most wheat production areas of the US can provide a source of inoculum for stem rust epidemics to develop (albeit later in the growing season). Still, the eradication of barberry greatly reduces an epidemiologically-important local and early-season source of initial inoculum.

Barberry serves another critical role in epidemiology, regarding genetic pathogen diversity. Peterson *et al*. (2005) reported that the diversity of races among uredinial collections of *P. gramins* f. sp. *tritici* from wheat fields in Minnesota declined sharply from 1912 to 1930, and remained low to 2002. The diversity in aecial collections however remained nearly constant for 90 years, indicating the importance of barberry not only as a local source of initial inoculum, but by also serving as an alternate host for sexual reproduction to generate greater pathogen diversity.

Alternative hosts. Alternative hosts are defined as other host species on which a pathogen can complete its disease cycle. Thus, they can serve as a source of initial inoculum by producing dispersal units (e. g. spores, sclerotia, viruliferous aphids, etc.) that can become infection units when they come in contact with another susceptible host species (e. g. crop or weed species) and the environment is favorable (or becomes favorable) for pathogen infection or transmission. For example, in the *Tobacco etch virus* (TEV)/bell pepper/aphid pathosystem in Northeast Georgia, six alternative weed hosts were found to be infected with TEV: four annual solanaceous species (apple of Peru, annual ground cherry, black nightshade, and jimsonweed), and two perennial species (horsenettle and perennial ground cherry) (Nutter, 1993). Epidemiologically, it would be important to eradicate the two perennial alternative TEV hosts because these weed hosts, if infected with TEV, can survive the winter and begin to produce new growth in early spring (early-to-mid March), that can serve as a source of TEV for virus acquisition by aphids before peppers are transplanted to the field (mid-May). While TEV incidence in horsenettle was found to range from 10 to 60%, almost every perennial ground cherry tested by ELISA was found to be infected with TEV.

Two annual alternative weed hosts (annual ground cherry and jimsonweed) were also important for the disease risk, because infection efficiency was 3-4 times higher in these two alternative hosts, compared to bell pepper. Acquisition efficiency by aphids was also 2 to 2.5 higher, when compared to bell pepper. Moreover, the presence of a single TEV-infected source within a 6 row × 8.3 m plot resulted in a shift in the disease progress curve of 7 days (earlier infection), resulting in an additional 19% yield loss. Thus, weed management (eradication of alternative weed hosts), within and in close proximity to pepper plantings, is of primary importance to reduce TEV disease risk and related crop losses.

2.3.3. Roguing of Diseased Plants (y_o and r)

Roguing is the practice of removing infected or diseased plants in a crop or orchard in an attempt to remove sources of initial inoculum. Thus this practice attempts to reduce y_0 and, to some extent, r. Roguing should be carried out at the earliest possible moment after symptoms become apparent (or soon as results of pathogens detection tools become available, i. e. through ELISA, PCR etc.). If roguing is done early, then the time (t) that host and pathogen populations interact is also shortened, which reduces alloinfection (pathogens spread from diseases plants to other plants, r).

Rouging is practiced in many perennial plantation crops in the tropics. This is because roguing (in some pathosystems) can delay the epidemic and hopefully add years to the life of the plantation. Roguing is commonly used in Africa to delay *Cassava mosaic virus* epidemics in cassava, and to delay yellow crinkle disease of papaya (caused by a phytoplasma transmitted by leafhoppers) in Africa and Australia (Esker *et al*., 2006b; Palti, 1981).

Roguing delays plant disease epidemics by reducing the incidence of infection in vegetable and plantation crops. If r is high, then roguing in itself may not be beneficial (see sanitation ratio, Section 1.2). However, in polycyclic pathosystems, roguing will reduce disease incidence (y) and therefore, dy/dt. Using the absolute rate equation for the logistic model: $dy/dt = ry(1-y)$, if y is successfully kept low by roguing, then dy/dt will be slowed because alloinfection will reduce "r".

However, there is one important epidemiological drawback when roguing is practiced in pathosystems involving insect-vectored plant viruses or phytoplasmas: the latent period of these organisms is shorter than the incubation period (as is the case with many human viruses). That is, plants infected with a virus or phytoplasma will become infectious before disease symptoms become apparent. Therefore, insect vectors will likely acquire the pathogen from infected asymptomatic plant hosts, and transmit the pathogen to a number of healthy plants before the infectious plant host becomes symptomatic and is rogued. Over time, $y_{\text{infected plants}}$ increases faster than y_{rogued} plants, resulting in little or no delay in the epidemic. However, if r is low, roguing can prove beneficial in terms of reducing y_0 and increasing the survival (production) time of crops.

2.3.4. Removal and Burial of Crop Residues (Debris), (y_o)

The removal or burning of crop residues is particularly effective in reducing initial inoculum (y_o) in pathosystems where the major source of inoculum is located in the above ground parts of infested crop residues (Fry, 1982; Palti, 1981). For example, most *Sclerotinia* spp. and *Sclerotium* spp. form their sclerotia primarily on the above-ground plant parts. Mol *et al*., (1995) reported that removal of potato and field bean residue reduced numbers of microsclerotia of *Verticillium dahliae* in the soil in subsequent years, but removal of barley straw had no effect. Epidemiologically, the physical removal or burning of infested crop residue debris acts to reduce y_0 (but not r).

Naylor and Leonard (1977) reported that sporulation of *Colletotrichum graminicola* (corn anthracnose) on corn stalks buried in the soil in November was severely reduced in samples collected just one month later, and that the pathogen could not be detected three months later (February). These results indicate that *C. graminicola* has very poor saphrophytic ability to compete and retain possession of corn residues that are buried. Thus, *C. graminicola*-infested corn residues are rapidly replaced by more competitive microorganisms in the soil, as occurs with many plant pathogens residing in crop residue.

Conversely, the practice of leaving corn residue on the soil surface (e.g. conservation tillage, minimum tillage, and reduced tillage), can provide an epidemiologically-important source of initial inoculum. Studies conducted by Lipps (1985) in Ohio showed that the incidence and severity of corn anthracnose decreased with respect to distance from corn residues left on the soil surface. The presence such disease gradients indicates the presence of a local source of inoculum, i. e. surface corn residue (Zadoks & Schein, 1979). Thus, burying or removing this residue greatly reduces disease risk, and leaving infested crop residues may allow some pathogens to remain in possession of residue substrates, thereby substantially increasing their survival time. Burying infested crop residues also physically prevents the dissemination of dispersal units (inoculum) to potential infection sites.

2.3.5. Pathogen Eradication Programs (y_o)

The global increase in trade and travel has increased the risk of invasive agricultural diseases and pests (Bandyopadhyay & Frederiksen, 1999; Gottwald *et al*., 2001). Such introductions can seriously compromise agricultural export markets (Nutter & Madden, 2005). The introduction and establishment of an invasive species indicates that pathogen/pest exclusion measures have failed. While the accidental or natural introductions of plant pathogens costs the US economy billions of dollars each year (Pimentel *et al*., 2000), the potential for deliberate biological attacks on U.S. agriculture using plant pathogens as weapons remains an Achilles heel that greatly threatens the U.S. agricultural economy (Nutter & Madden, 2005).

If an invasive pathogen or pest is: *i*) detected early, *ii*) limited in its extent (one or a few fields) and *iii*) dissemination has also been limited (so far), then complete eradication is often attempted (but is only sometimes successful). Moreover, if eradication is achieved, this often comes at a tremendous economic cost resulting from the complete destruction of infested/infected crops (loss of income) and from the infrastructure providing the labor necessary for an eradication program to achieve success (Fegan *et al*., 2004). For example, the detection of karnal bunt of wheat, caused by the fungus *Tilletia indica*, resulted in the regulatory branch of USAA, APHIS, spending more than $60 million dollars between 1996 and 1998 to finance a quarantine and eradication program in a relatively small infested area in Arizona. Moreover, it is estimated that growers in the affected areas lost well over $100 million from lost sales.

2.3.6. Flooding (y_o)

Flooding fields and orchards (also referred to as fallow flooding) is an ancient practice that is very effective in reducing initial inoculum (y_o) in some pathosystems. Flooding was probably first developed for crops grown in rotation with paddy rice and is believed to be a key reason why the incidence of soil borne diseases remain low in Chinese agriculture (Palti, 1981). In the Fusarium wilt/banana pathosystem, it is believed that the fungus that causes banana wilt (*Fusarium oxysporum* f. sp. *cubense*) does not survive well in oxygen-deprived environments thereby effectively reducing y_o .

2.3.7. Soil Solarization (y_o)

Soil solarization is the practice of covering moist soil with transparent polyethylene plastic for 2-8 weeks to allow the sun's energy to heat the top 5-10 cm of soil to temperatures (45-50 °C) that are lethal to most soilborne pathogen populations, thus reducing y_o at the source (Palti, 1981). This practice has proven cost-effective as an eradication tactic to reduce initial inoculum (y_o), particularly against fungi that cause damping-off or vascular wilts as well as controlling some nematode species. For example, Coelho *et al*., (1999) reported that soil temperatures under solarization treatments reached a maximum of 47 °C at the -10 cm depth, but only 41 °C at -25 cm depth. Solarization was found to be as effective as fumigation (methyl bromide) in reducing populations of *Phytophthora nicotianae* at the -10 cm depth, but had no effect at the -25 cm depth.

Heat applied in the form of hot water can be extremely effective in reducing initial inoculum (y_o) (Raychaudhuri & Verma, 1977). Several bacterial and fungal diseases are effectively controlled by hot water treatments. These include black rot of crucifers, blights of cotton, late blight of potato and loose smut of wheat. Hot water may be combined with other chemicals to enhance eradication efficiency (reducing initial inoculum). For example, on acidic soak method has been shown to effectively eradicate the causal agent of bacterial canker of tomato. Hot water and chlorine (1.2% NaOCl) treatments successfully eradicated

Cladosporium variabile (causal agent of Cladosporium leaf spot of spinach), and greatly reduced the incidence of *Stemphylium botryosum* (Stemphylium leaf spot of spinach), and *Verticillium dahliae* (Verticillium wilt) in spinach (Du Toit & Hernandez-Perez, 2005)

2.3.8. Eradication/Disinfestation by Heat Sterilization/Pasteurization (y_o)

Heat is commonly used to eradicate or reduce initial inoculum (y_o) in soil. Plant pathogen groups (fungi, bacteria, etc.) are killed by a 30-minute exposure to temperatures in the 50-80 °C range. Soil pasteurization (30 minutes of steam at 80 °C) is primarily used to eradicate plant pathogens in greenhouse and ornamental nursery populations. Moist heat (steam) is generally more effective as an eradicant than dry heat. The high value of ornamental plants makes this practice cost-effective and it is a mainstay to reduce y_o near zero for a wide range of plant pathogens. Another practice that relies upon heat to reduce initial inoculum is composting. When mulch is kept in same place for 2-8 weeks, temperatures in the center of the compost pile typically reach temperatures of 50-55 °C, which are sufficient to kill most soilborne plant pathogens. Moreover, microbiota antagonistic to soilborne plant pathogens will also increase.

2.3.9. Soil Fumigation (y_o)

Soil fumigation is the practice of injecting chemical fumigants into the soil to reduce initial inoculum (at the source). This practice is primarily used only in the production of high-value crops, such as strawberries (and other small fruits), ornamental and tree nurseries, and turfgrass. The use of chemical soil fumigants (such as methyl bromide) by producers is decreasing due to environmental (ozone-depleting compounds) and governmental safety concerns.

3. PROTECTION

3.1. Disease Management Principle IV: Protection (y_o and/or r)

When plant pathogens cannot be excluded, eradicated, or avoided, then the next line of defense against them is “Protection”, which can be defined as any tactic that provides a physical or chemical barrier to protect the crop. Such tactics generally reduce initial inoculum, but protection is directed at the point of crop infection (i.e. the protection of potential infection courts), and not directly at the source of inoculum in the field. In many cases, protection tactics may also reduce the rate of infection “r”.

3.1.1 Use of Physical Barriers to Protect Crops (y_o and r)

Physical barriers that attempt to reduce initial inoculum at the site of crop production include the use of plastic row tunnels to keep both fungal spores and insect vectors from entering the crop (Mueller *et al*., 2006). An example of the

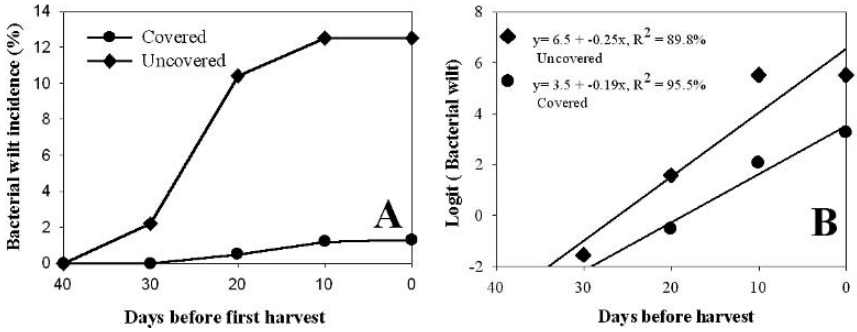

Figure 9. Percent incidence of bacterial wilt on muskmelon plants grown with or without plastic row covers in 2003 in Iowa (A) and the effect of plastic row covers (B) on the rate of disease progress. Using the logistic model, days before harvest explained 89.8 to 95.5% of the variation in the bacterial wilt incidence. Without plastic row covers, disease onset occurred 28 days before harvest, whereas with row covers its incidence did not reach the 5% level of disease onset.

efficacy of this practice is the protection of cucurbit crops from cucumber beetle vectors that can transmit *Erwinia tracheiphila,* the bacterial causal agent of blight of cucurbits (Mueller *et al*., 2006) (Fig. 9A). Again, this practice reduced initial inoculum (y_o) resulting in a delay in disease onset (time to reach y = 0.05 or 5% incidence) from 28 days before first harvest in plots that were not protected by plastic tunnels. Cucurbit plantings protected by plastic tunnels never reached disease onset (y = 0.05 or 5%). Moreover this practice also greatly reduced the rate of plant-to-plant spread r (due to reduced alloinfection) from 0.25 logits/day to 0.19 logits/day (Fig. 9B).

3.1.2. Use of Chemical Barriers to Protect Crops (y_o and r)

Seed treatment. The deployment of "chemical barriers" to protect a crop are often employed at the earliest stage of crop development. This involves the application of chemical (or biological) agents that protect the developing plant during seed germination and early seedling development, from soilborne pathogens and insects. Fungicide seed treatments are often employed to reduce the effectiveness of seed-borne and/or soilborne infection units (mycelium, conidia, sclerotia, etc.), thereby reducing the effectiveness of initial inoculum at the site of infection. Fungicide seed treatments can have "protectant" properties that provide a "sphere or zone of protection" around the developing roots. Other fungicide products may have "systemic" properties that protect seeds, the developing roots, crowns, shoots, and leaves well beyond the germination phase. For example, several fungicides offer protection to developing plants until the 5th leaf stage of crop development in corn or soybean crops.

Fungicide seed treatment products often consist of two or more different active ingredients to protect against a broad range of soil and/or seed-borne

pathogens. Some fungicide seed treatments can effectively eliminate fungal infection within the embryo (e.g. loose smut of wheat). Since the site of infection (embryo) and the source of inoculum (infected embryo) are the same, it could be argued that this tactic is also an example of reducing initial inoculum at the source (eradication).

Protectant versus systemic (eradicant) fungicides. Protectant fungicides are primarily meant to protect the plant surface with a chemical layer that is impenetrable by the fungus (Zadoks & Schein, 1979). Protectant fungicides reduce the infection efficiency of plant infection that would take place in the future if a fungicide barrier were not present beforehand. Eradicant fungicides "eradicate" infections that have already taken place.

Protectant fungicides usually remain on the plant surface, whereas eradicant systemic fungicides have to penetrate into plant and be transported systemically throughout plant tissues (Zadoks & Schein, 1979). An example of how increasing the dosage of the systemic fungicide tebuconazol reduced the infection efficiency of late leafspot of peanut (measured as the number of lesions/cm^2) is shown in Fig. 10. In this experiment, all but the upper three leaves of peanut plants were treated with different concentrations of tebuconazole, and then the top three leaves were inoculated 24-hours later with spores of *Cercosporidium personatum,* the causal agent of late leafspot of peanut. Dosage explained 98.6% of the variation in probit percent reduction in the infection efficiency of *C. personatum.*

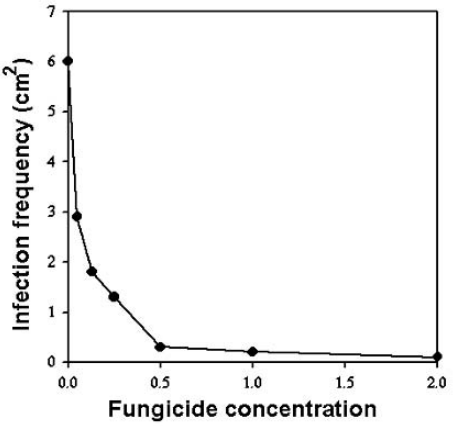

Figure 10. Effect of fungicide concentration (tebuconazole, × 0.42 kg a.i. / ha) on infection frequency (lesions / cm^2) of Cercosporidium personatum*, the causal agent of late leafspot of peanut. All but the top three peanut leaves of each plant were treated with fungicide 24 hours prior to inoculation (adapted from Labrinos & Nutter, 1993).*

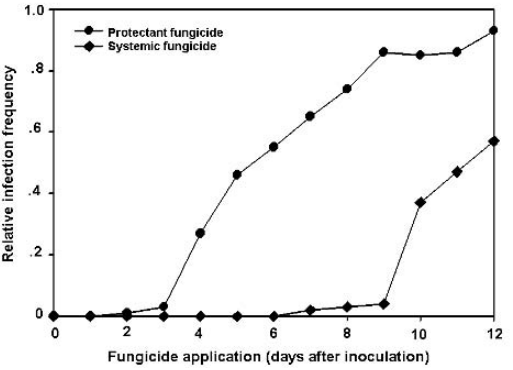

Figure 11. Effect of a protectant versus a systemic fungicide on reducing the relative infection efficiency of late leaf spot of peanut due to Cercosporidium personatum*, when fungicides were applied up to 12 days after peanut plants were inoculated. The systemic fungicide had greater eradicant (kick-back) efficacy compared to the protectant fungicide.*

In addition to reducing the infection efficiency of *C. personatum* on peanut, increasing dosage of this systemic fungicide also reduced lesion size, increased the length of the incubation period, and decreased sporulation (Labrinos & Nutter, 1993), resulting in a significant reduction in the rate of disease development (r) as well.

The "eradicant" properties of systemic fungicides can vary considerably and even protectant fungicides may possess limited eradicant properties. For example, when a protectant versus a systemic fungicide were applied separately to plants with respect to days after peanut plants were inoculated with a suspension of *C. personatum* spores, the protectant fungicide reduced relative infection efficiency to almost zero for up to three days after inoculation (Fig. 11). After three days, each day that the application of the protectant fungicide was delayed resulted in substantial increases in infection efficiency (more lesions per cm^2). However, the systemic fungicide reduced relative infection efficiency to zero, even when applied to plants up to six days after inoculation, and by day 10 infection was still less than half of that of the protectant fungicide (Fig. 11).

3.1.3. The Use of Organic and Reflective Mulches (y_o and r)

An example of a "protection" tactic that reduces both initial inoculum and the rate of an epidemic is the use of aluminum plastic mulch. In the bell pepper/*Tobacco etch virus*/aphid pathosystem, this tactic repels (reduces) viruliferous aphid populations (aphids that have already acquired a virus) from entering pepper crops, thereby reducing virus transmission frequency (i. e., initial inoculum). Aluminum mulch also lowers alloinfection (plant-to-plant spread within the crop), which reduces r as well.

3.2. Disease Management Principle V: Host Resistance

While some authors categorize host resistance under the disease management principle "Protection", the epidemiological effects of host resistance on initial inoculum (y_o), the rate of disease development (r), and/or the time that host and pathogen populations intact deserve separate recognition as a separate disease management principle.

Host resistances generally affects either initial inoculum by reducing y_o to zero (or nearly zero), or the rate (r) of an epidemic. When y_o = zero, then no epidemic can develop. For example, if disease (or pathogen) progress over time is best described by the logistic model, whereby the absolute rate of disease progress dy/dt = ry (1-y), then if disease resistance effectively reduces $y_o \rightarrow$ zero, dy/dt = zero. The same is true for other models (exponential and Gompertz) in which disease development is polycyclic.

It is important to keep the epidemiological effects of host resistance apart from the plant breeding/molecular methods that are used to obtain host resistance. It is more pragmatic (in epidemiological terms) to categorize host resistance either as rate-reducing resistance, that acts to slow the rate of disease development (infection), or as resistance that acts to reduce initial inoculum, thus avoiding the use of terms that are epidemiologically ambiguous, such as dilatory, durable, race-nonspecific vs. race specific, quantitative vs. qualitative, partial vs. specific, incomplete vs. complete resistance, polygenic, multigenic (or oligogenic) vs. monogenic (or single gene) resistance, etc.

3.2.1. Resistance Reducing Initial Inoculum (y_o)

When host resistance, at the cultivar or variety level, is effective in reducing initial inoculum to zero for some pathogen strains (races) but not others, then such resistance is defined as "strain-specific" and, epidemiologically, y_o is not reduced to zero.

The epidemiological impact of this type of resistance will differentially reduce the initial inoculum (delaying the epidemic), but race specific resistance will not reduce the rate of epidemics that develop in host cultivars that do not completely reduce initial inoculum to zero. Since $y_o > 0$, strain-specific resistance will behave epidemiologically in a manner similar to sanitation, resulting in a delay in time (t) that will be of little benefit in pathosystems where r is high.

Strategies to maintain effectiveness and durability include gene rotation in time, gene deployment, multilines and gene pyramiding (stacking) (Van der Plank, 1963, Zadoks & Schein, 1979).

3.2.2. Resistance Reducing the Rate of Infection (Disease Development)

Rate-reducing resistance is a good generic term to describe the epidemiological effects of host resistance that reduces the rate of a plant disease epidemics. Disease-specific terms have also been coined to describe this form of

resistance: slow blighting, slow mildewing, slow-rusting are some examples (Zadoks & Schein, 1979).

Host plant resistance reducing the rate of disease development (r) can have tremendous benefits in reducing disease risk (by lowering r), and increasing yields. In many crops, rate-reducing resistance can reduce r to the point where disease intensity in the crop is low, and the stage of crop development when individual plants become infected occurs well after flowering and fruit or seed set, thereby having little or no effect on yields. Delayed infection coupled with the fact that, the healthy plants in diseased crops can compensate for neighboring diseased plants, provides an even larger safety net in terms of not reaching economic injury thresholds (Nutter, 2001; Zadoks, 1985). With regards to integrated disease management, it is important to remember that as r is reduced, disease management practices that reduce y_o (sanitation) become more effective.

An example of resistance that reduces the rate (r) of disease development in a plant virus pathosystem is shown in Fig. 12 (Padgett *et al*., 1990). The change in disease incidence of *Tobacco etch virus* (TEV) in bell pepper with respect to days after transplanting was much slower in two rate-reducing pepper varieties (Asgrow and Tambel) compared to the susceptible variety Yolo Wonder, that was widely planted by growers at that time (Fig. 12A). The logistic model provided the best fit to quantify and explain the relationship between days after transplanting and the change in logit TEV incidence (r^2 values ranged from 85.4 to 99.1%) (Fig. 11B). The slopes of the regression lines are taken as measures of r. The rate of TEV infection (disease progress) in Yolo Wonder was 0.15 logits/day (moderately fast) with a doubling time of 4.6 days (time for

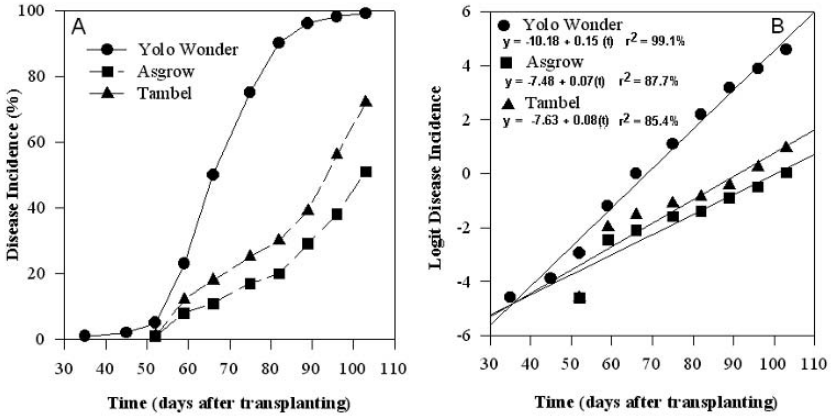

Figure 12. Resistance that reduces the rate of disease progress. Disease progress curves (A) for incidence of Tobacco etch potyvirus *(TEV) in three pepper varieties. Linear regression using the logistic model ln [y/(1-y)] versus time to transform incidence data (B).*

TEV disease incidence to double from 1% to 2%, 2% to 4%, etc.). The rate of disease development in Asgrow and Tambel, however, was 0.07 and 0.08 logits/day, i.e., it was cut in half and the doubling times increased to 9.1 and 8.7 days, respectively. Moreover, reducing r in these two pepper varieties resulted in a 45% reduction in final TEV incidence and the relative area under the disease progress curves were 42 to 68% lower in the rate-reducing varieties. This delay in the time of plant infection resulted in most pepper plants becoming infected with TEV well after fruit set with a minimal effect on yield, as evidenced by the fact that the time for TEV epidemics to reach 50% disease incidence (t_{50}) was delayed 23 to 37 days (Kuhn *et al*., 1989; Padgett *et al*., 1990). In the two rate-reducing pepper varieties, fruit yield increased by 24%, weight by 14%, and fruit numbers by 11.6% .

Thus, although the rate-reducing strategy allows some infection to occur ($y_o > 0$) within the crop, the delayed infection of individual plants and the plant population benefits derived from yield compensation make rate-reducing resistance a viable alternative to resistance strategies aimed at reducing y_o to zero (especially in annual crops).

3.2.3. Host Resistance Affecting Time (t)

Early maturing cultivars/varieties that reduce the time period (t) during which host and pathogen populations interact may greatly reduce the final level of disease intensity. Limiting the time of exposure to plant pathogens may also be considered under the disease management principle of "avoidance".

3.2.4. Molecular Technologies for Disease Resistant Plants

The goal of nearly all research programs that utilize molecular techniques to genetically modify host resistance is to reduce initial inoculum to zero. This strategy can place a new genetically-modified cultivar at risk because strong directional selection is created for pathogen strains that can overcome this type of resistance. This selective advantage will result in an increase in the frequency of a resistance-breaking strain within the pathogen population. This situation occurred in the papaya–*Papaya ringspot rirus* (PRV) pathosystem (Tenant *et al*., 1994). In this example, coat protein-mediated transgenic resistance that was developed using the coat protein from Hawaiian PRV strains provided excellent protection only against the Hawaiian PRV strains, (i. e., $y_o \rightarrow$ zero), but this form of resistance was ineffective against other PRV strains in other parts of the world ($y_o > 0$) (Tennant *et al*., 1994). Since this resistance was selected to reduce y_o to zero and not to reduce the rate of PRV epidemics in papaya plantations (high r pathosystems), this form of transgenic resistance initially had little effect on delaying PRV epidemics in papaya plantations. Subsequently, new transgenic lines of papaya resistant to PRV were developed, that remained effective in reducing y_o to zero (Bau *et al*., 2004; Davis & Ying, 2004).

A common practice in developing crops with improved disease resistance is that only the genetically modified lines that reduce initial inoculum to zero are being selected for crop development. These are host lines *i*) that do not exhibit symptoms when challenged by a pathogen, *ii*) on which the pathogen does not reproduce, and *iii*) cannot be detected beyond the site where it was introduced into the plant. The genetically-modified lines that do not satisfy these strict criteria (reduce initial inoculum to zero) are almost always discarded and are almost never evaluated for their ability to reduce the rate of plant disease epidemics (Padgett *et al*., 1990).

One exception is a study reported by Steinlage *et al*. (2002), concerning the development and selection of soybean lines transformed using the coat protein of *Soybean mosaic virus* (SMV). Soybean lines transformed with the coat protein of SMV were challenged by mechanical inoculation with SMV, and although some plants in each line were found to be infected with SMV, a few transgenic lines exhibited: *i*) a much lower incidence of SMV-infected soybean plants (reduced infection efficiency), *ii*) longer incubation periods (time from inoculation to the time that 50% of the infected plants displayed symptoms), and *iii*) reduced symptom severity compared to other transgenic lines (Wang *et al*., 2001). Since reduced infection efficiency and a longer incubation period are both considered components of resistance that reduce the rate of disease development (Johnson *et al*., 1986; Nutter, 1993; Parlevliet, 1979), the most promising transgenic lines based on these two disease components were selected to evaluate their epidemiological potential to reduce the rate of SMV epidemics in soybean fields (rather than discarding these transgenic lines because initial inoculum was not completely reduced to zero).

To detect and quantify the level of rate-reducing resistance in transgenic soybean lines, the incidence of SMV in 30-cm quadrats was plotted with respect to time (Fig. 5A) and the Gompertz model was found to best explain the relationship between the change in gompits (pathogen incidence) with respect to time (r^2 values ranged from 87 to 97%), indicating that time explained 87 to 97% of the variation (Fig. 5B). Two transgenic lines (3-24 and 7B-11) reduced the rate of SMV infection by more than half (from 0.04 gompits/day in the non-transgenic line 9341 to less than half that rate (0.015 gompits/day in line 3-24, and 0.017 gompits/day in line 7B-11).

The impact of the rate-reducing effects of these lines on SMV epidemics delayed epidemic onset (y = 0.05) an additional 23 days (from 202 to 223 days in line 3-24) and an additional 19 days in line 7B-11 compared to non-transgenic control (line 9341). Final SMV incidence in line 9341 was 68%, whereas final SMV incidence in the two rate-reducing transgenic lines was 23 and 26%, respectively. Most importantly, the majority of the quadrats infected with SMV in the rate-reducing lines were infected well after flowering and pod set, resulting in average yield gains of 25.7% over the non-transformed control (line 9341). It is advisable to field test genetically-modified plants for rate-reducing resistance if one or more components of resistance indicate that such lines may possess rate-reducing potential, even if genetically-modified plants do not reduce y_o to zero.

3.3. Disease Management Principle VI: Therapy (y_o and Sometimes r)

Therapy as a disease management principle primarily reduces y_o, and in some cases r as well. Therapy stands alone from the other disease management principles in that this principle comes into play only after a plant becomes infected and there is an attempt to "cure" or increase a plant's survival time (Esker *et al*., 2006a). This is somewhat analogous to the use of chemotherapy in humans to cure or increase the survival time of cancer patients (Nutter, 1999).

3.3.1. Heat Therapy (y_o)

One of the best examples of therapy is the use of "heat therapy" to cure an infected plant (Campbell, 1962; Rayhaudhuri & Verma, 1977). It has been proposed that nearly all viruses can be inactivated *in vivo* with the right combination of temperature, time and other factors. More than half of all virus-infected horticultural plants were already shown to be successfully eliminated by heat treatment. The main principle of heat therapy is to exceed the inactivation point of the pathogen, but not to exceed the heat tolerance threshold of the host tissue. Both hot air and hot water treatments have been used to eliminate plant pathogens from true seeds, tubers, cuttings, bulbs, budwood, dormant trees/saplings and plant sets. In addition to heat therapy, low temperature treatments were used to cure potato plants of *Potato virus Y*.

3.3.2. Antibiotic and Chemical Therapy (y_o)

Antibiotics and chemicals have been injected into trees infected by phytoplasmas or fungi primarily to increase survival time rather than to attempt to "cure" infected plants. Chemical injection of systemic fungicides was used to increase the survival time of elm trees infected by the fungus that causes Dutch elm disease.

3.3.3. Therapy Methods that Employ Radiation (y_o)

Other methods of therapy include the use of electromagnetic radiation (e.g. UV radiation, gamma radiation, X-rays, sonic radiation, etc.) (Chatrath, 1970). Due to its limited penetration power, ultraviolet (UV) radiation has proven to be particularly useful to control post-harvest diseases of fruit and vegetables. Ultraviolet radiation reduces y_o through its surface sterilization activity and not r, the rate of disease development.

3.3.4. Removal of Infected Plant Parts (y_o and r)

The physical removal of infected plant parts acts to reduce initial inoculum and r. For example, the removal of cankered tree limbs infected with fire blight acts to reduce initial inoculum (y_o) and the rate of infection (r) due to reduced alloinfection within an apple or pear orchard. The practice of ratooning (removal

of plant shoots infected with a plant pathogen that are replaced by the growth of pathogen-free shoots) has been proposed in the yellow crinkle disease of papaya/phytoplasma pathosystem as a means to manage this disease (albeit with limited success) (Esker *et al.*, 2006b). There are numerous other examples concerning the removal of diseased plant parts to increase the survival time of infected plants.

4. INTEGRATION OF IPM PRACTICES AT THE DISEASE COMPONENTS LEVEL

The concept of "Components of Resistance" was first set forth by Parlieviet (1979) to quantify the epidemiological effects of host resistance on disease "components" interacting to regulate the rate of disease development. This concept can be expanded to the other two sides of the plant disease (epidemic) triangle, namely the impact of the environment and the pathogen aggressiveness. For example, the "Components of Resistance" concept can be used to quantify the impact of the environment (including all management tactics) on "disease components", as well as quantifying these components from the perspective of the pathogen, i. e. concerning its aggressiveness.

When quantifying these epidemiological components with regards to the attributes of plant pathogens, they are referred to as "Components of Aggressiveness" (Zadoks & Schein, 1979). When these epidemiological components are used to quantify the effects of environment on components (e. g. leaf wetness, duration, relative humidity, temperature or even fungicides, etc.), then they are referred to as "Disease Components" (Nutter et al., 1990).

The relevance in extending the "components" concept to both the pathogen and the environment becomes clear when we couple the "components" concept with Lieberg's *Law of the Minimum* (Waggoner, 1980). In general, this law states that if several factors affecting a given outcome are present in abundance but one of them is deficient, adding more of the deficient factor will likely change the outcome with a great impact, whereas increasing the abundant factors will change the outcome very little.

With regards to the environmental effects on the rate of disease development (r), if the hours of leaf wetness are more limiting to one or more of the disease components shown in Table 3, compared to temperature, then it may be possible to significantly improve disease management by focusing management tactics on the disease components rather than on the effects of a variable. Improving management through e.g. more favorable temperatures will have relatively less impact on outcome, than by monitoring leaf wetness duration, which has a greater impact and is more limiting on disease components and r. Since temperature rarely limits infection efficiency of conidia of *Cercospora personatum* (the causal agent of late leafspot of peanut) during the growing season it is the variable accounting for the number of consecutive hours of leaf wetness that most inhibits infection efficiency. The effect of daily periods of leaf wetness shorther than 10 hours in reducing infection efficiency is

Table 3. Epidemiological components that interact affecting the rate of infection r (disease development) with respect to time.

Variable	*Dimension*
Infection efficiency	State/rate variable
Incubation period	Time
Lesion size/expansion	State/rate variable
Latent period	Time
Sporulation capacity	State/rate variable
Infectious period	Time
Number of dispersal units/infection	State/rate variable

just as effective as applying a protectant or systematic fungicide. Thus, according to the *Law of the Minimum*, it does not matter how favorable the temperature is to optimize infection efficiency, but it is the leaf wetness duration that is most limiting. Therefore, at the disease components level, fungicide is not needed when the number of hours of leaf wetness is not sufficient to elicit a response (i. e. an increase in the infection efficiency of *C. personatum* spores). However, when leaf wetness duration again becomes favorable (according to a disease forecasting-spray advisory model), then fungicides can be (and should be) deployed to keep infection efficiency close to zero. In either case, a reduction in infection efficiency ($y_o \rightarrow$ zero) due to either environment or by using fungicides that also reduce infection efficiency $\rightarrow$ zero would have "equivalent" affects on the rate of pathogen development.

The "equivalence" concept, however, is not new, as it was proposed at the strategic level (e. g., to reduce r). Equivalent environmental or fungicidal effects at the disease components level, however, represent a new concept that should help to develop more cost-effective integrated disease management programs. Van der Plank (1963) referred to this as the "*Equivalence Theorem*". Simply stated, changes toward a less favorable environment, an increased resistant host, and/or a less aggressive pathogen are equivalent in their effects in slowing the rate (r) of an epidemic. In the case of relying on a single tactic (i. e., the environment) to effectively reduce infection efficiency ($y_o \rightarrow 0$), then attempts to further reduce y_o infection by adding other tactics to further reduce y_o (e. g. fungicides) may result in a response that is less than additive and may not be cost-effective. Fry (1975) applied the *Equivalence Theorem* to the potato late blight (*Phytophthora infestans*) pathosystem, showing that fungicide concentrations could be reduced in potato cultivars that had higher levels of rate-reducing resistance and that a given amount of rate reducing resistance was worth a corresponding amount of fungicide. Fry (1977) also showed that the

interval between fungicide applications could be extended in potato cultivars with higher levels of rate-reducing resistance. Hence, the *Law of the Minimum* and the *Equivalence Theorem* are important concepts when applied at the disease components level.

In the era of crop biosecurity and risk assessment it is critical to understand that the converse may not be true: i. e. changes leading to a more favorable environment, decreased host resistance and/or a more aggressive plant pathogen population are not "equivalent" in their effects, but they are potentially additive or even synergistic. Why does this occur? Changes in the host that lead to greater susceptibility coupled with changes in increased aggressiveness of the pathogen and/or a more disease-favorable environment may interact to result in disease infection rates that are much greater than their "equivalent" additive effects. This is exactly what led to the infamous Southern Corn Leaf Blight Epidemic of 1970 (Ullstrup, 1972). First, changes from "normal" to Texas Male Sterile (TMS) cytoplasm resulted in enhanced susceptibility in corn hybrids carrying this cytoplasm and nearly 80% of corn planted in 1970 utilized TMS cytoplasm. Second, changes in pathogenic aggressiveness in the fungus that caused southern corn leaf blight (*Cochliobolus heterostrophus*) resulted in strong directional selection for a more aggressive race (Race T) when TMS-cytoplasm corn was planted. Components of aggressiveness for Race T resulted in greater changes in disease intensity versus time, since Race T caused larger lesions than Race O. Furthermore, Race T had a shorter latent period than Race O and produced higher numbers of conidia per lesion and unit time, than did Race O, resulting in a faster rate of infection (r) on either TMS or normal cytoplasm corn hybrids (Ullstrup, 1972). The 1970 Southern Corn Leaf Blight epidemic destroyed about 15% of the U.S. corn crop, resulting in a loss of approximately $1 billion (Ullstrup, 1972).

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CONCEPTS FOR PLANT PROTECTION IN CHANGING TROPICAL ENVIRONMENTS

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Abstract. Past changes and main climate features of the tropical regions are presented. Factors affecting plant protection in tropical environments are examined, in the light of environment and climatic changes. The effects of climatic changes on the efficacy of the plant protection technologies actually deployed in tropical agroecosystems are discussed, together with the mechanisms and possible consequences of climate variations on disease and pest epidemiology and management. Potentials and efficacy of some practical tools (i. e. modeling, monitoring, probability distribution maps) in plant protection are presented.

1. INTRODUCTION

Almost 75% of the world human population live in the Tropics, in regions covering approximately 50% of the Earth's surface, including some of the less favoured areas in the world (Thompson, 2000). The latitudinal differences among the population income distributions are striking and are the object of social, political and economic actions (Drèze & Sen, 1991; Ram, 1997; Sanchez, 2000). Although historical factors provide an explanatory framework for the Tropics low levels of economic and social development, it is generally recognized that these regions are under the pressure of high demographic growth rates, high prevalence of human diseases, low levels of food production and rural income, loss of natural resources and biodiversity, as well as severe climate variations.

Social partecipation to regional policies is considered as one of the main factors lowering the risk of food paucity and famine, through a direct link of a nation's political and social organization with food production and supply. In recent years, however, regional crisis and drought induced famine highlighted the potential threats linked to food storage, market prices and environmental changes (Anand & Sen, 2000).

The FAO hunger map, which shows the percent of undernourished population, (*http://www.fao.org/faostat/foodsecurity/FSMap/flash_map.htm*), highlights how, in spite of a general reduction trend observable during the last 30 years in some regions, several areas affected by hunger and starvation still remain in the Tropics. The peculiarities of the tropical agroenvironments, including climate extremes, and several features of tropical soils, i. e. their low mineral and organic matter content,

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were considered as factors responsible for the inverse linear relationship linking the latitude and the per capita income or other economic indicators (Ram, 1997). Assessing a cause-effect relationship among undernourishment and agricultural performance, soil fertility and productivity appears, however, a complex task, since several factors related to the environment or climate should be considered as cause and effect, acting at the same time.

In the last decades, food production and availability increased on a global scale (Fig. 1). Countries like India and China, characterised in recent years by high economic growth rates, experienced increasing trends for cereals or fruits and vegetables productions. Data show that the marginal increase of global cereals production reached, by the end of the twentieth century, a stable situation. A similar steady state is not yet evident, either worldwide and at the regional scale, for fruits and vegetables productions, which show growing trends at the beginning of this century. A food growing or stable production regime does not provide support for malthusian scenarios, but climate changes are expected to decrease yields at lower latitudes, with patterns more pronounced in the future, and significant impacts on the food system. However, some authors consider that prices and hunger risks will not be affected by the additional stress induced by climate changes (Parry *et al*., 1999).

In this chapter we focus on some environment factors involved in the protection of plants and food production agroecosystems in tropical regions and on the effects expected by environment and climatic changes. Dissecting the effects of climate on the efficacy of the plant protection technologies actually deployed is by itself a complex task. Inferring the main climate variations and identifying their possible consequences on integrated pests and disease management may, hence, appear as a theoretical exercise. Nevertheless, due to the impact of climate and plant diseases on agriculture, food production efficiency and the health of million human beings, identifying plants protection strategies and potential threats is worth the effort.

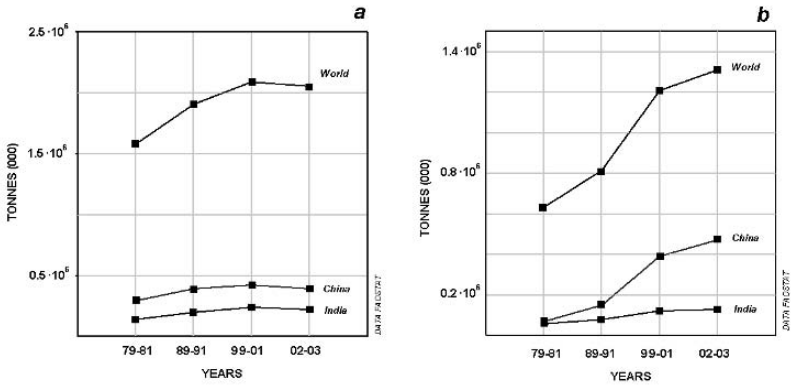

Figure 1. Production of cereals (a) and fruits and vegetables (b) for India, China and the world, in the period 1979-2003 (Source: FAO).

We will pay particular attention to the climate features and changes expected in some highly populated tropical regions and to their effects on crops production cycles and associated diseases. Our attention will focus on the mechanisms of climate variables on pests and diseases, on the management strategies applied, starting from potential scenarios and challenges determined by the climate variations expected during this century.

2. ENVIRONMENT AND CLIMATE CHANGES

Climate and seasons are subject to changes extending on different time scales, ranging from months to years, centuries or millenia. Climate changes are difficult to define, since a "change" requires an absolute, stable term of comparison and reference. On the medium term, climate is difficult to forecast, intrinsically chaotic (Lorenz, 1965; Zhang & Krishnamurti, 2000), and may be classified and studied on a statistical or historical basis, or through simulations and modeling. One useful concept required to treat such a difficult topic is, hence, "evolution", and we will use the term "climate change" always in reference to an evolutive path experienced in time, differing significantly from past trends.

We define a "stable" environment, and the boundaries of "acceptable" changes, as those introduced by man during the expansion of agriculture and the following industrialisation phases, with the recent process of intensive agriculture and deforestation. At this regard let's remember that the earth's surface is continuously "under construction", being influenced and re-shaped by several physical forces, including geological events (i.e. volcanism, floods, earthquakes), climate and man's action. In this view, for "evironment change" we intend any physical change, induced by man or climate, altering in a structural way the capacity of a given environment to remain stable in time and to sustain, directly or indirectly, different levels of complex food webs, including human societies.

2.1. Climate and Anthropogenic Changes

Major climate changes are expected in the course of this century. Two major forces are recalled in the climatological debate to explain expected changes: solar activity (in particular solar spots cycles) and other earth's related factors, and the atmospheric immissions of greenhouse gases, which include, apart of CO_2, also ozone (O_3), methane, sulfurs, NO_2 and chlorofluorocarbons (CFCs). The term "greenhouse effect" – the increase of the infrared solar radiation retained by the atmosphere – is generally used to describe the consequential increase of water and atmospheric temperatures, altering the biosphere in a wide range of scenarios and mechanisms (Houghton *et al*., 1992; Broecker; 1997; May, 2004; D'Orgeval *et al*., 2006). The increase in greenhouse gases is an historical event, which cannot be reversed or changed in the short term. For CO_2, the actual atmospheric content (358 ppm) results from an approx. 30% increase of the pre-industrial revolution levels. In the same period, ground level ozone almost doubled whereas methane concentration increased by more than 240% (Houghton *et al*., 1996; Vingarzan, 2004). Models of future climate variations during the next decades, although biased by uncertainties

concerning the magnitude of projected patterns (Wigley & Raper, 2001), forecast a global increase of oceanic surface water temperatures, with highest values in the tropical regions (Barsugli *et al*., 2006). Sea surface temperature (SST) is one of the main variables influencing global climate, and changes induced by increasing mean SSTs values are expected in the next future to affect the world climate through the Tropics oceanic circulation.

Global warming is already in act: the linear trend of recorded annual mean SSTs shows extensive warming in the Indian Ocean and West Pacific and the Eastern Tropical Pacific Oceans over the last 50 years. Data allowed trustable simulations and modeling, improving seasonal climate forecasting and estimation of the sensitivity of global climate to SSTs changes in the Tropics (Barsugli *et al*., 2006; Goddard *et al*., 2001). An insight about the effects of the possible future scenarios may be obtained examining the documents by the International Panel for Climate Change (IPCC), available at the internet address: *http://www.ipcc.ch/pub/sa(E).pdf*.

Changes related to temperature and rainfall are expected to interact with other factors of anthropic origin due to land use, urbanization, and water management. This complex of changes will affect agriculture and crops productivity in tropical regions, and the sustainability of several ecosystems as well. Although we cannot exclude that different changes may constructively interact each other, countebalancing other negative factors (i. e. increased rainfall levels in semi-desertic areas), we have to consider also the possibility of major negative events, like i. e. the consequences of extreme rainfalls regimes: their effects on crops should not only consider the direct damages induced, but also the loss of water supply and/or of agricultural land, due to floods or droughts.

Other consequences expected concern long term cumulative events, as pollution and environmental hazards, also related to human activities. Desertification, erosion and deforestation remain as "hot topics" of the biosphere anthropogenic changes. These actions interact with other man-induced damages, like the loss of natural resources, including wildlife and plant species, the development of irrigation and the loss in water quality, as well as other regional scale changes, i. e. landscape management, constructions, artificial lakes and urbanization.

Temperature changes will affect crops productivity and plants distribution, interacting with the epidemiology and spatial distribution of their pathogens and pests, either at the local and global scales. At the same time, the temperature increase and the demographic growth will affect the spread of pests and diseases as well as of their vectors, through mechanisms related to the exploitation of new land, commerce and commodities exchange. The evolution/conservation of agricultural practices and the related policies will require strategies to face severe changes including, among others, the development of databases on pests and pathogens behaviour and life-cycles and monitoring actions, following their spread to newly colonized areas through the modification of their distribution boundaries (Sutherst, 1998; Olfert & Weiss, 2006).

Although forecasting local trends in complex ecosystems is challenging, due to the complexity and chaoticity of the agroecosystems and, in general, of the environment itself, it is worth trying to develop and apply tools in order to evaluate, infer and produce, at the medium-large scale, environmental data related to human

activities and their impact (Mitra *et al*., 2005). At the regional, local scale as well, the application of models reproducing, simulating and analyzing climate trends was emphasized, in order to assess political priorities (based on costs/benefits analysis) and to provide strategic backgrounds for long-term research (Sutherst, 1998). When focusing on the effects of global changes on invertebrate vectors of human or cattle diseases, for example, target environmental components, subject to structural changes and deserving immediate action, were identified. Among them there were: land use and cover, microclimate and biodiversity; atmospheric CO_2 levels; travels and transports; biogeochemistry; genetic and climate changes and climate variability. In Australia, medium term forecasting based on the correlation of rainfall with the Southern Oscillation Index and El Niño data, although affected by extreme seasons, appeared enough informative for early warning decisions, in order to define applications in disease surveillance, management of pesticides inventories, cattle vaccination, release of biological control agents and monitoring (Sutherst, 1998).

2.2. Past Climate Changes in the Tropics

Due to the strong dependence of the biosphere on climate, experimentally reproducible data concerning the extent of climatic variations may result very informative on the general mechanisms of the expected climate variations. These data may be obtained through the study of past climates, as recorded by paleological research works.

Local, regional and large scale glacial records of climate changes, extending backward into the last glacial period, were made available in the Tropics through selected ice drilling sites (Thompson, 2000). Data records of global mean annual temperatures and El Niño Southern Oscillation (ENSO), as well as monsoon intensities, together with glaciological records from the Tibetan Plateau and the South America Andes, showed a progressive trend of climate warming occurring during the last century (Thompson, 2000).

Glaciological records are natural data archives, informative about the extent and severity of past climate variations and about the regional factors affecting climate, and are useful for potential estimations about future expected changes. The following examples provide an overview about the dimension of the problems related to climate changes and their global impacts.

In Asia and East Africa, monsoons have a strong, fundamental influence on agriculture and economy through the seasonal rainfalls. Understanding the main forces producing the observed paleoclimatic changes of monsoons intensities is important to assess future variations in strenghts, which will affect food production in densely populated regions (Overpeck *et al*., 1996). Also in this case, paleoclimatic data, on different timescales, may provide experimental evidence about the mechanisms responsible for changes and their long range consequences.

Records from the Tibetan Plateau showed that an increase of the snow cover of the Eurasian region is correlated to low monsoon intensities. The δ^{18} O isotopes records from the Dunde (China) cores also showed that the years in the period 1937-1987 were the warmest of the overall records covering the last 12K years.

Data from the Central Higher Himalayan/Tibetan Plateau are informative about the variations of the Asian monsoon intensities during the last 4000 years and their effects on vegetation (Phadtare, 2000). Peat cores from the Dokriani Glacier valley showed changes of the *Quercus* spp. and *Pinus* spp. pollens densities, whose ratio (Q/P) is indicative of climatic conditions ranging from cold-dry (higher Q/P) to warm–wet (lower Q/P). Monsoon intensities, as inferred by rainfall and temperature changes, were also deduced from grass pollen and algal spores, the latter correlated to a drop in moisture, indicative of rainfall or temperature increase (Phadtare, 2000). The data showed alternated climatic variations, with a significant decrease in summer monsoons in the period between 4000 to 3500 years B.P., characterized by a cold and dry climate. Correlation of this event with other concomitant records registered in the Indian subcontinent and western Tibet suggests that the dry, low monsoon period was a widespread event of the Holocene record in South–Central Asia. A decrease in summer monsoon was also registered between 1000 to 800 years BP. Data also showed a cooling phase around 800 BP, corresponding to the Little Ice Age, followed by a warming trend which continued until present (Phadtare, 2000).

Also peat cores data from Central China, based on ash content and humification, showed past climate variations affected by winter and summer monsoons (Xuefeng *et al*., 2006). Asynchronous variations gave rise, during the Holocene, to four phases of paleoclimatic variations, with alternating increase and decrease patterns for winter and summer monsoons. Last stage showed a trend, during until present, with decreasing intensities of summer monsoons and a corresponding gradual increase of winter monsoons. Also stratigraphic ^{14}C data from East Asian monsoon sensitive areas in Central and Northern China (Loess Plateau and Ordos sands, between the Yinshang and Qinling mountains) showed fluctuations in environmental conditions, with four phases of alternated dry and wet periods, occurring since the last deglaciation (Zhou *et al*., 2001). Paleoclimatic records showed variations during the last 20K yrs, with weaker monsoons during glacial times and stronger events in early Holocene. Two mechanisms were identified for millenial scale variations: 1) orbital forcing (effects of the earth's orbits) controlling the amount of heat reaching the earth and hence the plateau warming and 2) changes in ice volume, SST, albedo and atmospheric gases concentrations related to the ice cover, altering the monsoon response to astronomical forcing (Overpeck *et al*., 1996).

In the Tropical Andes, ice core data from Quelccaya, calibrated through the ash depositions of recent known volcanic activities, confirmed the global extent of the Little Ice Age (1520 – 1880 AD) (Thompson, 2000). Data from the Huascarán ice drills in Peru showed that the Late Glacial Stage conditions in the Tropics were cooler than today by 8-12°C, with higher amounts of dust in the atmosphere and a concomitant reduced extension of the Amazon forest basin. Data also showed that the last two centuries were the warmest during the last 6000 years. Progressive warming during the last two centuries, with droughts at 300 years intervals, were also revealed by the dust contents in the ice core records (Thompson, 2000).

The natural archives of paleoclimatic data are also informative about the vegetation changes and may provide useful indications about the changes expected for vegetation in the near future. In a review of the paleoclimatic data available from

the Central Mexico and Yucatán, Metcalfe *et al*. (2000) reported a variable warmer and wetter climate during the early Holocene, followed by a dry middle Holocene and a series of several alternated dry intervals during the late Holocene (2300-900 yrs BP), the most severe of which occurred 1000 BP. These authors identified alternating dry and wet phases since 26K yrs BP, as suggested by the alternance of Q/P pollen records, and the insurgence of early human influence on the pollen composition, as revealed by the maize pollen records (3500-3600 yrs BP) and by the effects of deforestation, abruptly shifting the pollen composition to herbaceous species. Diatom data from the Texcoco and other basins showed the alternation of dry and more humid periods during the Holocene, with sediments composition switching from shallow to deep lake waters. Higher amounts of Ca were indicative of periods of regional aridity and lower lake levels, as confirmed by diatoms data. Stable isotope records also were considered as indicative of a number of dry periods during the late Holocene. The δ^{18}O records from Lake Pátzcuaro showed an alternated series of dry and humid phases during the last 3500 yrs, with a final shift towards wetter conditions occurring 220 yrs BP (Metcalfe *et al*., 2000).

Aslo Yucatán data showed an early Holocene dry period, with wet conditions between 7000 and 3000 BP, possibly with an intermeditate dry interval. Late Holocene is marked by several dry periods, the strongest being recorded approx. 1000 yrs BP, probably responsible for the collapse of the Maya civilization (Metcalfe *et al*., 2000). In Northern Mexico the deserts were cooler and wetter during the early Holocene, with lakes in the present Chihuahua desert, winter rainfalls (actual rainfalls occur in summer) and woodland in areas today occupied by desert scrubs. Early and mid Holocene were warmer and wetter than today, and desert conditions started to appear about 4000 BP. Climate changes during the last Pleistocene and Holocene were probably the result of the ocean atmospheric changes. SSTs at the Bermuda level and the North America heating also played a fundamental role in climate regulation (Metcalfe *et al*., 2000).

Due to their long range effects, monsoons patterns are recognized also in East Africa. Pollen and foraminifera data from Arabian Sea cores showed, during the last 20 K yrs, an early increase of monsoon strengths occurring in two steps around 13-12.5 K years and 10-9.5 K years BP, with some century-long periods of weak monsoons punctuating longer phases with higher strengths (Overpeck *et al*., 1996).

Data from Van Campo *et al*. (1982) also show a rapid and abrupt increase of monsoons strength occurring during the early Holocene. The reaction of the monsoons intensities to orbital forcing and other ice boundary conditions are complex and non linear, as suggested by a 3000 years delay observed from the increase of Northern Hemisphere insulation (15K yrs BP), and by two later abrupt increases in strength, concident with two North Atlantic warming steps, affecting the Tibetan Plateau warming during the last deglaciation (Overpeck *et al*., 1996).

In conclusion, core data show that a number of changes affected climate in the Tropics on time scales lasting several centuries, with also some abrupt variations. Recent paleological data from different studies show a global warming trend, with a contemporary reduction of the ice caps and of the glaciers extent and depth. These changes will have an effect also in the short term, at the regional scale, producing a shortage of key available resources, i. e. the water supply available at lower

altitudes. For example, the reduction of the Qori Kalis glacier in Peru is indicative of the progressive speed reached in recent years by the ice melting, which produced new high altitude lakes (Thompson, 2000). Similar fast shrinking of glaciers were also observed in tropical mountains in Africa (Hastenrath & Kruss, 1992; Kaser & Noggler, 1991) confirming the global extent of the tropical warming process.

2.3. Present Climates

Glaciological records provide a reproducible and reliable amount of data describing the overall evolution of climate at the regional scale and its impact on ecosystems at different ages. Whatever the cause of climate changes (natural or human-induced), glaciological records show that significant temperature variations may also occur during relatively short periods of time, ranging from centuries to decades. Recent changes in climate at a scale of thousand years are well documented, as also the variations that characterized the last centuries, i. e. the increase in temperatures during the Middle Age (1000 - 1400 AD) followed by the Litle Ice Age (1400-1800 AD) with variations of up to 10 °C experienced during a millemium. Although the exact contribution of human activities on the world mean temperature is difficult to estimate, as cycles of solar activities also excerce a significant effect, it is nevertheless important to identify the main forces actually shaping the metereological regime in tropical regions, in order to estimate peculiar threats induced, at the regional scale, by climate evolution and changes of temperature and rainfall regimes.

Tropical climates are in general considered as wet and warm, but a great diversity of weathers and local climates exists. Using variables like the extreme air temperatures, the mean partial vapor pressure, the mean diurnal cloudiness, the wind speed and the occurrence of precipitation during the day, Lecha Estela (1998) identified up to 18 weather types in the Tropics.

The following sections provide some details about the major climate regulating regimes in main tropical agroecosystems, focusing on their sensitivity to changes and on the variations that they will experience in the next decades.

2.3.1. The Central Andes and South America

The metereological conditions of the Central Andean region and the Bolivian Altiplano have a strong impact on rainfall and moisture availability for the adiacent dry lowlands and their agriculture (Garreaud *et al*., 2003). Rainfall in the region is restricted to the austral summer season (November to March), expecially on its western side, with strong fluctuations experienced at an interannual scale. Rainfalls are mainly caused by increased amounts of lower levels and near-surface water vapour of eastern continental origin, whereas the moist and cooler air originating from the Pacific remains on the coast, due to the reliefs and to an upper atmosphere temperature inversion layer (Rutllant & Ulriksen, 1979). The inversion layer also produces severe arid conditions and deserts on the western

slope of the central Andes (Fig. 2), as the moist subtropical air does not contribute significantly to the Altiplano water cycle (Garreaud *et al*., 2003).

Rainfall variability in the Central Andes is observed at different time scales. Each year, moisture variability produces two seasons with alternated dry and moist periods, superimposed to a west-to-east moisture gradient. Moist air convection, linked to near-surface water vapour, gives rise to rainfalls alternated with low-convection and dry periods. Rainfall anomalies are also produced by the Bolivian High, a summer upper-level anticyclonic circulation, affecting climate in the region as well as in the subtropical Atlantic areas and southward up to central Argentina (Garreaud *et al*., 2003).

At the annual level, the regional circulation pattern, fuelled by surface heating, affects lowlands rainfall through changes in atmospheric moisture. Continental lowlands rainfalls are regulated by eastern air flows and western uplifts of coastal dry air, producing rainfalls after interacting with the moist atmospheric boundary layer. Seasonal cycles appear related to wind circulation and not to solar radiation, with easterly flows prevailing from December to March in the high troposphere over the Andes and Altiplano, with moisture transport and increased rainfall rates (Garreaud *et al*., 2003).

Long term variabilities are characterized by extreme fluctuations of summer precipitations, with extremely dry or wet seasons, the wet years corresponding to the ENSO cold phases. Data, however, suggest that the relationships between SST anomalies recorded in the tropical Pacific and the Andes precipitations system are

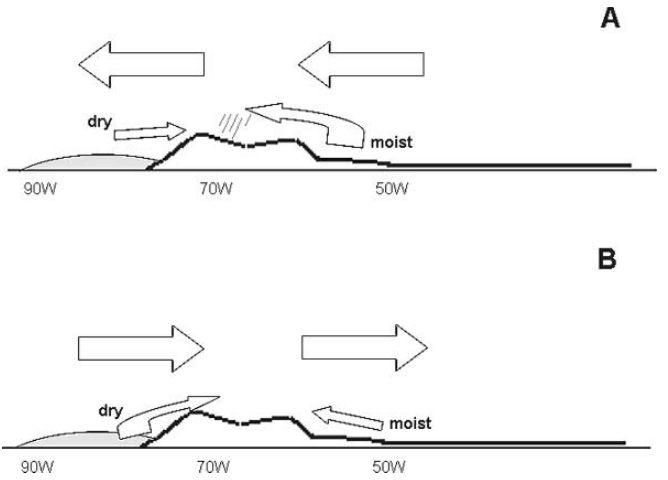

Figure 2. East to West section of the South America Andean region showing the effect of the upper atmosphere flow inversion (larger arrows) on lower moist and dry flows, originating wet (A) and dry (B) episodes on the Altiplano. Shaded areas show stationary cool and moist air on the Pacific coast (adapted from Garreaud et al., 2003).

complex. Garreaud *et al*. (2003) observed that on annual time scales easterly winds are correlated with the Andes wet summers, suggesting that annual precipitations variabilities reflect an increase of rainfall episodes rather than of their intensities. Finally, being estabilished a relationship between the SST anomalies and winds regime, a link was identified among tropical Pacific water cooling (warming), easterly winds increase (reduction) and Andean increased (reduced) precipitation regimes (Garreaud *et al*., 2003). Differences over the Altiplano and Central Andes also concern the Cordillera, with eastern sides still reached, during the dry seasons, by moisture proceeding from the Amazon basin.

Atlantic Trade winds affect the climate of the Amazon basin, but do not overcome the eastern slope of the Andes (Vuille *et al*., 2000). The rainy season in most of Brazil is associated with a summer monsoon regime in South America, with precipitation anomalies associated to La Niña events, with shortage of rainfall and consequent effects on crops (Grimm, 2004). In the year following the La Niña events, northeastern South America shows a tendency for more abundant precipitations, with a deficiency in southeastern areas of South America (parts of South Brazil, North Argentina and Uruguay) with interseasonal changes and anomalies mainly concentrated in January (Grimm, 2004).

2.3.2. The Caribbean and Tropical Pacific

The Caribbean and tropical Pacific regions are characterized by low seasonal temperature variations, high rainfall rates and seasonal cyclones. Differences in mean air temperatures between the warmest and coldest months are in the order of 6–8 °C. Cyclones are the effect of oceanic SSTs, which influence their intensities providing energy through the heat flux. They also produce some feedback effects, lowering SSTs up to 6-9 °C. This effect can in turn lower their intensity by more than 50 % (Zhu & Zhang, 2006).

Central America has a large diversity of climates, ranging from wet to dry (desert) regimes, resulting from several factors including altitude and latitude. Actual climate is influenced by the Trade winds, the sub-tropical high pressure and the Westerlies, which in winter produce dry conditions in most of the region. Summer flows bring moisture from the Gulf of Mexico and the tropical east Pacific and are enhanced by the effects of tropical cyclones, particularly in September. The seasonal wind reversal extending up to Southern USA is called “Mexican or Northamerican monsoon” (Douglas *et al*., 1996; Adams & Comrie, 1997). Moisture transport proceeds from the Pacific and from the Gulf of Mexico to the Sierra Madre Occidental and the Rocky Mountains (Tang & Reiter, 1984). The westerly upper flows may reach the highlands bringing cold conditions and occasional snowfalls. Under southerly flow conditions, cold air masses can penetrate Mexico from the Great Basin bringing light rain or even snow. In winter, outbreaks of cold polar air called “nortes”, connected to the eastern coastal margins of Mexico by the Sierra Madre Oriental, may produce heavy rains on the eastern slopes of the mountains, in Chiapas and Oaxaca.

Trade winds bring a deep easterly flow over most of Mexico from the south side of the Bermuda high. Moist air also proceeds from south-east to north-west, increased by convective storms due to the high plateau heating. Over the western slopes of the Sierra Madre Occidental and northwards along the lower Colorado valley and into Arizona and New Mexico, the eastern tropical Pacific is the main source of moisture. In the eastern part of Mexico, the summer rainy season can be disrupted for 2 to 4 months by a dry period called the "canicula", caused by air flows from eastern USA, Florida and Cuba, reaching the Yucatán peninsula. The canicula intensity is connected to the annual rainfall (Metcalfe *et al*., 2000).

Summer rainfalls show differences between western and eastern regions. Wet conditions in the west may be mirrored by drier conditions in the east related to the strength and location of the upper tropospheric monsoon anticyclone and changes in SSTs of the eastern tropical Pacific (Higgins *et al*., 1998; Metcalfe, 2000). ENSO events are also associated to wetter conditions in the west and north of Mexico, whereas the canicula appears stronger during La Niña than El Niño years. The Mexico latitude, topography and land-sea distributions produce a general decline in rainfall from the southern humid highlands to the deserts of the northern interior plateau and Baja California (Metcalfe *et al*., 2000).

2.3.3. The Asian Monsoon System

Central China climate is influenced by the East Asian and Indian monsoon systems, and by the westerly and Tibetan Plateau monsoons. Climatic variations reflect the global climate patterns, with a series of extremely cold periods correlated with North Atlantic and Indian Ocean circulations. Variations of summer monsoons in the eastern Tibetan Plateau also appear correlated to the solar radiation variations in the Northern Hemisphere (Xuefeng *et al*., 2006).

Indian agriculture is largely dependent on the southwest monsoon system whose impact reaches East Africa and Asia (Hastenrath, 1991; Overpeck *et al*., 1996). Monsoons have seasonal trends, with typical season winds and rainfalls occurring from June to September (Fig. 3). The flow is cross-equatorial, running through East Africa with the Somali jet and then with the westerly flow over the Arabian Sea, the Indian peninsula, the Bay of Bengal and further into Southeast Asia. Associated rainfall shows maxima on the west coast of India, the Bay of Bengal, in Bangladesh and northeast India (May, 2004).

Indian monsoons are due to the Tibetan Plateau heating during the Northern Hemisphere summers (Krishnamurti & Ramanathan, 1982). The low pressure over Asia and the higher pressure over the Indian Ocean are responsible for a low-level atmospheric pressure gradients generating the SW monsoon (Hastenrath, 1991). The monsoon intensity is regulated by the amounts of winter-spring snowfalls on the Tibetan plateau: stronger monsoons are observed in years characterized by lower snowfalls, which allow an earlier plateau warming, whereas weaker monsoons are observed in years with increased spring snowcover (Barnett *et al*., 1988; 1989; Douville & Royer, 1996). Long range effects of tropical Pacific waters on the South Asian monsoon transmitted through the east-west atmospheric circulation

(Walker Circulation) were also observed, with warmer Pacific SSTs reducing monsoons intensities (Rasmusson & Carpenter, 1983; Meehl & Arblaster, 2003).

2.3.4. Tropical Africa and Sub-Sahara

West Africa monsoons are seasonal events affecting the hydrologic cycle and intensity of the rainfall regime, extending their influence up to the Sahel region (Fig. 3). This area experienced a severe drought lasting since the early 1960s which now appears to be mitigated, with increasing rainfall trends observed during the last decade (D'Orgeval *et al.,* 2006). West African monsoons depend mainly on the displacement of a rain band localized to the south of the Inter Tropical Convergence Zone (ITCZ). They are characterized by strong variabilities over space and time, with three distinct regimes: an initial phase (March-June) with the extension of the rain band from the coast northward; the main rain period (July-September) with an abrupt shift of the core of the rain band from 5°N to 10°N by mid June and a final retreat of the rain band southwards, during September–November (Sultan & Janicot, 2000; Le Barbé *et al*. 2002).

West African monsoons are subject to four wind regimes: the southwesterly monsoon flow in the lower troposphere, the African Easterly Jet in the mid-troposphere (June-September), the Tropical Easterly Jet associated with the upper level outflow from the Asian monsoon from 5°N to 10°N, and the Subtropical Westerly Jet, from 30°N to 35°N (Le Barbé *et al*. 2002). Factors like

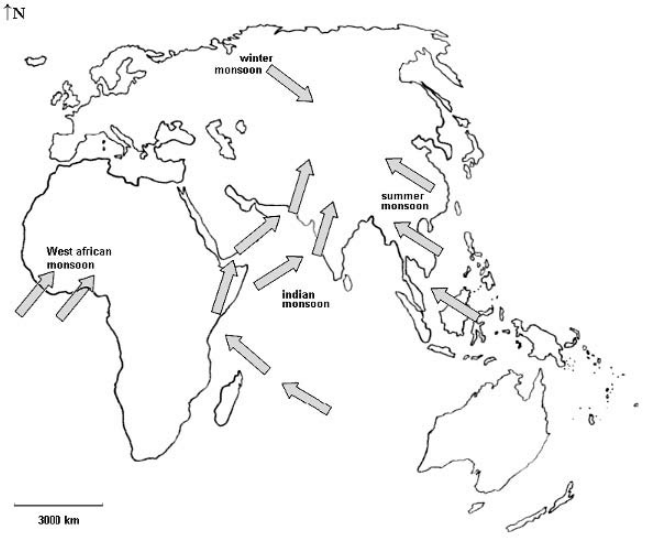

Figure 3. Summer and winter monsoon flows over Asia and West Africa (adapted from Xuefeng et al., 2006; Leuschner & Sirocko, 2003; Messager et al., 2004).

SST, land surface conditions and large-scale circulation affect monsoons variations (Sultan *et al*., 2003; Messager *et al.,* 2004).

2.4. Expected Scenarios

Due to the complexity of the climate changes expected during this century and the uncertainties of forecasting climate evolution on a small scale, any prediction about the extent of plant diseases or epidemics induced by the climate evolution remain largely unpredictable. We will, however, proceed to identify some general forces, which may be the object of future research and observations aiming at improving forecasting, with subsequent identification of the modeling and managing activities required for efficient crop protection.

2.4.1. Monsoon System

A large number of climate models inferred the potential impacts of the greenhouse gases increase on the Asian monsoons system. Simulations revealed a progressive overall warming in Southeast Asia with extremes temperatures expected in arid areas of Northwestern India. Temperature increase will be stronger over land (3 – 5 °C) than ocean areas (2 – 2.5 °C). In increasing CO_2 scenarios, increased Indian Ocean air moisture is expected to reinforce the rainfall strength (Douville *et al*., 2000; Meehl & Washington 1993; Meehl & Arblaster, 2003; May, 2004).

Although temperature increase alone is not considered as a good predictor of rainfall (Douville *et al*., 2000), the differential between land and SST variations will enlarge differences between Central India and ocean, with an increase in monsoons strength (May, 2004). Simulations of last decades climate correctly identified the present snowcovers on Himalaya, predicting for the second half of this century a future warming for Eurasia of +6 °C. This warming trend will produce a corresponding reduction in snowcover, with the exception of the North East Asian region: in this area, increased snowfalls in the Tundra region are expected, due to higher rainfalls with temperatures, in spite of global warming, still remaining below the freezing point (May, 2004). The Southern edge of the snowcovered area, however, is expected to move North by 5°, reducing its boundaries along the Himalayan edge, as well as in Eastern Europe and Southern Scandinavia. All these changes will enhance the plateau heating and, consequently, the monsoon flow and the rainfalls strength (May, 2004).

Simulations also reproduced some El Niño-like events, showing the effect of the relatively weak (or even negative) South Pacific warming, in areas like Indonesia and New Guinea. Future warming of the Pacific appears stronger in areas interested by El Niño events and weaker in opposite situations. In this case, a weaker Indian monsoon is expected, with warmer temperatures (> 2.5 °C) for the Arabian Sea and Bay of Bengal, and weaker SST (< 2.5 °C) in equatorial and Southern Indian Ocean (May, 2004). Warming of the eastern tropical Pacific also will affect the monsoon intensities, counterbalancing the increase in the monsoon flow with a corresponding warming of the Arabian Sea. Models also showed that increased precipitation

variability in the tropical Pacific will affect the intensity of the indian monsoon via the upper atmosphere Walker Circulation, enhancing the precipitation variability (Meehl & Arblaster, 2003; May, 2004).

Modeling of the hydrologic cycle of three indian basins (Ganges/Brahmaputra, Godavari and Indus), estimated a 25% enhancement of future mean annual discharges, with an expected one month delay in maximal discharges for the first two rivers. A different shape of the annual cycle was inferred for the Indus river, whose enhanced discharge showed two maxima, due to an earlier snow melting in June and to increased precipitations in September (May, 2004). For the Ganges/Brahamputra and Godavari basins, changes in their annual discharges appear related to the precipitations/evaporations balance, with a reduced soil water content in May - July and an increase between August and October (May, 2004).

An increase of the interannual variability of the monsoon rainfall was also forecasted for the Indian region, except the southern Indian Ocean and the northern part of the Indian peninsula, the western Bay of Bengal and, partially, on the Himalaya. Highest increase of the interannual variability is expected by the last quarter of this century (May, 2004). The interannual wind variability will be enhanced over the Arabian Sea and within the Somali jet, over India and Southeast Asia, with increased interannual variability of the cross-equatorial flow.

2.4.2. The Tropical Pacific

May (2004) proposed that the tropical Pacific SSTs anomalies will affect the Indian summer monsoon when exceeding a given threshold, which appears lower in future climates, inducing more variabilities. High (low) intensity ENSO events will be responsible of weaker (stronger) summer monsoons, affecting westerly winds from the Arabian Sea, southern Indian peninsula and the Bay of Bengal. El Niño will enhance the northerly flow over the Indian peninsula and the Bay of Bengal, reducing southerly winds and the inflow of moist air from the Bay of Bengal into eastern India and Bangladesh. An opposite effect is expected for La Niña (cold) events, which will enhance inflow of moist air into eastern India and Bangladesh.

Modeling showed, for a 2.2°C increase of SSTs in NW Pacific, a 5 to 12% increase in hurricane wind intensities and pressure (Knutson *et al*., 1998). Other models also showed, in a doubled CO_2 scenario, a reduction of storms frequency, expecially in the Southern Hemisphere (Bengtsson *et al*., 1996; Keim *et al*., 2004). High CO_2 simulations also showed a 28% increase of hurricane near-storm precipitations and a 2-3% increse in wind force (Knutson & Tuleya, 1999).

Tropical storms are expected to increase also in the Caribbean and tropical Pacific. The coasts and islands of the Caribbean will be affected with a major impact by the climatic variations, due to urbanization and infrastructural investments (Lewsey *et al*., 2004). Climate changes expected are mainly related to an increase in rainfall and storms frequency/intensity, as well as coasts degradation due to increasing sea levels, loss of vegetation and exploitation of natural resources (Ellison & Farnsworth, 1997; Lewsey *et al*., 2004).

2.4.3. West Africa

Changes in vegetation cover, evapotranspiration, albedo, soil moisture and tropical Atlantic SSTs will affect the West African monsoons rainfall patterns in several regions, including the semi-arid Sahel (Le Barbé *et al*., 2002).

The SSTs of the tropical and northern Atlantic and tropical Pacific are responsible of the interannual and interdecadal rainfall variabilities in West Africa and the Sahel as well. The application of a regional circulation model to recent climate data showed good agreement and reproduction of the monsoons dynamics and of the climatic variations observed, including the variabilities due to dry and wet trends (Messager *et al*., 2004). Modeling revealed sensitivity of the rainfall periods to the SST anomalies and the effects of surface interactions and orography, over highland areas, i.e. the Mount Cameroon, the Bauchi Plateau and the Eastern Fouta Djalon. The increase in rainfall along the coast is associated with an increase in meridional moisture transport in the lower troposphere, induced by warmer SSTs and larger evaporation, with consequent transport of moist air to the continental areas (Messager *et al*., 2004). In a regime of increasing SSTs, a rainfall increase and a lower incidence of droughts in adiacent areas are expected (Sheppard & Rioja-Nieto, 2005).

3. CLIMATE CHANGES AND PLANT PROTECTION

Plant pathogens and pests are among the most important limiting factors affecting crops productivity. Several historical accounts described the impact of plant diseases on the history of human populations and migrations. One of the most cited event is the series of Irish catastrophic epidemics of potato late blight caused by *Phytophthora infestans*. The disease plagued Ireland in the period 1845-1847, inducing famine and undernourishment, causing the loss of million lives and the eventual overseas migration (Chakraborty *et al*., 2000; Ristaino, 2002). Other large scale events often recalled are the Bengal famine in 1942 caused by a rice leaf blight epidemics related to increased moisture, the 1960 famine in China, due to a wheat stripe rust outbreak, and the recent epidemics of potato late blight caused by a new race of *Phytophthora infestans* (Chakraborty *et al*., 2000; Rosenzweig *et al*., 2001).

Plant pathogens and pests are extremely dependent on climatic and environmental conditions: several phases of a pathogen's life cycle strictly depend on the combination of two or more environmental variables. Optimal temperature and moisture are key elements for modeling and forecasting the extent of a disease or pest epidemics, or to deploy preventive crop protection or management strategies, i. e. through pesticide applications, spraying or release of biological control agents.

The effects of climate changes on the interactions among plants and pathogens or parasites received increasing attention in the last decades, and experimental data, mainly concerning the effect of CO_2 and other greenhouse gases on crops, were made available in the literature since the mid 90's (Phillips *et al*., 1996; Chakraborty *et al*., 2000; Downing *et al*., 2000; Olesen & Bindi, 2002).

3.1. Some General Concepts in Plant Protection

Two basic concepts underlay plant protection and pest management: *1)* there are no living organisms on earth that can be considered exempt of diseases or antagonism and *2)* cultivated crops represent the first permanent environment change induced by man, on a global scale. Cultivated fields are the first environment modified by man, through sowing, deforestation and soil clearing, or through the selection of most convenient vegetation covers. All these actions affect the natural composition of the local flora and thus the corresponding density and biodiversity of plants and their distribution in space. Actual cropping systems represent indeed the best fit of a long series of attempts, including plants selection, aiming at best productivity with a minimum crop damage, and transmitted for generations through farmers' traditionalism and conservation practices.

Crops are also artificial combinations of living factors (plants, soil microbial communities, pests and diseases, animals, including wildlife) whose balance, conservation and/or protection/suppression depend on management. Cropping systems, furthermore, do not share the same features throughout the world, since important physical and structural differences can be found among the environments or the technology and energy inputs of agricultural practices, among regions with different levels of industrialisation. Nor do they keep the same features in time, due to the changes in the rural economy induced by trade and industrialisation, during the last centuries, forces which are still active today.

The attention given to the complex of resources available in a cropping system is generally related to a structural analysis (based on a complex of elements and their relationships) of crops. In an ideal, undisturbed environment, i. e. a native forest, plant parasitism usually does not affect global biomass production, resulting in short term variations adjusted by the system biodiversity, by its self-balancing capacities and its related feedback mechanisms. The global structure resulting from the solar energy conversion by native species and their relationships can be deeply altered, on the opposite, by new (host switch) or external (introduced) parasites or diseases, not endemic in the system, i. e. proceeding from other continents or regions, and free from controlling agents or balancing mechanisms. This is the case, i. e. of the epidemics caused by the gypsy moth *Lymantria dispar,* a polyphagous lepidopteran introduced from Europe in North America around 1868 for recreational purposes. After introduction, this invasive species eventually escaped and adapted to the local climate and environment, spreading to forests and urban areas where it established as one of the most important introduced pests, and is still spreading today (Liebhold *et al*., 1989).

From this point of view, pests and disease epidemics should not be considered as the only factors responsible for crop losses, but also as the last and visible effect of changes originating i. e. in plants population genetics, transports, man's actions or goods movement, land management or related to a loss in biodiversity. These changes do not only result in a crop susceptibility, but also reflect historical or social factors.

A further example is given by the introduction of the soybean cyst nematode, *Heterodera glycines*, in the USA. Esterases polymorphisms comparison of Asian

and American nematode populations were used to identify the speciation area of the pest. Through the identification of specific carboxylesterase patterns it was shown that *H. glycines* was introduced into the USA from Japan with soil moved by the early XXth century to spread the symbiotic bacterium *Bradyrhizobium japonicum* in North America fields. Phylogenetic analysis and isozyme studies on biodiversity of *H. glycines* populations proceeding from China showed that the nematode originated in China and was later introduced into Japan and then into the USA (Noel, 1992; Noel & Liu, 1998). This event illustrates how crop protection is related to historical or social backgrounds, which may have severe long term consequences on crops productivity at a regional scale.

As a further example of structural changes let's consider the frequency of application of methyl bromide (CH_3Br), a fumigant applied to control root-knot nematodes and other soil pathogens. In Southern Italy this practices, progressively dismissed due to the CH_3Br ban, is often related to extremely high rates of nematode resurgence in the years following treatments, appearing in average six-eight months after the last treatment. Re-colonization of treated parcels is not the only factor responsible for the observed nematode population outbreaks, since observed rates of fungal parasitism rarely exceed, in these populations, 1% of eggs. Rates of up to 40% eggs parasitism are normally observed in untreated soils or in perennial crops, where a number of specific or generalist microorganisms may be found in eggs (Ciancio, unpubl. data). The management solution applied in treated soils appeared, in these circumstances, as the most probable cause of future problems, as soil sterilization leaves the roots unprotected due to the destruction of the surrounding soil microflora and nematode antagonists communities. Treatments based on general biocides alone appear, hence, unsuitable for long term conservation and management of soil fertility and productivity, also considering the multiple mechanisms (soil particles, water, air, seeds, mechanical tools, survival on weeds along the field boundaries) responsible for nematodes spatial spreading.

A structural view is useful to understand the mechanisms of biological control and natural regulation, and to identify the actions required to sustain a stable equilibrium among species through optimal management. This concept stays behind i. e. the search for specialised parasitoids in the areas of a pest speciation, for their subsequent introduction in newly invaded regions, or the search of resistant germplasm in the areas of a host plant speciation. Often these actions show only a partial success and must be integrated by a complex of practices, including the rational use of pesticides applied in different moments of a pest/disease cycle, or the planting of a resistant variety, in order to maximize control at an acceptable cost, either economic and environmental. These actions give rise to the integrated management (IM) approach, in which a complex of different tecnologies is employed for plant protection.

A wide literature covers already several aspects of biological and integrated management of plant diseases, from theoretical basis to field practices. In the next sections we will hence focus our attention on the effects of the environmental and climatic changes previously described, aiming at an adaptive approach to sustain and improve actual IM strategies applied in the tropical agroecosystems.

3.2. Crop Protection and Anthropogenic Changes

In this section we examine the threats caused to crop production by the expected climatic changes. A specific consideration concerns the fact that temperature extremes, affecting the behaviour and life cycle of pests in the temperate hemisphere, do not occur with similar extents in tropical regions, where main climatic variations concern changes of rainfall regimes and/or the alternation of dry-wet seasons. In this situation, phenomena like the spreading or changes in pests abundance, forecasted by models for introduced species in cold areas and crops, i. e. cereals in Southern Canada (Olfert & Weiss, 2006), should be expected with a lower incidence in the Tropics. Although risks analysis may correctly predict a change in the distribution areas of some pests, this variation should occur with different extents in the tropical regions, i. e. in areas characterised by cold/warm alternated seasons, i. e. the Andean region and the Altiplano. Temperature related changes should result negligible in warmer climates, with low excursion ranges expected during the year.

3.2.1. Changes Induced by Climate Variations

As previously stated, shifts in optimal temperatures for crops, plant pathogens and biological control agents may have different outcomes, depending on the regions and crops examinded (Olfert & Weiss, 2006). Similarly, changes in moisture regimes may have different beneficial or negative impacts, depending on the regional situations, climate and geography. In synthesis, the mechanisms that a particular climate or environment change may induce on a plant pest/disease and on the technologies actually applied for IM are herein summarized.

The effects of increasing temperatures on pests and crops may result in:

- Increased crops growth rates and yields
- Earlier germination of seeds, plant flowering or ripening
- Higher host plant carrying capacity
- Earlier emergence of pest/disease/vectors and crop attacks
- Longer life cycle and reduced pest/disease generation time
- Increased spatial spread towards new areas available for colonization
- Increased spatial spreading at higher altitudes
- Elimination of regional barriers for spatial spreading
- Shift to other host crops in adiacent or newly colonized areas.

Changes in moisture regimes may result in:

- Higher/lower water availablility for plants and crop yields
- Higher pest/disease density or increased prevalence
- Higher flood frequencies
- Increased spreading of water related diseases (i.e. acquatic fungi)
- Increased or earlier disease incidence (i. e. powdery mildew)
- Increased disease duration and/or pest or vector cycles/generations
- Lower/higher incidence of droughts.

Changes that temperature and moisture regimes may produce on biological control agents are:

- Increased densities due to higher yields and crops host carrying capacity
- Earlier emergence and outbreaks due to earlier pest emergence
- Longer life cycle and/or reduced generation time
- Increased spatial spread to newly colonized areas following pests spreading
- Increased spread of water related diseases (i.e. Entomophtorales)
- Higher incidence of hyperparasitism (i. e. microsporidians in parasitoids)

Other indirect mechanisms favouring a pest/disease insurgence or the spreading of an invasive species are (Goudrian & Zadoks, 1995; Fuhrer, 2003):

- Increased (reduced) leaf moisture in wet (dry) conditions
- Increased survival of propagules (spores, bacterial cells) on leaves and other host tissues
- Reduced (increased) plants resistance due to physiological adaptations to temperature changes (i. e. prolonged vegetation, lignification)
- Changes in the nutritive value of host plants tissues
- Increased density of alternated or secondary hosts (weeds)
- Spreading of new invasive plants and/or changes in floral composition.

Factors affecting the response of a variable (i. e. crop productivity, pest or disease prevalence) to a given climatic change are key elements and must be correctly identified, in order to yield reliable informations from modeling. They include also physical properties of soil, i. e. texture or water retention capacity, which may affect at different extents the response to changes of the hydrologic cycle (Wessolek & Asseng, 2006).

3.2.2. Marginal Benefit and Density Thresholds

Before affording in more detail some basic concepts required to evaluate the changes in IM strategies, whose introduction may result helpful to face emerging changes, we have to recall that cultivated fields are complex systems, and that absolute rules cannot be applied. As an example, let's consider the effect of an increasing rainfall regime: in arid climates an increase in water availability either in soil and/or reservoirs, i. e. natural or artificial lakes, may yield positive, structural consequences on agricultural practices, increasing crops productivity or their cultivated surface. Irrigation is indeed one of the most important factors increasing crops yields. Increasing rainfalls may also induce changes in the selection of varieties or cultivated species. In turn, these changes may produce a feedback effect, increasing the incidence of pests or diseases, switching either their species composition (replacement) or even increasing natural antagonists and prevalence levels, due to higher air moisture, affecting i. e. the spreading of antagonistic fungi or predatory insects.

On the opposite, in a wet climate, an increasing rainfall regime of the same magnitude may produce floods, with soil losses due to higher erosion, forcing changes in plant/cultivar choices or in the time of sowing. Higher amounts of water in soil will increase the incidence of aerial plant diseases (i. e. powdery mildew, *Botrytis*) or root pathogens or stress (i. e. tracheomycosis, or root asfixia). Catastrophic rainfalls may also affect some IM related agronomic pratices, like soil labour, solarization, sowing and fertilization or vanish the effects of chemicals.

In the search for optimal crops productivity, the general concept of marginal benefit may result useful as a basis for modeling and decision making. Marginal benefits are expected for crops reacting to any given factor affecting production: they approach to zero as the crop productivity function approaches its optimum. After this point no further benefit in productivity is observed, and negative increments are scored if the production factor is still increased (Fig. 4).

A rational IM decision must consider how far (and wheather) the crop response is, for a given factor, from its optimum. This approach is necessary to evaluate if the climatic or environment change expected will produce positive or negative implications, improving yields or acting in a detrimental way, thus identifying the action to be taken. This simple and general concept may be extended to any factor affecting plants productivity, including a crop response to i. e. a pesticide or irrigation. This relationship is useful when evaluating the costs/benefits ratio expected by actions aiming at pest prevention or eradication, with particular reference to invasive species (Fraser *et al*., 2006).

The study of invasive species behaviour may provide data informative about the problems and losses expected after a biological invasion favoured by altered climate or environment conditions. As previously cited, the gypsy moth epidemics in North America is one of the best studied cases of biological invasion. Recently, Johnson *et al*. (2006) discovered that the observed cyclic, pulsed progression of the moth distribution boundaries (expanding at an average speed of 21 km · year^{-1}) are affected by a density-related mechanism known as Allee effect, accounting for a minimum threshold number of individuals required, in new isolated colonies, for a successful establishment. The introduction of the

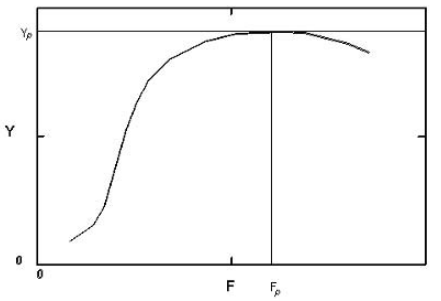

Figure 4. Increments in yields (Y) as a function of a general production factor F, showing decreasing marginal benefits after optimal yield (Yp) is reached at Fp.

Allee effect in models describing the moth expansion allowed the simulation of pulsed waves of emigrants settlings, originating from highest donor populations, peacking along the borders. The threshold in donors populations explained the peacks and the time required for the 4 years pulsing periodicity of the moths, corresponding to the time needed by the donor population to reach and exceed the donor threshold. The sum of the periods required by a donor population to reach a threshold and the time required, in a new settling, to reach an Allee threshold density fit the observed 9 years periodicity of moths outbreaks, suggesting that the donor threshold is reached when estabilished populations approach outbreaks levels. This study shows the importance of the knowledge about the main parameters affecting the mechanisms of a population spreading, since suppressing population peacks all along the distribution boundaries may help in slowering the moths epidemic progression (Johnson *et al*., 2006). Given the strong dependence of the epidemic progression on the moths densities, any induced change affecting the pest behaviour (i.e. longer life cycle, increased fecundity, longer deposition season, higher mortality or antagonists prevalence), should also be considered when forecasting an epidemics progression.

3.3. Effects of Climate and Environment Changes on Pests and Diseases

3.3.1. Insects and Mites

Exceeding critical climatic thresholds affect phenological and developmental stages of insects, including mortality, fecundity, oviposition and generation numbers (Kiritani, 2006). For example, the damage to rice caused by *Stenotus rubrovittatus* is becoming serious in northern part of Japan, as an increase of the annual mean temperature by about 1°C is enough to allow one additional pest generation (Kiritani, 2006). Adverse effects may also be expected: Patterson *et al.,* (1999) listed a number of species on which increasing temperatures produce adverse effects on fecundity, including bollworm, *Helicoverpa zea*, tobacco budworm, *Heliothis virescens*, beet armyworm, *Spodoptera exigua*, cabbage looper, *Trichoplusia ni*, saltmarsh caterpillar, *Estigmene acrea* and pink bollworm, *Pectinophora gossypiella*.

Both beneficial insects and pests will react to warmer conditions, and a general increase in insect abundance is expected at mid to high latitudes (Fuhrer, 2003). In tropical climates, temperature changes will affect either the insects behaviour and their life-cycle, with a strong influence on the resulting population dynamics. Differing from temperate climates in northern latitudes, the main effects in tropical regions will concern changes in survival, generation numbers and distribution boundaries (Fuhrer, 2003).

The latter changes are a major concern for tropical insects populations and the world agricultural system. The possibility that climatic changes and man activities may also open new ways for spatial spreading, reducing or eliminating natural barriers confining a species geographic range, is a further concern. Expected shifts

in insects distribution patterns concern upward (higher altitudes) and poleward shifts (Walther *et al*., 2002). In the Northern hemisphere, butterflies were observed to track decadal warming very quickly, following the upward and northward shifts in temperature isotherms (Parmesan *et al*., 1999). Porter *et al*. (1991) predicted for the European corn borer, *Ostrinia nubilalis*, a northward shift of 165-500 km per each 1°C raise in temperature, and an additional generation for each region in which it already occurs. In Japan, increasing damage to rice and fruit crops, simultaneous outbreaks and poleward spreads observed for six insect species were explained as effects of global warming. The winter mortality of adults of *Nezara viridula* and *Halyomorpha halys* were predicted to be reduced by 15% by each rise of 1°C (Kiritani, 2006). More than 50 species of butterflies showed northward expansions and ten species of previously migrant butterflies established on Nansei Islands in the period 1966-1987 (Kiritani, 2006).

Upward migration following the extension of crops to higher altitudes is a further possible outcome for several tropical insect pests, as well as invasion of new areas available for colonisation in temperate regions by milder winters.

A second change affecting insects is represented by increased UV-B irradiation. UV-B induces direct cell damage at the DNA and proteins levels, with increased production of free radicals and oxygen species (Caldwell *et al*., 1998). Although shielded by the cuticular layers, herbivorous insects may be affected indirectly, by the UV-B lowering of the leaf tissues quality and suitability for larval feeding, or directly, as UV-B affect eggs deposition or herbivory. In controlled tests, UV-B irradiation reduced the eggs deposition of *Plutella xylostella* L. (diamondback moth) on the model plant *Arabidopsis thaliana*, altering adult females selection of host plants and eggs deposition (Caputo *et al*., 2006).

UV-B irradiation may also interfere with host preference. A 50% increase in UV-B irradiation showed higher numbers of naturally occurring insect herbivores on *Salix* spp. Tests showed that the leaf beetle, *Phratora vitellinae*, a specialist herbivore of *Salix myrsinifolia*, was more sensitive to chemical changes induced by UV-B on its secondary host *S. phylicifolia*, than to changes induced in its primary host (Veteli *et al*., 2003).

In Argentina, increased UV-B irradiation, artificially induced on leaves of the southern beech tree *Nothofagus antarctica,* showed a reduction of field lepidopteran herbivory, mediated in part by the UV-B effects on gallic acid and flavonoids (Rousseaux *et al*., 2004). In soybean leaves exposed to solar UV-B, observed caterpillar survival and herbivory were lower and were considered as due to higher levels of soluble phenols and lower lignin contents (Mazza *et al*. 1999; Zavala *et al*. 2001). Higher N contents in leaves of the perennial herb *Gunnera magellanica* receiving solar UV-B were related to lower herbivory by lepidopteran larvae (Rousseaux *et al*. 1998).

Increasing CO_2 levels may also enhance insects feeding activity, through the effects on crops yields, plant physiology and composition, as well as their distribution and spatial ranges (Patterson *et al*., 1999). Effects of higher CO_2 levels on insect herbivory showed that altered C/N ratio in plants due to decreased N content enhanced insects feeding and increased food consumption (Fuhrer, 2003). Lower protein content of potato leaves, however, affected the development and

growth rates of the Colorado beetle, *Leptinotarsa decemlineata* feeding on potato leaves (Miglietta *et al*., 2000). Different responses may be expected by the type of herbivores, as leaf chewers increase their feeding on leaves to compensate lower N levels, whereas leaf miners and seed eaters show almost no effect (Bezemer & Jones, 1998). Increased thickness of leaves and stem tissues induced by CO_2 may also affect sucking insects and mites, although CO_2 enrichment was also found to increase density of *Tetranychus urticae* and several aphid species (Fuhrer, 2003). The effects of higher CO_2 are difficult to dissect from the effect of increasing temperatures, as both may interact constructively, with i. e. increasing insects herbivory and development, or negatively, through i. e. increased parasitoid numbers or higher stress levels, with complex relationships at the community level (Fuhrer, 2003).

High ozone levels increased ovipositioning of hornworm moth, *Manduca sexta,* on tobacco, increasing survival and growth. Having an opposite effect on N content in leaves, ozone was expected to reduce herbivory. However, accelerated female maturity and abundance were observed for mites feeding on clover, as the result of increased carbohydrate concentrations (Fuhrer, 2003).

Air moisture and intense precipitations may adversely affect , as reported for oviposition of the European corn borer, *Ostinia nubilalis*, although major adverse effects may be expected by the increase of soil saturation periods and spread of parasites and predators (Patterson *et al*., 1999).

Winds are used by some of the most important insect pests to reach new areas, to disperse and colonize plants and habitats. ITCZ as well as nocturnal wind jets are known to be used by *Bemisia tabaci* or locusts to disperse via the atmospheric transport in agricultural zones. Changes in circulation patterns may then produce severe consequences, favouring insects migration and dispersal of most invasive species over longer ranges (Patterson *et al.*, 1999).

Insects genetic adaptations to new selective pressures, as those introduced in urban environments or due to migrations to new habitats, were also documented, showing that insects have strong adaptive capacities and genetic potentials. Examples include the reports of natural selection of diamondback moth populations resistant to *Bacillus thuringiensis* (Tabashnik *et al.*, 1990), or the natural selection of *Drosophila* spp. observed in urban habitats (Patterson *et al.,* 1999).

3.3.2. Soil Food Webs

The response of soil nematodes communities to increasing CO_2 levels is complex, since different effects were observed on herbivorous, bacterial or fungal feeders and predatory species. No significant effects of high CO_2 levels were reported for nematodes from prairie soil (Freckman *et al*., 1991). Nematode numbers were observed to decrease in cotton rhizosphere (Runion *et al*., 1994) or to increase in aspen forest (Hoeksema *et al*. 2000), grasslands (Hungate *et al*. 2000) and pastures (Yeates & Orchard, 1993; Yeates *et al*. 1997; 2003). Soil complexity and structure of the food webs may also produce different responses to increasing CO_2 levels: nutrient availability in forests soils, for instance, may differ in their outcomes on

nematodes trophic groups. In two forests soils, increased CO_2 levels decreased nematodes total numbers, reducing bacteriovores and increasing the abundance of fungal feeders and predators (Neher *et al*., 2004). Increased numbers of predatory nematodes were also observed in prairie soils (Yeates *et al*. 2003).

A double atmospheric CO_2 content increased densities of arbuscular mycorrhizal fungi in soil, but showed no effect on bacteria, enhancing the numbers of soil arthropods. These changes were considered as a probable consequence of increased plants productivity and higher trophic availability in soil food webs, since several arthropod species are fungal feeders (Rillig *et al*., 1999). In a trial under poplar tree cuttings with an elevated (693 ppm) atmospheric CO_2 concentration and increased N fertilization, no effect was observed on microbial biomass, but numbers of protozoa increased, whereas the microarthropods and mycorrhizal content of soil doubled. These changes were interpreted as related to higher rates of bacterial turnover, due to protozoal predation (Lussenhop *et al*., 1998).

Jones *et al*. (1998) investigated a number of complex food chains in controlled environments, comparing actual levels of CO_2 with a 53% increased atmospheric content. They found that higher CO_2 fixation rates of plants allowed a greater amount of nutrients available belowground, with a 52% increase observed for the soil microarthropods decomposers, at the end of the food chain.

Air pollutant are considered to affect phytoparasitic nematodes through alterations induced in host plant physiology. Synergistic interactions between ozone or SO_2 and the root knot nematode *Meloidogyne incognita* were observed on tomato, with higher levels of foliar injuries scored on nematodes infested plants. Galls on roots were higher in nematodes infested plants exposed to 100 ppb ozone at 5 hours intervals every third day. Exposure to ozone, however, reduced the reproduction performance of *M. incognita*, as lower numbers of eggs and masses were observed at 50 and 100 ppb (Khan & Khan, 1997).

3.3.3. Plant Pathogens

Temperature and moisture are key variables influencing plant diseases through a number of mechanisms, starting from germination of infective propagules, and proceeding through all stages of infection until pathogens persistence in the environment.

Goudriaan & Zadoks (1995) pointed out that a disease thrieves where the host plant grows at its best, and that it may be possible to predict where a pathogen may reach its host plant in a new region, previously made available for cropping, through the use of geophytopathology principles. Also, the adaptability potentials and the range of genetic variability for several pathogens are not completely known. The identificaton of these adaptive boundaries is very important, since they may represent the basis for a number of future expansions and colonizations of new areas, following isothermal shifts.

Changes in moisture also affect the plant physiology and the host-disease interactions, particularly during the early process of host infection, as well as the survival of pathogen's resting propagules or infective spores. Long-term data on

indian chickpea crops showed that highest incidence of Ascochyta blight (due to *Ascochyta rabiei*) is largely dependent on relative humidity and temperature. Analysis of correlations between the disease incidence and a set of meteorological data, proceeding from historical series, showed that highest incidence of the disease, with catastrophic consequences on the crop yields, is expected during a late crop risky period of a few weeks, with more than 50 % afternoon relative humidities (indicative of longer night leaf moisture) and mean temperatures around 20°C, optimal for germination of pycnidiospores (Jhorar *et al*., 1997). Both variables were used to elaborate a model based on humid thermal ratio and disease indexes, useful to identify and anticipate the periods of crop exposure to the disease, requiring protection by fungicides. In presence of changes in temperatures and humidity regimes, the model may be readjusted in order to fit the meteorological variables to the crop disease index, keeping its value as a forecasting tool.

Increasing CO_2 levels affect several properties of leaves, altering their physiology and surfaces. In revising the effects of CO_2 on pathogens and host plant interactions, Coakley *et al*. (1999) highlighted two general mechanisms: a delay in pathogens establishment and/or infection progress, and a potential increase in a pathogen's reproductive rate. Both effects were observed for the fungal pathogens *Colletotrichum gloeosporioides* and *Maravalia cryptostegiae* or for *Erysiphae graminis* on barley. Increased CO_2 levels also affected the resistance of the pasture legume *Stylosanthes scabra* to anthracnose, caused by *C. gloeosporioides* (Pangga *et al*., 2004).

The pathogen target and its physiology may affect the host-pathogen interaction and the outcome of exposure to increased CO_2 levels. In a long term study in a controlled environment, Mcelrone *et al*. (2005) experimentally observed a reduction in disease incidence cased by the fungal pathogen *Phyllosticta minima* on leaves of *Acer rubrum*, grown in a high CO_2 atmosphere. Damage reduction was not related to the pathogen germination or infection rates, but to a lower stomatal opening of the host leaves, which showed altered chemistry and lower nutritive value, due to reduced N content and a higher C/N ratio (Mcelrone *et al*., 2005). A similar effect was also considered in a study of a monoculture of *Solidago rigida* under controlled CO_2 levels. Data from two growing seasons showed a lower incidence of leaf spot disease on plants exposed to higher (190 ppm) CO_2 levels (Strengbom & Reich, 2006).

Also the reaction of oat plants to viral (BYDV) infection appeared affected by the CO_2 amounts, since the plants exposed to higher levels showed higher growth, with the greatest response in diseased plants. Increased photosynthesis and higher water management efficiency due to reduced stomatal conductance were considered as responsible for the better performance of the virus infected plants (Malmstrom & Field, 1997).

The beneficial effects of higher CO_2 levels are also expected to counterbalance losses due to pathogens attacking plant organs other than leaves. In an experimental hydroponic assay on the effects of CO_2 on the tomato root pathogen *Phytophthora parasitica*, CO_2 at 700 ppm increased plant biomass by 30%, counterbalancing the losses induced by the pathogen (Jwa & Walling, 2001).

Also the effect of ozone on plant diseases may be strongly affected by factors like the plant stage and physiology, the levels of available nutrients and the concentrations of other atmospheric gases. Tests carried out to evaluate the effects of outdoor ozone concentrations, recorded in Central Germany, on plant disease susceptibility showed that wheat sensitivity to leaf blotch, caused by the fungus *Septoria nodorum*, was limited to some young or mature growth stages, and were enhanced in presence of high levels of N fertilization. Lower effects of ozone concentrations were observed during stem elongation. Plants exposed to ozone also showed low differences for powdery mildew (*Erysiphe graminis*). Also in this case the disease was enhanced in presence of high levels of N fertilization. The combination of two atmospheric gases increased the complexity of the plant response, since increased CO_2 levels reduced the effects of leaf rust (*Puccinia recondita*), but were hidden by the ozone hypersensitive response occurring at the cellular level. Leaf damage was detected only at the highest (600 ppm) CO_2 concentration (Tiedemann & Firsching, 1998).

Direct ozone damages may be observed experimentally. In simulation tests carried out in controlled chambers, high ozone concentrations strongly inhibited damage by leaf rust (*Puccinia recondita* f. sp. *tritici*) on spring wheat (*Triticum aestivum*) (Tiedemann & Firsching, 2000). At 90 ppb, however, wheat height and above-ground biomass generally decreased with ozone exposure and with increasing disease severity, but no synergistic effects were observed between them (Pfleeger *et al*., 1999).

Exposure for 257 days at high ozone concentrations (120 nmol mol^{-1}) increased susceptibility of coniferous trees (*Picea sitchensis*) to the root rot pathogen *Heterobasidion annosum*, reducing lignification and impairing antimicrobial defence (Pearce, 1996). A 6 weeks experimental exposure to ozone (100 ppb) increased the size of lesions induced by spores of the fungus *Marssonina tremulae* on leaves of *Populus trichocarpa* × *balsamifera*, with major effects on older leaves (Beare *et al*., 1998). A comparative study carried out from 1999 to 2001 on black cherry trees and milkweed stems in areas around the Lake Michigan with different chronic ozone levels, showed that ozone exposure (peak hourly concentration) was among the most important variable affecting plants. Black cherry branch elongation and milkweed growth and pod formation were significantly higher in low ozone areas. Exposures at concentrations greater than 13 ppm decreased cherry branch elongation by 18%, exposures greater than 93 and 98 ppb reduced milkweed stem height by 13% and pod formation by 11%, respectively (Bennett *et al*., 2006).

Tests in controlled conditions showed moderate ozone injury on *Sphaerotheca fuliginea*-inoculated cucumbers. Powdery mildew development was severe on the plants exposed to 50 ppb, as ozone exposures stimulated the conidial germination. At higher concentrations, however, there was a significant decline in fungus colonization, as conidia exposed to 100 or 200 ppb were smaller and showed poor germination. Ozone at 50 ppb and *S. fuliginea* interacted synergistically and caused significantly greater decrease in the number of fruits per plant, whereas at 200 ppb the mutual effects were antagonistic (Khan & Khan, 1999). Similarly, fungal colonization by *S. fuliginea*, causal agent of powdery mildew on bottle

gourd (*Lagenaria siceraria*), were higher at 50 ppb ozone concentrations, and decreased at 200 ppb (Khan & Khan, 1998).

UV-B effects may be observed in the plant-pathogen interactions but a few data are available to infer general rules in plant-pathogen systems (Paul, 2000). Tests carried out *in vitro* or in controlled environments on *Septoria tritici*, the causal agent of leaf blotch on wheat, showed that the fungus response to increased UV-B irradiation is isolate-dependent. Inhibition of *S. tritici* conidial germination was observed to vary not only among isolates proceeding from diverse geographical locations, but also among those originating from contiguous areas (Paul *et al*., 1998). Sensitive isolates reacted to the short-wavelength UV-B (280–320 nm), which were the most effective in inhibiting conidial germination and germ tube growth. Artificially inoculated plants exposed to UV-B irradiation after inoculation showed lower infections rates (Paul *et al*., 1998). Field data confirmed that leaf blotch was reduced by increased UV-B irradiation. The response was also affected by other environmental factors, with potential effects on the fungus epidemiology rather than on the disease incidence (Paul *et al*., 1998). Data on *Septoria* tritici infection of wheat suggested that UV-B increases due to ozone depletion are expected to be small compared with the effects of seasonal or cloud related UV-B variation. In general, increased UV-B showed an increase in subsequent disease, probably related to changes in host surface properties or composition (Paul, 2000).

3.4. Habitat Changes and Integrated Management

3.4.1. Rainforests

In general, rainforest clearing and disturbance are considered as an irreversible threat to insects, with consequent loss of biodiversity and increased probabilities of species extinctions, in particular at the small spatial scale (Hamer & Hill, 2000; Brook *et al*., 2003). However, habitats modifications may have different effects on the abundance and diversity of insect species migrating to agricultural land.

In Australia, endemic *Helicoverpa* spp. responded positively to rainforest clearing, and established as agricultural pests of cotton and chickpea. Recent and fast urbanization and agricultural conversion of cleared land largely reduced the Queensland coast rainforest to a mosaic of different habitats. Data on native endemic fruit flies species (mainly *Bractocera* spp.) showed that polyphagous species responded better than monophagous ones, adapting to local and exotic host plants. Rainforest clearing and its substitution with suburban habitats increased the total abundance of a single fruit fly species, thus reducing local biodiversity levels (Raghu *et al*., 2000).

In Nigeria humid rainforest agriculture, tests on aphid (*Aphis craccivora*) susceptible and resistant cowpea varieties showed significant interactions between watering regimes and varieties for aphid survival rates, biomass and fruit yields. Drought stress in the soil affected the increase of aphid population, with significantly lower densities on resistant varieties, which supported lower aphid survival rates compared with susceptible ones. Significant negative linear

correlations were found for watering intervals and aphid population, plant biomass and grain yield (Agele *et al*., 2006).

In traditional farming in the Amazonian region, with secondary forests resulting from long periods of fallow, agriculture rely on vegetation re-colonization for subsequent slash and burn clearing, allowing 1-2 years croppings before soil exhaustion. Fallow plays a very important role in sustaining productivity of this particular habitats, mainly based on acidic soils. A positive correlation between yields and length of the fallow period, with lower incidence of insect pests, was observed in these systems (Silva-Forsberg & Fearnside, 1997). Similar practices are common in other Tropical regions in West Africa and Philippines. In the Philippines rainforests habitats, insect pests were limited to adiacent cultivated land. None of the forest non-pest species were able to estabilish permanently in agricultural areas, facing extinction as the forest disappeared with the expansion of agriculture. Some pest and non-pest species were, however, recorded from the forest margins (Szinicz *et al*., 2005).

3.4.2. Hydrologic Cycles

Changes in the hydrologic cycle may affect crops and IM strategies directly, by increasing the susceptibility of plants to water sensitive diseases, or indirectly, through soil drought or saturation, root asphyxia, changes in levels of leaf moisture and exposure to fungal attacks. Water shortage and drought may be expected to decrease disease and pests incidence. Changes in the quality of aquifers (increased salts, pollutants) may also have significant consequences in crop protection.

The impact of greenhouse warming on soil moisture was studied comparing predictions of 15 global climate models. The models predicted summer dryness and winter wetness in only some areas from the northern middle and high latitudes, forecasting a worldwide agricultural drought with different magnitudes of soil moisture response (Wang, 2005). In the Tropics and Subtropics, a decrease of soil moisture was predicted over the southwest North America, Central America and the South Africa in all seasons, over much of the Amazon and West Africa in the June-August period and in the Asian monsoon region during the December-February season (Wang, 2005).

In tropical Asia, planting technology and water availability affect rice susceptibility to pests. Shifting from transplanting to water-saving irrigation practices, like direct-seeding, enhanced damage by brown spot (*Cochliobolus miyabeanus*) or planthoppers (*Nilaparvata lugens*) wherease injury due to stem rot (*Magnaporthe salvinii*), sheath blight (*Rhizoctonia solani*) and rice whorl maggot (*Hydrellia philippina*) were reduced. Water shortages and poor water management favoured sheath rot (*Sarocladium oryzae*), brown spot, neck blast (*Magnaporthe grisea*) and whiteheads (caused by stem borers: *Scirpophaga incertulas, S. innotata, Chilo suppressalis, Sesamia inferens*), with a suppressive effect on stem rot and sheath blight. 'Poor' water management, reducing plants growth, reduced the contacts among host tissues within the canopy, with lower probabilities for sheath blight spreading within and between rice plants. The level of water supply may

affect rice blast epidemics, through the release of spores, and subsequent germination and infection (Savary *et al*., 2005).

Water management also directly affects the microclimate, which in turn influences the pathogen life cycle and the plants susceptibility to the disease. For example, brown spot is favoured by a reduced water supply, enhancing infection and the rate of lesion expansion (Savary *et al*., 2005). The method of crop establishment and the level of water management will differ in their effects in the future, depending on rice pests and diseases: with water resource constraints and widespread use of direct-seeding technologies, diseases such as sheath rot, brown spot and neck blast might become more important, together with insect pests like leaffolder (*Cnaphalocrocis medinalis*), deadhearts and whiteheads. However, diseases such as stem rot and sheath blight would be negatively affected by these two factors (Savary *et al*., 2005).

In rainfed rice crops from Central Java, yield losses from pests were estimated as 56–59%, with low and unstable yields attributed to drought, nutrient stress and pest infestation management or to a combination of these factors (Boling *et al*., 2004).

Changes in frequency and intensitiy of rainfalls may also affect pests regulation by predators. Data from cassava crops in Benin, West Africa on the herbivorous mite *Mononychellus tanajoa* (accidentally introduced into Africa in 1971), and its predator *Typhlodromalus aripo* (a neotropical phytoseiid mite imported from Brazil and established in Africa since 1993), analysed through a time series analysis showed declining density of either the predator and prey. Mite regulation showed detrimental effects of spring droughts on predator water assumption, lowering predation efficiency. Intense rainfalls (May–October) caused substantial mortality of *M. tanajoa* through wash-off, low prey densities and indirect negative effects on predator numbers as well. Droughts (January–March) reduced oviposition and egg eclosion, increasing the mortality of immature and adult preys (Hanna *et al*., 2005).

3.5. Epidemics and Biological Control Agents

At the regional scale, communities of species often react in an asymmetric way to climate changes, with resulting shifts in their composition. In North America, for instance, warmer springs disrupt the synchrony of the oak winter moth phenology. The consequent mismatch between insect availability and predating birds have a consequence for insect population dynamics and the stability of the predator population (Visser & Holleman, 2001).

Earlier onset of a disease/pest epidemiology may result from a local increase in temperatures, with major effects related to the minimal values (Walther *et al*., 2002). Invasions, although frequently triggered by human activities, result by the species biology and reactivity to the new climatic conditions, allowing its establishment. Tropical and subtropical species will advance poleward continuosly, since they lack a diapause phase. On the opposite, temperate species, which need a winter diapause, will not advance poleward until enough warming will allow an additional generation, expanding their range stepwise (Kiritani, 2006). Further examples of

invasive species, and of the related concerns caused, are: the invasion of East Asia by the rice water weevil *Lissorhoptrus oryzophilus* which shifted to rice from wild gramineous hosts in North America and then spread on rice crops worldwide (Chen *et al*., 2005); the red palm weevil *Rhynchophorus ferrugineus* (Coleoptera: Curculionidae) that reached the Mediterranean regions (Sacchetti *et al*., 2006); the expansion of the mosquito-borne diseases (malaria, yellow fever, dengue) in high elevation areas in Asia, Africa and Latin America, considered as a consequence of annual temperatures rise (Epstein *et al*., 1998; Walther *et al*., 2002).

Global warming may be responsible for species substitution, like the recent decline in abundance of *Plutella xylostella* on *Brassica* plants in Japan, substituted by populations of *Helicoverpa armigera* and *Trichoplusia ni*. In general, global warming was considered to favour natural enemies by increasing their number of generations more than in corresponding hosts (Kiritani, 2006).

UV-A and UV-B radiations affected the survival of the insect pathogenic fungus *Metarhizium anisopliae* limiting its practical use as a biological control agent (Zimmermann, 1982). Growth tests showed that growth conditions and conidia production affect *M. anisopliae* UV-B tolerance and speed of germination, as isolates of *M. anisopliae* var. *anisopliae* were significantly more sensitive to UV-B, and germinated slower, when produced on insect cadavers. Nutritive stress greatly improved UV-B tolerance, but reduced conidial yield, whereas optimal growth conditions improved conidial yield, reducing UV-B tolerance (Rangel *et al*., 2004; Rangel *et al*., 2006).

3.6. Plants Reactions to Climate Changes

3.6.1. Reaction to Greenhouse Gases

There is a general agreement about the positive effects of increased CO_2 levels on plant productivity (Olszyk & Ingram, 1993; Goudriaan & Zadoks, 1995; Manning & Thedemann, 1995; Chakraborty *et al*., 2000). The correlation observed in controlled athmosphere between high CO_2 levels and plant productivity and conversion efficiency (Downing *et al*., 2000), suggests a global increase in crops and ecosystems productions, due to higher efficiency in water and N use and higher efficiency in conversion of radiation (Olesen & Bindi, 2002). Average yield increases by 28% were observed for wheat, at a double CO_2 concentration (Downing *et al*., 2000).

The response of crops to increasing CO_2 levels is largely due to the plant photosynthetic pathway, being higher in C_3 plants (i.e. cereals) whereas in C_4 plants (mainly tropical species, i. e. maize) the observed increase is lower (Allen, 1990). Increased CO_2 also affects plants stomatal aperture and density, lowering the transpiration efficiency, and dark respiration (Olesen & Bindi, 2002).

Reaction and productivity of crops to increased CO_2 levels and temperatures are also affected by climatic factors, mainly precipitations. Simulations for the US corn belt productive areas showed a 3% loss in yields with a mean 2 °C temperature increase alone, and a dependence of crop yields on rainfall regimes. A 10% increase

in rainfall appeared sufficient to balance the 2 °C temperature increase loss, with +17% and +27% yield increases for corn and soybean, respectively, expected in a 625 ppm CO_2 scenario (Phillips *et al*., 1996).

Simulations of maize production for South Africa smallholders showed that future climate scenarios of doubled CO_2, in presence or absence of a 10% rainfall increase, have the highest mean grain yields, with estimated increases of over 0.2-1 ton/ha, depending on the organic/manure fertilizer applied. The largest negative effects were observed for a mean 2 °C increase scenario, with losses of up to 5% of organic N in a doubled athmospheric CO_2 concentration (Walker & Schulze, 2006).

The effect of ozone on plants is considered detrimental, although forest vegetations in temperate areas, i. e. mediterranean forests, appear tolerant (Paoletti, 2006). Intermittent exposures of cucumber at 50, 100 and 200 ppb ozone concentrations showed necrotic lesions induced, at higher levels, on leaves and reduced plant growth and fruit-setting (Khan & Khan, 1999). Damage appears to be dependent on the length of exposure to high concentrations. Ozone damage also acts in a species-dependent manner on plants. At the cellular level, higher concentrations inhibit the chloroplast and cell nucleus functions, altering the expression/inhibition of transcripts of at least 40 sensitive genes related to the plant defensive pathways, including phytoalexins, cellular barriers, proteins involved in pathogenesis, signal compounds and anti-oxidative systems. As a consequence, the ozone activity acts in a way similar to pathogenic bacteria or fungi (Manning & Thedemann, 1995; Sandermann *et al*., 1998).

The inhibiting effects of ozone on plant photosynthesis and growth processes are known but the effects of increasing ozone levels on plant diseases are considered negligible, due to the lack of coincident periods with favourable disease conditions and peaks of ozone concentrations. Ozone, however, predisposed plants to enhanced parasitism by necrotrophic or root-rot fungi and bark beetles. Metabolic changes induced by ozone may persist longer than expected, with delayed symptoms (Sandermann, 2000).

3.6.2. Reactions to Irradiation

Atmospheric ozone concentrations increase in lower atmospheric levels due to industrial and urban concentrations. Ozone depletion, however, may affect crops also indirectly. The stratospheric ozone layer acts as a protective barrier for life on earth, filtering the solar ultraviolet radiation (UV-B) which is detrimental. Because of the reduction of ozone stratospheric concentrations due, among other factors, also to halogenated hydrocarbons like CFCs, an increase in the UV-B radiation at the ground level is expected (Kerr & McElroy, 1993).

Increased UV-B irradiation did not appear to directly reduce the productivity of plants in the Northern hemisphere (Allen *et al*., 1999; Olesen & Bindi, 2002) but extensive damage and ecological impact were observed in southern hemisphere ecosystems, directly exposed to a severe increase of UV-B irradiation, due to the opening of the Antartic ozone layer (Ballaré *et al*., 2001).

Plants response varies among species and varieties and depends on the UV-B to UV-A ratio (Teramura, 1983; Tevini & Teramura, 1989), with some species sensitive to present UV-B levels (Bogenrieder & Klein, 1982), while others appear less affected by higher irradiations (Becwar *et al*., 1982). One-third to one-half of all plant species tested are affected in a dramatic way by UV-B levels "above ambient" (Sullivan, 1992). Several studied also pointed out that the effects of UV-B irradiation mainly concern changes in biomass allocation, flowering patterns and plant height (Bornman, 1989; Teramura & Sullivan, 1991; Tevini & Teramura, 1989). Increased accumulation of flavonoids, increased leaf thickness and reflectance, growth reductions and direct damage to photosynthetic mechanisms were also reported (Bornman, 1991).

On cotton, field observations with supplemented UV-B irradiance increased by 9.5 % throughout the growing season, showed a negative impacts on growth with reductions in height by 14 %, in leaf area by 29 %, and in total biomass by 34%. Fiber quality was reduced and economic yield dropped by 72% (Gao *et al*., 2003).

4. EXPECTED CHANGES IN TROPICAL REGIONS

The diversity and complexity of ecosystems and the dimension of global changes represent a serious obstacle for any predictive analysis of expected consequences. Ongoing effects will vary regionally, in relation to the local diversity of climate and environment conditions (Walther *et al.*, 2002) with the most dramatic effects due to abrupt changes affecting one or more climatic parameters. On the basis of the actual knowledge about the response of marine and terrestrial ecosystems studied, environmental changes are expected to increase the likelihood of local or global extinctions for several species and/or to vary their distribution patterns, with a wide variability among species and regions.

In general, the dimension of the ecological niche occupied by a group of organisms affects their response to a climatic or environment shift, and appears informative in terms of distribution range and boundaries (Walther *et al*., 2002). Considering the complexity of the biosphere components, and in particular the links existing among plant disease epidemics, climate and environment, we can extend this statement to crops and their diseases or pests, suggesting that agroecosystems will react in a similar variable way, with significant differences recordable among crops and regions.

In the temperate regions, main effects of increasing temperatures on plants include an altered phenology, with earlier onsets of flowering and shooting. In Europe, recorded extensions of growing seasons, although regionally variable, showed an increase of 3.6 days per decade during the last 50 years, with greater responses for early blooming or herbaceous plants (Walther *et al*., 2002). In the Tropics, heterogeneous regional patterns of climate changes will be characterised mainly by abrupt increases of minimal rather than maximal temperatures, with a concurrent reduction of the corresponding excursions, favouring the germination and infection processes of several plant diseases.

Water availability will have a similar strong effect, since the rainfall season defines, in the Tropics, the cropping period and the consequent IM needs (Kickert *et al*., 1999). Major changes in rainfall regimes and the increased frequency of extreme events, like floods or hurricanes, will have a strong impact on tropical agriculture productivity, affecting the whole complex of agronomic practices and the strategies deployed to control pests and diseases.

As concerns the frequency of catastrophic events in space and time, historic records of global, worldwide epidemics of plant pathogens show that they appear at a time scale of several decades. For example, the first worldwide round of *Phytophthora infestans* occurred on potato around 1840-1860, whereas the last global epidemic event occurred more than a century later, in the period 1970-1990 (Ristaino *et al*., 2001). Altough future global-scale epidemics of plant diseases cannot be excluded, their frequency appears lower than those observed at a regional scale. Considering an inverse distribution linking the frequency of pest/disease epidemics to their extent in space (from local to regional and global), the incidence of climate changes on agricultural productivity (and consequent social impact) will be perceived mainly at local or regional scales. Considering that the most frequent small farm-scale epidemics have low economic and social significance and that the large scale, worldwide severe epidemics are rare (Zadoks, 2001), we will focus our attention on the regional scale and expected changes.

4.1. Central Andes and South America

Using a probability distribution map (PDM) several regions were identified in South America as potential new areas of colonisation for *B. tabaci,* a polyphagous pest, vector of geminiviruses (*Begomovirus*) on a wide range of cultivated and wild plants, including several horticultural or export crops (Hilje, *et al*., 2001).

The elaboration of PDMs for *B. tabaci*-prone areas, based on local climate and temperatures in geo-referenced points scored for disease outbreaks severity, showed that more than 50% of the *B. tabaci* and geminiviruses spots correspond to areas with alternation of wet and dry conditions (Morales & Jones, 2004).

Climate changes with increasing temperatures and variations in dry/humid conditions represent a threat either in the *B. tabaci* receptive areas not yet colonized by the vector and in those areas which may become suitable for future colonization. The broad distribution range of *B. tabaci* and geminiviruses in wild plants in the Tropics, and other agricultural, climate or biological changes, (i. e. the introduction of susceptible hosts, i. e. soybean in Brazil, or the insurgence of insect resistance due to pesticide spraying abuse), the spread of new *B. tabaci* biotypes and the occurrence of long dry periods with less than 80 mm monthly rains, are considered as conditions favouring the outbreaks of this pest and of geminiviruses epidemics on susceptible crops (Morales & Jones, 2004).

The selection of planting dates with *B. tabaci* unfavourable environment and climatic conditions is suggested as a possible management strategy, together with crop rotation and other field practices (i. e. mulching, weeds and crop residues removal) provided they are uniformly applied at a regional scale (Hilje *et al*., 2001).

PDMs may also be instrumental to alert countries for the potentialities of new outbreaks of pests not yet established, or to improve the detection/survey activities set to eradicate possible initial colonization foci (Morales & Jones, 2004).

Increased CO_2 levels may affect some pathogens, i. e. the soybean rust *Phakopsora pachyrhizi*, responsible of severe crop losses in Brazil (Yorinori *et al*., 2005). The reaction to changes may be complex, with possible reductions in the disease incidence due to a lower C/N ratio in plant tissues. These effects, however, may be counterbalanced by a higher pathogen efficiency, due to increased minimum temperatures on tolerant or resistant varieties, or by the intensification of other diseases, i. e. the soybean sudden death, caused by several *Fusarium* spp.

The effects of UV exposure on high altitudes crops productivity in the Andes, as well as on pests and diseases, are poorly known. A linear increase of the solar UV-B irradiation at higher altitudes and solar elevation was measured in the Andes, with differences in the transparency and optical properties of the atmosphere, due to aerosols, ozone and humidity. Changes in ultraviolet irradiations range by 2-23% per each 1000 m of altitude increase, and the penetration of UV-B is considered higher in drier zones than in humid areas (Piazena, 1996). Main food crops of the Andean highlands agroecosystems, like corn, barley and potato, may result affected by increased UV-B irradiations, given the altitude of cultivated fields, which often are higher than 3500 msl. Pest and disease management practices in these regions should consider the effects of increased UV-B irradiations at high altitudes, as well as the evolution and changing in clouding and humidity regimes, during the next decades.

Increased moisture levels and rainfalls due to El Niño and related climate changes may increase the likelihood and intensity of late blight epidemics on potato crops. These problems are mainly expected in the coastal cultivated areas, which are more affected by the ENSO phenomenon.

Changes in the rainfall regimes and temperature minima will also affect the behaviour of the Altiplano major insect pests, like the potato tuber moth, the lepidopteran *Phthorimaea operculella*, particularly destructive in storage tubers, and other pests i.e. the Andean potato weevils, *Premnotrypes latithorax* and *Rhigopsidius tucumanus*. These tuber borers may cause losses up to 50-100% of tubers, and represent a serious threat to subsistence agriculture, as farmers are often forced to abandone fields (Parsa *et al*., 2006).

Promising biological control agents were recently discovered for some of the endemic potato weevils in the Andes, including *Spodoptera exigua* (Hernández *et al.,* 2006; Parsa *et al*., 2006), and further studies are needed for practical exploitation in the field. Also the distribution boundaries of these pests are expected to be altered by increased minimum temperatures, with higher increases of tuber damage and losses recordable in the field rather than in storage conditions.

4.2. Caribbean and Tropical Pacific

Rainfall variability is also expected to produce extremes of floods or droughts, with consequent land losses due to erosion or desertification, and to affect the soil water

content, with a contemporary loss of water quality due to increased concentrations of salts in solution, and higher levels of air moisture.

Intrusion of saline water in coastal aquifers and estuarine areas due to increased sea levels will be particularly important in small islands agroecosystems, where local consumption agriculture mostly rely on rainfall and underground water (Singh, 1997). Increasing trends in salinity levels of different aquifers were recorded in Trinidad and Tobago over several years. Although affected by fluctuations mainly due to rainfalls and water pumping for civil or industrial use, salinity levels increased in some cases up to values higher than 4500 mg/l, which are above the threshold limit of chloride content in fresh water (Singh, 1997).

Increased hurricanes frequency represents a second major concern for Caribbean agriculture, since hurricanes are responsible for shaping the ecosystems and may abruptly affect crops productivity and vegetation over entire regions (Lugo, 2000). Known and potential effects of hurricanes on Caribbean vegetation include sudden and massive trees mortality as well as delayed tree deaths, changes in vegetation successions and alternative patterns of forests and natural ecosystems regeneration, higher species turnover and differential species substitution or composition, with faster biomass and nutrients turnovers (Lugo, 2000).

Vegetations plays an important role in tropical environments, and any related change may also produce some feedback effects on climate at the local as well as regional scales. Modeling the vegetation effects on climate showed seasonally dependent moderating effects, due to surface cooling through latent heat fluxes, with greater impacts during the dry seasons. Modeling showed that changes in soil parameters and drainage capacity affect climate sensitivity, soil hydrology and its interaction with vegetation, by altering runoff processes (Osborne *et al*., 2004).

4.3. Asian Monsoon Region

Long-term observations on climatic data in China were coupled with incidence of four main cereals diseases: wheat stripe rust (*Puccinia striiformis*) a cool-season disease; wheat powdery mildew (*Erysiphe graminis*); wheat scab (*Fusarium* spp.), a mid-season disease in central and southern areas and rice blast (*Pyricularia oryzae*), a disease in tropical and temperate regions. Data confirmed increasing temperatures trends, with dramatic increases for wheat scab and rice blast prevalence levels. Powdery mildew, almost not detectable before the ’70, became a leading yield-limiting factor whereas stripe rust showed decreasing trends in northern wheat-producing regions. The differences between monthly minima and maxima temperatures appeared related to changes in disease prevalence levels (Yang *et al*., 1998).

Climate change and warming are expected to favour the spreading and movement of the rice water weevil in China. This invasive species was blocked in its spreading northward by cold winter stress in Helongjiang and Jilin Provinces and to the west by high elevations encountered in Sichuan and Yunnan Provinces (Chen *et al*., 2005). As shown, one the expected consequences of warming in tropical climates is the possible expansion of pests distribution boundaries upward (higher

altitudes) or poleward. The species has a partial second-generation from early September to early October in the double-cropping rice region of Zhejiang Province in southeastern China. The possibilities of a further generation, if most favourable conditions are matched in these regions, should be actively monitored to prevent further spreading and crop damage.

Changes in climate conditions may also produce, at a regional scale, different outcomes in disease intesities and yield losses. Simulations of the effects of global warming on rice blast in China and other countries in East Asia, using historical daily weather data from 53 stations, showed that changes in temperatures affect blast epidemics and yield losses in most locations. In Japan, the simulated losses were, however, less than 1% even with a +3°C warming. In Korea and China the average losses were less than 2.5%, but higher temperatures appeared to decrease yields. However, lower temperatures showed incidence of more severe blast epidemics in most locations in the tropical countries (Philippines and Thailand), or Korea and China as well. In this country, in sub-humid sub-tropical regions (Wuhan, Nanjing and Hangzhou), modeling showed that lower temperatures did not change yield losses compared with normal temperatures.

In warm humid sub-tropical areas, (Guangzhou), a decrease in temperature increased yield losses. Increasing temperatures by 1°C and 2°C caused lower yield losses in Wuhan and Hangzhou. In tropical areas the risk of yield loss was higher with a temperature change of -3°C and lower with a temperature change of +3°C, with a maximum simulated 6% yield loss. In Thailand, a -3°C temperature change showed higher yield losses at most locations, but higher temperatures also lowered yields (Luo *et al*., 1998).

In general, the effect of climatic or disease/pest related variables should be considered in a more comprehensive way, since univariate evaluation or modeling do not appear suitable to produce informative outputs. Olszyk *et al*. (1996), for example, modeled the effect of rising UV-B irradiation on rice blast incidence, assuming that high UV-B levels would produce a 9-10% decrease in net assimilation rate. Simulations showed highest yield losses and maximum disease severity when UV-B exposures were concurrent with blast disease stress.

In India, the pearl millet downy mildew, caused by *Sclerospora graminicola*, is widespread and highly destructive, causing yield losses of up to 30%. Other important downy mildew species are *Plasmopara halstedii* and *Peronospora parasitica*, causing severe losses on some sunflower cvs., or rapeseed and mustard, respectively. Onion downy mildew (*Peronospora destructor*) in Himachal Pradesh accounted for up to 75% yield loss in the period 1988–1989 (Thakur & Mathur, 2002). These host-dependent obligate biotrophs survive during dry seasons as thick-walled and long-lived oospores resulting from their sexual phase, spreading through infective conidia or sporangia, which release infective zoospores during the suitable wet season. Changes in hydrologic cycles due to alterations in the monsoon regimes, affecting the occurrence and length of the host plants surface wetness (required for infection) and of the relative humidity (required for spore production), affect the disease spreading and prevalence. Resistant germplasm and chemical treatments (metalaxyl) provided the most suitable means of control. However, insurgence of metalaxyl resistant sub-populations in some species (i. e. *P. parasitica*) and the lack

of biological control effectiveness suggest that the most suitable management strategies will rely on the use of resistant germplasm (Thakur & Mathur, 2002), thus avoiding uncertainties related to rainfall regimes variabilities.

4.4. Africa and Sub Sahara

As for India, also in Kenya some lepidopteran species, i. e. *Plutella xylostella*, are among the main insect pests of cruciferans, with crop losses up to 14% and elevated risks of insurgence of insecticide resistance. A second cause of crop losses is *Xantomonas campestris* pv. *campestris*, followed by drought (Badenas-Perez & Shelton, 2006). Main management strategy for *P. xylostella* rely on the use of pesticides, mainly pyrethroids, and organophosphates applied in 4-6 treatments at pest detection or on a calendar basis. Analysis of farmers' attitude towards pest management showed main weakness in training and education required to establish biological control practice, with threats due to potential misuse of alternative technologies or pesticides. Chemicals, furthermore, produced several negative effects on parasitoids like *Diadegma semiclausum*, released for managing *P. xylostella* (Badenas-Perez & Shelton, 2006). Together with trap cropping and use of transgenic Bt-transformed cruciferous vegetables, biological control was considered as part of a general IM strategy aiming at reducing the environmental risks of widespread pesticide use.

Bananas and plantains represent a very important food source for million people in the Tropics. Monitoring the incidence of historical or new diseases is a strategic task which should be reinforced in a global climate changing scenario, due to the narrow genetic bases of the Cavendish banana actually used (Jeger *et al*., 1996). This variety replaced Gros Michel due to past Panama disease outbreaks, caused by race 1 of *Fusarium oxysporum* f. sp. *cubense*. In Central Africa, major banana productions occur in the higher elevation regions, characterized by abundant rainfalls, where several cultivar, including AAA and AB types, are grown for self consumption, beer, dessert or cooking (Speijer & Bosch, 1996).

The fungal disease black Sigatoka, caused by *Mycosphaerella fijiensis*, is one of the major constraints for plantain production in West Africa as well as in other tropical areas worldwide (Jeger *et al*., 1996; Ortiz & Akoroda, 1996). This pathogen is managed through the import of triploid, resistant cooking bananas (ABB) from Asia or by developing tetraploid resistant plantain hybrids (AAAB) through crossbreeding (Vuylsteke *et al*., 1993). One of the major concerns for the black Sigatoka is the loss of wild germplasm due to increasing deforestation in India, the area of speciation of Cavendish banana, as well as the disappearence of traditional local varieties, reservoirs of genes conferring resistance to the disease.

Monitoring for early detection and recognition at the sub-species level are considered very useful, in order to follow the spread of these pathogens and to prevent outbreaks through the use of fungicides (Jeger *et al*., 1996). Changes in minimum temperatures may increase the spatial spread as well as enhance the negative impact of both species on yields, as well as favour other minor pathogens to overcome the resistance levels of the germplasm actually in use.

The curculionid *Cosmopolites sordidus* is the main imported insect pest occurring on highlands banana and plantain in Africa. It is a severely destructive consumer, since immature stages live inside the plant and destroy the corm and vascular tissues. This species is not considered a pest in its speciation area in Asia where it is controlled by *Plaesius javanus* and other predators, capble to stabilize the host populations at low density levels (Abera-Kalibata *et al*., 2006). Since climate changes affect the insect pests distribution boundaries and their spread toward higher altitudes, this pest may become even more destructive in future decades. It appears, hence, very important to attempt the biological control of *C. sordidus*, through the import and release of its predator in the newly pest colonized regions (Abera-Kalibata *et al*., 2006).

Other banana threats include the nematode *Radophulus similis*, for which the best sanitary action actually deployed are based on prevention, through the use of *in vitro* produced material for new plantations. This species, highly destructive, was recently reported from Martinique on several weeds belonging to the Euphorbiaceae, Poaceae and Solanaceae (Quénéhervé *et al*., 2006). Careful management will then require the monitoring of native as well as new invasive weeds as potential hosts and field reservoirs. Nematode species include also *Helicotylenchus multicinctus*, *Pratylenchus goodey* and *Meloidogyne* spp., which appear with frequencies higher than *R. similis* (Speijer & Bosch, 1996). Changes in the farmers' preference for planted varieties were attributed to the nematodes susceptibility of some common cultivar, i. e. the East African Highland, highly affectd by *P. goodey* (Speijer & Bosch, 1996). Although some sources of genetic resistance were identified among *Musa* spp. (Sarah *et al*., 1997), export banana cultivars are all susceptible to *R. similis* (Jeger *et al*., 1996). Farmers have to rely on different management strategies based on chemicals, although the number of products available for management declined, as the toxicological hazards due to their use increased (Jeger *et al*., 1996).

The recent *Phytophthora infestans* world resurgence, with late blight epidemics caused by fungicide resistant pathotypes and new aggressive strains, originating through natural genetic recombinations, overcome in several occasins the potato germplasm already resistant (Gisi & Cohen, 1995; Fontem *et al*., 2005). Efforts to develop new resistant varieties will rely on a complex of resistant genes. The combined use of resistant potato varieties and fungicides, reducing the number of treatments, allows a satisfactory level of crop protection with lower environmental problems (Namanda *et al*., 2004). In the Tropics, severe late blight epidemics occur after several days of rain or high ambient moisture (8-10 hours per day), and changes in planting dates may improve the management of the disease, since mid-season late infections often have a lower incidence on crop yields (Namanda *et al*., 2004). The expected increase/decrease of the rainfall regimes variability must be taken into account when planning the combination of different (chemical, genetic) management strategies for late blight.

Several countries in Africa are also plagued by maize and sorghum downy mildew caused by *Peronosclerospora sorghi*. In Nigeria, the epidemics has a severe economic impact and its area almost doubled in the period 1980-1992, increasing at the rate of $1.5 \cdot 10^3$ km^2 per year (Bock *et al*., 1998). Typical epidemic areas are the

rainforest and the transitional zones, with 1200-1800 mm rainfall regimes and 300 to 1000 msl altitudes. The pathogen adapted to attack maize crops also in the drier, southern regions of Nigeria where it is a severe limiting factor of crops. It is also endemic in other central and southern countries in Africa. Since spores of *P. sorghi* require high moisture levels for production, spread and host plant infection, droughts affect the disease epidemics and prevalence. Changes in rainfall regimes in these areas may alter the disease epidemics, and managing through anticipated sowing may result in a lower incidence of the disease (Bock *et al*., 1998).

5. ADAPTIVE STRATEGIES FOR INTEGRATED MANAGEMENT

Tropical macroregions may face in future decades plant protection "emergencies" due to known or new pests and pathogens, with changes in species distributions and spreading, as well as potential abrupt climate shifts leading to higher risks of catastrophic events. These problems will be only partially balanced by the positive effects expected as a consequence of climate variations, i. e. the increase in plants growth rates and crop yields. Higher minimal temperatures, for example, even if increasing production, will also change the behaviour of several insect pests, whereas the expected higher incidence of droughts in the Tropics will counterbalance the increased plant productivity due to higher CO_2 levels.

The global agricultural system will undoubtly keep its intrinsic level of complexity and uncertainity, limiting by this way the value of past experience and learning. The identification of the potential "crisis" and the detailed monitoring of environment and climate changes, finalized to anticipate future needs, will require investments in monitoring and surveying, as well as in new genetic and technological knowledge, needed to implement appropriate plant protection strategies.

After revising the effects of climate changes on insects attacking humans, animals and plants, Epstein *et al.* (1998) concluded that actions like monitoring of montane areas and insect populations surveillance may result useful to prevent some "biological surprise" and recommended some preventive actions finalised to delay insect populations boundary shifts, including chemical control.

Modeling the effect of changes on the water demand and availability in some major agricultural areas of the world, Rosenzweig *et al*. (2004) found that there will be sufficient water supply for most of the relatively water-rich areas studied. However, some areas, i. e. Northern Argentina, could experience problems in future water supply due to variability in tributary stress, whereas other tropical areas, i. e. Southeastern Brazil, may allow an expansion of irrigated land use, under different climate conditions (Rosenzweig *et al*., 2004).

5.1. Adaptive Strategies and Disease Management

Research programmes aiming at introducing new varieties or at improving the long-term use or application of biological, genetic or instrumental factors (i. e. irrigation programmes, agronomic techniques, forest management or land use) related to

cropping systems in a given region should take into account the effects of the climate and environment changes described.

Particular attention must be paid at research programmes aiming at the management of plant diseases through the introduction of resistance genes in commonly used varieties, since an evaluation is needed about how long the resulting plant genetic pool will remain useful, on a scale of decades or even years. Protection of natural biodiversity and of plant genetic pools have in this sense a more practical and immediate justification. The term "adaptive strategy" in this sense reflects the need for a quick and flexible response, since some variations may occur rapidly. Expected changes are already in action and new varieties may display in a few years unsuitable agronomic traits, i.e. higher disease susceptibility due to increased air moisture or rainfall levels, or may become susceptible to newly colonizing or substitution pests or pathogens, thus vanishing long term research investments.

5.2. Tools and Technologies

Models are useful tools providing a rational basis for a number of different political, social or technological actions. In the case of invasive species, for example, the choice among different options (eradication, suppression, no action) may be supported by previous evaluation of the conditions necessary for a successful eradication action, with a positive return in economic and social terms (Fraser *et al*., 2006). Some examples of eradication campaigns are the UK Foot and Mouth Disease programme, the campaign for the potato beetle, *Leptinotarsa undecimpunctata*, (eradicated in repeated occasions after its first UK outbreack in 1901) and the destruction of garden plants for eradication of *Phytophthora ramorum* (Fraser *et al*., 2006).

Eradication is a costly strategy, requiring subsequent additional expenditures for monitoring and epidemic prevention, which must be lower than the economic losses expected by outbreaks. In alternative, other strategies (suppression or no action) may result more convenient. In this case too, marginal benefits progressively lower may be expected by increasing the efforts deployed for eradication. The marginal benefit which leads to eradication, however, has a sharp increase if the removal of the invasive species has an immediate market implication, due to the possibility of exports, once eradication is proved, to market areas free of the disease. Given the different situations encountered in planning an eradication campaign, the level of uncertainities (greater for eradication than suppression) and the value of the access to market, different options are available. Although suppression may be preferred to eradication because of lower costs, the eradication option may be preferred if exports benefits will be compensated by market access (Fraser *et al*., 2006).

In general, an insight about the possible outcomes of climate changes may be derived by the application of computer models. The sensitivity of these tools should, however, be checked with real data and scenarios, in order to verify their level of uncertainty and affordability, as well as their effective potentials in producing informative data in a reproducible manner, expecially for modifiable simulation models (Kickert *et al*., 1999).

One key issue in modeling is the level of data resolution achieved and its effects on forecasting. The asymmetric increase of minimum and maximum temperatures and the non-linearity in growth and development responses of plant pathogens require increased resolutions (in terms of hours or days) when modeling the effects of climate changes (Scherm & Van Bruggen, 1994). Best adherence to observed real data is obtained when sub-daily resolutions are used, since a bias is introduced when only mean temperatures are used, without considering the amplitude of the fluctuations (Scherm & Bruggen, 1994).

For a review of most used computer simulation tools, describing their properties and applications for agriculture and food supply, ecological conservation, protection of natural resources, social interactions, and including acronyms and sources, see Kickert *et al*. (1999).

Modeling plant diseases in a global change scenario includes the integration of a global climate model (GCM), with a number of sub-models accounting for plant diseases, growth and losses (Scherm *et al*., 1998). A similar approach, however, requires that GCM outputs be scaled down at the regional scale, that crops and diseases models be scaled up to the same resolution level and that a number of uncertainty measures be assessed, the preference being given in the near term to less complex empirical modeling approaches (Scherm *et al*., 1998) and to the effects of climate variability (Semenov & Porter, 1995). Modeling also may account for the different responses of crops to climate changes, identifying shifts in crops productivity expected at the near or mid term (Peiris *et al*., 1996).

Risk maps, generated through modeling of plant-scale conditions from regional-scale data, were used to represent a disease likelihood, i. e. primary infection for grape powdery mildew or downy mildew, covering large cultivated areas. When interacting with a GCM, they may also produce maps under estimated climate change conditions with different scenarios. They are useful for evaluating the risk of a disease epidemics within a region or the likelihood of invasion for areas previously free of the pathogen (Seem *et al*., 1998).

At the small scale, generic dynamic crop modeling may result informative about the effects of weather, variety, pests, soil and management practices on crop growth and yield, as well as on soil N and organic carbon dynamics in aerobic as well as anaerobic conditions. Data generated include pest induced yield losses, allowing a comparative analysis with different greenhouse gas emissions situations (Aggarwal *et al*., 2006). Weather variables introduced in modeling may result fundamental to explore the different outcomes of climate changes and hence forecast the spatial and seasonal dynamics of pests, i. e. fruit flies, in the mid term (Yonow *et al*., 2004).

A further tool is given by the probability distribution maps (PDM). Their use allows the identification of potential risks related to the distribution of pests, vectors or plant diseases. PDMs are calculated on the basis of the combined use of local records of pest or disease occurrence, climate data and subsequent statistical analysis. They show the areas susceptible of colonization or of endemism for the given organism (Morales & Jones, 2004). This tool has several practical advantages, including the possibility of early identification of areas susceptible of invasion, in a different climatic scenario, by a pest or disease or the possibility to anticipate the insurgence of epidemics for secondary pests, already present in areas

with sub-optimal conditions for their life-cycle. They require the monitoring of different climatic variables at the regional scale and a given resolution level, as well as the implementation of an early monitoring and detection support system.

6. CONCLUSIONS

As previously seen, it is possible that not all climate changes forecasted for the next decades will produce negative consequences for plant protection. It appears recommendable, however, to develop or implement, at the regional scale, a continuous monitoring of the most important agroecosystems and of the noxious species reservoirs, in order to prevent new emerging pests or diseases and to elaborate an effective IM strategy. Epidemiology plays a key role in prevention and management as well. Several examples of plant disease resurgence, with periods of intense epidemics alternated to temporary disease-free intervals, are reported in the literature (Zwankhuizen & Zadoks, 2002). For this reason, monitoring should also consider less severe or marginal pests or diseases, with historical and/or biological potentials for developing new epidemics, following their dynamics in space and time. Also, it appears wise to increase the investments in research on plant genetics and on the adaptive potentialities of traditional as well as less used varieties and accessions from germplasm collections, and to test the application of integrated management tools or of new biological control agents in sustainable agriculture.

The cooperation among producers, either at the regional and the national levels, may provide a first low cost network for monitoring, which may receive a further advantage by the diffusion of information technology and communications tools. Evaluating the effects of the forecasted changes on the traditional control methods adopted by farmers, and on pesticides use, also appears as a useful research field. Finally, quarantine efforts must be improved, with particular efforts required in less developed agricultural systems, through periodical concerted re-evaluation of the lists of organisms checked, and eradication or management plans, in order to provide a first barrier for target pests or diseases spreading, especially if affecting strategic crops of global interest.

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MANAGEMENT OF POSTHARVEST DISEASES IN STONE AND POME FRUIT CROPS

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Abstract. Diseases caused by fungal pathogens in harvested fresh fruit are one of the most serious losses of production. In this chapter we introduce the major postharvest diseases in stone and pome fruits and their infection process. Conditions affecting pathogen infection and disease development are illustrated. Approaches of postharvest disease control in fruit are discussed, particularly including recent biotechnologies, such as biological control through antagonistic yeasts and resistance induction by biotic and abiotic factors.

1. INTRODUCTION

Although quality deterioration of the harvested fresh fruit is the result of a number of different factors, diseases caused by fungal pathogens are by far the most important one (Sommer, 1985a). Substantial decay losses may occur during postharvest storage and shipment period if the product is not treated with an effective inhibitor of microbial growth or stored in an unfavorable environment to disease development. Therefore, postharvest diseases of fresh fruit have been considered to be one of the most severe sources of loss of production (Harvey, 1978).

Losses in postharvest can be remarkably high and their economic cost is proportionally greater than field losses (Eckert, 1978). Some reported values for disease losses show that approximately 10-30% of harvested fresh horticultural crops is lost to postharvest spoilage in developed countries, whereas losses are even greater, amounting to more than 40-50% in developing countries in which sanitation and refrigeration are lacking or minimal (Salunkhe *et al*., 1991). Skillful application of available technology can significantly reduce postharvest diseases to a fraction of the cited values, but the reduction in consumable units of produce is only the most evident of the losses. In addition, postharvest diseases are often the major concern in decisions influencing the consumer price, including requirements and duration of storage, mode of transportation and possible utilization of economizing practices.

The capability of a microorganism to initiate a postharvest disease, as well as its final outcome, depend on a number of factors that can conveniently be associated with 1) the micro-organism, 2) the host and/or 3) the environment. In general, a

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combination of infective pathogen, susceptible host and favorable environment is necessary for a disease to occur and develop optimally (Sommer, 1982). Therefore, an understanding of disease organisms, host commodity and their relation with handling methods is of critical importance. The integrative strategies for control of postharvest diseases include effectively inhibiting pathogens growth, enhancing resistance of hosts and improving environmental conditions resulting favorable to the host and unfavorable to the pathogen growth.

2. PRINCIPAL DISEASES AND INFECTION PROCESS

2.1. The Major Pathogens

Among postharvest diseases of fresh fruits, fungi are the most important and prevalent pathogens, infecting a wide range of host plants and causing destructive and economically important losses of most fresh fruit during storage and transportation (Sommer, 1985b). The major pathogenic fungi causing postharvest diseases in stone and pome fruits are shown in Table 1.

2.2. The Infection Process

There are two phases, a lag phase and a log phase, in infection process of fungal pathogens. When the fungus spores land on a medium suitable for growth, they swell and produce a germ tube after a few hours. Depending upon the fungus species, the lag phase may last from a few hours to several days at optimum temperatures, to weeks or months at temperatures near the minimum for fungus growth (Sommer, 1985c). The lag phase *in vivo* is usually longer than *in vitro* because of host resistance, resulting in a much less steep curve. The growth rate slows when much of the host has been invaded. When a steady state is reached, the very slow growth rate results in a much reduced slope of the log phase (Tian & Bertolini, 1995).

A high percentage of spore-contaminated wounds may not develop into lesions if low temperature occurs sufficiently prompt. Spore germination is extremely slow and may fail near the minimum temperature for fungus growth (Tian & Bertolini, 1996). When the pathogen is still in the early lag phase, low temperatures may consequently result in fewer fungal lesions and delay their development.

Many postharvest diseases usually have an obvious character, as at some stage between the arrival of the pathogen and the eventual development of a progressive disease, the growth of the pathogen is arrested (Sommer, 1985c). Such arrested infections are commonly described as “latent infection” or “quiescent infection”, indicative of a time lag between infection and overt symptoms production (Verhoeff, 1974). Verhoeff (1980) defined latency as a quiescent or dormant parasitic relationship which after some time changes to an active one. Latent infections result as an interruption in infection, after host tissues penetration.

Table 1. Major pathogenic fungi causing postharvest diseases in fresh fruit.

Pathogen	Disease	Minimum temperature for disease (°C)
Stone fruits [peaches and nectarines, *Prunus persica* (L.) Batsch, and cherry, *P. avium* L.]		
Monilia spp.	Brown rot	
Monilia fructicola (Wint.) Honey		-2
Monilia laxa (Aderh. & Ruhl.)		-4
Botrytis cinerea Pers. ex Fr.	Gray rot	-4
Rhizopus stolonifer (Ehrenberg ex Fries) Lind	Rhizopus rot	5
Penicillium expansum (Lk.) Thom.	Blue mold	-2
Cladosporium herbarium Link.	Cladosporium rot	-5
Alternaria alternata (Fr,) Keissler	Alternaria rot	-3
Pome fruits (apples, *Mulus pumila* Mill. and pear, *Pyrus communis* L.)		
Botrytis cinerea Pers. ex Fr.	Gray rot	-2
Penicillium expansum (Lk.) Thom.	Blue mold	-2
Anthracnose rots		
Cryptosporiopsis curvispora (Peck.) Grem. = *C. malicorticis (Cordl.)* = *Gloeosporium perennans* Zeller and Chids	Bull's-eye rot	-4
Colletotrichum gloeosporioides (Penz.) Sacc.	Bitter rot	-3 to 9
Phlyctaena vagabunda Desm. = *Gloeosporium album* Osterw	Lenticel rot	> 0
Alternaria alternate (Fr,) Keissler	Alternaria rot	-3
Cladosporium herbarium Link.	Cladosporium rot	-5
Cylindrocarpon mali (Alles.) Wollenw.	Eye rot	
Stemphylium botryosum Wallr.	Pleospora rot	-3

Data from Sommer (1985b), Tian & Bertolini (1999) and Tian (2001).

2.3. The Penetration Ways

There are two groups of postharvest pathogens with regard to their ability to gain entrance through the fruit peel into the fruit deeper tissues. A first group is called "wound infection", and includes fungi able to bypass the protective skin only through wounds. The other group is named "direct infection", with fungi able to form special morphological structures (appressoria) used to penetrate the fruit cuticle and epidermis (Sommer, 1985c).

2.3.1. Wound Infection

Wounds are the most common avenue of host entrance for postharvset decay fungi. Spores of all postharvest pathogens require high humidity or free water for a number

of hours for successful germination (Berg & Lentz, 1968). In wounds, the germinating spore may grow and colonize the exposed fruit tissues, provided the fungus is pathogenic (Sommer, 1982). For example, serious stem-end rots result from infection of wounds caused at harvest. *Diplodia natalensis* causes serious stem-end rots of citrus fruits, mangos and papayas. *Botryodiplodia theobromae* invade stem tissues of banana fingers whereas *Thielaviopsis paradoxa* invade pineapple fruits. Fruits with chilling injury may suffer serious losses from Alternaria stem-end rot (Snowdon, 1991).

2.3.2. Direct Infection

Spores landing upon a fruit, provided temperature and humidity conditions are satisfactory, will geminate within a few hours (Salunkhe *et al*., 1991). After germ tube complete formation, an appressorium will be produced. The appressorium and germ tube adhere tightly to the fruit surface. Eventually, a number of enzymes are excreted through the pore of appressoria walls on the fruit surface (Sommer, 1985a). Among these enzymes, cutinase is able to hydrolyze the cutin which overlays the epidermis. Through the appressorium pore, an infection peg penetrates the cuticle, which has been weakened by the enzymatic action. After penetration, the infection peg regains the normal size of the fungus mycelium, proceeding to branch and develop to thoroughly invade the fruit flesh (Eckert, 1978).

3. CONDITIONS AFFECTING PATHOGEN INFECTION AND DISEASE DEVELOPMENT

3.1. Environmental Conditions

It is common knowledge that most diseases appear and develop best during wet, warm days. The postharvest environmental factors that most seriously affect the initiation and symptoms development of infectious fruits are temperature, moisture and air composition (particularly O_2 and CO_2 concentrations).

3.1.1. Temperature

Storage temperature is so critical for controlling postharvest diseases that all other control methods are sometimes described as “supplements” to refrigeration (Sommer, 1985a). Low temperatures not only slow fungus growth and lesion development, but delay senescence and maintain fruit resistance (Tian & Bertolini, 1999). Meanwhile, the physiological and biochemical changes of fruit are usually slow at low temperatures so that the host can maintain high resistance to pathogens. The rate of deterioration increases by two- to three-fold for each 10°C increase above optimum (Kader, 1985). Obviously, temperature management is so critical to postharvest disease control that all remaining control methods can be described as supplements to refrigeration.

3.1.2. Humidity

Relative humidity is a very important environmental factor for harvested fruit in storage. The germination of some fungi, and direct penetration to fruit are aided by saturated atmospheres or liquid H_2O on the fruit surface (Hollier & King, 1985). In general, high humidity can promote diseases development if temperatures are favorable (Parker & Sutton, 1993). Fresh fruits need high humidity levels (>95% relative humidity) in storage environment in order to minimize the loss of moisture and preventing them from shrivel and losing tissues turgidity (Kader, 1985).

3.1.3. Atmosphere Control

Oxygen is required for normal respiration of both the fruit and its fungal pathogen. The effects of atmospheres with low O_2 and high CO_2 on postharvest diseases can be either direct or indirect (Tian *et al*., 2001). The maintenance of fruit in good physiological conditions may result in a fruit with considerable disease resistance. In general, increasing CO_2 above 5% and lowering O_2 to about 5% or below noticeably suppresses fruit respiration, maintaining fruit quality and resistance (Sitton & Patterson, 1992).

The effects of environmental conditions on disease may be evidenced through their influence on host growth and susceptibility, on the pathogen multiplication and activity, or on the interaction of host and pathogen (Spotts & Cervantes, 1991). Nevertheless, the occurrence and progress of postharvest diseases depend on both the properties of hosts and pathogens, together with their interaction with growing, harvesting and storage conditions (Eckert & Ratnayake, 1983). However, the speed of either infection or resistant action is significantly related to storage temperature, being the most important environmental factor. With the advent of higher temperatures, pathogens become active and, when other conditions are favorable, they can quickly infect hosts and cause serious disease.

3.2. Fruit Resistance to Fungal Attack

As fruit ripen they become susceptible to a variety of fungi, whose attacks they were capable to resist, during their development on the tree. Much of decay that develops in storage is derived from spores that collect on the surface during the growing season, but which are incapable of causing rotting until after harvest (Sommer, 1982). But fruit may resist fungal attack in several ways. The fruit skin, such as cuticle and epidermis, provides protection against pathogenic infection. Most fungi usually infect fruit by wounds, but some are able to penetrate the sound fruit, often highly depending upon favorable environmental conditions (Sommer, 1985a). Postharvest diseases also develop if fruit is, in some way, damaged allowing pathogens entry by the wound.

3.2.1. Maturity

Fruits are usually harvested before they are completely ripe, in order to secure sufficient time for long distance transportation and marketing. Harvesting well before the beginning of ripening ensures that fruits will have a higher resistance to certain diseases than fruits harvested later. Immature fruits always have a high degree of resistance, which is reduced very noticeably as the fruit begins to ripen (Eckert & Ratnayake, 1983). Ripe fruits are more susceptible to invasion by specific pathogenic micro-organisms as they are high in moisture and nutrients and no longer protected by the intrinsic factors which conferred resistance during their development. Many fruits become easily injured as they reach full maturing, and therefore, are more vulnerable to wounds pathogens. Some fungi, such as *Monilinia* spp., *Botrytis cinerea, Rhizopus* spp. or *Penicillium* spp., are most likely to invade after the fruit is completely ripe or has become senescent (Sommer, 1985c).

3.2.2. Biochemical Defense

It is believed that all living organisms normally have highly effective mechanisms for disease resistance (Van Loon, 1997). The resistance of the fruit to fungi comes from the biochemical action of tissues resulting in a single compound or a single mechanism (Eckert & Ratnayake, 1983). Immature fruits have a higher resistance due in part to fungi-toxic compounds already present at infection, belonging to the chemical group of polyphenols or tannins (Biale, 1964). Members of this group are responsible for the browning reaction when fruits are cut or bruised. In general, total polyphenols contents decrease as the fruit approach maturity and ripeness, which coincide with a decrease in disease resistance (Pérez, *et al*., 1999).

Polyphenols appear not as important as the presence of specific, highly fungi-toxic compounds, absent during ripening and produced as a consequence of the fungal attack (Bowles, 1990). The fungi-toxic compounds belong to the phytoalexins group and are often polyphenolic in nature. For example, apple fruits can produce benzoic acid in response to infection by *Cylindrocarpon mali*, causal agent of an important storage disease of apples (Snowdon, 1992). In general, the ability of producing phytoalexins is positively correlated to the tissue's vigor. Although phytoalexins are originally specific chemicals, elicited by one or more pathogens, some can be formed in response to plant wounds (Eckert, 1978). Such wound metabolites provide a type of healing that tends to prevent subsequent fungal colonization of the wound.

3.2.3. Wound Healing

Fungal spore often infect fruit by invading wounds, including cuts punctures, bruises and abrasions, which easily occur during harvest and handling (Snowdon, 1991). Fresh wounds can support nutrients and humidity for spore germination and colonization by fungi. For example, conidia of *Monilinia fructicola* need moisture and nutrients for spore germination and growth if deposited in fresh wound of stone fruit (Salunkhe *et al*., 1991). Fungal growth and lesion development follow when temperature conditions are favorable.

Healed wounds may no longer be highly prone to fungal invasion. Many studies of responses to wounding in a wide assortment of tissues including fruits, leaves, stems, tubers and roots showed that a biochemical wound healing process occurs, with common traits among plants (Mayer & Harel, 1978). When fruits are wounded cellular contents are mixed and exposed in the wound area. Enzymes, particularly polyphenol oxidases, mix with the polyphenols present in the cell sap (Wang *et al*., 2004). Living cells near wounds usually become very active, increasing their metabolic activities. Polyphenol synthesis may lead to the accumulation of further amounts of these compounds, (some of whom are highly toxic to fungi), which add to those already present. As a result of the biochemical cascade at the cell level, the spores germinating in such protected wounds are suppressed or killed (Eckert & Ratnayake, 1983).

4. APPROACHES OF POSTHARVEST DISEASE CONTROL

In order to extend the shelf life and improve the safety of fresh fruit, many preservation methods are actually used in many countries, aiming at the destruction of the pathogens or inhibition of their growth. They include refrigerated storage, controlled and modified atmosphere storage, low pressure or hypobaric storage (Kader & Ben-Yehoshua, 2000; Gorny & Kader, 1997; Tian *et al*., 2004).

Certain compounds, such as calcium, silicon and borate, which directly inhibit the growth of pathogenic fungi, have been highly successful in controlling a number of postharvest diseases (Conway *et al*., 1991; Qin & Tian, 2005; Qin *et al*., 2007).

The strategy of chemical control has concentrated on treatments that protect the product from infection or that inhibit the pathogen growth in incipient infections. Recently, some biotechnologies, such as biological control with antagonistic yeasts (Wilson & Wisniewski, 1989; Janisiewicz & Korsten, 2002) and resistance induction (Droby *et al*., 2002; Tian & Chan, 2004), have been used to control postharvest diseases in various fruits.

4.1. High-CO_2 Treatment

The use of high CO_2 atmospheres in fruit storage may be a promising tool, reducing decay without use of chemical fungicides (Kader, 1986). High CO_2 can act as a very effective fungistatic agent in stored fruit (Bonghi *et al*., 1999). Growth of *Monilinia fructicola* significantly declined with increased CO_2 concentrations, both *in vitro* and *in vivo*. CO_2 concentrations at 15-25%, provided a significant reduction in lesion size and, at 30%, completely prevented lesion formation at 25°C (Tian *et al*., 2001). On sweet cherries, fungal growth on PDA was completely suppressed and brown rot disappeared from inoculation sites in 10-30% CO_2 after 30 days at 0°C (Tian *et al*., 2001). Controlled atmosphere (CA) storage with CO_2 concentrations above 2.8% reduced the development of lesions by *Botrytis cinerea, Penicillium expansum* and *Pezicula malicorticis* in 'McIntosh', 'Diliciuos' and 'Golden Delicious' apples kept at 0°C (Sitton & Patherson, 1992). So, CA with high CO_2 treatment is effective in freshly harvest fruit to control postharvest diseases.

4.2. Heat Treatment

Postharvest heating, finalized at killing or weakening pathogens, offers a further pesticide-free method to control postharvest diseases. Immersion of peach and nectarine fruits in water at 46 – 50 °C for 2.5 min reduces the incidence of fruit decay from 82.8% to 59.3 and 38.8%, respectively (Margosan *et al*., 1997).

The strategy of combining heat treatment with calcium infiltration can reduce incidence of blue mould in apples (Falik *et al*., 1995; Conway *et al*., 1999) and control decay of pears (Spotts & Chen, 1987). Heat treatment of fresh fruits and vegetables can provide good control of decay, but the effect is not the same as that of fungicides (Barkai-Golan & Phillips, 1991). The use of polymer film wrap during treatment or the addition of nonpesticide chemicals to hot water may increase the effectiveness of heat treatments.

4.3. Chemical Fungicides

Chemical fungicides have been considered to be an effective method for control of postharvest diseases for long time (Eckert & Ogawa, 1988). The major approaches include spray, dip and fumigation. Preharvest application of iprodione is effective for control of postharvest brown rot decay and has been recommended for several years in the Pacific Northweast (Pscheidt & Ocamb, 1998). A single preharvest application of iprodione at 1.13 kg a.i. · ha^{-1} reduced brown rot in stored cherry fruits (Spotts *et al*., 1998). Significantly better control of brown rot was obtained when cherry fruits that received a preharvest iprodione application were also treated with a postharvest dip in a yeast suspensions producing 0.5-1.5 10^8 CFU · ml^{-1} (Spotts *et al*., 1998).

Dicarboximides such as iprodione inhibit conidial germination and mycelial growth. Sensitive strains of *Monilinia fructicola* are inhibited by less than 1 μg of iprodione · ml^{-1} and gave significant decay control (Elmer & Gaunt, 1994). The application concentration of iprodione for control of postharvest brown rot and Rhizopus rot is about 1000 μg · ml^{-1}.

Trifloxystrobin is a new strobilurin fungicide, active against a wide range of fungal plant pathogens. It is highly effective in controlling powdery mildews on field-grown apple, mango and nectarine trees and rust disease on prune trees (Reuveni, 2000). Therefore, trifloxystrobin is considered as a useful compound for efficient integration into control programs against fungal pathogens in apple, mango and stone fruit orchards.

4.4. Biological Control

Although synthetic fungicides have been widely used to control postharvest diseases, recent concerns with fungicide toxicity, development of fungicide resistance by pathogens, and potential harmful effects on the environment and human health have led to the necessity of searching means alternative to chemical control (Wisniewski & Wilson, 1992).

Several fungicides with an imidazole nucleus, i.e., thiabendazole, benomyl, and imazalil have provided excellent control of several postharvest diseases, but unfortunately some pathogenic fungi and all bacteria result tolerant to these compounds (Elmer & Gaunt, 1994).

A large number of studies showed that several microbial biocontrol agents are able to inhibit effectively postharvest diseases in various fruits. For example, application of the yeasts *Kloeckera apiculata* and *Candida guilliermondii* were effective and controlled postharvest diseases of grape, peach, and apple (Mclaughlin *et al*., 1992). *Pichia membranifaciens* at concentrations of $1 \cdot 10^8$ CFU· ml^{-1} of washed cells suspension can completely inhibit Rhizopus rot in nectarines during storage periods at 25, 15 and 3 ^oC (Fan & Tian, 2000). *Candida saitoana* is an effective antagonist for control of postharvest diseases of apple fruit under semi-commercial conditions (El-Ghaouth *et al*., 2000a).

Tian *et al*., (2002a) tested the biocontrol capability of the yeasts *Trichosporon* sp. and *Cryptococcus albidus* against *Botrytis cinerea* and *Penicillium expansum* in apple and pear fruits at 1°C in air and CA with 3% O_2 + 3% CO_2 or 3% O_2 + 8% CO_2. The results showed that apple and pear fruits treated with *Trichosporon* sp. and *C. albidus* had a lower incidence of gray mold rot than blue mold rot in the same storage conditions. *Trichosporon* sp. controlled gray mold and blue mold of apple fruits more effectively than *C. albidus*. In addition, biocontrol efficacy of the yeasts against gray and blue molds was better in apples than in pears (Fan & Tian, 2001).

Qin *et al.* (2004) reported that *Trichosporon pullulans*, *Cryptococcus laurentii*, *Rhodotorula glutinis* and *Pichia membranifaciens* were effective against several of the main postharvest pathogens (*Alternaria alternata*, *Penicillium expansum*, *Botrytis cinerea* and *Rhizopus stolonifer*) on sweet cherries at 25°C, with *T. pullulans* as the most effective control agent for all the diseases at 25°C. These authors also indicated that the activities of *C. laurentii* and *R. glutinis* against *A. alternata* and *P. expansum* were markedly enhanced by combination with CA conditions. Fruits treated with the two yeasts and stored in 10% O_2 + 10% CO_2 for 60 days showed better control for both diseases than fruits stored at 0 °C for 30 days (Qin *et al*., 2004). Actually, at least four products based on antagonistic yeasts and bacteria are commercially available under the trade names Aspire (Ecogen Inc., Langhorn, PA), YieldPlus (Anchor Yeast, Cape Town, South Africa), and Bio-Save 110 and Bio-Save 111 (EcoScience, Orlando, FL) (Droby *et al*., 2002).

Application of biological control agents alone, however, did not provide commercially acceptable control of fruit diseases. The biocontrol ability of these antagonists could be enhanced by manipulation of the environment, using mixtures of beneficial organisms, physiological and genetic enhancement of the biocontrol mechanisms and integration of biocontrol with other methods such as low doses of fungicides and CA storage (Spotts *et al*., 2002; Tian *et al*., 2002a). When applied in combination with other strategies the performance margin of biological control was increased. Biological control agents in combination with selected chemicals such as calcium chloride (Tian *et al*., 2002b; Wisniewski *et al*., 1995), chitosan (El-Ghaouth *et al*., 2000a), 2-deoxy-D-glucose (El-Ghaouth *et al*., 2000b; Janisiewicz, 1994), sodium bicarbonate (Wan *et al*., 2003), ammonium

molybdate (Wan & Tian, 2005), and salicylic acid (Qin *et al*., 2003) were demonstrated to get synergistic effects on controlling fruit decay.

Chand-Goyal and Spotts (1996) reported that using natural saprophytic yeasts and their combination with a low dosage of thiabendazole could effectively control postharvest diseases in pear fruits. Spotts *et al*., (2002) considered that the best control of brown rot of sweet cherry fruits was an integrative approach, including a preharvest fungicide, a postharvest yeast, modified atmosphere packaging and cold storage temperature. In recent years, our experiments showed that exogenous application of silicon (Si) in combination with *C. laurentii* provided synergistic effects against blue mold and brown rot of sweet cherry fruit caused by *P. expansum* and *M. fructicola* (Qin & Tian, 2005). Spore germination is strongly inhibited by Si as was the germ tube elongation of these fungi either *in vitro* (Fig. 1) and in the wounds of sweet cherry fruit (Fig. 2) (Qin & Tian, 2005). The modes of action of Si is through an enhancement of biological control efficacy, but it may be involved in its ability to stimulate the growth of *C. laurentii*, act directly on the pathogens, or induce defense mechanisms in the host.

Borate at 0.1% effectively inhibited conidial germination and germ tube elongation of *P. expansum* (Qin *et al*., 2007). After 17 h of incubation, when mycelial ramification was evident in the control, abnormal germ tubes and distorted mycelium were observed in culture medium supplemented with borate (Fig. 3; Table 2). Treatment with borate at 1% can completely inhibit blue mould rot caused by *P. expansum* in apple fruit 3 days after storage at 20°C (Fig. 4). Treatment of *C. laurentii* at $1 \cdot 10^8$ CFU $\cdot$ ml^{-1}, *C. laurentii* at $5 \cdot 10^7$ CFU $\cdot$ ml^{-1} in combination with methyl jasmonate (MeJA) with 200 µmol $\cdot$ l^{-1} resulted in a lower lesion diameter of brown rot and blue mold caused by *M. fructicola* and *P. expansum*, compared with controls in peach fruits (Yao & Tian, 2005).

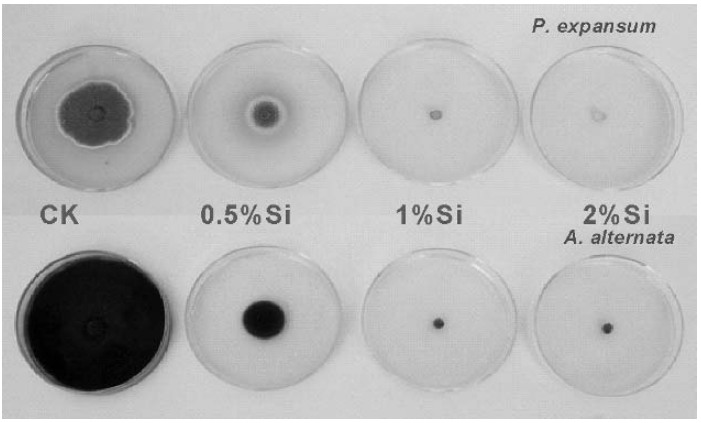

Figure 1. Inhibitory effect of Si on fungal pathogens growth on potato dextrose agar, after 7 days at 25 C (CK= untreated controls).

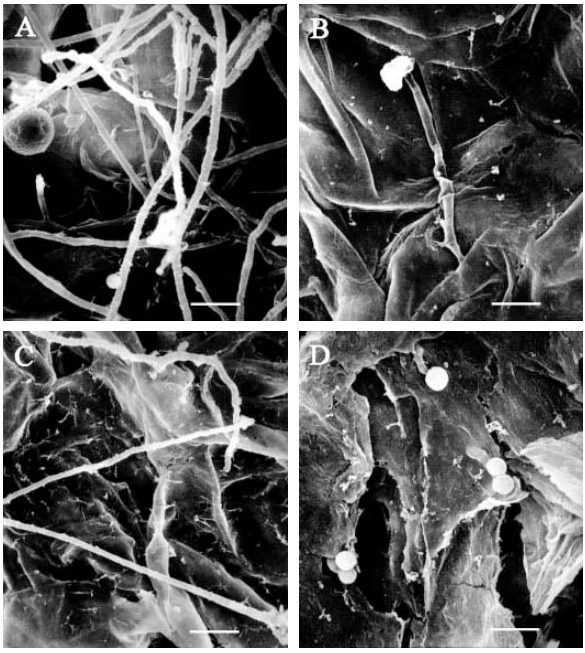

Figure 2. Scanning electron micrographs of P. expansum *(A, B) and* M. fructicola *(C, D) in the wounds of sweet cherry fruits. Fruits were wounded and inoculated with a conidial suspension of* P. expansum *or* M. fructicola *at 1 10^5 spores ml^{-1}. Dried wounds were treated with 1% (wt/vol) Si or sterile distilled water and stored at 20 °C. Fruit samples were taken from wounds 24 h and 48 h after* M. fructicola *and* P. expansum *inoculations, respectively. Scale bars: A and B = 15 µm; C and D = 30 µm. Five fruits samples per treatment were used. Adapted from* Qin & Tian, 2005).

Biocontrol efficacy of *Cryptococcus laurentii* and *Trichosporon pullulans* was enhanced by the addition of sodium bicarbonate (SBC) at 2% (Yao *et al*., 2004). Spore germination of *P. expansum* and *A. alternata* was completely inhibited and no decay was observed in pear fruits treated by *C. caurentii* combined with SBC at 2% (Fig. 5). Meanwhile, increasing intracellular trehalose content of *C. laurentii* and adding exogenous protectant (sugars + skimmed milk) could improve its viability and maintain its biocontrol efficacy on *P. expansum* in apple fruits (Li & Tian, 2006).

4.5. Induced Resistance

Induction of disease resistance in a number of crops following treatment with microbial agents has been demonstrated and provides protection against a wide range of pathogens (Lawrence *et al*., 1996; Sticher *et al*., 1997; Van Loon *et al*., 1998). In harvested commodities, the induction of disease resistance by microbial

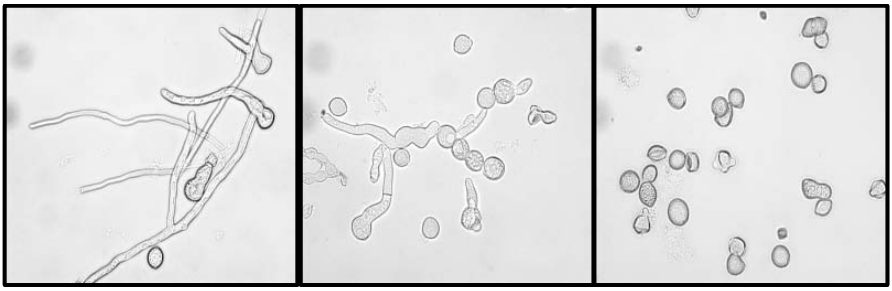

Figure 3. Microscopical observations of the inhibitory effect of different borate concentrations on growth of Monilinia fructicola *on PDB for 15 h at 200 rpm and 20°C.*

antagonists has been suggested in recent years (Droby *et al*., 2002). Induced resistance following treatment with biotic and abiotic factors has been considered to be a great potential approach for the control of postharvest diseases (Ippolito *et al*. 2000). Some antagonistic microorganisms and chemical elicitors proved to induce great efficiency by increasing resistance against postharvest diseases in fruit (De Capdeville *et al*. 2003).

Application of antagonistic yeast alone or in combination with some chemicals showed an effective potential in reducing postharvest decay of fruits, because of increasing defense-related enzymes activities (Fan *et al*. 2002; Qin *et al*. 2003). The immersion of sweet cherry fruits in *Pichia membranifaciens* (at $5 \cdot 10^7$ cells $\cdot$ ml^{-1} concentration) or in salicyclic acid (SA, 0.5 mM) for 10 min reduced the incidence as related to the synthesis of anti-oxydant enzymes and to total proteins induced (Chan & Tian, 2006).

Treatment of peach fruits with *C. laurentii* (at 10^7 CFU $\cdot$ ml^{-1}) combined with methyl jasmonate (MeJA, 200 μM) resulted in significant lesion diameters of brown

Table 2. Effect of borate on spores germination and germ tube elongation of P. expansum*.

Treatment	Spore germination (%)	Germ tube (μm)
Control	99.7 a	196.5 a
0.005 % borate	56.3 b	32.4 b
0.01 % borate	4.7 c	8.2 c

* Values on columns with the same letter are not significantly different according to LSD test ($P < 0.05$).

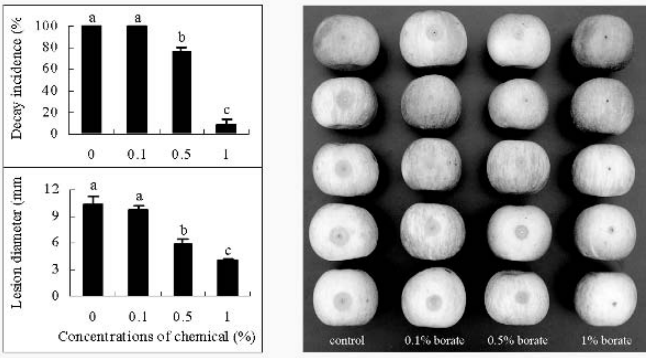

Figure 4. Control of P. expansum *by borate on apples at 20 C. Symptoms on fruits were observed 3 days after inoculation.* P. expansum *was used at 1 10 ^{5} spores ml^{-1}.*

rot and blue mold caused by *M. fructicola* and *P. expansum* lower than controls, due to higher activities of chitinase, β-1,3-glucanase, phenylalanine ammonia-lyase (PAL) and peroxidase (POD) induced in the fruit (Yao & Tian, 2005).

Recent results showed that SA, oxalic acid, $CaCl_2$ and antagonistic yeasts can significantly enhance the activities of defense related enzymes, such as β-1,3-glucanase, PAL, POD and polyphenol oxydase (PPO), reducing the disease incidence caused by *Alternaria alternata* in pear fruits (Tian *et al*., 2006).

Induction of pathogenesis-related (PR) proteins, capable of inhibiting pathogens development, also contributes to greater resistance (Fajardo *et al*. 1998). Fruit

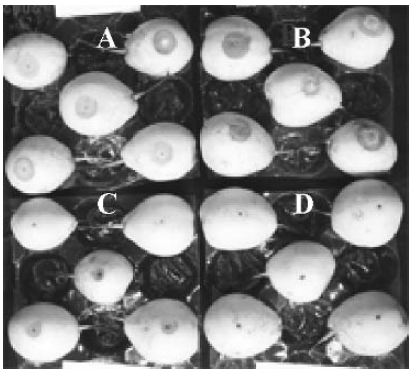

Figure 5. Control of P. expansum *(inoculated at 10 ^{5} spores ml^{-1}) by* C. laurentii *(at 5 10 ^{7} CFU ml^{-1}) alone or in combination with sodium bicarbonate (2% w/v) on pears at 20 °C. Symptoms on fruits are shown after 3 days after inoculation. A:* P. expansum*; B: $NaHCO_3$ +* P. expansum*; C:* C. laurentii + P. expansum*; D: (NaHCO3 +* C. laurentii*)* + P. expansum*.*

protection from invasion of fungal pathogens appears to result from the involvement of a highly coordinated biochemical and structural defense system that helps ward off the spread of several pathogens (Lawton *et al*., 1996). The onset of resistance is often correlated with the accumulation of defense-related enzymes, such as chitinases, β-1, 3-glucanases, PAL and POD (El Ghaouth, *et al*., 2003; Yao & Tian, 2005). Furthermore, the antifungal effects are synergistically enhanced when several enzymes are present.

Induced resistance holds promise as a new technology for the control of postharvest diseases in fruit and proved to be effective in the laboratory and in some field assays (Tian & Chan 2004). Efficiency of microbial biocontrol agents against fungal pathogens may be related to their ability to induce PR proteins in the host (Jijakli & Lepoivre, 1998).

In conclusion, disease infection and development in harvested fruits is a complex process, depending on a number of factors, such as physiological situation of the host, pathogenicity of fungal pathogens and environment conditions. Effective control of postharvest diseases in fruit crops needs an integrative strategy, including the combination of pre-harvest managements with postharvest treatments and/or the combined use of selected chemicals with biological control agents. In addition, beneficial postharvest handling and storage conditions (favorable temperature, relative humidity and control atmosphere) should also be required.

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INTEGRATED APPROACHES FOR CARROT PESTS AND DISEASES MANAGEMENT

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Abstract. Integrated approaches to the management of the main carrot diseases are reviewed, with particular attention to symptoms and field practices. Diseases caused by bacteria, foliar damages caused by fungi and parasitism by soil-borne fungi are described, together with the most important postharvest diseases. Management aiming at reducing the crop losses and damages induced by viruses, phytoplasmas and nematodes are also discussed.

1. INTRODUCTION

Wherever carrots are grown, a variety of diseases reduces both the yield and market value of the roots. Bunching carrots must have damage-free tops as well as roots. While tops are not an issue for bulk, cello-packed, or lightly processed carrots (e.g., the 'cut and peel' market), healthy tops are critical for harvest since in many areas the undercut carrots are mechanically picked up by the leaves. Thus, weak tops result in inefficient harvesting. Control of insects, diseases, and weeds, therefore, is extremely important for optimum carrot culture.

Because many pathogens of carrots are seedborne, the distribution of many diseases, including some of the most serious maladies, is worldwide. For example, Alternaria leaf blight and bacterial leaf blight, both of which can affect 100% of the acreage in a particular region, are seedborne and found wherever carrots are grown. Worldwide, Alternaria leaf blight is considered the most economically important carrot disease. Complete crop losses from Alternaria blight epidemics have been reported on individual farms.

Bacterial leaf blight, caused by *Xanthomonas campestis* pv. *carotae*, can also cause near 100% crop losses in areas with warm and rainy weather. In dry areas such as California, however, losses to bacterial leaf blight are rare, despite the almost ubiquitous occurrence of the disease.

The root-knot nematode (*Meloidogyne* spp.) is perhaps the next most serious disease pest of carrot worldwide. Carrots affected by nematodes often exhibit forking of the taproot, stubbing of the roots, and unsightly galls on the taproot and secondary roots. Because root-knot nematodes have a wide host range, they are difficult to manage. Growers often use costly control measures, such as expensive

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fumigants and/or nematicides, to reduce losses due to nematodes. Complete crop losses in carrots have been reported. Other nematodes cause local losses of carrots, but overall, losses are minimal.

In the United States, cavity spot, a soilborne disease, is considered the third most economically important carrot disease. This root malady has also been reported in Australia, Canada, France, Great Britain, Japan, and Norway. Although its occurrence is sporadic even in areas where it causes significant economic losses, it no doubt occurs in many more areas. In the United States, it occurs on about 50% of the carrot-producing acreage in California and Washington, 25% of the acreage in Colorado, and is sporadic in Wisconsin. Cavity spot occasionally causes complete crop losses in a field.

Pythium-induced diseases are often chronic and go unnoticed until the quality of the crop at harvest is assessed. These include root dieback, forking and stubbing, all of which result in a misshapened carrot that cannot be sold in the fresh market, and damping-off, which causes poor crop stands. Although the incidence of these diseases can be as high as 100%, generally the incidence is low. Occasionally, the majority of carrots in a field is misshapened and unsuitable for the fresh market.

In the United States, black rot is a serious local problem. In California, it causes an economically important disease of the crown neck area of carrots. In northern Europe and other carrot-growing regions with cool climates, black rot causes foliar blight and a postharvest decay of roots that are stored for long periods. Black rot is a seedborne disease; thus, it is found wherever carrots are grown. Areas all over the world where carrots are stored for long periods also suffer losses from crater rot, violet root rot, black root rot, among other diseases.

Most diseases of carrots, like diseases of all vegetables, are weather related and their occurrence is closely tied with the climate. For example, in warm and dry climates, powdery mildew is an economically important disease (Middle Eastern countries, for example). In more humid areas, cottony rot and downy mildew sometime cause significant losses.

Diseases caused by phytoplasmas and viruses occur where carrots are grown year-round or alternate hosts harbor the pathogens and their vectors. In some areas, phytoplasmas and/or viruses are the most important pests of carrots. For example, aster yellows is considered one of the most serious pests of carrots in Wisconsin, USA. In the state of Washington, another phytoplasma disease sometimes reaches epidemic proportions. In Australia, a newly discovered virus, carrot virus Y, threatens carrot production in that country.

Carrot diseases can be controlled by various methods, but perhaps the best method is one incorporating an Integrated Pest Management (IPM) approach. An IPM approach focuses on suppression or prevention of a disease problem. Techniques that make the pathogen less likely to cause a disease, such as improving sanitation, use of mechanical and physical control measures and use of genetically resistant varieties would all be a part of an IPM program. Pesticides are used in an IPM program when other measures fail to provide adequate control, and generally would be used only after careful monitoring of the fields show that they are needed according to established thresholds. When pesticides are used, only the ones that are least harmful to people, property, and the environment are used first.

Often times there is only one or few control options available for a particular disease. An IPM approach makes all control options available but chemical control measures are not used unless other available measures have been used first. The need to apply chemicals is also dependent on regular scouting of the field to determine if treated is warranted.

2. DISEASES CAUSED BY BACTERIA

2.1. Bacterial Leaf Blight

Bacterial leaf blight, a common disease of carrot worldwide, is caused by *Xanthomonas campestris* pv. *carotae* (Kendrick) Dye, an aerobic, Gram-negative rod. Symptoms on leaves include irregular, brown, watersoaked lesions surrounded by a yellow halo. In age, the halo disappears and the lesions become dry. The lesions are commonly observed on leaf margins, especially at the 'V' shaped junction of the leaflet lobes, resulting in leaf curling and distortion. In humid weather, a yellow-brown gummy exudate may be visible on infected leaves and petioles (Fig. 1). On infected flower stalks, copious bacterial ooze exudes from elongated lesions. Infected umbels may be completely blighted.

X. campestris pv. *carotae* is a common contaminant of carrot seed, which is an important source of primary inoculum as well as a means for long-distance dissemination. Both external and internal contamination is possible (Kendrick, 1934). In a study conducted in overhead-irrigated fields in the arid Central Valley of California, high levels of contamination (i.e., $>10^4$ CFU· $gram^{-1}$ of seed) were necessary for the development of symptoms (Umesh *et al.,* 1998). Relatively high rates of contamination (i.e., 10^7 CFU· $gram^{-1}$ of seed) were required for an epidemic to develop. However, lower levels of seed contamination could probably lead to disease development in areas with high rainfall and humidity.

The bacterium persists in soil in association with carrot debris and when the debris decomposes, the bacterium is apparently unable to survive. The bacterium is spread plant-to-plant by splashing rain and/or irrigation water as well as on insects and contaminated farm implements. On leaves, *X. campestris* pv. *carotae* grows epiphytically. When bacterial populations reach certain levels (e.g., $>10^6$ CFU· $gram^{-1}$ of leaf tissue), disease symptoms develop. Optimal temperatures for infection are 25 to 30ºC, but disease development can occur at warmer temperatures.

2.1.1. Integrated Management of Bacterial Leaf Blight

Effective control of bacterial blight involves an integrated strategy that begins with the planting of assayed seed. Contaminated lots should be treated with hot water (52ºC for 25 minutes) and re-assayed on a semi-selective medium (Kuan *et al.,* 1985) which increases ventilation between plants. Strategies that reduce hours of leaf wetness, such as reducing plant populations in the field, may reduce disease severity.

Copper-based bactericides are frequently used to slow the development of bacterial blight in the field, particularly if applications are initiated before infection occurs. Because *X. campestris* pv. *carotae* survives in crop debris, infected crop residue should be thoroughly incorporated to hasten decomposition. Crop rotations of 2 to 3 years should be practiced. Where feasible, the use of furrow rather than overhead irrigation can significantly reduce bacterial blight.

2.2. Scab

Scab, caused by *Streptomyces scabies* (Thaxter) Lambert & Loria, is usually a minor problem in carrots. However, it occasionally causes significant losses in carrot crops that may or may not follow potatoes, a commonly affected host (Hanson & Lacy, 1990; Goyer & Beaulieu, 1997). *S. scabies* infects carrot roots through wounds and natural openings, such as areas of lateral root emergence. It kills superficial cell layers and stimulates surrounding cells to form corky wound periderm. These scab lesions, which can be raised or crater-like, are typically oriented horizontally across the breadth of the root (Fig. 2). Numerous lesions result in carrots unsuitable for the fresh market.

In general, isolates of *S. scabies* are not host specific. In cross-inoculation studies, *S. scabies* isolates from carrot infected carrot, radish, and potato but not beets, and isolates from potato infected potato, carrot, radish, and beet (Goyer & Beaulieu, 1997). Artificial inoculation of carrots with potato isolates of *Streptomyces acidiscabies* Lambert & Loria and *S. caviscabies* Goyer, Faucher & Beaulieu also produced scab lesions (Goyer & Beaulieu, 1997; Lambert, 1991). However, these have not been reported from naturally infected carrots.

S. scabies survives indefinitely in soil in infected plant debris. The pathogen is disseminated by wind, water, infected potato seed pieces, or infested soil on machinery.

Figures 1-2. Bacterial ooze from carrot stem infected with Xanthomonas campestris *pv.* carotae *(1). Scab caused by* Streptomyces scabies *on carrot taproot (2).*

2.2.1. Integrated Management of Scab

Cultural controls include acidifying the soil since growth of the bacterium is inhibited by low pH. Disease incidence and severity are greatest in soils with a pH of 5.5 to 7.5. Incorporating sulfur into some soils effectively lowers the pH. If calcium fertilizers are necessary, gypsum should be used instead of lime since gypsum will not raise soil pH.

Because the disease is more severe in dry soil, adequate soil moisture should be maintained throughout the carrot cropping cycle. If carrots are grown with natural rainfall, supplemental irrigation may be necessary to maintain adequate soil moisture. Rotation with non-host crops, such as small grains, will reduce inoculum levels but should not be used as the sole method of control since damaging levels of inoculum may remain in the soil for many years.

2.3. Soft Rot

Soft rot is a common disease of most vegetables. In carrots, it causes disease both in the field and during storage. In the field, soft rot is often limited to low areas where water collects and its occurrence is erratic. However, severe, widespread outbreaks associated with warm temperatures and extended periods of soil saturation have been reported (Farrar *et al.,* 2000). In storage, soft rot is associated with wounds, contaminated wash water, or improper storage and transit conditions (Seagall & Dow, 1973).

Soft rot is characterized by pitting along the taproot or a soft decay of parts or all of the taproot (Fig. 1). Decay often progresses from the taproot tip to the crown. When pitting occurs, soft rot lesions are sunken and dull orange and the epidermis either rots or remains intact. The middle lamella between cells in affected tissues is dissolved by pectin degrading enzymes and the tissue often collapses into a soft mass. In some situations, a soft rot develops that leaves the epidermal tissue intact while the entire core rots (Tower & Beraha, 1976). The odor of infected tissue is generally not foul unless secondary organisms invade.

Soft rot is most commonly caused by the bacteria *Erwinia carotovora* subsp. c*arotovora* (Jones) Bergey *et al*. and *E. chrysanthemi* Burkholder, McFadden & Dimock. Both species are single-celled, Gram-negative rods that are motile by peritrichous flagella. They are facultatively anaerobic, oxidase negative, and catalase positive. They ferment glucose, reduce nitrate to nitrite, produce H_2S from sodium thiosulfate, grow at 36°C, and produce deep pits on selective media containing sodium polypectate. *E. carotovora* subsp. *carotovora* is phosphatase negative, insensitive to erythromycin, does not utilize malonate, and produces acid from trehalose. In contrast, *E. chrysanthemi* is phosphatase positive, sensitive to erythromycin, utilizes malonate, and does not produce acid from trehalose. Both bacteria have very wide host ranges.

E. carotovora subsp. *carotovora* is ubiquitous in plant tissue in soil and is found in many surface water sources. *E. chrysanthemi* is also widespread but its epidemiology is largely unknown. Both bacteria overwinter in crop residue. Bacterial cells enter the plant through wounds and natural openings and rapidly

degrade tissue under favorable temperatures (20 to 25°C for *E. carotovora* subsp. *carotovora* and 30 to 35°C for *E. chrysanthemi*). Long periods of soil saturation are necessary for field infection and symptom development. Post-harvest handling wounds, immersion in contaminated wash water, and unrefrigerated storage can increase the incidence of post-harvest soft rot.

2.3.1. Integrated Management of Soft Rot

Control of soft rot in the field includes proper irrigation and avoiding wounds. Because *E. carotovora* subsp. *carotovora* and *E. chrysanthemi* are facultative anaerobes, plant tissues deprived of oxygen, a condition that occurs in saturated soil, are especially susceptible to infection. Therefore, fields should drain well and should not be over-irrigated, especially during warm weather.

In general, soft rot incidence is correlated with increasing durations of soil saturation and increasing temperature (Farrar *et al.,* 2000). To prevent postharvest softrots, minimize wounds during harvest, chlorinate the wash water, regularly clean all processing lines, and store carrots close to freezing with 95% relative humidity. In one study, carrots stored at 2°C for 3 days followed by incubation at 21°C reduced soft rot compared to the incidence of rot in carrots held at 21°C (Segall & Dow, 1973). Because certain isocoumarins are produced after storage at cold temperatures, but not in freshly harvested carrots, these compounds may be partly responsible for the observed increased resistance to soft rot in cold storage (Sondheimer, 1957).

3. FOLIAR DISEASES CAUSED BY FUNGI

3.1. Alternaria Leaf Blight

Alternaria leaf blight, caused by *Alternaria dauci* (Kuehn) Groves & Skolko (synonym: *Alternaria porri* (Ell.) Neergaard f. sp. *dauci* Kühn), is a common foliar disease of carrot worldwide. Symptoms of the disease on leaves include dark brown to black lesions that may or may not be surrounded by a yellow halo and restricted by leaf veins. Lesions are often most numerous on leaf margins of older leaves. Because these symptoms are similar to those caused by bacterial leaf blight, laboratory analysis may be necessary for an accurate diagnosis. Under warm and humid conditions, lesions coalesce and cause severe foliar blight (Fig. 3). When about half of the leaf area is affected, the entire carrot leaf yellows, collapses, and dies. Petiole lesions are elongate and dark brown or blackish. Under optimal conditions, severe foliar epidemics develop rapidly, leading to loss of foliage and reduced yields. Leaf blight also indirectly reduces yields since roots are left in the ground when the weakened foliage breaks from the root during mechanical harvests.

Seed-borne inoculum in the form of spores on the surface of the seed and as dormant mycelia and conidia within the seed mericarp is important in the establishment of Alternaria leaf blight in new production areas (Strandberg, 1983). When damping-off results from contaminated seed, the fungus sporulates abundantly

Figure 3. Alternaria leaf blight caused by Alternaria dauci.

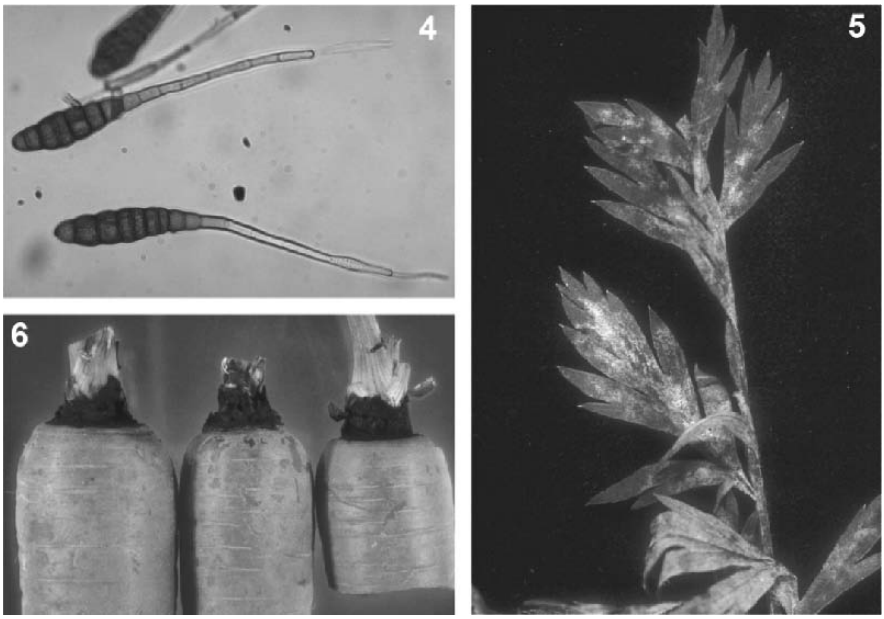
on the dead and dying seedlings. As humidity drops in the morning, spores are released and are spread by air turbulence to other plants and nearby fields (Langenberg *et al.,* 1977).

Alternaria dauci produces distinctive conidia and conidiophores. Conidia are borne singly or very rarely in chains of two. Mature conidia possess long terminal filamentous beaks that are often three times the length of the conidium body, which are ellipsoid to obclavate, brown, often minutely punctulate, and 50 to 100 µm long × 12 to 24 µm wide (Fig. 4). They have 5 to 11 transepta and one to several longisepta per segment. The olive-brown conidiophores form singly or in small clusters. On potato-dextrose agar, a distinctive light purple pigment diffuses from most colonies into the surrounding medium.

The fungus overwinters on seed, in crop residue, and on volunteer and wild carrots. Moderate to warm temperatures and prolonged leaf wetness favor infection. Incubation at 16 to 28°C in 100% relative humidity for 12 hours is required for infection of carrot leaves (Strandberg, 1988). Free water from dew, rain, or overhead irrigation prolong leaf wetness and greatly enhance disease development. Spray forecast models are available that estimate risks of infection based on temperature and duration of leaf wetness (Gillespie & Suttom, 1979).

3.1.1. Integrated Management of Alternaria Leaf Blight

The control of Alternaria leaf blight is optimized with an integrated program that uses several strategies. Because *A. dauci* can be seedborne, all seed lots should be assayed for the presence of *A. dauci* on blotter paper. If contamination occurs, the seed can be soaked in a warm (30°C) fungicide suspension to reduce seed contamination (Maude, 1966; Maude, 1992). Residue from affected carrot crops should be incorporated soon after harvest since the fungus will not survive in soil in



Figures 4-6. Conidia of Alternaria dauci *(4). Powdery growth of* Erysiphe heraclei*, the cause of powdery mildew, on carrot leaf (5) and crown rot of carrots caused by* Alternaria radicina*, the cause of black rot (6).*

the absence of host tissue. Some carrot cultivars are fairly resistant to Alternaria leaf blight and a wide range of disease tolerances exists among commercial cultivars.

In the field, fungicides are frequently used to control Alternaria blight but as the crop matures and the leaf canopy becomes increasingly dense, good coverage is difficult to obtain. In most cases, applications of fungicides should be initiated before the disease first appears.

Applications of gibberellic acid to carrot foliage may be used with or without fungicides. Gibberellic acid consistently increases the length of leaves and diameters of petioles, resulting in a more upright habit of the foliage, which may improve air movement through the canopy and thus reduce leaf wetness (Santos *et al*., 2000). Applications of gibberellic acid may provide an additional benefit by improving the harvestability of the crop since the majority of carrots destined for the fresh market are harvested by lifting the roots by their tops. Treated foliage is more robust and better withstands damage by pathogens as well as cold temperatures of winter.

3.2. Cercospora Leaf Blight

Cercospora leaf blight, caused by the imperfect fungus, *Cercospora carotae* (Pass) Solheim, occurs wherever carrots are grown. The tan leaf lesions, which are initially surrounded by a chlorotic halo, enlarge into brown necrotic spots. Lesions on the

leaf blades are circular to oval; lesions on leaf margins are elongated. Eventually, lesions coalesce, causing leaflets to die. Petiole lesions are elliptical and brown with a tan center but become grayish when the fungus sporulates.

Cercospora blight first occurs on young foliage, in contrast to Alternaria blight, which first occurs on older tissue. Severe infection of *Cercospora* blight results in the death of the entire leaf, which results in yield losses since taproots break from the foliage when gripped by mechanical harvesters. The mycelium of *C. carotae* is septate and hyaline to light brown. Conidiophores typically arise in clusters from a pseudostromata that develops in a substomatal cavity. Conidiophores are olivaceous brown and bear conidia successively at the tip as the conidiophore develops. Conidia are one- to multi-septate, filiform (40 to 110 µm long × 2.2 to 2.5 µm wide), and almost hyaline. Optimum growth of *C. carotae* is 19 to 28ºC. Infested seed, host debris, or wild carrots are sources of primary inoculum. Conidia are dispersed by wind, splashing rain and overhead irrigation, and on farm implements equipment and workers. Infection occurs in a minimum of 12 hours of leaf wetness at temperatures of 20 to 28ºC (Carisse & Kushalappa, 1990). A minimum of 24 hours of leaf wetness was necessary to induce severe infection. Symptoms may appear in as few as 3 to 5 days following inoculation under ideal conditions.

3.2.1. Integrated Management of Cercospora Leaf Blight

Control measures include planting pathogen-free seeds, crop rotation, and prompt incorporation of crop residue. Cultivar resistance has been identified and is available in commercial cultivars (Angell & Gabelman, 1968). Fungicides also effectively reduce Cercospora blight. In some areas, a fungicide spray program based on disease sampling and weather is used to optimize timing of fungicide applications (Kushalappa *et al*., 1989). In one scenario, disease incidence is determined at biweekly intervals, beginning at the five-leaf stage, by randomly sampling 50 plants in the field. Fungicidal sprays are initiated once 50% of the middle leaves exhibit symptoms. Subsequent applications should be made at 7 to 10 day intervals, provided temperatures exceed 16ºC and leaf wetness durations exceed 12 hours.

3.3. Downy Mildew

Downy mildew occurs on many plants of the umbelliferae family, although strains that infect carrot only occur in Europe. The causal agent is *Plasmopara umbelliferarum* (Caspary) Schröter ex Watenw, an obligate parasite that requires living host tissue to grow and reproduce. Synonyms that occur in the literature include *Plasmopara crustosa* (Fr.:Fr.) Jorst, *Plasmopara nivea* (Unger) J. Schröt., and *Peronospora umbelliferarum* Unger. There may be more than one species of *Plasmopara* on various umbelliferous plants since morphology of the fungus varies widely with the host plant (Constatinescu, 1992).

Symptoms are visible on the upper side of the leaves as chlorotic spots and as whitish sporulation on the corresponding underside of the leaf. Infection first appears on young foliage. During periods of high humidity, sporangiophores emerge in groups through stomata and release airborne sporangia (19 to 22 × 16 to 18 µm in

diameter), which germinate to produce motile zoospores that swim in free water on plant surfaces, eventually infecting through stomata. Sexual oospores, which are produced within the tissue, may survive the winter in crop debris or in seed.

3.3.1. Integrated Management of Downy Mildew

Several cultural practices can be adopted to manage downy mildew. Because the fungus may survive in seeds, only pathogen-free seed should be planted. Strategies that minimize the duration of leaf wetness, such as decreasing plant density, avoiding the use of excess fertilizers, and managing irrigation and drainage may reduce the incidence of disease. Carrots should be rotated with non-umbelliferous crops to reduce the inoculum load in the environment.

3.4. Powdery Mildew

Powdery mildew occurs wherever carrots are grown. Two species of powdery mildew attack umbelliferous crops. The most common one on carrots is *Erysiphe heraclei* DC. Synonyms of *E. heraclei* that appear in the literature are *E. polygoni* DC and *E. umbelliferarum* de Bary (Braun, 1995). The asexual stage is *Oidium. E. heraclei* produces white mycelium and sporulation, which are conspicuous and often dense. All above-ground plant parts, including leaves and petioles, as well as flower stalks and bracts, are susceptible and exhibit powdery fungal growth (Fig. 5). As spots enlarge on leaves, the foliage becomes chlorotic. Leaves can survive heavy infections, although they may senesce prematurely. The disease appears first on the older leaves and then spreads to the younger foliage. Depending on the crop, the severity of the disease, and the growth stage of the crop at disease onset, significant yield reductions can occur. Powdery mildew is particularly important in Mediterranean climates. *E. heraclei* occurs on many other umbelliferous crops, including anise, caraway, chervil, dill, parsnip, and parsley.

E. heraclei is ectophytic, i.e., it grows primarily external to the plant with only haustoria penetrating the host epidermal cells. Sporulation on carrot tissue occurs 7 to 14 days after infection. The mycelium of *E. heraclei* is highly branched and produces lobed haustoria. Hyphal cells are 55 to 85 μm long and 4 to 5 μm wide. The conidiophores are moderately long (60 to 140 μm) and straight. They possess a cylindrical foot cell that measures 20 to 35 × 8 to 10 μm followed by a longer cell and one or two shorter cells. Cylindrical conidia (25 to 45 × 12 to 21 μm) are formed singly. Germ tubes, which are located at the ends of conidia, form lobed or club-shaped appressoria. Cleistothecia, the sexual fruiting structures, are 80 to 120 μm in diameter with few to numerous appendages that are basally inserted, mycelioid, and brown. These appendages are mostly as long as the cleistothecial diameter and are usually irregularly branched, resulting in a coral-like appearance. There are three to six asci per cleistothecium (rarely as few as two or as many as ten) and three to five ascospores (rarely two or six) per ascus. Ascospores are relatively large (18 to 30 × 10 to 16 μm) and ovate to elliptic.

The other powdery mildew that occurs on carrot is *Leveillula lanuginosa* Fuckel (synonym: *Erysiphe lanuginosa* (Fuck.) Golovin), which is generally limited to the

Middle East, Armenia, India, Kazakhstan and other countries of Central Asia, Pakistan, and the Mediterranean regions of Europe and Africa. In addition to carrots, it infects anise, caraway, celery, coriander, dill, fennel, and parsley. It is sporadic and of minor economic importance.

Leveillula lanuginosa causes pale yellow areas on the upper leaf surface with associated whitish sporulation on the lower leaf surface. Infected areas may be limited by veins, thus giving the lesions an angular appearance. In advanced stages, sporulation also appears on the upper side of the leaf and the yellow areas turn brown. Severely affected areas eventually dry. Petioles are also infected. *L. lanuginosa* produces *Oidiopsis*-type conidia with mycelium that is both endophytic and external (as compared with the *Oidium*-type mildew, which is only external). Fungal growth is typically persistent, but is not as conspicuous as the *Oidium*-type mildew. The conidia of *L. lanuginosa* are cylindrical (around 40 to 80 × 13 to 20 μm) with distinctive rings near the ends. The conidiophores of *L. lanuginosa* are 200 to 250 μm long. Cleistothecia of *L. lanuginosa* are gregarious, sub-spherical, about 170 to 250 μm in diameter, and decorated with a few to numerous appendages on the lower half of the ascocarp. These appendages are typically shorter than the diameter of the cleistothecium, mycelioid, hyaline to yellowish, septate, often irregularly branched, interwoven with each other and with the mycelium, and measure about 4 to 10 μm wide. The asci are numerous (mostly more than 20 per cleistothecium), stalked, slender (75 to 100 × 25 to 35 μm), and two-spored. Ascospores are hyaline, one-celled, ovoid, and measure about 30 to 35 × 15 to 20 μm.

Conidia of both *Erysiphe* and *Leveillula* are light and can be carried long distances in the air. The spores are unique among fungal pathogens in their lack of a requirement for free water for germination. High humidity and moderate temperatures favor infection and disease development. Powdery mildew is more severe under shady conditions, as sunlight damages the spores and mycelium. Crops become more susceptible as they age. In Israel, the earliest age at which carrots were affected was 50 days after sowing (Palti, 1975). Rain or sprinkler irrigations tend to reduce disease severity. In general, powdery mildews tend to be more common and severe in warm, dry climates. This is particularly true of *Leveillula*. For example, in Israel, *Leveillula* on carrot occurs only in the driest part of the country.

Cleistothecia, if formed, may survive on debris and have been reported as contaminants in seeds of carrot, fennel, parsley, and parsnip, but transmission via seed has not been documented. In the absence of cleistothecia, infection of new crops probably depends on air-borne conidia from other crops or wild umbelliferous hosts.

3.4.1. Integrated Management of Powdery Mildew

Applications of sulfur are the most common chemical control but fungicides are not typically warranted unless the disease appears early in the growing season. Cultural controls include the use of tolerant cultivars, maintenance of good plant vigor while avoiding excess fertilization, and avoiding shady growing conditions and/or water

stress. In Israel, mulches applied to carrot crops to reduce drought stress significantly reduced severity of powdery mildew (Palti, 1975).

3.5. Rust

Rusts on umbelliferous crops are not economically important, although they are not uncommon in many areas. Initial symptoms of rust of carrot include a light green discoloration around infection sites. Later, the upper surface of the leaf becomes chlorotic around the lesion while yellow-orange pustules of spores often form on the underside of the leaf. Infected stems bend or arch and appear distorted or swollen. Severe infections may stunt plants.

Both autoecious and heteroecious rusts occur on umbelliferous plants. Autoecious rusts complete their life cycles on one host while heteroecious rusts typically produce their spermagonial and aecial stages on one host and their uredinal and telial stages on a second host, usually in a different plant family. In their natural habitat, rust fungi are obligate parasites, although a few can be grown on artificial media.

At least two rusts have been reported on carrot. *Uromyces graminis* (Niessl) Diet, which also occurs on fennel as well as other umbelliferous plants, produces aecidia on its umbelliferous hosts and uredinia and telia on *Melica* spp. It is found in Central Asia, Mediterranean regions, southern Russia, and South America. Urediniospores are globoid, golden, echinulate, and 22 to 30 μm in diameter. The pedicellate, one-celled teliospores are mostly ellipsoid or obovoid, a deep golden to clear chestnut-brown color, smooth, and 22 to 31 × 17 to 24 μm (Arthur, 1934; Wilson & Henderson, 1966).

Uromyces lineolatus (Desm.) Schroet. (synonym: *U. scirpi*), another heteroecious rust, occurs on carrots in Bermuda, Canada, Europe, and the U.S. Aecidia occur on umbelliferous species and uredinia and telia occur on *Scirpus* spp. in the family Cyperaceae. Urediniospores are yellowish brown in color and 16 to 25 × 22 to 35 μm in size. The wall of the urediniospore is 1.5 to 2 μm thick and minutely echinulate with three equatorial pores. Teliospores are brownish-black, clavoid, thickened apically, smooth, and 15 to 24 × 26 to 45 μm. They have persistent pedicels as long as or longer than the spore (Arthur, 1934; Wilson & Henderson, 1966).

3.5.1. Integrated Management of Rust

Little information is available on the epidemiology of rusts of umbelliferous crops, probably because they cause little economic damage. Because these fungi are obligate parasites, disease development depends on inoculum from alternate crops, wild hosts, and volunteers. Control measures include providing good field drainage to reduce humidity, removing nearby alternate hosts if the rust of concern is heteroecious, and the use of fungicides (systemic fungicides are reportedly more effective than protectant ones).

4. DISEASES CAUSED BY SOIL-BORNE FUNGI

4.1. Black Rot

Black rot occurs in most carrot production areas of the world. Although the disease is typically manifested as a black crown rot, it also causes seedling disease and a foliage and umbel blight. Where carrots are stored in bulk for extended periods, black rot is an important post-harvest disease.

A black decay of the lower petioles, which is often restricted to the petiole base and upper portion of the storage root, results in a diagnostic black ring of decay at the points of petiole attachment (Fig. 6). Decayed petioles that break during mechanical harvesting reduce yields. As a root rot, black sunken lesions develop on the taproot below the soil-line. Any stage of growth can be infected but older plants and senescent tissues are particularly susceptible.

Foliar blight symptoms begin with small necrotic spots that are often surrounded by a chlorotic margin. As lesions expand and coalesce, a black necrosis of the entire leaflet may result. Symptoms of umbel blight include necrotic lesions on the umbel stalk and on the inflorescence. Symptoms of damping-off, foliar blight, and umbel blight are similar to those of Alternaria leaf blight caused by *Alternaria dauci*.

As a post-harvest disease, black rot is characterized by dry, black sunken lesions on the surface of carrot taproots. Typically, margins of the lesions are distinct and clearly delineate diseased and healthy carrot tissue. Even in cold, moist conditions of storage, lesions can expand, coalesce, and decay the entire root. In bulk storage, the disease can spread from infected carrots to healthy ones.

Black rot is caused by the fungus *Alternaria radicina* Meier, Drechsler & Eddy (synonym: *Stemphylium radicinum* (Meier, Drechsler, & Eddy) Neergaard). Hyphae are subhyaline to olive-brown, septate, and 2.5 to 10 µm wide. The dark olive-brown conidiophores are 4 to 10 µm wide and 10 to 200 µm long. They are usually formed singly or in small clusters and are generally unbranched. One to three conidial scars are visible. Unlike many *Alternaria* species, conidia of *A. radicina* generally are borne singly, or occasionally in chains of two. In cultures less than 15 days old, conidia typically are dark olive-brown, broadly ellipsoid to ovoid, and 10 to 25 × 20 to 50 µm with two to five transepta and one to three longisepta in any or all segments, except the basal and apical segments, which are usually free of septa. Septa are well defined and darker than outer walls. Less frequently, conidia mature into long and narrow forms, which are broadly ellipsoid to obclavate and 15 to 20 × 50 to 65 µm with seven to eight transepta and one to two longisepta in most of the segments. In cultures older than 15 days, an increasing proportion of conidia become subspherical and very dark brown with numerous oblique septa. No sexual state is known.

On malt or potato-dextrose agar, colonies typically are gray-black to black with dense wooly mycelia and irregular margins. Colonies typically produce a yellow pigment that diffuses throughout the medium and white dentritic crystals that form beneath the mycelial mat. These crystals are composed of the mycotoxin radicinin, a keto-lactone that also has phytotoxic properties (Grove, 1964).

Alternaria radicina is a seed-borne pathogen, occurring as conidia on the seed surface and as mycelium in the inner layers of the pericarp or, occasionally, in the testa. The fungus has not been detected in the endosperm or in the embryo. The long-term consequence of planting infested seed is the introduction of the fungus into new fields and production areas. The pathogen also survives in association with crop debris and as free spores in the soil (Pryor *et al*., 1998). In long-term studies, the fungus survived eight years in soil in the absence of carrot cultivation (Maude & Shuring, 1972).

4.1.1. Integrated Management of Black Rot

Management of black rot in the field can be difficult. Because the disease often begins at the base of senescing carrot petioles or around the carrot crown, it is difficult to target fungicide applications at the base of the leaves once the crop canopy has closed. Relatively long crop rotations of 3 to 4 years are needed to effectively reduce soilborne inoculum levels since the fungus survives for long periods in soil. To minimize reproduction of the pathogen on carrot tissue, crop residues should be incorporated into the soil promptly after harvest. Commercial carrot cultivars with resistance to black rot are available (Pryor *et al*., 2000).

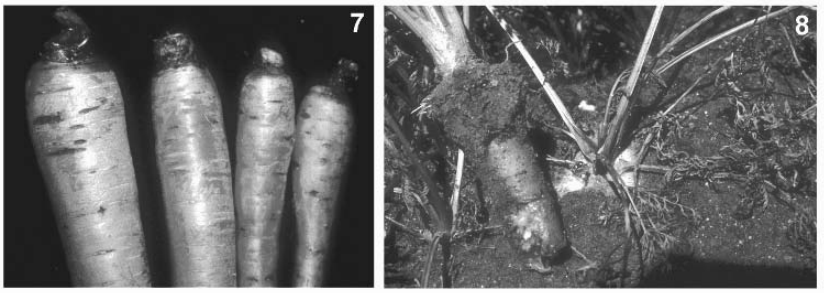
The use of pathogen-free (assayed) seed is probably the most important component in the integrated management of black rot, especially in new production areas (Tylkowska, 1992). Routine treatment of seed by a hot water dip (50ºC for 20 min) or a soak in a warm fungicide suspension may keep seed transmission to a minimum (Maude, 1966; Pryor *et al*., 1994).

To control black rot in storage, carrots should be washed and culled prior to storage to reduce inoculum in the storage facility. Wounding and breakage should be kept to a minimum. Maintaining proper temperature and humidity control during storage (0 to 1°C and about 95% relative humidity) will prevent carrot deterioration and reduce the opportunity for disease spread.

4.2. Cavity Spot

Cavity spot has been reported in most carrot-producing regions of the world, including Australia, Canada, France, Great Britain, Japan, Norway, and the U.S. The disease occurs on carrots grown in both mineral and organic soils. While the disease rarely reduces the actual yield of carrots, it can be economically important because affected roots are unsuitable for the fresh market.

The first symptoms of cavity spot are sunken, elliptical lesions oriented across the breadth of the root (Fig. 7). The lesions form under the intact periderm and are gray in color. Later, the periderm ruptures and dark, elongated lesions develop. These may occur randomly on the root or may be more dense on the upper half. Small vertical cracks are sometimes associated with the cavities. Cavities that are not infected by secondary organisms may become covered with callus tissue as the roots grow, leaving a clean, shallow, laterally elongated scar.



Figures 7-8. Cavity spot lesions caused by Pythium spp. (7). White mold of carrot caused by Sclerotinia sclerotiorum*. Note white mycelium growing from crown of infected taproot (8).*

Cavity spot can be caused by several species of *Pythium*. *P. violae* Chesters & Hickman and *P. sulcatum* Pratt & Mitchell, which are relatively slow-growing species (colony growth < 15 mm per day), are the most virulent. *P. violae* is the most important cause of cavity spot in California, Canada, France, and Great Britain, whereas *P. sulcatum* is more important in Australia and Japan. Other *Pythium* species capable of causing cavity spot symptoms are *P. intermedium* de Bary, *P. irregulare* Buisman, *P. sylvaticum* Campbell & Hendrix, and *P. ultimum* Trow.

Before the role of *Pythium* species in cavity spot etiology was firmly established, cavity spot was attributed to several causes, including infection by anaerobic bacteria, an excess of ammonia, the feeding of fungus gnat larvae, and a deficiency of calcium (Perry & Harrison, 1979). While some or all of these factors may affect carrot root health, or interact with *Pythium* spp., *Pythium* species appear to be the primary cause (Groom & Perry, 1985; White, 1988).

Little is known about the life cycle and population dynamics of *P. violae* and *P. sulcatum* in soil. These species can infect several different hosts and persist in the soil for a period of years. Germination of resting spores (oospores and hyphal swellings) probably occurs quickly in response to root exudates. Direct infection occurs through the unwounded surface of the root. Disease incidence and severity tend to increase as the plants approach maturity.

Intermittent heavy rains, poorly drained soils, and moderate temperatures favor disease development. Flooding of soil for a period of 24 hours increases the number of cavities; in dry soil, disease incidence is minimal. The optimum soil temperature for disease development is approximately 15ºC (Vivoda *et al*., 1991).

4.2.1. Integrated Management of Cavity spot

A number of cultural practices can reduce the incidence of cavity spot. Where possible, avoid carrot production in fields with a history of cavity spot. A serological method to detect the presence of *P. violae* in soil is sometimes used in Great Britain. Because the disease increases in cool wet soils, seeding in relatively warm soils with good drainage may reduce disease. Cavity spot is sometimes associated with excessive levels of fertilizer and regular soil testing should be practiced. In Britain,

cavity spot was scarce in fields with a soil pH over 8 (White, 1988). However, in a California study, disease severity was not related to soil pH, electrical conductivity, moisture-holding capacity, organic matter, total and exchangeable calcium, particle size distribution, or planting density (Vivoda *et al.,* 1991). Timely harvests can reduce disease incidence since the disease becomes more obvious later in the season and older roots are more susceptible. Long-term cold storage has little effect on disease incidence or severity.

Differences in the susceptibility of carrot cultivars to cavity spot have been identified, but no commercially available carrots are completely resistant. Although long crop rotations of at least 3 to 4 years are recommended, many *Pythium* species have wide host ranges. *P. violae*, for example, infects such diverse crops as alfalfa, broccoli, celery, cowpea, cucumber, sugarbeet, and wheat (Schrandt *et al*., 1994). Some of these crops may be asymptomatic. Because the pathogen has been reported on wild violet and other weeds, good weed management is also important.

The fungicide metalaxyl (or mefenoxam, one of the isomers of metalaxyl) is often used for the control of cavity spot. On sandy or loam soils it is most effective when applied more than once during the growing season. On soils with high organic matter content, a single application within six weeks of seeding may provide season-long control. Apparent failures of metalaxyl to control cavity spot can result from the rapid degradation of the fungicide in soil with a long history of repeated applications (Farrar *et al*., 2002).

4.3. Cottony Rot

Cottony Rot caused by *Sclerotinia sclerotiorum* (Lib.) de Bary occurs wherever umbelliferous crops are grown and is a significant problem both in production and in storage. At least 408 plant species in 278 genera representing 78 families are affected. A majority of these are herbaceous, dicotyledonous plant species, but several monocotyledonous and gymnosperm species are also affected.

On carrots, cottony rot begins as small, watersoaked, soft lesions on crowns and roots. Subsequently, characteristic white, fluffy mycelial mats develop all over the infected tissues, leading to further softening and decaying of affected areas (Fig. 8). Eventually, large, black sclerotia form in the rotted tissue.

Cottony rot is caused by *Sclerotinia sclerotiorum* (Lib.) de Bary. In culture, the fungus produces distinctive rings of white mycelium and dark sclerotia at the growing margin of the colony. The sclerotia measure 10 to 20 × 5 to 7 mm and are black outside and white inside, although very old sclerotia are black throughout. The sclerotial rind is composed of a layer of dark-walled globose cells two to six cells thick. *S. sclerotiorum* can survive in soil as sclerotia for up to 10 years (Ben-Yephet *et al*., 1993). Occasionally, it also may survive as active mycelium in living or dead plants.

After a two-week period of chilling (4ºC) and soil moisture near saturation, sclerotia of *S. sclerotiorum* located 2 to 3 cm below the soil surface germinate carpogenically by producing one to several white to tannish, cup-shaped apothecia (Dillard *et al*., 1995). Asci are cylindric-clavate, up to 130 × 10 µm, and contain eight spores. The apothecia produce and release millions of airborne ascospores,

which are dispersed by wind throughout the field and to adjacent fields. Ascospores are nonseptate, uniseriate, hyaline, and elliptical (9 to 13 × 4.6 μm). Ascospore release occurs over a period of 2 to 3 weeks. Daily release patterns during this period are cyclic; most spores are released between 10 AM and noon each morning and taper off to near nil by 2 PM. After the ascospores land on and colonize senescing or dead tissue, the fungus infects healthy tissue in the presence of free water for 48 or more hours.

4.3.1. Integrated Management of Cottony Rot

Fungicides are sometimes needed in carrot fields when conditions are cool and damp for extended periods of time. Soil fumigation is effective but is generally uneconomical in most areas (Ben-Yephet *et al*., 1986). There are, however, a number of cultural practices important in the management of *Sclerotinia* diseases (Subbarao, 1998). Irrigation manipulation such as subsurface-drip provides good control by keeping the top 5 to 8 cm of soil on the planting beds dry. Where feasible, soil flooding may provide acceptable levels of control. Deep-plowing removes sclerotia from the infection court, although plowing a second time may return sclerotia to the soil surface (Merriman *et al*., 1979). Sclerotia buried at a depth of up to 30 cm can survive for at least 15 months (Adams, 1975). Trimming carrot foliage allows greater air movement through the canopy and reduces free moisture, which may help to some degree in the management of the disease. Rotations with nonhosts, such as small grains, should be practiced. Resistance to *Sclerotinia* in most economically important crops, including carrots, is unavailable.

The key to controlling the disease in storage is to cull and clean the crop at harvest. Shipping and storage should be in sanitized containers at 0°C and near 95% relative humidity. During storage, condensation of moisture must be prevented.

4.4. Crown Rot

Crown rot is a sporadic problem of mature carrots, although the disease occasionally manifests itself early in the season as seedling damping-off. Lesions on the carrot crown or on the taproot can result in unmarketable roots. Crown rot is more severe on muck-grown carrots and in regions that have warm weather and wet conditions near harvest (Mildenhall & Williams, 1973).

Above-ground symptoms include premature senescence and death of foliage, which is sometimes apparent as patches of dying plants or disease foci in the field. A rotting of the petioles and crown tissues may develop. On roots, dark brown sunken lesions or cankers are visible near the crown and occasionally further down on the root. These can be mistaken for cavity spot lesions caused by various species of *Pythium*, especially when root lesions are not accompanied by decay of crown tissue. Although crown rot is generally a dry rot, secondary invasion by bacteria can produce a soft rot. Under moist conditions or high ambient relative humidity, web-like mycelia may be visible in crown lesions.

Thanatephorus cucumeris (Frank) Donk (anamorph: *Rhizoctonia solani* Kühn) is a widely distributed soil inhabitant that survives by forming dark brown,

undifferentiated sclerotia and by saprophytic colonization of plant debris. It produces hyphae that are relatively large, light brown, and septate. The many-branched hyphae are constricted at each point of branching. Isolates causing crown rot belong to anastomosis groups (AG) 2-2 and 4 (Grishham & Anderson, 1983; Mildenhall & Williams, 1970).

Infection of the crown tissue from overwintering mycelium and sclerotia can occur at any time during the growing season if adequate moisture is available and temperatures are warm (>18ºC). Unless visible decay occurs on the petioles and crown, early infections may not be detected until the roots are harvested. Disease can be enhanced by cultural practices that place infested soil or debris in contact with the crown and petiole tissues. In some cases, the incidence of disease is associated with high levels of colonized organic matter in the soil. The pathogen can spread from plant to plant in closely spaced carrots when the canopy is fully formed, which provides a humid microclimate. Lesions on roots may continue to expand during storage and secondary colonization by other organisms, especially bacteria, is common.

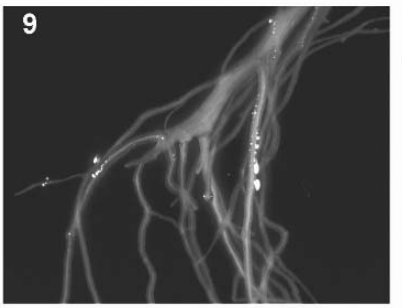
4.4.1. Integrated Management of Crown Rot

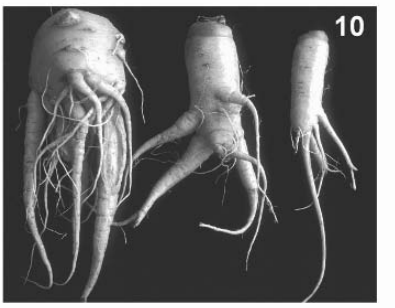
Cultural practices that minimize injury to the crown area and that enhance soil drainage and air circulation within the canopy are recommended. Before planting carrots, the residue from the previous crops should be allowed to decompose since the fungus colonizes fresh organic matter. Plantings of carrots following perennial crops such as alfalfa may suffer from severe crown rot infections. Late-season fungicide use may be required in situations where harvest is delayed by wet weather, although placement of the fungicide near the crown is problematic in mature carrots (Gurkin & Jenkins, 1985). Rotation of fields to small grains may help reduce inoculum levels.

4.5. Damping-off

Damping-off occurs wherever carrots are grown. The effects of damping-off are poor seed germination, root dieback due to the loss of the root apical meristem (Fig. 9), and seedling death. Symptoms include seed decay and pre-emergence and post-emergence plant death. Infected seeds are soft and discolored and may fail to germinate. Infection of emerging seedlings may occur along any point of the plant. The infection may rapidly spread, killing the seedling before it emerges from the soil. When seedlings are infected after they emerge from the soil, infection often occurs at the soil line, girdling the stem. The infected tissue is watersoaked and often discolored reddish-brown. Plants readily collapse. Above-ground symptoms include stunting and yellowing. The end result of damping-off is a poor stand.

Several *Pythium* spp., including *P. irregulare* Buisman, *P. mastophorum* Drechs., *P. ultimum* Trow, and others, cause damping-off of carrot seedlings. All are common soil inhabitants found wherever vegetables are grown. It has been observed that damping-off is more severe in soils with a history of successive carrot crops (Mildenhall *et al*., 1971).





Figures 9-10. Death of root apical meristem caused by Pythium *spp. (9). Branched and stubbed roots caused by* Pythium *infection of taproot (10).*

Pythium species grow vegetatively by aseptate, colorless hyphae and produce thick-walled oospores and sporangia or hyphal swellings. Exudates from host plants stimulate the oospores and sporangia to germinate. In culture, *P. irregulare* seldom produces sporangia but produces globose hyphal swellings up to 25 µm in diameter. Oogonia (about 18.5 µm in diameter) are smooth or ornamented with a varying number of short, conical or finger-like projections (mostly none to five per oogonium). Oospores are apleurotic and mostly 16 µm in diameter. Antheridia are usually monoclinous.

Rhizoctonia solani Kühn (AG-2 type 2 and to a lesser extent AG-1 and AG-4) primarily causes post-emergence damping-off (Grisham & Anderson, 1983). *R. solani* causes light to dark brown stem lesions at or near the soil line. The fungus produces septate hyphae that are relatively large (5 to 10 µm in diameter) and always some shade of brown at maturity. The hyphae are constricted at each point of branching and a septum forms near the origin of each branch. Small, brown sclerotia are often formed in association with plant debris in the soil. These sclerotia consist of tight masses of hyphae that are never differentiated into a rind and medulla.

Conditions that delay seed germination and slow seedling growth, such as cool, moist, poorly drained soils, favor seedling diseases. In some areas, damping-off caused by *R. solani* is more common in warm soils as opposed to *Pythium*, which is active in cool soils (Mildenhall & Williams, 1973). Both fungi are transported by water, contaminated soil on equipment, and movement of infected plant materials. Both have wide host ranges and exist indefinitely in most agricultural soils.

4.5.1. Integrated Management of Damping-off

Because damping-off is most severe when crops are grown in conditions not conducive to rapid seed germination and seedling emergence, avoid planting into cool, wet, and poorly drained soil. Fields should be prepared so that water does not stand. Although crop rotations do not eliminate the pathogens because of their wide host ranges, rotations with crops like small grains help

reduce inoculum levels. Seed treatments with appropriate fungicides may provide some protection from seedling diseases.

4.6. Itersonilia Canker

Among umbelliferous crops, Itersonilia canker is a minor disease of carrots. Roots, leaves, petioles, inflorescences, and seeds may be affected. On carrot roots, cankers primarily form on the crown and shoulder. Root cankers are reddish-brown with a roughened surface that becomes black in age. Cankers generally do not extend deeply into roots.

Itersonilia canker is caused by *Itersonilia perplexans* Derx., a basidiomycete that has affinities with the order Tremellales (Boekhout, 1991; Channon, 1963). The fungus is characterized by dikaryotic mycelium with clamp connections at most septa and the production and discharge of binucleate, kidney-shaped ballistospores from upright, narrow sterigmata. The hyphae are usually straight, septate at 50- to 120-μm intervals, and regularly branched. Ballistospores germinate either to form a mycelium or a secondary ballistospore. Ballistospores are 6 to 10.5 × 10 to 16 μm in size. Some isolates produce golden-brown, thin to thick-walled chlamydospores singly or in terminal clusters on short lateral branches. A yeast phase forms when cultures are submerged in water.

Itersonilia perplexans is widespread as a saprophyte of leaf surfaces of umbelliferous crops as well as many weeds and cultivated plants, particularly the composites. The fungus overwinters as mycelium in infected plants or as chlamydospores in soil (Smith, 1967). Spread within the field is by wind-borne ballistospores, which can infect foliage. New spores produced on the foliage fall to the ground and give rise to root infections. The fungus also infests seed.

Disease generally develops late in the growing season but may occur earlier if favorable environmental conditions occur. The fungus has an optimum temperature of 20°C. Disease development is enhanced in cool and wet weather and is limited in hot and dry conditions.

4.6.1. Integrated Management of Itersonilia Canker

Cultural management strategies include long rotations and good soil drainage. Deep plowing, which enhances the decomposition of host residues and exposes the fungus to antagonistic soil microorganisms, effectively reduces soilborne inoculum. The eradication of weeds will reduce other possible sources of inoculum. Fungicides are rarely needed to control Itersonilia canker in carrots. Control of the carrot rust fly is important because the larvae can predispose roots to infection.

4.7. Phytophthora Root Rot

Phytophthora root rot of carrots, also called rubbery brown rot, has been reported in Canada, France, Norway, Tasmania, and the U.S. Several species of *Phytophthora* have been associated with root rot, including *P. cactorum* (Lebert & Cohn) J. Schröt., *P. cryptogea* Pethybr. & Lafferty, *P. megasperma* Drechsler, and *P. porri* Foister

(Dowson, 1934; Ho, 1983, Stelfox & Henry, 1978). Although generally a minor problem, Phytophthora root rot can cause significant damage to carrot crops grown in waterlogged soils.

Symptoms of root rot of carrots generally occur near harvest in the spring or summer, although winter field losses have been reported. Both pre- and post-harvest losses have been associated with relatively wet soil conditions from excessive rain or irrigation. Infected portions of the taproot become dark brown to black and rubbery in consistency. The lesions may occur anywhere on the root in one or more bands. White mycelium of the pathogen is sometimes apparent on the lesions. As the lesions expand and age, a watery soft rot often permeates the root, usually in association with various bacteria and fungi.

Although the epidemiology of Phytophthora root rot of carrots is largely unknown, zoospores are thought to be the principal agents of infection. Periods of prolonged water saturation and cool temperatures during carrot growth, processing, or storage generally favor production and release of zoospores. However, other means of infection may occur. For example, mycelium of *P. porri* was implicated in carrot-to-carrot spread of the disease during post-harvest storage (Stelfox & Henry, 1978).

4.7.1. Integrated Management of Phytophthora Root Rot

To control Phytophthora root rot, soil water should be carefully managed. Providing good field drainage, adequate plant bed height, and timely irrigations that avoid extremes in soil water content are important strategies for reducing losses to root rot. Carrots should be stored at temperatures near freezing without condensation on root surfaces. Strict sanitation measures should be practiced in storage facilities.

4.8. Root Dieback

Root dieback, which results in forking and stubbing of mature taproots, occurs wherever carrots are grown. Although the incidence of the disease is sporadic and yield losses are generally low, occasionally the majority of carrots in a field are misshapened and unsuitable for the fresh market.

Root dieback causes excessively branched or stubbed roots (Fig. 10). If the root apex dies when the root is only a few millimeters long, apical dominance is removed and the taproot either fails to elongate (stubbing) or proliferates to form several functional taproots (forking) (Liddell *et al*., 1989). In severe cases, the root may not recover and the plant dies.

Although forking and stubbing can be caused by any agent that damages the root apex, such as soil compaction (Strandberg & White, 1979), nematodes, and excessive water (White & Strandberg, 1979), root dieback is often attributed to *Pythium* spp. (Howard *et al*., 1978, Mildenhall *et al*., 1971). In the United States, *P. irregulare* Buisman and *P. ultimum* Trow are the principal incitants of root dieback, although *Rhizoctonia solani* Kühn (anastomosis group 4) is occasionally associated with the disorder. Other causal fungi include *P. sylvaticum* Campbell & Hendrix and *P. sulcatum* Pratt & Mitchell, primary causal agents of dieback in carrots grown in organic soils.

All of these fungi are common soil inhabitants. When a carrot seed germinates, exudates from the seedling stimulate oospores and hyphal swellings of various *Pythium* spp. to germinate and infect the young taproot. If the fungus kills the taproot less than two weeks after seed germination, it reduces root length and/or stimulates multiple root formation. The severity of the disease may be dependent on the density of *Pythium* spores in field soils, in addition to other factors, such as very wet soil conditions or large amounts of fresh residue from previous crops. An increase in populations of *P. ultimum* by saprophytic utilization of plant nutrients after soil incorporation of fresh crop residues and the importance of partly decomposed plant residues as the base for propagules of *R. solani* have been described in other cropping systems. Disease caused by these fungi substantially decreases as residues from the previous crop decompose. Crop rotation also influences the severity of seedling root infections. In research trials, the incidence of root dieback (and subsequently forking and stubbing) was increased following alfalfa relative to the incidence of dieback in a carrot crop after a fallow field or barley, carrots, cotton, or onions (Davis & Nuñez, 1999).

4.8.1. Integrated Management of Root Dieback

Cultural practices such as good drainage are important in the management of root dieback. Other strategies include crop rotation to small grains, which might reduce soil populations of *R. solani* and some *Pythium* spp. Alfalfa in rotation with carrots should be used with caution since it may harbor relatively high populations of *P. irregulare* and *P. ultimum*. It is also a host of *Pythium violae*, the cause of cavity spot. Populations of *R. solani* also may increase following alfalfa. For optimum root quality, carrots should be grown in deep, friable, well-drained soils since all carrots, especially long-rooted cultivars, are adversely affected by shallow or compacted soils.

4.9. Southern Blight

Southern blight is a common disease of many vegetables, including carrots, grown in areas with warm climates. It derives its common name, southern blight, from its prevalence in the southeastern United States. The causal agent, *Athelia rolfsii* (Curzi) Tu & Kimbrough (anamorph: *Sclerotium rolfsii* Sacc), infects hundreds of plant species, including monocotyledons and dicotyledons.

On carrots, infection often begins on petioles at or near the soil surface. Infections typically arise at canopy closure because of increased humidity and foliar contact with the soil. Infected basal stems eventually brown and the entire plant may collapse. White, string-like mycelia, radiating out from the stem base, often develop on the soil surface around the infected crown region. Invaded tissues in carrot taproots are pale brown and soft but not watery, like bacterial soft rot. Infected carrots do not have a particularly unpleasant odor, unless the affected tissue is invaded by secondary organisms. The central core of carrots, held together by the woody conducting tissue, can occasionally be pulled free, leaving the outer portion of the root in the soil. In

severe cases, the whole root may disappear, leaving a cavity in the soil with the sides held firmly in place by the interwoven fungal threads. Numerous spherical tan to dark brown sclerotia, about the size of a mustard seed (0.5 to 1.5 mm in diameter), develop on and in infected tissues and surrounding soil.

A. rolfsii can survive for many years as sclerotia in soil and as a saprophyte on various host substrates. It produces cellulolytic and pectinolytic enzymes, which facilitate direct hyphal penetration of nonwounded tissues. Volatile compounds produced by senescent plant tissues appear to stimulate sclerotial germination. Mycelial growth develops at temperatures ranging from 8 to 40°C, but growth is greatly inhibited at temperatures below 15°C. In culture, optimium temperatures for sclerotia formation are 27 to 30°C. Mycelium is killed at freezing temperatures, but sclerotia can withstand temperatures as cold as -10°C. Moist soil conditions favor disease development, and serious outbreaks often are associated with unusually wet conditions. Southern blight is influenced by certain forms of fertilizer. Disease incidence may be reduced by the use of ammoniacal nitrogen sources and fertilizers containing plant-available calcium (Punja, 1985). The pathogen is disseminated by cultivation and tillage equipment, in irrigation and drainage water, and movement of infested soil or debris. The role of the sexual stage in the epidemiology of southern blight has not been firmly established.

4.9.1. Integrated Management of Southern Blight

The prolific growth, persistence in soil, and extensive host range of *S. rolfsii* make southern blight difficult to control, although it usually does not warrant specific control measures in cool to temperate regions. Freezing temperatures effectively destroy soilborne mycelia, limiting primary inocula in such regions to sclerotia. Although crop rotation by itself is not an effective or practical control method due to the large host range of *S. rolfsii*, rotating from carrots to a crop unaffected by the pathogen (e.g., corn or small grains) may result in less disease in subsequent years. Deep plowing to bury sclerotia and infested debris reduces inoculum viability (Gurkin & Jenkins, 1985; Jenkins & Averre, 1986).

4.10. Violet Root Rot

Violet root rot has been reported in carrot production areas of Europe, North America, New Zealand and Tasmania. The disease causes damage under field conditions and occasionally in storage. Above-ground symptoms in the field include leaf chlorosis, wilting, and patches of dying or dead plants. When infected plants are pulled up, soil often clings to the decaying roots and individual, firm, dark purple-brown lesions are visible on the taproot. As the infection progresses, a dense mycelial mat forms on the root surface, eventually reaching and extending from the carrot crown onto the adjoining soil surface. The mat is pink to brown and up to 30 cm long and 15 cm wide. In age, the mat develops a purplish to dark brown color and becomes leathery in consistency. An internal soft rot of the roots occurs, and as the roots are

pulled from the soil, only the external, leathery, outer layer of the root remains. The disease also develops on infected carrots in cold storage.

Violet root rot is caused by *Helicobasidium brebissonii* (synonym: *H. purpureum* Pat.) (anamorph: *Rhizoctonia crocorum* (Pers.) DC). The pathogen has a very wide host range, including trees, shrubs and vegetable crops such as asparagus, bean, beet, cabbage, potato, rhubarb, sea kale, sweet potato and turnip, in addition to umbelliferous crops (Valder, 1958). It causes a serious disease of alfalfa, and also infects clover, rapeseed, and saffron crocus. It has been isolated from numerous weed species, including broadleaf weeds and grasses.

Infection and disease development occur slowly. In culture, *R. crocorum* grows between 9 and 39ºC, with an optimum of 26ºC. Infection of carrots can occur between 5 and 30ºC, with an optimum of 20ºC (Whitney, 1954). While infection probably takes place in the spring, symptoms on the roots appear later in the season. Wounds are not required and any part of a carrot plant is susceptible to infection. Infection can occur from mycelium or sclerotia residing in soil or on weed hosts or other susceptible crops. The major means of spread within and between fields is by movement of infested soil on farm implements and by movement of infected plants. High soil moisture levels and low pH increase the severity of the disease.

4.10.1. Integrated Management of Violet Root Rot

Fields with infected plants should be harvested early to prevent late-season development of the disease. Rotations with nonhosts, such as cereals, are recommended. Good drainage, proper fertilization, and liming to raise the soil pH may reduce the extent of infection. Equipment should be thoroughly cleaned to avoid movement of infested soil to clean ground. In some areas, management of violet root rot is achieved by maintaining low soil moisture levels. In one study, Chantenay-type carrots were less susceptible to violet root rot than other carrot types, but resistance could not be confirmed (Dalton *et al*., 1981; Whitney, 1956).

5. POSTHARVEST DISEASES

5.1. Black Root Rot

Black root rot is generally considered a post-harvest disease, but seedlings and occasionally mature carrots in the field are affected. The disease is usually noticed after carrots have been washed, graded, and packaged in polyethylene bags. Under conditions of high humidity and warm temperatures (25ºC or higher), a blackening of wounded root surfaces develops from masses of dark brown to black chlamydospores (Fig. 11). Spread of the pathogen to adjacent roots within the package may occur. The disease is more serious on carrots grown in muck soils than carrots grown in mineral soils.

Chalara elegans Naj, Raj & Kendrick (synonym: *Thielaviopsis basicola* (Berk. & Broome) Ferraris) is a dematiaceous hyphomycete that produces multicelled, thick-walled, melanized chlamydospores (aleuriospores) as well as large numbers of

Figures 11-12. Masses of black chlamydospores of Chalara elegans *are characteristic of black root rot (11). Thread-like leaflets of plant affected with carrot thin leaf (12).*

single-celled, rectangular-shaped, phialospores (endoconidia) produced within phialides. Both spore types are common in culture and on diseased tissues. A sexual state is unknown.

Chalara elegans has a wide host range, including ornamentals, vegetables, and field crops, and is found in soils worldwide. Since saprophytic growth in soil is minimal, its survival is dependent on the longevity of chlamydospores or reproduction on host roots (Gayed, 1972). Inoculum levels are higher in acidic soils containing high levels of organic matter. The pathogen can be detected using carrot root discs as baits or on semi-selective media. Contact of infested soil with wound sites on the carrot roots during or after harvest results in infection (Punja *et al.,* 1992). Carrots damaged during harvest or grading and left for prolonged periods without cooling are predisposed to infection. When the disease occurs on taproots in the field, it is always associated with wounds of some type.

5.1.1. Integrated Management of Black Root Rot

Since infection primarily occurs after harvest and during grading, attempts to minimize wounding accompanied by rapid removal of field heat, such as dipping carrots in chlorinated hydrocooled water, are recommended (Punja *et al.,* 1992). Storage of carrots at temperatures below 10ºC minimizes pathogen growth. Good disease control is achieved when harvested carrots are dipped in solutions of potassium sorbate and propionic acid (Punja & Gaye, 1993).

5.2. Crater Rot

Crater rot is a postharvest disease of carrots placed in long-term storage. Often there are no visible symptoms of disease until 1 to 2 months of storage (Rader, 1948). Under typical storage conditions of high humidity and cool temperatures, sunken lesions (craters or pits) gradually form on root surfaces. White patches or aggregates of mycelium closely appressed to the root surface develop and small dark brown sclerotia may be evident. The carrots develop a dry rot, but if secondary invasion by bacteria occurs, soft rot can result.

The cause of crater rot is *Rhizoctonia carotae* Rader. The associated teleomorphic state, *Athelia arachnoidea* (Berk.) Jülich, has been found on decaying forest litter and does not seem to play a role in disease of carrot (Adams & Kropp, 1996). In culture, optimum growth occurs at 16 to 20ºC and no growth occurs at 28ºC (Punja, 1987).

Incipient infections of carrots from soil inoculum adhering to roots or mycelium in crown tissue occur prior to harvest (Punja, 1987). Late-harvested carrots with senescent tissues at the crown may harbor higher infection levels. Mycelium on contaminated wooden crates used to store carrots also may initiate disease. Spread of the pathogen can occur to adjacent roots held in crates or bins. The pathogen subsequently develops on the roots in cold storage at temperatures as low as 2 to 3ºC. High humidity or a film of water on the root surface enhances disease development.

5.2.1. Integrated Management of Crater Rot

Since lesions are difficult to detect on roots at harvest, the implementation of disease management strategies requires prior knowledge of the occurrence of the pathogen in the field. If disease pressure is high, carrots can be dipped in fungicides or inorganic salt solutions prior to long-term storage (Ricker & Punja, 1991). Washing roots in water also may reduce disease by removing inoculum attached to roots. Sanitary measures, including disinfestation of crates or lining them with polyethylene, minimize pathogen spread. Proper cold storage regimes that prevent temperature fluctuations and avoid moisture condensation on the root surface are essential to reduce infection and prevent dehydration. Timely removal of carrots from storage can reduce losses.

5.3. Licorice Rot

Licorice rot is one of the most important diseases of carrots held in cold storage. The disease is common in Europe and sporadic in North America. Licorice rot affects at least 90 other hosts, including other umbelliferous crops such as caraway, dill, parsnip, and numerous ornamental plants.

On carrots, lesions on stored roots are small and inconspicuous for at least several weeks. The disease is most common in the crown and root-tip regions but sometimes occurs around lateral root scars. Later, lesions extend deep into the root tissue, causing an extensive, soft, watery, black decay. Unlike black rot lesions

caused by *Alternaria radicina*, licorice rot lesions typically do not have discrete margins separating healthy from diseased tissues.

Licorice rot is caused by the imperfect fungus, *Mycocentrospora acerina* (Hartig) Deighton (synonym: *Centrospora acerina* (Hartig) Newhall), a soilborne pathogen that overwinters in soil as chlamydospores. These spores may remain viable in the soil for at least two years but the wide host range of the pathogen suggests that it may persist in production areas for longer periods. The fungus has been identified on peas, spinach, sugarbeet, and numerous weeds (Hermansen, 1992). It is possible that it may be saprophytic on decaying leaves of many plants.

In areas of intensive carrot production, chlamydospores and short lengths of pigmented mycelium may be abundant in the rhizosphere of growing roots (Davies *et al*., 1981). Infection can occur at all stages of plant growth but the fungus rarely causes disease in the field. Infection first occurs on petiole bases, taproot wounds, and at lateral root scars, but lesion expansion typically develops only after 5 to 6 weeks in storage, when tissue has begun to senesce. Conidia are not generally formed on carrots in the field but may form under conditions of high humidity during storage. The fungus is favored by cool temperatures; in culture, maximum growth occurs at 16ºC (Neergaard & Newhall, 1951).

Unlike most other postharvest fungal pathogens of carrot, the spread of *M. acerina* from diseased to healthy plants is somewhat limited. Intact plant tissues are strongly resistant to the fungus; in carrot, this resistance is likely associated with the antifungal, polyacetylenic compound falcarindiol, which is present in periderm tissue at high concentrations (Davies & Lewis, 1981; Lewis *et al*., 1981).

5.3.1. Integrated Management of Licorice Rot

Careful handling of the produce during harvest and storage, proper storage conditions, and sanitation during storage are the most important control measures. Removing soil from the surface of carrots prior to storage reduces much of the initial inoculum. Exposing carrots to high temperatures and humidity for a short period prior to storage may reduce the incidence of disease by allowing callus to form on wounds. Maintaining temperatures near freezing and high humidity without surface free water help preserve carrot quality.

6. DISEASES CAUSED BY VIRUSES AND PHYTOPLASMAS

6.1. Carrot Motley Dwarf

Carrot motley dwarf (CMD) is a widespread disease of carrots grown in cool climates, including Canada, Germany, Japan, New Zealand, United Kingdom, and the United States. Symptoms of infected carrots vary with the environmental conditions and age of the plant at infection. In cool weather (15 to 20ºC), plants infected young develop reddening and yellowing of leaves and overall stunting, symptoms that can be confused with nutritional disorders. Roots also may be severely stunted. Plants of very susceptible cultivars may die if infected when very

young. Foliar symptoms of older-infected plants and plants growing under warmer conditions are less severe and the roots are nearly normal-sized. At temperatures above approximately 24ºC, infected carrots may be symptomless.

The etiology of CMD consists of two unrelated viruses, the *Polerovirus*, *Carrot redleaf virus* (CRLV) and the *Umbravirus*, *Carrot mottle virus* (CMoV).

The CRLV virion is isometric, approximately 25 nm in diameter, and contains a single-stranded genomic RNA of approximately 5.6 kb (Murant *et al*., 1985). Virions of CMoV have not been identified. The CMoV genome, which does not encode a capsid protein, consists of a single-stranded RNA measuring approximately 4.2 kb in size.

Although each virus is capable of infecting plants alone, CMD only results from the mixed infection of CRLV and CmoV (Waterhouse & Murant, 1983). Together, both viruses are transmitted plant-to-plant in a circulative, nonpropagative manner by the willow-carrot aphid, *Cavariella aegopodii* (Scopoli). If CRLV alone is present in plants, the virus can be efficiently transmitted by its aphid vector but cannot be mechanically transmitted; however, CMoV alone in plants can be mechanically transmitted but not transmitted by the aphid. Therefore, vector transmission of CMoV requires the presence of the helper virus, CRLV. In doubly-infected plants, the CMoV single-stranded genomic RNA becomes encapsidated by CRLV capsid proteins (a process called genomic masking or transcapsidation), and thereby gains the ability to be transmitted by *C. aegopodii*.

A third virus-like RNA was identified in CMD-affected carrots from California. This CRLV-associated RNA (CRLVaRNA) is a small genomic RNA (approximately 2.8 kb) that encodes for its own RNA-dependent RNA polymerase but no capsid protein (Watson *et al*., 1998). Like CMoV, the CRLVa RNA obtains capsids composed of CRLV capsid proteins from mixed infections, and as a result gains aphid transmission by *C. aegopodii*, along with CRLV and CMoV, to new plants. It is not known if the CRLVaRNA affects symptoms on infected plants.

CRLV and CMoV have relatively narrow host ranges. Under natural conditions, CMD appears to be limited to umbelliferous plants, including carrot, wild carrot, cilantro, cow parsley, cow parsnip, dill, and parsley. In addition to these plants, the viruses causing CMD can be transmitted experimentally by aphids to bean, chervil, crimson clover, petunia, and several *Nicotiana* and *Chenopodium* spp.

Because most studies indicate that the CMD viruses are primarily associated with carrots, alternate crop or weed hosts do not appear to be important in the epidemiology of the disease. In addition to the narrow host ranges of the viruses, the aphid vector likewise has a narrow host range and prefers to feed and reproduce on carrot, although *C. aegopodii* populations also will increase on celery, chervil, and, to a lesser extent, on fennel and parsley.

Primary inoculum sources are, therefore, most often old carrot plantings or overwintered carrots that are infected with the CMD viruses and harbor *C. aegopodii*. If new carrot plantings are established near or downwind from old, infected carrot crops, *C. aegopodii* can readily vector the CMD viruses from old to new plantings.

6.1.1. Integrated Management of Carrot Motley Dwarf

CMD can be controlled in many areas by strategically selecting locations of new carrot plantings away from overwintered carrot fields and carrots grown for seed. Volunteer carrot plants should be destroyed. In certain areas, good control is achieved by planting new fields at least a mile from overwintered carrot fields (Watson & Falk, 1994). If new plantings cannot be placed at a substantial distance from old fields, applying insecticides to the old fields may be warranted to reduce vector populations. Carrot cultivars exhibit a wide range of responses to CMD, and genetic resistance to the disease is available.

6.2. Carrot Thin-leaf

Carrot thin leaf is a minor problem of carrots in California, Idaho, and Washington in the United States (Falk *et al*., 1991). The disease is sometimes common but it generally does not affect carrot yield or quality. In a few areas, yield reductions are economically significant, especially when the virus occurs with other diseases.

Symptoms vary by carrot cultivar and growth stage when infection occurred. In general, leaflets are thread-like and twisted, giving the foliage a narrow and distorted appearance (Fig. 12). Leaves also may exhibit faint mottling and yellow vein-banding. When plants are infected at a young age, the leaflets may be extremely thin, hence the name of the disease.

Carrot thin leaf is caused by the *Potyvirus*, *Carrot thin leaf virus* (CTLV). Polyclonal antisera have been produced against CTLV virions; in SDS-immunodiffusion and DAS-ELISA tests, CTLV was not closely related to other common potyviruses such as *Lettuce mosaic virus*, *Tobacco etch virus*, *Zucchini yellow mosaic virus*, or *Potato virus Y* (Howell & Mink, 1976). There is no information on strain variability of CTLV. Long, flexuous, rod-shaped virions typical of other potyviruses can be readily identified from extracts of CTLV-infected plants by using transmission electron microscopy. Virions measure 11 nm in width and 550 to 820 nm in length.

The natural host range of CTLV appears to be limited to carrots**.** In laboratory studies, some *Nicotiana* spp. and other common virus indicator plants and a few commercial umbelliferous crops, such as coriander, parsley, and parsnip, have been infected with CTLV (Howell & Mink, 1976). However, because these crops are grown on such a limited acreage and infection is not known to occur naturally, they are not considered a reservoir for the virus. In some cases, CTLV survives in volunteer carrots, which serve as the primary source of inoculum for subsequent plantings. In greenhouse experiments, aphids such as the green peach aphid, *Myzus persicae* (Sulzer), and the willow-carrot aphid, *Cavariella aegopodii* (Scopoli), efficiently transmit the virus in a non-persistent manner. Other natural vectors are not known.

6.2.1. Integrated Management of Carrot Thin-leaf

Control strategies are not commonly implemented due to the limited economic impacts of CTLV on carrot. However, removal of volunteer carrots near newly planted carrot fields probably eliminates primary inoculum. Planting near older fields also should be avoided.

6.3. Carrot Virus Y

Carrot virus Y causes serious losses in carrots in Australia. Disfiguration of roots of plants infected when young renders the crop unmarketable. Symptoms on foliage include chlorotic mottle, marginal necrosis or reddening, and general chlorosis. An increased subdivision of leaflets gives the top of the plant a feathery appearance. Infected plants may be slightly stunted. Carrot taproots on plants infected when young are stubby, severely distorted, and knobby. Roots on plants infected later develop limited distortion (Latham & Jones, 2000).

The disease is caused by the *Potyvirus, Carrot virus Y* (CVY). Flexuous filamentous virions, typical of other potyviruses, are readily identified in extracts of CVY-infected plants using electron microscopy. The virions are about 11 nm in width and 770 nm in length. Sequence analysis has revealed that CVY is distantly related to celery mosaic virus. General potyvirus monoclonal antibodies detect the virus in DAS-ELISA (Moran *et al*., 1999).

The known natural host range of CVY is limited to carrots. Substantial CVY epidemics occur in areas where carrots are grown in sequential plantings year-round. Previous plantings and volunteer carrots infected with CVY are sources of inoculum for new plantings. In greenhouse experiments, the green peach aphid, *Myzus persicae* (Sulzer), efficiently transmits the virus in a non-persistent manner. Transmission by other aphid species and seed transmission are unknown.

6.3.1. Integrated Management of Carrot Virus Y

Control of this virus can be achieved by destroying volunteer carrots, planting new crops in isolation from old crops, and using an annual carrot-free period. Managing aphid vector populations with insecticides is unlikely to be effective. Manipulating sowing dates to avoid peak aphid populations when carrots are young may be beneficial.

6.4. Aster Yellows and BLTVA (Beet Leafhopper-transmitted Virescence Agent) Yellows

Aster and BLTVA yellows affect a wide variety of wild and cultivated plants, including more than 300 species of vegetables, weeds, and ornamentals. Losses in umbelliferous crops are sporadic. Aster yellows occur worldwide in many umbelliferous crops; BLTVA yellows has been reported in carrots only in the western U.S.

In carrots, initial symptoms of aster yellows infection include yellowing of the veins of young leaves, which are often narrower than healthy leaves. The yellowing progresses until the entire leaf is chlorotic. Dormant buds in the crown then break to form upright, chlorotic, adventitious shoots. Older leaves often turn bronze, red, or purple. These older leaves may break off, making bunching and mechanical harvesting difficult. The taproots of affected plants are long and thin with a

proliferation of roots. The taproot is reduced in both size and quality. Premature flowering in the first growing season due to aster yellows infection is rare. In carrots grown for seed production, the flowers of aster yellows-infected plants exhibit virescence (greening of the flowers) and phyllody (development of leaf-like flower petals) (Fig.13). The umbels are often stunted and chlorotic, and some infected plants may die before seed is produced.

Symptoms of BLTVA include a mild chlorosis and red or purple lower leaves. The taproot is usually thin, woody, and hairy from the proliferation of roots. Many infected plants prematurely flower in the first year. The abnormal virescent and phyllodied flowers proliferate to form multiple compound leafy umbels.

Aster and BLTVA yellows are caused by two genetically distinct phytoplasmas (previously known as mycoplasma-like organisms or MLOs) (Lee & Davis, 1988). Phytoplasmas are small (0.5 to 1 μm in diameter) prokaryotes that reproduce by division or budding in the phloem sieve cells of host plants as well as in the bodies of their leafhopper vectors. They are pleomorphic in shape and lack a cell wall. Phytoplasmas have never been cultured *in vitro*. Because of their size, they can only be visualized by electron microscopy or fluorescent DNA staining (DAPI) techniques. Detection of phytoplasmas can be confirmed using bioassay, ELISA, PCR, or DNA hybridization methods (Kuske *et al*., 1991). Aster yellows- and BLTVA-infected carrots have been found in the same field, but the two phytoplamas have never been detected in the same plant.

Although the aster yellows phytoplasma is vectored by many different species of leafhoppers, the most important vector is the aster leafhopper, *Macrosteles fascifrons* Stål. After an incubation period, leafhoppers transmit the phytoplasma in a persistent manner and remain infective for life. During the spring in the northern Midwest of the U.S., infected aster leafhoppers migrate into carrot-growing areas on

Figure 13. Phyllody (development of leaf-like flower petals) of carrot infected with a phytoplasma.

prevailing winds from the south central U.S. These leafhoppers have previously acquired the aster yellows phytoplasma by feeding on infected weeds and crops. Soon after arriving (some having already completed the transmission incubation period), the aster leafhopper transmits the phytoplasma into carrots. Leafhoppers that have overwintered locally as eggs can acquire the phytoplasma from weed hosts or infected crops, but these leafhopper populations mature weeks later and are usually less infectious. In the far western and eastern U.S., where aster leafhopper migration is not known to occur, local leafhopper vectors acquire the phytoplasma from infected crops and weed hosts (e.g., dandelion, plantain, Russian thistle, sowthistle, wild lettuce, and many others) and transmit it to carrots.

BLTVA is acquired and transmitted by the beet leafhopper vector, *Circulifer tennellus* Bak. In the far western U.S., the leafhopper apparently acquires the phytoplasma from infected wild plants in the hills bordering farmlands (Golino *et al*., 1987). After their wild food source dries during the seasonal summer drought, the leafhoppers move into irrigated valleys on prevailing winds in search of green plants, including carrots. *Circulifer tennellus* transmits the phytoplasma in a persistent manner and remains infectious for life after an incubation period. Carrot to carrot transmission of BLTVA by *C. tennellus* does occur in greenhouse studies, but it is unknown whether this occurs frequently in the field. BLTVA is not transmitted by the aster leafhopper. Neither BLTVA or the aster yellows phytoplasma is seed transmitted, nor is either transmitted from infected female leafhoppers to their offspring.

6.4.1. Integrated Management of Aster Yellows and BLTVA

Control measures for these diseases include removal of weed reservoirs and planting away from infected crops. Controlling the insect vector with insecticides gives some control of aster yellows. In the northern Midwest of the U.S., where aster yellows is a recurring economic problem, the infectivity of migratory and local aster leafhoppers is monitored using a bioassay of captured aster leafhoppers on China aster. Using a combination of the relative susceptibility of the carrot cultivar, the number of leafhoppers present, and the percentage of infective leafhoppers, an aster yellows index (AYI) can be calculated (Mahr *et al*., 1993) as:

$$\text{AYI} = (\text{P} \cdot \text{N}) / 100 \text{ sweeps}$$

where P = percent infectivity of the aster leafhopper population and N = the number of aster leafhoppers present.

Insecticidal treatment is only recommended if the AYI is 50 for susceptible, 75 for intermediate, and 100 for resistant carrot cultivars. Because the infectivity of the migratory leafhoppers can vary yearly, applying insecticides only when the index value is reached can reduce the amount of sprays needed for leafhopper control. Applications of insecticides are not needed later than three weeks before harvest because three weeks are needed for symptom development. Once a plant is infected with either the aster yellows or BLTVA phytoplasma, there is no known control.

7. DISEASES CAUSED BY NEMATODES

7.1. Cyst Nematodes

The carrot cyst nematode, *Heterodera carotae* Jones, is an obligate parasite of cultivated and wild carrot (*Daucus carota* L.), its wild relative, *D. pulcherrimus* (Willd.) Koch ex DC, and the umbelliferous weed, *Torilis* spp. The nematode has been found in carrot producing regions of Cyprus, former Czechoslovakia, England, France, Germany, Holland, Hungary, India, Ireland, Italy, Poland, Russia, Scotland, Sweden, Switzerland, and Michigan, U.S.

Symptoms include plant stunting and patches of weak and undersized plants in the field. Leaves often turn yellowish-red, with older parts exhibiting necrosis. Infected plant stands do not cover the planting beds as fully as healthy stands. Below ground symptoms include smaller than normal taproots and a proliferation of lateral feeder roots, giving the plants a 'bearded' appearance. Taproots of infected plants may become distorted or restricted in length and lignify prematurely. Numerous brown cysts are apparent on the root surfaces. Carrot cyst nematodes are detected in the field by examining roots of damaged plants for the presence of the pin-head sized white females and brown cysts. While cysts and egg sacs may occur on the root surface, many females remain buried within the root tissue. These females are only visible when infected roots are cut open or teased apart.

Heterodera carotae has one of the narrowest host ranges of any plant-parasitic nematode. Only exudates from carrot roots act as a hatching stimulant for *H. carotae* eggs that are produced within cysts (Greco & Brandonisio, 1986). *H. carotae* is spread locally on farm equipment and more widely by the movement of cysts adhering to taproots. The weed, *Torilis* spp., supports the nematode in the absence of carrot, and may serve as a source of inoculum. Eggs are deposited into a large egg sac containing a gelatinous matrix or they accumulate within the female body, which becomes the leathery cyst. Eggs in cysts may remain viable for several years. After one molt within the egg, the second-stage juvenile hatches and migrates into the soil until it locates a root tip. Juveniles may orient towards roots along gradients of attractant molecules in the rhizosphere. These juveniles usually penetrate a root just behind the root cap. Once they come to the eventual feeding site, they become sedentary and initiate the formation of a feeding site called a syncytium. Comprised of several coalesced cells formed by partial cell wall degradation, the syncytium contains several nuclei from the component cells. This site acts as a transfer cell from which the nematode withdraws water and nutrients during feeding. Depending on the time of planting and seasonal temperature, one or two generations of *H. carotae* may be completed in a season. Optimum temperatures range from 15 to 20ºC for hatch and development. Hatch occurs above 5ºC but is inhibited at 25ºC.

7.1.1. Integrated Management of Cyst Nematodes

Soil fumigation and some nonfumigant nematicides lower the nematode population density in soil to or below an economic threshold. Measurable yield reduction occurs at population densities of about 80 eggs per 100 cc of soil

(Greco & Brandonisio, 1980). Marketable carrots may not be obtained at densities greater than 6,400 eggs per 100 cc of soil. Crop rotation to nonhosts or a long fallow is useful for reducing soil population levels since *H. carotae* has a very narrow host range. However, the persistence of viable eggs in cysts requires nonhost rotation periods of 4 to 6 years. Adjusting planting and harvest dates can be beneficial in managing *H. carotae.* High summer temperatures delay root invasion and may limit the nematode to a single generation. No sources of resistance to *H. carotae* have been identified.

7.2. Root-knot Nematodes

Root-knot nematodes are obligate sedentary endoparasites of carrots and many other plants. The most conspicuous and diagnostic symptom of root-knot is round to spindle-shaped swellings (galls) on feeder roots, for which the disease is named (Fig. 14). The galls induced by *Meloidogyne hapla* Chitwood tend to be smaller and more spherical or bead-like than galls induced by other root-knot species, which tend to produce larger galls that often coalesce along roots.

White to dark brown egg masses (about 0.5 to 1 mm in diameter) are found on the surface of the galled roots. When galls are cut open, mature females, which appear as white 'pearls' no more than about 1.5 mm long, are often visible within the root tissue. Infected roots are usually short and have few lateral roots and root hairs. Galling causes a disfiguration of the carrot taproot due to the swellings, resulting in unmarketable roots. Another characteristic symptom of root-knot infection is forking of the taproot, which occurs when the developing root apex at the seedling stage is damaged. Additional symptoms, such as erratic plant stands, plant stunting, yellowing,

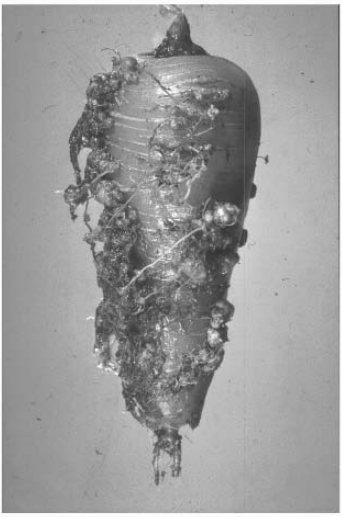


Fig. 14. Galls on carrot taproot infected with the root-knot nematode Meloidogyne hapla.

and even wilting, may result from the loss of plant vigor. Typically, affected plant stands do not cover the planting beds as fully as healthy stands.

Meloidogyne arenaria (Neal) Chitwood, *M. chitwoodi* Golden, O'Bannon, Santo & Finley, *M. fallax* Karssen, *M. hapla* Chitwood, *M. incognita* (Kofoid & White) Chitwood, and *M. javanica* (Treub) Chitwood have been reported as parasites of carrot. Two races of *M. arenaria*, three races of *M. chitwoodi*, and four races of *M. incognita* have been identified by differential hosts. Different races may reproduce at different rates and cause different symptoms on carrot.

Eggs are deposited into a gelatinous matrix by the female nematode, which is partially or completely embedded in a root of the host plant. The egg mass may contain more than 1,000 eggs and may be larger than the female body. After one molt within the egg, the second-stage juvenile hatches and moves randomly within the egg mass or migrates into the soil until it locates and enters a root tip just behind the root cap. Once inside, the second-stage juveniles migrate, become sedentary, and initiate the formation of hypertrophied, multinucleate giant cells. The nematodes enlarge, undergo three additional molts, and develop into mature females entirely embedded within the root tissue. Males leave the root after the fourth molt and become adults. The proportion of males to females is increased under conditions of environmental stress. However, because the females are self-fertilizing, males are not required for completing the life cycle.

Depending on the root-knot species and seasonal temperature, one to three generations may be completed within a season. Optimum temperatures range from 15 to 25ºC for *M. chitwoodi*, *M. fallax*, and *M. hapla*, and 25 to 30ºC for *M. arenaria*, *M. incognita*, and *M. javanica*. There is very little activity by any *Meloidogyne* species above 38°C or below 5°C.

Root-knot generally is more severe in sandy-textured and muck soils than in clay soils. Apparently, this is related to the size of the pore and the greater mobility of the nematode in water in larger, aerated pore spaces. When soil moisture is maintained at an adequate level for plant growth, the nematode may have little effect on overall plant health, but in carrot the distortion of the taproot still can be severe. Damage to carrot is positively correlated to the size of the initial nematode population.

7.2.1. Integrated Management of Root-knot Nematodes

Various soil fumigants, including the novel fumigant methyl iodide, provide effective control of root-knot (Hutchinson *et al*., 1999; Roberts *et al*., 1988). Some nonfumigant preplant nematicides also protect carrots from *M. hapla* infection in peat and muck soils by lowering the nematode population density to below an economic threshold. In practice, the threshold for carrot is considered to be at or below the root-knot nematode detection level in soil. This is referred to as a 'zero tolerance' threshold, i.e., the presence of root-knot juveniles in soil at the start of the season will result in some crop loss. This is especially true for carrots grown in Mediterranean and sub-tropical climates in the presence of *M. arenaria*, *M. incognita*, and *M. javanica*, where multiple generations during the growing season intensify damage. For *M. hapla*, economic threshold levels have been defined

at about 30 second stage juveniles per 100 cc of soil in the Netherlands, 9 per 100 cc of soil in organic soils in Canada, and 2 per 100 cc of soil in the state of Washington, U.S. (Vrain, 1982).

Crop rotation to nonhosts or a long fallow effectively reduces nematode populations. However, the extensive host ranges of root-knot species make rotation difficult to implement. Resistant cultivars to certain species of *Meloidogyne* are available in alfalfa, common bean, cotton, cowpea, pepper, and tomato. All carrot cultivars should be considered susceptible, although several sources of genetic resistance to root-knot nematodes have been identified (Simon *et al.,* 2000).

The adjustment of planting date is an effective management approach for root-knot nematode on carrot. This tactic is based on avoiding the planting of carrots when nematode juveniles in soil are active. In California, a delay in the autumn planting until soil temperatures fall below 18ºC (the *M. incognita* activity threshold), avoids significant root infection (Roberts, 1987). On *M. hapla*-infested organic soil in Quebec, Canada, early spring plantings in May (soil temperatures of 6 to 8ºC) increased marketable yields by 20 to 50% compared to mid-June plantings (soil temperatures of 15ºC) (Belair, 1987).

8. CONCLUSIONS

A diverse group of pathogens causes diseases of carrots that may result in direct yield losses or reduced market value. Because many pathogens are seedborne, the occurrence of these diseases is often worldwide. Rootknot nematodes, Alternaria leaf blight, Xanthomonas leaf blight, cavity spot, and *Pythium*-induced diseases are responsible for most economic losses; in some cases complete crop losses have been reported. The occurrence of specific diseases is dependent on local weather conditions. For example, Xanthomonas and Alternaria leaf blights are more prevalent in areas with warm and rainy weather patterns, whereas powdery mildew is of concern in drier areas. Cultural, biological, genetic, and chemical control strategies are all used to manage carrot diseases. Usually the optimum strategy is an Integrated Pest Management approach, where several methods are used to prevent economic losses with minimum input and minimum harm to the environment. Pesticides are used in conjunction with other strategies and sometimes only when all the other measures fail to provide adequate control. Where possible, chemicals are used after careful monitoring and according to established threshold levels.

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Section 2

Emerging Technologies in IPM/IDM

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INTEGRATED AGRICULTURAL PEST MANAGEMENT THROUGH REMOTE SENSING AND SPATIAL ANALYSES

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Abstract. Modern agriculture is influenced by both the pressure for increased productivity and increased stresses caused by plant pests. Geographical Information Systems and Global Positioning Systems are currently being used for variable rate application of pesticides, herbicide and fertilizers in Precision Agriculture applications, but the comparatively lesser-used tools of Remote Sensing and Spatial Analyses can be of additional value in integrated pest management practices. The tools provide valuable information in an integrated pest management context, allowing for a complete understanding (via remote mapping or spatial modeling) of the spatial complexity of the abiotic and biotic characteristics of a field and its crops, and providing information about pest populations that are present, or likely to occur. This chapter details some of the advances in Remote Sensing and Spatial Analysis as applied to integrated agricultural plant pest management, and outlines some of the remaining challenges that farmers have to face in their adoption.

1. INTRODUCTION

Modern agriculture is influenced by increasing human population and the consequent pressure for increased agriculture productivity (Seelan *et al.*, 2003). In addition, plant pests are increasing worldwide due to globalization and a ready exchange of pests, weeds and disease material, with increasing costs to nations (Mack *et al.*, 2000; Oerke, 2006; Vitousek *et al.*, 1996). For example, the potential losses due to pests worldwide are estimated to range from 50% in wheat to 80% in cotton production (Oerke, 2006). Efforts to combat pests on crops while maintaining farm profitability and productivity are not new, and Integrated Pest Management (IPM) approaches are important additions in the fight. There are many definitions of IPM (the term was first coined in 1967), the one adopted by the National Coalition on Integrated Pest Management is used in this chapter: "a sustainable approach to

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managing pests (animal pests, pathogens, and weeds) by combining biological, cultural, physical, and chemical tools in a way that minimizes economic, health and environmental risks" (Jacobsen, 1997). Farmers and growers practicing IPM use natural predators and parasites, pest-resistant crop varieties, cultural practices, biological controls, various physical techniques, and try to minimize pesticide and herbicide application (Kogan, 1998). In IPM, the emphasis is placed on acceptable pest levels and encouragement of natural predators and parasites (biological controls) rather than pest eradication, and on monitoring and regular observation of crop condition and pest populations.

The emphasis on monitoring and thorough knowledge of field conditions prescribed by IPM make new imagery sources and integrative geospatial technologies, particularly global positioning systems, remote sensing, geographical information systems and spatial analysis, powerful tools to assist in the management of pests (Barnes *et al.*, 1996); and these tools can be considered part of an IPM system. Global Positioning Systems (GPS) and Geographic Information Systems (GIS) are by far the more commonly used of the geospatial tools (Whipker & Akridge, 2006), and have been revolutionary technologies for agriculture (Seelan *et al.*, 2003).

The recent wide-scale adoption of GPS across all economic sectors exemplifies a broad-scale technological transition from expert system to broad use. GPS is a satellite-based navigation system made up of a network of 24 satellites placed into orbit by the U.S. Department of Defense. Originally intended for military applications, the U.S. government made the system available in the 1980s for civilian use (Johnson & Barton, 2004). The technology has become increasingly used in precision agriculture applications; and when differential correction is implemented, mapping- and survey-grade GPS have been used to accurately map fields for variable fertilization application (Fleming *et al.*, 2000; Robert, 2002), targeted weed control (Tian *et al.*, 1999) and currently form the backbone of many precision agriculture applications (Reyniers *et al.*, 2006; Thomas *et al.*, 2002).

GPS integrates well with other technologies that rely on accurate and precise spatial location information like remote sensing and GIS, computer and analysis systems designed to store, analyze and output spatial data that are linked to non-spatial information (Burrough & McDonnel, 1998). GIS are increasingly used across a range of scientific fields, and are used now as databases to store multiple "layers" of overlapping spatial data to integrating spatial data from numerous sources, to performing complex analysis of spatial patterns across landscapes.

There are numerous examples demonstrating the benefits of adding precise and accurate spatial data and analytical techniques to agricultural management. Precision Agriculture (PA), also called site-specific management (SSM), uses site specific knowledge of field biotic and abiotic conditions to precisely and comprehensively map crop yield, and to target amendments such as applications of fertilizers, pesticides and herbicides (Bongiovanni & Lowenberg-DeBoer, 2004; Morgan *et al.*, 2002; Senay *et al.*, 2000). Such targeted management can reduce chemical applications and nutrient loadings while maintaining farm profitability (Beeri & Peled, 2006; Bongiovanni & Lowenberg-DeBoer, 2004; Delgado *et al.*, 2001; Fitzgerald *et al.*, 2006; Khosla *et al.*, 2006), and be thus an important component in agricultural sustainability.

In the context of IPM, early detection and accurate mapping of incipient disease and insect infestations can assist managers in optimizing within-field placement of agricultural practices (variable rate technology) (Pinter *et al.*, 2003; Scotford & Miller, 2005; Weisz *et al.*, 1995) with numerous environmental benefits. Such mapping can assist farmers to more accurately apply preventative measures such as pesticides and fungicides, matching chemical application to pest density across a field (Weisz *et al.*, 1995; Zhang *et al.*, 2003), and assist in the detection of weeds for targeted herbicide application (Christensen *et al.*, 2003; Heisel *et al.*, 1996; Langner *et al.*, 2006). Such focused applications can reduce the amount of chemical used, reducing overall costs, as well as the potential for pest resistance (Bongiovanni & Lowenberg-DeBoer, 2004; Hatfield & Pinter, 1993). Labor costs incurred for plant monitoring and removal can also be reduced (Kobayashi *et al.*, 2001). There are also studies showing that targeted application of pesticides over a field can control pests while increasing beneficial parasitoids and predators (Fleischer *et al.*, 1995; Midgarden *et al.*, 1997; Weisz *et al.*, 1996).

Geospatial tools are increasingly seamless in integration: GPS are used to capture precise and accurate information in the field, remote sensing imagery are used to map conditions across a broad area over time, spatial analyses are used to understand the patterns of plant stress or mortality, and all data are stored in, and in many cases analyzed within a GIS environment and integrated into the Internet (Kelly *et al.*, 2004).

While there are examples of such comprehensive and integrated geospatial tools in the agricultural setting (e.g. Senay *et al*., 1998; Seelan *et al*., (2003) and Morgan *et al*. (2002), all of whom describe examples of remote sensing, GIS and GPS use to answer a broad suite of farm-related questions), most PA applications rely on GPS and GIS rather than remote sensing and spatial analysis (Holmstrom *et al.*, 2001; Thomas *et al.*, 2002).

A recent annual report on the adoption of geospatial tools in the agricultural sector describe two levels of technology adoption in the United States (Whipker & Akridge, 2006) which are largely distinguished by the use of remote sensing imagery and analysis. They describe a "high tech" category, which includes multi-nutrient variable rate application machinery, satellite and/or aerial imagery and analysis, variable seeding with GPS, all integrated through a GIS, and a "low tech" category, which includes single variable rate application with support from field mapping and soil sampling with GIS and GPS. They also describe the large numbers of farmers who use none of the geospatial tools, but still perform site specific farm management, including manual variable rate application and variable rate seeding without GPS; and the farmers who use no site specific management (Whipker & Akridge, 2006).

This chapter details some of the advances in the comparatively lesser-used geospatial tools of RS and SA in their application to integrated agricultural pest management, and outlines some of the remaining challenges farmers face in the adoption of geospatial technologies.

2. REMOTE SENSING

Remote sensing and digital image analysis are methods of acquisition and interpretation of measurements from a remote target without physical contact between the measuring device and the object (Nilsson, 1995a; Nilsson, 1995b). The object can be analyzed many times, non-invasively and without damage (Jensen, 1996; Jensen, 2000b). Remote sensing platforms can be field-based, or mounted on aircraft and satellites; and the data they capture is often characterized by four resolutions: (1) spatial (what the smallest resolvable unit is on the ground, also called the pixel), (2) spectral (how sensitive the spectra is sampled), (3) temporal (how often the data can be captured) and (4) radiometric (the ability to discriminate very slight differences in reflected or emitted energy). Common pixel sizes are wide-ranging: weather satellites have pixel resolutions larger than 1 km; the AVHRR sensor, an early multispectral sensor still in use has a 1km pixel size; the series of Landsat sensor have 30 m pixels, and there are a range of newer commercial satellites (e.g. Quickbird and IKONOS) that have near and under 1 m spatial resolution. Sub-meter resolution imagery is increasingly common, especially with the use of aircraft-borne sensors. The spectral information contained in imagery can include multispectral (<10 bands of spectra, covering the visible and NIR portion of the spectrum), hyperspectral (10s to 100s of bands, covering a wider range of the spectrum) and thermal spectra (covering longer wave infrared emittance spectra).

Management of crop health and detection of stress from pests, diseases and weeds can make use of remote sensing technology. Weeds can sometimes be mapped directly in imagery, and plant stress from disease or insect infestation can be expressed by a plant in many ways. Stress can influence stomata closure and transpiration rates, and impede photosynthesis (Barnes *et al.*, 1996; Luquet *et al.*, 2003; Nilsson, 1995b). Other stress symptoms include morphological changes such as leaf curling, change in leaf angle, wilting or stunting, and chlorosis, necrosis, or premature abscission of plant parts (Nilsson, 1995a).

Detection and rapid accurate quantification of early symptoms are important in an IPM context, and efforts at remotely detecting plant stress due to disease or insect activity utilize principles of biophysical remote sensing outlined in several sources (Jensen, 1983; Jensen, 2000a). Plants stressed by disease display changes in absorption and reflectance in the visible and near infrared (NIR) light due to decreases in chlorophyll content, changes in other pigments, and changes to the internal cellular structure of the leaves (Zhang *et al.*, 2003). Plant stress usually results in an increase in visible reflectance (due to a decrease in chlorophyll and a resulting decrease in absorption of visible light), and a decrease in NIR reflectance from changes in the internal leaf structure (Hatfield & Pinter, 1993). Weed mapping relies on weed plant spectra being different from the crop target (Ustin *et al.*, 2002).

Platforms used for vegetation mapping typically are sensitive in the following spectral regions: near infrared (NIR) (725 – 900 nm), green (550 nm), red (650 – 690 nm) and thermal (8,000 – 12,000 nm) (Barnes *et al.*, 1996). Utilization of these characteristic alterations in absorption and reflectance captured with aerial

photography and ground-based sensors began in the 1930s, and until the Landsat satellite launch in 1972, analysis of visible and infrared aerial photography dominated the science (Bawden, 1933; Brenchley, 1968; Colwell, 1956; Hatfield & Pinter, 1993; Kobayashi *et al.*, 2001). Bawden (1933) first used visible aerial photography to detect viral disease in potato and tobacco crops, and Colwell (1956) showed infrared photography could capture disease-related changes to internal leaf structure in cereal crops. Since then, both visible and infrared photography have been used across a broad spectrum of crops and diseases, including potato blight (Brenchley, 1968; Manzer & Cooper, 1967), bacterial blight on beans (Jackson & Wallen, 1975; Philpotts & Wallen, 1969), cotton root rot (Henneberry *et al.*, 1979; Toler *et al.*, 1981), and spot blotch on barley and powdery mildew on wheat (Clark *et al.*, 1981).

Moderate resolution imaging applications, beginning with the launch of the Landsat sensors in the 1970s, provided support for large scale plant disease and insect damage mapping and monitoring. The first Landsat-based sensor, the Multispectral Scanner (MSS) sampled the earth at 79 m resolution with 7 visible, near-, mid- and thermal-infrared bands. This was quickly followed by a series of other Landsat launches with the very successful Thematic Mapper sensors which had similar spectral resolution, but had 30 m spatial resolution. Most recently, the Enhanced Thematic Mapper sensor onboard Landsat 7, launched in 1999, provides comparable spatial and spectral resolution, and includes a 15 m resolution panchromatic band. While more commonly used in forestry applications (Price & Jakubauskas, 1998; Radeloff *et al.*, 1999), there are broad-scale remote sensing examples from the agricultural sector (Apan *et al.*, 2004; Nagarajan *et al.*, 1984; Nutter *et al.*, 2002). Landsat 2 imagery was used to discriminate between cabbage and potato fields for subsequent evaluation of clubroot disease (pathogen: *Plasmodiophora brassicae*) (Torigoe *et al.*, 1992). Nagarajan *et al.* (1984) used Landsat MSS data to detect leaf rust (pathogen: *Puccinia recondita* f. sp. *tritici*) and yellow rust (pathogen: *Puccinia striiformis*) over large areas of wheat in Pakistan in a preliminary study of the efficacy of remote sensing for disease detection. Nutter *et al.* (2002) used a combination of Landsat 7 and high spatial resolution multispectral imagery to map damage caused by soybean cyst nematode (*Heterodera glycines*) in crops near Ames, IA. They also found the imagery useful for indicating soybean crop quality and yield. The Hyperion sensor, on board the new E0-1 satellite provides continuation of broad spatial coverage with increased spectral sensitivity (over 200 bands from 0.4 – 2.5 nm) that can help in plant disease or pest damage discrimination. Apan *et al.* (2004) report that the inclusion of the longer wavelength moisture sensitive bands (e.g. 1660 nm) on the Hyperion satellite increased their ability to map orange rust disease (pathogen: *Puccinia kuehnii*) on sugarcane in Australia, over the use of visible and NIR reflectance alone.

The recent advent of high spatial resolution satellite and aircraft-borne imaging instrumentation has proved to be a boon to agricultural applications (Barnes *et al.*, 1996; Liu *et al.*, 2005). Such multispectral instruments typically capture reflectance in three visible and the NIR band, and thus their imagery is often used to map vegetation. One increasingly commonly used system is the high spatial resolution

multispectral imaging system called Airborne Data Acquisition and Registration (ADAR), which has been used with some success to map and monitor crop health (Qin *et al.*, 2003; Seelan *et al.*, 2003). The ADAR camera is digital, and captures reflectance in blue, green, red and near infrared. Because it can be mounted on aircraft, flight altitude and spatial resolution can be controlled. Qin *et al.* (2003) used multispectral ADAR imagery to detect sheath blight (pathogen: *Rhizoctonia solani* Kunh (anamorph), *Thanatephorus cucumeris* (Frank) Donk (teleomorph)) on rice in Arkansas, USA. They had better success in discriminating severe infestation levels, and had more trouble discerning early stages of the disease. Seelan *et al.* (2003) used this imagery, flown low over potato, wheat and sugar beets, to map numerous stresses on crops. In the high spatial resolution (70 cm) imagery wind damage, fertilizer skips and disease were visible.

Sudbrink *et al.* (2003) used an aircraft-borne multispectral imaging camera to monitor insect infestations on beet and cabbage plants. They report a significant negative correlation between beet armyworm (*Spodoptera exigua*) hits and transformed normalized difference vegetation index (NDVI) values on two dates across the growing season. This association may be useful in the development of future sampling plans or site-specific management techniques that direct insecticide applications for beet armyworm. They had less success detection via remote sensing an infestation of cabbage looper (*Trichoplusia ni*) (Sudbrink *et al.*, 2003).

The greater spectral discrimination provided by hyperspectral imagery combined with high signal to noise ratio allows differentiation between vegetation characteristics over small spatial areas (Zhang *et al.*, 2003; Fitzgerald *et al.*, 2004; Zhang *et al.*, 2005). For example, Kobayashi *et al.* (2001) used ground-based spectrometer reading to examine the progression of rice panicle blast disease (pathogen: *Magnaporthe grisea* Barr (anamorph *Pyricularia grisea* Cavara)), the most important disease on rice in Japan, on individual plants. They found that early in the disease infestation, changes in visible reflectance are most indicative of the disease, and as the disease progresses, changes in NIR reflectance are more useful. Mirik *et al.* (2006a, 2006b) used field-based spectrometry and digital camera imagery to investigate the relationship between reflectance and damage caused by greenbug (*Schizaphis graminum* (*Rondani*) (Hemiptera*:* Aphididae)) on winter wheat. The report greenbug density was positively correlated with visible reflectance, and negatively correlated with NIR reflectance (Mirik *et al.*, 2006a; Mirik *et al.*, 2006b). Zhang *et al.* (2002) investigated spectral changes to tomato plants in the Salinas Valley, CA USA as a result of tomato late blight (pathogen: *Phytophthora infestans*) using ground-based spectrometer. In follow-up studies, they used the Airborne Visible/Infrared Imaging Spectrometer (AVIRIS) to map crop stress associated with tomato blight (Zhang *et al.*, 2003), and multispectral ADAR imagery to separate healthy from diseased tomatoes (Zhang *et al.*, 2005). In most cases, they had better success classifying late-stage disease plants than early-stage infestations (Zhang *et al.*, 2002; Zhang *et al.*, 2003; Zhang *et al.*, 2005), and note the complications in extending ground-based spectrometer information of individual plants to canopy-based imaging. Fitzgerald *et al.* (2004) used AVIRIS imagery at 18 m ground resolution to identify the position, spatial extent and severity of strawberry

spider mite (*Tetranychus turkestani*), infestation on cotton crops in Shafter, CA USA. They used spectral mixture analysis (SMA) technique, a tool based on the assumption that each pixel is a physical mixture of multiple components (endmembers) and the spectrum of this mixed pixel is a linear combination of the endmember spectra (Fitzgerald *et al.*, 2004).

Hyperspectral imagery, especially when imaged at high enough spatial resolutions to capture plants or clusters of plants, has been successful at mapping weeds in agricultural settings. Remote sensing of weeds can take advantage of both spatial and temporal patterns (Goel *et al.*, 2003; Lopez-Granados *et al.*, 2006). Weeds discrimination in agricultural fields can make use of differing maturation times between crop and weed. For example, Lopez-Granados *et al.* (2006) report that with high spatial resolution multispectral imagery acquired 2-3 weeks before crop senescence, grass weed patches could be mapped in wheat fields. When weeds occur in patches, or are patchy across a field, remote sensing can help map them and target eradication efforts (Goel *et al.*, 2003; Karimi *et al.*, 2006; Thorp & Tian, 2004). Compact Airborne Spectographic Imager (CASI), an aircraft-borne hyperspectral imaging sensor, has been used in a number of cases to map weeds. Goels *et al.* (2003) used 72 band hyperspectral CASI data to map weeds over experimental corn fields in Quebec, Canada. The fields were controlled for level of nitrogen fertilizer rate application and amount of weeds present. While there was some confusion in teasing apart the fertilizer and weed treatments, discriminating weeds from weed-free areas was possible. Karimi *et al.* (2006) had better success with similar CASI imagery in a similar experimental design; they were able to differentiate the treatment of weeds and fertilizer in corn fields using a different (and newer) classification algorithm called Support Vector Machines (Cristianini & Scholkopf, 2002; Guo *et al.*, 2005; Rogan *et al.*, 2003). Thop & Tian (2004) provide a comprehensive review of remote sensing of agricultural weeds.

Plants under stress also display characteristic thermal changes as well, and leaf temperatures can be used to indicate water deficit stress (Fitzgerald *et al.*, 2006; Inoue *et al.*, 1997; Leinonen & Jones, 2004). As a plant transpires through leaf stomata, the evaporated water cools the leaf surface. Water disruption to leave tissues results in stomata closure, impeded photosynthesis, reduced evapotranspiration and increased leaf surface temperature (Barnes *et al.*, 1996; Luquet *et al.*, 2003; Nilsson, 1995b; Thorp & Tian, 2004). Thermal imagery has been used in combination with hyperspectral and multispectral imagery to characterize plant water capacity or potential canopy transpiration, for example, both Inoue *et al*. (1997) and Leinonen & Jones (2004) used a combination of thermal, HIS and MSI imagery to map water status in crops (cotton and soybean respectively). Leinonen & Jones (2004) cautions that measures of leaf temperature alone are insufficient to discern plant stress, as plant temperature can be influenced by a range of other factors like time of day, wind, air temperature and sun/sensor configurations (Leinonen & Jones, 2004).

While useful, all remote sensing applications must contend with variable ground conditions that can influence reflectance and emittance spectra. Canopy characteristics, plant architecture and leaf orientation, growth stage, time of day,

plant row orientation and crop cover proportion can impact remote sensing results (Luquet *et al.*, 2003; Nilsson, 1995b; Riley, 1989). For example, both thermal emittance and visible reflectance can be highly responsive to background soil conditions (the soil is usually hotter and can be brighter than the plants in it) and time of day among other factors (Luquet *et al.*, 2003). And it must be noted that all remote sensing applications measure the effects of disease or pests, and cannot identify the specific pathogen or stressor, but only map stress or mortality patterns, giving clues to likely cause (Nilsson, 1995b). Follow up ground verification must be performed for diagnostic identification of pathogen or pest.

3. SPATIAL ANALYSIS

Natural variations in soil characteristics (texture, organic matter), biological characteristics (soil microbial populations, available nutrients), weed populations, presence of pathogens and insects, and the interactions among these factors combine to influence crop quality and yield (Delgado *et al.*, 2001; Midgarden *et al.*, 1997; Midgarden *et al.*, 1992; Willers *et al.*, 2005). While remote sensing can map some of these factors, not all of these can easily be detected remotely, and often field measurements are critical for establishing spatial heterogeneity of field conditions and characteristics. Consequently, in cases where remotely sensed technology is not applicable or remotely sensed data are unavailable, field sampling is required to study crop disease dispersal or insect damage, and Spatial Analytical (SA) techniques can often lend added understanding of the field and plant conditions, and processes controlling pest distribution. Specifically, SA methods can be used to interpolate point samples to create a continuous surface, or to describe spatial pattern, or to investigate the spatial co-occurrence and relationships between complex factors. The tools can help farmers map the pattern of a pest across a field from samples, help managers guide pest sampling efforts, and aid researchers develop epidemiological hypotheses about pest establishment and spread, and the effects of control (Van Maanen & Xu, 2003).

Measurements from the field are often gathered as point data (e.g. individual plants), and can be interpolated into a 2-dimensional disease distribution map or 3-dimensional surfaces using well-defined analytical methods called spatial interpolators (Ellsbury *et al.*, 1998; Panagopoulos *et al.*, 2006; Park & Tollefson, 2005; Wollenhaupt *et al.*, 1997). For example, Wu *et al.* (2001) applied the inverse distance weighting method to interpolate the incidence of lettuce downy mildew (*Bremia lactucae*). The interpolated results were the classified into two disease levels (low incidence and high incidence) for further study. Park & Tollefson (2005) used the kriging interpolator to predict the spatial distribution of corn rootworm (Coleoptera: Chrysomelidae), and found that adult counts in the ear zone at peak population densities during the one year could predict adult emergence in the following year. Similarly, Ellsbury *et al.* (1998) used a geostatistical approach to characterize spatial variability in western (*Diabrotica virgifera virgifera*) and northern (*Diabrotica barberi*) corn rootworm adult emergence patterns, and applied semivariograms based on spherical spatial models to produce contour density maps

of the adult populations in the fields. Additionally, Heisel *et al.* (1996) used kriging to map the density of weeds in winter wheat.

There are many interpolator methods, and it should be noted that when there are abundant data, most interpolation methods will produce similar results. However, when data are limited, choosing the best interpolator is important and may significantly influence the final prediction result. Most interpolation methods assume spatial autocorrelation (i.e. nearby samples provide information about the unknown samples) exists. Kriging methods are often recommended to interpolate point data since kriging is based on a statistical model that minimizes the error variance using a weighted linear combination of the data. For example, Panagopoulos *et al.* (2006) compared kriging, inverse distance weighting and Theissen polygons to interpolate total mineral nitrogen, phosphorus, potassium, pH, electric conductivity and saturated soil hydraulic conductivity across a lettuce field and concluded kriging was the most accurate method. Others have had similar results; however Wollenhaupt *et al.* (1997) reviewed different interpolation methods, and concluded that no single interpolator is best for all the data.

An alternative to spatial interpolation (which uses a sample of data to model across a field), is to analyze completely sampled data in order to reveal pattern structures. This requires more thorough sampling of the population, and different SA methods. Spatial pattern analysis is often used as a precursor to analyze spatial data to reveal if the phenomena under observation displays random, clustered, or regular distribution (Bailey & Gatrell, 1995). These patterns can reveal underlying spatial process and controls. For example, researchers often use randomly located plots or arrays of one-dimensional samples to analyze pattern. Such data can be analyzed using such spatial statistics tools such as Ripley's K statistic, which is defined as the expected number of individuals within a distance of a randomly chosen individual in a population (Cressie, 1993; Kenkel, 1988; Kenkel, 1994; Ripley, 1976). The method has been used to study vegetation mortality patterns and tree interactions in numerous systems (Andersen, 1992; Cole & Syms, 1999; Eccles *et al.*, 1999; Szwagrzyk & Czerwczak, 1993; Vacek & Leps, 1996). The method is not ideal in all situations, as it requires a complete enumeration of the target (e.g. all plants in a field must be analyzed).

Spatial pattern analysis can also aid in guiding sampling strategy for a field. Standard sampling protocols are designed to estimate mean density, and do not capture spatial variations across fields (Weisz *et al.*, 1995), which is neither linear nor regular, thus field sampling should be guided by spatial complexities (Bongiovanni & Lowenberg-DeBoer, 2004). Gent *et al.* (2006) used transects to survey commercial hop yards to characterize the spatial heterogeneity of the incidence of hop cones with powdery mildew (*Podosphaera macularis*). They found that a beta-binomial distribution provided a good fit to the data, and indicated an aggregated pattern of disease displaying significant spatial autocorrelation. They used these results to construct sampling curves to better estimate the incidence of powdery mildew on cones, which in turn improved the sampling methods for disease monitoring and management. Others report similar procedures; Willers *et al.*

(2005) and Midgarden *et al.* (1992) discuss the importance of understanding the spatial complexity across fields to guide sampling for pests on cotton fields.

Spatial analysis can be a useful tool to explore the spatial distribution of pests, and help to formulate and test epidemiological hypothesis of pest establishment and spread (Groves *et al.*, 2005; Perring *et al.*, 2001; Wu *et al.*, 2001). The co-occurance over space of pests and different aspects of hosts can help farmers and managers understand pest dynamics. Wu *et al.* (2001) applied geostatistical analyses to study the spatial variability of the lettuce downy mildew in coastal California. The relatively short disease influence range, which was estimated by a semivariogram, suggested that the role of inoculum availability in the disease epidemics is less important than environmental variables. Perring *et al.* (2001) applied spatial analysis together with ANOVA analysis to study Pierce's disease (caused by the pathogen *Xylella fastidiosa*) in Temecula Valley, CA vineyards, and found that proximity to citrus orchards has influenced the incidence and severity of Pierce's disease. This was an important result, guiding potential management strategies for the vector of the disease, the glassy-wing sharpshooter (*Homalodisca coagulata*). In another study dealing with the same pathogen, but a different crop, Groves *et al.* (2005) used semivariograms to map the differing spatial pattern of almond leaf scorch over several different almond cultivars. Their results document both random and aggregate patterns of disease spatial distribution and illustrate how cultivar susceptibility influences the distribution patterns of the disease (Groves *et al.*, 2005).

However, results from spatial analysis can be inconclusive without support from other independent data sources and analysis. Two factors can contribute to the difficulties of interpreting spatial analysis results. First, spatial pattern interpretation is not always straightforward. For example, a commonly misused spatial index is the dispersion index (ratio of variance and mean). A random pattern will yield a dispersion of one, but this is not a unique fit (Dale, 1999): a non-random pattern can also yield a dispersion of one, and researchers who use the index to measure pattern can be mislead. Second, a spatial pattern may be caused by different processes. For example, Real & McElhany (1996) describe the example of a clustered point pattern which could be caused by a "true" contagion or an "apparent" contagion. The presence of a "true" contagion means that the patchiness develops in the location of initial infected individuals because of limited dispersal capabilities of the pathogen, while an "apparent" contagion means that the patchiness is generated by clustered pattern of host population or the environment. Consequently, other independent tests (e.g. field measurements) are often needed to further support the results of the spatial analysis (Real & McElhany, 1996).

4. REMAINING CHALLENGES

While clearly useful for agriculture, geospatial technologies have uneven adoption rates, even across the United States, and to investigate their use in Integrated Pest Management we must borrow results from surveys conducted determining adoption of PA techniques. Griffin *et al.* (2004) present the most comprehensive statistics on PA adoption, charting rates across four technologies – yield monitoring, yield

mapping with GPS, georeferenced soil maps, and remotely sensed imagery – and across four crops – corn, soybean, wheat and cotton in the United States from 1996 through 2002 (Griffin *et al.*, 2004). The adoption of technology varies by crop, region and tool; for example, remote sensing is consistently the least utilized tool, yield monitoring was consistently the most utilized; georeferenced soil maps increased in use across all crops between 1996 and 2002; and GPS use leveled off in 2000 with corn and soybean, and was not used in wheat or cotton farming. An alternative report lists that in 1998, 70% of farmers surveyed were unaware of PA technologies, 25% were aware but were not adopters, leaving less than 5% of farmers using PA tools (Daberkow & McBride, 2003).

The National Academy of Sciences in a 1997 report comment that the adoption of any farming technology is unlikely to be universal because of the high degree of heterogeneity across the agricultural sector (National Research Council, 1997). Differences in climate, soils, topography, water availability, government programs, and other factors produce a variable agricultural landscape across the United States and globally, and can explain some of the variations in technology adoption. Robert (2002) further explains this uneven adoption, and characterized the challenges faced by farmers implementing geospatial technology as socio-economic, agronomic, and technological. The socio-economic barriers of costs, considered excessive by many, and the difficulty in attaining necessary software and hardware expertise keep much of this work in the research domain. Lack of good base data including soils maps, and digital elevation models confound the problem in many areas. Other barriers include the lack of compatibility of much farm equipment and GPS, although others report active research and develop efforts in the development of GPS-compatible farm machinery (Tian *et al.*, 1999).

Profitability is the likely the most important consideration in long-term of adoption of geospatial and other technologies in agriculture (National Research Council, 1997). Additional factors that might influence adoption of technology are accessibility of satellite and other imagery libraries, commercially-available high spatial resolution imagery in near real time, comprehensive digital weather and elevation data, and increased access to GPS and GIS technologies will influence technology adoption (Thomas *et al.*, 2002). The distribution of data, tools and results over the Internet has increased the number of individuals who can take advantage of these tools (Thomas *et al.*, 2002).

Robert (2002) suggests an age preference to technology adoption, and anticipates that a younger generation of farmers might influence adoption of technology. In a different study, Roberts and colleagues (2004) found that younger, more educated farmers who operated larger farms and were optimistic about the future of precision farming were most likely to adopt site-specific information technology. The probability of adopting variable-rate input application technology was higher for younger farmers who operated larger farms, owned more of the land they farmed, were more informed about the costs and benefits of precision farming, and were optimistic about the future of precision farming. Computer use was not important, possibly because custom hiring shifts the burden of computer use to agribusiness firms (Roberts *et al.*,

2004). Interestingly, awareness of technology is not always a barrier to adoption. Daberkow & McBride (2003) found that profitability, large farms, full-time farms, and computer literacy were factors in technology adoption, and that awareness of technologies was not a constraint (Daberkow & McBride, 2003). For outreach efforts to succeed in increasing adoption of a suite of integrated geospatial tools in an IPM setting will likely require clear examples of economic benefits to farmers, a commitment over several growing seasons, customization of applications, more real time access to imagery and data, and attention paid to spatial resolutions (Seelan *et al.*, 2003).

5. CONCLUSIONS

Precision Agriculture is built on the ability of GPS integrated with GIS to promote variable management practices according to variable field characteristics, (Holmstrom *et al.*, 2001; Seelan *et al.*, 2003; Thomas *et al.*, 2002) and adoption of these spatial tools in agriculture for Integrated Pest Management and variable rate application of pesticides, herbicide and fertilizers will likely continue (Seelan *et al.*, 2003; Whipker & Akridge, 2006). Remote sensing and spatial analyses are of additional value in planning crop management practices, but these technologies are used less often for several reasons. First, high spatial resolution imagery is not easily available for all rural areas and hyperspectral imagery usually requires contracting; second, there is a lack of technical knowledge about remote sensing and spatial analysis by farmers and consultants; and finally, much of the benefit of remote sensing and spatial analysis depends on time-sensitive mapping and near real-time image acquisition and product delivery (Moran *et al.*, 1997; Seelan *et al.*, 2003). Despite these challenges, remote sensing and spatial analysis can provide valuable information in an IPM context, allowing for a complete understanding (via remote mapping or spatial modeling) of the spatial complexity of the abiotic and biotic characteristics of a field and its crops, and providing information about the disease and pest populations that are present, or likely to occur.

The transition to the utilization of a full suite of geospatial tools for integrated pest management is agricultural sector is mirrored in the realm of forestry, where increasing and large-scale pest and disease attacks are increasingly reported, and where the spatial pattern across landscape-scales of pest hosts, pest and pathogen population dynamics and landscape structure interact to at times promote pest establishment (Holdenrieder *et al.*, 2004). As in agricultural settings, geospatial technologies are making forestry management more precise and spatially comprehensive: and a better articulation of resources across space yields new insights to yield, pest and control dynamics. New access to data and technology will likely promote the transition of these tools from a research to an applied domain across both sectors.

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APPLICATIONS OF INFORMATION TECHNOLOGY IN IPM

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Abstract. This chapter discusses the impacts and applications of information technology (IT) in pest management. A brief review will be given to the history of IT applications in pest management with an emphasis on recent developments. The discussion will focus on two IT technologies, the World Wide Web (the Web) and databases, within the context of their applications to pest management. Some of the applications will be highlighted to illustrate the potential impacts of the IT technologies in pest management in the near future. Finally, a detailed consideration of the application of these technologies in a decision support system for plant disease management will be presented.

1. INTRODUCTION

The application of information technology (IT) in pest management has been one of the most exciting developments in the field over the last decade. IT has improved the efficiency of data collection and analysis, pest identification, control agent selection, and pesticide field applications. Additionally, IT has enhanced our ability in research, training, education, and information dissemination and management (Stinner, 1999).

The pace of IT adoption in pest management has been so swift that even experts can't predict what will happen in the near future. In fact, just a little over 10 years ago, many experts claimed that CD-ROM technology would play a central role in pest management by the year 2000 (Scott & Gilmore, 1992). As it turned out, CD-ROM technology was superceded by newer advances in the field by the year 2000. Many "new" technologies such as the World Wide Web which were not widely known when the experts made the prediction, have played far more important roles than the CD-ROMs in pest management.

2. IT AND PEST MANAGEMENT

The use of computers and computer software in certain fields relevant to pest management has quite a long history. Mainframe computers were used for data collection, simulation, and modeling in ecology in the early 1960s (Pielou, 1969; Watt, 1963). Later, expert systems became another pest management field where researchers relied on the power of computers for simulations (Kinnucan, 1984; Jones *et al*., 1986; Batchelor *et al*., 1989). However, these early applications of computers

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were mainly limited to research and academic arenas, and were utilized by few highly trained professionals. There are few successful cases in which computers or computer software helped in solving real world pest management problems (Jones, 1989). Readers wishing to know more about these early IT applications in pest management can refer to other review articles such as Scott & Gilmore (1992).

Emergence of the Internet and related technologies, such as the World Wide Web and database technology, in parallel with increases in computing speed, data storage, and computational power in the 1990s, made a broad array of IT tools available for pest management uses. In fact, pest management came into a new era in terms of data and information distribution, education, training, and collaboration during this period. The number of information systems relating to pest management (either online, internal, or on CD-ROM) has rapidly increased. These information systems help users solve many real problems in pest management. For example, users can obtain grasshopper forecast and outbreak data from the USDA online information system: *http://www.sidney.ars.usda.gov/grasshopper/Extras/index.htm*, learn how to use new and advanced technologies like GIS for IPM at *http://www.colostate.edu/Depts/IPM/tmp/gis_ag/ipmgis.html*, find or review a pesticide material safety data sheet (MSDS) at *http://www.msds.com*, find pest identification information at http://www.bug-net.co.uk or buy biocontrol agents at *http://www.groworganic.com/a/a1.html?sCategory=431&sMax=All&sDisplay=Brief.*

A milestone in using IT for pest management during this period was the formation of several centralized pest management information resource centers, such as the CABI Crop Protection Compendia (Compendia hereafter) and the many information systems at the NSF Center for Integrated Pest Management (CIPM), where the authors are associated. The Compendia are the direct result of an international workshop on using information technology for crop protection held in Wallingford, UK, in 1989 (Harris & Scott, 1989; McGillivray & Scott, 1999). Compendia on Crop Production, Forestry, and Animal and Livestock Production are each comprehensive information systems containing information useful for pest management. The compendia are one of the most important sources for obtaining pest management information in many countries around the world. They are available in CD-ROM and online (http://www.cabi.org/compendia/cpc). In the United States, the USDA's Cooperative States Research Extension and Education Service (CSREES), in cooperation with the Regional Pest Management Centers and the National Plant Diagnostic Network, have developed an agreement with CABI that allows faculty and staff at all U. S. land-grant universities to have free online access to these compendia (see *http://www.ipmcenters.org/CABI*).

CIPM has been partly funded by CSREES to develop the pest management information system for the USDA's Regional Pest Management Centers mentioned above. This system has evolved into a comprehensive pest management information provider with over 50 international, national, and regional information systems and databases funded by government agencies, non-profit organizations, and private sections (*http://cipm.ncsu.edu/Websites.cfm*).

Because of the vast increase in scope of IT applications in pest management since the 1990's, it is impossible for us to cover all these aspects in a chapter like this one. Several good review articles are available on the subject. Readers can refer

to Ellsbury *et al*. (1999) and Zhang *et al*. (2002) on precision agriculture/GIS/GPS, Jones (1989) on expert systems, Scott & Gilmore (1992), Knight (1999), and Foulds (2000) on many other applications. Two technologies: the Web and databases, are playing an increasingly important role. Except for Stinner's article (Stinner, 1999), there are few review articles on these topics so far. A good understanding on these technologies will help IPM practitioners to better use the information systems developed from these technologies. Therefore, we will deepen this topic in the rest of this chapter. We will then review two recent applications and development from the Web and database technology, with examples from data sharing and bioterrorism threats, to understand how new technologies might shape the future of pest management in a broader scope. Finally, we will review the concepts and application of decision support systems (DSS) in plant disease management. DSS has been widely used in IPM recently. These systems are also highly relevant to databases and the Web technology.

3. THE WORLD WIDE WEB AND DATABASE TECHNOLOGY: APPLICATIONS IN PEST MANAGEMENT

Since Tim Berners-Lee invented the World Wide Web (we use World Wide Web and Web interchangeably hereafter) in 1989 (http://www.w3.org/People/Berners-Lee), the Web has been rapidly adopted for delivering and sharing IPM related information (Stinner 1999). Typing "Integrated Pest Management" into the Google search engine, will turn over 860,000 Web sites containing these words. Therefore, the actual number of pest management related Web sites should exceed 1 million if one considers that: 1) Google indexes less than half of all Web documents (*http://msnbc.msn.com/id/4297156/*), and 2) many pest management related Web sites may not contain any of the three words above. Obviously, it is beyond our capacity to review the plethora of applications in this chapter. Therefore, we will present the fundamentals of the technologies, and explain two categories of information systems: the static Web application and the dynamic or database-driven Web applications.

3.1. The World Wide Web

Many people may not separate the Internet from the Web. The Internet is a client/server type of network that uses a series of protocols collectively called TCP/IP (Transmission Control Protocol/Internet Protocol) for transferring electronic data (Comer 2000). A client is any computer or software application requesting a document. The server is the computer or software application accepting the request and returning the requested document. The Web is a collection of computer protocols and standards by which a computer or software accesses data. Three computer standards that define the Web are: Uniform Resources Locator (URL), Hypertext Transfer Protocol (HTTP), and Hypertext Markup Languages (HTML). The URL's are used for locating Internet documents, HTTP is the primary protocol for transferring documents, HTML is the programming language for creating Web

documents. More detailed information about these standards and other related standards and specifications can be found at the World Wide Web Consortium (*http://www.w3.org*).

Although the protocols and standards discussed above are the cornerstones of the Web, it might not be nearly as popular as it is today without the development of applications called "browsers". The first Web browser, DOSLynx, was developed by the University of Kansas in 1992, it is still available through FTP (*ftp://ftp2.cc.ukans.edu/pub/WWW/DosLynx*). The first graphical browser, Mosaic, became available in 1993 (*http://livingInternet.com/w/wi_browse.htm*). Graphical browser programs provide the users with easy access to multimedia graphics, audio and video files from anywhere on the Internet. Other applications utilizing the interactivity of the Internet are rapidly being developed.

3.2. Database Technology

In non-technical terms, a database can be described as a structured collection of data. The flat file database, which was very common in early database history, is an early example (Elmasri & Navathe, 1994). The flat file database was mainly used for data storage and had few features for managing data. Documents in a flat file database are usually stored as text files and these files typically contain few or no inter-relationships. Flat file databases can be queried using basic text search methods. These files can be transformed and converted to other data formats (Jungfer *et al*., 1999). It is however, almost impossible to perform more complicated operations, such as grouping data from several databases or updating data automatically. Additionally, developing an application that works with flat files can be cumbersome because the structure of files has to be known precisely (Bellahsene & Ripoche, 2001). Due to these drawbacks, flat file databases are rarely used for any large-scale application.

A modern database can be described as a structured collection of data managed by a DataBase Management System (DBMS) (Elmasri & Navathe, 1994). Like the Web browser, a DBMS is a software application. Some well-known DBMSs include Oracle, Sybase, DB2, and SQL Server. In these modern databases, the data are linked by relationships or associations, depending on database type. The relational nature of this type of data structure is the key feature that makes a database powerful and efficient. Databases allow users to store, retrieve, or modify data easily and efficiently regardless of the amount of information being manipulated. Another outstanding feature is that databases usually use a query language to interact with the data in it. A query language is like simplified English language with a very limited vocabulary. Query languages can query many tables in a database or even across databases. Consequently, a large amount of data can be examined simultaneously. Data from different databases can be combined, summarized, and searched together.

One of the major benefits in using databases for data storage is data sharing. Sharing data is extremely important in the scientific community. It helps in the education of new researchers, enabling the exploration of topics not envisioned

initially by investigators, permits the creation of new datasets by combining data from multiple sources, analysis of data from disparate sources, and many other benefits (*http://grants.nih.gov/grants/policy/data_sharing/data_sharing_faqs.htm*). For example, CIPM maintains the USDA's National Agricultural Statistics Service's Agricultural Chemical Use Database, (*http://www.pestmanagement.info/nass*). Using programmatic manipulation and by altering the database configuration, we can make the whole database accessible to other users worldwide from a single source. Users can then analyze the data or subset of data based on their need or interest. We will examine in more details data sharing in the following paragraphs.

3.3. Applications of the Web and Database in IPM

Like in many other fields, using the Web and databases for IPM related information delivery, data management and sharing, training, and education is still a new phenomenon. Ten years ago, few IPM practitioners would be able to find pest information on the Web or search an online information system to find an expert for a particular pest. Today, it is hard to find any IPM practitioner who has not used the Web or a database for his/her professional works.

In spite of the vast number of IT applications in pest management, we can divide almost all these applications into two categories: static Web application and dynamic or database-driven Web application. Nearly all early IPM-related Web applications were static. Static Web applications are basically online versions of the printed document, plus the unique feature that HTML can provide --hyperlinks. Even today, the majority of pest management related Web applications are static. Readers can refer to some static Web applications at the CIPM Web site, such as: The International Association for the Plant Protection Sciences (*http://www.PlantProtection.org*), Biological Control Virtual Information Center (*http://cipm.ncsu.edu/ent/biocontrol/biocontrol.html*), and Insecticide Resistance Management Training (*http://www.plantprotection.org/irmtraining*).

One drawback to static Web applications is that this type of information system is limited in the amount of data it can store and present. It may also lack the flexibility, richness, and features needed to support today's complex information needs. One simple example is to develop a Web site providing information about all IPM specialists around the world. If this were done in a static Web application, information about a specialist's name, country, specialty, and other information would be presented on the page formatted in a fixed way. One may find it difficult to use the site if he or she searches for a plant pathologist who is specialized in tobacco blue mold in Japan. The situation will only get worse as the volume of information or complexity of data on the Web increases.

This example demonstrates the need for another type of Web application called dynamic or database-driven Web application. It combines both Web and database technologies together to create a comprehensive information system. The Web is for presentation only, and the database is for maintaining and managing the data, information, or knowledge. Users deal with the presentation sides (the Web) only, the

presentation side handles the communication with the databases. This combination of Web and database makes a powerful and flexible information system.

By combining Web applications and database technologies together, a large number of comprehensive online pest management information systems, such as Compendium mentioned above, has been developed within the last ten years or so. In the U. S., there are statewide IPM information systems such as California IPM program (*http://www.ipm.ucdavis.edu*) and IPM information systems maintained by professional or other governmental organizations i.e. the U. S. Environmental Protection Agency's IPM for School (*http://www.epa.gov/pesticides/ipm*). There are also IPM information systems for the most important pests or crops (*http://pmo.umext.maine.edu/potatoes/potato.htm*). In addition, there are some major national IPM information systems such as Database of IPM Resources (*http://www.ippc.orst.edu/cicp*), developed by Oregon State University, and the National Integrated Pest Management Network (*http://www.ipmcenters.org*) developed by the Regional IPM Centers. Both systems contain a large amount of IPM related data and knowledge.

4. WEB SERVICES AND THEIR APPLICATIONS IN PEST MANAGEMENT

4.1. The Role of Web Services in Data Sharing

The primary model of how users interact and request information from the Web can be described as being *human-centric* (Cerami, 2002; Dertouzos, 2002). In this model, a human (using a Web client) initiates a request for information from the Web (server). The user must know how to access the information they need (query) and subsequently process the data returned into some meaningful set of results. This process can be quite time consuming and tedious if a large amount of information needs to be processed by the individual requesting the data.

The overall inefficiency of this method of data discovery and information processing has led to the formation of a new set of technologies (Web services) that enables machine-to-machine communication and data discovery as easily as that between Web browsers (clients) and servers (Cerami, 2002).

Web services are defined as a set of programmatic interfaces, e.g., Simple Object Access Protocol (SOAP), Web Service Description Language (WSDL) and Uniform Data Discovery Interface (UDDI), that enables platform independent application-to-application communication via Internet (*http://www.w3.org/2002/ws*). Gartner, Inc. defines Web services as (Smith, 2002):

> "software components that employ one or more of three technologies - SOAP, WSDL and UDDI - to perform distributed computing. Use of any of the basic technologies constitutes Web services. Use of all of them is not required."

A key feature of this system is the use of a standardized, structured data format that permits the exchange of a variety of data and data types over the Web. Extensible Markup Language (XML) is a simple, text format used for expressing data elements in Web service documents (*http://www.w3.org/XML*). The use of Web

services and XML thus enables interoperability among heterogeneous computer platforms, operating systems and programming languages.

The flexibility, power and extensibility of Web services and XML have lead to their rapid adoption for business transactions. For example, both Amazon.com (*http://www.amazon.com/gp/aws/landing.html*) and providers like Google.com (*http://www.google.com/apis*) offer Web services that permit users to directly access information from their own computer systems. A growing number of vendors now use Web services to process credit card transactions over the Internet. The Department of Homeland Security also implemented Web services and XML messaging, i. e. the URL *http://www.dhs.gov/dhspublic/getAdvisoryCondition* which returns an XML document containing the current Homeland Security Threat Level.

4.2. Web Services and their Role in IPM

A search of the Web will reveal numerous static Web sites that have attempted to collect and consolidate data on pests and their management (e.g., *http://www.DiscoverLife.org*). Collecting and managing these data by manually querying the Web and parsing the information into a meaningful result set is a time consuming and labor intensive task. Tools that attempt to automate these tasks have been developed (e.g. web scrapers), but in general, this is typically a manual process. Furthermore, when a Web site adds or modifies their data, the updated data will not be reflected on the consolidator's site *unless* the data from that site is refreshed periodically. Web services can overcome this lag in data currency by querying Web sites in real-time and dynamically retrieving information on demand.

Because Web services are an emerging technology, they have been slow to be adopted within much of the scientific community. There are however, a few instances where information relating to IPM, invasive species, and species biodiversity has been made available using Web services. The following examples illustrate the application of Web service technology towards achieving data interoperability and data sharing.

4.2.1. Consumer/Provider Interoperability via Web Services

At it's simplest, Web services can be viewed as a custom integration between two or more computer systems for the specific purpose of sharing distributed information using XML messaging. In this system, the provider supplies the consumer with a mapping to their data using WSDL (Web Services Definition Language) or SOAP (Simple Object Application Protocol). The consumer uses this definition to query the provider system and return the requested data in standard XML format. The XML message is then parsed to extract the data.

One example of a consumer/provider Web service is the integration of the Plants Database (USDA, NRCS. 2004) and the USDA ARS Systematic Botany & Mycology Laboratory (*http://nt.ars-grin.gov/SBMLWeb/homehtml.cfm*). The Plants Database exposes their plant taxonomic data via Web services to the SBML. Plants Database Web services are under development for InvasiveSpecies.gov, National

Soils Information System (NASIS), Grazing Spatial & Analysis Tool (GSAT), and the Ecological Site Information System (ESIS) (S. Peterson, *pers. comm.* 2004.).

The NSF Center for Integrated Pest Management (CIPM) has also developed Web services for the USDA Regional Pest IPM Centers. A Crop Profiles Web service provides a searchable interface into crop data sheets containing information on production practice for numerous commodities and states and the ability to search Pest Management Strategic plans for critical commodities and regions (*http://cfmx1.ent.ncsu.edu/cropprofiles/wservices/insertcropprofileswddx.cfm*). The four regional IPM Centers (and the national site) use this Web service to query the centralized database for crop profiles and strategic plans. Each regional IPM Center is then able to display and present the information on their Web site in a format suitable to their needs. As an example, compare the Northeastern IPM Center (*http://www.nepmc.org/rese_profiles.cfm*) with the North Central IPM Center (*http://www.ncpmc.org/CropProfiles/index.html*). Both of these sites draw their information via Web Services from the national database, but present the information in different orders and styles, and for states in their respective regions only. In this approach, each data entity is tagged with a resource identifier and authority in order to acknowledge authorship of the cited information.

4.2.2. Web Services Registries and their Impact on IPM

The promise of truly cross platform, distributed computing in IPM will only become a reality with the creation of centralized directories of IPM Web services. The Universal Description, Discovery and Integration (UDDI) specification, based on internets standard (XML, HTTP, and DNS) proposed by the W3C (*http://www.w3.org*) and IETF (*http://www.ietf.org*), was developed in order to standardize web registry creation (http://www.uddi.org/specification.html). A registry of IPM services would contain a listing of IPM nodes and their Web services. For example, queries sent to a pesticide usage registry would search the listing of available IPM nodes and use their Web service definitions (WSDL) to retrieve data requested from each remote site.

Regrettably, this technology has yet to be widely adopted within the IPM community. However, a few groups have initiated efforts to create registries for the sharing of invasive species information. A brief review of a selected number of these sites will serve to illustrate how these technologies can be developed for the express purpose of sharing IPM data.

The Global Biodiversity Information Facility (GBIF; *http://www.gbif.org*) is a registry and clearing house for distributed data related to species biodiversity. GBIF maintains a UDDI registry for the express purpose of registering and advertising the data and services of participating GBIF member nodes. In order to become a GBIF data provider, you must use one of two data provider packages that they support: 1) DiGIR (Distributed Generic Information Retrieval; *http://digir.sourceforge.net*) or 2) BioCASE (Biological Collection Access Service for Europe; *http://www.biocase.org/provider*). Presently, users can search for species information using the GBIF search portal. Alternately, users can query the GBIF

UDDI registry, download the Web service interface (WSDL) description for the site and create a Web service to a specific provider site to retrieve biodiversity data. In the future, GBIF plans to provide a global metadata registry of the available biodiversity data sites having open interface architectures. Users will then be able to build a Web service to query the GBIF global registry and return biodiversity data directly to the requestor site.

On April 5-8, 2004, an experts meeting on implementing a Global Invasive Species Information Network (GISIN) was held in Baltimore, Maryland, USA (*http://invasivespecies.nbii.gov/as/gisin.htm*). One of the primary goals of this meeting was to discuss the feasibility of developing a portal and/or a global registry of invasive alien species data providers. A draft plan for implementing the GISIN model was formulated and will be further refined and developed over the next two years. The success of GISIN will depend on a number of factors: i.e., funding, cooperation among data providers, agreements on metadata standards, etc. that have yet to be developed.

Unless Web sites serving up IPM data increase their capacity for data sharing and adopt Web services and XML messaging as standards, the creation of IPM data portals, UDDI registries, and a distributed Web for IPM information will never fully reach its potential as a truly interoperable, distributed computing environment.

5. THE IT ROLE AND IMPACT ON DEFENCE

Within the United States, the threat of bioterrorism as it pertains to the health of the vast agricultural resources and food systems has prompted numerous efforts to improve and strengthen the country's ability in the areas of biosurveillance and biosecurity. Homeland Security Presidential Directive #9 (HSPD-9) explicitly establishes a national security policy to defend the health of the United States agriculture and food systems (*http://www.fas.org/irp/offdocs/nspd/hspd-9.html*).

Several categories of IT technology are associated with fulfilling this mission. The General Accounting Office report GAO-03-139 identifies these IT categories as: detection, surveillance, diagnosis and clinical management, communication and supporting technologies. Within the framework of pest management, systems have been designed to facilitate the communication and sharing of detection and surveillance information within various branches of government at the federal, state and local levels. Here within, we describe one system, the Offshore Pest Information System (OPIS).

HSPD-9, directive 8 (Awareness and Warning) directs the departments of Interior, Agriculture, Health and Human Services, the EPA, and any other appropriate Federal departments and agencies to: *a)* develop robust, comprehensive, and fully coordinated surveillance and monitoring systems, including international information, for animal disease, plant disease, wildlife disease, food, public health, and water quality that provides early detection and awareness of disease, pest or poisonous agents and *b)* develop systems that, as appropriate, track specific animals and plants, as well as specific commodities and food. The Plant Protection and Quarantine (PPQ) section of the United States Department of Agriculture, Animal,

Plant Health Inspection Service (USDA APHIS) has developed the Offshore Pest Information System in part to fulfill its mission of safeguarding U. S. agriculture from bioterrorism threats.

OPIS is a secure Web-based system that reports information about "targeted" exotic (i.e., not present or in limited distribution within the U. S.) plant and animal pests and diseases. The OPIS system database stores information on pest occurrences, outbreaks, as well as other emerging pest developments gleaned from a number of domestic and international sources. These data are then analyzed and the results used to focus international monitoring efforts and activities.

IT will play an even more important role in safeguarding the U. S. against bioterrorism as additional tools come online. The ability to integrate and share data and information among distributed stakeholder systems will be instrumental in coordinating those efforts which will lead to the development of a national biosecurity network.

6. USING IT AS IPM DECISION SUPPORT SYSTEM

6.1. What is a Decision Support System?

A Decision Support System (DSS) integrates and organizes all types of information required for production decisions (Petersen *et al*., 1993). DSS tools vary in complexity. Examples include rules, schedules of management, equations, combinations of decision aids (Seem & Russo, 1984), and expert systems (Travis & Latin, 1991; Travis *et al*., 1992). The type of DSS is determined by the cooperative efforts of a multi-disciplinary team of knowledge specialists, the technical and financial resources available, the degree of industry organization and support, and the expectations of end users (Travis & Latin 1991). The selection of an appropriate DSS for a given cropping situation often depends upon the pathogen/pest complex as it interacts with crop and grower preference factors.

A DSS is often considered to be simply a piece of computer software or a tool but it is important to remember that it is also a methodology. Figure 1 is a conceptual diagram of a DSS for disease management. The four components of a DSS are the crop environment, data, analysis and decision. Each component can be thought of as a method with a set of associated tools. For example, the data component is associated with the collection method, which has several tools including automated weather stations and site-specific weather products. The data need be analyzed with respect to a pathogen's life cycle to determine likely disease incidence and severity if no action is taken. After analysis, interpretation is the formulation of appropriate management options for a disease or the spectrum of diseases and pests. Finally, the decision step is choosing between the management options. In the following discussion, we will briefly describe each step of the DSS process and then describe methods for the delivery of DSS.

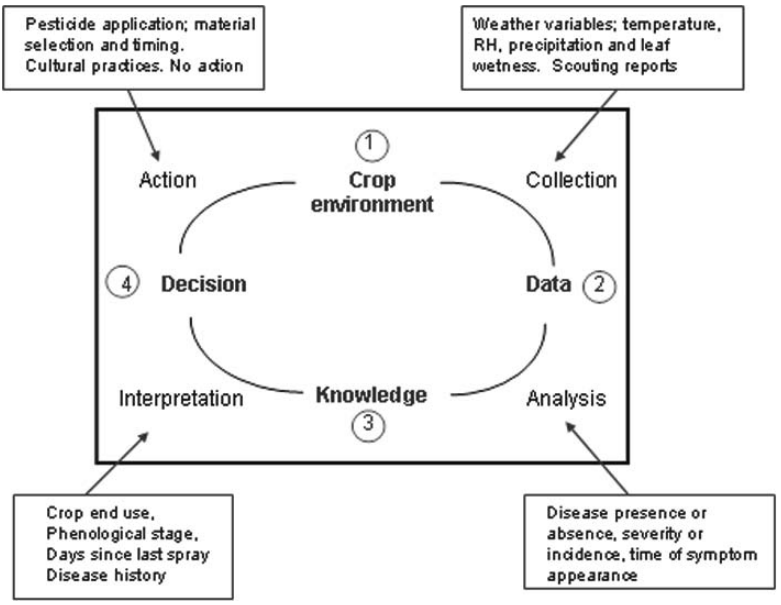

Figure 1. Overview of a DSS (adapted from Magarey et al., 2002).

6.1.1. Data Collection

The DSS process begins with the collection of data in the crop environment. Field observations collected by a scout represent one of the most effective methods of data collection. The use of personnel data assistants equipped with global positioning provides an efficient mechanism for survey data collection. However, for plant diseases, some significant epidemiological events can not be observed without a trained technician and a microscope (Seem & Russo, 1984). Weather data can be used as a surrogate to observation for the prediction of many pathogenic life stages or processes as will be discussed below. Commonly collected variables include air temperature, leaf wetness, relative humidity and precipitation. Since the mid-1980s, automated weather stations (AWS) have become more frequently used as an alternative to manual weather data collection. Although a great improvement upon the manual process, many AWSs are expensive, some require considerable maintenance, and there are problems associated with the calibration and standardization of sensors. Additionally for some users, AWSs may be too complex to operate and download (Magarey *et al*., 2002).

Sensors require constant maintenance, including repair, cleaning and calibration. Since the mid-1990s simulated site-specific weather data including simulated observations and forecasts have been a real alternative to automated weather stations (Magarey *et al*., 2001; Russo, 2000). Simulated observations are

generated by spatial interpolation procedures using data from weather observations made by local, state or national weather station networks or services. High-resolution atmospheric models are used to create site-specific forecasts. Growers, as monthly subscribers, can receive the site-specific data on a daily basis by either facsimile (fax) or electronic mail (e-mail) (Russo, 2000; Travis *et al*., 1999) or more recently over the Web. Usually model output is also included in the site-specific forecasts. No on-site weather measurement was required, thus freeing the user from the constraints of collecting data.

6.1.2. Analysis

After data have been collected it needs to be analyzed. It is important to understand the epidemiological basis by which a decision support system leads to the correct biological interpretation. A simplified representation of a pathogenic life cycle begins with survival spores in the soil, in leaf litter, in a vector or on a plant part (Fig. 2). For some pathogens, preconditioning may be related to degree accumulation for fruiting structures to mature. For example, apple scab (*Venturia inaequalis)* has a predictable pattern of ascospore maturity related to day degree accumulation (Gadoury & McHardy, 1986). Once the spores discharge they can be dispersed to a growing plant surface where infection takes place. Infection is the process by which the pathogen enters the plant.

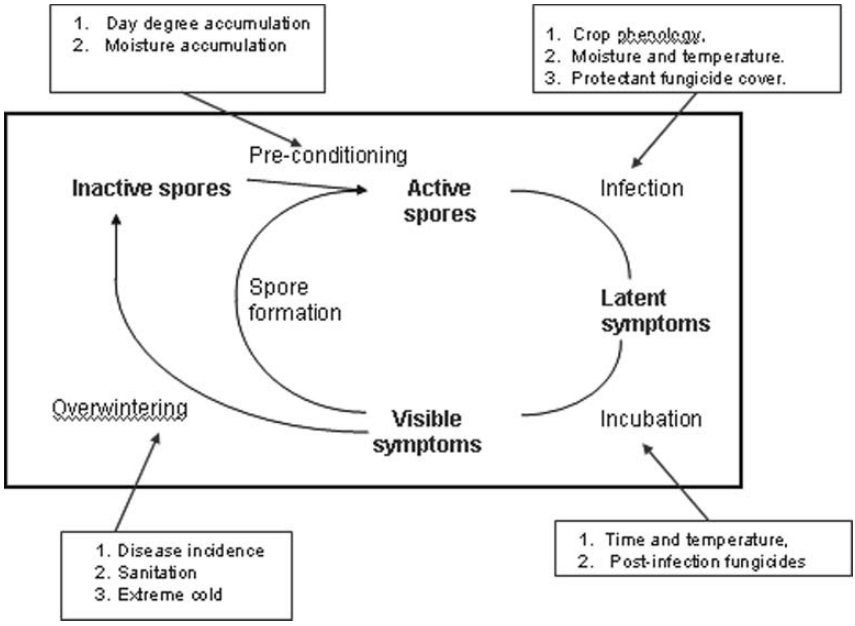

Figure 2. A simplified life-cycle diagram.

Phenology is one of the most important criteria for infection as many diseases have a relatively narrow window during which the fruit or economic plant part is susceptible. For example, grape clusters are only susceptible to powdery mildew during the period from capfall to two weeks after fruit set (Ficke *et al.*, 2003). Remembering the classic disease triangle, when disease inoculum is available and the phenological stage of the host is susceptible the next consideration is a favorable environment.

For most pathogens, infection is limited first by moisture and second by temperature. Often rain is required for dispersal to susceptible host surfaces and a film of wetness is required for infection itself. The duration of moisture required for infection is dependent upon temperature and can vary from a few hours to several days (Huber & Gillespie, 1992).

Finally, after all these biological considerations, a protective fungicide cover is a management factor that limits infection. After infection, there is usually an incubation period before visible symptom expression. The incubation period is temperature dependent and the minimum time at optimum temperature can be as little as 3 days for soft rotting pathogens, and greater than 14 days for some anthracnose pathogens. Knowledge of the duration of the incubation period is critical if field scouts are to look for symptoms at the correct time and if the incidence of disease in the field is to be correctly estimated. After new lesions have appeared on the plant surface there is opportunity for increased spread of disease. For most diseases, spore formation (aka sporulation) requires free moisture and high humidity. This is not true for powdery mildews but especially true for downy mildews, while other pathogens fall in between (Huber & Gillespie, 1992). While some fungicides may reduce the sporulation potential or prevent the appearance of latent symptoms, the most effective time for their use is as a protectant prior to infection.

Another important component of the life-cycle is the overwintering (or oversummering) survival spores. Usually these spores are in a dormant or inactive state but this is not always the case. The density of survival spores is mostly determined by the severity of disease in the previous season. For this reason, maintaining a clean field, orchard or vineyard is one of the most effective methods for disease control. Effective sanitation such as the natural or artificial removal of leaf litter also reduces the density of survival spores. Another natural factor is extreme hot or cold weather, events that may kill spores but overwintering (or oversummering) spores are resistant to extreme temperatures.

6.1.3. Interpretation

After analysis of the biological data, interpretation will lead to the best management options. Interpretation depends upon host factors including *a)* phenological stage and canopy size, *b)* remaining time to harvest or ontogenic resistance, and *c)* cultivar susceptibility; pathogenic factors *d)* disease history, *e)* scouting observations, *f)* destructiveness of disease; environmental factors *g)* predicted infection periods and *h)* soil type and accessibility after rain; management factors *i)* growers perception and willingness to take risk or manipulate spray timing, *j)* effectiveness of cultural

practices, *k)* ease of arresting disease development, *l)* pesticide label restrictions, *m)* safety considerations (e.g. re-entry time), *n)* fungicide price and *o)* crop value and end use (Bailey, 1999; Travis & Latin, 1991). The automated interpretation of some host, environmental, and disease factors by a DSS may save a user considerable time. However many management factors with the exception of fungicide timing may be too complex to incorporate into a DSS with existing technologies and they may be more easily interpreted manually.

Some management factors can be incorporated into a DSS by establishing a crop profile (Travis *et al*., 1992). These factors are setup at the start of the year and do not change during the season. Interpretation is best made on a daily basis although models themselves may run from hourly data. It is important for the DSS to display the phenological stage along with which diseases are active on a daily basis. Each active disease could be listed with incidence as observed by a field scout and predicted incidence as measured by the cumulative number of infection periods.

The number of 'missed' infection periods may also be a useful indicator of likely disease incidence. Missed infection periods are those that occurred when effective fungicide cover was absent. Although it is not included in many DSS at present, a history button showing comparisons of these variables with data from previous seasons based on scouting history or climatological averages would be useful. Effective fungicide cover is determined from a degradation curve, usually based upon elapsed time since last spray, but may also include other factors such as rainfall (Bruhn & Fry, 1981) or rate of foliage or tissue expansion. For text based summary of this information a last effective spray date is a useful and easily understood concept (Bailey, 1999). After determining the spectrum of diseases requiring control, fungicide selection involves a comparison of spectrum of activity, label restrictions on use, resistance management and price. This can be simplified by having a consultant create pre-determined fungicide mixes and programs prior to the start of the season.

6.1.4. Delivery

To facilitate decision making, a user needs to be able to see the analysis in visual form. Complexity should be avoided since many users have limited time allotment for the use of a DSS (Magarey *et al.*, 2002). However, the flip side is that some DSSs are so simple that they do not answer questions that real users want to know. The time it takes to use a DSS is also important. Many users want to be able to interrogate a DSS in a very short time. Information in a DSS is often summarized in text messages, tables, graphs, maps or calendars. For many growers, a simple text message including the last effective spray date may be appropriate. However, text messages are often limited in the amount of information that can be quickly absorbed by a user.

Tables may often present too much information and confuse users. Often it is best to use simple symbols rather than complex arrays of numbers. Graphs are a nice method to present information but may have limited application for certain types of information. Maps need to be interpreted with care since there may be greater spatial

uncertainty in disease predictions than those associated with point forecasts. Nevertheless maps often provide a good method to present disease forecasts at a regional scale or to provide high resolution site-specific information. Where maps are used they can be linked to a calendar providing a quick, easy and familiar method for a user to move through space and time. With the increasing importance of precision and site-specific agriculture it is possible that the map and calendar approach will become one of the most valuable tools for presenting disease forecasts.

The Web represents one of the most popular methods of delivering information but there are also many other methods (Fig. 3). The choice of delivery system depends upon the choice of local or regional scale; information content site-specific or generic; and accessibility push, pull or plug (Russo, 2000). Push and pull systems provide DSS information from a remote source. Push systems deliver information to the user, while pull systems require the user to request the information. Push systems work best for supplying daily information, updates and other types of dynamic information. Pull systems work best for allowing users to access specific databases or resources. Plug systems provide DSS information built into an on-site device, for example a disease predictor (Magarey & Western, 1999). They allow users to maintain hands-on contact with the data source. Push is analogous to turning on a faucet (tap), while pull is drawing water from the reservoir with a pump and plug is like installing bottled water.

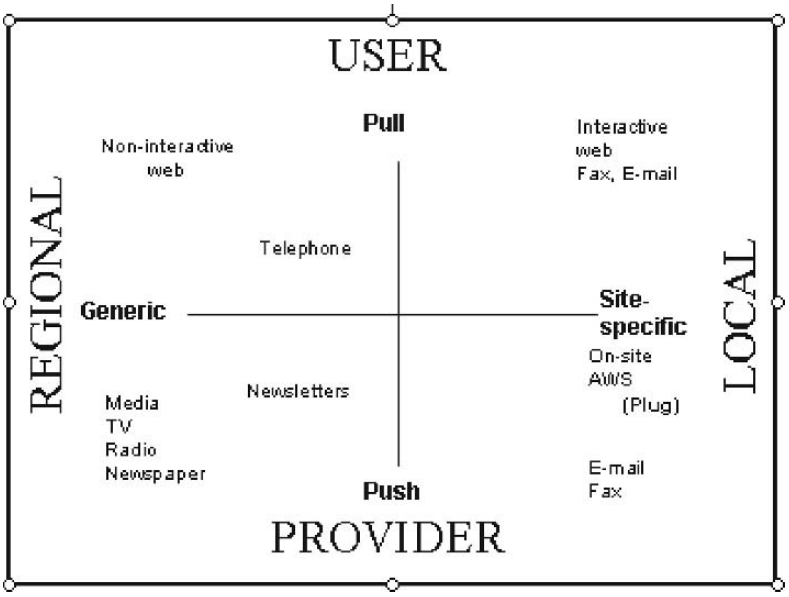

Figure 3. Delivery of decision support systems (modified from Magarey et al. 2002).

Some of the distribution systems can work in more than one way. For example, faxes can be used in both push and pull modes. One distribution problem in the pull mode is that users can easily become discouraged as they search for desired information among poor quality sources. A successful distribution system should be easy to use, reliable, cheap and updated frequently. It should be able to carry both text and graphical information. For example, faxes have an advantage over many electronic techniques, in that paper is quick and easy to use (Felland *et al*., 1997; Gershenfield, 1999).

6.2. Limitations and Future Development

Historically, the application and use of decision support systems has been limited by several factors including a) the lack of sufficient computer power for data analysis and interpretation, b) the cost of collecting site-specific weather data and disease observations, c) cost and speed of the communications, and d) the ease of DSS use. Technological advances have greatly reduced many of these problems but the cost of collecting disease observations may continue to be a significant limitation for some DSS applications. Remote sensing in the future will become a more useful method but its application at present has been limited by the spatial and temporal resolution of the images. For many diseases, the action threshold is so low that the use of image technology is especially problematic. There is great need for the development of a new range of sensors to enable the precision treatment of crops. These sensors could be mounted on a tractor or on a robotic droid. One of the most promising approaches is the use of sensors that detect volatiles released by pathogens or by a plant-pathogenic interaction (R.C. Seem, *pers. comm.* 2003). While such a technique may not improve upon the sensitivity of a trained scout, it may ultimately be a method to automate scouting.

As a final word, the cost of developing and maintaining DSS remains an important problem (Rajotte *et al*., 1992). Services provided by universities and state departments of agriculture have seen substantial budget cuts over the last few years. We see that increasingly DSS's will increasingly be built with the following ingredients of organizations and personnel (J. Russo, *pers. comm*., 2003). Funding for system development will be provided by small or large agricultural supply companies or cooperatives. In exchange, the DSS provides a mechanism for the company to market their products or coordinate their management protocols. The DSS will be built by information technology companies. The advantage of using a company is that they provide a sustainable mechanism for support and software development. In contrast, universities too often rely upon student programmers who come and go. In addition, it allows the costs of building a DSS to be shared between different agricultural industries or cooperatives. It also frees university specialists of the burden of maintaining the DSS and the associated databases. Instead, the university specialists can spend their time on model validation, documentation and other issues not related to software or information technology.

Consultants and extension agents will act as retailers or distributors of information to growers and provide the system with scouting observations. The

scouting observations are in turn used by the specialist to formulate alerts or evaluate model output. This framework provides an equitable method to vertically share information. As a final layer in this structure, it also creates a mechanism to share information with government phyto-regulatory agencies. The agency will provide the DSS distributors with information about exotic pests including fact sheets and scouting protocols. In turn, the DSS distributors can provide scouting observations in the event of an exotic pest outbreak

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BIOLOGY AND APPLICATIONS OF *BACILLUS THURINGIENSIS* IN INTEGRATED PEST MANAGEMENT

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Abstract. The application of *Bacillus thuringiensis* is revised, with description of its biology, ecology and potentials for insects management. Co-evolution of the crystal proteins structure with insect hosts is reviewed, with data on the classification and nomenclature. The function of various Cry proteins and their role in insect parasitism are described together with the mechanism of action of the toxins. Applications for control of mosquitoes and blackflies and formulations are reviewed. Factors related to the development of resistance and potentials in the integrated pest management are discussed.

1. INTRODUCTION

Bacillus thuringiensis (Bt) is a Gram-positive, spore forming, entomopathogenic bacterium characterized by the production of proteinaceous crystalline inclusions during the stationary phase of its growth. Bt has been found to be an inhabitant of various ecological niches including soil, insect cadavers, dust of stored products and plant surfaces (Delucca *et al*., 1979; Martin & Travers, 1989; Smith & Couche, 1991; Carozzi *et al*., 1991; Hostowo *et al*., 1992; Meadows *et al*., 1992; Kaelin *et al*., 1994; Chaufaux *et al*., 1997). The entomopathogenicity of Bt has been largely or completely attributed to the parasporal crystal inclusions, also known as insecticidal crystal proteins (ICPs), Cry proteins or as δ-endotoxins.

Several strains of Bt have been identified worldwide from numerous screening programmes. Bt isolates have narrow specificities against various insects, but together they span a wide range of orders including Lepidoptera (beetles and moths), Diptera (mosquitoes and blackflies), Coleoptera (beetles and weevils), Hymenoptera (wasps and bees), with some isolates also active against nematodes, mites and protozoa (De Barjac & Sutherland, 1990; Becker &

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Margalith, 1993; Estruch *et al*., 1996; Rang *et al*., 2000; Donovan *et al*., 2001; Moellenbeck *et al*., 2001; Sayyed *et al*., 2001; LeConte, 2002).

The wide majority of genes encoding for these proteins appears to reside on self-transmissible mega plasmids that allow their transfer between related cells within natural populations, providing the basis for a higher diversity (González *et al*., 1981; 1982; Kronstad *et al*., 1984; Lereclus *et al*., 1984). Several *cry* genes encoding for the insecticidal crystal proteins have also been expressed in economically important crop plants, to provide effective protection against insect predation (Nayak *et al*., 1997; Alam *et al*., 1998; Maqbool *et al*., 1998; Tu *et al*., 2000; Ye *et al*., 2001). Formulations of Bt have been used as biological insecticides to control numerous species of insect pests in agriculture and forestry, as well as against vectors of human and animal diseases. The Cry proteins that have been studied so far are toxic to various pests, including vectors of human diseases, but are also non-pathogenic to non-target insects, mammals, birds, amphibians and reptiles. Apart from delta-endotoxins, Bt strains also produce other enzymes and secondary metabolites such as phospholipases, heat labile beta-exotoxins, proteases, chitinases and vegetative insecticidal proteins (Vip) that contribute to their levels of virulence (Lövgren *et al*., 1990; Zhang *et al*., 1993; Estruch *et al*., 1996).

These applications are largely responsible for an increased practical and commercial interest in this bacterium and its inclusions. The present review emphasizes on the biological properties of Bt that make it a useful alternative and/or supplement to chemical pesticides and also describes challenges facing its applications.

2. ECOLOGY AND PREVALENCE

Bacillus thuringiensis is a member of the genus *Bacillus*, consisting of more than twenty different Gram-positive, spore-forming bacteria. Other important species of this group are *B. anthracis*, *B. cereus* and *B. subtilis*. Bt can be differentiated from the other closely related members of the family based on their biochemical, nutritional and serological analyses and by the presence of crystalline inclusions in Bt cells, visible by light microscopy.

Bt is common to terrestrial habitats including soil, living and dead insects, granaries, and plant surfaces (Carozzi *et al*., 1991; Smith & Couche, 1991; Meadows *et al*., 1992) and occurs predominantly as spores dispersed in the environment. Bt spores can be disseminated widely in space and their persistence under laboratory and field conditions has been well studied (West, 1984). Vegetative cells are sensitive to ultraviolet radiations and disappear rapidly from the environment upon exposure to sunlight. Bt spores are resistant to adverse conditions such as heat and drought, thereby enabling them survive periods of stress and allowing the bacterium to re-germinate under favorable conditions (Benoit *et al*., 1990; Chiang *et al*., 1986).

Conjugation between different Bt strains can be observed in the soil environment as well as within insects and is believed to be responsible for heterogenous distribution of *cry* genes among various isolates (Thomas *et al.,* 2000).

Although the true ecological role of Bt is still a matter of debate, it can be best described as an opportunistic pathogen (Schnepf *et al*., 1998).

3. EVOLUTION

Co-evolution of Cry proteins and insects has been hypothesized and interaction of Cry toxins with their receptors may play an important role in the selection of new varieties. Phylogenetic studies suggest that Cry proteins have evolved from a common origin.

Differences in the physiological conditions of the insect gut such as pH, proteolytic activity and receptor recognition may have been important selective forces for the diversification of proteins. Solubilization of long protoxins depends upon the highly alkaline midgut environment in the lepidopteran and dipteran pests, in contrast to the rather acidic coleopteran midgut (Dow, 1986). The main digestive proteases of Lepidoptera and Diptera are serine proteases, whereas those of Coleoptera are cysteine and aspartic proteases (Terra & Ferreira, 1994). Sequence divergences and homologous recombination, leading to interchange of domains among different toxins, could represent another cause of the high diversity observed among these proteins. DNA recombination, transposition and self-transmissibility of plasmids could also have been relevant to the evolution of Bt toxins (Gonzalez *et al.,* 1982; Jarret & Stephenson, 1990; Lecadet *et al.*, 1992).

The Bt delta-endotoxins are closely clustered into groups of related sequences, and share significant homologies at the nucleotide level and may permit re-assortments of the corresponding gene sequences through homologous recombinations (Bravo, 1997; De Maagd *et al*., 2001). Most of the Bt toxin genes are located close to sequences that appear related to transposition (Mahillon *et al*., 1994), providing an obvious mean for mobilizing the toxin sequences within the resident plasmids and/or between plasmids and host chromosomes. Such transposition may allow new toxin gene assortments within individual Bt strains. Plasmid transfer between different Bt strains, already shown either *in-vitro* and *in-vivo* could have also contributed to the evolution of Bt strains (Thomas *et al*., 2000; 2001).

4. CLASSIFICATION AND NOMENCLATURE

Several thousands of Bt isolates obtained from numerous screenings worldwide made it necessary to have simple and reliable tools for classifying strains according to significant criteria. Vegetative cells of Bt have two major antigens on their surfaces: *i*) the flagellar (H) antigen and *ii*) the heat stable somatic (O) antigen (HSSA) (Zhang *et al*., 2002). Both phenotypic methods used (H-serotyping and biochemical characterisation) contributed to the establishment of a classification system for Bt isolates.

The differentiation of these strains into various serovars, developed on the basis of flagellar antigens by De Barjac & Bonnefoi (1968), was in use ever-since and more than 80 serotypes have been identified so far. New H-serotypes are numbered

and registered at the International Entomopathogenic Bacillus Centre (IEBC) Collection at Institute Pasteur, Paris, France. Since the cloning of first insecticidal crystal protein gene from Bt (Schnepf & Whiteley, 1981), several such genes have been isolated (Ellis *et al*., 2002).

The first systematic attempt to organize the nomenclature was based on the insecticidal activities of crystal proteins (Hofte & Whiteley, 1989) and provided a useful framework for classifying the ever-expanding set of known genes. Inconsistencies arose when new genes highly homologous to known genes did not exhibit a similar insecticidal activity spectrum.

Crickmore *et al*. (1998) suggested a revised nomenclature for *cry* genes based solely on amino acids identity of toxins. In the revised nomenclature system, toxins are classified on the basis of amino acid sequence homology and each protoxin acquires a name consisting of mnemonic Cry (or Cyt) and four hierarchical ranks consisting of numbers, capital letters, lower case letters and numbers (e.g., Cry2Aa1) depending upon its place in the phylogenetic tree. Thus proteins with less than 45% amino acid identity differ in primary rank (Cry1, Cry2, Cry3 etc.). Proteins with 45-78% sequence homology differ in their secondary rank (Cry1A, Cry1B, etc.) whereas proteins with 78-95% sequence homology differ in their tertiary rank (Cry1Aa, Cry1Ab, etc.). Finally, proteins with greater than 95% sequence homology are given a new quaternary rank (Cry1Aa1, Cry1Aa2, etc.).

A full list of all the *B. thuringiensis* toxins discovered so far is available at http://www.biols.susx.ac.uk/Home/Neil_Crickmore/Bt/Index.html.

5. STRUCTURE AND FUNCTION

Alignment of amino acid sequences of various Cry proteins has revealed the presence of up to five conserved blocks among a majority of Cry proteins as well as diversity in the length of different protoxins (Crickmore *et al*., 1998). These differences, which are in the form of C-terminal extensions, are believed to play a role in the crystal formation, as they are not part of the active toxin generated, due to proteolytic degradation inside the host insect gut (Schnepf *et al*., 1998).

The three-dimensional structures of activated forms of three different Cry proteins, Cry1A, Cry2A and Cry3A were predicted by X-ray crystallography (Li *et al*., 1991; Grochulski *et al*., 1995). These structures are remarkably similar, each consisting of three domains: I, II and III. The domain I consists of seven α-helices in which six amphipathic helices surround a central core helix. Domain I is involved in membrane insertion and pore-formation and has shown structural similarities with other pore-forming bacterial toxins such as hemolysin E and colicin A (Schnepf *et al*., 1998).

The roles of domain II and domain III in receptor recognition/binding and therefore insect specificity have been demonstrated by various studies (Aronson *et al*., 1995; Lee *et al.* 1995a; De Maagd *et al*., 1996; Schnepf *et al*., 1998). The domain II, also known as beta-prism, has a three-fold symmetry consisting of three β-sheets having a greek-key formation. It plays an important role in receptor interactions (Aronson *et al*., 1995; De Maagd *et al*., 1996; Schnepf *et al*., 1998;

Jenkins & Dean, 2000), with a remarkable similarity to the topology of some carbohydrate binding proteins. The functional relevance of domain II's similarity to the topology of carbohydrate binding proteins and the fact that putative Cry receptors are glycosylated proteins suggest that carbohydrate recognition may play an important role in this interaction (Griffitts *et al*., 2001; Jurat-Fuentes *et al*., 2002). The C-terminal domain III consists of two antiparallel β-sheets in a jelly-roll assemblage, and is involved in receptor binding and pore-formation.

6. PCR SCREENING

The identification of *cry/cyt* gene content in a Bt strain can be correlated, to some extent, to the spectrum of its insecticidal activities. Initially, the Bt collections were screened by testing their insecticidal activities against different insect species and looking for the presence of new genes in terms of host range or potency. However, this method of screening novel toxin encoding genes was a tedious process, thereby necessitating for simpler and less time consuming approaches (Hofte & Whiteley, 1989; Krieg *et al.*, 1983).

The polymerase chain reaction (PCR) is a rapid and more reliable method, compared to traditional bioassays-based screenings of Bt collections. The efficacy of PCR in identifying large *cry* genes families relies on the presence of conserved and/or variable nucleotide regions. The first PCR-based identification of *cry* genes (Carozzi *et al*., 1991) could identify the presence of *cry1A*, *cry3A* and *cry4A* gene types in their collection, which were predicted to be effective against lepidopterans, coleopterans and dipterans, respectively. The PCR-based prediction of insecticidal activity of these isolates was further confirmed by performing bioassays. The PCR-based screening of large Bt collections has been further expedited in the form of multiplex-PCR, extended-PCR, PCR-RFLP and exclusive-PCR techniques (Gleave *et al*., 1993; Chak *et al*., 1994; Ceron *et al.,* 1995; Ben-Dov *et al*., 1997; Guerchicoff *et al*., 1997; 2001; Bravo *et al*., 1998; Ferrandis *et al*., 1999; Ben-Dov *et al*., 2001; Ejiofor & Johnson, 2004).

Although various PCR techniques greatly simplified the screening of large Bt strains collections, the method is mostly limited to detection within previously known *cry* genes subfamilies. Also, another commonly associated problem to PCR based screenings is the extreme sensitivity of this technique, resulting in the occurrence of false positives, due to the presence of reaction contaminants.

7. MECHANISM OF ACTION

The primary action of Cry toxins is to lyse midgut epithelial cells in the target insects by forming lytic pores in the apical microvillar membranes (Schnepf *et al*., 1998). Unlike most chemical insecticides that function on contact, Bt insecticides in the form of a spray or a Bt crop must be ingested by the target organism to be effective. The mechanism of action of Cry toxins can be broadly divided into three steps: *i*) solublization and activation of protoxin; *ii*) receptor recognition and binding, and *iii*) formation of lytic pores and cell lysis.

Upon ingestion, the Cry protoxins are solubilized in the alkaline/acidic gut environment of the target insect, followed by its activation by specific proteases present in gut. The activated toxin then binds to specific receptors present on the midgut epithelium which further leads to the formation of lytic pores, cell lysis and consequently death of the host. Each step can modulate the activity of a Cry toxin against particular insect and hence the toxins overall specificity. Studies demonstrated the presence of aminopeptidase-N (APN) and cadherin-like receptor molecules on the epithelium of insect gut (Knight *et al*., 1994; Garczynski & Adang, 1995; Gill *et al*., 1995; Vadlamudi *et al*., 1995). The interaction of Cry toxins with these high affinity receptors is a key factor in their high specificity. Large differences in the gut physiology among insect orders can also be responsible for the variations in the proteolytic activity of target pests and, hence, in the different Cry toxins affinities (Bradley *et al*., 1995; Lambert *et al.*, 1992).

8. APPLICATIONS

Managing insect pests affecting economically important crops and/or vectors of human diseases is a major concern worldwide in food production and human health. The overwhelming use of chemical pesticides led to many harmful effects such as environment accumulation of potentially carcinogenic chemicals, contamination of ground water, development of resistant insect populations and indiscriminate destruction of beneficial species. Bt-based biopesticides proved to be suitable as commercial biological control agents for agronomically important pests, as well as for vectors of human diseases with various associated advantages, including the narrow host specificity, their environment-friendly nature and the low cost of production. The specificity of Bt have long been documented as well as its degree of diversity, testified by more than 80 different serotypes and hundreds of subspecies, isolated so far.

8.1. Control of Mosquitoes and Blackflies

Vector borne diseases are still rife and a cause of worldwide concern. The non-selective mode of action of synthetic insecticides and the development of resistance in insects shifted the focus of present day research to the role of entomopathogenic bacteria in vector control. The discovery of the mosquitocidal activity of *B. thuringiensis* subsp. *israeliensis* (Bti) dates back to 1976 (Goldberg & Margalit, 1977) and the first trials of Bt-based products were carried out in the early ‘80s for mosquitoes and blackflies control in temperate and tropical countries (Hougard *et al*., 1997; Gray *et al*., 1999; Becker, 2000). Since then, significant amount of laboratory and field efforts were devoted to the understanding of the biology, genetics and mode of action of Bti.

The ICPs of Bti are composed of four major polypeptides viz. Cry4A, Cry4B, Cry11A, and cytosolic CytA (Schnepf *et al*., 1998) that interact synergistically (Poncet *et al*., 1995). Among them, CytA is of particular interest as it is highly cytolytic *in-vitro* to several vertebrates and invertebrates. CytA also showed to act

synergistically with Cry proteins present in Bti, thereby delaying development of resistance in insects to Bti formulations. In general, the mode of action of these ICPs is similar to the lepidopteran specific ICPs. Once ingested by the aquatic larvae of mosquitoes and blackflies, the protoxins in the crystals are solubilized/activated under the combined action of the gut alkaline pH and the proteinases present therein (Gill *et al*., 1992). The activated toxin then binds to the apical microvillae of midgut cells (Davidson, 1988; Ravoahangimalala *et al*., 1993), causing formation of lytic pores, swelling of midgut epithelium cells, cell lysis and, finally, the insect death (Davidson & Titus, 1987; Charles, 1987; Knowles & Ellar, 1987; Poncet *et al*., 1995).

Apart from *B. thuringiensis* subsp. *israeliensis*, Bt subsps. *morrisoni* and Bt subsps. *jegathasan* are other isolates that exhibited strong mosquitocidal properties**.** Bt subsp. *morrisoni* discovered in the Philippines is similar to Bti in terms of toxin contents and toxicological properties except for the presence of a 144 kDa, lepidopteran-active Cry1 toxin. In the case of Bt subsp. *jegathesan,* a complex of seven Cry and Cyt proteins is produced, several of which are related to Bti but differ in their toxicological properties. In fact one of the Cry toxins, Cry11D, an 80 kDa protoxin present in Bt subsp. *jegathesan* was found to be 10 times more toxic than the related Cry11A present in Bti (Delécluse *et al*., 1995).

8.2. Formulations

Formulations in the form of sporulated Bt cultures have a long history of safe use for pest control and sprays of Bt subsps. *israeliensis* were successfully used to control disease carrying mosquitoes and blackflies. Bt-based formulations are now the most widely used bio-pesticides, representing about 2.0% of the total global insecticide market (Lambert & Peferoen, 1992).

Bt products for the forest industry have been based on Bt subsp. *kurstaki* HD-1 (Dulmage, 1970) that produces Cry1Aa, Cry1Ab, Cry1Ac and Cry2Aa toxins. In the forests of United States, Bt formulations have become the major pesticide against gypsy moth (Lepidoptera). The other target pests of *Bt* include spruce budworm (Canada), the Asian gypsy moth (US, Canada and Far East), the pine processionary moth (Spain and France) and the European Pine short moth (South America) (Van, 1990; Van *et al*., 1993). The annual worldwide distribution of Bt amounts to 2.3×10^{6} Kg. There are about 200 registered Bt products in the United States and Canada. Culture supernatant fluids of certain Bt cultures have also been shown to possess potent insecticidal properties against black cutworm, fall armyworm and beet armyworm (Estruch *et al*., 1996). This insecticidal property has been attributed to the presence of vegetative insecticidal proteins (Vip3A) that has no homology with known Cry proteins.

Despite the high specificity of Bt ICPs to target insects and safety to the non-target organisms, the use of Bt biopesticides has not increased in the desired way due to lack of stability, failure to penetrate all parts of the plants and tissues, narrow host range and human health considerations. Degradation by ultraviolet radiation remains the single biggest drawback to the use of Bt sprays. Insecticidal crystal

protein and/or spores exposed on plant surfaces can be easily washed off by rain and are highly sensitive to UV light, thereby necessitating frequent applications. Insects such as sap-sucking and piercing insects, root-dwelling pests or pests that rapidly burrow or bore into plant tissues immediately after hatching, remain unaffected due to the failure of Bt sprays to penetrate these parts of the plants. In addition, crops are also subject to predation by a large variety of pests that can not be controlled by a single product. Genetic improvement of Bt strains, construction of transconjugants, transfer of *cry* gene to other environmentally more persistent microbes and root-dwelling bacteria, fermentation and media optimization and multipronged integrated pest management (IPM) are some of the approaches that resulted in the development of novel pesticides with broader spectra, enhanced potency and improved persistence (Carlton & Gawron-Burke, 1993; Carlton, 1996; Bora *et al*., 1994).

8.3. Bt-Transgenics

Besides their long-term use as a biological insecticide in the form of sprays of spore-crystal mixtures, individual Cry toxins have been expressed in plants to render crops resistant to insects. Bt crops are engineered to express ICPs throughout all parts of the plants. Since ICPs are present in high concentrations in most or all parts of the plant, this not only eliminates difficulties in targeting pests that burrow inside plants but also help cutting down expenses associated with spray applications.

The early efforts to express full-length and truncated *cry* genes in economically important crop plants yielded plants showing some measure of protection but with gene expression levels too low to confer sufficient protection under field conditions. *Cry* genes are typical bacterial genes that have a high A/T content compared to plant genes. The high A/T rich regions may contain transcription termination sites (AATAAA), cryptic mRNA splicing sites and mRNA instability motifs (ATTTA) making *cry* gene codon usage inefficient in plants. Partial or complete removal of such interrupting sequences in combination with various plant promoters has led to 100 folds higher levels of expression of these genes (Vaeck *et al*., 1987; Perlak *et al*., 1990; 1991; Koziel *et al*., 1993a).

Over the last decade, the success in producing insect-resistant crops through transfer of modified *cry* genes has been impressive. Several modified or chimeric *cry* genes have been introduced into economically important crop plants, including tomato, potatoes, tobacco, cotton, maize, rice and soybean (Barton *et al*., 1987; Umbeck *et al*., 1987; Vaeck *et al*., 1987; Delannay *et al*., 1989; Perlak *et al*., 1990; Koziel *et al*., 1993a; Schuler *et al*., 1998). In most cases foreign Cry toxin has provided effective protection against various insects attack under laboratory and field conditions. *Cry1Aa*, *cry1Ab* or *cry1Ac* isolated from lepidopteran active Bt subsp. *kurstaki* were introduced into tobacco (Vaeck *et al*., 1987), tomato and potato by *Agrobacterium*-mediated transformation and their expression conferred some degree of protection against *Manduca sexta* on tobacco, (Vaeck *et al*., 1987), *Heliothis virescens* and *Helicoverpa zea* on tomato, and the potato pest *Phthorimaea operculella*. Transformed cotton lines

have been shown to provide good field protection against cotton bollworm and pink bollworm (*Pectinophora gossypiella*) (Wilson *et al*., 1992).

Another δ-endotoxin encoding gene *cry3A* (Perlak *et al*., 1993) isolated from coleopteran active Bt subsp. *tenebrionis* was transferred into potato plants conferring protection from Colorado potato beetle (*Leptinotarsa decemlineata*) under high levels of natural field infestations. The initial attempts were restricted to dicotyledonous plants. Expression of *cry* genes in monocotyledonous plants was advanced when Koziel *et al*. transformed elite cultivars of maize with a truncated *cry1Ab* gene (Koziel *et al.*, 1993b). These transgenic maize plants provided excellent protection against European corn borer (*Ostrinia nubilalis*) in field conditions under insect pressure 100 folds higher than natural infestations. In addition to cotton and vegetable crops, forestry associated trees like poplar have also been transformed with *cry* genes. Robison *et al*. (1994) showed that transgenic poplar (*Populus* spp.) trees expressing Cry1Aa toxin provided nearly complete protection against larvae of *Malacosoma disstria* and *Lymantria dispar*.

Concerns have been raised against the introduction of Bt transgenics regarding their effects on non-target organisms, gene flow to other organisms and above all development of resistance in insects to Bt toxins. Laboratory and field studies have failed to show any detrimental effect of Bt crops on non-target organisms or their predators (Dale *et al*., 2002). In fact studies in the US, China and Australia have documented larger populations of predatory bugs, spiders and ants as well as an enhanced biodiversity of beneficial insects in Bt crop fields as compared to fields treated with chemical insecticides. Development of novel technologies, that can restrict pollen fertilization and seed germination and might also reduce outcrossing between their wild types, are also underway (Schernthaner *et al*., 2003).

9. DEVELOPMENT OF RESISTANCE AND ITS MANAGEMENT

Insects are highly adaptable and have developed resistance to many chemicals due to their incorrect application and overuse. Over 500 species of insects have become resistant to one or multiple synthetic insecticides. In this context Bt toxins are no exceptions, and extensive application of Bt in the laboratory or field led to the isolation of resistant colonies among various insect populations.

Several species with different levels of resistance to Bt crystal proteins were obtained by laboratory selection experiments, using either laboratory-adapted species or insects collected from wild-populations (Tabashnik, 1994). The indian-meal moth (*Plodia interpunctella*) (McGaughey, 1985), the almond moth (*Cadra cantella*) (Beeman & McGaughey, 1988), the Colorado potato beetle (*Leptinotarsa decemlineata*) (Whalon *et al*., 1993), the cottonwood leaf beetle (*Chrysomela scripta*), the cabbage looper (*Trichoplusia ni*), (Estada & Ferré, 1994), the cotton leaf worm (*Spodoptera littoralis*) (Müller-Cohn *et al*., 1996), the beet armyworm (*S. exigua*) (Moar *et al*., 1995), the tobacco budworm (*H. virescens*) (Stone *et al*., 1989; Gould *et al*., 1992; Lee *et al.* 1995b) and the European corn borer (*O. nubilalis*) are some of the examples of laboratory selected strains.

The occurrence of resistance in field populations in response to extensive applications of Bt sprays was also reported. The first case of field-selected resistance to *Bt* was reported from Hawaii, where populations of diamondback moth (*Plutella xylostella*) showed different levels of susceptibility to a formulated Bt product up to a 30-fold dose resistance level (*Tabashnik et al.,* 1994). Laboratory selection rapidly increased resistance to greater than 1000-fold (Tabashnik *et al.,* 1993). Diamondback moth populations resistant to *Bt* subsps. *kurstaki* and *Bt* subsps. *aizawai* were reported from Indonesia and several states within the continental United States. Development of resistance to multiple toxins is another interesting reported effect (McGaughey & Whalon, 1992). The pattern of cross-resistance in some cases is clearly related to the Cry protein composition to which the insects were exposed.

Considering the sequence of several steps between protoxin ingestion and toxin membrane insertion, it is perhaps not surprising that there would be several alternative ways of achieving resistance. Mechanisms affecting the binding of toxins would be selective, whereas those affecting the steps utilized by all toxins (i.e., proteolysis of protoxins and membrane insertion) may result in cross-resistance. Interaction of toxins with high affinity binding sites (receptors) and crucial role of this receptor binding for toxicity were demonstrated in many species (Hofmann *et al*., 1988; Schnepf *et al*., 1998).

It was emphasized that the insects that developed resistance to a Cry toxin often had reduced or no binding to their receptor. Receptor binding studies with ^{125}I-labelled toxin and purified BBMVs prepared from susceptible and resistant insects, *P. interpunctella* and *P. xylostella*, indicated that toxin-binding sites are altered or modified in resistant strains (Oei *et al*., 1992).

Increased/altered protease activity may also render toxin inactive. Oppert *et al*. (1994) reported that midgut proteolytic activity of Bt resistant *P. interpunctella* larvae is significantly reduced compared to midgut juices from susceptible insects, thereby suggesting that altered or inadequate processing of Cry toxins is a possible factor in the resistance mechanism. However, in the case of another Cry-toxin resistant *H. virescens* strain, neither receptor binding affinity nor binding site concentration are affected further, suggesting that the mechanism of resistance is complex and that post-binding events, such as integration into membrane or ion-channel activity, may also be altered in some cases (Gould *et al*., 1992).

9.1. Resistance Management

Insects demonstrated a high capacity to develop resistance to a wide variety of chemical insecticides. Field and laboratory populations of insects showed to be equally apt at developing resistance to microbial sprays and transgenic plants based on Bt δ-endotoxins. The success of Bt crops and Bt based biopesticides, to a large extent, depends on the management of pests resistance to Cry toxins.

Various strategies to tackle the problem of resistance development were reported. Strategies for managing resistance to Bt in sprays include non-treated refuges, high dosage, mixture of insecticidal toxins and rotation or alteration of Bt toxins.

Most frequently, the practiced resistance management for commercialized Bt crops relies on the application of high doses combined with structured refuge strategy. In this technique, Bt transgenic crops are engineered to express high concentrations of ICPs in the tissues on which insect feeds so as to achieve near total lethality to the insects. At the same time 50% of the acreage is planted with non-engineered crop as a refuge for target insects that seem to sustain susceptible alleles within the insect population. Through random mating, rare recessive resistance genotypes are diluted within the populations of susceptible hosts.

10. INTEGRATED PEST MANAGEMENT (IPM)

Integrated pest management (IPM) strategies also showed to be helpful in countering development of resistance in insects towards various insecticides. Bt is also well suited to IPM strategies, due to its compatibility with insect parasitoids or fungal pathogens, polyculture and conventional chemical pesticides. Dextruxins from the entomopathogenic fungus *Metarhizum anisopliae,* serine protease inhibitor and bacterial endochitinases were found to act synergistically with Bt toxins (Brousseau *et al*., 1998).

The multiple toxin approach, the use of synergists, the rotations among toxins and ultrahigh doses in combination with refuges are some of the tactics patterned after those proposed and used in managing chemical insecticide resistance. They take into account some unique features of Bt, as well as traits shared with other insecticides.

When a mixture is used, individual pests are exposed to more than one toxin simultaneously (Gould, 1986). The use of mixtures to retard evolution of resistance is based on the idea that if resistance to each component in a mixture is rare, then individuals with resistance to all components will be exceedingly rare or absent. One condition that is necessary, but not sufficient, for the success of mixtures is the lack of cross-resistance between mixture components (Tabashnik, 1989). The broad-spectrum cross-resistance of *H. virescens* clearly violates this principle. Results from *P. xylostella* and *P. interpunctella* show that even when cross-resistance among components is not present, insects can readily evolve resistance to mixtures of Bt toxins.

The use of synergists has been proposed for managing resistance to *B. thuringiensis* (MacIntosh *et al*., 1990). This interaction between a synergist and an insecticide causes increased toxicity even though the synergist may have very little toxicity by itself. Serine protease inhibitors showed to synergize with Bt against species of moths and *L. decemlineata* (MacIntosh *et al*., 1990).

The use of temporal rotations (i.e. alterations) of insecticides is based on the assumption that the frequency of individuals resistant to one toxin declines while a different toxin is applied. Rotations among toxins conferring cross-resistance have little value, while it can be especially useful when large fitness costs are associated with resistance.

In theory, a dose that is sufficiently high to kill resistant homozygotes would not select for resistance because all individuals exposed to such a dose will die. However, such a high dose may not be feasible. Also, reduced binding affinities for toxins is a primary mechanism of resistance in some species (Ferré *et al*., 1991; Bravo *et al*., 1992). If binding affinity approaches zero, attempts to kill resistant insects with high doses may be futile. If survival is low but greater than zero, this approach could rapidly produce resistance in target and non-target pests. A very attractive management tactic is the combination of high dose strategy with the use of refugia (toxin-free areas). The principle is to express Cry toxins at such a dose that all heterozygotic carriers of resistance alleles will be killed. Survivors will most likely mate with the sensitive insects harbored in the nearby refuge. Consequently, a population of homozygous resistant insects would unlikely emerge.

11. CONCLUSIONS

The scale of both economic and environmental costs of insects control and of losses incurred in-spite of such measures is high. The increasing importance of alternatives to overwhelming use of synthetic chemical pesticides, as a result of greater environmental awareness, food safety concern and human health considerations provided a major niche for the development of Bt and its δ-endotoxins. Transgenic microorganisms or plants, and microbial formulations based on Bt offer a range of possibilities for insect control and have a safety advantage of precluding the environmental hazards such as possibility of spray drift and ground water contamination. The introduction of Bt crops and sprays led to a reduction in insect pest damage in agriculture and forestry and to the simultaneous elimination of chemical pesticides to a reasonable extent.

It is anticipated that engineered forms of Cry proteins showing improved potency or yields, in-spite of their host, will make Cry-based pesticides a more attractive and practical alternative to synthetic chemical control agents. It is expected that an increased understanding of the complex interplay among Cry toxins, their hosts, their target organisms and the ecosystem they share will allow, for the long-term, effective use of Cry toxins for pest management. Given the specificity of Cry toxins, their lack of toxicity for other animals, humans or plants and their environment friendly nature, there is a considerable potential for exploiting these bacteria as biological control agents. The concern about insects rapidly becoming resistant to Bt toxins received a great deal of attention. Knowledge of how management tactics affect rates of resistance development is surely needed. In principle, field experiments are the best way to evaluate tactics for managing resistance. Environmental concerns regarding the field use of Bt centered on the release of spores, particularly in the water catchment areas. However, Bt has been widely used for many years in a number of different environments with no reports of adverse effects. Similarly, Bt does not pose a risk to mammals and can be safely used in environments in which human exposure will occur. Concerns were raised about the negative effects of toxins on non-target animals such as predators of parasites that feed on pests. According to most published reports, Bt toxins have no

detrimental effects on natural enemies because of their high specificities and low persistence. The surest way to conserve the efficacy of Bt is to use it in a rational way, in conjunction with other control methods as a part of Integrated Pest Management strategies.

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MYCORRHIZAE IN THE INTEGRATED PEST AND DISEASE MANAGEMENT

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Abstract. Plant diseases cause serious losses in crop production and pesticide applications are currently the main way deployed for control. Due to severe environmental problems, achieving a sustainable agriculture will require avoidance of chemical pesticides/fungicides. Mycorrhizal fungi provide an effective alternative method of disease control, especially for those pathogens which affect below ground plant organs. In mycorrhizal fungi lies an enormous potential for use as biocontrol agents for soil- and root-borne diseases. Some species are also effective control agents against phytoparasitic nematodes and others are also reported as effective for control of leaf spot diseases. For efficient and persistent disease management the need is to evaluate the mycorrhizal symbionts in the natural system under field conditions. The use of mixed inocula of mycorrhizal symbionts can be more effective and yield better results than the use of a single species.

1. INTRODUCTION

There is a worldwide growing awareness regarding the negative repercussions of the indiscriminate use of chemical pesticides, which are not only toxic to human life but also lead to environmental as well as ecosystem pollution. The long term use of broad spectrum chemical pesticides has been identified as one of the major causes of environmental pollution and contributes to the deterioration of agricultural land and ecosystem as a whole.

The present needs concern the use of safer biocontrol measures that are easily degradable, environmentally friendly with greater selectivity, requiring low dosage rates with less harmful effects on non target organisms.

The magnitude of the problem can be minimised or avoided by fully exploiting chemicals in combination with biological, physical and cultural control measures in integrated pest and disease management programmes. Integrated pest management (IPM) is an effective mean for disease control and holds tremendous potentials in the future (Mukerji *et al*., 1996). The use of any microbial-based biotechnology in IPM appears of great advantage, since it is both environmentally friendly and ecologically sound. The most important issue in the use of biological control resides in developing biodegradable, consistent, persistent control measures coupled with high performance.

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In recent times, several different microorganisms which show natural antagonism to pathogens have been identified and experimented for their potential as biological control agents (BCA) (Whipps, 2001). One group of microorganisms showing this ability is represented by the arbuscular mycorrhizal fungi (AMF). They are an economically and ecologically important group of symbiotic fungi, which colonise the roots of over 80% of plant species and are present in all soil ecosystems (Norman *et al*., 1996; Trotta *et al*., 1996; Cordier *et al*., 1998; Azcón-Aguilar & Barea, 1996; Vigo *et al.,* 2000).

Research priorities for alternative management practices, compatible for sustainable agriculture and the environment, include the use of beneficial microorganisms as biocontrol agents. Biological control of plant pathogens is currently accepted as a key practice in sustainable agriculture and can be defined as the directed, accurate management of common components of ecosystems, aiming at protecting plants against pests and pathogens. Biological control preserves environmental quality by reducing chemical inputs, and is characteristic of sustainable management practices (Altieri, 1994; Barea & Jeffries, 1995).

The mycorrhiza is a mutualistic symbiotic association between a fungus and a plant root. The felicitous comment "most woody plants require mycorrhizae to survive and most herbaceous plants need them to thrive" is possibly the most apt generalization in the contest (Trappe & Fogel, 1977). Biological control of any disease can be achieved through the manipulation of resident microorganisms or by introducing antagonists in order to reduce the amount of inoculum or disease producing activity of the pathogen (Chet, 1987; Cook & Baker, 1983; Deacon, 1983; Mukerji & Garg, 1988a, b; Mukerji *et al*., 1996). At this regard, mycorrhizal fungi provide an effective alternative method for disease control, especially for those pathogens which affect below ground plant organs. Mycorrhizal fungi have enormous potentials for use as BCAs for soil-borne diseases, as root diseases are one of the most difficult targets to manage, causing losses in disturbing proportions (Mukerji, 1999; Xavier & Boyetchko, 2002).

The role of mycorrhizae in disease control is better observed in arbuscular mycorrhizae than in ectomycorrhizal associations. Both systems are herein presented and their potentials in IPM illustrated and discussed.

2. ECTOMYCORRIZAE

The ectomycorrhizal symbiosis has the ability to increase the level of roots resistance to infection by potential pathogens, acting as a first physical and biological barrier. A pathogen attacking an ectomycorrhizal root system is initially confronted externally with highly interwoven networks of fungal mycelium (mantle), and then internally with cortical cells whose walls are surrounded by fungal hyphae (Hartig's net) (Fig. 1). Both mantle/matrix and Hartig's net provide a protecting physical barrier and promote the formation of favourable microbial populations in the soil around the mycorrhizal roots (Schisler & Linderman, 1987). This change in soil microflora is associated with release of organic compounds including volatile substances around the

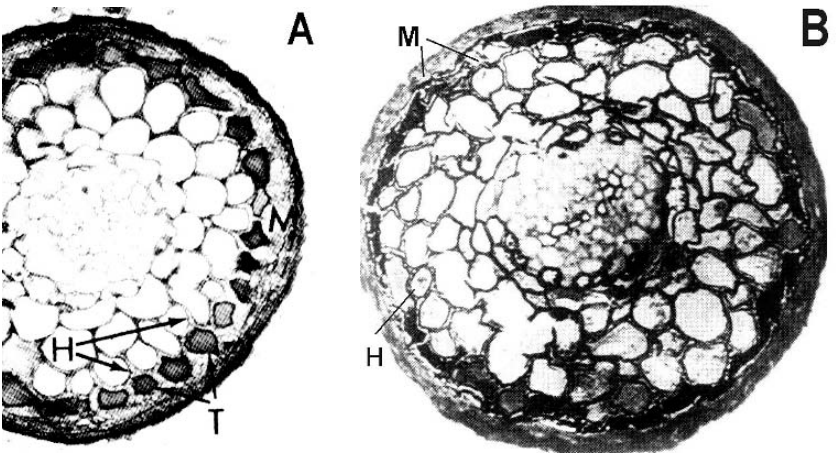

Figure 1. Transversal sections of ectomycorrhized roots of Pinus contorta *(A) and* P. caribaea *(B). M = mantle; H = Hartig's net; T = tannin layer (courtesy H. S. Thaper).*

ectomycorrhizal roots. In the rhizosphere there is an abundance of beneficial bacteria including fluorescent pseudomonades (Linderman, 1988). Some species are known to produce plant growth promoting substances (PGPR), which also help in developing suppression of fungal pathogens. Stain F113 of *Pseudomonas fluorescens* showed a biological control activity whose primary determinant was identified in 2,4-diacetylphloroglucinol (2,4-DAPG). This phenolic compound has some antibacterial and antifungal activities and results from the export of secondary toxic metabolites produced in the bacterial cells (Shanahan *et al*., 1992). The capacity of *P. fluorescens* strain CHA0 to act as a biological control agent of soil borne pathogens also depends on the production of 2,4-DAPG (Dowling & O'Gara, 1994; Keel & Défago, 1997). Phenolic compounds, produced in the plant tissues in response to the presence of mycorrhizal fungi, also improve the hosts levels of resistance against pathogens (Sylvia & Sinclair, 1983). Some ectomycorrhizal fungi also exert direct antibiotic effects against pathogens (Kope *et al*., 1991).

The role of ectomycorrhizal fungi in the control of plant diseases was reviewed in the last years by several authors (Duchesne, 1994; Duchesne *et al*., 1989a,b; Gasper *et al*., 1991; Simoneau *et al*., 1996; Mukerji, 1999; Munzenberger *et al*., 1997; Hodge *et al*., 1995). Duchesne *et al*. (1989a, b) pinpointed the following characters in determining the potential success of ectomycorrhizal fungi for effective biological control: *i*) longer shelf life, *ii*) amenability to storage and transportation, prior to field applications, *iii*) little manipulation required in field applications, *iv*) protective effect of selected isolates, displayed soon after inoculation, *v*) efficient competition with the resident soil microflora, *vi*) roots colonisation and mycorrhiza formation, at rates faster than the pathogen invading the roots, *vii*) suppressive action against most pathogenic species, *viii*) absence of any effect disruptive of the normal beneficial soil processes, with no disturbance of the microbial equilibrium,

ix) compatibility with natural pesticides/fungicides, *x*) possible genetic engineering for enhanced disease suppression and *xi*) use in IPM in conjunction with other means of plant protection (Duchesne, 1994).

3. ARBUSCULAR MYCORRHIZAE

Arbuscular mycorrhizae (AM) are characterised by two main features recognizable within the plant roots: *i*) an internal hyphal system connected to an external hyphal network through initial entry points, *ii*) presence of intracellular arbuscules, which are dichotomously branched, tree-like structures enclosed by host plasmalemma and appearing as a site of nutrient exchange between the fungus and the plant. In some species, the presence of vesicles, which are terminal and/or intercalary, thin walled expanded structures, not delimited by a septum and containing large amounts of lipids may be observed (Fig. 2).

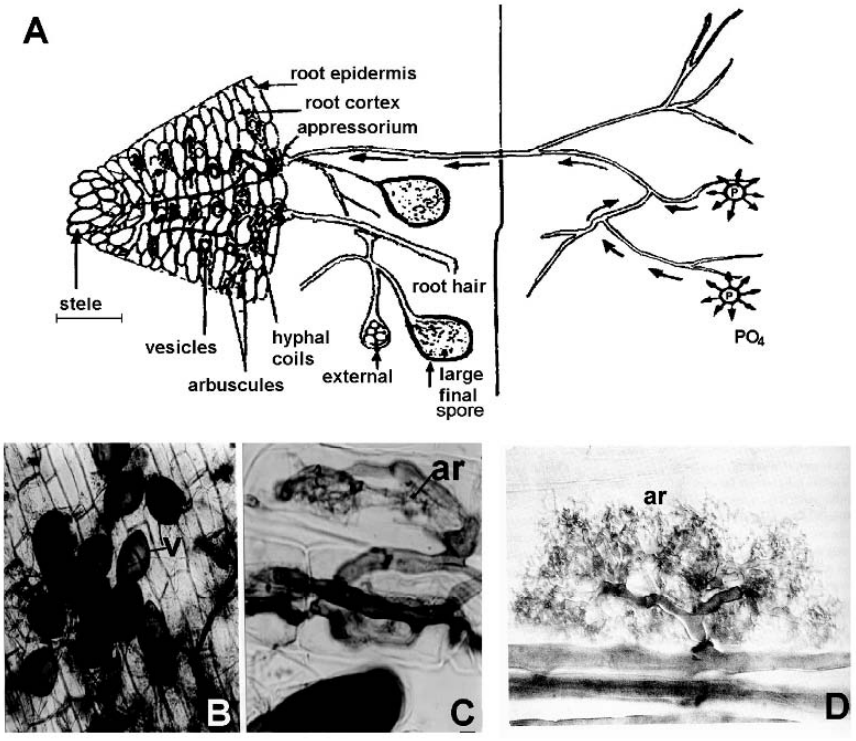

Figure 2. Diagrammatic representation (A) of AM root colonisation, showing mechanism of P uptake. B: vesicles (v). C: arbuscules (ar) in root cell. D: enlarged arbuscule (B and C courtesy R. Kapoor).

AM colonization begins with the development of appressoria on the root epidermis, for subsequent penetration and growth within the root cortex. This phase may follow two patterns, known as the *Arum* type and the *Paris* type (Smith & Read, 1997). In the former, the hyphae grow in the intercellular spaces between root cortical cells, and then they penetrate cells to differentiate the branched, terminal arbuscules, with a single hyphal penetration per arbuscule-containing cell. In the *Paris*-type AM, several hyphae spread from cell to cell with minimal intercellular development and more common penetration of plant cell walls. Colonized cells of *Paris*-type AM develop complex coils with arbuscule-like branches (arbusculate coils). The hyphae of both morphotypes do not penetrate the plant plasma membrane and all phases of development (intercellular hyphae, arbuscules, coils, and arbusculate coils) are confined within an apoplastic compartment outside the plant protoplast (Smith & Read, 1997; Gao *et al*., 2004).

The arbuscular mycorrhizal (AM) fungi are ubiquitous soil inhabitants and form mutually beneficial symbiotic associations with the plant roots. AM fungi are an integral component of the root system, supporting the growth of complex assemblages of different microorganisms. The soil microflora and mycorrhizal associations have significant effects on the host plant growth. The extramatrical hyphae of AM fungi exude substances that cause soil and organic fractions to cloth or aggregate, giving rise to a microenvironment in which microorganisms flourish. The extramatrical hyphae spread in the bulk soil and increase the activity of the root system for nutrient and water absorption and soil microfaunal activities. The relationship between AM fungi and soil biota is well known (Curl & Truelove, 1986; Linderman, 1991; 1992; Mukerji, 2002a).

The possible mechanisms of mycorrhiza in biocontrol (Norman & Hooker, 2000; Huang *et al*., 2006) are: *i*) enhanced plant nutrition, *ii*) biochemical changes in plant tissues, *iii*) anatomical changes, *iv*) alleviation from stresses predisposing plants to disease, *v*) microbial changes in the rhizosphere (mycorrhizosphere), *vi*) induced changes to the root system morphology, *vii*) direct competition between the AM and the pathogens for physical space or resources, and *viii*) induction of systemic resistance (Cordier *et al*., 1998).

The study of the plant-mycorrhiza interactions received a great enhancement by the application of molecular biology methods and mutant plant lines deprived of AM colonization, both revealing a deep effect of AM on roots gene regulation. For example, gene expression data showed that in barley (*Hordeum vulgare*) roots colonized by the AM fungus *Glomus intraradices*, higher levels of endogenous jasmonic acid (JA) and jasmonate coniugated isoleucine were present. These products resulted from the cell-specific expression of genes controlling the JA biosynthesis pathway, active in the arbuscule-containing root cortex cells (Hause *et al*., 2002). JA and related compounds are plant hormons regulating the response to abiotic and biotic stresses. They are activated upon local wounding of leaves (Ryan, 2000) and are involved in plant defense reactions, mediated through the production of several compounds, including proteinase inhibitors, phytoalexins, vegetative storage proteins, thionins and defensins (Creelman & Mullet, 1997; Farmer *et al*., 1998; Ryan, 2000).

Using mutant and wild-type tomato lines, Gao *et al*. (2004) observed that the expression of several defense-related genes was low in *Arum*-type interactions, whereas *Paris*-type colonizers, i. e. the members of the family Gigasporaceae, produced a substantial increase in the expression of some plant defense related genes. However, the extent of root colonization for both AM morphotypes suggested that defense gene products do not limit the development of the mycorrhiza. When the fungus could not penetrate the root, i. e. in the mutants and AM interactions, no accumulation of defense gene mRNAs was observed (Gao *et al*., 2004).

A complex set of chemical signals marks the interaction of plants with AM fungi. Superoxide dismutases (SOD) are a group of enzymes active in a series of cell detoxification mechanisms. They are actively involved in the plant hypersensitive response to invading pathogens, or in the prevention of cell membrane damages due to reactive oxygen species, representing a common mechanism of plants defence. A CuZnSOD was identified in the AM fungus *Gigaspora margarita* (Lanfranco *et al*., 2005). Experimental data concerning the analysis of the fungus cDNA in two host plants, *Lotus japonicus* and *Medicago truncatula*, showed that it encodes a functional enzyme conferring tolerance to oxidative stress and that the highest amount of transcripts occurs when the fungus is inside the root cells. The CuZnSOD of *G. margarita* is differentially expressed during the fungus life-cycle and is up-regulated inside the root tissues. The enzyme is considered to act during the host colonization phase, through the inactivation of defense-related reactive oxygen species or in an apoptosis-like process, related to the arbuscules deactivation (Lanfranco *et al*., 2005).

3.1. Mycorrhizosphere

AM fungi influence plants growth and nutrients uptake, in addition to increasing the absorptive surface area of the whole host root system. The mycorrhizal hyphae, outside and inside the root, increase the surface area available for the interactions with other soil microorganism and provide an important pathway for the translocation of energy-rich plant assimilates to the soil (Johansson *et al*., 2004). The rhizosphere is characterised by increased microbial activity which is stimulated by the leakage and exudation of organic substances from the roots (Grayston *et al*., 1997; Mukerji, 2002a, b; Gutpa & Mukerji, 2002).

Mycorrhizae also significantly alter the physiology and/or morphology of roots and plants in general, leading to altered root exudation (Bansal & Mukerji, 1994; 1996). The changes in root exudates affect the microbial communities around the roots and so the rhizosphere microflora composition results distinctly influenced. This microenvironment surrounding the mycorrhizal roots is known as "mycorrhizosphere", which identifies the zone affected by the root and the fungus, whereas the "hyphosphere" indicates the zone surrounding the fungal hyphae (Figs. 3, 4) (Kapoor & Mukerji, 1998; Mukerji, 2002b; Mukerji *et al*., 1997; Linderman, 1988; 1991; 2000; Giri *et al*., 2005; Paulitz & Linderman, 1991; Bansal *et al*., 2000; Johansson *et al*., 2004).

In the mycorrhizosphere, exudates may selectively enhance the population density of single species of soil bacteria. Some AM species may also increase the density of bacterial populations as a whole, probably because of increased amounts of aminoacids exudated. The stable aggregation of soil particles was shown to increase the density and species composition of the soil around roots, with changes observed among different fungal species. The hyphosphere qualitative composition was also observed to excerce a role on bacteria populations, stronger than the effects of the fungal biomass amount in soil (Joansson *et al*., 2004).

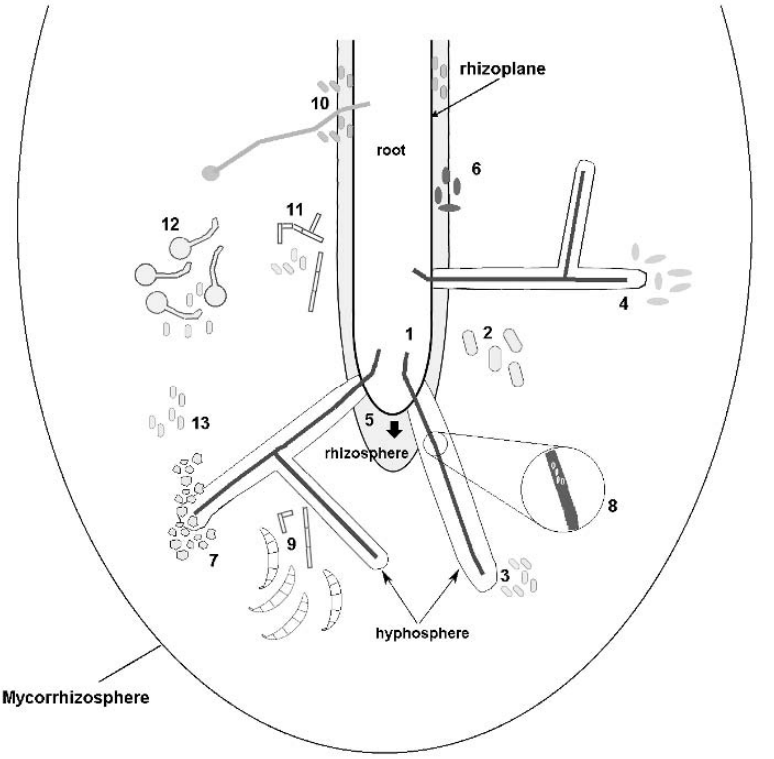

Figure 3. Schematic view of the interactions among different components of the mycorrhizosphere. Effects of AM fungi on bacteria: energy supply (1), pH changes(2), competition for nutrients (3), release of inhibitory or stimulatory compounds (4), root growth stimulation (5), changes in root exudates (6) and effects on soil structure (7). Effects on endophytes or soil pathogens: growth of AM endobacteria (8) and effects on pathogenic fungi. Further interactions include the effects of mycorrhizosphere bacteria on roots AM receptivity (10), fungal growth (11), propagules germination (12) and soil chemistry (13) (adapted from Johansson et al., 2004).

3.2. Impact of Biocontrol Agents on AM Formation and Disease Control

AM fungi deserve special attention as they are mainly responsible for the solubilization of phosphate and for plant growth promotion. The microbial solubilisation of insoluble phosphates through release of organic acids is often accompanied with release of other metabolites involved in biocontrol of soil-borne phytopathogens, mainly siderophores, phytohormones and lytic enzymes (Dar *et al*., 1997; Filion *et al*., 1999; Johansson *et al*., 2004; Vassilev *et al*., 2006; Chincholkar *et al*., 2007).

Pseudomonas strain F113 used as biocontrol agent does not exhibit antifungal activity against AM fungus *Glomus mosseae* rather it has a significant stimulatory effect on mycelia development from *G. mosseae* spores and on the overall processes involved in the formation of AM association in soil (Barea *et al*., 1998). This associated state is more effective against soil-borne fungal pathogens, since it is not only directed against the target fungal pathogen but also concerns the ecological impact of biocontrol agents on beneficial resident soil microbial populations in the

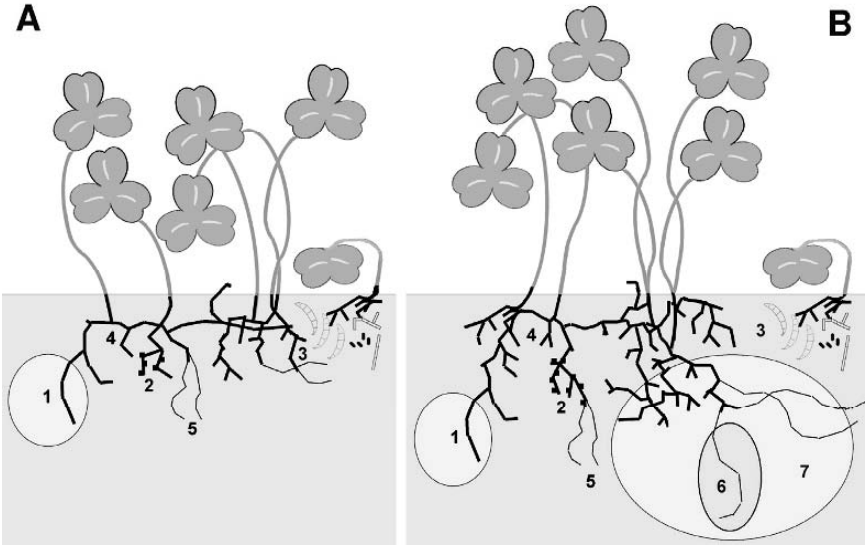

Figure 4. Schematic representation of the mycorrhizosphere concept in contrast to the rhizosphere. The drawing shows the traditional agricultural soil (A) with high inputs of pesticides and fertilizers, and the soil under sustainable management (B), with higher hyphal biomass and biological control agents, low fertilizers inputs and higher microbial diversity. The scheme shows in a simplified way the rhizosphere (1), the symbiotic N fixation in soil (2), the roots pathogens (3), the endophytes (4), the AM fungi (5), the hyphosphere (6) and the mycorrhizosphere (7) (adapted from Johansson et al., 2004).

rhizosphere (Andrade *et al*., 1997; 1998; Paulitz & Linderman, 1989; Barea *et al*., 1998; Atkinson *et al*., 2002). AM fungi increased colonisation of sugar cane roots with plant-growth promoting bacteria (PGPR) in mixed inocula (Boddey *et al*., 1991; Bianciotto *et al*., 1996a,b) improving the mineral nutrition, and the levels of disease suppression and phytohormone production (Brock & Vanderleyden, 1995; Défago & Keel, 1995). The association between fungi and bacteria may result very helpful. *Pseudomonas flourescens* strain CHA0, a well known biocontrol agent of soilborne plant pathogens (Voisard *et al*., 1994) associates well with AM fungi, improving the plant nutrients uptake and allowing a better disease control (Calvet *et al*., 2000; Bianciotto *et al*., 2001).

Some other bacteria can directly influence the physiology of the plants by increasing root cell permeability, influencing the mycorrhizal relationship and/or plant growth (Garbaye, 1994; Vivas *et al*., 2003; Artursson *et al*., 2006; Barea *et al*., 2002). This association also may allow an enhancement of the root branching, resulting in better nutrient uptake (Gamalero *et al*., 2004). Specific bacteria together with AM fungi may create a more indirect synergism that supports a better plant growth (Barea, 1997) as well as the inhibition of plant pathogenic fungi (Budi *et al*., 1999; Linderman, 2000). Artursson & Jansson (2003), found that the Gram-positive bacterium *Paenibacillus brasilensis* was inhibitory to plant pathogenic fungi, i. e. *Fusarium moniliforme* and *Diplodia macrospora* (Von der Weid *et al*., 2005), stimulating the growth of certain AM species (Artursson *et al*., 2006) and the formation of a stable AM association.

Finally, certain fungal biocontrol agents like *Trichoderma* sp. and *Gliocladum* sp. are compatible with the formation and functioning of AM fungi, becoming more effective, in association, against soil-borne pathogens (Barea & Jeffries, 1995; Barea *et al*., 1993; Calvet *et al*., 1992; Paulitz & Linderman, 1989; 1991). In cooperation with AM associations, *Trichoderma* sp. may confer the mycorrhized plants higher resistance levels towards pathogens. *Trichoderma* sp. when used as BCA exerts positive effects on plants, including an increase of the plant growth rates (biofertilisation) and the stimulation of some plant-defense mechanisms (Benitez *et al*., 2004). Some antagonistic effect and mycoparasitic behaviour were, however, reported for *Trichoderma* spp., which affected the ectomycorrhiza *Laccaria bicolor* preventing the mycorrhiza formation and the host root colonization (Summerbell, 1987).

4. SOIL AND ROOT BORNE DISEASES

There is increasing evidence that AM fungi can reduce disease incidence and propagule number of several soil-borne pathogens like *Aphanomyces*, *Fusarium*, *Rhizoctonia*, *Phytophthora*, *Pythium* and *Verticillium* (Caron *et al*., 1986; Cordier *et al*., 1996; Hwang *et al*., 1992; Liu, 1995; Filion *et al*., 2003; Mcallister *et al*., 1994; Slezack *et al*., 1999, 2000; St-Arnaud *et al*., 1994; 1997; Vigo *et al*., 2000; Declerck *et al*., 2002; Guillon *et al*., 2002; Norman & Hoorker, 2000; Demir & Akkopru, 2007; Utkhede, 2006; Sharma & Adholeya, 2000; Singh *et al*., 2000, Sharma *et al*., 2004; 2007).

Bioprotection with mycorrhized plants is the outcome of a number of complex interactions occurring among plant, pathogen and AM fungi (Figs. 3, 4). Different mechanisms are possibly involved in the development of an effective bioprotection, acting separately and/or together (Harrier & Watson, 2004). They are: *i*) enhanced crop nutrition, *ii*) alteration in root architecture and longevity, *iii*) competition with the pathogen for infection and colonisation sites by AM fungi, *iv*) alteration in the root anatomical structure, *v*) competition between the pathogen and the AM fungi for host photosynthesis products and/or nutrients, viii) rhizosphere deposition i. e. of root exudates, *ix*) damage compensation – nutrients in mycorrhizal plants can compensate the loss of root cells or lower root function, due to infection, *x*) enhanced rhizosphere microflora in mycorrhizal plants (Marschner *et al*., 2001; Burke *et al*., 2002; Timonen & Marshner, 2006); *xi*) activation of plant defence responses.

Several genes are activated and corresponding protein products are produced in AM plants. These include phytoalexins, callose deposition, hydroxyproline–rich ghycoproteins, phenolics, peroxidases, chitinases, β-1-3 glucanases and PRs (pathogenesis related proteins) (Pozo *et al*., 1998; 1999; 2002; Slezack *et al*., 1999; 2000; 2001; Guillon *et al*., 2002; Salzer *et al*., 2000; Ruiz-Lozano *et al*., 2001; Garmendia *et al*., 2006; Shaul *et al*., 1999; Mukerji, 1999; Zeng, 2006; Bestel-Corre *et al*., 2002; Harrier & Watson, 2004). In biocontrol of Verticillium wilt on pepper, the AM association induced formation of specific and new isoforms of acidic chitinases and superoxide dismutase (SOD), together with enhanced peroxidase and phenylalanine ammonialyase (PAL) activities, observed two weeks after pathogen inoculation (Garmendia *et al*., 2004a,b; 2005; 2006).

Pathogen suppression due to mycorrhizal association has been ascribed to both physiological changes in the mycorrhized plants as well as to direct interactions between AM fungi and the pathogen (Heungens & Parke, 2001; Thygesen *et al*., 2004). AM symbiosis, reported to affect plant-water relations by increasing drought resistance and/or tolerance, also improves the management efficacy of disease control (Augé, 2001).

Beneficial effects of mycorrhizal symbiosis on plant growth and physiology is more relevant in dry conditions. AMF can improve drought resistance by uptake of soil water by AM hyphae (Davies *et al*., 1992; Augé, 2001) and/or increasing the root to shoot ratio (Davies *et al*., 2002). In general, the AM symbiosis protects plant from drought though a number of physical, nutritional, and cellular effects (Ruiz-Lozano *et al*., 2001; Ruiz-Lozano, 2003). Pathogens become aggressive in water deficit conditions while their impact may be suppressed in mycorrhizal plants (Garmendia *et al*., 2004a, b; 2005; Goicoechea *et al*., 2000).

Several plant-mycorrhiza associations provided evidence for a suppressive role of the symbiosis against soil pathogens, although in some relationships detrimental effects were also reported. Roots of *Pinus resinosa* mycorrhized with the fungus *Paxillus involutus* showed a suppressive effect on *Fusarium oxysporum* f. sp. *pini* (Duchesne *et al*., 1987a,b; 1988a,b). Wacker *et al*., (1990) reported the beneficial effects of *G. fasciculatum* against *F. oxysporum* parasitizing asparagus. Efficient protection of black spruce seedlings (*Picea*

mariana) against Cylindrocladium root rot (*Cylindrocladium floridanum*) was observed for some ectomycorrhizal fungi. In Petri dishes studies, *Tricholoma* sp. and, with a higher intensity, *P. involutus* and *Hebeloma cylindrosporum* inhibited growth of *C. floridanum*. A direct effect was also reported for *P. involutus* and *H. cylindrosporum*, which were capable to deteriorate the hyphae of the pathogen in the contact zone. A detrimental effect was, however, observed for *L. bicolor,* which appeared inhibited and covered by the mycelium of *C. floridanum*. The inoculation of *P. involutus* reduced the infection of the black spruce seedlings by almost 50%, as the numbers of infected plants was negatively correlated with the mycorrhiza formation (Morin *et al*., 1999). In a field study, inoculation of onion (*Allium cepa*) with *Glomus* sp. against onion white rot (*Sclerotium cepivorum*) delayed the insurgence of the disease epidemic by 2 weeks. Mycorrhization protected plants against the disease for almost three months after transplanting, as compared with untreated controls, with an increase of 22% in yield, regardless of the presence of the white rot pathogen (Torres-Barragán *et al*., 1996).

5. LEAF PATHOGENS

Mechanisms of plant disease control by mycorrhizal associations were reviewed from time to time (Singh *et al*., 2000; Xavier & Boyetchko, 2002; Sharma *et al*., 2007; Demir & Akkopru, 2007; Zeng, 2006). Mycorrhiza generally leads to control of soil and root-borne pathogens but evidences have come which show suppression of leaf attacking pathogens also. Mycorrhizal tomato plants had significantly less symptoms of leaf spots due to *Alternaria solani* than in non-mycorrhizal plants. The protective effect of mycorrhiza towards development of leaf spots due to A. salani on tomato leaves has been paralleled to induced systemic resistance (ISR) mediated by rhizobacteria. Both AM and rhizobacteria being root-associated organisms are together effective against necrotrophic pathogens (Durrant & Dong 2004; Fritz *et al*., 2006). Zaidi & Mukerji (1983) also observed that diseases were suppressed in arbuscular mycorrhizal plants (Mukerji *et al*., 1997), with more spores found around roots of healthy plants than around roots of diseased plants (Table 1).

Protective effects induced by a direct action of AM fungi against a phytoplasma of the Stolbour group were observed in infected tomato plants. The disease appeared less severe when the plants harboured AM fungi. In these plants some morphological parameters (shoot and root fresh weight, shoot height, internode lenght, leaf numbers and adventitious root diameters) were observed to be closer to those of healthy individuals. Lower incidence of nuclear senescence was also observed in AM colonized plants infected with phytoplasmas (Lingua *et al*., 2002).

Table 1. Number of AMF spores in the roots of diseased and healthy plants (Zaidi & Mukerji, 1983).

Host	*Disease*	*Pathogen*	*Spores ·10 g^{-1}*	
			diseased	*healthy*
Melilotus indicus	Downy mildew	*Peronospora trifoliorum*	24	46
Melilotus indicus	Powdery mildew	*Erysiphe trifolii*	58	156
Brassica napa	Powdery mildew	*Erysiphe chicoracearam*	25	48
Euphorbia pulcherrima	Rust	*Melampsora euphorbii*	44	86
Brassica campestris	White rust	*Albugo candida*	12	116
Triticum aestivum	Loose rust	*Ustilago tritici*	22	76
Triticum aestivum	Leaf spot	*Drechslera graminearum*	18	71
Triticum aestivum	Brown rust	*Puccinia graminis*	13	92
Coriandrum sativum	Stem gall	*Protomyces macrosporus*	26	84

6. PLANT PARASITIC NEMATODES

The protective effect of AMF against plant parasitic nematodes is documented in several reviews (Hussey & Roncadori, 1982; Saleh & Sikora, 1984; Cooper & Grandison, 1986; Hallmann & Sikora, 1996; Diedhiou *et al*., 2003). It is known that changes in root exudates of AM plants influences the attack of roots by some phytoparasitic nematode species (Mukerji *et al*., 1996; Mukerji, 1999). AMF improve host plant vigour and reduce yield losses due to nematode infections, particularly in phosphorus deficient soils, whereas physiological changes in AM roots may impart resistance to nematodes by increased production of inhibitory substances.

Tomato plants colonized by *G. fasciculatum* showed significantly lower numbers of giant cells produced by the root-knot nematode *Meloidogyne incognita*, although roots did not prevent the penetration by the nematode juveniles. Root extracts from mycorrhized plants showed almost 50% mortality of the nematode larvae in four days (Suresh *et al*., 1985). Improving plant health, AMF confer protection, with prophylactic effects increasing the host tolerance to nematode penetration and parasitism (Francl, 1993; Pinochet *et al*., 1996; Jaizme-Vega *et al*., 1997).

AM applications appeared particularly useful in the management of nematodes parasitising tree crops and seedlings. On commercial *Prunus* spp. rootstocks, susceptible to root-knot nematodes parasitism, mycorrhizal inoculation with *G. intraradices, G. etunicatum* and *G. mosseae* showed significant increases in stem diameter, plant height, fresh and dry shoot weights. Significant effects were however observed on artificially inoculated plants only, when planted in pasteurized soil and mainly during the first growing season.

Similarly, a lower incidence of root galling induced by *M. javanica* was observed in the first growing season after transplant. Nematodes were not observed to produce negative or suppressive effects on the natural or artificial mycorrhizal colonization (Calvet *et al*., 2001).

Tests on the effects of *G. etunicatum* and the burrowing nematode *Radopholus similis* on the growth of rough lemon (*Citrus limon*) seedlings showed that the mycorrhizal was significantly greater the growth of seedlings and that the mycorrhizal stimulation of seedling growth was inhibited by the nematode attack. When seedlings were inoculated with both organisms, a lower suppression of seedling growth by *R. similis* was observed on VAM seedlings than on nonmycorrhizal controls, whereas nematode-infected roots showed reduced vesicle formation and mycelia growth (O'Bannon & Nemec, 1979). Under dixenic culture conditions, a 50% reduction in the reproduction of *R. similis* was reported (Elsen *et al*., 2001).

Tests on ectomycorrhization of *Acacia mangium* and *A. holosericea* with the basidiomycetes fungi showed that the mycorrhizal association with *Scleroderma dictyosporum* decreased the numbers of *Hoplolaimus pararobustus* on *A. holosericea*. However, an opposite effect was found for *Scutellonema cavenessi* parasitizing *A. mangium,* since the nematodes density was significantly higher when *Pisolithus* sp. was inoculated (Founoune *et al*., 2002).

Inoculation with spores of *Gigaspora margarita* or *Glomus etunicatum*, reared on *Sorghum vulgare*, increased the level of tolerance of peanut to *M. arenaria* and reduced the impact of nematode parasitism, at low levels of phosphorus fertilization. However, the fungi and phosphorus additions increased galling and *M. arenaria* eggs production, increasing the peanut susceptibility to the nematode attack. The root weights of plants grown at higher levels of phosphorus fertilization appeared to be enhanced by *M. arenaria* parasitism, in both mycorrhized and non-mycorrhized plants, because of the development of galls rather than assimilative roots. Both VAM development and fertilization increased nematode galling at the highest levels of phosphorus fertilization, with higher magnitudes for mycorrhized plants (Carling *et al*., 1996).

The interactions between mycorrhizae and sedentary nematodes appear complex, and may range from direct nematode parasitism to indirect effects related to the nutrients, (mainly phosphorus) assimilation. Spores of *G. fasciculatum* were found within cysts of the soybean cyst nematode, *Heterodera glycines*, whose populations showed field prevalence levels up to 24% of cysts. The hyphae were observed to penetrate the female nematode cuticle shortly after the rupture of the root epidermis, filling eggs and giving rise to the sporogenic phase. Isolates of *G. fasciculatum* infected nematode eggs in experimental pot tests, lowering the number of first-generation females by 26%, compared with nonmycorrhizal control, with a positive effect on the soybean plant biomass production (Francl & Dropkin, 1985). In some microplot and field experiments, inoculation of *M. incognita* infested soil with *G. intraradices* and *G. margarita* showed significantly lower population densities of nematode juveniles in the cotton microplots receiving *G. intraradices*, and up to three months after planting. The mycorrhizae reduced yield losses due to *M. incognita*, but an

opposite effect was observed for phosphorus fertilization. In the following year the microplot yields negatively correlated to nematode inoculum densities with a lower incidence in soil inoculated with *G. intraradices*. In the seedlings roots, the nematode densities increased linearly with the original inoculum and appeared favored when mycorrhizal fungi or superphosphate were added. The mycorrhizae appeared to increase the roots tolerance to *M. incognita* in field conditions acting as a biological control agent in soils at high AM population densities (Smith *et al*., 1986).

Inoculation with either *G. margarita* or *G. mosseae*, two weeks prior to inoculation with *M. incognita*, did not alter the tomato parasitism compared with nonmycorrhizal plants, regardless of soil phosphorus levels. Nematode penetration and reproduction did not differ between mycorrhizal and nonmycorrhizal plants, but plants grown in soil with high phosphorus contents showed greater root weights, increased nematode penetration and egg production, with lower levels of colonization by mycorrhizal fungi, than plants exposed to low phosphorus levels. In a plant split-root systems assay carried out in double-compartment containers with different combinations of phosphorus levels, although the fungus increased the root inorganic phosphorus content to a level similar to that of plants receiving higher fertilization, no difference was observed in the nematode penetration and reproduction rates. In other assays, the nematode development was not affected by *G. margarita* or high soil phosphorus contents, suggesting that supplement of phosphorus may alter nematode parasitism more than *G. mosseae* and *G. margarita* (Thomson Cason *et al*., 1983). Further synergistic effects, detrimental to root-knot nematode parasitism, were observed when mixing AM with some organic soil amendments (Rao *et al*., 1996; 1998).

7. CONCLUSIONS

The use of mycorrhizal fungi provides an effective alternative method of disease control particularly in soil or root-borne diseases. The mycorrhizal fungi in a controlled system may need to be very specifically tailored for each host-pathogen interaction. In the biological control of diseases with the help of mycorrhizae, the results being host mediated, provide some hope that in future mycorrhizal fungal technology could be used as a potential biocontrol agent. Certain antagonistic groups – *Trichoderma*, *Gliocladium, Pseudomonas*, *Bacillus* and PGPR in integration with mycorrhizal fungi are more effective as bioprotectant agents. Mycorrhizae are also promising and effective agents against leaf-borne pathogens and sedentary plant parasitic nematodes.

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Section 3

Molecular Aspects in IPM/IDM

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INTEGRATED MANAGEMENT OF INSECT BORNE VIRUSES BY MEANS OF TRANSMISSION INTERFERENCE AS AN ALTERNATIVE TO PESTICIDES

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Abstract. Viruses are important plant pathogens responsible of yield and quality losses in many crops. Most plant viruses are spread in nature surpassing plant defence barriers with the help of vector organisms, mainly insects. The application of pesticides is an insufficient strategy to stop virus dissemination and, in turn, it can cause important environmental damages. As a consequence, an active area of research is currently devoted to explore alternatives to the abuse of pesticides including, for instance, attempts to unravel the molecular mechanisms operating during insect transmission of plant viruses. All these efforts are aimed to design strategies of interference with the transmission process, which will eventually become part of Integrated Disease Management programmes for the control of virus pathogens. The present chapter reviews the available and potential means to interfere with transmission, and the prospects of such strategies.

1. INTRODUCTION

Plant viruses are important pathogens causing economic losses whose severity places them only second after fungi in the ranking of most damaging plant pathogens, worldwide (Baker *et al.*, 1997; Strange & Scott, 2005). For several crops, virus pathogens constitute real limiting factors, lasting for extended periods of time in many territories. Furthermore, the continuous appearance of emerging and re-emerging plant viruses keeps viruses among the most serious concerns of farmers and plant scientists, nowadays.

One of the features that makes viruses a serious threat in modern agriculture is the efficient system followed by most of them for spreading, relying on vector organisms for transmission (Brunt *et al.*, 1996; Hull, 2002). Since the early identification of a leafhopper vector of rice dwarf virus (Takami, 1901), several arthropods and a few other organisms have been described as putative vectors of

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plant viruses. Most of the known plant viruses depend indeed on vectors for their transmission and survival. As a consequence, all integrated management approaches dealing with the diseases they cause must consider the effective control of vector organisms as one of the action priorities.

The organisms capable to transmit plant viruses include fungi, plasmodiophorids, nematodes and arthropods. In the latter category we found for instance mites but, in numerical terms, insects (mainly in the orders *Hemiptera, Coleoptera* and *Thysanoptera*) rank as the first group of vectors (Gray & Banerjee, 1999). Almost 60% of all known plant viruses are transmitted by insects within the order *Hemiptera*. Among them, aphids in the family *Aphididae* (Blackman & Eastop, 2000) are considered the principal vectors, transmitting about half of all insect-borne plant viruses (Nault, 1997; Hooks & Fereres, 2006). The importance of aphids as pests relies in their ability to quickly increase their population size, as they reproduce by parthenogenesis and spread to new hosts. In many cases damages caused by viral transmission surpass their instrinsic economic interest as herbivorous pests. Although less important in numbers, other pierce-sucking hemipteran insects such as leafhoppers, planthoppers, grasshoppers and whiteflies constitute other important groups of vectors. Finally, other insects such as epidermal-feeding thrips and chewing coleopterans can also transmit certain viruses between plants.

The extensive use and abuse of insecticides represented for a long time the main strategy to stop plant viruses spread through control of their vectors. However, this strategy is frequently ineffective and environmentally unacceptable. The pressing need for finding alternative means of viruses control is one of the main priorities in plant health programs. The present review focuses on insect-borne viruses, reflecting the major importance of this kind of vectors in spreading plant diseases and actual control strategies. Current research and future prospects aiming at exploitation of interference with the transmission process, as an alternative to the use of pesticides, are illustrated.

2. MODES OF TRANSMISSION

The retention sites of viruses in the vector and the length of acquisition and inoculation periods represent the major parameters used for classification of plant viruses transmission modes (Walkey, 1985; Gray & Banerjee, 1999; Hull, 2002). Considering these characteristics, transmission modes can be first categorized as circulative or non-circulative. Viruses transmitted in a circulative manner must reach internal tissues of the vector to be transmitted, while viruses transmitted in a non-circulative manner are retained at the beginning of the digestive tract, without the requirement of crossing cellular membrane barriers, inside the vector.

Three different periods can be distinguished during the transmission process: acquisition, latency and retention. Their duration also may be used as a criterium to determine the mode of transmission. The acquisition period is the time required for the vector to feed on an infected plant and to acquire enough virus particles to be able to transmit the disease. The latency period corresponds to the time in which the vector has acquired the virus but is not able to transmit it. Finally, the retention

Table 1. Main characteristics of transmission modes described for plant viruses.

Transmission characteristics		*Modes of transmission*			
		Non Persistent (NP)	*Semipersistent (SP)*	*Circulative Non Propagative (C-NPr)*	*Circulative Propagative (C-Pr)*
Virus location in the vector	Crossing of cellular barriers required	No	No	Yes	Yes
	Virus replication in insect cells	No	No	No	Yes
Duration	Acquisition	Seconds to minutes	Minutes to hours	Hours	Hours
	Retention	Minutes	Hours to days	Days or lifetime	Days or lifetime
	Latency	No	No	Hours	Days or weeks

period is the time in which the vector remains competent to transmit the virus. The transmission process ends with the inoculation of the virus in a new plant. Combining these criteria, four main modes of transmission can be distinguished, as summarized in Table 1.

As shown, insects capable of transmitting plant viruses belong mainly to the order *Hemiptera*. Thanks to their pierce-sucking mouthparts, working as needle-like structures capable of penetrating the plant cell walls without causing major damage to the plant tissues, these insects are very suitable vectors of plant viruses. Interestingly, and despite the similarities in the mouthpart anatomy and feeding behaviour among different hemipterans, aphids are the only vectors that transmit viruses in a non persistent manner. In the remaining categories, both aphids and other insects can be identified.

One important feature useful for classification of insects vectored viruses is the dependency for transmission on auxiliary accessory factors, known as helper components (HC) (Pirone & Blanc, 1996), or the existence of transmission-active specific structures in viral particles, such as the read-through (RT) extensions of the coat protein (CP) (Wang *et al.*, 1995; Gray & Gildow, 2003) or the "rattlesnake" (RS) portions of the particle (Agranovsky *et al.*, 1995; Peremyslov *et al.*, 2004).

These factors are virus-encoded products which facilitate transmission, and often the vectors specificity is associated with their presence. Table 2 lists the plant virus taxa known to be transmitted by insect vectors, with indication of their mode of transmission and the need for accessory factors.

Table 2. Modes of transmission and principal vectors of plant viruses.

Family	*Genera*[a]	*Main vectors*[b]	*Mode of transmission*[c]	*Auxiliary factors.*[d]
Bromoviridae	*Alfamovirus*	Aphids	NP	
	Bromovirus	Beetles	C*	
	Cucumovirus	Aphids	NP	
Bunyaviridae	*Tospovirus*	Thrips	C-Pr	
Caulimoviridae	*Badnavirus*	[Mealybugs, leafhoppers]	SP	HC
	Caulimovirus	Aphids	SP	HC
	Tungrovirus	Leafhoppers	SP	
Comoviridae	*Comovirus*	Beetles	C*	
	Fabavirus	Aphids	NP	
Closteroviridae	*Ampelovirus*	Mealybugs	SP	RS?
	Closterovirus	Aphids	SP	RS
	Crinivirus	Whiteflies	SP	RS
Flexiviridae	*Carlavirus*	[Aphids, whiteflies]	NP	
	Potexvirus	[Aphids]	NP	
Geminiviridae	*Begomovirus*	Whiteflies	C-NPr	
	Curtovirus	Leafhoppers, treehoppers	C-NPr	
	Mastrevirus	Leafhoppers	C-NPr	
Luteoviridae	*Enamovirus*	Aphids	C-NPr	RT
	Luteovirus	Aphids	C-NPr	RT
	Polerovirus	Aphids	C-NPr	RT
Nanoviridae	*Nanovirus*	Aphids	C-NPr	HC
Potyviridae	*Ipomovirus*	Whiteflies	SP	HC?
	Macluravirus	Aphids	NP	HC?
	Potyvirus	Aphids	NP	HC

Table 2 (continued)

Reoviridae	*Fijivirus*	Planthoppers	C-Pr	
	Phytoreovirus	Leafhoppers	C-Pr	
	Oryzavirus	Planthoppers	C-Pr	
Rhabdoviridae	*Cytorhabdovirus*	Planthopper, aphids	C-Pr	
	Nucleorhabdovirus	Planthopper, aphids	C-Pr	
Sequiviridae	*Sequivirus*	Aphids	SP	HC
	Waïkavirus	Leafhoppers, aphids	SP	HC
Tombusviridae	*Carmovirus*	[Beetles]	C*	
Tymoviridae	*Marafivirus*	Leafhoppers	C-Pr	
Non assigned	*Sobemovirus*	[Beetles, aphids]	C* SP	
Non assigned	*Tenuivirus*	Planthoppers	C-Pr	
Non assigned	*Umbravirus*	Aphids	C-NPr	

[a] Taxonomy according to ICTV (Fauquet *et al.*, 2004) and subsequent online updates at ICTVdB - The Universal Virus Database, version 4. http://www.ncbi.nlm.nih.gov/ICTVdb/ICTVdB/.
[b] Brackets indicate that not all species in the genus are transmitted by insects, or by the same type of vectors.
[c] Modes of transmission: Non persistent (NP), Semipersistent (SP), Circulative non propagative (C-NPr), Circulative propagative (C-Pr). The circulative relationship with coleopteran vectors is indicated by C*.
[d] Existence of transmission-specific auxiliary components or structures: helper component (HC), capsid readthrough (RT), rattlesnake particles with two CP forms (RS).

Although the extensive description of the transmission modes is out of the scope of the present review, a brief outline of current knowledge is needed to introduce transmission interference.

2.1. Non-circulative Transmission

Viruses using the non-circulative transmission mode do not infect the vector, neither need to cross cellular membranes to be effectively transmitted. Regarding the mechanism, viruses are supposed to be associated specifically with putative receptors placed at the exterior surface of the internal mouthparts and anterior digestive tract, mainly in the lining cuticle of the food and salivary canals or in the foregut (Figure 1). As indicated, these viruses can be divided in non-persistent and semipersistent species.

Non-persistent transmission is characterized by brief acquisition and retention periods, by the lack of a latency period and by the loss of retained virus particles, when the vector moults. By referring to this mode of transmission as "stylet-borne", early studies reflected the belief that the virus was merely contaminating the outside of the stylet, although it was later found that this was not the case. All non-persistent viruses are vectored by aphids, and include important groups of pathogens such as cucumoviruses and potyviruses (Ng & Falk, 2006). During non-persistent transmission, the virus is acquired during short probes that the aphid makes with the stylet to determine whether or not the plant is an adequate host. The probes are mostly limited to the epidermal leaf cells (Lopez-Abella *et al.*, 1988; Powell & Hardie, 2000; Pirone & Perry, 2002). Virus particles remain associated temporarily to the aphid stylet epicuticle (Figure 1) and can be retained during only a few minutes, being inoculated in the following probes in a new plant. Transmission efficiency decreases when the acquisition period increases, and it is favoured by previous aphid starvation. Electrical monitoring of aphid behaviour supported an ingestion-salivation mechanism proposed for the uptake and inoculation processes (Martin *et al.*, 1997; Powell, 2005).

In semipersistent transmission, the efficiency increases when acquisition and retention periods last longer, and the virus is retained during hours or even days. Aphids, whiteflies and leafhoppers are known vectors of plant viruses that are transmitted in a semipersistent manner. In semipersistent transmission, most acquisition and inoculation processes are considered to occur from and to plant phloematic tissues. Caulimoviruses (Blanc *et al.*, 2001; Drucker *et al.*, 2002) and closteroviruses (Martelli *et al.*, 2002; Dolja *et al.*, 2006) are the best characterized semipersistent viruses. Closteroviruses are mostly found in the phloem, while caulimoviruses are present in most plant tissues, although acquisition for transmission occurs also from the phloem (Palacios *et al.*, 2002). The exact location of the cuticle receptors in the mouthparts of vectors of semipersistently transmitted viruses remains unclear, with controversy between the stylet tip vs more internal locations, such as the foregut (Ng & Falk, 2006).

Two main strategies, known as capsid-only and helper-dependency, have been described for non-circulative viruses (Pirone & Blanc, 1996; Froissart *et al.*, 2002). In the first case, the CP is the only viral product involved in transmission, while in the second case an auxiliary factor, or helper component, is required in addition to virions.

The requirement of a transmission factor has been postulated as an adaptive system to deal with bottlenecks forced on virus populations by the transmission process (Pirone & Blanc, 1996). Two well-studied cases of viruses depending on helper elements for transmission are potyviruses and caulimoviruses. In the first case, a single factor known as HC-Pro is required (Berger & Pirone, 1986; Atreya *et al.*, 1992; Atreya & Pirone, 1993) acting in retention to aphid stylets (Wang *et al.*, 1996). For caulimoviruses, two viral products are operating, P2 and P3, along with virions (Leh *et al.*, 1999). In the case of semipersistent viruses, an interesting system described for closteroviruses is represented by the existence of a 'rattlesnake' structure in the virus particles, with a long body and a short tail, formed by a different version of the CP (known as CPm), located in one portion of the particle

(Agranovsky *et al.*, 1995). This structure appears to be involved in transmission, although the complexity of closterovirus particles (Tian *et al.*, 1999) still leaves unknown some details of the mechanism.

2.2. Circulative Transmission

Viruses transmitted in a circulative manner must be internalized by the vector to be successfully transmitted, and the virus needs to be transported across cell membranes (Figure 1). These species are further divided into two subgroups: propagative and non-propagative.

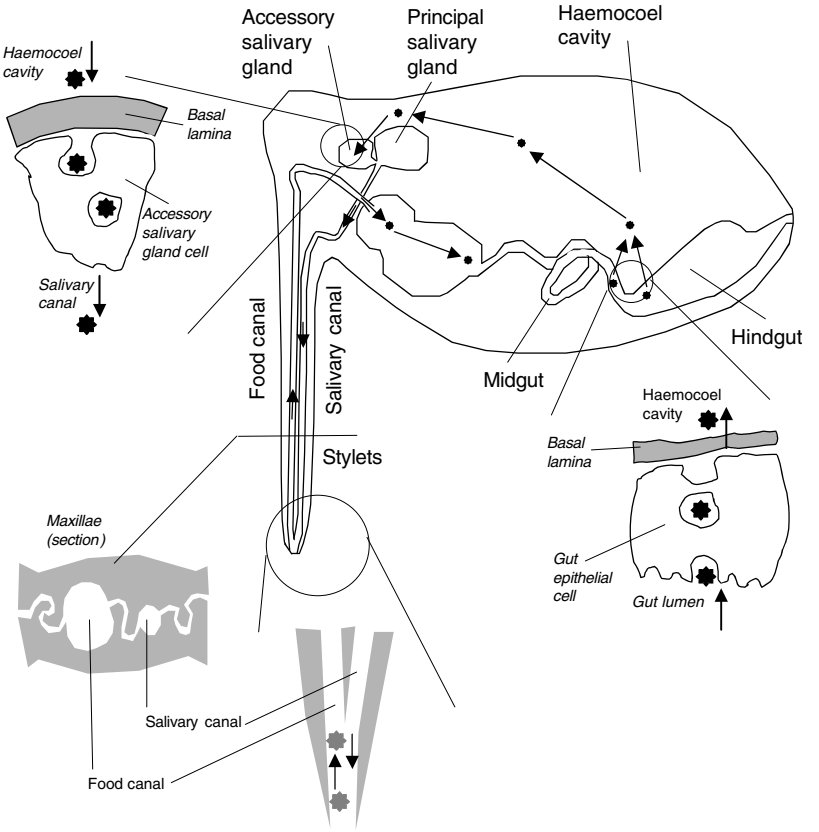

Figure 1. Schematic representation of the interactions between non-persistent and circulative plant viruses within an insect vector (in this case an aphid). Virus particles corresponding to a circulative virus (black symbols), are shown at different stages during circulation inside the insect body. Details of acquisition at midgut or hindgut, and inoculation through the accessory salivary glands, are shown. The particles of a non-persistent virus are also shown (grey-coloured) in their presumed reversible retention site near the stylet tip, where food and salivary canals become connected.

In the circulative propagative transmission the virus replicates in the vector cells and becomes a parasite of both organisms, the plant and its animal vector. Almost all the genera of plant viruses that replicate in their insect vectors have phylogenetic relatives among viruses that infect other arthropods or even vertebrates. It was postulated that these propagative viruses might have evolved from arthropod viruses by acquiring the ability to replicate in organisms on which their initial arthropod host feed, such as plants or vertebrates (Lovisolo *et al.*, 2003). Although this is a certainly attractive hypothesis explaining the evolution of these pathogens, no conclusive evidences are still available at this regard.

The general circulative pathway of virus movement through the insect is similar for both subgroups and involves the entry of the virus through ingestion into the gut followed by association with epithelial cells and uptake by endocytosis. This process may occur at the midgut or the hindgut, depending on the virus species (Gildow, 1987; Gildow, 1993; Garret *et al.*, 1993; Rouze-Jouan *et al*., 2001; Reinbold *et al*., 2003). Afterwards, virus particles are released into the haemocoel, or secondarily infect other tissues. The virus must be able to survive in the insect internal tissues and haemolymph, a highly aggressive environment that also involves escaping from the insect immune responses. It has been postulated that for surviving within the insect, viruses rely on the association with endosymbiotic bacteria (Van den Heuvel *et al.*, 1994), such as those belonging to the genus *Buchnera* and present in aphids (Baumann *et al.*, 1995). These bacteria produce a chaperonin protein denominated symbionin, a homologue of the *Escherichia coli* GroEL protein, whose role in aphid metabolism is still unknown. Interestingly, it was found that symbionins bind to viruses (Filichkin *et al.*, 1997), but the actual implication of these interactions during the transmission process is still unclear. A similar association also exists between begomoviruses and their whiteflies vectors (Czosnek *et al.*, 2001).

All circulative viruses must associate eventually with the accessory salivary glands and be released into the salivary ducts, from where the virus can be inoculated into another plant. The interactions of the virus with the basal lamina of the accessory salivary glands are highly specific, for instance in the case of luteoviruses, specific insect receptors are implicated in virus identification at this point (Gildow & Gray, 1993; Peiffer *et al.*, 1997). Viruses enter the salivary glands by receptor-mediated endocytosis *via* coated vesicles, and then the virus particles are transported in endosomes and released into the salivary duct. The RT domain of the virus coat protein is the structure that seems to be specifically recognised at the salivary gland surface by cell receptors (Gildow & Gray, 1993). Besides, the importance of this RT domain is highlighted by its capacity to interact with symbionin in the haemolymph (Van den Heuvel *et al.*, 1997). The involvement in transmission of the major and minor CP of poleroviruses has been deeply studied (Brault *et al.*, 1995; Brault *et al.*, 2000; Reinbold *et al*., 2001; Brault *et al.*, 2003; Brault *et al.*, 2005). Existence of helper factors in other circulatively transmitted viruses has been proposed (Franz *et al.*, 1999). Finally, also the case of tospoviruses and their thrips vectors was investigated (Whitfield *et al*., 2005).

3. PRACTICES TO CONTROL VECTORS AND VIRUS SPREAD

Since insect vectors are responsible for transmission and dissemination of most plant viruses, considerable efforts are made to control their numbers and their ability to transmit viruses. One important implication of the intrinsic characteristics of the different modes of transmission described above, is that the effectivity of the control strategies may depend on the different transmission routes. This is specially important for strategies dealing with blockage of insect transmission, and can be illustrated with the different effectivity of insecticides. Indeed, pesticides are being used extensively to kill plant virus vectors, and the reduction of their populations densities served in many cases to minimize the incidence of circulative viruses. For non-persistent viruses, however, the success of these kind of measures is far from being common (Perring *et al.*, 1999).

3.1. Use of Insecticides in Virus Control: Drawbacks

The frequent use of insecticides is a common practice in modern agriculture. It is important to recognize the value of chemical control measures as part of integrated virus disease management programs. Except the chemicals-free organic farming, a prudent use of pesticides can be very beneficial for virus control. Unfortunately, in many occasions growers tend to abuse, arriving to certainly absurd situations like the periodical use of prophylactic treatments in high-value crops, which turn out to be a rather costly and inefficient strategy. On the other hand, vectors monitoring and forecasting is actually possible thanks to new technologies, for instance Geographic Information Systems. These technologies may help to precisely describe field distribution and spreading of insects, and therefore can serve to target pesticide applications in space and time, leading to new management strategies. This approach is known as site-specific or precision pest management (Weisz *et al.*, 1995), and could significantly reduce the amount of pesticides needed to achieve effective control.

As mentioned, even the extensive use of insecticides does not guarantee success in virus control. In particular, insecticide treatments are largely ineffective in control of non-persistent viruses (Raccah, 1986; Perring *et al.*, 1999) and only products that result in a reduced vector probing activity can contribute to the management of non-persistent viruses, with a reasonable efficacy (Irwin, 1999). Even worse, insecticides can be counterproductive, since they might paradoxically contribute to the spread of viral diseases by inducing greater vector activity and mobility. The importance of vector mobility on transmission is obvious, and there are abundant examples in the literature. For instance, in many cases it was reported that colonising aphid species contributed less to total virus transmission than non-coloniser ones (Raccah *et al.*, 1985; Harrington *et al.*, 1986; Garzo *et al.*, 2004). The decision about when to use insecticides is therefore complex, specially in the case of non-circulative viruses. Furthermore, the insects behaviour might be very different when probing or feeding on different plant species, and in many cases there is not enough information to know in advance if insecticides might be counterproductive or not. Studies on vectoring capacity are often contradictory, and only careful

experimentation, including analysis of insect behaviour, can clarify the real contribution of particular insects to the transmission spread of given viruses (Fernandez-Calvino *et al.*, 2006).

Another serious concern when using insecticides is the possibility of the appearance of resistance in the target insect populations. This factor can threaten effectiveness even for persistent viruses (Perring *et al.*, 1999). A good example of this event can be found in potato crops, where a reasonable amount of information is available (Robert *et al.*, 2000). In this case, the appearance of aphid populations resistant to insecticides in recent years forced the continuous adoption of new products without solving the problem (Parker *et al.*, 2006).

Finally, even in cases where pesticides are effective to control virus diseases, there are abundant unwanted side-effects caused by their use, such as the accumulation of toxic residues, or some negative impacts on non-target insects and beneficial species like honey-bees and other pollinating insects. Natural enemies of pests, including predators and parasitoids, may also be destructively affected. As a result of these forces, the use of pesticides might modify any equilibrium situation towards the emergence of new pests and/or pathogens. Therefore, we can conclude that, in spite of a presumably more rational use of pesticides in the future, the development of non-chemical management strategies will remain a need for controlling insect borne viruses.

3.2. Alternative Control Strategies

Apart from the use of pesticides, several management strategies are being used nowadays to control insect vectors, and the viruses they transmit. The concept of integrated measures was proposed to merge all available strategies into a combined effort, expected to be more efficient that each single measure alone. Evidently, the concept is dynamic, and it needs a permanent revision and decision-taking criteria to result adequate. This topic has been extensively reviewed recently (Jones, 2004; Thresh, 2006b). Here we will simply present a list of commonly used practices:

Cultural practices

- Use of certified virus-free plant material (seeds and propagation plants).
- Phytosanitary measures, including sanitation and cleaning of farming equipment and tools, elimination of plant residues, use of virus-free soil substrates and water supplies.
- “Roguing”, or physically removing symptomatic plants, including elimination of possible reservoirs (weeds, volunteer plants).
- Modification of sowing dates and crop-free periods, trying to uncouple crop presence in field from periods of peak activity of vectors.
- Confinement measures, applied to protected crops (greenhouses, plastic covers).
- Reinforce isolation, by means of distance between crops, or using barriers (non-host plants).

Biological control

- Use of natural enemies (predators, parasitoids) to reduce vector populations.
- Cross protection, using mild virus strains to protect against potential arrival of severe virulent strains.

Genetic resistance

- Against viruses.
- Against vectors (including resistance for the transmission process).

The adoption of genetically resistant crop plants is undoubtly one of the most desirable objectives among these measures. When available, the use of resistance traits, targeted against viruses, vectors or the vector transmission process itself, is an effective and safe measure of control. By recognizing this, the scientific community is currently devoting important efforts to explore, find and implement the use of genetic resistance. As a first consequence of these efforts, our understanding of the resistance mechanisms against viruses is improving greatly. There are recent works that review this topic in depth (Soosaar *et al.*, 2005; Kang *et al.*, 2005). For similar reasons, also numerous genes confering resistance against insect pests are being described. Recent developments dealt with the mechanisms of action operating in insect resistance (Moran & Thompson, 2001; Weng *et al.*, 2004; Klingler *et al.*, 2005; Alvarez *et al.*, 2006).

It is interesting to remark that in many cases the responses to insects attacks are very similar to the defensive traits activated by plants against pathogens (Kaloshian, 2004; Kaloshian & Walling, 2005). The importance of such traits for virus control has been recognized (Herselman *et al.*, 2004). In fact, the most interesting traits when looking at insect vectors are those that directly affect the transmission process. The *Vat* gene controlling resistance to cucumber mosaic virus (CMV) in melon (Lecoq *et al.*, 1979) is an excellent example. This gene was identified for its effect on virus transmission by *Aphis gossypii*, and its action is specific for this particular vector, being not active for instance with *M. persicae* (Lecoq *et al.*, 1980). The behaviour of both aphids has been studied, finding that the observed differences between them cannot explain the inhibition of transmission, and thus a mechanism of action during inoculation through blockage of stylet tips has been proposed (Chen *et al.*, 1997; Martin *et al.*, 2003).

Other genes affecting transmission are being studied (Diaz-Pendon *et al*., 2005). The recently characterized *Mi* gene of tomato has been implicated in resistance against nematodes, aphids (Rossi *et al*, 1998; Vos *et al.*, 1998), and also against whiteflies (Nombela *et al.*, 2003). Furthermore, these genes are homologues to virus resistance genes (Brommonschenkel *et al.*, 2000), suggesting that a shared or comparable signal transduction pathway might be operating during resistance against both viruses and vectors.

Unfortunately, and in spite of these advances, the slow pace of gene discovery, the absence of adequate sources of resistance in many crops, the long and costly

processes of breeding, and the risk of low durability (Lecoq *et al.*, 2004; Thresh, 2006a) constitute important disadvantages compromising the broad application of natural genetic resistance against viruses and vectors.

Deployment of transgenic plants against virus pathogens is a very attractive alternative. Several recent articles review extensively this topic (Goldbach *et al.*, 2003; Prins, 2003; Ritzenthaler, 2005), including safety issues (Tepfer, 2002). In some cases, the effect of virus-resistant transgenic plants on transmission was studied (Lapidot *et al.*, 2001; Shao *et al*., 2003; Jimenez-Martinez & Bosque-Perez, 2004). Finally, it deserves to be mentioned that non-transgenic approaches to achieve resistance exploiting RNA interference were recently described (Tenllado *et al.*, 2004).

To summarize, at the present moment the available strategies to control plant viruses, including the use of pesticides, are clearly insufficient for an adequate control in most cases (Jones 2004; Thresh, 2006b). Frequently, a combination of measures is required. The most adequate combination of practices depends on many factors, and for each particular case (pathosystem of crop plant, virus, vector and cultural conditions) a complete study needs to be implemented before deciding which control measures should be adopted to optimize effectivity and minimize costs and overall adverse impacts (Thresh, 1988; Irwin *et al.*, 2000). In this scenario, strategies aiming at transmission interference represent an attractive possibility, concentrating significant research resources.

4. INTERFERENCE WITH TRANSMISSION

When looking at the available strategies to interfere with transmission, there are two main targets for action: *i*) the whole insect; and *ii*) the molecular interactions required for the transmission process. In the first case, interference is based on affecting the insect behaviour through attraction, repellency or confusion. Another possible interference can be provided by non-toxic elements that impede or make difficult the insect feeding. In the second case, the possibility of blocking the molecular interactions during transmission can be, at least theoretically, exploited to interfere with virus dissemination.

4.1. Interference with the Insect

Manipulations that interfere with the landing responses of vectors can disrupt host selection and restrict virus spread, for instance through visual interference. This strategy can be implemented using UV-filtering plastic covers (Antignus, 2000). It is also known that aphids and other insects can be attracted by yellow traps (Cohen & Marco, 1973) and repelled by highly reflective surfaces (Kring, 1970), and therefore these elements have been used to control virus diseases. Recent examples of successful application of these methodologies to reduce virus incidence in crops are: *i*) the use of straw mulch against aphid-transmitted potyviruses in legumes (Jones, 1994) or potatoes (Saucke & Döring, 2004); *ii*) the high degree of protection against aphid transmitted viruses provided by polymer webs in peppers (Avilla *et al.*, 1997);

and *iii*) the effectivity of UV-blocking plastic covers in the control of aphid-transmitted potyviruses and thrips-transmitted tospoviruses in lettuce crops (Diaz *et al*., 2006).

Another system to interfere with insects relies on the application of semiochemicals and feeding deterrents (Herrbach, 1992). These products are intended to modify vector feeding behaviour, and putatively serve to decrease virus transmission. Biological control of vectors with natural enemies is not considered an effective management tool to reduce the spread of viruses in most cases, specially in non-persistently transmitted viruses. As already mentioned, just a few individuals of a non-colonizing vector species are needed to spread the disease. However, the study of these systems is interesting from a practical point of view. For instance, when aphids are attacked by predators or parasitoids, they release an alarm pheromone which causes other aphids in the vicinity to stop feeding and to move away (Hardie *et al.*, 1999).

Also sex pheromones are molecules with potential use in insect vectors control (Birkett & Pickett, 2003). Available data indicate that unknown components emitted by whiteflies-infested plants might contribute to localization of the insect host by parasitoids (Birkett *et al*., 2003). On the other hand, recent data suggest that the identification of plants infected by poleroviruses can be mediated by unknown volatiles that provoked attraction and arrest responses in aphid vectors (Eigenbrode *et al*., 2002). All these examples demonstrate that our understanding of chemical communication is improving, and that the acquired knowledge might lead soon to develop new practices of virus control. Table 3 summarizes a selection of recent results of research on insect vectors pheromones.

Table 3. Examples of insect vectors pheromones with potential use in virus control.

Insect	*Molecule(s) used*	*Type*	*Possible uses*	*Reference*
Aphids (*Myzus persicae, Acyrthosiphon pisum, Sitobion avenae*)	(E)-ß-farnesene (derived from the essential oil of *Hemizygia petiolata*)	Alarm pheromone	Repelency	Bruce *et al*., 2005
Aphids (*Aphis glycines*)	(1R, 4aS, 7S, 7aR)-nepetalactol + (4aS, 7S, 7aR)-nepectalactone	Sex pheromone	Mass trapping	Zhu *et al*., 2005
Mealybugs (*Planococcus ficus*)	Lavandulyl senecionate	Sex pheromone	Mating disruption	Walton *et al*., 2006
Thrips (*Frankliniella occidentalis*)	Decyl acetate + dodecyl acetate	Alarm pheromone	Landing reduction; increased take-off	MacDonald *et al*., 2002

The application of synthetic pheromones on leaf surfaces has an important drawback in the chemical instability and high volatility of the used propheromones, a fact that has stimulated the search of other ways to apply these non-toxic chemical products. Transgenic plants expressing a pheromone that elicited potent effects on behaviour of *M. persicae* (alarm and repellent responses) and its parasitoid *Diaeretiella rapae* (an arrestant response) have been already produced (Beale *et al.*, 2006), and might contribute to the development of new strategies of vector control.

Barrier plants are used as another management tool, useful in virus control (Shelton & Badenes-Pérez, 2006). Plants differing from the main crop are used to border or separate fields to control the spread of viruses. Barrier plants act as a natural sink for non-persistent viruses and have proved to be an effective crop management strategy to protect against virus infection (Hooks & Fereres, 2006). Effective barrier plants must be non-host for the virus and the vector, but appealing to vectors landing. These plants should be attractive to vector natural enemies too, allowing sufficient residence time for vector probing before they take-off. Examples of the effectivity of different barrier plant in protection of several crops against viruses are abundant, and they have been extensively reviewed recently, focusing on the mechanisms operating and on the integration of barriers use along with other management strategies (Hooks & Fereres, 2006). Table 4 illustrates some recent examples of successful use of barriers against virus spread in integrated disease management.

Oil sprays are effective low-toxicity products used against vector transmission of non-persistent viruses (Webb & Linda, 1993; Powell *et al.*, 1998). Studies with aphids have shown that mineral oils alter the surface structure of aphid stylets and thus interfere with the ability to retain virus particles reducing transmission efficiency (Wang & Pirone, 1996). Recently, the use of kaolin-based particle films has been reported to effectively reduce the damage caused by whiteflies (Liang, & Liu, 2002) or aphids (Eigenbrode, 2006). Although still limited, the preliminary available information about effects of kaolin treatments on virus transmission is very promising. Significant lower incidence of *Beet curly top virus* was observed on kaolin-treated chilli pepper plants than on untreated controls (Creamer *et al*., 2005).

Table 4. Examples of barrier plants used to control insect vectors of plant viruses [a].

Insect vector and viruses	*Crop*	*Barrier plant*	*Reference*
Aphids (cucumoviruses)	Chilli pepper	Maize, sorghum, sunflower	Anamdam & Doraiswamy, 2002
Aphids (cucumo- & potyviruses)	Pepper	Maize, vetch, sorghum	Fereres, 2000
Aphids (potyviruses)	Lupins	Oat	Jones, 2005
Thrips (tospoviruses)	Lettuce	Cabbage	Coutts *et al*., 2004

[a] Adapted from Hooks & Fereres (2006).

Drawbacks of these management tools are the weather parameters that may affect the efficiency and persistency of mineral oil sprays or kaolin films. Furthermore, young plant tissues growing after spraying or films application remain unprotected.

4.2. Virus Specific Receptors in Insects

The identification of insect receptors implicated in the transmission process would provide insights into the molecular mechanism of virus transmission, and thus facilitate the development of new strategies to interfere with the process and limit virus spread. The basis of such strategies would be the disruption of one key interaction, for instance using artificial molecules that mimic one of the molecular elements (virions, auxiliary factors or vector receptors) in the interaction, and either bind irreversibly or compete with the natural product.

The putative virus specific receptors in the vector are first candidates to be blocked. Although almost no information is available about receptors for non-circulative viruses in the aphid mouthparts, studies with circulative viruses identified several proteins as possible receptors along the pathways followed by virus particles in the aphid (Gildow & Gray, 1993; Van den Heuvel *et al.*, 1997; Li *et al.*, 2001; Reinbold *et al*, 2001; Seddas *et al.*, 2004).

Several studies rely on far Western-blot methodologies to identify vector components that interact with virus particles. This approach was used successfully to detect potential virus receptors in aphids and other insects. For instance, several proteins within *M. persicae* bind *in vitro* to particles of a polerovirus, including symbionin from a bacterial endosymbiont (Van den Heuvel *et al.*, 1997). Two proteins were identified in extracts from *Sitobion avenae* heads that bound specifically to purified particles of a luteovirus (Li *et al.*, 2001). Seddas *et al.* (2004) also showed *in vitro* interaction between polerovirus particles and *M. persicae* proteins that may be involved in the epithelial transcytosis of virions in the aphid vector. In this system, particles glycosilation seems to play a role in the process (Seddas *et al.*, 2006).

For other insects, such as thrips, experimental evidence supports the existence of a specific interaction between glycoproteins of Tomato spotted wilt tospovirus (TSWV) with cellular receptors in the thrip midgut (Ullman *et al*., 1992; 2005). Specifically, a 50 KDa thrip protein, detected by far-Western analysis (Bandla *et al.*, 1998) and by immunoprecipitation assays (Medeiros *et al.*, 2000), binds to TSWV particles, although its low abundance precluded further characterization. This situation is common in the study of virus–vector interactions, both in plant and in animal systems.

In the case of non-persistent transmitted viruses, the virions are retained in the lining cuticle of the aphid stylets (Ammar *et al.*, 1994). However, it is not known if this retention is mediated by a single receptor or by a more complex structure. The roles that the aphid saliva might play, alone or together with the plant sap acquired during the feeding process, is also unknown. In these viruses, a presumed interaction of those hypothetical receptors with virions or with helper factors could be competed, interfering with the transmission process.

The strategies described require an adequate structural knowledge of the molecules involved. Therefore, solving the structure of virions and of auxiliary transmission factors is a topic of the outmost interest. Recent advances in this field were obtained for instance with CMV, a virus transmitted following a CP-only strategy (Liu *et al.*, 2002; Ng *et al.*, 2005). In the case of viruses that use helper-strategies, structural data of the auxiliary factors are available for caulimoviruses and potyviruses. The association of P3 protein to caulimovirus virions, mediating the binding to P2, has served to propose a mechanism of action (Plisson *et al.*, 2005). For potyviruses, the structure of HC-Pro aggregates is actively pursued (Plisson *et al.*, 2003; Ruiz-Ferrer *et al.*, 2005). Interestingly, a recent report showed the presence of HC-Pro associated to one end of virions, a fact that could have important implications on transmission (Torrance *et al.*, 2006).

5. PROSPECTS

Although there is a significant amount of literature data describing the various virus-arthropod associations, there is still insufficient information about the molecular mechanisms that regulate the transmission processes and determine its efficiency. Nowadays, molecular biology tools allow the genetic manipulation of viruses, plants and insects. These possibilities encourage new studies to better understand such mechanisms. The basic information, generated by ongoing research programs, would eventually facilitate the development of strategies to interfere with the process and limit virus spread.

Effects of climate changes (global warming) and other alterations of the current situation regarding vector populations and virus problems are new issues that we must face in the near future. Indeed, cases of emerging diseases spread by new and/or old vectors in a changing environment are frequently reported. These unexpected and unforeseen situations would continue to appear, and our response should be based on judicious scientific knowledge to follow the most effective and rational control strategies. Also the adoption of new agriculture policies might result in disappearance of traditional crops and their substitution with novel and less studied crops, such as plants devoted to energy (biofuels) production. All these changes would create new challenges that we need to accept and deal with.

In addition to the described advances on identification of resistance genes in plants and use of transgenic approaches, manipulation of plant genomes may represent another way to deal with pests infestation, and indirectly, with virus transmission. Some results on defence-related mechanisms suggest that alterations of key genes (Vancanneyt *et al.*, 2001) might serve to this purpose. Experimental systems to apply genomic tools to insect/plant interactions are being explored actively (Moran *et al.*, 2002; Divol *et al.*, 2005; Hunt *et al.*, 2006). Also transgenic approaches, such as the mentioned use of plants expressing alarm pheromones (Beale *et al.*, 2006), await future developments.

An exciting field of research that might be applied in plant virology in the future is provided by innovative studies with mosquito vectors of animal viruses (Sanchez-Vargas *et al.*, 2004). In one of these pathosystems - the human dengue virus

transmitted by *Aedes aegypti* mosquitoes - genetically modified insects were produced to transgenically express inverted repeats targeting, through RNAi mechanisms, specific genes that lower the virus transmission capacity of the vector (Franz *et al.*, 2006). The expected outcome of such approach will be the development of vector populations replacement strategies to control virus transmission. Although public acceptance for this sort of approaches might be difficult to obtain in cases that do not involve human health threats, at least theoretically we can envisage similar strategies applicable to vectors of plant viruses.

To test if modification of a vector transmission capacity is feasible, a great deal of basic research will be initially needed, for instance to select target genes, or to develop transformation systems for the different types of vectors. Again, the mosquito system might provide a guidebook of alternatives (Carlson *et al.*, 1995), including, for instance, the use of entomopathogenic viruses as expression vectors in insects (Carlson *et al.*, 2006). As an attractive possibility, we can envisage the use of circulative-propagative plant viruses for a similar purpose, adapting them to the genetic manipulation of insect vectors, and to the control of virus diseases. As a precedent, the small RNA viruses of insects are being considered as potential sources for resistance against insect pests by engineering transgenic plants (Gordon & Waterhouse, 2006).

The impact of genomics, transcriptomics, proteomics and other approaches to address complex biological problems is gaining momentum in recent times. In addition to the available completed plant genomes and the ongoing programmes dealing with different plant species, we can also envisage that these new tools could be potent driving forces in the study of insect mediated transmission of viruses. Indeed, genomic programmes for aphids are under way (Sabater-Munoz *et al.*, 2006), which could facilitate the identification of genes of interest in these important vectors.

6. CONCLUSIONS

Control of plant pathogenic viruses is one of the major challenges of agriculture, necessary for both traditional crop systems as well as for modern and high-tech plant production. Prevalence of viral diseases over time indicates that most current control measures are clearly insufficient. This is specially true for the chemical control of vectors. Besides, the demand for clean and safe products, together with the need of preserving the environment, requires the use of alternative measures.

We briefly presented herein the available and future strategies for interfering with transmission. Some of them are already in use, specially those acting at the individual insect level, and include insect confusion, vision interference, or induction of difficult feeding. It is envisaged that new products and procedures with better performances might be developed in the near future. The recent advances on molecular biology are disclosing new control strategies, aiming to block the required molecular interactions between viruses and insects. It is difficult to predict which of these strategies might result useful in the long run, but it is sure that exciting new

advances will be attempted in the near future, and that some of them will eventually serve to develop and deploy better control measures.

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NOVEL TENSIO-ACTIVE MICROBIAL COMPOUNDS FOR BIOCONTROL APPLICATIONS

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Abstract. Several microorganisms are known to produce tensio-active compounds (biosurfactants). They have emerged out as successful alternative to synthetic surfactants. The enormous diversity of biosurfactants makes them interesting for application in several areas. Rhamnolipids are one such heterogeneous group of compounds which has been studied as a model system and acquired a status as potential performance-effective molecules in various fields, like production of speciality chemicals, additives for environmental remediation and biological control agent.

1. INTRODUCTION

Advances in the era of industrial globalization have increasingly directed several industries towards biotechnology. While the world market for biotechnology products was US $ 1.7 billion in 1992, it has increased beyond US $ 500 billion (Muller *et al.,* 1997). Surfactants constitute an integral part of chemical feedstock inventory to many industries and are mainly synthesized from petrochemicals. Their world-wide production is estimated to exceed four million tonnes and by US $ 9-10 billion per year (Banat *et al.,* 2000). Surfactants are basically amphipathic compounds which partition at interface between fluid phases and hydrogen bonding. These characteristics allow the reduction of the surface and interfacial tension, leading to formation of micro-emulsion. Such characteristics impart better wetting, spreading, foaming, detergency and emulsifying traits, rendering them most versatile process chemicals (Banat *et al.,* 2000). However, with increased environmental awareness among consumers, rapid progress in biotechnology, ecological acceptability of biosurfactants and new stringent legislation(s) aiming at ecosystem protection, stimulated consideration of biosurfactants as alternatives to chemical surfactants (Banat *et al.,* 2000; Kim *et al.,* 2000a; 2000b).

2. BIOSURFACTANTS

World-wide interest in biosurfactants significantly increased in the recent years due to their ability to mitigate most requirements of chemical surfactants (Banat, 1995; Chhatre *et al*., 1996). Biosurfactants are a group of heterogeneous secondary

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metabolites, synthesized by a variety of microorganisms during their growth on water immiscible substances. These secondary metabolites are complex, amphiphilic molecules, possessing a hydrophilic moiety (amino acids or peptides, anions or cations, di- or polysaccharides) and hydrophobic region (saturated or unsaturated hydroxylated fatty acids or fatty alcohols) (Lang & Wullbrandt, 1999).

The main properties, having considerable applied interest, are *i)* reduction of the surface tension, *ii)* stabilization of emulsions, *iii)* promotion of foaming, *iv)* induction of flocculating action, *v)* increasing wetting, spreading and penetrating action(s) and *vi)* enhancement of microbial growth and metal sequestration (Kosaric, 2001). Their production and properties were reviewed by several researchers (Cooper, 1986; Fiechter, 1992; Georgiou *et al.,* 1992; Kosaric, 1993; 2001).

Biosurfactants are gaining prominence by virtue of commercial applicability (Banat *et al.,* 2000; Kourtkoutas & Banat, 2004) due to their *i)* biodegradability, *ii)* lower toxicity, *iii)* environment-friendly characteristics, *iv)* feasible fermentative production on economical renewable resources, *v)* functionality under extreme conditions (temperature, *p*H, salinity) and in small quantities, *vi)* specificity of application and *vii)* potential of tailoring to suit specific applications. Due to these potential applications, ranging from biotechnology to environmental cleanup, biosurfactants became a unique commodity (Kosaric, 1996; 2001; Ishigami, 1997; Banat *et al.,* 2000).

These applications are summarized in Table 1. Several low or high molecular weight biosurfactants are mainly categorized into *i)* glycolipids, *ii)* lipo-amino acids and lipopeptides, *iii)* lipo-proteins and lipo-polysaccharides and *iv)* phospholipids, mono and diglycerides and fatty acids (Desai & Banat, 1997; Karanth *et al.,* 1999; Kosaric, 2001). While many microbes synthesize biosurfactants, this review places particular emphasis mainly on rhamnolipids and their role, particularly as a biocontrol agent.

3. RHAMNOLIPIDS

Rhamnolipids are among the most effective biosurfactants known today (Kosaric *et al.,* 1987; Lang & Wullbrandt 1999; Maier & Soberon-Chavez, 2000). The production of rhamnolipids by *Pseudomonas aeruginosa* was initially described by Jarvis & Johnson (1949). The synthesis of these surfactants by cell-free extracts and their secretion by microorganisms during a stationary growth phase were subsequently described (Hauser & Karnovsky, 1958). Duynstee *et al.* (1998) chemically synthesized rhamnolipid in six stages with phenyl-3,4-O-(2,3-dimethoxybutane-2,3-diyl)-1-thio-α-L-rhamnopyranoside and phenacyl-(R)-3-hydroxydecanoate.

Basically, rhamnolipids are glycolipids produced by different bacterial species, like *P. aeruginosa, Bacillus* sp. AB-2, recombinant *P. putida* and *Escherichia coli*, growing on cost effective hydrocarbon substrates (Banat, 1993; Arino *et al.,* 1996; Lee *et al.,* 1999; Lang & Wullbrandt, 1999; Banat *et al.,* 2000; Benincasa *et al.,* 2002; 2004).

Table 1. Applications of microbial surfactants and functional properties.

Function	*Area of application*
Biodegradation and bioaugmentation activities	Bioremediation of soil, oil contamination
Biological control	Agriculture
Detergents	High-tech and agricultural products
Deemulsification	Waste treatments
Dispersants	Paints, coal-water/oil slurry
Emulsification	Pharmaceutics, cosmetics, paints and food, drug delivery, perfluorochemicals, vaccine adjuvants
Flour additives and shelf life improving agent	Food industry
Foam formation	Cosmetics, floatation
Microbial growth promotion	Oily sludge processing, fermentation processes
Metal sequestration	Mining
Micelle formation (micro emulsion)	Pharmaceutics
Resources recovery agents	Tertiary oil recovery
Sweetening agent	Food
Thickening agents	Paints, foods
Therapeutics	Chemotherapy by intratumoral injections
Viscosity reducing agents	Post fermentation recovery, pipelines, filtration
Wetting agents	Pharmaceutics, paints

3.1. Structure of Rhamnolipids

Several species of *Pseudomonas* are known to produce primarily two forms of rhamnolipids viz. rhamnosyl-β-hydroxydecanoy-β-hydroxydecanoate (mono-rhamnolipid) and rhamnosyl-rhamnosyl-β-hydroxydecanoyl-β-hydroxydecanoate (di-rhamnolipid), which are consisting of 1 or 2 rhamnose residues and 2 molecules of β-hydroxydecanoic acid (Edwards & Hayashi, 1965).

Jarvis & Johnson (1949) demonstrated a glycosidic linkage between the hydroxyl group of one of the acids with the reducing end of two rhamnose residues, whereas the hydroxyl group of β-hydroxydecanoic acid is associated with ester formation. One of the free carboxylic groups yields rhamnolipid anions above *p*H 4.0. These molecules are synthesized by two sequential rhamnosyl transfer

reactions, each catalyzed by a specific rhamnosyl transferase and are excreted into the culture medium. Recently, up to 28 new rhamno-surfactants were detected by HPLC-MS (Abalos *et al.,* 2001; Benincasa *et al.,* 2004). The homologues of rhamnolipids are formed by one or two rhamnose molecules linked to one or two alkyl chains of saturated or unsaturated fatty acids, between C_8 and C_{12}. However, the type of rhamnolipid produced depends on the bacterial strain, the C source used and the metabolic strategy (Lang & Wullbrandt, 1999; Déziel *et al.,* 2000; Haba *et al.,* 2003).

3.2. Physiological Role of Rhamnolipids

Several physiological aspects are not yet clearly understood. Rhamnolipid secretion by *P. aeruginosa* (100 $g \cdot L^{-1}$) occurs under limiting concentrations of N, P and Fe during late exponential and early stationary growth phases (Ochsner *et al.,* 1995; Arino *et al.,* 1996; Lang & Wullbrandt, 1999; Chayabutra *et al.,* 2001).

Rhamnolipids change reversibly their morphology as a function of *p*H changes. Their molecular aggregates transform from vesicles under acid conditions, to lamellae, lipid particles and micelles under weakly acidic conditions within a narrow *p*H range of about 5-7. Thus, they have an anionic character at *p*H 6.8, whereas they are almost totally protonated and exhibit nonionic behavior at *p*H 5.0 (Ozdemir *et al.,* 2004). Rhamnolipids display further physiological properties, remarkably they may *i)* reduce the surface tension of water from 72 to < 30 $mN \cdot m^{-1}$ (Abalos *et al.,* 2001), *ii)* minimize interfacial tension of water and oil systems from 43 $mN \cdot m^{-1}$ to < 1 $mN \cdot m^{-1}$, *iii)* exhibit a critical micelle concentration (CMC) of 10-30 $mg \cdot L^{-1}$ and *iv)* demonstrate excellent emulsifying power with a variety of hydrocarbons. Ozdemir *et al.* (2004) observed that intermolecular interactions between rhamnolipid molecules allow foam formation, but its stability depends on the air flow rates as affected by the elasticity of the monolayer, whereas its volume and durability depend on the *p*H.

Finally, rhamnolipids were found to *i)* increase dispersion of hydrophobic compounds in water (Zhang & Miller, 1992), *ii)* enhance recovery of polyaromatic hydrocarbons (PAH) from soils (Van Dyke *et al.,* 1993) and *iii)* stimulate uptake and degradation of PAH, indicating a physiological role to enhance bioavailability of molecules otherwise recalcitrant. Structure networking, behaviour, multiple functions and determinants may throw light on their precise physiological role in the secreting organism and outside its cells.

4. MICROBIAL PRODUCTION OF RHAMNOLIPIDS

Several authors pointed that a prerequisite for rhamnolipid production is growth limitation, which is induced by limiting N, P or multivalent ions and excess C (Desai & Banat, 1997; Lang & Wullbrandt, 1999; Chayabutra *et al.,* 2001). Among the C sources, glycerol, glucose and n-alkanes are used (Arino *et al.,* 1996; Lee *et al.,* 1999; Rahman *et al.,* 2002). However, economic production depends on the use of low cost raw materials and development of cost-effective processes. Alternative

cost-effective substrates like molasses, whey, distillery wastes, effluent from olive oil or soybean oil processing and ethanol were explored to minimize the cost of production of raw material and to compete with their chemically synthesized counterparts (Patel & Desai, 1997; Sim *et al.,* 1997; Daniel *et al.,* 1999; Haba *et al.,* 2000; Abalos *et al.,* 2001; Rahman *et al.,* 2002). Use of water immiscible substrates like oil, hydrocarbon contaminated waste, waste frying oil, soap stock and vegetable oil were also reported (Haba *et al.,* 2000; Abalos *et al.,* 2001; Benincasa *et al.,* 2002; Haba *et al.,* 2003).

Rhamnolipids are commercially produced at the level of 100 $g \cdot L^{-1}$ (Matsufuji *et al.,* 1997; Lang & Wullbrandt, 1999). At such productivity level, their cost becomes competitive with chemical surfactants. Besides, cost-effective production of rhamnolipids can be achieved by batch fermentation under growth limiting or resting cell conditions (Wagner *et al.,* 1984; Gruber *et al.,* 1993), by immobilized cells (Wilson & Bradley, 1996), or by a continuous process with free cells (Fiechter, 1992; Ochsner *et al.,* 1996). All these aerobic processes, however, suffer from heavy foaming, which is fast and stable even at low product concentration (Wu, 1997). To alleviate this constraint Chayabutra *et al.* (2001) suggested denitrification of production medium or alternative respiration routes to destabilize foam formation, possible process contamination and consequent economic losses.

5. APPLICATIONS

Rhamnolipids with CMCs in the range of 5-200 $mg \cdot L^{-1}$ are capable of reducing the surface tension of water to 25-30 $mN \cdot m^{-1}$. These values are favourable compared to those for the chemically derived surfactants, which display higher CMC levels and lower efficiency. Because of these properties rhamnolipids (Table 2) are widely used in cosmetic, pharmaceutical formulations, agriculture and food industries (Banat, 1995; Maier & Soberon-Chavez, 2000).

Rhamnolipids have several industrial and environmental applications, including in the production of fine speciality chemicals (eg. rhamnose as a flavouring agent) and characterization of surfaces and surface coatings (Maier & Soberon-Chavez, 2000; Ozdemir *et al.,* 2004). They are implicated in the attachment of bacterial cells to surfaces and in the maintenance of biofilm architecture (Davey *et al.,* 2003). They are also used in cosmetic industry, because to their compatibility with skin, to reduce dermal irritation (Haba *et al.,* 2003). In the food industry, emulsification allows the formation of phase dispersion, consistency and texture.

Recently, rhamnolipids attracted increased interest within the petroleum industry for the prospects of enhanced oil recovery. Because of their ability to emulsify hydrocarbon-water mixtures and complexion, rhamnolipids are used as an additive for environmental remediation of dispersed hydrocarbons (Rahman *et al.,* 2002). The use of biosurfactants promoted 94% oil recovery in 77 hrs, compared to 81% oil recovery in 112 hrs in control (Banat *et al.,* 2000). Biological activity of biosurfactants also raised a considerable interest in the agriculture and industrial

Table 2. Potential applications of rhamnolipid biosurfactants based on functional properties.

Property	*Application*
Emulsifying activity	Cosmetics, food and pharmaceutics (liposomes)
Growth stimulating activity	Hydrophobic hydrocarbons/oil or environmental bioremediation from soil
Emulsifying and solubilizing	Enhanced oil recovery and bioremediation
Antimicrobial activities (antibacterial, antifungal, antiviral)	Biocontrol in agriculture, food and pharmaceuticals
Additives, strengthening agents	Cement concrete
Flavouring agent (rhamnose)	Processed food (precursor feed stock)
Resource recovery	Oil spill recovery
Hydrophilic carrier	Difficulty soluble drugs
Complexing agent	Heavy metals recovery, decontamination of polluted areas
Adjuvant & supplementing	Vaccines and pharmaceutics (pulmonary surfactant)
Flushing agent, flushing additives and dispersant	Environmental bioremediation of hydrocarbons, pesticides, metals

sectors (Lang & Wullbrandt, 1999). The subsequent section explores the biological activity of rhamnolipids and their potentials in crop protection.

6. BIOLOGICAL ACTIVITIES

Widespread application of pesticides and agro-chemicals to intensive cropping results in their accumulation in soil, water and ecosystem. These recalcitrant chemicals not only entered the food chain, but equally proven to be toxic and at times carcinogenic. Therefore, their further release in water, soil and food must necessarily be reduced. Towards this application, use of effective biocontrol agents could offer better alternatives to existing strategies adapted for pest control. Being environmentally compatible, negligibly toxic, biodegradable and effective at extreme temperatures or *p*H, rhamnolipids acquired a status of potential performance-effective biocontrol compounds for crop protection.

6.1. Fungicidal Activity

For biological control of plant pathogenic fungi, potential of rhamnolipid produced by *Pseudomonas* was recently recognized. On the background of efficacy of synthetic surfactant to control *Olpidium brassicae* and *Pythium aphanidermatum* (causal agent of the Big vein disease in hydroponically grown lettuce), use of rhamnolipids was promoted against the zoosporic stages (no cell wall) in the plant pathogen life-cycle (Maier & Soberon-Chavez, 2000). A similar activity was

investigated in root infection of cucumbers and peppers caused by *Pythium aphanidermatum* and *Phytophthora capsici* (Stanghellini *et al.,* 1996), through monitoring lysis of fungal zoospores by rhamnolipids. Whether purity of rhamnolipids had any role was examined at concentrations ranging from 5-30 $\mu g \cdot ml^{-1}$. Cessation of motility and lysis of entire zoospore population of both pathogens within < 1 min was observed (Stanghellini & Miller, 1997). In fact, introduction of rhamnolipid secreting strains into the recirculating hydroponic system also demonstrated to produce an effective control of *P. capsici*. Kim *et al.* (2000a,b) consolidated these observations by demonstrating that rhamnolipids have not only zoosporicidal activity, but also inhibit spores germination and hyphal growth of several pathogens. Thus, by the application of rhamnolipids, mycelial growth of *P. capsici* and spore germination of *Colletotrichum orbiculare* were inhibited, with suppression of the disease caused by these fungi on pepper and cucumber plants.

Similarly, De Souza *et al.* (2003) confirmed the lysis of zoospores of multiple oomycetes, including *Pythium* sp., *Albugo candida* and *Phytophthora infestans* in 30-60 sec. These investigations established that zoospore taxis is an essential part of the pre-infection process and a susceptible target for controlling diseases caused by oomycetes and zoosporic fungi.

Recently, the U.S. Environment Protection Agency (2004) approved a biofungicide formulation (Zonix ^{TM}) containing 8.5% rhamnolipids for controlling zoosporic plant pathogenic fungi, including downy mildew, *Pyhtium* and *Phytophthora* spp. on a variety of crops, including tubers and vegetables, citrus fruits, ornamental plants, trees, shrubs, bedding plants and turf grasses. It was observed that rhamnolipids disrupt the cell membrane via a physico-chemical surfactant action, which destroys permeability, with loss of motility and rapid lysis of zoospores. Aqueous solutions of the biofungicide are applied at concentrations between 85 and 125 ppm. The mode of application is conventional (spray, fogging, drenching, chemigation, hydroponics) to saturate the crop, soil or growth medium being treated. The volume consumed depends on plant density and soil conditions.

Rhamnolipids showed excellent antifungal properties against *Aspergillus niger, Gliocadium virens* (16 $\mu g \cdot ml^{-1}$), *Chaetonium globosum, Penicillium crysogenum, Aureobasidium pullulans* (32 $\mu g \cdot ml^{-1}$), whereas the growth of phytopathogenic fungi like *Botrytis cinerea* or *Rhizoctonia solani* was inhibited at 18 $\mu g \cdot ml^{-1}$ (Abalos *et al.,* 2001). The spectrum of activity broadened when Benincasa *et al*. (2004) observed that rhamnolipid-like compounds are active against *Penicillium, Alternaria, G. virens* and *C. globosum* at concentrations of 32 $mg \cdot L^{-1}$.

6.2. Antiviral Activity

In addition to fungicidal activities, rhamnolipids were also been investigated for controlling viral infection in crop plants. Haferburg *et al.* (1987) successfully used 1% rhamnolipids emulsion for treatment of *Nicotiana glutinosa* leaves infected by *Tobacco mosaic virus* (TMV) and for control of *Potato virus X* disease, while,

Bunster *et al.* (1989) used rhamnolipids to improve wettability of leaf surface, which probably involved detachment of the hydrophobic waxy layer.

7. CONCLUSIONS

Rhamnolipids being produced in high yield and commercial scale, their application will be economically competitive with synthetic surfactant alternatives. These compounds have low US EPA regulatory requirements which paved the way to enhance their utility as biological control agents for agriculture crops. Demand for rhamnolipids in agriculture will steadily increase provided they are still produced at an economical scale and yield. Due to their versatile properties, rhamnolipids could acquire in the near future several potential applications not only in the specific field of plant protection as low cost, biological control agents but also in associated sectors related to their wide range of potential applications.

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MOLECULAR DETECTION IN INTEGRATED PEST AND DISEASE MANAGEMENT

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Abstract. The basic principles of detection applied to IPM of plant pests and diseases are described, including immunodetection, monoclonal antibodies, DNA-based detection procedures and molecular fluorescent probes. Applications in disease and pest management are revised, in reference to field detection of plant pathogens, detection in vectors and pathogens identification from soil through DNA extraction and identification. The role of detection in quarantine of invasive species, pests and diseases epidemiology and identification of biological antagonists, including parasitoids and biological control agents, is revised. Finally, the application of molecular markers in IPM strategies based on plant resistance is discussed.

1. INTRODUCTION

Pests and pathogens are the result of an historical adaptation of different organisms to the plant genetic changes induced by man through the domestication of ancient wild species. Several evolutive and adaptive processes are responsible for pests and pathogens specialization and insurgence, including co-evolutive adaptation and/or host switch. In any case, pests and pathogens were always associated to the evolution of agriculture, producing recurrent epidemics and outbreaks of different magnitudes, in function of the anthropic intervention and the historical or technical crop backgrounds.

Having knowledge in advance about the presence of a pest or pathogen, in an infected plant material, vector or natural reservoir, is the first requirement for a successful management strategy or a better understanding of pests or diseases behaviour. The principal goal is to assure the sustainable management and protection of the crop and its environment, as well as the production of healthy and safe agricultural products, avoiding pests or pathogens dispersal through infected propagation material (Martin *et al*., 2000; Rowhani *et al*., 2005).

Since the spatial dispersal of plant pests or pathogens cannot be completely circumvented but can be controlled, their early and rapid detection is fundamental for prevention and exclusion strategies (Jones, 2001; 2004; Louws *et al*., 1999; Rowhani *et al*., 2005). The basic goal is the identification of the most efficient

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control measures and the development of management programs limiting, at different scales, the spread of species into areas not previously invaded, either at the farm, regional or national levels. For these reasons, methodologies relying on i. e. DNA-based technologies, gene sequencing or polymerase chain reaction (PCR) (Mullis & Faloona, 1987), actually support the most useful and advanced detection applications, as they improved either the quantitative and qualitative identification of almost any kind of plant pest or pathogen. For any control programme, either chemical, biological, physical or cultural, accurate and early identification is of the utmost importance, in any agricultural context.

In this chapter we review some fundamental principles of molecular detection, including some basic concepts in molecular tools applications, and their possible involvement in pest and disease management strategies, as well as their potentials in the identification of useful characters in plants, as provided by molecular markers and genetic studies.

2. BASIC PRINCIPLES OF DETECTION

2.1. Conventional Tools

Traditional methods of plant pathogens identification were basically descriptive, relying on careful measurement and comparison of symptoms or morphological traits, which must be agreed upon by the corresponding community of plant pathologists and/or technicians and taxonomists.

Many plant pathogens or pests are indeed difficult to identify, as in the case of insects, nematodes and fungi, and their correct identification requires special trainings. When dealing with microscopic organisms such as phytoviruses, any morphological identification is unpractical, as detection would require the study with advanced electron microscopy techniques. Detection was previously performed through specific visual traits of the disease symptoms shown by attacked plants. Furthermore, phytoviruses are frequently prone to a fast evolution, occurring within a few years (Aranda *et al*., 1993; Finetti Sialer *et al*., 2002; Seal *et al.,* 2006), giving rise to more virulent strains characterised by different symptoms displayed by affected plants. These also include the absence of any phenotypic change, as in the case of virus latency (Boulton *et al*. 1991; Hammond *et al*., 1999; Shepherd *et al*., 2005). In this latter case, plants visual inspection fails in detecting the presence of the virus, with potential risks in quarantine prevention or deriving by a possible host switch and eventual loss of virus latency. This selective pressure is especially evident in RNA viruses, allowing them to face unpredictable environmental changes (Koonin & Dolja, 1993).

In the case of plant pathogenic bacteria accurate identification often requires the use of complementary methods, as the pathogen must be isolated, purified and characterised by a series of biochemical, physiological and pathogenicity tests (Braun-Kiewnick & Sands, 2001). Similar considerations hold for plant parasitic fungi, although they frequently offer enough morphological features leading to a

morphology-based description and identification. Host pathogenicity tests are, however, required for detection of different races or *formae specialis*.

2.2. Molecular Tools

The diagnostic techniques available nowadays are mainly based on molecular and DNA sequence data. They are rapidly evolving, as data accumulation, consequence of many sequencing projects of plant pathogens genomes, allows the elaboration of new and accurate detection reagents, improving speed and reliability. At the same time, last generation detection technologies permit the identification of pathogens from lower or even minute quantities of sample material and are particularly relevant for pathogens characterized by low or non-immunogenic reactions. Furthermore, molecular identification techniques are useful in revealing new diseases of unknown aetiology. They offer the advantage of the possible discovery of mutations or recombination events in hosts and/or pathogens, as well as the detection of specific genomic elements and their reassortment. These technologies do not only reveal the presence of the pathogen, but could even be used, with higher specificity and sensitivity, for the accurate quantification of its biomass in a given environment (i. e. host plant tissues or soil).

The comparative analysis of genomic sequences allows the phylogenetic reconstruction of the pests and pathogens relationships, at different taxonomic levels (Brunner, *et al*. 2002). This possibility may highlight differences among pathogenic and not pathogenic species, improving the detection resolution, relying on the sequence databases actually available for interrogation and study (Altschul *et al*., 1990). The most common diagnostic procedures actually applied for detection are: serologic techniques, like the enzyme-linked immunosorbent assay (ELISA) with the production of monoclonal or polyclonal antibodies, mainly applied to viruses and bacteria; molecular probes, especially designed to recognize specific sequences and applied to a wide number of diverse targets; hybridization techniques, like dot-blot and other electrophoretic techniques, restriction fragment length polymorphisms (RFLP), or variable number of tandem repeat (VNTR); *in-situ* hybridization and fluorescence detection techniques, and several applications based on the polymerase chain reaction (PCR).

One of the main constraint in the exploitation of any diagnostic technique is given by the previous knowledge required about the magnitude of the target diversity range, directly affecting the resolution level of the technology chosen. The term “biodiversity”, although initially referred to the number of species present on a given space, actually embraces also the concept of “genetic diversity” and is commonly used also to indicate the degree of divergence among given sequences in a taxonomic group, at the inter- or intra-species levels.

2.2.1. Immunodetection

Since Clark & Adams (1977) described a method for the detection of plant viruses with ELISA, the antibody-basic diagnostic method was expanded to several plant

pathogens, including bacteria, fungi and phytoplasmas. Basically, the technique relies on the capacity of the immune system of higher vertebrates to produce antibodies after an infection. The antibodies are small proteins with a typical Y shape, having a specific biological activity as they bind to and inactivate their own specific target, represented by a molecule (antigen) of an invading pathogen.

The serologic detection is based on the reaction between the antigen and its self homologue antibody, produced by i. e. rabbits or horses, previously immunized. The antigenic capacity of the pathogen is provided by some specific molecules, i. e. in the case of phytoviruses, by the capsidic proteins. The reconnaissance capacity is given by the reaction of the polyclonal antibodies produced by the animal, and persists also in other contexts outside the producing organisms, conferring a high utility for the *in-vitro* serologic detection. The bulk of polyclonal antibodies produced in this way represents the issue of a generalized immunitary response of the infected animal, and is composed by a variable population of diverse antibodies, reacting with different antigenic determinants.

There are several assays revealing the antigen-antibody reaction *in-vitro*. The first tests were the direct ones, developed on agar medium, in which the reaction was revealed by a precipitation band visible by a simple visual inspection of the dish. The second generation of assays was based on the amplification of the antigen-antibody reaction, performed through the use of latex beads. A significant improvement of the techniques was achieved through the introduction of the ELISA assay, allowing a higher sensibility and the contemporary testing of large numbers of samples, with low costs and commercial scale applications.

In the double antibody sandwich (DAS)-ELISA technology, a test is performed on a solid phase in which each reagent acts in succession, initially anchoring and then detecting the target antigen on the same substrate (Fig. 1).

The specific antibody is adsorbed to the walls of a polystyrene wells plate, and then the sample with the antigen to be detected is added. A second specific antibody linked to an enzyme molecule, alkaline phosphatase (AP) is then sequentially introduced. Finally, the reaction is revealed through the spot (colour) produced by

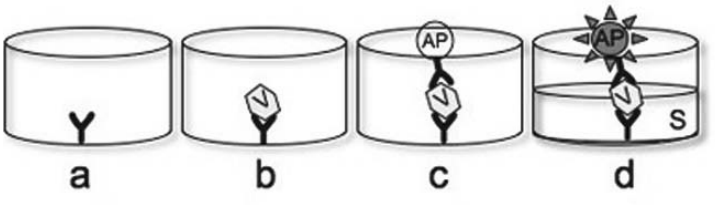

Figure 1. A schematic drawing showing the DAS-ELISA detection procedure. Antibodies (Y) are adsorbed to the well plate (a). Sap sample is then added (b) and the antigen (V) binds to the antibody. The antibody with enzyme attached (AP) is added (c). Finally the enzyme reacts with its substrate (S), and the reaction is revealed through colorimetric signal (d).

the enzymatic reaction of a specific substrate (streptavidin-HRP), degraded by the AP (Clark & Adams, 1977). Several applications of this method were developed for sensitive detection of virus and bacteria from different plant tissues and organs, including asymptomatic potato tubers (Lin & Cheng 1985; Caruso *et al*., 2002).

2.2.2. Monoclonal Antibodies

Differing from polyclonal antibodies, the monoclonal antibodies (Mabs) are specific towards a single epitope or antigen determinant. They are produced *in-vitro* by hybridomas, which are cell lines resulting from the fusion of a spleen B-lymphocyte, producing the antibody, with mieloma tumor cells (Koheler & Milstein, 1975). The advantages of this technology are related to the immortalization of the hybridomas, whose cultures allow long-term Mabs production and reproducible results, without losses in specificity. Further advantages include the possibility to produce Mabs from low antigen amounts, and their use in mixed reagents, to increase the detection range, as well as the high molecular standardization and affinity of the reagent (Huguenot *et al*., 1989; Gallitelli & Boscia, 1995; Martin *et al*., 2000). The possibility of producing synthetic polypeptides (SP) partially mimicking the original organism and recognized by specific Mabs, offers the advantage of using SPs instead of the original pathogen as internal positive control, avoiding risks of contaminations or allowing tests when the target organism is not available in the area of interest (Geysen *et al*., 1984).

A wide range of applications is described in the literature, concerning Mabs detection of viruses (Halk *et al*., 1984; Halk & de Boer, 1985; Chen *et al*., 1997; Boonham & Barker, 1998; Boscia *et al*., 1997; Cambra *et al*., 1991), fungi (Banowetz *et al*., 1984; Torrance, 1995; Martin *et al*., 2000), bacteria (Benedict *et al*., 1990; Gorris *et al*., 1994; Griep *et al*., 1998; Caruso *et al*., 2002; Alvarez, 2004) and phytoplasmas (Lin & Chen,1885; Musetti *et al*., 2002). In spite of the potentialities of Mabs-based diagnostics, some negative constraints include the identification of the single lines, specifically detecting the target of interest, among thousand hybridomas, and the need to use several Mabs to avoid false negative signals (Gugerli & Fries, 1983; Hajimorad *et al*., 1990; Massalski & Harrison, 1987). Further disadvantages concern the strict specificity (Chen *et al*., 1997), or the lack of qualitative discrimination, as some Mabs may display a reduced discriminatory capacity, yielding quantitative data only (Banowetz *et al*., 1984).

2.2.3. Molecular Detection

Several molecular methods flanked or substituted antibodies-based techniques, including DNA-based technologies, offering higher specificity reliability and lowering costs at the single test level (Tyagi *et al*., 1998; Singh *et al*., 1999; Whitcombe *et al*., 1999; Thelwell *et al*. 2000). When detecting differences at the isolate level, antibody mediated recognition cannot identify changes occurring at the DNA level, i. e. when nucleotide polymorphisms do not affect the amino acid composition of the target proteins (Finetti Sialer & Ciancio, 2005).

The detection techniques based on DNA analyses yield the possibility to explore the entire genome of a pathogen. They are versatile and require only a small amount of material, revealing the presence of the pathogen within a myriad of another unspecified items, and often result much faster than cultured-based methods (Fanelli *et al*., 2007). These techniques allow identification of changes in strains, races and isolates of the pathogen to be monitored or pest population (Brown, 2000; De Barro *et al*., 2003). Furthermore, DNA-based diagnostics offer a higher flexibility in the choice of the target DNA regions, together with a variety of detection protocols.

2.3. Molecular Probes

Advances in molecular biology permit the synthesis and cloning of nucleic acids in large amounts of copies, almost from any source. Following the manipulation of the DNA fragments, it is possible to synthesize molecular probes adapted to a wide range of specific scopes. PCR is the method of preference in target DNA amplification, nevertheless the downstream analyses (electrophoresis, hybridization and/or amplicon sequence) impeded its general use in several large scale or applied detection procedures.

2.3.1. Fluorescent Probes

The advent of fluorescent probes, gave raise to the development of homogeneous assays (Nazarenko, 1997; Tyagi *et al.,* 1998; Whitcombe *et al*., 1999; Thelwell *et al*., 2000). The amplified product is detected in real time through the fluorescence emitted during the PCR amplification. All the reaction occurs in a closed tube format, thus reducing the risk of contamination and the time needed to get results. This procedure maintains the same sensitivity of PCR, improving its specificity and, most important, permits the assay multiplexing. The use of multiple probes labelled with different fluorophores, furthermore, allows the detection of multiplex PCR amplification products, thus recognising several targets in the same reaction.

2.3.1.1. Molecular Beacons

This type of probes belongs to the group of self-hybridization fluorescent molecules. A hairpin-like DNA single strand (complementary to the target and provided with complementary ends) holds a fluorescent group, (i. e. fluorescein) and a quenching molecule linked to the opposite end. The latter molecule captures any fluorescence emitted by the probe in the self-hybridization state as heat. When the probe matches its complementary DNA target, the aperture and elongation of the hairpin structure and its hybridization splits the fluorescent group far from the quencher, allowing the emission of light under UV excitation (Fig. 2A). In case of a single nucleotide mismatch, the self-hybridized conformation is preferred, as the mismatches near the probe center and flanked by G:C pairs are more unstable than those near the oligoprobe ends flanked by A:T pairs (Tyagi *et al*., 1998; Mackay *et al*., 2002). This

property forces the probe to react to a single nucleotide mismatch, conferring a high degree of sensitivity and fidelity in the target hybridization reaction, due to the affinity of the molecule for self-folding (Thelwell *et al*., 2000). The molecular beacons (MB) were the first generation probes used to identify single nucleotide changes, proving enough reliability to function as an alternative DNA sequencing, without any need for cloning and subsequent sequencing protocols (Tyagi *et al*., 1998, Whitcombe *et al*., 1999).

2.3.1.2. Scorpions™

The Scorpion probes differ from MB in that the fluorescent molecule becomes integrated in the amplified product, giving rise to an intramolecular detection mechanism. The basic elements are a PCR primer, a PCR stopper to prevent the read-through of the probe, a specific sequence representing the true probe and the fluorescence detection system, containing the fluorescent group and the quencher, as in MB (Fig. 2B).

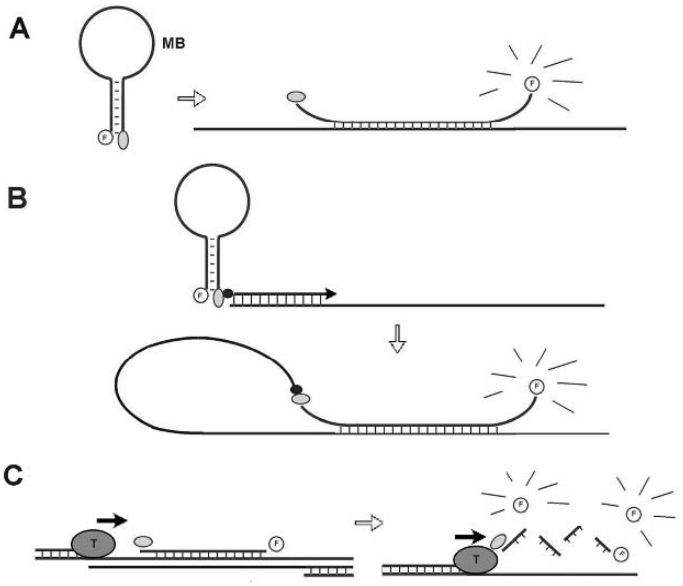

Figure 2. Schematic drawings showing the mechanisms of the amplicon detection of molecular beacons (A), Scorpion probes (B) and Taqman (C).The emission of the fluorophore (F) is captured as heat by the quencher molecule (grey). In the Scorpion mechanism, the probe is part of the amplicon produced by the primer (arrow) and the probe is protected from copying by a stopper (black dot). In Taqman, the nuclease activity of Taq polymerase (T) cleaves the probe, separating the fluorophores (F) from quenchers.

2.3.1.3. Taqman

The Taqman probes (Fig. 2C) are oligonucleotides labelled with a fluorophore at the 5' end and a quencher molecule at the 3' end. The probe is designed to hybridise to the middle of the amplified fragment and is degraded during primer extension, due to the 5'-3' exonuclease activity of Taq DNA polymerase. In this case the fluorescence is produced by the probe cleavage, indirectly accounting for the amplicon DNA hybridization.

2.3.2. Hybridization Techniques

Molecular hybridization techniques were initially developed to overcome some limitations of the serologic procedures and represent practical, low cost methods, alternative to detection through DNA amplification. Among DNA hybridization techniques, dot-blot analysis is a fast and simple procedure often applied in routine clinical diagnostic tests or for detection of plant pathogens, i. e. viruses, fungi or nematodes (Astruc *et al*., 1996; Schurko *et al*., 2004; Uehara *et al*., 1999).

Non radioactive dot-blot hybridization assays offer a specific and sensitive detection capability, and can be applied for detection directly from crude plant sap or from squashes of i. e. virus transmitting vectors, with resolutions of up to 20 μg of infected tissues per spot. The method consists in the serial deposition, on a nylon membrane, of droplets of sample solutions with the nucleic acid of interest. The target (as single RNA or double DNA strands, later denatured) is anchored to a positively charged nylon membrane, and then hybridized with a nucleic acid probe labelled with digoxigenin. The heteroduplex is revealed by the autoradiographic spots of the reaction signal, produced by an enzyme (linked through an anti-digoxygenin antibody to the probe) with its own substrate (Fig. 3). This simple protocol allows the analysis of a large number of samples in batches and may provide a first indication about the amounts of nucleic acids present, based on spots intensities and protocol sensitivity (Finetti Sialer *et al*., 1997).

2.4. Immunofluorescence and In-situ Hybridization

Fluorescent *in situ* hybridization (FISH) proved to be a strong tool for the detection of bacteria in environmental samples (Amann *et al.*, 1990; 1990a; Bottari *et al.*, 2006) allowing detection and localisation, inside the bacterial cell, of nucleic acids, protein and bio-polymers (Li *et al*., 1997).

FISH also additionally enables compartmentalisation studies. The technique unifies the simplicity of microscopy observations with the high specificity of hybridization. However, to carry out a detection *in-situ*, the cells must be permeabilized, allowing the probe acces to the tissue at the single cell level. The protocol comprises an oligonucleotide probe labelled with a fluorescent molecule coupled with an indirect immunofluorescence procedure. The specificity of the assay depends on the region selected to design the probes and the stringency of the hybridization used, that is a function of the base complementary between the probe and the target sequence.

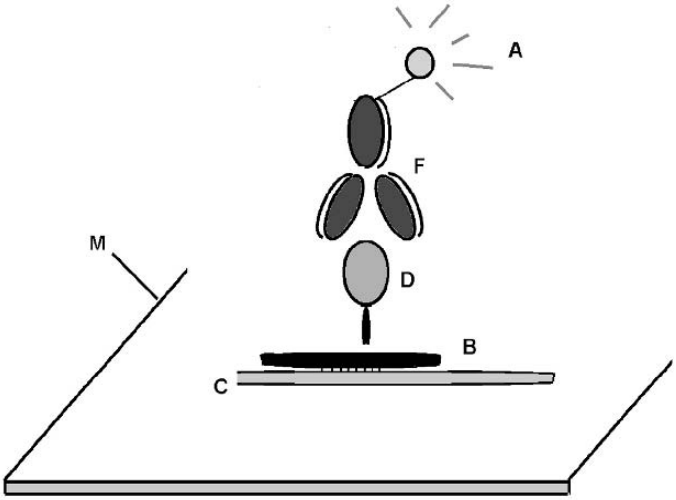

Figure 3. A schematic drawing showing the non-radioactive dot-blot hybridization detection procedure. The reaction occurs on a nylon membrane (M), with the blotted nucleic acid (C) hybridized to the probe (B), labelled with digoxigenin (D). The reaction is immunodetected with anti-DIG (F), conjugated to alkaline phosphatase (A). The enzyme then reacts with the chemiluminescence substrate in solution, for subsequent film impression.

The assay has been used for detecting and identify bacteria of agricultural importance like *Clavivacter michiganensis* subsp. *sepedonicus* and *Ralstonia solanacearum* (Wullings *et al.*, 1998). The protocol requires skilled laboratory personal with experience in microscopy. Further applications are mainly confined to laboratory studies, i. e. in search for differential subnuclear localisation of RNA strands of opposite polarity in viroids, within host plant tissues (Qi & Ding, 2003).

3. APPLICATIONS IN DISEASE AND PEST MANAGEMENT

3.1. Field Detection of Plant Pathogens

Tissue and dot blot, or closely related techniques, are generally used in large scale analyses plans, in order to identify the spreading patterns of diseases in the field or in quarantine or certification schemes. Samplings may be carried out in the field, using a single drop of plant sap, immobilized on a membrane pre-treated with NaOH/EDTA, or using a stem fresh cut or even a whole leaf print (Fig. 4). In a subsequent step the material is shipped to the competent laboratory, where the nucleic acids hybridization will be performed with the corresponding probe for later immuno or chemio-luminescent detection of the hybrid formed. Several

Figure 4. Some examples of blots from infected material. Tomato leaf (A) and transversal sections of tomato stems blots (B) from infected plants hybridized with a Cucumber Mosaic Virus *probe labelled with digoxigenin. Dot blot (C) of drops of tomato plants sap from infected material (black spots reveal probe hybridization with* Tomato Spotted Wilt Virus*).*

probes may be used in multiplex assays in a set of one or more samples (Gallitelli & Saldarelli 1996; Finetti Sialer *et al*., 1997).

3.1.2. Biosensors

Farming requires detailed knowledge about the distribution of diseases within the field, but manual inspections may result time consuming and expensive. In a precision farming system, sensors can enable targeted applications of pesticides and optimise the use of agrochemicals, through the detection and spatial identification of specific microorganisms. If limited at on-site analyses, assays could be performed by untrained personnel.

Biosensors are defined as analytical devices incorporating a biological sensing element associated with a transducer (Eggins, 1996) giving quantitative informations instead of yes/no. They should display high sensitivity and specificity, low detection limits, and stability. Pathogens should even be tracked to produce temporal maps defining the disease spreading in the field. Ideally, a field biosensor system should be designed to perform a similar highly specific and sensitive quantification. The results should be achieved rapidly in real-time, without the need for additional sample preparation (Velasco-Garcia & Mottram, 2003). Several project developed prototypes of immuno- or DNA-based biosensors, targeting various microorganisms, including viruses and fungi (Kintzios *et al*., 2001), nevertheless the most diffuse biosensors are for clinical and pharmaceutical markets. Although this technology represents the cutting hedge of research in high intensive agriculture, it requires further efforts for integration with information technology applications, like GIS and communication technologies (Xia *et al*., 2007).

3.2. Virus Detection in Vectors

Many control strategies rely on pests and pathogens monitoring in the agricultural environment, principally through the control of their dispersal mechanisms, including invertebrate vectors (Klerks *et al*., 2006). Factors like the potential risk of acquisition, pathogens load, transmission efficiency and retention time in the vector organism are of crucial importance, and require precise and accurate technologies.

Several procedures for detection a pathogen inside its vector are reported in the literature, varying from the initial and long-used applications based on the sensibility of bait plants (very efficient but also time consuming), to the more recent molecular probes. Some of these methods, however, appear limited to laboratory applications only, as they could not be transferred to the field or multiplexed application levels. In the case of plant viruses, i. e. immunosorbent electron microscopy was used for detection of *Grapevine fanleaf virus* (GFLV) in its vector, the nematode *Xiphinema index* (Roberts & Brown, 1980). This method is a direct assay but the use of electron microscopy limited its practical application.

The use of DAS-ELISA or B-A (biotin-avidin) ELISA was subsequently reported for detection of the virus in its natural vector, with a threshold of ten nematodes for reliable detection (Esmenjaud *et al*., 1993). Some later studies on GFLV detection focused on RT-PCR and other techniques applied to specimens of *X. index* (Esmenjaud *et al*., 1994). The advent of further DNA-based probes allowed the identification of the virus in single nematodes, with a potential resolution at the virus strain level (Fig. 5 A,B) (Finetti Sialer & Ciancio, 2005).

Compared to traditional methods, fluorescent probes like Scorpions and molecular beacons offer some advantages, including the fast and single-base sensitive recognition of the target sequence, sparing subsequent sequencing time and costs. These methods show a high degree of flexibility, since their reliability largely depends on the sequence selected for detection, and not on the general structure and arrangement of the molecules involved. Further examples include TaqMan probes, a technology successfully applied for virus detection from single thrips specimens (Boonham *et al*., 2002). The direct analysis of specific DNA motifs yields a higher performance in detection time. If the assay is coupled to a fragment specifically amplified from the vector, the parallel identification of the pathogen and its vector appears affordable (Finetti-Sialer & Ciancio, 2005).

3.3. Soil DNA Extraction and Microbial Detection

Microbial communities in soil affect to a great extent plants health. Known functions related to plant health improving microrganisms are: biological control, antagonism and pathogenicity, plant resistance regulation, plant stress reduction and nutrient cycling and acquisition (Martin, 1977). Many soil microrganisms are non-culturable and the exact role of soil microbial communities in regulation of disease insurgence and suppression is largely unknown. The identification and detection of these species is of paramount importance, either for plants health and for soil safety and fertility management.

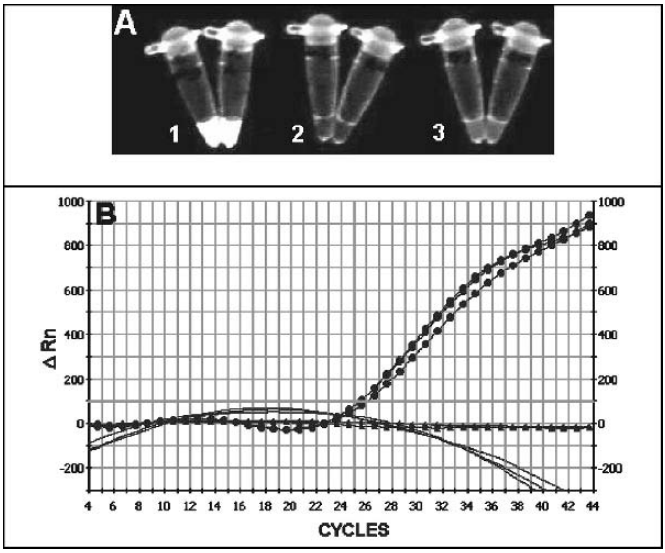

Figure 5. Detection of GFLV single nucleotide polymorphisms by molecular beacons in vials as revealed by UV irradiation of fluorescent amplified DNA (A, 1) and lack of emission in controls (A 2,3). Real-time PCR fluorescence readings during amplification (B), showing GFLV detection from its vector Xiphinema index *(dots).*

Many different molecular techniques for microbial pathogens detection rely on amplification of target DNA. These techniques are a prerequisite especially when targets are present in very low amounts or densities (Yeates *et al*., 1997; Zhang *et al*., 2006). When DNA amplification methods are applied, the purity of the nucleic acids extracted from soil is essential for microbial detection and quantification (Barthelet *et al*., 1996; Cheryl *et al*., 1998; Jacobsen, 1995). In presence of PCR inhibitors, the PCR reaction cannot proceed or can yield negligible amounts of undetectable amplification products, giving rise by this way to risks of false negatives (Gao *et al*., 2004).

Soil organic matter represents the major source of inhibitors, which may be co-extracted from soil with the microbial DNA. In particular, humic acids pose a considerable problem as they interfere in the enzymatic manipulations of DNA (Holben *et al*., 1988; Steffan *et al*., 1988; Tsai *et al*., 1992). DNA polymerases have been found to be inhibited by as little as 1 µl of undiluted humic-acid-like extract, regardless of the amount of DNA present (Tsai *et al*., 1992). The humic materials in soil have similar size and charge characteristic of DNA, resulting in their co-purification, evident by extractions being brown in colour (Holben, 1994). Humic contaminants also interfere in DNA quantitation since they exhibit absorbance at both 230 and 260 nm, the later used to quantify DNA. This characteristic can be used to determine the level of contamination of humic material by examining absorbance ratios. A high 260/230 ratio (>2) is indicative of pure DNA, while a

lower ratio is indicative of humic acid contamination. A high 260/280 ratio (>1.7) is indicative of pure DNA, while a low ratio is indicative of protein contamination.

When the target microorganism is present in low amounts in soil it is necessary to increase its accessibility treating the sample, i. e. with a complete dissolution of soil aggregates swirling the sample in distilled water with subsequent soil concentration, or through the use of filters or sieves, to retain and eliminate the largest particles, thus increasing the density of the target miocroorganism in the volume.

Methods for DNA extraction from soil may consider a traditional chloroform extraction followed by an ethanol precipitation in presence of cations. An alternative method considers the use of magnetic beads binding the DNA in suspension. The beads are then removed from the suspension through the use of a magnetic support, with subsequent DNA release through a specific buffer solution.

Quantitative PCR is a sensitive method for detection of plant pathogens, especially useful to detect and quantify non-culturable or slow-growing organisms. Nested PCR may increase the sensitivity of detection in real time PCR from soil.

3.4. Quarantine Detection of Invasive Species

Plant pest quarantine has been imposed to prevent casual introduction and/or limit the spread of agricultural invasive species. Quarantine regulations limit the production, movement or presence of plants, plant products, animals or their products, or any other articles or material, through national boundaries. It also applies to anthropic activities that could give raise to unintentional introduction or fortuitous spread of a specified pest(s). Quarantine represents a legal instrument properly prescribed by a governmental department in order of abate and, if possible, avoid a pest threat. The European Union apply laws, concerning quarantine, in order to accept products that only enter the EU market if they comply with the standards required. Those requirements are of paramount importance in the case of viruses, which are not amenable to curative control measures, and for which the most efficient control methods still rely on exclusion protocols (Rowhani *et al*. 2005).

Examples of invasive species include the introduction of the pine wood nematode, *Bursaphelenchus xylophilus* in Japan: conifers in North America are quite resistant to the pathogen, whereas Japanese species are susceptible. From Japan the nematode later spread to other Asian countries (Li *et al*., 1983). Introduction in EU through infested wood was detected in Portugal (Mota *et al*., 1999). *B. xylophilus* was intercepted in different occasions on sawn wood, round wood and wood chips imported from USA and Canada.

Further examples are the fungi responsible of different strawberry diseases. Among these, *Colletotrichum acutatum* was associated with field outbreaks in which the principal infection foci were plants originating from nurseries. These reports gave rise to the classification of the pathogen as a quarantine organism, for which the early detection and the exclusion of contaminated planting material is of primary importance (Mertely, 2004).

Quarantine plays a particular role in the actual scenario of climate changes, due to the insurgence and outbreaks of new emerging pests and diseases, whose epidemics may have an enormous impact on food production at the global scale (Anderson *et al*., 2004).

3.5. Epidemiology and Detection

In the agricultural system, the species enduring human intervention are those able to withstand the challenging of new habitats and niches. In addition to the cultivated plants we found weeds, pathogens and pests well adapted to the artificial communities created and maintained by man. This forced environment is unstable, prone to widespread risks of epidemics and outbreaks. The right management strategies aim at extracting the appropriate information from epidemiological data, in order to develop the most appropriate pest or disease control measures (Jones, 2004). Furthermore, the cognition of spatial distribution of plant diseases helps assuming the management decisions, in particular mode for the application of site specific management as in precision agriculture, avoiding the indiscriminate application of control measures i. e. pesticides, in the field plots where the problem is not present. Applications include data collection and introduction in models describing or forecasting a pest or disease epidemic at the field or regional scale, integration of biosensors with information technologies for early detection, monitoring and use of decision support systems.

3.6. Detection of Biological Antagonism

3.6.1. Parasitoids

Techniques based on molecular markers are useful for the broad exploration of genetic diversity, especially in organisms like insects, which comprise the largest species composition in the animal kingdom (Behura, 2006). Insects include the majority of the species lacking molecular keys suitable for identification. Furthermore, revealing insects parasitism is still a complex task, mainly due to the small size and the insufficient morphological characters effective for parasitoid taxa (Agustí *et al*., 2005; Greenstone, 2006).

Nowadays entomologists are employing protein or molecular markers to face identification problems. Apart from the molecular markers already described, other novel approaches are: transposon display, sequence specific amplification polymorphism (S-SAP), and repeat-associated PCR. These methods are being utilised as alternative markers system in insect studies (Behura, 2006).

Specific-PCR techniques represent an optimal choice due to their reproducibility, the shortest latency required for detection and the possibility of parasitism rates estimation. Additionally, in biological control applications, the accurate detection and identification of natural enemies became essential in agricultural practices. A region of the mitochondrial cytochrome oxidase I (COI)

gene has been successfully used to follow a range of parasitoids (belonging to tachinid species) and host species (*Ostrinia nubilalis*, the European corn borer) for the evaluation of parasitism in natural populations (Agustí *et al*., 2005). Jones *et al*. (2005) used PCR for parasitoid identification and assessment of parasitism rates in grain cereal aphids, successfully identifying the immature parasitoid to the species level for reliable prevalence evaluation.

3.6.2. Biological Control Agents

Biological control agents of insects, nematodes and fungi represent an important tool in IPM with potentials for future developments. Microbial enemies of insects include mainly entomopathogenic fungi (EF), whose potential as biological control agents is widely recognised. Some EF are already used as biopesticides, alone or in combination with cultural practices. Further insects enemies include also several bacterial species and viruses.

The use of mycoinsecticides as an alternative to chemicals must face several problems including mass production of mycelia and spores, as well as the identification of the best formulation and storage conditions, the method of application, taking care to maintain the product virulence and efficacy, compared to chemical insecticides.

Similar problems are encountered for formulations based on selected nematodes antagonists. Many nematode-trapping fungi developed a wide range of adaptations to capture or parasitize nematodes through either adhesive knobs and constricting or non-constricting rings. Some species may produce toxins, whereas others are capable to penetrate the body cuticle (or the eggshell). However, not all species appear suitable for practical exploitation. Several nematode antagonists (mainly bacteria) are also unculturable, due to obligate parasitism, and their detection with traditional methods is often difficult. Due to their easier isolation and cultivation, nematophagous fungi represent the main organisms used for biocontrol of plant parasitic nematodes, with some successful cases of natural suppression shown in certain conditions (De Leij *et al*., 1992; Kerry, 1984).

Considering a pest or disease biological control, it might be worth to examine the introduction of mycoinsecticides in IPM programs. The biocontrol agent acts at different levels ranging from an antagonistic activity (indirectly competing for nutrients and/or space), through the production and release of antagonistic compounds, i. e. hydrolitic enzymes (Ippolito, 2000), til the large scale reduction of a pest population density because of direct parasitism or predation.

IPM strategies require that the applied biocontrol agent should be detected throughout its whole life-cycle, starting from its release though its final recovery. For this purpose several survey activities are needed, and the traditional methods of identification, based on morphological and biochemical tests, result difficult to achieve and time consuming. In general, the application of any biological control agent requires specific monitoring strategies in order to evaluate its ability to colonise the niche and its tolerance to environmental changes. Furthermore, the risk for unpredicted environmental consequences

must be carefully evaluated when compared with the benefits gained by the activity of the released organism (Gandeboeuf *et al*., 1997).

Also in this case, the validation of the species used may benefit from biotechnology advances. The use of molecular markers to distinguish between indigenous and introduced strains at the DNA level appears promising for the correct and fast identification and quantification of the released organisms in the field. Fast and reliable detection systems appear necessary for quality control, patent protection and environmental monitoring and persistence. A PCR-based system was used to identify strains of the fungus *Beauveria bassiana* within infected insects (Hegedus & Khachatourians, 1996; Castrillo *et al*., 2003), or where deliberate release into insect population occurred. At the same time, PCR-SSCP (Single Stranded Conformation Polymorphism) allowed monitoring of the fungus isolate released against a background of others insects. Molecular methods also may shed light on particular associations active between different organisms, like the identification of unusual virus-insect mutualism association. For example, Espagne *et al*. (2005) found that parasitism of *Manduca sexta* by the parasitoid wasp *Cotesia congregata* was favoured by the concomitant host infection by a bracovirus, expressing a cystatins-homologue gene, counteracting the host's immune system reaction. It is worth to recall at this regard that any IPM strategy implemented in the field must rely on a deep knowledge concerning the organisms involved.

As concerns fungi, the genus *Trichoderma* encompasses a great number of species known for their antagonistic role against several plant pathogens, i. e. phytopathogenic fungi (Elad, 2000; Heremans *et al*., 2005) or even root-knot nematodes (Suarez *et al*., 2004). The capabilities of *Trichoderma* spp. as biocontrol agents was translated into commercial products actually availale, and different molecular markers were developed to estimate genetic variations among strains.

There is an increased interest towards the elucidation of antagonism mechanisms at genetic level and the use of molecular methods is making this task possible. The analyses of an expressed sequence tag database provided some information on gene expression in *T. harzianum,* revealing genes involved in parasitism processes as well as a set of 673 novel genes (Liu & Yang, 2005; Zeilinger *et al*., 2005). When evaluating sixteen strains of *T. asperellum, T. atroviride, T. harzianum, T. inhamatum* and *T. longibrachiatum*, Hermosa *et al*. (2001) obtained distinctive markers for the identification of an isolate of *T. atroviride*, and successfully identified SCAR markers to accurate distinguish strain 11 from other closely related *Trichoderma* strains.

4. MOLECULAR MARKERS AND RESISTANCE

Since the early 80s, the techniques based on the use of molecular markers developed rapidly, allowing the construction, for several plant species, of genetic maps with high markers density. In general, a molecular marker is any fragment of nucleic acid or protein sequence useful for reporting a genetic difference. For tomato, for example, it is now available a RFLP map including more than 1000 markers, on which several resistance genes were localised, allowing the pyramidal arrangement

of a large number of genes (Kelly *et al*., 1995). Several classes of molecular markers are available, allowing the identification and marking of genomic identities and/or differences, including gene modifications and polymorphisms, typical of any species. By this way, it is possible to drive the response to selection, which for this reason is called "marker-assisted selection". Saturated maps were produced for several plants, with genes localised on the chromosomes, known as markers of utility characters (Kumar, 1999).

Molecular markers allow the identification of the most useful parental lines for a breeding programme and the early selection within the progeny of individuals carrying the genetic trait(s), object of selection (Michelmore *et al*., 1991). This work would result more difficult, if only conventional selection methods had to be applied, due to the effects of epistasis, or of environmental influence or plant sanitary status (Kumar, 1999).

Table 1. Some examples of molecular markers application for the detection of disease resistance genes in plants.

Plant	*Pathogen or pest*[*]	*Marker*	*Reference*
Triticum aestivum	*Puccinia striiformis* f. sp. *tritici*	SSR	Imtiaz *et al*., 2004
Triticum aestivum	*Puccinia recondita* f.sp. *tritici*	STS	Singh *et al*., 2004
Triticum aestivum	*Fusarium graminearum*	AFLP, SSR	Zhou *et al*. 2004
Triticum aestivum	*Erysiphe graminis* f. sp. *tritici*	RFLP, MS[**]	Liu *et al*., 2001
Hordeum vulgare	*BaMMV, BaYMV, BaYMV-2*	SSR	Werner *et al*., 2003
Hordeum vulgare	*BaMMV*	STS, SSR	Werner *et al*., 2000
Oryza sativa	*Rhizoctonia solani*	RFLP, RAPD, AFLP, SSR	Che *et al*., 2003
Lolium multiflorum	*Pyricularia* sp.	AFLP, EST-CAPS	Miura *et al*., 2005
Cicer arietinum	*Ascochyta rabiei*	RAPD, SCAR, STMS	Iruela *et al*., 2006
Phaseolus vulgaris	*Apion godmani*	RAPD, STS	Blair *et al*., 2006
Arachis spp.	*Meloidogyne arenaria*	RAPD, SCAR, RFLP	Garcia *et al*., 1996
Glycine max	SMV	SSR	Fu *et al*., 2006
Glycine max	SMV, PMV	RFLP, RAPD	Gore *et al*., 2002
Vasconcellea parviflora × *V. cundinamarcensis*	PRSV-P	RAF,CAPS	Dillon *et al*., 2006

[*] *Papaya ringspot virus* type P (PRSV-P); *Barley yellow mosaic virus* (BaYMV); *Barley mild mosaic virus* (BaMMV); *Soybean mosaic virus* (SMV); *Peanut mottle virus* (PMV).
[**] MS: microsatellites sequence tagged microsatellite site (STMS).

Several plant characters like individual productivity, yield quality, maturity or resistance to biotic or abiotic stresses are polygenic, meaning that they are controlled by several genes having an additive action, termed Quantitative Trait Loci (QTL). One of the most useful applications of molecular markers was the construction of linkage maps (Collard *et al*., 2005).

The use of molecular markers linked to or located within several loci, coding for quantitative characters, provides information improving the efficiency of selection. Molecular markers fall within two main categories: those revealing polymorphisms at the protein level (i. e. isoenzymes) and those accounting for the nucleic acid polymorphisms. The latter include AFLP (Amplified Fragment Length Polymorphism), CAPS (Cleaved Amplified Polymorphic Sequences), EST (Expressed Sequence Tag), IPCR (Inverse Polymerase Chain Reaction), IRAP (Inter-Retrotransposon Amplified Polymorphism), ISSR (Inter-Simple Sequence Repeat amplification), PCR, RAPD (Random Amplified Polymorphic DNA), REMAP (Retrotransposon-Microsatellite Amplified Polymorphism), RFLP, SCAR (Sequence Characterized Amplified Region), SNP (Single Nucleotide Polymorphism), SSCP, SSR (Simple Sequence Repeat), STS (Sequence Tagged Site) (IAEA, 2002). Some applications of molecular markers are shown in Table 1.

5. CONCLUSIONS

Actual advances in detection technologies allow the identification of future trends in field applications, including the development of real time pathogen–specific biosensors for continuous monitoring and farm-scale information technologies, which may take advantage by GIS and GPS applications.

The scale of the detection procedures will range from local to regional, national or continental, depending on the goals and needs of the monitoring schemes implemented. For this purpose, more DNA data, produced through the concerted actions of technical or scientific organizations, are required for effective broad range surveys and accurate detection. This view should be encouraged, also in consideration of global risks related to the emergence of new plant pests and disease epidemics and/or to the appearance of new strains of “ancient” pathogens (i. e. the recent *Phytophthora infestans* epidemics). The biosphere biodiversity is not yet fully explored, and the microbiologists community considers that a large number of microbial species and/or populations still remain undiscovered. At this purpose, actions aiming at prevention, detection and management of plant epidemics require the reinforcement of basic and applied molecular and genetic studies, covering the whole range of agroecosystems on a global scale.

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