

# **INTEGRATED PEST AND DISEASE MANAGEMENT IN GREENHOUSE CROPS**

# **Developments in Plant Pathology**

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VOLUME 14

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# Integrated Pest and Disease Management in Greenhouse Crops

*Edited by*

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## FOREWORD

The International Centre for Advanced Mediterranean Agronomic Studies (CIHEAM), established in 1962, is an intergovernmental organization of 13 countries: Albania, Algeria, Egypt, France, Greece, Italy, Lebanon, Malta, Morocco, Portugal, Spain, Tunisia and Turkey.

Four institutes (Bari, Italy; Chania, Greece; Montpellier, France; and Zaragoza, Spain) provide postgraduate education at the Master of Science level. CIHEAM promotes research networks on Mediterranean agricultural priorities, supports the organization of specialized education in member countries, holds seminars and workshops bringing together technologists and scientists involved in Mediterranean agriculture and regularly produces diverse publications including the series *Options Méditerranéennes*. Through these activities, CIHEAM promotes North/South dialogue and international co-operation for agricultural development in the Mediterranean region.

Over the past decade, the Mediterranean Agronomic Institute of Zaragoza has developed a number of training and research-supporting activities in the field of agroecology and sustainability of agricultural production systems. Some of these activities have been concerned with the rational use of pesticides and more particularly with the implementation of integrated control systems in order to gain in efficacy and decrease both the environmental impact and the negative repercussions for the commercialization of agricultural products. Stemming from the organization of a course on “Integrated Pest and Disease Management in Protected Crops”, and as a consequence of the enthusiasm of the lecturers who took part in the course and its scientific co-ordinators, we decided to publish a book based on the contents of the course to provide professionals interested in updating their knowledge with a comprehensive vision of the state of the art of IPM.

Several objective reasons convinced us of our decision. On one hand, the growing economic and social importance of protected crops in the countries of the Mediterranean area. On the other, the fragility of the ecosystems on which they are grown, very often close to areas of urban concentration and tourist development. Therefore, integrated management must be incorporated into the present production systems and appropriate research and experimentation programmes must be developed in order to generate a pest and disease control technology adapted to the ecological conditions and predominant species in each circumstance. We felt that this book could contribute in this task. The Mediterranean Agronomic Institute of Zaragoza has experience from similar publications arising from their professional-training programmes and this also encouraged us to undertake this ambitious project.

The magnitude of our ambition only became clear to us when, compiling the book, we were confronted with the large number of authors, their diverse specialities and origins (from researchers to extensionists, from both the public sector and private firms), and the multidisciplinary nature of the approach, addressing both basic and applied aspects. Accommodating such diversity into the different parts of the book has been our most difficult task. Therefore, it is with great satisfaction and gratitude that we acknowledge and thank the editors, R. Albajes, M.L. Gullino, J.C. van Lenteren and Y. Elad for their inspired and efficient work in orienting and co-ordinating the book. Likewise, we would like to express our gratitude to each and every one of the 62 authors for their contribution to this team effort.

The design and development of this book are yet another example of the results that can be achieved through co-operation, and as such, contributes to CIHEAM’s objective of promoting co-operation for the development of the agro-food sector in the

Mediterranean area. We hope this example will encourage the same co-operative attitude amongst readers.

Finally we should like to express our satisfaction of the efficacious collaboration from Kluwer Academic Publishers and wish to thank them for their interest in this project.

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## PREFACE

This book originated from an international course that was organized on “Integrated Pest and Disease Management in Protected Crops” at the Mediterranean Agronomic Institute of Zaragoza of the CIHEAM. Thirteen guest speakers lectured to some thirty participants, and the idea of publishing the contributions to the course arose as a result of their enthusiasm. The project soon became more ambitious with the purpose of enriching the publication’s objectives and contents. Thus, the variety of ways in which protected crops are cultivated world-wide demanded the collaboration, not only of European authors, but of authors from all those regions that have developed the greenhouse crop industry. Likewise it was necessary, on this occasion, to count on the multi-disciplinarity of integrated control, therefore new entomologists and plant pathologists working in different disciplinary environments, such as ecology, molecular biology, statistics, information systems and plant breeding, were incorporated into the project. It was also considered necessary to count on the collaboration of specialists from the public and private sectors involved in the different links of the chain necessary for the technological innovation of integrated control: researchers, extensionists, natural enemy producers, consultants. This diversity of authors is probably what we are most satisfied with as editors. Nevertheless, this has also complicated the edition work as we have tried to keep a maximum of homogeneity without falling into too much uniformity. As the basic elements of integrated control need to make use of local conditions favourable to pest and disease control, one cannot expect the points of view, practices, even scientific backgrounds to be common throughout all the chapters of the book when very often the authors work in areas which are geographically very different. Whenever possible, we have entrusted each chapter to authors whose activity and perspectives could be complementary: entomologists together with pathologists, from both public and private sectors, differentiated geographical areas, etc. It is our sincere belief that no text published to date has offered such a diverse yet integrated approach to pest and disease control in greenhouse crops.

The book opens with an initial chapter describing the scenario where integrated pest and disease control operates, that is, the greenhouse and its environment. Ensuing chapters provide the basic strategies and tactics of integrated control, with special reference to greenhouse crops. Further chapters include the different facets of biological pest and disease control – its scientific bases, its development in practice, its commercialization and quality control. The pre-eminence of biological control in the book is not surprising since without a doubt it is the cornerstone of integrated insect pest control and is also becoming increasingly more important in disease control. The concluding chapters of the book show us the present situation of integrated pest and disease control in the most important greenhouse crops world-wide. This final section opens with a chapter discussing the technology transfer process from research to the consumer; this chapter is by no means superfluous, as the lack of an efficient technology transfer is often the main cause of the slow adoption of integrated control.

This book is neither a manual nor a guide. We have attempted to provide post-graduate and professional readers already familiar with the subject, with a means to acquire deeper knowledge on integrated control of pests and diseases in greenhouse crops and furthermore suggest possible roads to take in future tasks. It is evident, however, that each situation and each problem requires a particular solution. Integrated control in greenhouses first developed in England and The Netherlands in the 60s. The success reached in both countries led the research, extension and application of this type of control system to become generalized throughout northern Europe in the 70s and 80s.

This experience, so positive in the North of Europe, stimulated the adaptation of integrated systems for other areas such as the Mediterranean, North America, Oceania and Asia at various rates and degrees of success. It has been shown that a mere transposition of northern European solutions is not valid in other parts of the world. Each new situation demands further research, development, extension, training and new forms of application. Without this local effort, it will be very difficult for integrated control to progress at a faster rate. We trust that this work will contribute to stimulating and guiding this effort.

We have many people to thank. The Mediterranean Agronomic Institute of Zaragoza organized and hosted the course that gave rise to this book and subsequently undertook the co-ordination of the edition and technical editing. Had we not been able to count on their experience, professionalism and enthusiasm, we would not have been able to embark on this endeavour. The participants in the mentioned course have also permitted us to enrich the content of this work with their suggestions and constructive criticism. The authors have shown at all times a great patience and comprehension on reacting to our requests and revisions with good will and wisdom. The IOBC/WPRS, "International Organization for Biological and Integrated Control of Noxious Animals and Plants, West Palaearctic Regional Section" likewise deserves a special mention of gratitude. In two of their working groups on "Integrated Control in Greenhouse Crops", these editors and many of the authors have been collaborating and continue to do so, thus facilitating the edition of the book.

To publish a book is an arduous task. The mere conviction of the need to divulge and teach what has been learnt from others and our own sense of duty can compensate such an undertaking. Fortunately, we are convinced that the effort of the hundred people who have collaborated, in one way or another, in this book has been worthwhile. Another decisive stimulant for this endeavour was the realization of the growing need to incorporate integrated systems of protection from arthropod pests and diseases for the thousands of hectares of protected crops in the world. Both the fruit, vegetable and ornamental plant markets and the technical and economic efficiency of crop protection require these integrated control systems.

Ramon Albajes  
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### **SETTING THE STAGE: CHARACTERISTICS OF PROTECTED CULTIVATION AND TOOLS FOR SUSTAINABLE CROP PROTECTION**

M. Lodovica Gullino, Ramon Albajes and Joop C. van Lenteren

#### **1.1. Protected Cultivation and the Role of Crop Protection**

Attempts to adapt crop production to the environment with protective devices or practices date back to ancient times. Structures for crop production were first used in the early period of the Roman Empire, under Emperor Tiberius Caesar, 14–37 AD. Such structures consisted of mobile beds of cucumber placed outside on favourable days and inside during bad weather. Covers were slate-like plates or sheets of mica or alabaster (Dairymple, 1973). Greenhouses in the UK and The Netherlands developed from glass structures built to protect plants imported from tropical Asia and America in the 16th and 17th century during the winter period. However, such methods of cultivation ceased with the decline of the Roman Empire and it was not until the 15th to 18th centuries that simple forms of greenhouses appeared, primarily in England, The Netherlands, France, Japan and China. By the end of the 19th century, commercial greenhouse crop production was well-established (Wittwer and Castilla, 1995).

The purpose of growing crops under greenhouse conditions is to extend their cropping season and to protect them from adverse environmental conditions, such as extreme temperatures and precipitation, and from diseases and pests (Hanan *et al.*, 1978). Greenhouse structures are essentially light scaffolding covered by sheet glass, fibreglass or plastic. Such materials have a range of energy-capturing characteristics, all designed to maximize light transmission and heat retention. Crops may be grown in groundbed soil, usually amended with peat or farmyard manure, in benches, in pots containing soil or soil mixtures or soil substitutes, and in hydroponic systems, such as sand or rock wool cultures and flowing nutrient systems, without a matrix for the roots.

Modern technology has given the grower some powerful management tools for production. Generally, added-value crops are grown under protection. Most of them are labour-intensive and energy-demanding during cold weather. Greenhouse production therefore normally requires a high level of technology to obtain adequate economic returns on investments. Quality is a high priority for greenhouse crops, requiring much care in pest and disease management, not only to secure yields but also to obtain a high cosmetic standard. Although technological changes are ultimately intended to reduce production costs and maximize profits, precise environmental and nutritional control push plants to new limits of growth and productivity. This can generate chronic stress conditions, which are difficult to measure, but apparently conducive to some pests and diseases. Historically, not enough attention has been paid to exploiting and amending production technology for the control of pests and diseases. This makes the control of pests and diseases in protected crops even more challenging, with many important

problems being unresolved and new ones arising as the industry undergoes more changes in production systems.

Additionally, the international trade in ornamental and flower plants facilitates the spread of pests and diseases around the world and their establishment in new areas. In Europe, for example, at least 40 new pests have been recorded in protected crops in the last 25 years. The increasing complexity of pest and disease problems and the high cosmetic standards of vegetable, ornamental and flower products have led growers to apply intensive preventive chemical programmes, which result in pests and pathogens becoming resistant to the most frequently used pesticides in a few years, which, in turn, increases control costs. In southern Spain, the average cost of pesticide application in 1992 in protected vegetables was estimated as **US\$0.14/m<sup>2</sup>** (16.5% of the total production cost) (Cabello and Cañero, 1994), and several whitefly, thrips, aphid and fungus species are suspected to be resistant to several active ingredients. A similar figure is valid for Italy, where the most sophisticated structures are located in the northern part of the country: pesticides are widely applied and pest and disease resistance is quite widespread (Gullino, 1992). In The Netherlands, pest and disease control costs for vegetables are still limited and are normally below 3% of the total costs to produce a crop (van Lenteren, 1995).

As control costs increase, pesticide-resistance spreads and consumers become aware of the risks of pesticide-residues in fresh vegetables, a strong demand for non-chemical control methods is emerging in many countries. Integrated systems for greenhouse pest and disease control have been developed and implemented in northern Europe and Canada, but implementation is still cumbersome in other parts of the world.

## **1.2. Importance of Protected Crops for Plant Production**

During the late 50s and early 60s the use of greenhouses spread: initially they were mostly used for vegetable production, with an emerging cut-flower and ornamental plant industry starting, particularly in the UK and in The Netherlands. By 1960, The Netherlands had the most concentrated production of glass-house grown crops, estimated as 5000–6000 ha (75% of which grew tomatoes). At the same time, the UK had 2000 hectares of greenhouses (Wittwer, 1981). Hydroponic cultivation started in The Netherlands in the 60s and spread to many countries. In the USA, hydroponic cultivation became widespread (Jensen and Collins, 1985): in the late 60s and early 70s, there were more than 400 ha devoted to hydroponic vegetable production (tomato, followed by cucumber and lettuce), although this surface area has diminished to less than 100 ha today (Wittwer and Castilla, 1995). Moreover, there has been a strong shift from vegetables to ornamentals grown in glasshouses. Nowadays, in the USA, of the total greenhouse production (estimated as 2000 ha), 95% is represented by flowers, potted plants, ornamentals and bedding plants (Wittwer and Castilla, 1995). There has also been a shift in northern Europe, with a delay of about 15 years compared to the USA, from vegetables to added-value ornamental crops (Wittwer and Castilla, 1995). For example, more than 80% of the greenhouses in The Netherlands were used for vegetables in the 60s, whereas now 60% of the approximate 10,000 ha are used for production of ornamentals.

By 1980, there was an estimated 150,000 ha of greenhouses (glass, fibreglass, plastic) world-wide producing high-value crops (Wittwer, 1981). In 1995, the surface area had increased to about 280,000 ha (Bakker, 1995; Wittwer and Castilla, 1995) (Table 1.1). New areas, particularly in Asian and Mediterranean countries, showed a strong increase in protected areas, attracted by cultivation of high-value vegetable crops. The expansion in plasticulture in the Mediterranean area is still going on, again with a gradual transition from the production of vegetables to ornamentals. Spain and Italy have been the leading countries in the 80s and 90s. At present, the North African countries are experiencing a very rapid increase in the area covered with plastic houses, often with very simple structures. This development has been accompanied by a spread in drip irrigation (Wittwer and Castilla, 1995). At the same time, the use of plastic row tunnels, covers and plastic soil mulches has expanded world-wide. These structures will not be discussed further in this book, but it is interesting to know that, for example, in China an area of more than 2.8 million ha of crops was covered with plastic soil mulch in 1995 (Wittwer and Castilla, 1995).

**TABLE 1.1. Distribution of protected cultivation world-wide (from Wittwer and Castilla, 1995)**

| Structure                     | ha (× 1000) in geographical area |                            |                     |              |       |
|-------------------------------|----------------------------------|----------------------------|---------------------|--------------|-------|
|                               | Asia                             | Mediterranean <sup>1</sup> | North/South America | North Europe | Total |
| Direct cover (floating types) | 5.5                              | 10.3                       | 1.5 <sup>2</sup>    | 27.0         | 44.3  |
| Low tunnels (row-covers)      | 143.4                            | 90.5                       | 20.0 <sup>2</sup>   | 3.3          | 257.2 |
| High tunnels                  | –                                | 27.6 <sup>3</sup>          | –                   | –            | 27.6  |
| Plastic-houses                | 138.2                            | 67.7                       | 15.6                | 16.7         | 238.2 |
| Glass-houses                  | 3.0                              | 7.9                        | 4.0                 | 25.8         | 40.7  |

<sup>1</sup>Including France

<sup>2</sup>Figures are crude estimates

<sup>3</sup>High tunnels are often taken together with plastic-houses in countries other than Mediterranean

The world greenhouse area is now estimated as 307,000 ha, 41,000 ha of which is covered with glass, 266,000 ha with plastic. The global status of protected cultivation (*sensu lato*) is reported in Table 1.1. The distribution and types of crops grown in greenhouses are outlined in Table 1.2. Vegetable crops are grown in about 65% of greenhouses, and ornamentals in the remaining 35%.

### 1.3. Type of Structures Adopted for Protected Cultivation and their Impact on Cultivation Techniques and Crop Protection

Structures adopted for covering crops vary a lot, from the simple to the sophisticated:

- (i) Low tunnels (row-covers). These are small structures that provide temporary



protection to crops. Their height is generally 1 m or less, with no aisle for walking, so that cultural practices must be performed from the outside. Their use enhances early yields and yield volume; they also protect against unfavourable weather. Thermal films of infra-red polyethylene (PE), ethylene vinylacetate (EVA), copolymer, polyvinylchloride (PVC) and conventional PE are used.

(ii) High tunnels (walk-in tunnels). Such structures use the same cover materials as low tunnels and are high enough to perform cropping practices inside. Moderately tall crops are grown. Statistics concerning high tunnels are often included in the same category as low cost plastic houses (Table 1.1) since the materials used are similar.

(iii) Greenhouses. These differ from other protection structures in that they are sufficiently high and large to permit a person to conveniently stand upright and work within (Nelson, 1985). Greenhouses appeared when glass became available for covering. Later, the introduction of plastic films permitted world-wide expansion of the greenhouse industry.

**TABLE 1.2. World distribution of crops most commonly grown under protected structures (tunnels, greenhouses) (modified from Wittwer and Castilla, 1995)**

| Crop  | Leading countries  |
|---|--|
| Cucurbits                                   | Argentina, Belgium, Canada, Chile, China, Egypt, Finland, France, Germany, Greece, Hungary, Israel, Italy, Japan, Morocco, Turkey, Korea, Poland, Portugal, Saudi Arabia, Scandinavia, Spain, Taiwan, The Netherlands, Tunisia, Ukraine, former URSS, USA  |
| Strawberry                                  | Argentina, Belgium, Canada, China, Finland, France, Greece, Israel, Italy, Japan, Jordan, Morocco, Portugal, Spain, Tunisia, Turkey, USA   |
| Solanaceous + green beans                   | Algeria, Argentina, Belgium, Bulgaria, Canada, Chile, China, Denmark, Egypt, Finland, France, Germany, Great Britain, Greece, Hungary, Israel, Italy, Japan, Jordan, Korea, Morocco, Poland, Portugal, Saudi Arabia, Scandinavia, Spain, Taiwan, Tunisia, Ukraine, The Netherlands, Turkey, former URSS, USA |
| Grapes and tree fruits                      | Italy, Japan, Morocco, Portugal, Spain   |
| Lettuce, cabbage, celery, radish, asparagus | Belgium, China, France, Germany, Great Britain, Hungary, Italy, Japan, Korea, Poland, Spain, The Netherlands, former URSS, USA   |
| Flowers, ornamentals                        | Argentina, Belgium, Canada, Denmark, Egypt, Finland, France, Germany, Great Britain, Hungary, Israel, Italy, Japan, Poland, Scandinavia, Spain, The Netherlands, USA   |
| Bedding and potted plants                   | Belgium, Canada, Denmark, Finland, France, Germany, Great Britain, Hungary, Israel, Italy, Japan, Scandinavia, Spain, The Netherlands, USA   |

Greenhouses protect crops against cold, rain, hail and wind, providing plants with improved environmental conditions compared to the open field. In greenhouses, crops can be produced out-of-season year-round with yields and qualities higher than those produced in the open field. Greenhouses have also allowed the introduction of new crops, normally foreign to the region (Germinating, 1985).

There are two basic types of greenhouse. The first type seeks maximum control in an environment to optimize productivity. In Europe, optimal conditions for year-round production are provided in the glasshouses of The Netherlands, Belgium, the UK and Scandinavia. The other type of greenhouse, which is very common throughout the Mediterranean area, provides minimal climatic control, enabling the plants grown inside to adapt to suboptimal conditions, survive and produce an economic yield (Enoch, 1986; Tognoni and Serra, 1989; Castilla, 1994).

The choice of greenhouse depends on location, crop and financial resources. There is a strong relationship between local conditions, greenhouse design, cladding materials and insulation needs.

The structure of a greenhouse depends on the climate and the cladding used. There are various roof, space and height geometries with single-span materials such as bamboo, used in low cost structures, particularly in China and in semi-tropical and tropical areas. Cladding materials were limited to glass until the middle of the 20th century. From 1950, plastic films, because of their low cost, light weight and adaptability to different frame designs, became available, permitting world-wide development of the greenhouse industry, particularly in the semi-tropical areas (Nelson, 1985). But plastic covers are not acceptable in northern Europe because of low light transmission compared to glass.

A full range of conventional and modified plastic films is now available (Giacomelli and Roberts, 1993): all coverings can perform well, depending on the desired use and location. Single plastic films prevail in warm climates; inflated double plastic film or rigid single plastic panels are more common in cool areas. A combination of high and low technology may be seen in countries such as Korea and Israel.

Nets are used in tropical areas or during hot weather in temperate zones: they may reduce pest damage and the extremes of temperature and air humidity. Moreover, nets have a windbreak effect and reduce the damage from heavy rain and hail (Castilla, 1994) (see Chapter 18 for a further description of the use of nets for pest control).

The greenhouse design (particularly its height, shape, opening systems and cladding material) strongly influences climatic conditions inside, thus having a profound impact on pest and disease development. Plastic houses almost always have a more humid climate, large diurnal temperature variation and are more difficult to ventilate. Typically, they result in more problems with high humidity-dependent diseases, such as grey mould, downy mildews and rusts (Jarvis, 1992). Regulating the atmosphere throughout the day and night is important for disease control and for reducing the total amount of chemicals sprayed. This has been demonstrated in the case of grey mould (*Botrytis cinerea* Pers.:Fr.) in tomato (Gullino *et al.*, 1991) and cucumber (Yunis *et al.*, 1994), and of downy mildew (*Bremia lactucae* Regel) in lettuce (Morgan, 1984).

With respect to the cladding material used, in some cases a possible effect on diseases has been reported, mostly through the direct influence of radiation on sporulation (Jarvis, 1992). Certain UV-absorbing plastic coverings for greenhouses that absorb light at 340 nm have been exploited to inhibit the sporulation of *Sclerotinia sclerotiorum* (Lib.) de Bary (Honda and Yunoki, 1977), and species of *Alternaria* and *Botrytis squamosa* J.C. Walker (Sasaki *et al.*, 1985). Reuveni *et al.* (1989) observed a reduction in the number of infection sites of *B. cinerea* on tomato and cucumber when a

UV-absorbing material was added to polyethylene film to increase the ratio of blue light to transmitted UV light. Recently, blue photoselective polyethylene sheets have been suggested for their ability to reduce grey mould on tomato (Reuveni and Raviv, 1992) and downy mildew on cucumber (Reuveni and Raviv, 1997). Green-pigmented polyethylene reduced the conidial load and grey mould in commercial tomato and cucumber greenhouses by 35–75%. *Sclerotinia sclerotiorum* on cucumber, *Fulviafulva* (Cooke) Cif. (= *Cladosporium fulvum* Cooke) on tomato and cucumber powdery mildew were also reduced (Elad, 1997).

The technologies for environmental control in the most sophisticated greenhouses have been characterized by many new developments over the past three decades. The variables of light, temperature, air and soil humidity, and  $\text{CO}_2$  content of the atmosphere are computer-programmed 24 h a day to achieve maximum crop yield (Nederhoff, 1994). Further refinements and improvements for adjusting the greenhouse climate to optimal crop productivity can be expected. In the less sophisticated structures of the sub-tropical and tropical regions, it is much more difficult to manipulate the greenhouse climate (Gullino, 1992). In tropical and subtropical areas greenhouses often simply have an umbrella effect, using just roofs, with sides left open.

The influence of greenhouse structures and covers on greenhouse climatic regimes may have strong consequences for pests and their natural enemies, as they have for diseases. A typical case of climate influence on pests and natural enemies concerns the spider mite and its predator *Phytoseiulus persimilis* Athias-Henriot: low humidity regimes may constrain effective use of *P. persimilis* (Stenseth, 1979). In high-tech greenhouses, regulation of temperature and water pressure deficit enables the creation of conditions less favourable to pathogens and, in some cases, more favourable to biocontrol agents. The use of heating to limit development of a number of pathogens is well known (Jarvis, 1992): however, heating is not economically feasible in all greenhouse systems. Recently, with the development of soilless systems, the effect of managing the temperature of the circulating solution has been studied, and has proven to be effective against certain pathogens. The use of high root temperatures in winter-grown tomatoes in rock wool offers a non-chemical method of controlling root rot caused by *Phytophthora cryptogea* Pethybr. & Lafferty. The high temperature was shown to enhance root growth while simultaneously suppressing inoculum potential and infection, and, consequently, reducing or preventing aerial symptoms (Kennedy and Pegg, 1990). Careful control of the temperature also proved important in the case of hydroponically grown spinach and lettuce, in which it prevented or reduced attack by both *Pythium dissotocum* Drechs. and *Pythium aphanidermatum* (Edson) Fitzp. (Bates and Stanghellini, 1984). Recently, attacks of *P. aphanidermatum* on nutrient film technique (NFT) grown lettuce in Italy were related to the high temperature (>29°C) of the nutrient solution. Root rot was inhibited by reducing the temperature below 24°C (Carrai, 1993).

Much less exploited are the effects of temperature and water pressure deficit on biocontrol agents, although the first models, resulting in advice for optimal climate control for insect natural enemies, are now becoming available (van Roermund and van Lenteren, 1998). In the case of biological control of plant pathogens, most of the studies carried out are related to the effect of environmental conditions on *Trichoderma*

*harzianum* Rifai, used as biocontrol agent of *B. cinerea* and of several hyperparasites of *Sphaerotheca fusca* (Fr.) Blumer. [= *Sphaerotheca fuliginea* (Schlechtend.:Fr.) Pollacci]. In the case of *T. harzianum*, populations of the antagonist are promoted by low vapour pressure deficit; in commercial greenhouses significant control of grey mould of cucumber has been correlated with low water pressure deficit but not with conditions of air saturation and dew deposition (Elad and Kirshner, 1993). In the case of *Ampelomyces quisqualis* Cesati:Schltdl., hyperparasite of *S. fusca*, a period of 24 h with low vapour pressure deficit is necessary (Philipp *et al.*, 1984). Low vapour pressure deficit also favours the activity of *Sporothrix flocculosa* Traquair, Shaw & Jarvis (Hajlaoui and Bélanger, 1991). More studies in this field are necessary, both in order to keep conditions close to the optimum for biocontrol agents within the greenhouse and for selecting biocontrol agents more adapted to the greenhouse environment (Elad *et al.*, 1996).

Greenhouses were initially built in areas with long, cold seasons to produce out-of-season vegetables, flowers and ornamental plants. Northern Europe is the paradigm of pioneering areas of greenhouse cultivation. The development of international exchanges of agricultural products and the availability of a variety of cheap plastic materials for covering simple structures has led to a spectacular increase in the area of protected crops in warmer regions like the Mediterranean basin and East and Southeast Asia (Wittwer and Castilla, 1995). These new regions are commonly characterized by low or irregular annual precipitation and poor vegetation development. The insertion of greenhouse patches leads to drastic changes in the structure and ecology of the landscape. In early stages of greenhouse cultivation in a new area, greenhouses are isolated spots, like oases, where some phytophagous insects find good seasonal conditions for rapid increases in density. But optimal weather and host-plant conditions rarely last throughout the year and for a few months – usually the hottest – the increase in the herbivore population is interrupted. When greenhouses become more common in the area, the mosaic pattern may evolve to a large area of protected crops, with a succession of crops throughout most of the year and with polyphagous pests. These pests are able to feed on many agricultural plants and migrate between greenhouses. Additionally, field crops may be excellent refuges for pests in hot seasons, when the temperature is too high for greenhouse cultivation. This has several consequences, as the immigration of pests into the greenhouse causes sudden and largely unpredictable pest density increases.

Exotic pests quickly become established, especially if ornamental plants are cultivated. Polyphagous pests (like whiteflies, spider mites, thrips, leafminers, several aphids species, especially *Aphis gossypii* Glover, leaf-eating caterpillars and soilworms), which may exploit several crops successively, become prevalent. As pest densities increase, crops are increasingly sprayed with insecticides, native natural enemies become very rare, and natural control loses effectiveness. Unexpected and high pest pressure from the outside makes biological control very difficult. Under such conditions, a more holistic approach would consider the fields outside the greenhouse and the crop inside the greenhouse as a single entity for applying integrated strategies against pests and diseases. Programmes for conserving native or introduced natural enemies in the area should both lower pest pressure on greenhouse crops and

incorporate beneficial fauna into the outside-inside greenhouse cycle of the pest-natural enemy complex.

#### 1.4. Cultural Techniques Used in Protected Cultivation

In most greenhouses of northern Europe continuous cropping is practised, without a fallow crop-free interval. This has profound implications for diseases and pests. In the case of plant pathogens, it leads to the build-up of soilborne pathogens and an increased importance of foliar pathogens with a broad host-spectrum (i.e. *B. cinerea*). The same can be said for insects that pupate in the soil such as leafminers and thrips.

Greenhouse crops are grown in various soils and soilless media whose physical and chemical properties are adjusted to obtain maximum productivity. These properties, such as heat conservation, water-holding capacity, fertilizer levels and pH can also be manipulated to reduce the amount of inoculum of pests and pathogens and the probability of infection (Jarvis, 1992). Systems for growing crops in the greenhouse vary widely in terms of complexity. The most common rooting media are soil and various soil mixtures, incorporating peat, vermiculite, perlite and several other materials which are added to the soil in order to modify its structure.

In the 60s, bench cultivation was adopted for high value crops (i.e. carnations), permitting better results in soil disinfestation. In the 80s and 90s, soilless substrates gained more and more importance, particularly in the northern European countries, because they eliminate or reduce the need for soil disinfestation. Among soilless substrates, rock wool has been widely used in northern Europe, while in the tropics and sub-tropics cheaper substrates have been exploited. The nutrient film technique, originally devised to improve precision in crop nutrition, reduces soilborne diseases and removes the cost of soil disinfestation. In fact, it confers relative freedom from diseases, although severe epidemics can still occur (Stanghellini and Rasmussen, 1994).

During the past two decades, various composted organic wastes and sewage sludges have partially replaced peat in container media used for production of ornamentals. Recycling of these wastes has been adopted for economic and production reasons. The cost of these composts can be lower than peat. Production costs may also be decreased because some of the compost-amended media, particularly those amended with composted bark, suppress major soilborne plant pathogens, thus reducing plant losses (Hoitink and Fahy, 1986). As discussed later, not only chemical and physical, but also biotic factors affect disease suppressiveness (see Chapter 23). The low pH of sphagnum peat, pine bark and composts could theoretically have beneficial side effects for some plants. For example, Phytophthora root rot of rhododendron (*Phytophthora cinnamomi* Rands) is suppressed at pH<4.0, because the low pH reduces sporangium formation, zoospore release and motility. This may be important during propagation of rhododendron cuttings under mist. Moreover, chemical inhibitors of *Phytophthora* spp. have been identified in composted hardwood bark. These inhibitors do not affect *Rhizoctonia solani* Kühn (Hoitink and Fahy, 1986).

Soilless cultivation can affect pests that need the soil/substrate to complete their development, as in the case of leafminers or thrips.

The thermal and gas exchange properties of rooting media affect the growth of roots as well as the activities of pathogens. Peat, a common rooting medium, used either alone or in mixture, often suppresses pathogen activity, depending on its origin (Tahvonen, 1982). However, pathogens, including species of pathogenic *Pythium* and *Fusarium* (including *Fusarium oxysporum* Schlechtend.:Fr. f. sp. *radicis-lycopersici* W.R. Jarvis & Shoemaker) have been isolated from commercial peat compost (Couteaudier *et al.*, 1985; Gullino and Garibaldi, 1994).

The design of benches is important due to the effect on the ventilation of seedling trays and potted plants.

Correct spacing prevents the establishment of a microclimate conducive to foliar diseases and the rapid spread of pathogens and pests from plant to plant in crops grown in groundbeds. Altered greenhouse and bench design can improve air movement, thus reducing the risk of diseases. Bottom heating of benches, a traditional means of avoiding Phytophthora, Pythium and Rhizoctonia root rots, is enhanced in cutting and seedling trays with upward air movement between the young plants. Through-the-bench air movement is perhaps the most neglected and simplest means of reducing seedling rots in tangled plant masses (Jarvis, 1989).

Every crop species and cultivar requires a special fertilizer regime in order to obtain maximum productivity and to prevent stress on the plant. Fertilizer requirements change as the crop ages from seeding to harvest. In general, excessive nitrogen leads to excessive foliage that is intrinsically more succulent and susceptible to damage and necrotrophic pathogens, such as *B. cinerea*, and also stimulates development of pests such as aphids and leafminers. Nitrogen generally has to be balanced with potassium; for many diseases, susceptibility decreases as the potassium-nitrogen ratio increases. Calcium generally enhances resistance, due to its role in the integrity of the cell wall.

No general practical recommendations can be made for controlling diseases by adjusting the fertilizer levels supplied to plants: each host-pathogen combination reacts differently. However, optimal, instead of maximal fertilization, results in lower pest and disease pressure. General recommendations can be given concerning irrigation. First of all, the factors that determine irrigation demand in greenhouse crops can all be closely regulated. From a general point of view, overhead irrigation must be carried out early in the day and should be limited late in the afternoon in order to avoid long periods of leaf wetness, which favour diseases such as downy mildews, rusts, grey mould, leaf spots, etc. When it is necessary to wet foliage for any reason (including pesticide spraying), it is always essential to maintain environmental conditions under which the foliage can dry out within a very short period of time. Also, it is important to avoid excess water in the soil: this creates conditions that are very favourable for the development of root rots. The effects of irrigation on pests are mainly through the relative humidity of the environment or through the water-status of the plants. For instance, plants under stress are more easily colonized by thrips and spider mites.

### **1.5. Factors Favourable to Pest and Disease Development**

Well-grown and productive crops are generally less susceptible to diseases, but in many

cases compromises have to be made between optimum conditions for economic productivity and conditions for disease and pest prevention. Well-fertilized and irrigated crops are, however, often more sensitive to pests, like aphids, whiteflies and leafminers.

Groundbed crops are rarely rotated, so soilborne pathogens and pests pupating in the soil accumulate if the soil is not disinfested. Soil disinfestation, although effective, creates a “biological vacuum” (Katan, 1984) (see Chapter 10). Major changes in cultural techniques include the use of hydroponic and soilless cultures and artificial substrates controlled by computerized systems. Although these changes are ultimately intended to reduce production costs and maximize profits, precise environmental and nutritional control that pushes plants to new limits of growth and productivity can generate chronic stress conditions, which are difficult to measure, but are apparently conducive to diseases caused by pathogens such as *Penicillium* spp. or *Pythium* spp. (Jarvis, 1989). Some soil substitutes and soilless systems do not always provide sufficient competition for pathogens, due to their limited microflora.

High host plant densities and the resulting microclimate are favourable to disease spread. Air exchange with the outside is restricted, so water vapour transpired by the plants and evaporated from warm soil tends to accumulate, creating a low vapour pressure deficit (high humidity). Therefore, the environment is generally warm, humid and wind-free inside the greenhouse.

Such an environment promotes the fast growth of most crops, but it is also ideal for the development of bacterial and fungal diseases (Baker and Linderman, 1979; Fletcher, 1984; Jarvis, 1992), of insects vectoring viruses and of herbivorous insects. For bacteria and many fungi (causal agents of rusts, downy mildews, anthracnose, grey mould, etc.) infection is usually accomplished in a film or drop of water on the plant surface. Unless temperature, humidity and ventilation are well regulated, this surface water can persist in the greenhouse until infection becomes assured.

Many of the energy saving procedures adopted during the past three decades are favourable to disease development, since they favour increases in relative humidity (Jarvis, 1992), but they may lead to pest suppression as temperatures are generally somewhat lower (see Chapter 8).

Most greenhouse crops are labour-intensive, and for long periods require daily routine operations (such as tying, pruning, harvesting). The risks of spreading pathogens through workers and machinery are increased by the risks deriving from accidental wounds and from the exposure of large areas of tissues by pruning.

Greenhouses are designed to protect crops from many adverse conditions, but most pathogens and several pests are impossible to exclude. Windblown spores and aerosols containing bacteria enter doorways and ventilators; soilborne pathogens enter in windblown dust, and adhere to footwear and machinery. Aquatic fungi can be present in irrigation water; insects that enter the greenhouse can transmit viruses and can carry bacteria and fungi as well. Once inside a greenhouse, pathogens and pests are difficult to eradicate.

## 1.6. Factors Stimulating Sustainable Forms of Crop Protection in Protected Cultivation

Protected cultivation is an extremely high-input procedure to obtain food and other agricultural products per unit of land, although inputs are the lowest when related to the yield per area. Crop protection activities contribute to the total input in variable proportions mainly through the application of pesticides. Several features of protected cultivation are delaying the adoption of more sustainable ways to control pests and diseases. In areas where protected cultivation is most intensive, crop protection costs rarely exceed 5% of the total production costs. In these circumstances, growers are not stimulated to make decisions based on economically founded criteria, and chemicals are frequently applied to prevent pest occurrence rather than to control real pest problems. This is particularly true in ornamental and flower crops, which can lose their value at extremely low pest densities (see Chapter 34). In addition, pesticides may be applied easily and little expertise is needed to spray or to recommend pesticides so that no specialized advisory personnel is usually employed by growers who rely on this “simple” technology.

Consequently, innovative crop protection methods become difficult to implement in practice. From a general point of view, vegetable crops, due to their limited diversity, are most suitable for IPM (see Chapters 30–33). In the case of ornamentals, the enormous crop diversity and the many cultivars per species grown make the development of IPM strategies more complicated (see Chapter 34).

Several stimuli are pushing growers to use less pesticides and to adopt more sustainable ways to protect crops from noxious organisms as world marketing becomes more global. Among the factors stimulating sustainable forms of crop protection are the following:

(i) Consumer concern about chemical residues. This is a general stimulus for growers wishing to adopt IPM systems (Wearing, 1988), but it is particularly relevant in fresh-consumed products like the majority of vegetables grown in greenhouses. Consumers not only demand high quality products, but are also concerned with how they are grown to judge them from the environmental aspects. Food marketers and European regional administrations are developing auditing procedures to sell vegetables under IPM or Integrated Production (IP) labels. In some cases, a surplus price is achieved by growers who produce vegetables under established IPM/IP technology.

(ii) Pesticide-resistance in pests and pathogens. As protected cultivation allows pest and pathogen populations to increase faster than in the open air, and as protected crops receive a great number of pesticide treatments, pesticide-resistance develops rapidly. Dozens of greenhouse pests or pathogens are suspected to have developed resistance to the most common active ingredients and this has been observed in many pests (aphids, whiteflies) and pathogens such as *B. cinerea* (see Chapter 11).

(iii) Side-effects of chemical application are increasingly observed in old and new growing areas (see Chapter 11). Because society in general and governments in particular are aware of the impact of chemicals on soil, water and air, several initiatives to restrict the use of chemicals in Europe and North America are being undertaken (van Lenteren, 1997).



(iv) Efficacy. Some pests and diseases are difficult – sometimes impossible – to control if an integrated approach is not adopted. On the other hand, natural control can prevent several pests from building-up high populations under the action of predators, parasitoids and entomopathogens that naturally establish on greenhouse crops if chemicals are not intensively applied, and several cultural practices allow enhancement of their effectiveness (see Chapters 18 and 19 for the role of parasitoids in leafminer control and polyphagous predators for a potentially broader effect on pests).

A first step towards sustainability in greenhouse crop protection is to analyse why and which phytophages and pathogens are able to increase their population densities until reaching damaging levels. Methods to improve the accuracy and speed of diagnosis are needed, particularly for diseases, and may be one of the most useful applications of biotechnology. Once the pest or disease is correctly diagnosed, environmental factors that allow or prevent such a pest or pathogen to reach economic injury levels should be identified.

Such knowledge may help us to design integrated methods to take advantage of the whole environment. If an action threshold is determined, accurate techniques for pest and disease sampling and monitoring should permit intervention at the best moment (see Chapters 6 and 7) and prevent unnecessary treatments. The identification of key factors governing pest or pathogen population dynamics may allow modification of the greenhouse and crop environment – including greenhouse-surroundings – to adversely affect a pest or pathogen or to favour the effectiveness of the natural enemies or antagonists.

Sometimes this can be achieved cheaply – in both economic and energetic terms – by means of correct crop and management practices (see Chapter 8). As mentioned before, the most damaging pests and many pathogens in greenhouses are polyphagous; although they are able to develop on many host plants, their negative effect on yield varies with host plant species and cultivar. The development of cultivars which are less susceptible to pests and diseases or that favour the activity of pest natural enemies is undoubtedly one of the most sustainable ways to control diseases in greenhouses and its potential for pests has been shown in a few but significant cases (see Chapter 9).

Many of the arthropod pests and diseases that affect greenhouse crops are exotic and became established in greenhouse growing areas from accidental importation of infested crops, mainly ornamentals. In some cases, as for *Liriomyza trifolii* (Burgess) and *Liriomyza huidobrensis* (Blanchard), native natural enemies have been able to greatly contribute to the natural control of these pests, but in other cases exotic parasitoids or predators have to be released in the environment to control them, as is done for *Trialeurodes vaporariorum* (Westwood) by means of *Encarsia formosa* Gahan. Natural and biological control is nowadays the basis of most of the integrated pest management strategies adopted in northern Europe (van Lenteren, 1995) and its practical achievements are particularly emphasized in this book (see Chapters 13–22). The history of biological control of diseases in greenhouses is more recent, but significant advances have also been achieved here in the last few years (see Chapters 23–28). Given the very high cosmetic demands and the low pest and disease thresholds applied by greenhouse growers, the progress in application of Integrated Pest and Disease Management is remarkable, as described in Chapters 30–34. Until recently,

biological and integrated control was seen as a cost factor. Nowadays, however, it is considered as a beneficial marketing factor.

### 1.7. Concluding Remarks

The greenhouse industry faces many new crop protection problems as a consequence of modification of production procedures and crops. The major changes will include more widely adopted mechanization and automation systems for improved crop management and the use of biotechnology in plant production. These modifications will affect the severity of pests and diseases.

Strong cooperation among plant pathologists, entomologists and horticulturists is necessary in order to assure that new management practices have a beneficial effect on plant health. Methods to improve the accuracy and speed of diagnosis are needed and may be one of the best applications of biotechnology. Improved and widely used monitoring and diagnosis systems to determine the degree of infestation and economic thresholds of pathogens and pests will enable rational management decisions. A high priority should be given to the production of pathogen and pest-free propagation material, obtained through sanitation. The use of pest and pathogen-free material, and growing media disinfested with steam or naturally suppressive to soilborne pathogens will help to reduce the impact of important pests and diseases considerably.

When all such measures are integrated with the use of resistant germplasm, with modern techniques for applying pesticides and with biological control of several diseases and pests, a greatly reduced input of chemicals becomes realistic for protected cultivation.

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## VIRAL DISEASES

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### 2.1. Introduction

Viruses are a major problem in greenhouse crops especially in temperate regions. However, most efforts in programmes for integrated pest and disease management are focused on pest and fungal or bacterial disease control and few recommendations are given for viral diseases. In general, viruses are not considered at all or are treated in a very simplistic manner. The main reason for this is the lack of information about viral disorders to give recommendations to deal with plant virus problems. In addition, in contrast to pests, fungi, or bacteria, no direct control methods can be used against viruses. Nevertheless, in recent years a significant progress in knowledge on plant viruses has occurred and valuable information has been obtained that will facilitate the development of control strategies. Because of difficulties and costs of reducing the spread of viruses by controlling their vectors and sources of infection, the introduction of resistance to a particular virus into commercially useful cultivars is the best control method but, unfortunately, the exception. Most virus management programmes involve the integration of indirect measures to avoid or reduce the sources of infection and dispersal of the virus, or the minimization of the effect of infections on crop yield. When confronted to a virus problem, the understanding of the ecology and epidemiology of the disease will provide the information needed to make strategic decisions for virus disease control.

In many circumstances control strategies are based on the dispersal procedures used by viruses in nature and similar control measures are recommended for viruses with equivalent dispersal manners. Therefore, virus dispersal mechanisms and the deduced control methods will be briefly reviewed in the next section before major diseases caused by plant viruses in protected crops are described.

### 2.2. Plant Virus Dispersal Mechanismr

The ability of a virus to be disseminated and perpetuated in time and space depends upon which methods are used for dispersal. Figure 2.1 summarizes the main transmission mechanisms of plant viruses; one or several of them can be exploited by a specific virus. The knowledge about the main dispersal procedures of a virus in nature will provide a means to prevent and control viral diseases: to minimize sources of infection, to reduce dissemination during growing practices, and/or to limit spread by vectors. Some aspects of virus dispersal and their importance in virus control are analysed below.

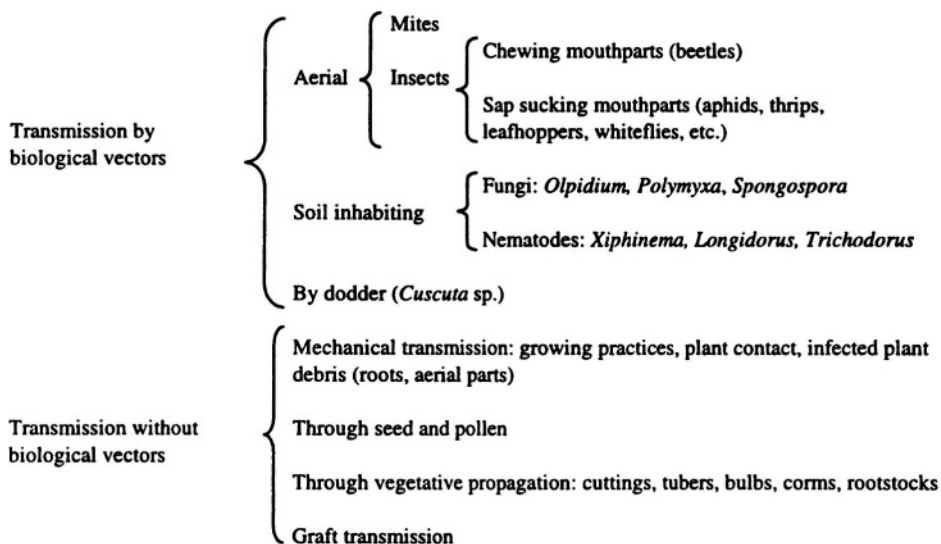


Figure 2.1. Main transmission strategies used by plant viruses.

### 2.2.1. SOURCES OF INFECTION

As a general rule, virus-infected plants are sources for secondary spread by mechanical or biological vector means and, therefore, should be eliminated as soon as possible. When existing, mechanical transmission is one of the most dangerous dispersal methods for viruses in protected crops due to the frequent handling of plants during the intensive cropping practices. Some viruses are extremely important in protected crops because of their efficient transmission by mechanical inoculation during cultural operations. If plants infected with some of these viruses are suspected to be present in a crop, secondary spread can be reduced by adequate treatment of hands and implements during plant handling. In these cases, plant debris in soil and greenhouse structures are important sources for primary infections in subsequent sensitive crops and, therefore, as long as possible, they should be eliminated and soil and structures disinfected.

The propagation material used for planting can be a very effective means of introducing viruses into a crop at an early stage, giving randomized foci of infection within the planting. If other transmission methods (e.g. mechanical, insects) are coupled, which may rapidly spread the virus within the crop, then infected seeds, plantlets, etc. can be of significant importance in the epidemic of the disease. In these cases, certified virus-free material should be used as the basis to control the virus.

Approximately 18% of the known plant viruses are seed-transmitted in one or more hosts (Mink, 1993; Johansen *et al.*, 1994). The rate of seed transmission is very variable depending on the virus/host combination and is not necessarily a good indicator of the epidemiological importance; low transmission rates combined with efficient secondary spread can be very important epidemically. Tolerance levels in a seed certification

programme will depend, therefore, on the kind of secondary spread. For example, only very low infection levels are permitted in lettuce seed lots for an effective control of lettuce mosaic virus (LMV) because of its efficient secondary spread by aphids; good control was obtained in California if less of one seed in 30,000 was infected (Grogan, 1980; Dinant and Lot, 1992).

For many vegetatively propagated crops like ornamentals (carnation, tulip, etc.) the main virus sources are infected plants themselves and their vegetative derivatives (cuttings, tubers, bulbs, connns, rootstocks). In these cases, control may be done by using virus-free stocks and certification schemes to produce propagation material free of virus.

Soil may be another source of virus infection. Soilborne viruses can be transmitted by fungi or nematodes or can have no biological vector like tobamoviruses, that are very stable and are maintained in infected plant debris mixed with the soil. Control usually is through soil disinfection if no resistant cultivars are available.

The maintenance of virus-sensitive crops continuously throughout the year will ensure the permanent presence of significant levels of inoculum and, then, of virus infection. Therefore, crop rotations should incorporate non-sensitive species. However, although a rupture of the infection cycle is done, the presence of alternative hosts for the virus in the surroundings of the protected crop can be of special relevance to perpetuate the virus. The management of these hosts will help to the control of the virus.

### 2.2.2. VECTOR TRANSMISSION

Many important viruses in protected crops are transmitted from plant to plant by invertebrates. Sap-sucking insects are the main vectors, mostly Homoptera, and among them, aphids are the most important, transmitting 43% of known viruses.

Control of insect-transmitted viruses has been traditionally done by spraying insecticides to reduce the vector populations. However, the effectiveness of treatments in controlling the virus depends on virus/vector transmission relationships. Table 2.1 summarizes the main properties of the different kinds of relationships based on the feeding times needed by the vector to acquire (*acquisition time*) and inoculate (*inoculation time*) the virus, on the *latent period* from acquisition until the vector is able to transmit the virus, and on the *retention time* during which the vector remains infective following inoculative feeding without further access to the virus. This classification is mainly based on aphid-transmitted viruses. No evidence for virus in hemocoel or salivary system exists in the *noncirculative* transmission. In the *circulative transmission*, virus is acquired by feeding, enters the hemocoel via the hindgut, circulates in hemolymph, and enters the salivary gland. Inoculation results from transport of virus into the salivary duct, and introduction of saliva into the plant during feeding. If virus multiplies in the insect cells then the transmission is called *propagative*.

Insecticide treatments may be ineffective in controlling nonpersistently-transmitted viruses (short acquisition and inoculation times, no latent period, Table 2.1) because acquisition, latent, and inoculation times are so short that the virus is acquired and

transmitted before the vector can be affected by most insecticides. However, especially in protected crops, chemical treatments can help to reduce the overall vector populations and therefore secondary spread of the disease. For nonpersistently-transmitted viruses, oils or tensioactive film-forming products have been reported to be effective in controlling virus acquisition and inoculation in outdoor crops. Insecticidal treatments used to control semipersistently- (long acquisition and inoculation times, no latent period, Table 2.1) or circulatively- (long acquisition and inoculation times, latent period, Table 2.1) transmitted viruses can be effective in controlling the virus because longer acquisition, inoculation and/or latent times are needed, and the vector may die before the virus can be transmitted. In any case, it should be noted that the small percentages of insects that usually survive the treatments are enough to cause important infections if virus sources are present. Accurate knowledge of disease epidemiology in a certain region will provide information about the critical periods of infection, which will facilitate decisions on when treatments should be done, or the adjustment of planting dates to avoid high vector populations in young plantings (Zitter and Simons, 1980).

**TABLE 2.1. Classification of virus/vector relationships based on time needed for acquisition and inoculation of the virus, time after an acquisition feed for which the vector is unable to transmit (latent period), and time of retention of inoculativity following inoculative feeding, without further access to virus**

|                  | Noncirculative |                | Circulative (Persistent) |               |
|------------------|----------------|----------------|--------------------------|---------------|
|                  | Nonpersistent  | Semipersistent | Nonpropagative           | Propagative   |
| Acquisition time | Seconds        | Minutes/hours  | Minutes/hours            | Minutes/hours |
| Latent period    | 0              | 0              | Hours/days               | Hours/days    |
| Inoculation time | Seconds        | Minutes/hours  | Hours                    | Hours         |
| Retention time   | Minutes        | Hours          | Days                     | Entire life   |

### 2.3. Major Virus Diseases in Greenhouse Crops

Table 2.2 summarizes the characteristics of the main virus species that cause diseases in protected crops, for which comprehensive reviews are available (Smith *et al.*, 1988; Dinant and Lot, 1992; German *et al.*, 1992; Coffin and Coutts, 1993; Shukla *et al.*, 1994; Murphy *et al.*, 1995; Brunt *et al.*, 1996). Some of these species have been further reviewed in the text.

#### 2.3.1. APHID-TRANSMITTED VIRUSES

##### *Cucumber Mosaic Virus (CMV)*

*Description.* CMV is the type species of the genus Cucumovirus of the family Bromoviridae of plant viruses. CMV virions are 29 nm icosahedral particles that



TABLE 2.2. Major virus species affecting protected crops

| Natural transmission <sup>1</sup> | Family         | Genus <sup>2</sup> | Species <sup>2</sup>                    | Acronym | Main infected crops <sup>3</sup> |
|-----------------------------------|----------------|--------------------|---|---------|----------------------------------|
| Aphids (np)                       | Bromoviridae   | Cucumovirus        | Cucumber mosaic virus                   | CMV     | cu, le, me, pe, to, wa, zu       |
| Aphids (np), seed (bc)            | Potyviridae    | Potyvirus          | Bean common mosaic virus                | BCMV    | bc, fa                           |
| Aphids (np)                       | Potyviridae    | Potyvirus          | Bean yellow mosaic virus                | BYMV    | bc, fa                           |
| Aphids (np), seed (le)            | Potyviridae    | Potyvirus          | Lettuce mosaic virus                    | LMV     | le                               |
| Aphids (np)                       | Potyviridae    | Potyvirus          | Potato virus Y                          | PVY     | pe, to                           |
| Aphids (np)                       | Potyviridae    | Potyvirus          | Papaya ringspot virus-W                 | PRSV-W  | cu, me, wa, zu                   |
| Aphids (np)                       | Potyviridae    | Potyvirus          | Watermelon mosaic virus <sup>2</sup>    | WMV2    | cu, me, wa, zu                   |
| Aphids (np)                       | Potyviridae    | Potyvirus          | Zucchini yellow mosaic virus            | ZYMV    | cu, me, wa, zu                   |
| Aphids (np)                       | Potyviridae    | Potyvirus          | Zucchini yellow fleck virus             | ZYFV    | cu, me, wa, zu                   |
| Aphids (p)                        | - <sup>4</sup> | Luteovirus         | Beet western yellows virus              | BWYV    | le, cu, pe, to, wa, zu           |
| Aphids (p)                        | -              | Luteovirus         | Cucurbit aphid-borne yellows virus      | CABYV   | cu, me, zu                       |
| Thrips (p)                        | Bunyaviridae   | Tospovirus         | Tomato spotted wilt virus               | TSWV    | fa, le, pe, pea, to              |
| <i>Bemisia tabaci</i> (p)         | Geminiviridae  | Geminivirus        | Tomato yellow leaf curl virus           | TYLCV   | to                               |
| <i>Bemisia tabaci</i> (sp)        | -              | -                  | Cucumber vein yellowing virus           | CVYV    | cu, me                           |
| <i>Bemisia tabaci</i>             | -              | Closterovirus      | Lettuce chlorosis virus                 | LCV     | le                               |
| <i>Bemisia tabaci</i> (sp)        | -              | Closterovirus      | Lettuce infectious yellows virus        | LIYV    | le, me, wa, zu                   |
| <i>Bemisia tabaci</i> (sp)        | -              | Closterovirus      | Cucurbit yellow stunting disorder virus | CYSDV   | cu, me                           |

<sup>1</sup>np, non persistent; sp, semipersistent; p, persistent

<sup>2</sup>In bold are indicated those species or virus genus that are reviewed in this chapter

<sup>3</sup>bc, French bean; cu, cucumber; eg, eggplant; fa, faba bean; le, lettuce; me, melon; pe, pepper; to, tomato; wa, watermelon; zu, zucchini squash

<sup>4</sup>Not defined

TABLE 2.2. Major virus species affecting protected crops (cont.)

| Natural transmission <sup>1</sup>     | Family        | Genus <sup>2</sup> | Species <sup>2</sup>               | Acronym | Main infected crops <sup>3</sup> |
|---------------------------------------|---------------|--------------------|------------------------------------|---------|----------------------------------|
| <i>Trialeurodes vaporariorum</i> (sp) | <sup>4</sup>  | Closterovirus      | Beet pseudoyellows virus           | BPYV    | cu, me, le                       |
| <i>Trialeurodes vaporariorum</i> (sp) | -             | Closterovirus      | Tomato infectious chlorosis virus  | TICV    | to                               |
| <i>Trialeurodes vaporariorum</i> ,    | -             | Closterovirus      | Tomato chlorosis virus             | ToCV    | to                               |
| <i>Trialeurodes abutiloneus</i> ,     |               |                    |                                    |         |                                  |
| <i>Bemisia tabaci</i>                 |               |                    |                                    |         |                                  |
| Beetles (np)                          | Comoviridae   | Comovirus          | Squash mosaic virus                | SqMV    | me, wa, zu                       |
| <i>Olpidium bornovanus</i>            | Tombusviridae | Carnovirus         | Melon necrotic spot virus          | MNSV    | cu, me, wa                       |
| <i>Olpidium brassicae</i>             | -             | -                  | Lettuce big vein virus             | LBVV    | le                               |
| <i>Olpidium brassicae</i>             | -             | -                  | Lettuce ring necrosis virus        | LRNV    | le                               |
| Fungus                                | -             | -                  | Pepper yellow vein disease         |         | pe                               |
| Mechanical                            | -             | <b>Tobamovirus</b> | Tobacco mosaic virus               | TMV     | pe, to                           |
| Mechanical, seed (to)                 | -             | Tobamovirus        | Tomato mosaic virus                | ToMV    | pe, to                           |
| Mechanical, seed (pe)                 | -             | Tobamovirus        | Pepper mild mottle virus           | PMMV    | pe                               |
| Mechanical, seed (cu)                 | -             | Tobamovirus        | Cucumber green mottle mosaic virus | CGMMV   | cu, me, wa                       |
| Mechanical, fungus                    | -             | Potexvirus         | Potato virus X                     | PVX     | to                               |
| Unknown (soil)                        | Tombusviridae | Tombusvirus        | Tomato bushy stunt virus           | TBSV    | eg, pe, to                       |
| Unknown (soil), seed (cu)             | Tombusviridae | Tombusvirus        | Cucumber leaf spot virus           | CLSV    | cu                               |

<sup>1</sup>np, non persistent; sp, semipersistent; p, persistent

<sup>2</sup>In bold are indicated those species or virus genus that are reviewed in this chapter

<sup>3</sup>be, French bean; cu, cucumber; eg, eggplant; fa, faba bean; le, lettuce; me, melon; pe, pepper; to, tomato; wa, watermelon; zu, zucchini squash

<sup>4</sup>Not defined

encapsidate a single-stranded RNA genome of messenger sense divided in three molecules, RNA 1, 2 and 3 ( $1.3 \times 10^6$ ,  $1.1 \times 10^6$ , and  $0.8 \times 10^6$  daltons, respectively). Some CMV isolates encapsidate an additional small RNA called satellite RNA ( $0.1 \times 10^6$  daltons), that depends on virus for replication, encapsidation and movement. RNA satellites are able to modulate the symptoms induced by CMV (Palukaitis *et al.*, 1992).

A great variability among CMV isolates has been reported. According to biological properties of symptomatology, thermosensitivity *in vivo*, molecular and serological characteristics, most CMV isolates have been assigned to two main groups.

*Transmission, Host Range and Diseases.* In nature, CMV is transmitted in a nonpersistent manner by more than 60 aphid species including *Aphis gossypii* Glover, *Macrosiphum euphorbiae* (Thomas) and *Myzus persicae* (Sulzer). Variable rates of seed transmission have been described in 20 species including some vegetable crops like bean or spinach, or weeds like *Stellaria media* Cyrill. There is no evidence of seed transmission in cucurbits. CMV can be mechanically transmitted in experimental conditions.

CMV has an extremely wide host range that comprises more than 1000 species of dicotyledons and monocotyledons. Host range includes many important vegetable crops like melon, cucumber, zucchini squash, watermelon, tomato, pepper, eggplant, lettuce, carrot, celery, spinach, pea, etc.; ornamentals like anemone, aster, dahlia, delphinium, geranium, lily, periwinkle, primula, petunia, viola, zinnia, etc.; and woody and semiwoody plants like banana, ixora, passion fruit, etc. Symptoms are extremely variable depending on the CMV isolate, host species or cultivar, plant age at infection time, and environmental conditions. Early infected plants can show marked stunting. Symptoms in leaves are mosaic, mottle and/or distortion. Necrosis is induced by certain isolates. Flower abortion and fruit discoloration and malformations are caused.

*Economic Importance and Control.* CMV is distributed worldwide, predominantly in temperate regions but with increasing importance in tropical countries. It causes serious diseases in many important crops grown in the open but also in protected conditions (tomato, pepper, cucurbits, etc.) (Jordá *et al.*, 1992). Yield reductions are mainly due to decreased fruit set, and production of non-marketable fruits because reduced size, or presence of symptoms like mosaics, malformations or necrosis. Control of CMV is difficult because of the wide host range and its rapid natural transmission by aphids. Integrated control measures are recommended in protected crops to reduce CMV incidence: (i) elimination of infected plants; (ii) avoidance of aphid entrance in the greenhouse by covering entrances with aphid-proof nets; (iii) reduction of aphid populations by using insecticides; (iv) use of virus-free seeds (for example in bean and spinach); and (v) elimination of alternative spontaneous hosts present in and around the crop. Resistance to CMV is available in cucumber and programmes are in course in melon using Korean and Chinese varieties. Sources of resistance or tolerance have been found in most cultivated or related species. However, in most cases resistance or tolerance is not absolute, and is overcome by some CMV species. Aphid vector tolerance or resistance incorporated in the plant can be combined with other control methods. Transgenic melon, cucumber and squash plants expressing the coat protein gene of CMV offer a good level of resistance to several strains of the virus.

### *Potyvirus Genus*

*Description.* The Potyvirus genus of the family Potyviridae is by far the largest of the plant virus groups. Many members cause important economic losses in protected crops and can be a major limiting factor for production. Virus particles are elongated and flexuous (680–900 × 11 nm) with one molecule of messenger sense single-stranded RNA ( $3.0\text{--}3.5 \times 10^6$  daltons) attached covalently to a protein. The genomic RNA codes for a large polyprotein that is proteolytically cleaved to yield the mature viral proteins. Virus infections are associated with characteristic cytoplasmic and nuclear inclusions, pinwheels, bundles and laminated aggregates (Shukla *et al.*, 1994).

*Transmission, Host Range and Diseases.* Potyviruses are transmitted in nature by aphids in a non-persistent manner. Some aphid species (especially those of the genera *Myzus*, *Aphis* and *Macrosiphum*) are associated with high virus incidences in crops. Seed transmission is important epidemiologically in certain potyviruses, like bean common mosaic virus (BCMV) in French bean, or LMV in lettuce. Potyviruses can be transmitted experimentally by mechanical inoculation.

In nature, most potyviruses have relatively narrow host ranges, few species within one genus or closely related genera; for example: BCMV is restricted to *Phaseolus* species; potato virus Y (PVY) to members of the Solanaceae; watermelon mosaic virus2 (WMV2), zucchini yellow mosaic virus (ZYMV) and zucchini yellow fleck virus (ZYFV) mainly to species of the Cucurbitaceae; and LMV to species mainly in Compositae.

Potyviruses can induce severe diseases in important crops. Symptoms may vary depending on host species, virus strain, environmental conditions and plant age at infection time. Potyviruses like ZYMV, WMV2 and papaya ringspot virus-W strain (PRSV-W) can cause severe diseases in zucchini, squash, melon, cucumber and watermelon, inducing stunting, chlorosis, mosaic, leaf malformation, flower abortion, and fruit and seed malformation. Vein clearing, mosaic, yellow mottling and growth reduction are often observed in LMV infections of lettuce, endive and spinach. Legume infecting potyviruses like BCMV cause abnormal formation of seeds that are smaller, discoloured and/or distorted.

*Economic Importance and Control.* The Potyvirus genus is the most devastating among plant viruses. Damaging members like BCMV, bean yellow mosaic virus (BYMV), ZYMV, WMV2, PRSV-W and PVY are spread worldwide and cause economically important problems where present. Several authors reported losses up to 100% in squash, cucumber and watermelon caused by ZYMV. Potyviruses are mainly a problem in outdoor crops, however, can also be a severe threat in protected crops.

Control should be done by an adequate management of the crop, integrating different control measures: if seed-transmitted, the use of certified virus-free seeds is the basis for effective control; use of virus-free plantlets will avoid primary infections; because transmitted in a nonpersistent manner, spraying insecticides is not effective for preventing virus spread, however, effective control has been obtained in some cases by spraying with light mineral oils in outdoor crops. Successful breeding programmes for

resistant cultivars have been done in lettuce to LMV (resistance breaking strains have recently been described), in French bean to BCMV, and in melon to PRSV-W. Transgenic approaches have also been explored, overcoming difficulties associated with conventional breeding methods. Cross protection using an attenuate poorly aphid-transmissible strain of ZYMV (ZYMV WK) have been successfully used to control ZYMV in cucumber, melon and squash.

### *Luteovirus Genus*

*Description, Transmission, Host Range, Diseases and Economic Importance.* There are a number of yellowing diseases transmitted in nature by aphids that are caused by viruses in the Luteovirus genus. This is the case of beet western yellows (BWYV) and cucurbit aphid-borne yellows (CABYV) viruses. Viral particles are 25–30 nm icosahedral, and encapsidate a monopartite, single stranded, messenger sense RNA genome. Transmission in nature is by aphids in a circulative, nonpropagative, persistent manner. BWYV infects lettuce, cucumber, watermelon, squash, sugarbeet, carrot, spinach, pepper and tomato, symptoms being mild chlorotic spotting, yellowing, thickening and brittleness of older leaves. It has been reported in North America, Europe and Asia, and is probably distributed worldwide. CABYV causes a yellowing disease of melon, cucumber and zucchini squash; symptoms are initial chlorotic patches, leaf thickening and general bright yellowing of leaves. In melon and cucumber important yield losses are reported, due to reduced number of fruits per plant caused by flower abortion but not by altering fruit shape or quality. It was first described in France in outdoor and protected crops and has been found through the Mediterranean area, Asia, Africa and California.

*Control.* Disease management should be by integrating measures to reduce aphid populations within the greenhouse via avoidance of insect entrance (nets in windows) and chemical spraying, with measures to reduce infection foci (virus-free planting material, elimination of infected plants). Sources of resistance have been found for CABYV in melon germplasm, and for BWYV in lettuce (Dogimont *et al.*, 1996).

## 2.3.2. WHITEFLY-TRANSMITTED VIRUSES

### *Tomato Yellow Leaf Curl Virus (TYLCV)*

*Description.* TYLCV is a member of the geminivirus genus of plant viruses whose virions have twin isometric particle morphology that encapsidate a circular, single-stranded, monopartite DNA genome. Based on its transmission by the whitefly *Bemisia tabaci* (Gennadius) to dicotyledons, TYLCV belongs to the subgroup III of geminiviruses. Similar to other monopartite geminiviruses of this group, TYLCV genome contains six partially overlapping open reading frames (ORFs) organized bidirectionally, with two ORFs (V1 and V2) in the virion-sense, and four (C1, C2, C3, and C4) in the complementary sense. These ORFs encode proteins involved in replication, movement, transmission and encapsidation of the virus, and are separated

by an intergenic region of approximately 300 nucleotides that contains signals for replication and transcription of the viral genome.

*Transmission, Host Range and Diseases.* TYLCV is transmitted from plant to plant by *B. tabaci* in a circulative manner (Mehta *et al.*, 1994); propagation in insect cells is still under discussion. TYLCV has a very narrow host range that covers some solanaceae species like tomato, *Datura stramonium* L. and different *Nicotiana* spp., and has also been described in French bean and *Malva parviflora* L. (Mansour and Al-Musa, 1992; Cohen and Antignus, 1994). In nature, TYLCV-caused diseases mainly affect to tomato crops. Symptoms in tomato consist in stunting, curling of leaflet margins with or without yellowing, reduction in leaf size and flower abortion.

*Economic Importance and Control.* TYLCV causes devastating damages in tomato crops of the Mediterranean basin, subtropical Africa and Central America. Losses are caused by reduced fruit yield and by the limitation of the economically feasible growing areas and periods. Effective control through crop management measures to avoid the vector and inoculum sources is possible in greenhouse crops. In the semiprotected crops typical of the Mediterranean regions, chemical control of vectors is ineffective to limit the spread of TYLCV. In these cases, control should be based on crop management following recommendations derived from the epidemiological knowledge of the disease and/or the use of the resistant/tolerant cultivars commercially available.

#### *Closteroviruses and Closterovirus-like Viruses*

*Description, Transmission, Host Range, Diseases and Economic Importance.* In recent years there is an emerging threat in worldwide agriculture, particularly in temperate regions, that is caused by a number of viruses that are transmitted by whiteflies and induce yellowing symptoms in plants. This is probably related to the increasing importance of whitefly populations worldwide and to changes in the relative predominance of existing species. These viruses are not generally well characterized, however most of them seem to be members of the Closterovirus genus of plant viruses. This is the case of beet pseudo yellows virus (BPYV) and tomato infectious chlorosis virus (TICV), transmitted by *Trialeurodes vaporariorum* (Westwood), and of cucumber yellow stunting disorder virus (CYSDV), lettuce infectious yellows virus (LIYV) and lettuce chlorosis virus (LCV), transmitted by *B. tabaci* and *Bemisia argentifolii* Bellows & Perring (Célix *et al.*, 1996; Duffus, 1996a,b). A semipersistent transmission manner has been demonstrated in certain cases. Whitefly-transmitted closteroviruses have flexuous particles of variable length depending on species (900 × 12 nm). The genome is composed by two molecules of single stranded, messenger sense RNA, with a size of about 8 kilobases each. This is opposed to the monopartite genome characteristic of the aphid-transmitted closteroviruses such as beet yellows (BYV) or citrus tristeza (CTV) viruses. Most of these viruses have been first described in USA and cause important diseases in outdoor and protected crops. Symptoms usually consist in interveinal yellowing of the leaves, stunting and/or necrosis. LIYV infects lettuce, sugar beet, melon, squash, watermelon and carrot; yield losses of up to 50–75% occur in lettuce

affected crops. TICV was found infecting field and greenhouse tomato crops in California. LCV infects lettuce crops and does not infect cucurbits. CYSDV is present in the Mediterranean area, causing disease in cucurbits, and has not been described in America.

*Control.* Integrated management of the disease in protected crops should be based on the early elimination of primary infected plants, avoidance of entrance of whiteflies, and rationale insecticide treatments to reduce overall vector populations in the greenhouse. In melon, resistance to BPYV has been described few years ago and, recently, to CYSDV (Gómez-Guillamón *et al.*, 1995).

### 2.3.3. THRIPS-TRANSMITTED VIRUSES

#### *Tomato Spotted Wilt Virus (TSWV)*

*Description.* TSWV is the type species of the Tospovirus genus of the family Bunyaviridae. TSWV has isometric, membrane-bound particles of approximately 80 nm in diameter that contain two ambisense, S (small) and M (medium), and one negative sense, L (large) linear single stranded RNA segments. The L RNA encodes the viral RNA polymerase, the M RNA encodes a non-structural (NsM) protein and a precursor to the G1 and G2 glycoproteins associated with the lipid membrane of the virus particle, and the S RNA encodes an additional non-structural (NsS) protein and the nucleocapsid (N) protein.

*Transmission, Host Range and Diseases.* TSWV is transmitted by several species of thrips of which *Frankliniella occidentalis* (Pergande) is the most important worldwide. Transmission is circulative and propagative and is unique in that the virus is only acquired by first stage larvae and is transmitted by second stage larvae and adults. Adults are the most important epidemiologically because are more mobile and remain viruliferous for their entire life (German *et al.*, 1992; Aramburu *et al.*, 1997).

TSWV has a wide host range, infecting more than 250 species in 70 different families of both monocotyledons and dycotyledons including important cultivated species (Edwardson and Christie, 1986). The symptomatology vary from no symptoms to chlorotic or necrotic local lesions, ring spots, line patterns, mosaic, mottling, bronzing, chlorosis, necrosis, leaf or stem malformation, and stunting. Flower abortion is observed and fruits can exhibit malformation, necrosis and abnormal coloration. Symptoms vary depending on host-virus isolate combination, plant age at infection time and environmental conditions.

*Economic Importance and Control.* TSWV causes serious diseases worldwide in both outdoor and protected economically important crops. Significant yield losses are caused in vegetable crops like tomato, pepper or lettuce, and in different ornamental species.

Control of TSWV is difficult because of the wide host ranges of both the virus and the vector and the efficient natural transmission by thrips. The use of insecticides to reduce virus incidence by controlling the vector is ineffective and crop management

practices are difficult to implement. In this situation, the use of resistant cultivars is the best solution. Genetic resistance to TSWV has been difficult to identify, characterize and incorporate into commercial cultivars. Some important progress has been done in this field in tomato, where resistant cultivars are available, and in pepper and lettuce. However, the durability of resistance depends upon the biological variability that seems to exist among TSWV isolates (Roca *et al.*, 1997). The development of genetically-engineered virus-resistant plants is also under investigation. While efforts to produce resistant crops are going on, control in protected crops should be done integrating measures to limit the spread of the disease using certified virus-free vegetal material, roguing infected plants, and by biological or chemical control of thrips.

#### 2.3.4. BEETLE-TRANSMITTED VIRUSES

##### *Squash Mosaic Virus (SqMV)*

*Description.* SqMV is a member of the Comovirus genus. Virions are 30 nm isometric particles that encapsidate two single-stranded RNA segments of  $1.6 \times 10^6$  and  $2.4 \times 10^6$  daltons, respectively. Comoviruses produce polyproteins from which the non-structural and structural proteins are generated by proteolytic cleavage. RNA1 carries all information for RNA replication, including the polymerase. Non-structural proteins include a putative cell-to-cell movement protein (encoded by RNA2), an NTP-binding motif-containing protein, a Vpg, a proteinase, and a polymerase. Two coat polypeptides are encoded by the RNA2. SqMV has several pathogenically different strains. Isolates could be grouped into 2 serological groups that differ in seed transmissibility and, to a certain extent, in host range and symptomatology (Campbell, 1971).

*Transmission, Host Range and Diseases.* SqMV is naturally transmitted by chewing insects, especially chrysomelid beetles, in a nonpersistent manner, and, like all comoviruses, is seed-borne (embryo-borne). Subgroup 1 isolates are seed-transmitted in pumpkin, squash, melon and watermelon, and subgroup 2 isolates in pumpkin and squash. Mechanical transmission easily occurs by plant contact and during cultural operations. Commercial and experimental seed lots generally yield about 1–10% infected seedlings but up to 94% transmission has been reported in melon. Natural host-range is narrow, restricted to the Cucurbitaceae, in which most species are susceptible. Experimentally, it also infects plants in other families. In cucurbits, SqMV cause symptomless infection or may induce ringspots, systemic mosaic, malformation and vein-banding, depending on virus strain, host and environmental conditions. Symptoms in fruits vary from small chlorotic areas to severe malformation with dark green areas. Isolates in subgroup 1 cause severe symptoms in melon, and mild ones in pumpkin; some strains infect watermelon. Subgroup 2 isolates do not infect watermelon and cause mild symptoms in melon and severe in pumpkin.

*Economic Importance and Control.* SqMV is widely distributed in the western hemisphere and also occurs in other countries throughout the world, probably introduced through seed lots. Control is achieved by testing seed lots to prevent seed



transmission (Nolan and Campbell, 1984). If present, mechanical transmission should be avoided by elimination of symptomatic plants, and reducing handling and pruning transmission possibilities.

### 2.3.5. FUNGI-TRANSMITTED VIRUSES

#### *Melon Necrotic Spot Virus (MNSV)*

*Description.* MNSV belongs to the genus Carnovirus of the family Tombusviridae. Virions are 30 nm icosahedral particles that encapsidate a monopartite, single-stranded RNA genome ( $1.5 \times 10^6$  daltons). Two putative proteins (p29 and its read-through p89) are expressed from the genomic-length RNA, and another two (p7A and its read-through p14) from a 1.9 kilobases (kb) subgenomic RNA. Coat protein is expressed from a 1.6 kb subgenomic RNA (Riviere and Rochon, 1990).

*Transmission, Host Range and Diseases.* MNSV is naturally transmitted by the zoospores of the fungal vector *Olpidium bornovanus* (Sahtiyanchi) Karling (= *Olpidium radicale* Schwartz & Cook fide Lange & Insunza). Seed-transmission is reported: 10–40% of the seedlings from seeds of muskmelon affected plants became infected when grown in presence of *Olpidium* contaminated soil. Mechanical transmission is possible experimentally and has been reported during cultural operations. MNSV isolates have a narrow experimental host range mainly restricted to cucurbits and differ in the systemic infection of certain hosts: watermelon isolates failed to infect melon and cucumber plants systemically, melon isolates systemically infect melon plants but not watermelon and cucumber, and cucumber isolates infect melon and cucumber plants systemically and inoculated but not uninoculated leaves of watermelon plants. In melon, cucumber and watermelon, MNSV causes small chlorotic spots in young leaves that turn into necrotic spots and large necrotic lesions. In melon and watermelon, necrotic streaks appear along the stems and petioles and sometimes are the only visible symptoms. In fruits, discoloration, necrosis and malformation both externally and internally are observed.

*Economic Importance and Control.* MNSV has been found as a natural pathogen in melon, cucumber and watermelon protected crops in Japan, USA and Europe in which it causes significant yield losses. Apart from recommended control methods for soil-, seed- and mechanically-transmitted viruses (soil, seeds and tools disinfection, etc.), grafting on immune *Cucurbita ficifolia* Boucé rootstocks has been used in cucumber to control MNSV. Melon cultivars resistant to this virus are commercially available.

### 2.3.6. MECHANICALLY-TRANSMITTED VIRUSES

#### *Tobamovirus Genus*

*Description.* The genus Tobamovirus of plant viruses includes species that cause devastating diseases in protected crops. Virions are elongated rigid rod-shaped particles

about  $300 \times 18$  nm that encapsidate one molecule of single-stranded RNA of messenger sense ( $2 \times 10^6$  daltons). The type member is tobacco mosaic virus (TMV): the genome contains five open reading frames, four of which encode proteins (126K, 183K, 30K and 17.5K) found *in vivo* that have been associated with replication, encapsidation, movement and symptoms induction. A fifth protein (54K) is obtained by *in vitro* translation but has not been found *in vivo*. Homologous genetic organization and genome expression is found in the tobamoviruses that have been sequenced to date.

*Transmission, Host Range and Diseases.* In nature, tobamoviruses are the most infectious and persistent disease agents; they are transmitted and easily spread between plants by contact, and during cultural operations, through contaminated implements. The viruses can survive over years in plant debris that may be source for new infections via the roots or aerial parts if infected remains are present in the greenhouse structures. In certain cases (Table 2.2), these viruses are seed-transmitted: the virus is carried in the external seed surface, testa, and sometimes in the endosperm (Johansen *et al.*, 1994). Seed samples with endosperm infection can remain infected for years. No natural vectors are known; presence in irrigation water has been reported for tomato mosaic virus (ToMV). Tobamoviruses are easily transmitted experimentally by mechanical inoculation.

Natural host range is very narrow, usually restricted to specific hosts; however, experimentally can be transmitted to numerous species of different families. For example, pepper mild mottle virus (PMMV) naturally infects pepper, ToMV tomato and pepper (Brunt, 1986), and cucumber green mottle mosaic virus (CGMMV) some cucurbits like cucumber, watermelon and melon, and spontaneous perennial hosts like *Lagenaria siceraria* (Molina) Standl. (Okada, 1986).

Tobamoviruses cause severe diseases in susceptible species especially in protected crops because of the intensive production that implies high density of plants and frequent cultural operations which favour mechanical transmission. PMMV induces a faint mosaic in pepper leaves whereas fruits are severely malformed with distorted coloration and often exhibit depressed necrotic areas. ToMV causes a wide range of symptoms on tomato depending on virus strain, cultivar, plant age at infection time, and environmental conditions: mottle or mosaics are observed in leaves, that are malformed, plants are stunted, and fruits show external mottling and, sometimes, internal browning. In pepper, symptoms vary with cultivar and can be mosaics, systemic chlorosis, necrotic local lesions, leaf abscission, and/or systemic leaf and stem necrosis. In cucurbits, CGMMV causes more or less prominent leaf symptoms (mosaic, mottling, malformation), stunting, flower abortion, and fruit mottling, distortion, and/or internal discoloration.

*Economic Importance and Control.* Tobamoviruses are a first order problem in protected crops. Most tobamoviruses are easily distributed worldwide via infected seeds. PMMV is one of the most destructive pathogens of protected pepper crops; infections may reach 100% of the plants and the yield of marketable fruit be drastically reduced. ToMV has been for years a virus of great economic importance in protected tomato crops; however, the development of resistant cultivars has reduced considerably

the incidence of the disease, but it is still a serious threat where resistant cultivars are not grown. In pepper, ToMV can also cause severe losses on susceptible cultivars (Brunt, 1986).

In first term, control methods are addressed to eliminate or reduce primary inoculum sources. Virus-free seeds should be used: sanitation of seeds can be done by soaking seeds in different solutions of active reagents (trisodium phosphate, hydrochloric acid, sodium hypochlorite) or by dry heat treatment (Rast and Stijger, 1987). Removal of plant debris from previous susceptible crops and steam treatment of the soil and greenhouse structures will aid to avoid primary infections. Secondary spread can be reduced by washing hands and implements with soap and water before and during plant handling, and/or frequent dipping into skim milk solutions. Cross protection has been largely used in greenhouse tomato crops to control ToMV by inoculation of tomato seedlings with an attenuated strain obtained by Rast (1972) in The Netherlands, thus avoiding ulterior infection with virulent ToMV strains. Other solanaceous crops that are susceptible to the mild strain (like pepper) must not be grown in proximity. Resistant genes have been described and incorporated in commercial tomato against ToMV, and in pepper against different tobamoviruses [TMV, ToMV, PMMV, and paprika mild mottle virus (PaMMV)]. However, resistant breaking strains can be detected (Tenllado *et al.*, 1997).

#### **2.4. Current Perspectives for Plant Virus Control within Integrated Management of Greenhouse Crops**

Greenhouse crops represent a singular case for disease management. They are closed systems where external exchanges are reduced to the minimum, although the intermediate situation present in the protected crops grown under the simple and less hermetic structures typical of the Mediterranean area, should also be considered. The most damaging viruses in protected crops are soilborne viruses [MNSV, PMMV, ToMV, tomato bushy stunt virus (TBSV)], or those imported via contaminated seed (TMV, ToMV, PMMV, CGMMV, SqMV, MNSV, BCMV, LMV, etc.), or contaminated plantlets. The precise knowledge about which virus problems are affecting in a specific crop, the dispersal mechanisms, and the epidemiology of the disease induced will help to make strategic management decisions within an integrated control strategy.

The means to prevent and control viral diseases based on the knowledge of their dispersal mechanisms have been discussed in Section 2.2. Other strategies for virus control are focused to the minimization of the impact of the infection on crop yield; breeding for resistance and cross protection are two of these strategies. When possible, the best control method against plant viruses would be the development of resistant cultivars (Sherf and Macnab, 1986). However, experience has shown that breeding for resistance or the development of transgenic plants is unlikely to give permanent solutions for any particular virus and crop. Variable virus populations may be present (Pink *et al.*, 1992; Luis-Arteaga *et al.*, 1996; Tenllado *et al.*, 1997) and/or virus can mutate (Aranda *et al.*, 1997) in the field with respect to virulence and the range of crops

and cultivars they can infect. Cross protection is based in that mild virus strains can be used to protect plants against infection by severe strain(s) of the same virus. Basic criteria for selection of cross protection as a disease control strategy are well known (Fulton, 1986; Gonsalves and Garnsey, 1989). Mildness of a strain is usually relative to a certain target crop and this should be taken into account if cross protection want to be used in greenhouses where other crops that may be sensitive to the protective virus strain are grown simultaneously. The same applies for precautions to be taken to avoid dispersal of the mild strain to sensitive crops grown in the vicinity of the protected greenhouse crop. Due to possible virus mutations, the reversion of the mild strain used in the cross protection programme to a severe one must be continuously verified. When using cross protection, the risk of coinfection with other virus(es) that may have synergistic effects with the protective strain should also be evaluated. Cross protection alone is not enough to give a high level of control of the disease because protection depends on the homology of the severe strain and on challenge pressure (Gonsalves and Garnsey, 1986). Therefore, the combination of various virus management practices compatible with an integrated management of the greenhouse is often desirable. Indirect measures for virus control have been discussed, e.g.: (i) adjustment of planting dates to avoid high vector populations in young plantings if epidemiological data of the disease are available; (ii) use of virus-free propagation material; (iii) disinfection of soil and greenhouse structures; (iv) minimization of external entrance of insects; (v) rapid elimination of virus-infected plants; (vi) adequate plant handling; and (vii) avoidance of overlapping or continuous cultivation of sensitive species in the rotation.

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## FUNGAL AND BACTERIAL DISEASES

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### 3.1. Introduction

Greenhouse cropping is the most intensive agricultural industry. It is suitable wherever land is limited or where early produce is required under adverse environmental conditions. Greenhouse cropping poses complex challenges in the field of plant protection. In such an intensive cropping system several factors, explained in other chapters of this volume, favour the development of a large number of fungal and bacterial diseases; if no proper control measures are taken in time, losses may be very high. This chapter provides information relevant to the diagnosis and biology of the pathogen, and epidemiology of several diseases, key information if control strategy is to be effective. Disease control is dealt with in other chapters and so is only briefly discussed here. Diseases are grouped arbitrarily and the main characteristics of each group are described. Due to the huge number of diseases reported in greenhouse crops, several of them, of minor or local interest, have been omitted. Additional information can be found in books dealing specifically with greenhouse diseases (Fletcher, 1984; Jarvis, 1992) or in books on vegetables or floral crop diseases (Strider, 1985; Sherf and Macnab, 1986; Blancard, 1988; Horst, 1989; Blancard *et al.*, 1991; Jones *et al.*, 1993).

### 3.2. Fungal Diseases

#### 3.2.1. DAMPING OFF–CROWN AND ROOT ROTTS

Plants in seedbeds may be diseased, either before or after their emergence from the soil, and the disease is called pre- or post-emergence damping off, respectively. In the first case, seedlings do not emerge in patches of the seedbeds. In the second case, plants rot quickly and drop down on the soil. Low temperatures and very wet soils, which delay the growth of the plants, favour infection. A large number of fungi may cause damping off, but *Pythium* spp., *Phytophthora* spp., *Fusarium* spp. and *Rhizoctonia solani* Kühn are the most common. Nowadays, due to the use of improved technology, damping off is no longer a severe disease in greenhouses (Sherf and Macnab, 1986; Blancard, 1988; Blancard *et al.*, 1991). However, root rots and crown rots are still destructive in soil, though not in soilless cultures (Davies, 1980). The most widespread diseases are as follows.

#### *Pythium and Phytophthora Rots*

Various *Pythium* spp. and *Phytophthora* spp. may damage the lower part of tomato, pepper, cucumber, carnation, poinsettia, gerbera, etc. both in soil and soilless cultures.

In tomato a root and crown rot extending a considerable height above the soil level may

occur. The infected area has a dark discoloration and the pith is usually destroyed. *Phytophthora nicotianae* Breda de Haan var. *parasitica* (Dastur) G.M. Waterhouse is the most common pathogen. In pepper a similar disease caused by *Phytophthora capsici* Leonian is very common. Collar, stem and fruit rot as well as leaf spots may occur. In cucumber a soft rot of the young plants at the soil level may occur soon after transplanting. Infected tissues shrink and in wet weather a white mycelium develops. Infected plants wilt and die quickly. Poinsettia grown in pots also suffers from Pythium rot. Severe root rot, extending above ground in succulent plants, and quick death are the main symptoms. In cucumber and poinsettia, *Pythium ultimum* Trow, *Pythium irregulare* Buisman, *Pythium debaryanum* Auct. Non R. Hesse and *Pythium aphanidermatum* (Edson) Fitzp. are mostly involved (Tompkins and Middleton, 1950). Carnations infected by *Pythium* and *Phytophthora* species develop soft rot at the collar and in the root system, resembling Rhizoctonia stem rot

#### *Rhizoctonia Stem Rot* (*R. solani*)

This infects a large number of plants, such as tomato, carnation, poinsettia, etc. causing symptoms resembling Pythium or Phytophthora rots. Rhizoctonia stem rot is mainly confined to the collar. Carnation is very susceptible. Infected plants show pale brown dry lesions, with circular rings, at soil level. Growth is stunted and leaves become dull green. Complete wilting soon follows. Strands of the pathogen develop on the lesions and stems break easily at the infected area (Parmeter, 1970).

All *Pythium* and *Phytophthora* species as well as *R. solani* are common soil inhabitants. They survive in the soil. Infection usually takes place at the time of planting and symptoms appear very soon in Pythium and Phytophthora rot or several weeks later in Rhizoctonia stem rot *Rhizoctonia solani* may infect at moderate soil moisture levels, but *Pythium* spp. and *Phytophthora* spp. infect only in water-saturated soils (Strider, 1985).

#### *Corky Root Rot of Tomato* (*Pyrenochaeta lycopersici* *R. Schneider & Gerlach*)

The pathogen damages mostly tomato, but also eggplant, melon, etc. Initially tomato leaves turn dull green and growth is stunted. Later, leaves take on a bronze colour and curl downwards. Necrosis of the leaflets follows. Young roots are brown and poorly developed. Scattered lesions appear on the surface of the larger roots which become corky with cracks of different sizes. Yield may be severely reduced. The pathogen survives on the infected root debris due to the presence of minute sclerotia. It is a cool weather disease. In subtropical countries it progresses during the winter and plants start to recover by early spring (Ebben, 1974; Malathrakis *et al.*, 1983).

#### *Crown and Root Rot of Tomato* (*Fusarium oxysporum* *Schlechtend.:Fr. f. sp. radialis-lycopersici* *W.R. Jarvis & Shoemaker*)

In plastic greenhouses, a yellowing of the lower leaves appears in infected plants during late winter, when many fruits have already set. In severe infections the whole plant becomes chlorotic and wilts. A dry lesion up to 10 cm long appears on part of or all around the collar. There is a brown discoloration on the root system, predominantly at the end of the main root, the base of the stem and the vascular region of the central root

A large number of microconidia, which disseminate the pathogen, appear on the infected stem. The fungus survives by chlamydospores which develop in the soil. The disease is favoured by cool weather (Jarvis *et al.*, 1975,1983).



*Black Root Rot of Cucurbits* (*Phomopsis sclerotioides van Kestern*)

The disease has been recorded in several countries of northwestern Europe and elsewhere. It infects cucumber and melon, causing a brown rot in the cortical tissue of the root system. Soon a large number of sclerotia develop and the infected tissues turn black. Severely infected plants wither and die. Infection is favoured by cool weather. The pathogen survives in the soil for several years by means of sclerotia (Blancard *et al.*, 1991).

*Control*

Effective control of the above-mentioned diseases may be obtained selectively by the following means: (i) use of naturally or artificially suppressive substrates; (ii) early drenching by effective fungicides; (iii) soil disinfestation; (iv) use of resistant cultivars (cvs); (v) grafting on resistant rootstocks; and (vi) biological control (Ginoux *et al.*, 1978; Jarvis *et al.*, 1983; Hoitink and Fahy, 1986; Tjamos, 1992) (see Chapter 23).

## 3.2.2. WILTS

All major greenhouse crops suffer from one or more wilts. In several crops wilts are the main diseases due to the damage they cause and the difficulty of controlling them.

*Fusarium Wilt* (*F. oxysporum*)

The most common *Fusarium* wilts in greenhouses appear on: tomato [*Fusarium oxysporum* Schlechtend.:Fr. f. sp. *lycopersici* (Sacc.) W.C. Snyder & H.N. Hans.], cucumber (*Fusarium oxysporum* Schlechtend.:Fr. f. sp. *cucumerinum* J.H. Owen), melon (*Fusarium oxysporum* Schlechtend.:Fr. f. sp. *melonis* W.C. Snyder & H.N. Hans.), carnation [*Fusarium oxysporum* Schlechtend.:Fr. f. sp. *dianthi* (Prill. & Delacr.) W.C. Snyder & H.N. Hans.], gladiolus [*Fusarium oxysporum* Schlechtend.:Fr. f. sp. *gladioli* (L. Massey) W.C. Snyder & H.N. Hans.], cyclamen (*Fusarium oxysporum* Schlechtend.:Fr. f. sp. *cyclaminis* Gerlach) and chrysanthemum (*Fusarium oxysporum* Schlechtend.:Fr. f. sp. *chrysanthemi* G.M. Armstrong, J.K. Armstrong & R.H. Littrell).

Wilt, yellowing, chlorosis, drooping (mostly of the lower leaves), stunting and brown discoloration of the vascular bands up to the top of the stem are the dominant symptoms. Wilting of the lateral shoots and large lesions on the lower part of the stem are also common in *Fusarium* wilt of carnation and melon. All the above *F. oxysporum* formae have more than one race. Each of them infects cvs of one host but may colonize the root system of other plants as well. They survive in the soil for several years, due to the production of thick-walled chlamydospores, but inoculum is reduced over the years. *Fusarium* wilt in tomato, watermelon, carnation, cyclamen, chrysanthemum and gladiolus is favoured by higher temperatures than *Fusarium* wilt in melon (Walker, 1971; Nelson *et al.*, 1981; Strider, 1985; Sherf and Macnab, 1986).

*Verticillium – Phialophora Wilt* [*Verticillium dahliae* Kleb., *Verticillium albo-atrum* Reinke & Berthier, *Phialophora cinerescens* (Wollenweb.) van Beyma (= *Verticillium cinerescens* Wollenweb.)]

This infects a huge number of plants and among them the majority of the plants grown in greenhouses. It is more severe in Solanaceae such as tomato, eggplant and pepper. Of the

floral crops, chrysanthemum seems to be more susceptible. Symptoms are very similar to those of Fusarium wilt. Verticillium wilt is favoured by moderate temperatures. *Verticillium dahliae*, which is more common, survives in the soil for many years due to the abundant production of black resistant microsclerotia, while *V. albo-atrum* survives by producing dark dormant mycelium. A similar wilt caused by *P. cinerescens* damages carnations in several areas (Stricter, 1985; Sherf and Macnab, 1986).

### 3.2.3. POWDERY MILDEWS

Powdery mildews are very destructive of several greenhouse crops. The following are some of the powdery mildew fungi which most attack greenhouse-grown plants: (i) on cucurbits *Sphaerotheca fusca* (Fr.) Blumer. [= *Sphaerotheca fuliginea* (Schlechtend.:Fr.) Pollacci], *Erysiphe cichoracearum* DC. and *Leveillula taurica* (Lév.) G. Arnaud (only on cucumber); (ii) on solanaceous plants *L. taurica* and *Oidium lycopersicum* Cook & Masse (only on tomato); (iii) on roses *Sphaerotheca pannosa* (Wallr.:Fr.) Lév.; (iv) on begonia *Microsphaera begoniae* Sivan.; and (v) on gerbera *E. cichoracearum*.

Powdery mildew fungi, except for *L. taurica*, may attack all green tissues. Initially, white powdery spots, which enlarge and coalesce to cover large areas, are the dominant symptoms. *Leveillula taurica* infects only leaves. Light yellow or yellow-green spots on the upper leaf surface, which later become brown, and scarce white mould on the lower surface are the main characteristics. Powdery mildew-infected plant parts may be chlorotic and distorted. Premature defoliation and poor growth are common features of severely infected plants (Palti, 1971; Sitterly, 1978; Braun, 1995).

Infections take place by conidia. Under favourable conditions powdery mildew progresses rapidly. By the end of the season some powdery mildew fungi, such as *S. fuliginea*, *E. cichoracearum*, etc. may develop cleistothecia with ascospores, but these do not play an important role in the epidemiology of the disease (Braun, 1995).

Conidia are mostly discharged and transferred by wind currents. Animal pests may also disseminate conidia in greenhouse crops. Young conidia readily germinate on plant surfaces depleted of nutrients. The relative humidity (RH) favouring infection by powdery mildew fungi and development of the disease differs from species to species. For instance, high RH is more favourable for *S. fuliginea* than for *E. cichoracearum*. Therefore, the first fungus is more frequent on greenhouse cucurbits than the second. High RH may favour spore germination of powdery mildew fungi, but free water may be deleterious. RH at 97–99% is optimal for spore germination of *S. pannosa* and *S. fuliginea*. At RH below 75% spores of *S. pannosa* do not germinate, but mycelium development and sporulation may occur at RH as low as 21–22%. Powdery mildew fungi overwinter on cultivated plants or weeds, which survive in or outside the greenhouse (Coyier, 1985a,b).

Chemicals such as demethylation inhibitors (DMIs) (triadimefon, fenarimol, etc.), pyrimidines (ethirimol, bupirimate, etc.), pyrazophos and dinocap remain the main means of controlling powdery mildews in greenhouses. Biological control agents have also been effectively tested against *S. fuliginea* and *S. pannosa*. Finally, fully resistant cvs of melon and partially resistant cvs of long-type cucumber are available (Coyier, 1985b; Molot and Lecoq, 1986).

### 3.2.4. DOWNY MILDEWS

Downy mildews of tomato [*Phytophthora infestans* (Mont.) de Bary], cucurbit [*Pseudoperonospora cubensis* (Berk. & M.A. Curtis) Rostovzev], lettuce (*Bremia lactucae* Regel), rose (*Peronospora sparsa* Berk.) and snapdragon (*Peronospora antirrhini* J. Schröt.) are the most destructive in greenhouse-grown plants.

In tomato, leaves and young shoots are infected first. Fruit infection starts mostly near the stalk and spreads very quickly to the whole fruit. Infected tissues of fruits and shoots are firm and brown (Sherf and Macnab, 1986).

In cucurbits downy mildew appears as yellow, angular or circular spots on the upper surface of the mature leaves of the plant. Soon the tissues at the centre of the spots die and become light brown. Cucumber and melon are more susceptible than watermelon.

Downy mildew of lettuce causes scattered light-green to yellow spots on the upper leaf surface. Old spots become brown and dry up.

Downy mildew of rose damages all green plant parts, but leaves are more susceptible. Leaf infection resembles the effect of toxins. Infected leaves have purplish red to dark-brown irregular spots and shed readily (Strider, 1985). Snapdragon plants infected by *P. antirrhini* are stunted and the top internodes of the young plants are short. The borders of the lower leaves curl down and then dry. Eventually the entire plant dies (Garibaldi and Rapetti, 1981). A white fungal growth (brown for cucurbit downy mildew) on the infected tissues under moist conditions is typical of all downy mildews.

Plant infection takes place through stomata and mycelium develops intercellularly. Soon branched conidiophores are produced and protrude through the stomata. Infection progresses in the periphery of the spot which gradually enlarges. Conidiospores of downy mildews are ovoid and hyaline, except for *P. cubensis* which are brown. They are discharged by hygroscopic changes and disseminate in greenhouses by wind currents and water splashes. Initial infection may take place by spores transferred long distances on the wind. Abundant oospores of *P. antirrhini* develop on dead plant stems. Oospores of *P. sparsa* also very often develop on infected roses, whereas *P. cubensis* and *P. infestans* oospores are rare.

*Phytophthora infestans* survives on seed potato tubers and spreads to young potato plants after they have been planted. Inoculum is disseminated from potatoes to neighbouring tomato crops. Cucurbit downy mildew can infect all year round several species of cucurbits, grown either in greenhouses or open fields. There is evidence that *P. sparsa* survives as a dormant mycelium on the infected stems of roses. *Peronospora antirrhini* perennates as dormant thick-walled oospores in dead plant parts and soil (Garibaldi and Rapetti, 1981; Sherf and Macnab, 1986).

Free water on plant tissues is necessary for downy mildew fungi to cause infection. High RH is also required for good sporulation. *Peronospora antirrhini* is favoured by low temperature and high RH. Free water or high relative humidity is not often a factor limiting downy mildew development in plastic greenhouses. It seems that temperature is more critical. For instance, *P. cubensis*, with a high maximum temperature for development and infection, may, under certain conditions, infect all year round, whereas *P. infestans* and *P. sparsa* do not infect during the hot period of the year. Downy mildews complete a cycle within about 6–8 days. Thus, under favourable weather conditions they may have several cycles and spread rapidly (Palti and Cohen, 1980; Strider, 1985).

Chemical fungicides remain the major means of control of downy mildews. Dithiocarbamates, chlorothalonil and the systemic phenylamides (metalaxyl, etc.) are the most commonly used in greenhouses. There are some tomato cvs fully resistant to downy mildew and some partially resistant cucumber cvs suitable for greenhouses, but all rose cvs grown for cut flowers are susceptible to downy mildew. Ventilation of the greenhouses may also effectively prevent infection (Palti and Cohen, 1980; Fletcher, 1984; Strider, 1985).

### 3.2.5. BOTRYTIS DISEASES

*Botrytis cinerea* Pers.:Fr., *Botrytis tulipae* (Lib.) Lind and *Botrytis gladiolorum* Timmermans are *Botrytis* spp. that most damage greenhouse crops.

*Botrytis cinerea* causes grey mould on a large range of hosts, including nearly all the major greenhouse plants. All plant parts at different growth stages may be damaged. Due to the diversity of the infected plant parts, several types of symptoms appear on one or on various hosts. On young stems, leaves, flowers and fruits, initially water-soaked spots occur, which rapidly enlarge under favourable weather conditions. In tomato fruits green-white circular spots called “ghost spots” also appear. On hard plant parts, such as stems and collars, *B. cinerea* causes cankers and parts above them may die. These symptoms are very common on vegetables such as tomato, eggplant, pepper and cucumber. Infected tissues die soon and a grey mould which consists of conidiophores with clusters of spores develops on their surface. In plants, like tomato, black sclerotia develop inside the infected stems. *Botrytis cinerea* also causes very characteristic collar rot in lettuce. The infected plants usually develop large brown necrotic lesions on the stem near the soil surface and the lower leaves. The infection gradually progresses upwards. Infected plants may wither and die in a short time (Sherf and Macnab, 1986).

*Botrytis tulipae* causes tulip fire blight. Spots of various types on leaves and flowers, lesions on the stem, blossom blight and bulb rot are the dominant characteristics. *Botrytis gladiolorum* damages gladiolus and some other Iridaceae. Large spots on leaves and the stem, pinpoint spots on the flowers, neck rot and soft rot of corms are the most common symptoms. *Botrytis* spp. also infect all types of propagating material, which are either destroyed before planting out or become weak plants which may die before or after emergence. Finally, *Botrytis* spp. may cause severe post-harvest losses in plant products during storage or transportation (Trolinger and Stider, 1985).

*Botrytis cinerea* develops and sporulates profusely on any organic material. Spores are disseminated by wind over long distances or by water splashes. Healthy plants are infected through wounds, senescent tissues, directly through the epidermis and rarely through stomata. Symptoms may appear very quickly or infection may remain quiescent and symptoms appear later when tissues age or during storage. In greenhouses, initial infection depends on spores transferred from outdoors. Later, the inoculum established in the greenhouse is the main source of infection. In plants grown in non-heated greenhouses, low temperature, high RH and low light intensity, prevalent from late November till late March, create good conditions for infection by *B. cinerea* (Elad *et al.*, 1992; Jarvis, 1992).

Botrytis-incited diseases are prevented by ventilation and heating of greenhouses. Fungicides, mostly benzimidazoles and dicarboximides, are also used extensively. Nowadays, due to the predominance of resistant strains of the pathogen, they are only

marginally effective and growers are advised to combine dicarboximides with other means of control such as biocontrol preparations. New fungicides have recently been released, but in greenhouses they are used on a limited scale. Formulations of biological control agents such as Trichodex (*Trichoderma harzianum* Rifai T39) are also available (Elad *et al.*, 1992; Gullino, 1992).

### 3.2.6. SCLEROTINIA ROT [*Sclerotinia sclerotiorum* (Lib.) de Bary and *Sclerotinia minor* Jagger]

This is a common greenhouse disease that damages lettuce, eggplant, tomato, cucumber, pepper, etc. Infection on lettuce begins close to the soil, where a water-soaked area appears. Infection may spread downwards to the roots or upwards to the heart of the plants. Infected leaves fall onto the soil and dry up. The other plants are infected along the stem, leaves, flowers and fruits. Infected areas become water-soaked. Stem infection is more severe. Leaves above the infection area become yellow, wither and die. In wet weather a white mass of mycelia appears on the infected areas, which gradually develops into black sclerotia. *Sclerotinia sclerotiorum*, which is the most common pathogen, produces sclerotia up to the size of bean seeds, whereas *S. minor* produces smaller sclerotia. Sclerotia fall onto the soil where they can survive for several years. When weather conditions are favourable they germinate to produce apothecia which release ascospores and cause new infection. High RH and moderate temperature is required for infection (Purdy, 1979; Fletcher, 1984).

The elimination of sclerotia and the control measures recommended against grey mould are effective against Sclerotinia rot as well.

### 3.2.7. ALTERNARIA DISEASES

The following diseases, caused by *Alternaria* spp., seriously affect vegetable and floral crops in greenhouses.

#### *Tomato Early Blight* (*Alternaria solani* Sorauer)

A collar rot of the young plants before or after transplanting may be the first symptom. In mature plants small irregular brown spots, with or without a yellow halo and concentric rings, appear mainly on leaves. Severely infected leaves are ragged and senescent. Similar spots without a yellow ring appear along the stem, leaf stalks, peduncles and the calyx. On fruits, brown to black spots with a leathery surface appear at the stem end. Severely infected plants may be defoliated (Sherf and Macnab, 1986).

#### *Alternaria Branch Rot and Leaf Spot of Carnation* (*Alternaria dianthi* Stev. and Hall.)

This mostly infects carnation cuttings during mist propagation and in wet parts of greenhouses. Small purple spots on the leaves are the first symptoms. Soon they enlarge, and their centre turns brown and then black due to the masses of spores which develop. Stem infection usually appears on the knots (Strider, 1978).

#### *Alternaria diseases of minor importance for greenhouses*

As well as the *Alternaria* diseases described above, strains of *Alternaria alternata* (Fr.:Fr.)

Keissl. have been recorded: (i) causing cankers in tomato crops; (ii) causing leaf spotting in cucumber; and (iii) causing mostly post-harvest rotting on tomato fruits. Also *Alternaria cucumerina* (Ellis & Everh.) J.A. Elliot may on occasion infect cucumber, melon, watermelon and squash (Grogan *et al.*, 1975; Fletcher, 1984; Vakalounakis and Malathrakis, 1987). At present, none of them has any economic impact on greenhouse crops.

All *Alternaria* species are facultative parasites mostly infecting weak plants. They survive in the soil on plant debris, but their black spores may also survive on several surfaces in greenhouses. *Alternaria solani* may survive on potato, which is an alternative host. Spores growing on dead material or on host plants are easily disseminated by wind or by splashed water. Plant infection takes place through stomata or directly through leaf surface. Spore germination and subsequent infection take place under a wide range of temperature. RH needs to be higher than 97% for rapid germination, but germination may take place in some cases at RH >75%. Senescent tissues are preferentially infected. The optimal temperature reported for *A. solani* is 18–25°C and for *A. cucumerina* 20–32°C. However, temperatures prevailing during the growing period of the respective hosts are not a factor limiting infection.

#### Control

*Alternaria* diseases can be prevented by dithiocarbamates, chlorothalonil, iprodione, etc. Hygienic measures and use of healthy propagating material are very important, especially when crops are grown in the soil. Inoculum surviving on plant debris in the soil and spores remaining on the greenhouse frames should be eradicated.

### 3.2.8. DIDYMELLA DISEASES

Two very severe diseases of greenhouse crops are caused by *Didymella* spp.: *Didymella* stem rot or canker in tomato and eggplant [*Didymella lycopersici* Kleb [teleomorph of *Phoma lycopersici* Cooke (= *Diplodina lycopersici* Hollós)]] and gummy stem blight in cucurbits [*Didymella bryoniae* (Auersw.) Rehm [anamorph *Phoma cucurbitacearum* (Fr.:Fr.) Sacc.]].

Both diseases damage all aerial plant parts of their hosts in greenhouses when weather is cool and RH high. They may infect the collar and root system causing yellowing and withering of the plants, which may later die. Cankers along the stem and the petioles are also very common. Plant parts above cankers may die. Both diseases cause large spots on the leaves which may cover the entire leaf surface. Tomato fruits are infected at the stem end. Initially, the infected area is light brown but it soon turns pink due to the large amount of pycnidio-spores released. Infected parts may cover one third of the fruit surface. Infection of cucumber and melon fruits by *D. bryoniae* appears mostly at the blossom end. Infection may occur only inside the fruit without being visible on the surface. Soon after infection, a lot of pycnidia appear on the infected areas and their colour turns dark brown. Dark perithecia also appear a little later than pycnidia produced by *D. bryoniae*, while those of *D. lycopersici* are rare (Anonymous, 1971; Blancard *et al.*, 1991).

The inoculum remains in plant residues inside and outside greenhouses. In the first case infection starts through the collar. There is good evidence that infection of the aerial parts by *D. bryoniae* is initiated by ascospores released from infected plant material left outside greenhouses. In greenhouses the two diseases are rapidly spread by water splashes and

cultural practices. Soil disinfestation, destruction of plant residue and strict hygienic conditions delay the outbreak of the diseases. However, disinfested soil is readily reinfested. The fungicides commonly used in greenhouse against other fungal diseases are also effective. Moreover, the reduction of the RH and of free water on the leaf surfaces is very effective (Anonymous, 1971; Sherf and Macnab, 1986).

### 3.2.9. RUST DISEASES

These are a very important group, with many common characteristics. The following are the main rusts affecting greenhouse crops.

*Carnation Rust* [*Uromyces dianthi* (Pers.:Pers.) Niessl (= *Uromyces caryophyllinus* G. Wint.)]

The disease is more severe on leaves, but other green plant parts are infected as well. Initially, small light green spots appear. They gradually turn to powdery brown blisters due to the urediospores developed. Severely infected plant parts are twisted.

Healthy crops are infected by urediospores transferred from neighbouring crops. They are wind or water-splash disseminated and germinate readily on free water. The cycle of the pathogen lasts about two weeks. In greenhouses, where leaves may remain wet for several hours, there may be many disease cycles per crop season (Strider, 1985).

*Rose Rust* [*Phragmidium mucronatum* (Pers.:Pers.) Schlechtend.]

The disease is easily identified by the yellow orange rust pustules which develop profusely on the lower surface of older leaves. In greenhouses it is not very destructive. Several species of *Phragmidium* have been reported to infect rose, but *P. mucronatum* is the most common. It is an autoecious, macrocyclic fungus producing telia by the end of the crop season in the same place as uredospores. They serve as overwintering structures and initiate infection during spring. Free water and temperature 9–27°C are necessary for the uredospores to germinate (Horst, 1989).

*Chrysanthemum Rust* [*Puccinia tanacetii* DC. (*Puccinia chrysanthemi* Roze)] and *White Rust of Chrysanthemum* (*Puccinia horiana* Henn.)

Pale yellow flecks on the leaves followed by dark brown pustules with urediospores are the dominant symptom. Leaves with several pustules may wither and die. No stem infection has been reported. It is a low to moderate temperature disease requiring free water for infection. It survives on infected leaves and is disseminated by wind. Chrysanthemum white rust is a new and destructive disease of chrysanthemum in Europe and the Mediterranean. Initially, circular white or yellow cushions develop on the lower leaf surface and then soon turn brown. The disease is favoured by high RH and moderate temperatures (Strider, 1985). Snapdragon rust (*Puccinia antirrhini* Dietel & Holw.), geranium rust (*Puccinia pelargonizonalis* Doidge), etc. are also destructive diseases, but the respective crops are not grown in large acreage (Strider, 1985).

Regular applications of protective fungicides, such as dithiocarbamates and chlorothalonil, or systemic fungicides, such as oxycarboxin and members of the DMIs, are mostly recommended for rust control. Prevention of water condensation is also very effective (Strider, 1985; Horst, 1989).

### 3.2.10. CLADOSPORIUM DISEASES

*Tomato LeafMould* [*Fulvia fulva* (Cooke) Cif. (= *Cladosporium fulvum* Cooke)]

This causes light green to yellow spots on the upper surface of mature leaves. Soon the sporulating fungus growth appears as an olive-green velvety growth on the underside of the yellow spots. The pathogen survives for several months on the greenhouse frame, on the materials used for cropping and in plant debris. It is disseminated by wind or splashed by water drops. The optimal temperature for infection is 20 to 25°C. If weather conditions are favourable, leaf mould has several cycles in a season and can destroy the crop completely. There are several races of the pathogen (Blancard, 1988; Jones *et al.*, 1993).

*Cucurbit Scab* (*Cladosporium cucumerinum* Ellis & Arth.)

This mostly attacks cucumber, but also squash, melon, etc. It causes nearly circular or angular leaf spots on the leaves, which look water-soaked. Fruit infection is more serious. Initially, water-soaked lesions about 1 cm long, with gummy exudations, develop. A corky tissue usually develops around the lesions, which finally develop a scabby appearance. The pathogen survives on plant debris and spores are air-disseminated. Temperatures of about 15 to 25°C and RH over 86% favour the disease (Sherf and Macnab, 1986; Blancard *et al.*, 1991).

#### *Control*

For both diseases, greenhouse ventilation is the best control measure. The disinfestation of greenhouse soil and frames is also very important. Regular application of dithiocarbamates, iprodione, benzimidazoles, etc. are recommended as well. There are several resistant cvs against some races of the pathogens.

## 3.3. Bacterial Diseases

Several bacterial diseases damage all types of greenhouse crops. The most common are the following.

### 3.3.1. WILTS

*Tomato Bacterial Canker* {*Clavibacter michiganensis* (Smith) Davis et al. *ssp.* *michiganensis* (Smith) Davis et al. [= *Corynebacterium michiganense* (Smith) Jensen] *ssp.* *michiganense* (Smith) Jensen}

Initially, infected plants show a sudden unilateral wilting of leaflets, entire leaves or shoots. Young plants are more susceptible to wilting. Stem vessels at the side of the wilted leaves develop a yellow-brown discoloration. In the more severely infected places, the cortex splits and cankers several centimetres long may develop. Such plants usually die prematurely. Systemic fruit infection leads to yellow or brown discoloration of vascular strands and infected seeds are often shrivelled and black. Birds-eye spots, up to 6 mm in diameter, often appear on fruits.

The pathogen is a typical seed-borne organism. It can also survive for several months on



cultivation equipment, on plant debris and in the soil. It can also maintain large populations on leaves of tomato and other plant species. It may infect at 16–36°C, with optimum at about 24–28°C. It is disseminated by seed or transplants, which remain symptomless until transplanted. In greenhouses, it spreads mostly during cultural practices (Strider, 1969; Gleason *et al.*, 1993).

*Slow Wilt, Bacterial Stunt of Carnation* [*Erwinia chrysanthemi* Burkholder, McFadden & Dimock *pv.* *dianthicola* (Hellmers) Dickey]

Infected plants become grey-green and may be stunted without any obvious wilting. Plants eventually wilt and in a period of 6–8 months may die. Vascular tissues, and pith mainly at the base of the stem, may show a yellow discoloration. Occasionally, stem cracks and root rot may occur (Fletcher, 1984).

#### *Control*

Soil disinfection, use of resistant cvs, grafting on resistant root stocks, use of clean propagating material and application of strict hygienic conditions are recommended against wilts (Walker, 1971; Ginoux *et al.*, 1978; Sherf and Macnab, 1986).

### 3.3.2. ROTS

*Tomato Soft Rots* [*Erwinia carotovora* (Jones) Bergey *et al. ssp.* *carotovora* (Jones) Bergey *et al.*, *Erwinia carotovora* (Jones) Bergey *et al. ssp.* *atroseptica* (van Hall) Dye, *Pseudomonas viridiflava* (Burkholder) Dowson] and *Tomato Pith Necrosis* [*Pseudomonas corrugata* (*ex* Scarlett *et al.*) Roberts & Scarlett, *P. viridiflava*, *Pseudomonas cichorii* (Swingle) Stapp]

Infected plants are stunted, their lower leaves show yellowing at the edges and on the veins and become flaccid. Initially the pith turns yellow to light brown, but later it disintegrates. The stem becomes hollow, splits and may exude bacterial slime. Brown to black blotches may also appear along the stem and the leaf stalks. A yellow to light-brown discoloration usually appears along the vascular system. Plants with severe stem rot may wilt and die, but very often even plants with split stems survive and yield normally. Several reports indicate that the above bacteria can cause similar symptoms under similar conditions in tomato plants. Plants with lush growth, grown under conditions of high RH, are more susceptible. Infection starts from leaf scars on the lower part of the stem, but may also appear in plants which have never been pruned (Scarlett *et al.*, 1978; Malathrakis and Goumas, 1987).

*Bacterial Blight of Floral Crops* (*Pathovars of E. chrysanthemi*)

This causes various rotting, necrotic and systemic diseases of several floral crops, such as chrysanthemum, cyclamen and saintpaulia, in greenhouses. The pathogen comes from affected stock plants and is disseminated by cultural practices. Infected plants should be discarded and knives disinfected (Fletcher, 1984).

### 3.3.3. LEAF AND STEM SPOTS

*Tomato speck* [*Pseudomonas syringae* van Hall *pv.* *tomato* (Okabe) Young *et al.* and

*Bacterial Spot of Tomato and Pepper* [*Xanthomonas vesicatoria* (*ex Doidge*) *Vauterin et al.*]

Bacterial speck causes small dark brown spots with bright yellow halo on tomato leaves. Necrotic tissues tear off and leaves appear ragged. Small dark brown spots develop on stem and petioles. Spots may coalesce to cause dark brown-black blotches on the surface of the infected plant parts. Small (up to 1 mm) black spots also appear on the fruits. Severely infected leaves turn yellow and finally dry out. The symptoms of bacterial spot are similar to those of bacterial speck. The spots on the fruits are initially raised and at the end look scabby. Both pathogens survive on plant debris in the greenhouse or outdoors, as well as on seeds. They are splashed from plant to plant by water drops from condensation and infect plants through stomata and injuries. Infection requires free water on plant surfaces (Schneid and Grogan, 1977; Goode and Sasser, 1980; Gitaitis *et al.*, 1992).

*Angular Leaf Spot of Cucurbits* [*Pseudomonas syringae van Hall pv. lachrimans* (*Smith & Bryan*) *Young et al.*]

This mostly damages cucumber, zucchini and melon causing small, angular, light-grey leaf spots. They may coalesce to cover large areas. Severely infected leaves become chlorotic; infected areas tear off and appear ragged. Water-soaked spots also appear on the stem and fruits. In humid conditions, tear drops form on leaves, stem and fruit spots. The causal organism survives on the infected plant debris and in the seed coat. The bacterium is splashed from the soil by water and infects plants. It spreads from plant to plant during the cultural practices (van Gundy and Walker, 1957; Fletcher, 1984).

#### *Control*

Strict hygiene, soil disinfestation, use of healthy seeds and reduction of the wetness period are recommended measures against bacterial diseases in greenhouses. Reduction of nitrogen fertilizers is also important for tomato soft rot. Copper fungicides are the most effective chemicals. Some resistant cvs have also been released for tomato speck and bacterial spot of tomato, but none of them is suitable for greenhouses (van Gundy and Walker, 1957; Fletcher, 1984).

### **3.4. Future Prospects**

Bacterial and fungal diseases will remain serious problems in protected crops in the future, particularly in the case of plastic-houses. The severity and even the relative importance of diseases may vary as a consequence of the introduction of new crops/cultivars and/or cropping systems.

The shift to control strategies which rely less on chemicals and the application of the most recent fungicides with a specific mode of action favoured, in some cases, the development of some foliar pathogens, formerly of secondary importance.

Disease control is complex and necessarily relies on the integration of several measures. While fungicides played a major role in the past, recently, for technical, economical and environmental reasons, a big effort has been made to integrate disease management. Such an approach is also necessary because fewer and fewer chemicals

are now registered for use on crops such as most of those grown under protection, which are considered “minor”.

Better diagnostic tools, for early and quick disease detection, a wider use of resistant cultivars, a more considered adoption of cultural practices, coupled with the use, whenever possible, of biocontrol agents, will enable our dependence on chemicals to be reduced in the near future.

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**PRINCIPLES OF EPIDEMIOLOGY, POPULATION BIOLOGY, DAMAGE RELATIONSHIPS AND INTEGRATED CONTROL OF DISEASES AND PESTS**

Aleid J. Dik and Ramon Albajes

**6.1. Introduction**

Epidemiology and population biology study the development and spread of plant diseases and arthropod pests and the factors affecting these processes. The level of disease or pest infestation is the result of many interacting factors and this level determines the yield loss that the grower suffers from the pathogen or pest. In many respects, the methodology of research and the underlying concepts are very similar for bacterial and fungal plant pathogens, insects, mites, viruses and nematodes. In this chapter, we will: (i) introduce the reader to these concepts; (ii) explain how they can be used in integrated control; and (iii) illustrate how damage relationships can be established.

**6.2. The Disease/Pest Tetrahedron**

The disease/pest tetrahedron is used to envisage the interaction of diseases and pests with their environment. The tetrahedron consists of four components, which can all influence each other and together determine the level of the disease or pest. The four components are the plant pathogen/pest, the host plant, the environment, and human activity. Generally, it can be said that chemical control is only aimed at influencing the pest or pathogen directly, whereas integrated control may reduce the level of disease or pest by influencing any or several of the four components of the tetrahedron. A thorough knowledge on the influence of different factors on pests and diseases offers the basis for integrated control. The four components of the tetrahedron will be discussed below.

**6.2.1. THE PATHOGEN/PEST**

*Infection Cycle of Plant Pathogens*

The infection cycle of fungal plant pathogens consists of the following phases (Zadoks and Schein, 1979): (i) infection (germination of spores, penetration of plant tissue and colonization of plant tissue); (ii) sporulation (production of spores and maturation of spores); and (iii) dissemination (spore liberation, spore dispersal and spore deposition). Bacterial pathogens and viruses generally have a similar but simpler infection cycle. Some pathogens only complete one infection cycle per season, but most pathogens complete several or sometimes many cycles per season. The amount of disease that

develops is the sum of the successes of the different phases in the cycle. All phases are subject to influences from the environment, the host plant and human activity.

### *Life Cycle of Pest Organisms*

Although pest organisms belong to various taxonomic groups and consequently their life cycle varies accordingly, a general pattern for phytophagous arthropods may be described. Dispersal usually occurs at adult stage; adults look for an exploitable habitat and then a plant to feed and oviposit on. Once a plant is recognized as suitable for feeding and ovipositing, an adult female lays eggs (ovipary) or deposits nymphs or larvae (vivipary). Progeny commonly feed and develop on the plant where oviposition took place or on neighbouring host plants until reaching the mature stage. The adults may feed and oviposit on the same plant or move to younger and more suitable host plants. Many arthropods spend all their life on a plant but others, particularly among holometabolous insects, either have soil-inhabiting stages or the adults feed on a different host plant than the immatures did. Most greenhouse pests are multivoltine (several generations within a year), and univoltinism (one generation per year) is rather rare.

Research on disease epidemiology and pest population biology, can study the effect of different factors on the phases in the infection/pest cycle or can determine the effect on the resulting amount of disease or pest density directly. Studying the effect on each of the processes described – for instance germination and spore production in the infection cycle, or host plant selection and exploitation in a pest cycle – often yields a good understanding of the effect of a factor on a disease or amount of damage caused by a pest. This kind of research is best done under controlled conditions, which allows the fluctuation of only one or two factors. Research on epidemiology of a disease or pest population biology in a whole crop is usually based on monitoring many factors and analysing their respective effects through regression analysis. It is important that in such research all the relevant factors are monitored.

## 6.2.2. THE HOST PLANT

Traditionally, the host plant has been considered as a more important factor for the development of a disease than for determining the damage caused by an arthropod pest. This may be one of the reasons why plant resistance has been used more frequently to control diseases than to control pests. In fact, the tetrahedron scheme is common in plant pathology texts but rare in applied entomology books. However, this situation has changed recently as entomologists have intensified studies on insect-plant relationships.

Different cultivars may vary in their susceptibility to certain diseases or pests, even if none of them is completely resistant. This partial resistance may influence disease development by decreasing the number of successful infections by the pathogen, by increasing the latency period, by reducing the rate of lesion expansion or sporulation, or by any combination of these processes. The result will be a slower development of the epidemic. Similarly, different cultivars may influence the host plant selection by a pest and/or its oviposition, development and survival, and thus the rate of population increase. Many partially resistant cultivars express the same resistance during their

entire life, but some resistance may depend on the physiological age of the plant. Age-related resistance can be either adult plant resistance or young plant resistance. On another scale, certain parts of the plant may be more or less susceptible to disease because of their age (see Chapter 9 for the use of plant resistance in IPM).

The physiological status of the plant is affected by temperature, humidity and nutrition. Nutrient deficiencies or certain climatic conditions may predispose plants to the development of diseases and pests, but unbalanced fertilization may also increase this kind of problems. For example, excess of nitrogen amendments renders the plant more susceptible to *Botrytis cinerea* Pers.:Fr. and enhances the population increase of homopteran pests like aphids and whiteflies. A thorough knowledge of the factors affecting the incidence of diseases and pests through their host plant may help to prevent outbreaks by applying correct crop management practices (see Chapter 8 for crop management practices and pest and disease control in greenhouses).

Because the host plant is such an important factor in disease epidemics and pest population dynamics, it is important to carefully monitor the host plant in any epidemiological research. This means not only that cultivar and planting date should be recorded, but also plant spacing, nutrition, development stage of the plant and plant growth. Recording plant growth is also important for another reason: whether the disease is assessed as a percentage of total plant area – for example leaf area covered – or as a pest density – for example, the number of insects per leaf area – it is important to know the size of the host plant. Also, a count of the number of lesions for example may yield the same number for different crops, but if the number of leaves or other aspects of the size of the host plant are different, the impact of this number of lesions will be different and results difficult to compare between crops.

### 6.2.3. THE ENVIRONMENT

Many components of the environment can indirectly influence the severity of disease and pest injury through the host plant or by a direct effect on the pathogen and pest. Here, only the direct impact of the environment on the pathogen and pest population is dealt with.

The main influences on pathogens stem from temperature, relative humidity (RH), radiation and wind. Pathogens are often affected by climatic conditions in most of the phases of the infection cycle. Germination of spores and superficial germ tube growth often show an optimum curve for temperature. For most pathogens, germination only occurs above a certain (high) level of RH or in the presence of wetness on the plant surface. Lesion expansion is often influenced by air temperature, since this also affects the temperature of the plant tissue, but less by RH. Sporulation is affected by temperature and RH, whereas spore dispersal is often mostly influenced by air humidity and movement. It is important to realize that all these environmental factors will be different at different crop heights. It is therefore necessary to measure microclimatic conditions at a height where the pathogen is expected to attack the host plant. Manipulation of the environment by, for example, changing radiation with different covers, and/or changing temperature, RH and ventilation by using different heating and/or ventilation regimes, may influence diseases and pests. This will be discussed in more detail in Chapter 8.

Concerning pests, their density is directly affected by many biotic and abiotic factors of the environment other than the host plant, such as competing herbivores, natural enemies and climatic factors. Among the latter, temperature, RH, light (quantity, quality and periodicity) and air movement are usually the most decisive in determining behaviour, phenology, survival, fecundity and dispersal of pests. All these abiotic factors may also affect natural enemy populations and, therefore, pests in an indirect, but important, way. Understanding all these complex interactions is crucial for detecting the most decisive factors that govern pest population dynamics and managing the environment for pest control accordingly.

#### 6.2.4. HUMAN ACTIVITY

Humans can affect both the host plants, the greenhouse environment and the pathogen or pest organism by cultural practices and by application of chemical, biological and other types of control. Cultural practices may be aimed at rendering the plant less susceptible or acceptable to diseases and pests, or eliminating (or at least reducing) infection sources. Furthermore, any cultural practice changing plant growth, such as leaf area, will influence the microclimatic conditions for the pathogens and pest organisms in the crop. Greenhouse crops tend to be labour-intensive and this can lead to a more frequent spread of pathogens and pests over the crop by workers than in field crops.

### **6.3. Disease Epidemics and Pest Population Dynamics: Bases for Intervening in Agroecosystems to Reduce Losses**

The amount of disease or the pest density resulting from the interactions between environment, plant and pathogen/pest, and the influence of humans on these factors, is the subject of plant disease epidemiology and pest population dynamics research. Plant disease epidemiologists and agricultural entomologists have approached these studies in seemingly different ways. However, given that both types of scientist deal with populations of living organisms that are subjected to common phenomena such as birth, death, development and migration, approaches of plant disease epidemiology and pest population dynamics are basically similar. Whereas the plant pathologist deals mainly with the effects of the harmful agent (the pathogen), that is the disease, the entomologist is more concerned with the agent itself (the pest). Consequently, plant pathologists usually measure the disease incidence or severity, whereas entomologists generally estimate the number of pest individuals.

#### 6.3.1. DISEASE EPIDEMICS

The amount of disease changes over time. The curve of the amount of disease against time is called the disease progress curve (DPC). It is typically of a sigmoid shape, with a slow increase in the beginning, followed by a logistic increase and a levelling off at the maximum level of disease. Vanderplank (1963) showed that DPCs can be described by logit:



$$y_t = \text{logit}(y_0) + r * t$$

where  $\text{logit}(y) = \ln [y/(1 - y)]$ ,  $y_t$  = disease on time  $t$ ,  $y_0$  = initial disease on time 0,  $r$  = rate of increase, and  $t$  = time. The logit transformation will turn a sigmoid curve into a straight line, which enables an easier comparison of DPCs than the original sigmoid curves. For some diseases, transformation by gompits is better than logits:

$$\text{gompit}(y) = -\ln [-\ln(y)]$$

Disease can be reduced by reducing initial inoculum ( $y_0$ ), lowering the rate of increase of the disease ( $r$ ) or limiting the duration of the epidemic ( $t$ ) by delaying its start through preventive measures. Reduction of initial inoculum is the purpose of sanitation measures before planting the new crop, but inoculum can also be reduced during the epidemic by removing diseased plants or plant parts. Reduction of inoculum will delay the epidemic. It depends on the kind of pathogen whether this delay provides sufficient control. In the case of a pathogen whose spores are abundantly present in the environment, for example powdery mildew fungi, the reduction of initial inoculum will only give a small delay in disease progress. In the case of rare inocula, sanitation may provide almost complete control. Sanitation takes place both in the nurseries and in the greenhouses.

Most manipulations of either the pathogen, the environment or host-plant susceptibility are aimed at a reduction of the rate of disease progress. This is achieved by slowing down any of the processes in the infection cycle.

### 6.3.2. PEST POPULATION DYNAMICS

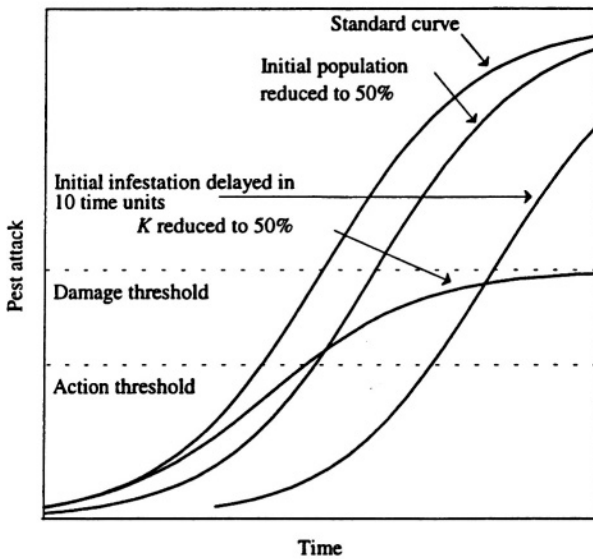
Malthus' equation, initially developed to describe human population growth, was soon adopted by entomologists to study insect demography. The equation predicts that a population will grow exponentially according to:

$$N_t = N_0 e^{rt}$$

where  $N_t$  is the number of pest organisms at a specified time,  $N_0$  is that number at an initial time (0),  $e$  is the base of Napierian logarithms,  $r$  is the rate of population increase, and  $t$  is the elapsed time. If  $r$  is assumed to be constant and independent of conditions that affect pest development, survival and reproduction, population growth is unlimited. This rate of increase  $r$  is also called intrinsic or maximal rate of increase and as such is referred to as  $r_m$  and depicts the rate at which the population would increase under permanently favourable conditions. In nature, however, favourable conditions are never indefinitely maintained and several – usually many – factors limit or retard population growth. To reflect this, the so-called Verhulst' model predicts that populations will grow until reaching a maximum following a logistic or sigmoid curve that can be mathematically expressed by:

$$N_t = K / \{ 1 + [(K - N_0) / N_0] e^{-rt} \}$$

where  $K$ , called *carrying capacity* (the maximum population size that the environment's resources can sustain), is the asymptote of the sigmoid curve and the rest of elements as in the formula of exponential growth. The parameter  $K$  is a measure of the global effect of all environmental factors that limit the growth of a population, the so-called environmental resistance. The shape of population growth in Verhulst's model is represented in Fig. 6.1. Note that it can also represent the logistic model of Vanderplank and, although biologically unrealistic, it allows to show how pest control procedures may prevent pest populations to reach damaging densities. Like in disease control, pest population growth may be reduced by decreasing or delaying immigration of first pest individuals into crop plants (lowering  $N_0$  or  $t$ ), or by decreasing the rate of population increase via integrated enhancement of environmental resistance, for example by release of natural enemies (lowering  $K$ ). In the chapter on plant resistance (Chapter 9), the reader will find a discussion on how different effects on pest development and reproduction influences  $r_m$ .



**Figure 6.1.** The logistic curve of population increase and the different procedures to prevent pest population to reach damage threshold. Initial population can be reduced (e.g. by planting uninfested seedlings) or  $K$  can be lowered (e.g. host-plant resistance or biocontrol) or initial infestation delayed (e.g. sanitation).

#### 6.4. Damage Relationships

Knowledge of the impact of diseases and pests on crop yield is needed for decision-making in disease/pest control. Decision-making is based on the damage relationship, that is, the level of yield loss associated with different amounts of disease or levels of pest attack. Yield loss can be either loss of quality or loss of quantity or both. Usually,

damage relationships for a certain pathosystem or pest/plant interaction are expressed in quantity of yield, either in relative or absolute units. Most frequently, damage relationships are assessed empirically and analysed with regression analysis to estimate yield or yield loss from observations on the amount of infestation. The main drawback of this approach is that the resulting models are descriptive and do not take into account the physiological processes underlying the yield. Despite this, the descriptive models can be very useful in integrated control. Following Campbell and Madden (1990), five descriptive models to calculate the amount of disease and yield loss relationship may be distinguished:

(i) The single-point models or critical-point models. These models estimate yield loss by determining the amount of disease on one given moment, usually determined by the physiological status of the crop, for example the onset of flowering. Less frequently, these models have been developed with time variables, for example the number of disease-free days or the time until a certain level of disease is reached. Single-point models have been developed mainly for diseases in cereals. Their use is limited in crops in which yield accumulates over a longer period of time or harvesting takes place more than once, as for example in greenhouse vegetables.

(ii) Multiple-point models. These models use several disease assessments to estimate yield loss. This type of model is most useful in situations where disease progress can be highly variable, depending on the host plant or the environment.

(iii) Integral models. These models use the summed disease pressure over a specific period of crop growth which is relevant to yield. This is determined by calculating the area under the disease progress curve (AUDPC). These models can not distinguish between an early moderate epidemic and a more severe epidemic which starts later with the same AUDPC. This can be overcome by assigning weighting factors to the disease assessments made on different times or by incorporating another factor, for example the number of disease-free days, into the model.

(iv) Response surface models. These models predict yield loss by using two different types of variables, for example disease severity and crop growth stage.

(v) Synoptic models. They are multivariate models that estimate yield loss by incorporating all independent variables in one equation.

Much of the conceptual framework to estimate the relationship between amount of disease and yield loss may be applied as well to damage relationship concerning arthropod pests. For decision-making purposes, a linear function of the yield response to insect infestation is generally assumed. In case the crop is able to compensate limited injury, there is a level of tolerance associated with low pest density. Crop tolerance to pest attack may be relatively high when pests injure the leaves of fruit vegetables like tomato, pepper, cucurbits or egg plants; often even 30–40% of leaf injury does not result in yield reduction. Sigmoid yield responses to pest infestation are more difficult to fit, but logarithmic or power transformations may linearize the damage relationship. The consideration of more than one pest or disease and crop variables render complex polynomial relationships (the synoptic models mentioned above) which are difficult to interpret and to use in decision-making. If a linear yield response may be assumed or derived, damage relationship can be “easily” found with field data as it has been mentioned above in single- and multiple-point models. When pests are multivoltine and

their numbers are quite variable along the season, the use of insect\*days instead of seasonal mean insect densities may be more meaningful as noted in the above-mentioned integral models. Methods and techniques for this kind of studies may be found in Teng (1987).

A different approach to determining crop loss is the use of dynamic simulation models. Generally, a model for the development of the pest or disease is combined with a model for crop growth and production. This approach generates explanatory models, which are expected to have a greater predictive value than descriptive models. However, the development of simulation models requires a lot more basic information on the physiological processes and on the effect of environmental parameters on epidemics, and are therefore more difficult to develop than models based on regression analysis.

### **6.5. Damage and Action Thresholds**

In combination with the damage relationship for a given pathosystem or pest/crop interaction, it is important to assess the damage and action thresholds. The damage threshold is the maximum level of disease or pest attack below which yield losses do not occur. The action threshold is the level of disease or pest attack at which control action should be taken to prevent the epidemic or pest to reach the damage threshold. Because there are often no fully effective techniques to control a disease or a pest immediately, the action threshold is lower than the damage threshold. Generally speaking, action thresholds for diseases lie below the logistic increase in the DPC.

Damage and action thresholds are an important tool in integrated control when several control alternatives are available. Whereas the damage threshold essentially depends on the disease/pest level and yield loss relationship, the action threshold may greatly vary according to the efficacy of each of the control alternatives and how long they take to be effective. Action threshold to control a disease – or a pest – will be probably higher if we choose a quick acting pesticide than with biological control, where natural enemies need time to react. For example, the action threshold for greenhouse whitefly control may be up several adults per leaf if we rely on insecticides, whereas it is around one adult per leaf when the parasitoid *Encarsia formosa* Gahan must be used in seasonal inoculative releases for the biological control of the whitefly. The knowledge and application of action thresholds generally reduce the amount of control inputs compared to general practice.

Determination of the thresholds is not always easy. Yield loss can be defined as loss in weight of the harvested product or the loss in economic value. This potential economic loss can be compared to the cost of a control measure. The translation of yield loss in weight to economic yield loss depends on the expected price of the harvested product and is therefore difficult to perform. In greenhouse crops that are harvested continuously, this is further complicated by the fluctuating prices within one growing season. For example, one kilo yield loss in cucumber or tomato in The Netherlands will be much more costly for the grower at the beginning (early spring) and end (late fall) of the season when prices are higher than in summer. Further complexity

in the determination of the action thresholds leads to some other considerations: (i) long term consequences of the current decisions for the disease or pest levels (instead of considering just one generation or infection cycle); (ii) influence of control actions on crop revenue (it may be different if the decision is made at farmer or regional level); and (iii) the risk attitude of the grower. Regarding the latter, stochastic models (in which an occurrence probability is associated to each decision) are more useful thresholds for quantifying risks than the deterministic damage and action model. Readers specially interested in the subject may consult the book by Norton and Mumford (1993).

### 6.6. Damage Relationships and Thresholds in Greenhouse Crops

Despite the importance of knowing damage relationships and damage and action thresholds for integrated control, very limited specific information is available for most greenhouse diseases and pests. This can be explained in flower and pot plant crops for the extremely low tolerance of their aesthetic value to the most common diseases and pests. When known, damage thresholds in ornamental crops are near zero, as in the case of powdery mildew in roses, where the damage threshold is only **5 pustules/m<sup>2</sup>** (Pieters *et al.*, 1994). The same pest may have quite different damage thresholds if vegetable or ornamental crops are considered. For example, tomato may tolerate relatively high leafminer infestation (e.g. several dozens of mines per plant) with no yield loss, whereas 1–2 mines on chrysanthemum leads to cosmetic damage.

Some thresholds are available for greenhouse vegetable diseases and pests. Currently, the damage relationship for powdery mildew fungi in greenhouse vegetables is determined in The Netherlands. For cucumber, the best fitting model to describe the damage relationship for powdery mildew caused by *Sphaerotheca fusca* (Fr.) Blumer. [= *Sphaerotheca fuliginea* (Schlechtend.:Fr.) Pollacci] was an integral model using AUDPC. The slope of the regression line between yield and AUDPC was similar in several seasons and for different cultivars. In this case, early disease was also compared to late, more severe disease. Similar AUDPC values and similar yield losses were found for these situations (Dik, 1995), so the inability of integral models to distinguish between early and late epidemics does not seem to be very important here.

An additional problem for the determination of damage relationships is the fact that some pests can inflict more than one type of damage concurrently. This is the case of greenhouse whitefly, that feeds on phloem sap, with the consequent debilitating effect on the plant, but it also damages leaves and fruits by producing honeydew on which sooty mould develops, hampering photosynthesis and respiration and rendering fruits unmarketable. Damage thresholds may be quite different depending on which type of damage is considered to first occur as whitefly populations increase and this is decisively influenced by RH. In very humid greenhouse environments – particularly common in northern Europe – damage by sooty mould development occur at whitefly densities lower than those needed for damage derived from whitefly feeding activity. A density of 2500 greenhouse whitefly nymphs per leaf has been reported to cause yield reduction on tomatoes as direct consequence of phloem extraction, whereas a much

lower density of 60 nymphs/leaf has been observed to produce sufficient honeydew to induce sooty mould development on tomatoes if RH reaches at least 80% for eight hours (Hussey and Scopes, 1977). For *Bemisia* species, a third type of damage is known which is that related to their ability to transmit tomato yellow leaf curl virus (TYLCV). This adds even more complexity to the pest density and yield loss relationship. The damage threshold is highly dependent on the amount of virus inoculum present in or near the greenhouse. Examples of damage thresholds for thrips pests in vegetable crops may illustrate the variability of values that can be found in the literature. For *Thrips palmi* Karny on aubergine the damage threshold is 0.08 individuals/leaf, whereas the same author (Kawai, 1990) gives a value of 4.4 thrips/leaf for cucumber. For sweet pepper, Kawai (1986) gives, for the same pest, a threshold of 0.11 thrips/flower (all details in Parrella and Lewis, 1997). There is unlikely to be a single damage threshold for a given pest on a given crop, but many, depending on the market and climatic conditions (Pedigo *et al.*, 1986). Even more variable are the action thresholds of greenhouse pests in various IPM programmes implemented in the world. If natural control must be considered in biological control of greenhouse pests, abundance of naturally occurring predators and parasitoids is an additional element to monitor and consider in decision-making. This is the case of action thresholds to release *Diglyphus isaea* (Walker) in Mediterranean greenhouses for the control of leafminers; the standard action threshold can be lowered if the parasitoid, that occurs naturally in the area, comes into greenhouses and establishes early in the season (Albajes *et al.*, 1994).

## 6.7. Research on Damage Relationships

In order to establish damage relationships, several issues should be considered.

### 6.7.1. MONITORING AND SAMPLING: WHAT, HOW AND WHEN?

A decision has to be made on what and how to monitor and sample. Accurate assessment of disease severity or pest density is essential. Decisions have to be made on the size, method and frequency of sampling. This topic will be discussed in more detail in Chapter 7. Besides monitoring the pest or disease, it is also important to monitor the host plants and the environmental parameters. Plant growth may vary from season to season and thus potential yield in a crop free of pests and diseases will vary. Often, disease or pest infestation are assessed as number of lesions, pustules or insects, for example, without monitoring the size of the host plant. From a physiological viewpoint, the amount of healthy plant tissue is more important than the amount of affected plant tissue, since the healthy tissue produces the yield.

### 6.7.2. REGRESSION ANALYSIS: WHICH VARIABLES TO USE?

When yield loss is expressed as a percentage of the yield in the uninfested control, a problem may arise when comparing different growing seasons or predicting future losses. When the yield in the uninfested control varies, the slopes of the regression lines

describing percentage yield loss will be different from each other, since both lines are forced through the origin. When the damage relationship is described as yield compared to the control, the level of the lines will vary from season to season, but the slopes of the regression lines will be similar and give a more accurate description of the actual situation (Pace and MacKenzie, 1987).

Modern computer programs enable fairly easy stepwise multiple regression analyses. However, the choice of parameters should be restricted to those that can logically be expected to play a role in the damage relationship in order to provide a more predictive relationship.

### 6.7.3. HOW TO CREATE DIFFERENT EPIDEMICS/PEST DENSITIES FOR DETERMINING THRESHOLDS?

Various methods can be used to create different epidemics or pest densities. The time of inoculation/infestation can be modified, which will result in different levels of disease and pest attack at any given time point. However, climatic conditions will also vary and may interfere with an adequate analysis of the impact of severity on yield. Furthermore, it is possible to use different levels of inoculum/initial pest density or create a gradient of disease/pest attack by putting infested plants on one side of the greenhouse. Disease or pest level can also be modified by a variation in environmental parameters, but this method is not preferred because the environmental parameters may influence yield regardless of disease or pest. The most frequently used method is the utilization of selective pesticides. A disease or pest is allowed to reach a certain predetermined level at which time it is stopped with a chemical pesticide.

### 6.7.4. SIZE OF THE EXPERIMENTS

The design of the experiments largely depends on the type of model to be developed. For descriptive models based on regression analysis, the experiment should resemble commercial practices as much as possible. To assess yield, the plots should be large enough to rule out significant edge-effects. Sometimes, damage relationships are derived from a comparison of different greenhouses. However, this is not recommended, since factors other than the level of disease or pest may also vary.

For the development of simulation models, the experiments are usually of smaller scale and can partly be done under controlled conditions. The effect of one or two factors on plant physiology and on pests or pathogens can thus be determined and this effect is then quantified and incorporated into the model. Thus, prediction of yield is done by using information from an immediately lower level.

## 6.8. Integrated Control

Knowledge of the epidemiology of plant pathogens and population biology of pest organisms in greenhouse crops enables the development of integrated control measures. More than in field crops, cultural practices and the environment can be manipulated to

prevent epidemics. Until now, growers were mostly interested in high yields and therefore, cultural practices and greenhouse climate regimes would not primarily be chosen for disease/pest damage prevention. However, the increasing awareness of the need to limit the input of energy and chemical pesticides, as well as the increasing problems with pesticide resistance, have made growers more willing to consider adaptations to limit diseases and pests. It is important that this knowledge is available.

Integrated control can consist of any combination of control measures, including chemical control. Usually, chemical control is limited to an absolute minimum in IPM systems and it is considered as the last defensive barrier. Integration of cultural practices, such as cultivar choice, the composition of the nutrient solution and climate control, together with biological control measures offer good perspectives for the future. It depends on the crop and on the greenhouse facilities to determine which measures can be incorporated into an IPM programme. In general, all components of the tetrahedron may be modified. As long as the control measures have no negative influence on each other, generally speaking, the amount of control will be greater when more than one component of the tetrahedron is modified. Biological control will generally be enhanced by cultural practices that prevent a too explosive disease epidemic or pest outbreak, or by practices that favour the activity of natural enemies. For example, biological control of powdery mildew fungi is more suitable in partially resistant cucumber cultivars than in very susceptible cultivars (Dik *et al.*, 1998), and the biological control of greenhouse whitefly works better in cultivars that are less prone to pest development. In heated greenhouses, biological control can be combined with a climate regime that promotes the development of the biocontrol agents (see Chapter 14). This combination of climate control and biological control is also a form of IPM.

As mentioned before, integrated control is more complicated than chemical control because more components of the tetrahedron are usually involved and more detailed knowledge on interactions is needed. However, several successful integrated control programmes have been developed (see Chapters 30 to 34).

## 6.9. Concluding Remarks

In this text, several aspects of plant disease epidemiology and population biology of pest organisms have been discussed, mainly to show how many factors play a role in the occurrence and management of an epidemic or pest. The factors which should be studied largely depend on the disease or pest concerned. In general, a combination of small-scale experiments under controlled conditions and larger-scale experiments under semi-commercial conditions gives a good insight into the relationships between the host plant, the environment, the pathogen or pest and human activity. The knowledge of such relationships is fundamental to understand why and when a pathogen or pest population may grow and cause yield loss. The identification of the factors and relationships that permit a species to achieve pest status can help the researcher to design and evaluate methods to manipulate such factors in an integrated way to prevent diseases and pests from reaching the damage threshold.

Damage thresholds are a basic tool for decision-making in integrated pest



management. Reliable damage thresholds are derived from a full understanding of damage relationships. However, the complexity of damage relationships means that relatively few damage thresholds are nowadays available, even in greenhouse crops in which several IPM programmes have been successfully implemented. Further knowledge on damage relationships would permit pest control decisions to be based on a cost/benefit analysis. This is particularly relevant for diseases and pests that leave visible injuries on the plant and force growers to spray chemicals – or to adopt any other control measure – unnecessarily. Furthermore, awareness of the plant tolerance to certain levels of diseases and pests would help to apply control methods – like plant resistance or biological control – that do not seek to eradicate the disease or the pest, but to optimize their control in an economical, social and environmental context.

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## **SAMPLING AND MONITORING PESTS AND DISEASES**

Laurent Lapchin and Dan Shtienberg

Integrated Pest Management strategies require detailed studies which can be broken down into three steps: (i) a precise description of pest population dynamics in space and time in order to assess damage thresholds, to determine key points for control (possibly by modelling), and to evaluate control efficiency; (ii) a general survey to estimate the variability in the first step between seasons or across a region; and (iii) the control strategy, including a survey by the grower of population dynamics. Each of these steps requires particular sampling methods that differ in accuracy: precise measurements for detailed studies, less precise measurements but which can be used on a larger scale for variability evaluation in the second step, and quick and simple methods for final use by the growers.

Pest and disease intensity may be quantified using two different measurements: (i) estimation of the population size, e.g. number of aphids per leaf, number of fungal spores in a cubic meter of air, etc.; and (ii) quantification of the injury caused to the host plant, e.g. the proportion of leaf tissue infested by larvae, the relative leaf area covered with disease symptoms, etc. The methods should be easy to use, allow rapid estimation, be applicable over a wide range of conditions, and most of all, be accurate and reproducible. This chapter presents some of these methods which may be used for greenhouse crops.

### **7.1. Insect Pests**

#### **7.1.1. ESTIMATING INSECT NUMBERS IN SAMPLES**

We will examine different ways of reducing pest assessment time. Methods based on visual abundance indices will be developed in particular, and examples of their application to insect pests will be given. Since many authors have developed methods which may be used with species other than those that they have studied, the references do not always concern greenhouse species.

At each step of a study, the spatial distribution of most pests will be very patchy. Sampling plans will thus require numerous data to reach the required level of accuracy. Particular attention should be given to the evaluation of pest densities at each sampling point. This is a bottleneck which will define the "cost" of the sampling. Methods are often available to reduce the cost of counts, but, except when automatic counting (i.e. picture analysis) is possible, these methods lead to a drastic decrease in accuracy. This loss of accuracy is cumulated with the error induced by the sampling itself, to define the final value of the density estimates. Moreover, several methods that are used to

accelerate pest counts (e.g. field visual observation) underestimate pest densities per area or volume. Such systematic bias, as well as the accuracy of estimates, must be evaluated before using this kind of method.

The most accurate way of obtaining quantitative data on a pest population is to collect the substrate (e.g. host plants) and to take these samples to the laboratory where individuals may be isolated and counted under a stereoscopic microscope (see, for instance, Baumgärtner *et al.*, 1983). As this method is time-consuming, numerous authors have attempted to reduce the time required. The first step is the mechanical extraction of the individuals from the substrate. They can be extracted by washing (Banks, 1954; Taylor, 1970; Halfhill *et al.*, 1983; Raworth *et al.*, 1984; McLeod and Gonzalez, 1988), by flotation in high-density medium such as saccharose (Lapchin and Ingouf-Le Thiec, 1977), or by the use of Berlesse-Tullgren funnels (Wright and Cone, 1983, 1986). Once the insects are separated from mud, sand or plant fragments, the clean extract can be fractionated into sub-samples (Banks, 1954; Waters, 1969; Taylor, 1970; Raworth *et al.*, 1984). Both steps give results of varying precision, depending on the medium surrounding the insects and the species involved. The time required for counting the insects is reduced two to five times, but is often still too great (e.g. up to two hours per leaf, including washing, sub-sampling and counting, for aphids on cucumber).

Collecting insect substrate is not efficient for species such as thrips, which are very mobile. In this case, the extraction has to be made directly in the field, by using sweep-nets (Cuperus *et al.*, 1982; Senanayake and Holliday, 1988), direct picking, mouth vacuum devices (Lapchin *et al.*, 1987) or vacuum nets like the Dietrick vacuum (D-vac) (Rohitha and Penman, 1981; Cuperus *et al.*, 1982; Dewar *et al.*, 1982; Hand, 1986). The numbers are then calculated from the part of the population which can be recovered. This part is often highly variable and the precision of the method is difficult to evaluate. However, successive sampling at the same sites may enable insect density to be estimated with accuracy. The method of Suber and Le Cren (1967), frequently used to evaluate fish densities in rivers (Laurent and Lamarque, 1974), was adapted to benthic insect counts by Lapchin and Ingouf-Le Thiec (1977), and then by Lapchin *et al.* (1987) to estimate larval and adult coccinellid densities in wheat fields. It has recently been used in cucumber greenhouses by Boll *et al.* (1997a) to estimate the number of thrips on leaves after several successive strikes.

### 7.1.2. ESTIMATING INSECT POPULATION DENSITIES

Insect densities may be estimated *in situ*, without collecting samples (see, for instance, Dewar *et al.*, 1982). The characteristics of the species distribution on the host plants should be taken into account and the most representative leaves or stems should be observed (Addicott, 1978; Hull and Grimm, 1983; Bues *et al.*, 1988; Steiner, 1990). This method eliminates the laboratory stage of density estimation, but does not significantly reduce counting time.

Observation time may sometimes be drastically decreased if rough counting is performed. This method is useful if the strong systematic under-estimation of the numbers it produces is constant or depends on known parameters. In the previous

example of *Coccinella septempunctata* L. in wheat fields, Lapchin *et al.* (1987) developed a “quick visual method”, i.e. the observer walked within the **25 m<sup>2</sup>** sub-plot for 2 min and counted all the adult coccinellids he saw. The numbers obtained by this method correlated well with the density estimated with the Seber and Le Cren method, and allowed the development of a sequential sampling plan for adult coccinellids in wheat fields (Iperti *et al.*, 1988). However, such a quick method is not automatically appropriate, even for closely related species, as Frazer and Raworth (1985) did not find that “walking counts” of adult coccinellids in strawberry fields were reliable.

Variance of insect numbers is closely related to the mean (Taylor, 1961). This property implies that variations in numbers may be more easily perceived by observers who follow a geometric rather than arithmetic scale of density. In the field, such orders of magnitude can be easily translated into abundance classes. This was probably the reason why the use of categorical data was soon considered to be a good way of drastically reducing sampling costs. In the case of aphid populations, precise density estimates of *Myzus persicae* (Sulzer) were related to the proportion of infested leaves in different parts of potato plants (Broadbent, 1948). This method was used on other host plants and statistically developed by several authors (Tamaki and Weiss, 1979; Hull and Grimm, 1983; Ward *et al.*, 1985a,b, 1986; Bues *et al.*, 1988). As far back as 1954, presence-absence methods were improved by use of a set of abundance classes (Banks, 1954). Such classes can be purely arbitrary and, for instance, the sampling units distributed into poor, medium or heavy infestation classes (Srikanth and Lakkundi, 1988). Several authors used more precisely defined classes, according to the number of colonies, their size and their localization (Banks, 1954; Baggiolini, 1965; Leclant and Remaudière, 1970; Anderson, 1981; Lapchin, 1985; Lapchin *et al.*, 1994). Different kinds of abundance class systems may be developed, according to the insect studied and its environment.

#### *Building a System of Abundance Classes*

Three main types of class can be considered. Firstly, there are classes whose limits are defined by the number of individuals that are seen during one sample unit of observation. A logarithmic scale of these limits was first used by Leclant and Remaudière (1970) to estimate *M. persicae* densities on peach trees. Another scale which is based on the approximate powers of  $\sqrt{10}$  has successive classes such as: no insect seen, 1 to 3, 4 to 10, 11 to 30, etc. This scale was used by Ferran *et al.* (1996) to evaluate the density of the rose aphid [*Macrosiphum rosae* (L.)] on rose bushes, by Boll and Lapchin (1997) for *Macrosiphum euphorbiae* (Thomas) in tomato greenhouses, and by Lapchin *et al.* (1997) to estimate mummified *Aphis gossypii* Glover on cucumber plants. Secondly, purely qualitative classes, based on size and number of insect patches (Lapchin, 1985) or on the percentage of contaminated shoots (Lapchin *et al.*, 1994), may be used. Such classes are generally used for large sampling units such as trees. Finally, there are intermediate systems which are based on the number of sub-units (e.g. leaves of a plant) in each class of a set of qualitative classes. This system was used by Lapchin *et al.* (1997) to evaluate non-mummified *A. gossypii* on cucumber plants and by Boll *et al.* (1997b) for *A. gossypii* in open-field melon crops.

A visual class system must be both simple and complete. Ease of use depends on the

number of classes and therefore there should be a sufficient number to describe accurately the trends of variability in insect density, but not so high as to be difficult to remember. An optimal class number is generally between five and eight. Another condition required for the use of visual classes is that there must be a biological basis for their definition. To be representative, a qualitative class set must cover the different kinds of patchiness of the species which may be encountered in the field. For example, on cucumber plants isolated colonies of *A. gossypii* a few centimetres in diameter will first develop around winged immigrants (slightly infested leaves). After several days of development, the colonies suddenly spread all over the leaf area (heavily infested leaves). These simple characteristics, which are associated with the size of the leaves that are heavily infested, define the classes of abundance. Simplicity of the class system determines both the robustness of the results and the time required for field observations. In the example cited above, the observation of one sampling unit takes approximately 30 sec for each cucumber plant.

The class system must cover the whole range of insect densities per sampling unit which may be encountered within the observation period and under different conditions. Thus, this range must be evaluated either from previous knowledge or from trials prior to defining the classes.

#### *Calibration of Visual Abundance Classes*

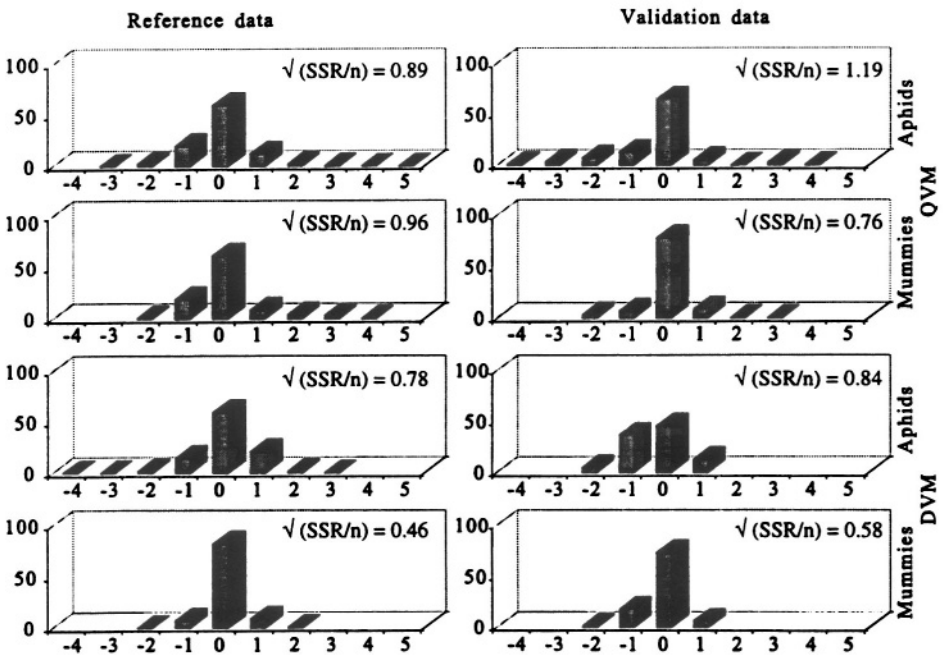
The results of visual observations may often be used without any reference to the number of individuals that they represent and, as such, these ranked qualitative data may be analysed using a large set of non-parametric statistic tools. This method has been used, for example, to evaluate the efficiency of biological control of the rose aphid on rose bushes in public gardens (Ferran *et al.*, 1996). When more precise data are needed, each visual class must be calibrated by computing the mean and variability of the number of individuals actually present in the sampling units. This step is very time-consuming because a large set of precise counts must be gathered so as to represent accurately the variability of the situations in which a given class may be chosen.

#### *Improving the Calibration of the Classes Using Environmental Descriptive Variables*

Calibration of the classes may be viewed as a statistical model having as a response variable the density of aphids, and as a categorical explanatory variable the visual abundance classes. A complex multivariate regression method, "projection pursuit regression", was adapted to this statistical data (Lapchin *et al.*, 1997). Predictions of these models may be further improved by complementary explanatory variables. This work, including calibration with complementary variables, was performed with four different class systems which were used to evaluate the density of the aphid *A. gossypii* and its parasitoid *Lysiphlebus testaceipes* (Cresson) on cucumber plants in greenhouses. Two visual methods were used to estimate densities: "the detailed visual method" (DVM) for a leaf, and the "quick visual method" (QVM) for the whole plant. The class sets of DVM and QVM were built according to the apparent numbers of individuals in the observed sampling units. When the QVM was applied to healthy aphids, the four classes were based on the proportion of the area of leaf infested and on the size of the leaves.

Precise counts were also made on the same sampling units and used as the response variable, and the data were divided into reference and validation sets. The reference sets were used to develop the regression models, and the validation sets to test their robustness.

The choice of complementary explanatory variables was crucial to the development of these regression models. These variables were selected for their influence on the goodness of fit of the models as well as for the time required for collecting. For example, when using the detailed visual method and when the target population of the model was either healthy or mummified aphids, seven explanatory variables were used: (i) the visual class of the leaf; (ii) the visual class of the non-target population on the leaf; (iii) and (iv) the visual classes of the target population on the upper and lower neighbouring leaves on the same plant; (v) the vertical rank of the leaf on the plant; (vi) the number of leaves on the plant; and (vii) the number of leaves infested by the target aphid on the plant. Such data can be easily gathered during sampling without significant additional cost. QVM sampling of whole plants yielded a mean error of approximately one class per plant (the limits of each class are in the ratio of  $\sqrt{10}$ ). The DVM had a mean error of less than one class (Fig. 7.1). The range of the residuals was generally the same for both the reference and the validation data sets, confirming the robustness of these models.



**Figure 7.1.** Robustness of the projection pursuit regression models used to fit precise counts of healthy or mummified aphids on cucumber plants (QVM) or leaves (DVM) against visual abundance classes and environmental complementary variables. Differences between observed and fitted values are expressed in number of classes, according to the scale based on the powers of  $\sqrt{10}$ . SSR: sum of squared residuals.

The same method has now been used to calibrate visual class systems for different pest species on vegetable crops in greenhouses (thrips on cucumber plants, aphids on tomato, melon, eggplant and sprout). Each time that a new regression model is tested, particular attention must be given to the development and sampling of the reference data sets, i.e. they must include the same combinations of variables which will be used in further field sampling.

### 7.1.3. REDUCING THE TIME OF SAMPLING

Reducing the time spent on evaluating insect densities in sampling units has a cost, which is a decrease in the precision of the estimation. However, the time saved allows the observer to increase the number of units taken into account in a sampling plan, and thus to increase the precision of the mean and variance estimates of the density.

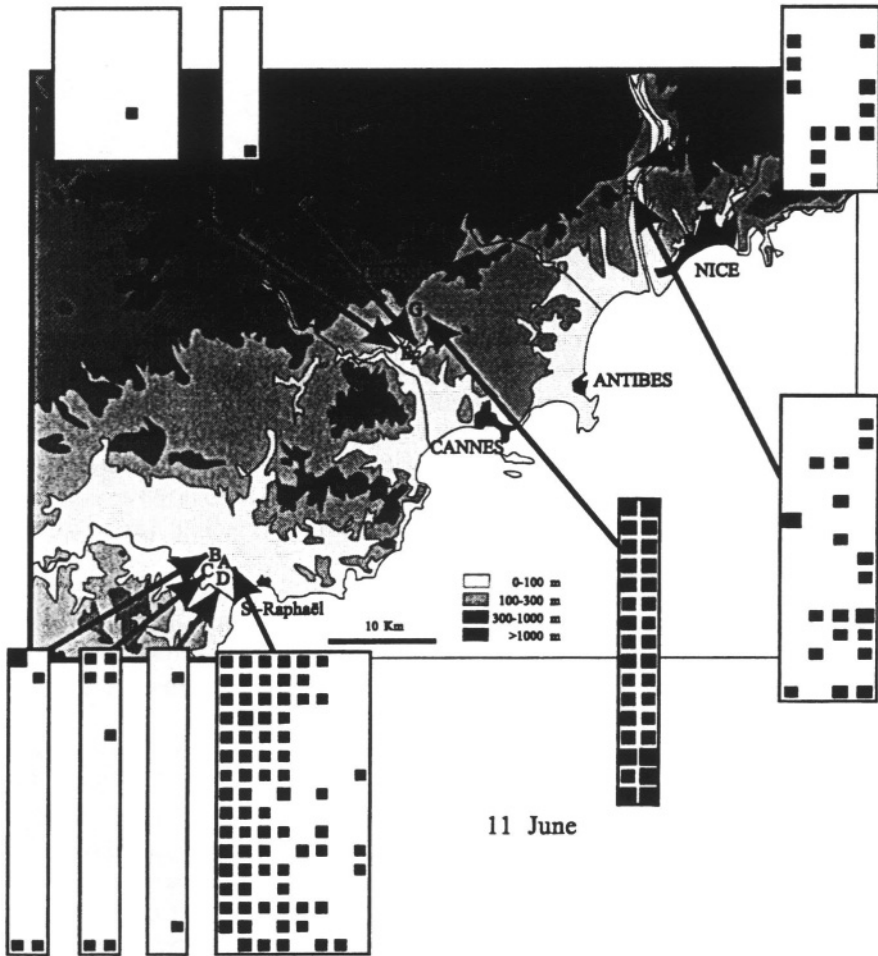
The gain in time is greatly increased when visual methods are used (a ratio of 1:10, when compared with precise counts). Mechanical methods, such as washing (ratio of 1:2), are much slower. However, the evaluation of the precision of visual methods requires a time-consuming calibration of the classes, which must be repeated for each study that deals with a different species, a different environment of the insects or a different scale of observation (i.e. plant or leaf). The decision to undertake such work will depend on the chance of building a “good” visual system. We can summarize the four criteria of this evaluation as follows: (i) insect density must be highly variable from one sampling unit (often a host plant) to another, and from one sampling date to another (this condition is easily met for numerous phytophagous insects whose densities vary on logarithmic scales from one host plant to another); (ii) most insects must be visible (for instance, such methods cannot be used for certain aphid species mainly located inside rolled leaves); (iii) the visual classes must be simple and distinct, i.e. their boundaries have to be easily recognizable in the field; and (iv) these boundaries must be stable in time and space (for example, independent of the host plant growth stage).

The benefit of building such calibrated visual scales depends on which species is being observed and its environment. The scales are particularly useful for most aphid species, as they can reach very high densities and have strongly aggregated distribution patterns. Since the sampling units are not destroyed, crops can be easily monitored and the population dynamics studied separately in different fields. Such a method permits large-scale surveys. An example is given in Fig. 7.2 (Boll *et al.*, 1994): a set of cucumber greenhouses in Provence (France) was sampled weekly by using visual abundance classes and number modelling (see Section 7.1.2). A regular sampling grid was used in every greenhouse and required less than one hour of observation by two people on each sampling occasion.

### 7.1.4. PEST MONITORING

Most phytophagous insects are highly aggregative. Thus, the number of elementary units that are needed in a sampling plan to reach a given reliability of density estimates is drastically increased. This problem is particularly serious at the beginning of the crop season when insects are clumped around early immigrants and for species with a very

high rate of increase. This is the case for most insect pests that exist under greenhouse conditions. Moreover, the efficiency of a biological control will often depend on the detection of such initial foci.



**Figure 7.2.** Example of regional cartography of *A. gossypii* infestation in cucumber greenhouses in Provence (France) on one sampling date. The squares are proportional to the number of aphids per sampling plant, estimated by projection pursuit regression modelling.

Early trials have been performed in tomato greenhouses to develop whitefly sampling schemes that are compatible with the time constraints imposed by the grower. Eggenkamp-Rotteveel Mansveld *et al.* (1978) used stratified random sampling in which absolute counts were performed on 0.6% of the plants, spread evenly through the



greenhouse. These data were compared with an enormous set of absolute counts obtained from the 18,000 plants in the greenhouse. The results demonstrated that the random sampling did not accurately reflect the actual numbers and distribution of the whitefly and also that, in practice, absolute counts were not useful. The same conclusions were drawn by Ekbohm (1980). She suggested that some device should be used to detect at an early stage the presence of whiteflies. This was tried out by Guldemont and den Belder (1993) in chrysanthemum greenhouses. They simultaneously used yellow sticky traps and incidence counts (percentage of infested plants) to detect the moment and the level of the attacks by the major pests of the crop: leafminers, thrips, aphids, whiteflies, spider mites and caterpillars. They concluded that traps were still useful for monitoring the number of leafminers and thrips during the entire season and for aphids in the winter season, but that less emphasis should be placed on the use of traps and more on crop sampling. The low density of pests and their aggregated distribution, however, makes the use of fixed sampling sites less suitable.

Different approaches to aphid sampling have been tested, but unfortunately most of these experiments were performed in open-field cereal crops, and not in greenhouses. The spatial heterogeneity of populations was incorporated into the sequential sampling plans, based on the relationships of variance and the mean of density (Ba-Angood and Stewart, 1980; Ekbohm, 1985; Elliott and Kieckhefer, 1986). The sample size may be adapted according to the reliability desired. More recently, incidence counts (percentage of infested/noninfested tillers) have replaced precise counts (Ekbohm, 1987). Incidence counts will improve aphid sampling efficiency if there is a strong relationship between the incidence and the precise counts at the scale of the sampling unit, and if the loss of precision for each unit is more than compensated by the increase in the number of units observed in a given time. It is useful to combine the errors that are induced by the representativeness of the sampling scheme and by the use of incidence counts, as has been done for aphid predators in cereal crops (Iperti *et al.*, 1988).

The monitoring of insect pests in greenhouses thus remains a complex problem. The most accurate and least expensive methods need to be developed in each situation and then adjusted to give the necessary precision for each particular biological question to be answered.

## **7.2. Plant Pathogens**

### **7.2.1. MEASURING DISEASE INTENSITY**

The intensity of disease may be estimated by two distinct measurements: disease incidence and disease severity. Disease incidence is defined as the number of units infected, expressed as a proportion of the total number of units assessed, e.g. the percentage of infected plants, leaves, fruits, tubers, twigs, etc. This is a quintal measurement (i.e. the unit is infected or it is not infected). Disease severity expresses the intensity of the symptoms, e.g. the area of plant tissue affected by disease expressed as a proportion of the total leaf area, number of lesions per plant unit, etc. (Horsfall and Cowling, 1978).

Measurement of disease intensity in a crop is fundamental for IPM. Disease incidence is generally easy to assess with considerable accuracy, but accurate estimates of the severity of many diseases are much more difficult to obtain. Moreover, a farmer concerned about his crop readily overestimates severity. Thus for decision-making, disease incidence rather than disease severity is the preferred measurement. However, disease severity generally correlates better with yield and crop loss. Because of the relative ease of obtaining most incidence values with accuracy, many attempts have been made to correlate severity to incidence. At low disease levels, good correlation between disease severity and incidence has been found (Seem, 1984). At high disease levels, the relationship between incidence and severity becomes insufficient. When the correlation is significant, the similarity of the two measurements is confirmed and more easily measured incidence values for disease assessment may be used. When this relationship is not linear, an appropriate transformation may be employed. A square root transformation of the severity values is often used to create regression equations that predict severity from incidence (Seem, 1984). Thus, many schemes that warn against pests and diseases depend on enumeration rather than estimation procedures.

#### *Estimating Disease Severity in Field Situations*

Visual estimation of disease severity is almost exclusively used for estimating disease severity in the field. Methods for visual assessment of disease generally fall into two categories (Lindow, 1983). The first category contains descriptive keys that utilize arbitrary scales, indices, ratings, grades or percentages to quantify disease (James and Teng, 1979). Such keys have been successfully used to estimate disease severity of host plants with differing disease resistance, or of host plants subjected to different environmental conditions or cultivation procedures. For example, disease can be described using categories of 1–5 to denote incidence (none to extreme) or severity (none to heavy). It is not appropriate to perform mathematical manipulations such as averaging on these records because values between two adjacent categories have no meaning (Berger, 1980).

The second category for visual assessment of disease involves the use of standard area diagrams. Pictorial representations of the host plant with known and graded amounts of disease are compared with diseased leaves to allow estimation of disease severity. Estimates of disease severity are proportional to the absolute area of the leaf that is diseased, and are not expressed as a percentage of an arbitrary maximum severity value. In contrast to descriptive keys, standard area diagrams allow estimation of intermediate levels of disease severity by comparing a diseased plant with diagrams that show both more and less disease (Lindow, 1983).

Horsfall and Barratt (1945), while noting the Weber-Feckner law, emphasized the limitation of the eye in the assessment of plant disease. The Weber-Feckner law states that the visual acuity of the eye is proportional to the logarithm of the intensity of the stimulus. These authors also noted that in visually estimating disease severity, the observer actually assesses the diseased proportion of leaves having <50% injury and the healthy portion of leaves having >50% injury (Horsfall and Cowling, 1978). Horsfall and Barratt (1945) developed a disease-rating scale that contained 12 equal divisions of disease severity on a logarithmic scale with a median value of 50%. Thus, divisions of

this scale included decreasing ranges of disease severity when either increasing or decreasing from 50% disease severity (Horsfall and Cowling, 1978). This scale and many standard diagrams constructed thereafter account for the logarithmic decrease in acuity of the eye in estimating severities approaching 50% by their selection of representative keys. Estimations of disease severity intermediate between two keys are made by careful interpolation.

#### *Accuracy, Repeatability and Reliability of Disease Assessments*

Visual estimation of disease severity can differ significantly from the actual amount of disease. If the observer is not aware of the limitation in visual acuity at the midrange of disease severity, estimated disease severity and actual disease severity will be linearly related, and the variance of estimates will be independent of disease severity. However, the Weber-Feckner law indicates that the true confidence interval of estimates of disease severity will approach the expected linear relationship at both low and high disease levels, but will increasingly depart from this line with increasing disease severity, with a maximum variance at 50% disease (Lindow, 1983).

Inter-rater reliability has been operationally defined as the ratio of true variance to total variance, which includes a variance component for the error among raters (Shokes *et al.*, 1987). Although improved sampling designs and increased sample size can lower the actual and total variance, limited resources often restrict sample size. In addition, when more than one rater is involved, it is difficult to quantify the bias attributable to any one individual. Shokes *et al.* (1987) proposed measuring intra-rater repeatability with the test-retest correlation procedure. The correlation coefficient ( $r$ ) provides a statistical measure of the relationship between repeated assessments of the same sampling units by the same individual or instrument. However, correlation analysis between two variables cannot be used to infer a cause-and-effect relationship, nor can one variable (repeated assessments) be used to predict the value of another variable (first-time assessments).

Least-squares regression can be used to determine if there is a significant linear relationship between disease assessment performed by different raters and whether there is a statistical relationship between related assessments performed by the same individual (Nutter *et al.*, 1993). Regression-equation parameters, such as the slope and the intercept, could be used to evaluate and compare the accuracy and precision of disease assessment raters and methods. Slopes that are significantly different from one indicate the presence of systematic bias among rates, whereas intercepts significantly different from zero indicate the presence of a constant source of error among raters.

### 7.2.2. DISTRIBUTION OF DISEASE

Spatial distribution of diseased units in a pathosystem is the most important factor affecting the field estimation of disease intensity. Spatial distribution includes the way in which disease lesions are distributed among healthy units and the way in which diseased host units are distributed among healthy units. Distribution of diseased units may be random, aggregated or regular (Teng, 1983). With randomly distributed disease, the variance is theoretically equal to the mean. In aggregated patterns, the variance in

the number of lesions per leaf is greater than the mean number of lesions per leaf, but when there is a regular pattern the variance is smaller than the mean.

When a large number of host units are sampled for disease, a frequency distribution showing the number of diseased units in each severity category may be determined. The sample frequency distribution can be compared with theoretical distributions using the goodness-of-fit test, and the parameters of the empirical distribution may be defined. Theoretical distributions applied to biological systems include the normal, log normal, Poisson, Weibull, Gamma and negative binomial ones. Knowledge of the frequency distribution is essential for the design of sound sampling procedures.

When estimating disease intensity per field, the sampling unit, sample size, sampling point, sampling fraction and sampling method must be considered. In most disease assessments, the sampling unit is a plant. Often, only selected parts (such as individual leaves) may be assessed for disease intensity. For each field, a predetermined number of sampling units is selected to give a mean value representative of that field; this is the sample size. Sample size is determined by the cost of sampling, the precision required and the available time, as well as by the spatial distribution of the disease; that is, the sample size should be empirically defined.

Many sampling methods have been reported for plant disease assessments. Samples may be taken at intervals along predetermined lines in the field or greenhouse and these may be either one diagonal, both diagonals (forming a big letter X), or (if a more representative sample is required) a large W or Z pattern. With a disease that is randomly distributed, all the above methods will give comparable results and reduced variance in the sample mean may be better achieved by increasing sample size. If the diseased units are aggregated, the sampling method will be more important than sample size, and the large X or W sampling pattern is preferable to the single diagonal (Teng, 1983).

### 7.2.3. MONITORING PATHOGEN POPULATIONS

Monitoring the pathogen, primarily by trapping air-borne spores, has been used as a measurement of disease intensity and development and could serve either as an alternative, or a complement, to disease assessment. Given the current technology, the use of spore counts of pathogen populations for field measurement of disease is unlikely to replace the main conventional methods of measuring disease severity (disease symptoms), unless its accuracy can be shown to override the ease and low cost of symptom assessment (Teng, 1983).

The monitoring of pathogen populations may serve another purpose. As fungicides still remain an important tool for control of plant pathogens in the greenhouse, it is important to monitor populations of the pathogen for their resistance to potential fungicides. The term “monitoring resistance” is used to denote testing for sensitivity of target organisms in field populations. This can range in scope from continuous surveillance programmes over several years and involving many locations to short-term investigations into individual cases of suspected resistance. Good monitoring is the cornerstone of fungicide resistance research. Without such work, we would know virtually nothing about the occurrence of resistance in crop pathogens. Moreover,

resistance monitoring, together with monitoring for changes in practical performance, is a vital component of integrated resistance management (Gullino and Garibaldi, 1986; Brent, 1988). Several tools have been developed for such a purpose. For example, a tool for estimating the resistance of populations of *Botrytis cinerea* Pers.:Fr. to common fungicides has recently been developed (Elad and Shtienberg, 1995). Tested fungicides are added to a selective medium in Petri dishes. The plates are exposed in the greenhouse at approximately midday, when *B. cinerea* conidia are released into the air. Plates are exposed for 30–60 min, according to the intensity of the disease in the greenhouse and then incubated for 4–7 days. Counts of typical *B. cinerea* colonies in the media supplemented with the fungicides are compared with those from fungicide-free plates. The data may then be used to make a recommendation on fungicide use.

### 7.3. Concluding Remarks

Studies on population dynamics of insect pests or beneficials and plant pathogens, which have been performed to improve the efficiency of IPM, have followed parallel paths and run into similar obstacles. Because of the speed of the dynamics and the strong spatial heterogeneities of these populations, control strategies have had to be designed to include the large amounts of data that may be generated over different temporal and spatial scales. In both disciplines, methods have been designed to evaluate quickly insect densities or levels of disease injury in large and frequent samples. Moreover, the need to sample commercial crops to take into account large-scale variations implies the use of non-destructive methods. Pathologists and entomologists have independently concluded that visual indices could be practical and efficient. Initially, both of these groups have tried the two-class (presence/absence) indices, and then later the several-class indices. After it was discovered that the logarithmic scale is a natural tool of the human eye discriminating among different kinds of intensities, statistical approaches were developed to evaluate the precision of such evaluations.

In the future, many new methods will need to be constructed to advance IPM strategies. A good idea would be to synchronize some of these developments in the two disciplines (i.e. for all the major insects and pathogens of a given protected crop), and to pool the statistical approaches which, as a matter of fact, deal with very similar problems. Such an integration would allow the pathologists and entomologists to propose standardized “toolboxes” to professional and technical partners.

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## MANAGING THE GREENHOUSE, CROP AND CROP ENVIRONMENT

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### 8.1. Introduction

Greenhouses vary in structural complexity from simple plastic film-covered tunnels, with no assisted ventilation, to tall, multispans, glass or plastic-covered structures covering several hectares and having sophisticated, computer-controlled environments. Essentially, however, all have climates inside that are rain-free, warm, humid and windless, ideal for raising crops but at the same time also ideal for many diseases and arthropod pests (Hussey *et al.*, 1967; Jarvis, 1992).

Though it is restricted, the climate within the greenhouse forms a continuum with the climate outside the greenhouse, and there are gradients in temperature, humidity, light and carbon dioxide. Depending on the needs of the crop, the need to exclude pests and pathogens, and the need to implement biological control programmes, these gradients can be manipulated to certain extents by such devices as screening, shading, cooling, heating and ventilation. At the other end of the scale, the climate at the immediate plant surface, the so-called boundary layer (Burrage, 1971), whether of shoots or roots, is of paramount importance in the avoidance of pests and diseases. It extends 1–2 mm for arthropod pests, about **30  $\mu\text{m}$**  for fungi and even less for bacteria. Its climate, the true microclimate, forms a continuum with the climate within the intercellular spaces of leaves on the one hand, and with the macroclimate of the greenhouse and its environs on the other hand. While most stages of most arthropod pests and beneficial insects are free to enter and leave the boundary layer if it is inimical to their activity, most micro-organisms enter passively and leave as wind-dispersed or water-splashed secondary propagules. In order to escape arthropod pests and pathogens, the microclimates of phyllosphere and rhizosphere must be made inimical to their activity but at the same time biological control organisms have to be encouraged with appropriate microclimates. It is often overlooked that biological control organisms have their own hyperparasite and predator chains extending theoretically indefinitely and acting alternately counter to effective biological control on the crop or beneficially with it (Jarvis, 1989, 1992). They also have their own adverse environments. It is apparently an insoluble task to manage boundary layer microclimates without detriment to the crop or to biological control, at the same time not permitting primary pests and diseases to become established.

### 8.2. Managing the Greenhouse

The local climate, the external disease and insect pressures, the greenhouse structural design, the climate-control equipment available, and the skill level of greenhouse workers have a major bearing on how a greenhouse is managed to control insects and diseases.

From the outset, it is important to have the input of a greenhouse manager to ensure that the physical facilities are properly designed for IPM when building a new greenhouse operation. Once a greenhouse is in operation, greenhouse managers have to be forever mindful of how activities in and around a greenhouse will affect IPM.

### 8.2.1. SITING AND ORIENTATION

On a world-wide basis, commercial greenhouse production is concentrated in regions between 25° and 65° latitude where the climate is moderate and local weather patterns are favourable. At high latitudes solar irradiance is low, day length is short and temperatures are low during the winter months resulting in poor growth and increased susceptibility to disease. Under such conditions, diapause of predatory insects may make biological control difficult. Large inputs of energy are required to maintain greenhouse temperatures, and humidification is often necessary to overcome the drying effect of continual heating. At low latitudes, high solar irradiance stresses crops making them more susceptible to disease. More outside ventilation air is required which brings with it more pathogen propagules and insect pests.

Within the most favourable latitudes, greenhouse production is concentrated in maritime areas where large bodies of water moderate the local climate. In continental areas, large swings in outdoor temperature and maximum solar-irradiance levels (Short and Bauerle, 1989) on a day-to-day basis create crop stresses that make greenhouse management more difficult. In summer, cooling of greenhouses is difficult if ambient air temperatures are above the desired greenhouse temperature, and if the relative humidity is so high that evaporative cooling is not effective.

Within any given region, the siting of a particular greenhouse operation makes a significant difference in the management of disease and insect problems. Field crops and natural vegetation growing in close proximity to a greenhouse create disease and insect pressure, especially if those crops and the vegetation are susceptible to the same disease and insect pests as the greenhouse crop. This pressure is intensified when pathogen propagules are stirred up by field operations, or when the outdoor crop is harvested or senesces and insects are forced to find a new host. Low temperatures force insects to seek out warmer climates indoors. On the other hand, freezing outdoor temperatures reduce pest pressures by inactivating pathogens and arthropod pests. Insects and pathogen propagules are carried into greenhouses through vents and doors by wind. By locating a greenhouse away from and/or upwind of outdoor crops, many pest problems can be reduced to manageable levels.

Out of concern for maximizing productivity and crop uniformity, greenhouses are oriented for maximum light penetration. This usually means an east-west orientation for free standing greenhouses and gutter-connected complexes (Harnett and Sims, 1979). Achieving good lighting uniformity over the course of a day is also important for IPM because insects and diseases proliferate in shaded areas and on stunted plants. In addition to orientation for optimal lighting, greenhouses should be oriented to take advantage of the prevailing winds. High wind speeds, if not reduced by windbreaks, increase heat loss and increase static pressures against which ventilation fans must operate. Moderate wind velocity at right angles to ridge, gutter and side vents is optimal for natural ventilation air movement through vents.

As said before, the environs of the greenhouse may be reservoirs of pathogens and pests. Greenhouses are often in an arable area, with trash piles, weeds and crops botanically related to the crop being grown in the greenhouse to provide ample inoculum and infestations of pathogen vectors (Harris and Maramorosch, 1980; Jarvis, 1992). Entry into the greenhouse can be rapid and on a massive scale: wind-blown dust carries spores and bacteria, air currents with or without forced ventilation carry spores and viruliferous insects from trash piles and weeds, water run-off into the greenhouse can carry soilborne pathogens such as *Pythium* and *Phytophthora* species and chytrid vectors of viruses, and dirt on feet and machinery carries pathogens. A foot bath containing a disinfectant reduces this latter risk when placed at the doorway. To surround greenhouses by a 10-m band of weed-free lawn and to eliminate trash piles may prevent or delay pest and pathogen inoculum entrance into greenhouses. Though whitefly-proof screens can keep out most insects (and keep in pollinator insects) fungal spores and bacteria cannot be excluded. Diseases of tomato such as Verticillium wilt, Fusarium crown and root rot, and bacterial canker are often first noticed directly beneath root vents or just inside doorways, as is the *Diabrotica*-borne bacterial wilt of cucumber [*Erwinia tracheiphila* (Smith) Bergey *et al.*].

Overlapping of cropping, i.e. raising seedlings and transplants alongside production crops, is unsound hygiene, inviting infection and infestation of the new crop from large reservoirs in the old crop.

## 8.2.2. STRUCTURES AND EQUIPMENT

The structural complexity of successful greenhouse operations tends to increase with time as older structures are replaced with more advanced designs, as the operations increase in size, as profits are reinvested, and as the need for improved climate-control becomes apparent. The low cost, low height, plastic film-covered structures that are often first built by growers provide some protection from outdoor weather and pests, but without any means for climate-control, conditions inside are often more favourable for diseases and pests than outside. Higher structures with more substantial framing members are required to accommodate climate-control equipment.

The trend in greenhouse structural design in recent years has been towards large gutter-connected complexes with high (4–5 m) gutter heights. As the size of operations under one roof has increased, increased gutter heights have become necessary to create the chimney effect needed to ventilate these structures naturally. With increased air space between the crop and the greenhouse cover, the uniformity of horizontal and vertical air movement has improved, temperature gradients in the crop canopy have been reduced and the uniformity of lighting of the crop has improved because shadows cast by higher overhead structural members move around more throughout the day. Increased gutter heights have also been beneficial for IPM because they increase the height that insects and pathogen propagules must be transported by wind to find their way into greenhouses through vents.

With larger complexes and the economies of scale they provide, it is feasible to incorporate features in a greenhouse design that favour IPM. With large-scale operations, it is practical to build header-house facilities that restrict access to the greenhouse. Separate shower and lunch room facilities, foot baths, refuse handling facilities, concrete floors, etc., may reduce the transport of insects and pathogen propagules into the growing areas and can be justified. The costs of pressure washing equipment and specialized potting

and growing medium sterilizing equipment are easier to justify. Also, for large scale operations, it is feasible to have separate propagation facilities (Section 8.3.2) specially designed for the production of disease-free transplants. On the other hand, because of the increased number of nooks and crannies, it is more difficult to eradicate insects and disease propagules from large complexes once they have gained a foothold.

### *Covers*

The radiation transmission characteristics and the air tightness of greenhouse cover materials have a major effect on the climate for IPM inside a greenhouse. Ideally a cover material should have a high photosynthetically active radiation (PAR) transmission to maximize productivity and solar gain, low infra-red (IR) transmission to minimize radiation heat loss, and low ultraviolet (UV) transmission to inhibit sporulation of fungi (see Section 8.4.4). Unfortunately, no material has all these radiation transmission characteristics. Depending on latitude and local climate, some cover materials have been found better than others for IPM.

Glass is the preferred greenhouse cover material at high latitudes, where winter light levels are limiting and outdoor temperatures are low, because of its high PAR and low IR transmission characteristics. Glass, however, does transmit the UV radiation necessary for the sporulation of fungi and has relatively high air leakage which can lead to very low humidity during cold periods with high heat demand. During these periods it is necessary to humidify glass greenhouses to ensure the continued activity of biological control agents.

Polyethylene is the preferred greenhouse cover material at lower latitudes where high PAR transmission is not as critical and where retention of humidity for IPM is important. Some manufacturers include admixtures in their polyethylene films to block the UV wavelengths necessary for sporulation of fungi. The effectiveness of these blockers decreases as the films age. Polyethylene-film covered greenhouses are tighter than glass houses and therefore are better at retaining humidity during hot dry periods. During cool wet periods, high humidity and condensation on the underside of polyethylene films is a problem that can lead to indiscriminate dripping and spread of diseases in the crop. Surfactant sprays have been developed for polyethylene films that cause a film-wise condensation and runoff at the gutter. In recent years, roof arches used for polyethylene greenhouses have been modified from a semi-circular shape to a gothic shape to enhance film-wise condensation and runoff at the gutter.

### *Heating Systems*

A carefully designed heating system to maintain air and root zone temperatures close to recommended levels is essential for an effective IPM programme in greenhouses. In the northern hemisphere greenhouse heating systems should be designed to maintain the desired indoor temperature when the outdoor temperature is at the 2.5% January design temperature (i.e. the temperature below which 2.5% of the hours in January occur on average) for a given location. If it is expected the greenhouse will be heated from a cold start in January, then it is common practice to add another 25% of pick-up capacity to the calculated 2.5% January design heating load so that the greenhouse can be fully warmed up before plants are transplanted.

Centralized hot-water or steam pipe heating systems are the most practical for commercial greenhouses. Fan-forced unit heaters are practical for small greenhouses or in

greenhouses where it is only desirable to maintain temperatures above freezing, but heat delivery from fan-forced units is too costly and very non-uniform on a large scale. With hot-water or steam heating systems, heat is delivered to the base of the plants via radiation pipes running between the crop rows approximately 15 cm above floor level. Low-level positioning of heat pipes is important to provide heat to the root zone and to induce vertical air movement via natural convection. The temperature of water circulating in hot-water heating pipes is adjusted from 40 to 90°C depending on heating demand, thus heat is always applied at the base of the plants for a uniform temperature distribution. The flow of steam at 100°C through steam pipes is cycled on and off as required to maintain air temperature. This cycling leads to a non-uniform heating of the base of the plants and more temperature variability in steam-heated greenhouses. During very cold weather, operation of additional heating pipes around the perimeter and under gutters in hot-water and steam heated greenhouses is required to prevent cold spots where diseases are prone to develop. In hot-water heated greenhouses, especially those with tomato crops, an additional small-bore heating pipe is often used to apply heat at the growing tip of the plants to enhance growth and to prevent condensation on developing fruit.

#### *Misting Systems*

A common reason for failure of biological disease and insect controls early in the greenhouse growing season, and later on when outdoor conditions become hot and dry, is very low humidity levels in the greenhouse air. Under these conditions, transpiration of the crop is not adequate to maintain humidity levels in the optimum range for biological controls and it is necessary to add humidity to the air. Under hot and dry conditions, addition of humidity to the greenhouse has the added benefit of evaporatively cooling the greenhouse air. The theoretical and practical management of greenhouse humidity has been discussed by Stanghellini (1987) and Stanghellini and de Jong (1995).

The best humidification systems for greenhouses are those that create small water droplets that evaporate before they have a chance to settle out on leaves where they could provide the moisture necessary for germination of fungal spores. High-pressure (4–7 MPa) misting systems with 10 µm diameter nozzles and sonic misting systems that require a compressed air supply have been developed to create 10 µm diameter water droplets for greenhouse humidification. When properly maintained, these systems create a fog that gradually disperses as the water droplets evaporate in the air.

#### *Ventilation Systems*

Intake of outdoor air and exhaust of indoor air is necessary to prevent excessive solar-heat gain or humidity build-up inside greenhouses. Most large scale greenhouse operations are passively naturally ventilated through vents in the roofs and side walls. Small greenhouses, and polyethylene covered structures that are not equipped with roof vents, are actively ventilated with fans. Gutter vent systems have recently been developed for polyethylene covered greenhouses that allow them to be ventilated passively. Ventilation rates required for summertime temperature control are 0.75–1.0 air changes per hour (ASAE, 1989). Winter ventilation rate requirements are typically 10–15% of summer requirements. The relationships between greenhouse geometry, vent geometry, wind speed, wind direction, temperature and natural ventilation rates have been established by Kittas *et al.* (1997).

When greenhouse vents are closed, natural convection air movement inside

greenhouses is often not sufficient for good air mixing and mass transport in the crop canopy. At low wind speeds leaf boundary layer resistance increases, resulting in decreased transpiration (Stanghellini, 1987) and increased relative humidity at the leaf surface. In large greenhouse complexes overhead fans strategically placed above the crop are required to bring horizontal air velocities up to approximately 0.5 m/s for good air mixing and to minimize boundary layer effects.

Air pressure differentials between inside and outside are necessary to move air actively through greenhouses. In actively and passively ventilated greenhouses, the pressure differential between inside and outside is usually negative, and it is easy for airborne pathogens and insects to enter the greenhouse, particularly if doors and ventilators are left open in hot weather. In special circumstances where it is essential to exclude pests and disease propagules, it may be necessary to maintain a positive pressure differential. With such a ventilation system, air can be filtered as it is drawn into the greenhouse to remove insects (Section 8.2.3) but removing airborne fungal spores and bacteria is impracticable. With a positive pressure differential, there is less tendency for infiltration of insects and disease propagules from outside through cracks in the greenhouse cover.

Regardless of type of ventilation system, any obstructions that reduce the vent openings increase the pressure differential and/or reduce the air flow through vents. If screens are placed over vent openings (Section 8.2.3) then the area of the vent openings must be increased by a factor equal to the reciprocal of the percent free area of the screen material to maintain the same pressure differential. If screens are used in established greenhouses, it would be necessary to build boxes over vents, add screened-in bays or screen the entire head space of a greenhouse to provide adequate intake air for good ventilation.

#### *Thermal/Shade Curtains*

Thermal curtains and shade curtains are generally beneficial for IPM because they reduce the extremes in climate that stress the crop and biological controls. Thermal curtains, aside from saving energy in the winter, reduce the net radiation from leaves through a greenhouse cover to a clear sky. For this reason leaf temperatures are higher and condensation on leaves is less under thermal curtains.

Shading of greenhouses is necessary in hot climates to reduce solar radiation and heat stress on crops. Paints can be applied on the exterior surface of the greenhouse cover (Grafiadellis and Kyritsis, 1978) or shade curtains can be deployed inside or outside (Willits *et al.*, 1989) to attenuate the radiation reaching the crop. Moveable shading systems (Jewett and Short, 1992) are also useful for acclimatizing crops and biological controls to rapidly changing solar radiation conditions.

#### *Control Systems*

The climate inside a greenhouse at any given time is determined by a complex interaction between outside climate variables, status of the crop and operating state of the climate-control equipment. Because of highly variable solar energy fluxes, the climate can change rapidly and climate-control equipment has to be manipulated quickly and frequently to maintain optimum conditions. The complex climate-control requirements of modern greenhouses can realistically only be met with computer-control systems.

Climate-control computers have been specially developed to meet the demanding

requirements of greenhouse operations. The hardware used in greenhouses has been specially designed to withstand the high humidity and high levels of electrical noise. Special temperature and humidity sensing systems have been designed to monitor the inside and outside climate for control purposes. These sensors are shielded from the sun and are aspirated so that control is based on measurements of true ambient air temperature and relative humidity.

The software in commercial greenhouse computers has been specially developed to be fault-tolerant and to integrate the operation of climate-control equipment. In most cases the software has to be configured and control loops for each piece of climate-control equipment have to be tuned by the installer to give satisfactory performance. Currently available greenhouse control software enables greenhouse operators to schedule climate setpoints for the conditions that they believe are best for production and IPM. The actual climate-control achieved is limited by the capabilities of the climate-control equipment and the operator's skill and knowledge.

### 8.2.3. INSECT SCREENING

In the Mediterranean basin, protecting crops from arthropods is regarded as more important than protecting them from the weather, so the physical exclusion of insects from the greenhouse should help in reducing the incidence of direct crop damage and also of insect-transmitted virus diseases, theoretically this exclusion can be done by fitting fabric screens of mesh aperture smaller than the insects' body width over ventilators and doorways, or by insect-repellent fabrics, but in practice there still can be significant insect penetration. Moreover, screens impede ventilation and reduce light transmission, so compromises in the management of light, temperature and humidity are necessary to avoid adverse effects on crops and their susceptibility to diseases.

Screens do not suppress or eradicate pests, they merely exclude most of them; therefore, they must be installed prior to their appearance, and supplementary pest control measures, such as biocontrol, are still required (Berlinger *et al.*, 1988). Insect parasitoids and predators that are smaller than their prey can still immigrate through pest screens into the greenhouse but larger ones have to be introduced. Since they offer an economical method of biological control of pests, they must be preserved, and destructive insecticides should be avoided. Screens impede ventilation (Robb, 1991; Price and Evans, 1992; Baker and Shearin, 1994), resulting in overheating and increased humidity. Increased humidity necessitates more frequent fungicide sprays than were required previously in an unscreened greenhouse. In Israel, 5–6 sprays per season (as opposed to 2–3 previously) are required in screened greenhouses (Y. Sachs, pers. com.). To minimize these harmful effects, growers add forced ventilation but this only helps to pull whiteflies through the screen, while exhausting air from the screenhouse increases the intake of small insects. Application of positive air pressure, pushing air into the structure through an insect-proof filter, reduces whitefly influx (Berlinger and Lebiush-Mordechi, 1995).

Thus, while screens can reduce immigrant populations of pests, they also reduce the immigration of beneficial arthropods. In neither case is exclusion total. Screens are disadvantageous in that temperatures and humidities tend to rise, promoting plant stress and susceptibility to diseases, and they also reduce light. Access to the greenhouse by workers and machinery is more difficult.

### *Types of Screens*

Various types of screens and plastic covers have been developed to protect crops from insects; the challenge for the grower is to match the proper type of screen to local insect populations.

*Woven Screens.* The conventional woven screens are made from plain woven plastic yarns. Weaving leaves gaps (slots) between the yarns both in the warp and in the weft. In commercial screens the slot is rectangular whose width must be smaller than the whitefly's body size, about 0.2 nun, but it must allow maximum air and light transmission. Elongating the slot to improve ventilation is not feasible, since the threads slide apart, allowing insect penetration.

Bethke and Pain (1991) found that screens designed to exclude *Bemisia tabaci* (Gennadius) still permitted some to penetrate, and they failed to exclude *Frankliniella occidentalis* (Pergande). They did, however, exclude most larger insects such as moths, beetles, leafminers, aphids and leafhoppers, and they retained bumble bee pollinators.

*Unwoven Sheets.* These are made of porous, unwoven polyester and polypropylene or of clear, microperforated, polyethylene fabric. All are very light materials which can be applied loosely and directly over transplants or seeded soil, without the need of mechanical support. They have been used primarily in the open field, in early spring, as spun-bonded row covers, to enhance plant growth and to increase yield. At the same time they also proved to protect plants from insects. A polypropylene perforated sheet protected tomatoes from Tomato Yellow Leaf Curl Virus (TYLCV) transmission by *B. tabaci* (Berlinger *et al.*, 1988).

*Knitted-Screens.* Because of irregularity in the shape of the holes, whiteflies are not excluded (Berlinger, unpublished). Reducing slot size to block whiteflies reduced ventilation to an impractical level. However, knitted screens can exclude larger insects.

*Knitted-Woven Screen.* This plastic screen is produced by a technique that combines knitting and weaving. The slot is almost 3 times longer than in the commercial woven screen, while the width is smaller than the whitefly body size. The insect cannot pass, but ventilation is improved. A laboratory test confirmed the screen's high blockage capacity for whiteflies, which was similar to that of a conventional screen (0.1% vs. 0.5% penetration, respectively; Berlinger, unpublished).

*UV-Absorbing Plastic Sheets.* These are claimed to protect crops from insect pests and from virus diseases vectored by insects, by modifying insect behaviour (Antignus *et al.*, 1996) but Berlinger (unpublished) was unable to confirm those claims. Nevertheless, these UV-absorbing plastic sheets have become available for commercial use. Their role in controlling diseases is discussed in Section 8.4.4.

### *Whitefly Exclusion*

The sweetpotato whitefly (*B. tabaci*) is a small insect, about 0.2 mm wide, which transmits TYLCV, and has become the limiting factor in vegetable and flower production in Israel (Cohen and Berlinger, 1986; Zipori *et al.*, 1988). Its physical exclusion from greenhouses



is crucial, and accordingly whitefly-proof screens were developed (Berlinger *et al.*, 1991). While the rate of whitefly exclusion is generally proportional to the screen's mesh ( $R^2 = 0.85$ ) (Berlinger and Lebiush-Mordechi, 1995), the insect's ability to pass through any barrier could not be predicted solely from thoracic width and mesh size (Bethke and Pain, 1991). There is an unexpectedly high rate of whitefly penetration resulting from a great variability among the samples of the same screen resulting from uneven and slipping weave (Berlinger, unpublished).

#### *Thrips Exclusion*

Whitefly-proof (50 mesh) woven screens are by far the most widely used covers for the exclusion of whiteflies and bigger insects. In laboratory tests, thrips, with a body width of only **245  $\mu\text{m}$** , moved freely through this screen. However, in the field, a high proportion (50%) are excluded, possibly because of the optical features of the plastic (Berlinger *et al.*, 1993).

Western flower thrips are strongly affected by colour. A loose shading net of aluminium colour, through which even whiteflies penetrated freely in the laboratory test, was tested in the field and in a walk-in tunnel. The aluminium screen reduced thrips penetration by 55% over an identically shading net but white in colour (Berlinger *et al.*, 1993). The closer aluminium fabric is placed around the entrance the more effectively it works (Mcintyre *et al.*, 1996).

### 8.2.4. OPERATION AND MAINTENANCE OF EQUIPMENT

Proper operation and maintenance of climate-control equipment is essential for healthy crops and avoidance of disease and insect problems in greenhouses. Mistakes in climate-control settings or failures of key pieces of equipment can lead to devastating losses in a matter of minutes. Even if such events do not cause immediate crop losses, physiological, disease and insect problems often show up some time later. The key to avoiding such problems is skilled operators and preventive maintenance programmes. Regardless of the level of equipment sophistication and maintenance, alarm systems together with backup power units and fuel supplies are essential to guard against losses during equipment break-downs or service interruptions.

Computer-control systems have taken much of the manual labour out of operating greenhouse climate-control equipment. A greenhouse manager should review climate data collected by the computer on a daily basis and make adjustments to setpoints to keep the climate conditions within desired ranges. It is critical that the temperature and humidity sensors used as the basis for control in each greenhouse compartment be cleaned and checked on at least a monthly basis. Greenhouse boiler systems need to be kept on line and in peak operating condition, not only during the winter heating months, but also in the summer months when it may be necessary to provide heat in the morning hours to avoid condensation on the crops. Vents and vent drives have to be kept in good working order to ensure they open when needed or close under high wind conditions when they could be damaged. Misting systems require stringent water treatment programmes to prevent nozzle blockages. The mechanisms for thermal and/or shade curtains have to be kept in alignment so that the curtains can be deployed quickly without snags or tears of the material. Insect

screens have to be repaired if damaged. Also, insect screens have to be cleaned periodically to prevent blockages of light and air flow.

### 8.2.5. WORKER EDUCATION

For an effective IPM programme, greenhouse workers have to be trained to recognize nutrient deficiencies and disease and insect problems, and to take appropriate action. Personal protective gear, disinfectants, disposal bins, markers, etc. have to be made available to workers so that they can play their part in an IPM programme. In large operations, it is necessary to have a large site map of the greenhouses and a good record-keeping system so that disease and pest outbreaks as well as control actions that have been taken can be noted for the information of all greenhouse staff. New decision-support software programs (Clarke *et al.*, 1994) (Chapter 12) offer great potential for education of workers and record-keeping of all greenhouse activities, including IPM.

## 8.3. Managing the Crop

### 8.3.1. SANITATION

After genetic resistance, prophylaxis is by far the most effective and cheapest way of escaping major disease epidemics and pest infestations. It reduces the need for multiple applications of pesticides (which stress the crop), the risks of pesticide resistance, and pesticide contamination of the produce, the operator and the environment. Physical screening against immigrant pests has already been discussed (Section 8.2.3), which, coupled with aggressive control of insects in the environs of the greenhouse and in adjacent weeds and field crops, is very effective prophylaxis against both direct damage and insect-transmitted diseases. Some growers rely on old crop prunings to perpetuate populations of biocontrol insects. This is not a good practice because they constitute a reservoir of pathogens and non-parasitized pests. New introductions of biocontrol insects are a better practice.

Reducing inoculum is also important in early crop management (Baker and Chandler, 1957; Jarvis, 1992), with such tactics as quarantine, seed disinfestation, the use of healthy mother plants for cuttings, micropropagation, removing and properly disposing of all previous crop debris, pasteurizing or solarizing soil and soilless media, and disinfesting the greenhouse structure, benches, trays, stakes and other materials.

Disinfestants include formaldehyde (as formalin) and hypochlorites but both materials are hazardous to humans and residues are phytotoxic. A persulphate oxidising agent (Virkon®; Antec International), however, destroys viruses and micro-organisms without such side effects (Anonymous, 1992; Avikainen *et al.*, 1993; Jarvis and Barrie, unpublished results).

### 8.3.2. CROP SCHEDULING

Seeding, pricking-out and sticking cuttings should all be done in a greenhouse separate from the main production areas, and on mesh or slatted benches allowing through-the-

bench ventilation (Section 8.4.6). The benches should be well above the level of soil-splash and there should be no overhead pots from which contaminated soil and drainage-water fall.

Where there is risk of diseases more destructive in cool soils, for example, *Fusarium* crown and root rot and corky root rot of tomatoes (Section 8.4.1), transplanting should be delayed until the root zone has warmed up, and insulating mulch materials put down later.

Where two or more monocrops are grown each year, overlapping of transplant production and marketable crop production means that pest and pathogen populations are perpetuated unless special care is taken to keep the young and cropping plants entirely separate. There is further risk if adjacent field crops constitute a reservoir of pathogens and pests.

### 8.3.3. SPACING

Close horizontal and vertical spacing of plants both on the bench and in the ground bed invites rapid plant-to-plant spread of walking insects, and of pathogens as diverse as *Pythium* spp., tomato mosaic virus, *Clavibacter michiganensis* (Smith) Davis *et al.* ssp. *michiganensis* (Smith) Davis *et al.* [= *Corynebacterium michiganense* (Smith) Jensen ssp. *michiganense* (Smith) Jensen], the downy mildews and *Botrytis cinerea* Pers.: Fr. (Burdon and Chilvers, 1982; Trolinger and Strider, 1984; Burdon *et al.*, 1989). The agents of virus spread are mainly water and soil splash, insects, and workers handling plants with contaminated tools and fingers (Thresh, 1982). Since air movement is restricted in dense plantings, the movement of airborne propagules is restricted, giving patchy distribution of diseases (Burdon *et al.*, 1989) and insects. Moreover, close spacing results in undue interplant competition for water, nutrients, light and **CO<sub>2</sub>**, and undue damage by workers.

### 8.3.4. THE GROWING MEDIUM

Growing media cover a wide spectrum of substrates: soil and soil-mix composts, organic materials such as sawdust and coconut fibre, inorganic materials such as rockwool and synthetic foams and aggregates, and the nutrient film technique (NFT). Soilborne diseases are no less prevalent in soilless substrates than in soil (Zinnen, 1988; Jarvis, 1992). All substrates must be substantially free of insects and pathogens at planting and must be kept so throughout the life of the crop, thus demanding a high standard of hygiene.

Soils are usually heavily amended with peat, farmyard manure, straw or crop residues. Ploughing or rotovating the soil should be done in order to comminute plant root debris and other organic matter, and so expose pathogen propagules to natural biological control. Getting the soil into good tilth with optimum temperature, water content and aeration promotes this microbial activity. Soils also harbour several insects, such as pupae of leaf miners and thrips, as well as fungus gnat and shore fly larvae, both of which vector *Pythium* and *Fusarium* spp. Their populations, as well as populations of predatory microarthropods, are determined by soil organic matter, soil type and pore size (Vreeken-Buis *et al.*, 1998). Populations of omnivorous collembola and non-cryptostigmatic mites, for example, are enhanced by the organic matter usually plentifully added to greenhouse soils. Fungal parasites of insects and nematodes are also encouraged in soils of good tilth. The root-knot nematode *Meloidogyne incognita* (Kofoid & White) Chitwood, however, survives at 1–2 m, well below soil disturbance levels (Johnson and McKeen, 1973).

Most substrates can be fumigated or heat-sterilized but pasteurization to about 70°C (Baker, 1957) or serialization to about 40–55°C (Katan, 1981) is preferred over total steam sterilization to 100°C because it preserves thermophilic biocontrol organisms. The whole greenhouse can be closed in sunny conditions for solarization of both substrate and superstructure (Shlevin *et al.*, 1995; Jarvis and Slingsby, unpublished). High temperature and vapour pressure deficit in closed greenhouses can kill the western flower thrips (*F. occidentals*) but unfortunately also its predator *Neoseiulus* (= *Amblyseius*) *cucumeris* (Oudemans) (Shipp and Gillespie, 1993; Shipp and van Houten, 1996).

As with the original ideas that soilless cultivation would eliminate soilborne pathogens, crops in rockwool or other inert substrate, or in NFT are no less free of soilborne arthropods. Fungus gnats, leafminers and thrips are numerous in rockwool and shore flies are always present in pools of water on plastic sheets. Even if soil is covered with plastic sheet, there are always gaps around stems, and tears and displacement of the cover readily permit insect access.

### 8.3.5. NUTRITION

Deficiencies and excesses of macro- and micronutrients, and imbalances in relative amounts of fertilizers can predispose plants to most diseases (Schoenweiss, 1975; Jarvis, 1977, 1992; Engelhard, 1989). In addition, fertilizers that increase foliage density at the expense of flowers and fruit not only reduce yield but tend to lower the vapour pressure deficit (VPD) in the boundary layer by restricting transpiration and wind-assisted evaporation, and consequently increase the risks of infection.

High nitrogen rates in fertilizers generally increase foliage density and softness, with increasing susceptibility to leaf and flower pathogens. For example, Hobbs and Waters (1964) found a quadratic increase in grey mould (*B. cinerea*) in chrysanthemum flowers (*Dendranthema grandiflora* Tzvelev) with nitrogen supplied with 1.5, 3.8 and **6.0 g/m<sup>2</sup>**. Nitrate nitrogen combined with liming gives excellent control of Fusarium wilt of several crops (Jones *et al.*, 1989). Because of its role in the integrity of cell walls, calcium imparts resistance if balanced with potassium in a high ratio. A low Ca:K ratio permits susceptibility to *B. cinerea* in tomato (Stall *et al.*, 1965). The K:N ratio is important in the susceptibility of tomato stems to the soft rot bacterium *Erwinia carotovora* (Jones) Bergey *et al.* ssp. *carotovora* (Jones) Bergey *et al.* (Dhanvantari and Papadopoulos, 1995). The incidence of soft rot was low at a K:N ratio of 4:1, increasing at 2:1 and 1:1. Verhoeff (1968) noted similar trends in tomato stems infected by *B. cinerea*. Paradoxically Verhoeff noted that high soil nitrogen can delay the development of latent lesions of *B. cinerea* in tomato, possibly because stem senescence is delayed.

Over-luxuriant foliage is conducive to greater damage by sap-sucking insects such as aphids (Scriber, 1984).

### 8.3.6. PRUNING AND TRAINING

Pruning and training tall staked and wire-supported crops like peppers, tomatoes and cucumbers not only modify the microclimate by altering spacing (Section 8.3.3) but pruning alters the fruit:foliage ratio and hence source-sink relationships in photosynthates (Section 8.3.7) and the disease-susceptibility of various tissues.

Removal of leaves bearing prepupal and pupal stages of pests can reduce their populations, but premature removal of leaves bearing parasitized stages can result in loss of biocontrol.

### 8.3.7. FRUIT LOAD

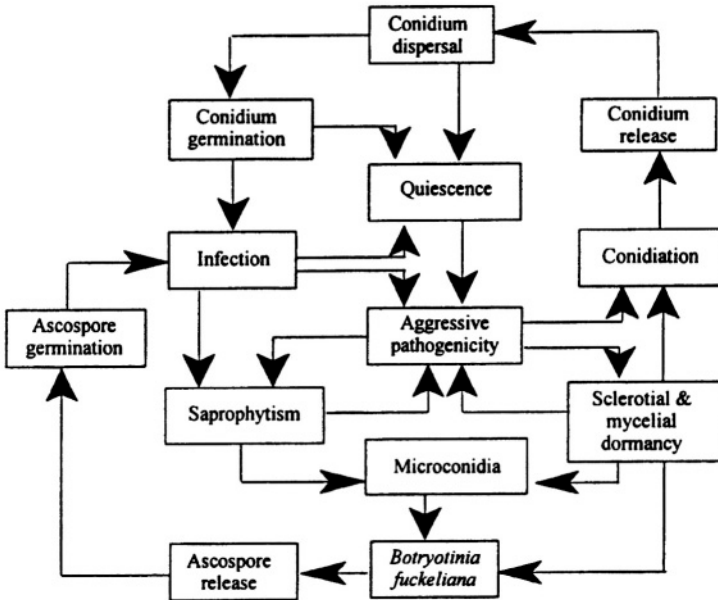
Closely related to the management of pruning is the distribution of photosynthates in heavily cropping plants (Gifford and Evans, 1981) in relation to the susceptibility of tissues to fungal and bacterial pathogens (Grainger, 1962, 1968). As Jarvis (1989) pointed out, modern technology has increased yields of greenhouse vegetables several-fold in the last two decades, with accompanying source-sink stresses on cultivars that have not changed very much. Thus, diseases such as *Fusarium* crown and root rot (*Fusarium oxysporum* Schlechtend.:Fr. f. sp. *radicis-lycopersici* W.R. Jarvis & Shoemaker) of tomatoes and *Penicillium* stem and fruit rot (*Penicillium oxalicum* Currie & Thom) of cucumbers have become serious in that same period. Both have been shown to be stress-related (Jarvis, 1988; Barrie, unpublished; Jarvis, unpublished) and there has been a resurgence in the incidence of corky root rot (*Pyrenochaeta lycopersici* R. Schneider & Gerlach) of tomatoes that might be related to a diminished flow of photosynthates to roots (Jarvis, unpublished observations). Grainger (1962, 1968) referred the “plunderable” carbohydrates available to certain pathogens – the so-called high-sugar pathogens (Horsfall and Dimond, 1957) – which include *B. cinerea*, whereas other pathogens, notably *Fusarium* spp., are classed as low-sugar pathogens principally attacking tissues starved of photosynthates. It is therefore incumbent on the grower to manage the nutrition, light and pruning of fruit and foliage so that a balanced partition of assimilates is attained without unduly compromising yield.

### 8.3.8. MANAGING PESTICIDES

Pesticides are a component of integrated pest management systems but are used too freely as insurance applications rather than judiciously as almost agents of last resort. Pesticides are significant agents of stress (Schoenweiss, 1975) whose over-use leads to problems of resistance (Regev, 1984; van Lenteren and Woets, 1988), to interference with microbial, insect biocontrol organisms (see Chapter 11) and bee pollinators, and so to an increase in iatrogenic diseases, diseases normally held in check by indigenous biological controls (Griffiths, 1981).

Unlike the pesticides on crops outdoors, pesticides in the greenhouse remain unweathered and persist longer, thus putting edible produce at risk of exceeding legally-tolerated residues, and exposing workers to higher concentrations for longer. There are no well-established economic threshold populations of insect pests and pathogens and the grower must thus rely largely on his own experience and on the experience of his advisors. It is at present difficult, if not impossible, to predict the course of disease epidemics in the greenhouse because the complex sequence of events in the life cycles of pathogens is dependent on a succession of different microclimates occurring in the correct order (Fig. 8.1). At best, therefore, fungicides can be used only in expensive and often unnecessary insurance programmes or within a very few hours of the requisite microclimate for spore germination occurring. On foliage this can usually simply mean leaf wetness (Section 8.4.2).

Pesticides are discussed at length in Chapter 11.



**Figure 8.1.** The life cycle of *B. cinerea*. Each stage is differently affected by microclimate factors, and control of grey mould is achieved by interrupting as many pathways as possible with environmental and cultural manipulations. Reprinted with permission from Jarvis (1992).

## 8.4. Managing the Crop Environment

### 8.4.1. TEMPERATURE

In very general terms, diseases as well as arthropods can be said to have optimum temperatures for their dispersal and development (Avidov and Harpaz, 1969; Jarvis, 1989, 1992; Chase, 1991) but these cardinal points are the integral of the optima of several growth phases of the pathogen as well as of different defence reactions of the host. Jarvis (1992) cited different temperature optima for different growth processes in the grey mould pathogen *B. cinerea*: mycelium growth, sporulation, conidium germination, germ tube growth, appressorium formation, sclerotium formation and sclerotium germination. All have different temperature optima, most of which lie above the general optimum range for grey mould development, 15–20°C. In most of its hundreds of hosts, resistance to *B. cinerea* is probably least within that range.

The temperatures of leaves and fruit can vary markedly from ambient air temperatures as determined by conventional greenhouse instruments, and so the temperature within the boundary layer can be assumed also to be different. At night, energy lost by radiation from leaves can result in temperatures 1–3°C cooler than ambient air and temperatures

frequently reach the dew point. In crops transpiring well, evaporative cooling can also reduce leaf temperature but insulated leaves not transpiring can become considerably warmer, by as much as 2–8°C, than ambient air (Curtis, 1936; Shull, 1936).

Similarly, Schroeder (1965) found that the temperature of red tomato fruits rose from about 20 to over 50°C in air that rose from 26 to 37°C in the same period. On the other hand, green fruits exposed to the same conditions remained 4–8°C cooler than the red ones.

Temperatures of leaves, flowers and fruit can be considerably decreased by shading from direct sun and by increasing evaporative cooling by adequate ventilation and forced air flow (Carpenter and Nautiyal, 1969; von Zabeltitz, 1976). Eden *et al.* (1996) discussed the possibilities of raising flower truss temperatures in tomato crops to avoid grey mould. Whereas higher temperatures resulted in increased numbers of flowers infected by *B. cinerea*, the fungus was less likely to grow proximally to the main stem where the damage would be far more severe than one infected flower. On the other hand, higher temperatures (20–25°C) resulted in fewer infections of stem wounds than at 15°C. Eden *et al.* (1996) interpreted these results in terms of changing balances between fungal aggression and host defence reactions.

Just as with diseases of shoots, temperatures can be to some extent selected to minimize diseases of roots; for example corky root rot (*P. lycopersisci*) of tomato can be largely avoided by transplanting into warm media at 20°C (Last and Ebben, 1966), as can Fusarium crown and root rot (*F. oxysporum* f. sp. *radicis-lycopersici*) (Jarvis, 1988). By contrast, the optimum temperature for the expression of Fusarium wilt [*Fusarium oxysporum* Schlechtend.:Fr. f. sp. *lycopersid* (Sacc.) W.C. Snyder & H.N. Hans.] is 27°C. Similarly, *Pythium aphanidermatum* (Edson) Fitzp. is most pathogenic to spinach in hydroponic culture at 27°C, whereas *Pythium dissotocum* Drechs. is most pathogenic at 17–22°C (Bates and Stanghellini, 1984). It is therefore important to know exactly which of closely related pathogens is present.

Insects and mites, like diseases, have also an optimum temperature for their activity, dispersal and development. Generally, greenhouse pests are thermophilic and perform best within 20–30°C night-day ambient temperatures. The preferred temperature for aphids and the greenhouse whitefly is somewhat lower, 15–25°C. The interaction between temperature and VPD on the survival of western flower thrips was determined by Shipp and Gillespie (1993).

Of course, temperature affects not only arthropod pests but also their natural enemies. Natural enemies may perform poorly if temperatures are too high or too low which may occur during summer and winter respectively in the Mediterranean area. Then, the more temperature-tolerant *Diglyphus isaea* (Walker) or *Dacnusa sibirica* Telenga can be used according to thermal regimes expected in greenhouses. Excessive heat, combined with high VPD is a serious constraint for *Phytoseiulus persimilis* Athias-Henriot in warmer Mediterranean areas. Shipp and van Houten (1996) determined optimum temperatures and VPD for the use of *N. cucumeris* in Canadian cucumber houses, and these types of studies serve as guides to more intelligent biological control.

#### 8.4.2. HUMIDITY

The effects of humidity on greenhouse crops have been reviewed by Grange and Hand

(1987), and their direct and indirect effects on diseases by Jarvis (1992). Uncertainty about VPD and temperatures in the boundary layer raises considerable suspicion about the validity of countless experiments on the infective abilities of fungal spores and disease prediction systems at low VPDs and inadequately measured or inadequately controlled temperatures (Schein, 1964). Fungal spores and bacteria require a wet substrate in which to initiate infection, and the water on leaves and fruits is provided by dew, guttation or overhead irrigation. This last can be discounted in well-managed greenhouses as an invitation to pathogens. Fogging systems cooling the air by evaporation are permissible if all the droplets evaporate before they land on plants (Section 8.2.2).

Measuring the onset and disappearance of dew is very difficult without the sensors themselves altering the boundary layer microclimate by heat conduction, shading, etc. (Wei *et al.*, 1995a). Wei *et al.* (1995a), however, developed a copper-coated polyamide film sensor that could be wrapped around a tomato fruit and which had a response time of only a few seconds from dry to wet, and a response of less than 2 minutes to Peltier cooling of the surface to dewpoint. Connected to microclimate modifiers (heating, ventilation), this device could obviate much of the risk of infection.

Predicting the onset of condensation and its evaporation is even more difficult using atmospheric variables such as relative humidity, temperature, airspeed and radiation. Most predictions have errors in excess of 0.8 h and as much as  $\pm 3$ h (Wei *et al.*, 1995b). Clearly this is unacceptable in a cucumber house where infection of flowers by *Didymella bryoniae* (Auersw.) Rehm can occur in 1–2 h (van Steekelenburg, 1985). Modelling the duration of dew in situations other than greenhouses has been done but with wide differences between predicted and observed durations of wetness (Wei *et al.*, 1995b). When the dewpoint temperature of the air falls below the temperature of the plants in a greenhouse, they become covered with water droplets and films, perhaps with hydrophilic fungal spores as nuclei, especially in still air at low VPD. Wei *et al.* (1995b) developed a model from heat transfer theory that accurately simulated condensation and evaporation from tomato fruits still attached to the plant:

$$E_L = \frac{\rho_a C_P [e_a - e_s(T)]}{r_d \gamma}$$

where  $E_L$  is the latent heat flux,  $\rho_a$  is the density of air,  $C_P$  is the specific heat of air,  $e$ , and  $e_s(T)$  are vapour pressures of air and saturated vapour pressure of air, respectively at  $T^\circ$ ,  $r_d$  is the boundary layer resistance to vapour transfer between the wet surface and the air, and  $\gamma$  is a psychrometric constant. Using the wetness sensor of Wei *et al.* (1995a), Wei *et al.* (1995b) obtained excellent agreement between simulated and measured fruit surface temperatures during condensation and evaporation, within 0.3–0.5°C (standard deviation 0.4°C). The model predicted wetness within 5 minutes of its detection, and dryness came as predicted. Clearly, this precision gives ample time for preventive action to be taken against most fungal infections.

While free water and low VPD are to be avoided if pathogens are present, those very conditions are needed to establish epidemics of fungal pathogens of insects, such as *Verticillium lecanii* (A. Zimmerm.) Viégas, *Beauveria bassiana* (Balsamo) Vuillemin and *Paecilomyces fumosoroseus* (Wize) Brown & Smith (Quinlan, 1988) (see Chapter 21).



Similar contrary indications have been obtained for arthropod pests and their predators. While spider mites are most active at relatively high temperatures and low VPDs, their predator *P. persimilis* is inhibited in those same conditions. Optimum humidity conditions for the predatory activity of *N. cucumeris* has been established by Shipp and van Houten (1996).

#### 8.4.3. WATER STRESS

Guttation results when the rate of water supply osmotically pumped by the roots exceeds the rate of water lost by transpiration and used in growth (Hughes and Brimblecombe, 1994). To prevent guttation, the osmotic potential of the root xylem must be more negative than that of the nutrient solution (Kaufmann and Eckard, 1971; Bradfield and Guttridge, 1984). In poorly-managed greenhouses, guttation frequently happens at night when VPDs are low and root temperatures maintain high metabolic activity and root pressure. Tissues become waterlogged (oedema) and water guttates from stomata and from hydathodes at leaf margins with profound effects on the phylloplane micro-organisms (Frossard, 1981). Water continuous with the surface and substomatal vesicles facilitates the entry of bacteria into leaves of for example *Pelargonium* spp. (Lelliott 1988), particularly when resumed transpiration leads to resorption of the water. Wilson (1963) described how reversal of transpiration flow permits conidia of *B. cinerea* to enter tomato stem xylem, there to remain a latent inoculum.

Water alternately accumulating and evaporating from hydrothodes leaves toxic deposits of salts (Curtis, 1943; Ivanoff, 1963), a ready entry point for necrotrophic pathogens (Yarwood, 1959a,b). Lesions of gummy stem blight (*D. bryoniae*) are frequently seen originating from such points on cucumber leaves.

Guttation damage can easily be eliminated by regulating atmospheric humidity, ventilating effectively, reducing evening watering and adjusting the osmoticum of nutrient solutions (Slatyer, 1961).

#### 8.4.4. LIGHT

Setting aside the effects of daylength on flowering in florists' crops, photosynthetically active radiation (PAR) (400–700 nm) is the part of the spectrum with the greatest effect on crop growth and productivity (Cockshull, 1985). Low and high light intensities are important agents of stress in crops (Schoenweiss, 1975) that induce physiologic strains predisposing the crops to disease. Particularly important is the effect of light combined with crop management procedures, such as plant spacing, row orientation, training and pruning systems, irrigation and nutrition, on the partition of assimilates, and the relative susceptibility of different tissues and organs to disease (Yarwood, 1959b; Grainger, 1968; Jarvis, 1989, 1992).

Daylength, however, is important in determining diapause in both arthropod pests and their predators. Early diapause may be a major constraint in their use. Non-diapausing strains can, to some extent, overcome this problem.

Light also has direct effects on fungal sporulation, germination and sclerotium formation. In *B. cinerea*, most isolates are stimulated to form conidia by light in the near-UV band (320–380 nm), an effect temporarily reversed by blue light (Epton and Richmond, 1980). Some isolates, however, form conidia in the dark (Hite, 1973;

Stewart and Long, 1987). All fungi grow mycelium in the dark, and *B. cinerea* forms its sclerotia in darkness, or in yellow or red light, or when irradiated for less than 30 min with near-UV light (Tan and Epton, 1973).

The requirement of *B. cinerea* and some other fungi for near-UV light for sporulation has led to the development of greenhouse covering materials that screen out that band as a means of disease control. Tuller and Peterson (1988) found fibreglass to transmit much less light of 315–400 nm than did polyethylene but in a comparative assessment of grey mould in Douglas fir seedlings [*Pseudotsuga menziesii* (Mirb.) Franco] it was concluded that the principal effect of low irradiance transmitted by fibreglass was in inducing needle senescence in dense canopies and thus susceptibility to grey mould, rather than on any direct effect on fungal sporulation. In both types of greenhouse, the mean intensity of light that inhibited sporulation (430–490 nm) exceeded that that promoted sporulation (300–420 nm). In those greenhouses, too, predisposing conditions of temperature (15–20°C) and humidity (>90% RH) persisted 14.5 times longer in fibreglass than in polyethylene-covered houses.

Humidity effects also seem to have outweighed effects of light wavelength in a series of trials with coloured cloches covering strawberries (Jordan and Hunter, 1972). Grey mould was most severe under pink and blue plastic covers, where VPDs were lower (0.41 and 0.64 kPa, respectively) than under clear plastic (1.14 kPa), or under glass (1.74 kPa). The effects of light are evidently not simple. Nevertheless, attempts have been made to filter out the near-UV light that induces sporulation in some fungi. Reuveni *et al.* (1989) incorporated hydroxybenzophenone into polyethylene, which increased the ratio of inhibitory blue light (480 nm) to UV (310 nm), and reduced the sporulation of *B. cinerea* in polystyrene petri dishes. Under the treated plastic, grey mould lesions were fewer in tomato and cucumber (17 and 15, respectively) than under untreated plastic (41 and 29, respectively) (Reuveni *et al.*, 1988). Similarly, plastic coverings absorbing light at 340 nm inhibited the sporulation and reduced the incidence of grey mould lesions on cucumber and tomato (Honda *et al.*, 1977) as well as white mould lesions caused by *Scerotinia sclerotiorum* (Lib.) de Bary (Honda and Yunoki, 1977). Many isolates of *Alternaria solani* Sorauer also depend on near-UV light for sporulation, and Vakalounakis (1991) used vinyl films filtering out light of <385 nm to reduce the incidence of early blight in tomato greenhouses to less than 50% of that under unamended vinyl film.

Except as an agent of stress on the host, light has little direct effect on the rhizosphere microflora.

#### 8.4.5. CARBON DIOXIDE AND OXYGEN

Carbon dioxide enrichment is a standard procedure in many commercial greenhouses (Porter and Grodzinski, 1985) but because it necessarily involves some restriction in ventilation to achieve the concentrations of **CO<sub>2</sub>** required, of the order of 1000 vpm, there is often increased danger of unmanageable low VPD (Watkinson, 1975; Ferare and Goldsberry, 1984). The concentrations of **CO<sub>2</sub>** that impair the growth of *B. cinerea* are 2–3 orders of magnitude greater than those found even in **CO<sub>2</sub>-enriched** greenhouses (Brown, 1922; Svircev *et al.*, 1984) and so reports, for example, of Winspear *et al.* (1970), of increased incidences of grey mould in **CO<sub>2</sub>-enriched** greenhouses, can be interpreted in terms of enhanced levels of assimilates (Grainger, 1962, 1968), or a denser canopy, with its increased risks of disease-susceptible wet plants (Grange and Hand, 1987).

While  $\text{CO}_2$  is a prominent component of the rhizosphere atmosphere as a product of root and microbial respiration, it has little direct effect on pathogens.

Oxygen deficiency stress readily occurs in compacted and waterlogged soils and in over-warm hydroponic solutions in which both increasing temperature and increasing solute concentration decrease oxygen solubility. Further, increased temperatures lead to higher root and microbial respiration rates which further deplete oxygen tensions (Stolzy *et al.*, 1975). Low oxygen tension has been advanced as an explanation for physiological root death (Daughtrey and Schippers, 1980; van der Vlugt, 1989) as well as decreased host resistance to root pathogens.

#### 8.4.6. AIR MOVEMENT

The primary purposes of directing and regulating air movement in the greenhouse are: (i) to reduce the steepness of gradients in temperature, vapour pressure deficits and  $\text{CO}_2$ ; (ii) to assist in the evaporation of infection droplets; and (iii) to induce thigmomorphogenesis in bench-grown crops. This last results in sturdier plants (Biro and Jaffe, 1984) and resistance to *Fusarium* wilts (Shawish and Baker, 1982).

Through-the-bench air movement and plant spacing on the bench are important factors in escape of forest seedlings (Peterson *et al.*, 1988) and *Exacum affine* I.B. Balf. ex Regel (Trolinger and Strider, 1984) from grey mould.

Counter to the generally beneficial effects of air movement are its effects on pathogen spore dispersal. Most fungi sporulate best in still air at VPD of 1.2–0.6 kPa but fungi of the Peronosporales, like *Bremia lactucae* Regel and *Pseudoperonospora cubensis* (Berk. & M.A. Curtis) Rostovzev sporulate on wet surfaces (Rotem *et al.*, 1978; Crute and Dixon, 1981). Airborne conidia are often liberated from conidiophores by hygroscopic mechanisms (Ingold, 1971) and are dispersed by air currents. Both mechanisms rely on disturbance of the microenvironment such as is readily provided by worker activity (Peterson *et al.*, 1988; Hausbeck and Pennypacker, 1991).

The same mechanisms that control the liberation and dispersal of pathogen spores also apply to spores of biocontrol fungi when control is by enhancement of indigenous populations (Jarvis, 1992).

Air movement also effects the passive transport of spider mites on webs floating through the air and being trapped on neighbouring plants (Avidov and Harpaz, 1969). Forced air flows can transport larger insects into the greenhouse, even through some screens (Section 8.2.3). Aggregation of insects is controlled by airborne semiochemicals, while the dispersal of pheromones on excessive air currents can interfere with mating disruption as a means of biological control, or attraction into sticky traps.

#### 8.4.7. INTEGRATION OF ENVIRONMENTAL FACTORS

Epidemics of diseases are the result of a complex sequence of biological events each with a different set of permissive environments that have to occur in sequence, and coupled with hosts in a receptive state. Jarvis (1977, 1992) outlined the complexity of those events in the case of grey mould epidemics (Fig. 8.1). Beginning with sporulation, conidia are formed at temperatures around 15°C and in moderate VPD; they are liberated by hygroscopic movements of the conidiophore in rapidly changing conditions of humidity,

and are dispersed on air currents or by water-splash; infection occurs on wet surfaces at 15–20°C; and colonization of the host is fastest at 25–30°C. Marois *et al.* (1988) found that epidemics of grey mould on rose depend as well on inoculum concentration, a relationship that was different in winter and summer, and affected by temperature, relative humidity and VPD, the latter the far more meaningful parameter for describing epidemiology of *B. cinerea* in roses.

It has been possible to construct working models of grey mould epidemics in cucumber (Tunis *et al.*, 1990, 1994; Elad *et al.*, 1992; Elad and Shtienberg, 1995; Shtienberg and Elad, 1997); tomatoes (Eden *et al.*, 1996; Shtienberg and Elad, 1997); gerbera and rose (Salinas *et al.*, 1989; Keressies, 1992); and conifer seedlings (Zhang and Sutton, 1994a,b). The value of epidemic models such as BOTMAN (Shtienberg and Elad, 1997), an integrated chemical and biological control program, in predicting the onset and course of epidemics, however, is severely compromised by the rapidity with which infection occurs – 9–10 h for grey mould (Yunis *et al.*, 1994) and only 1 h for gummy stem blight in cucumber flowers (van Steekelenburg, 1985; Arny and Rowe, 1991) – and by the wide variability of the greenhouse climate typically served by only one psychrometer in several hundred cubic metres of space (Jarvis, 1992). Shtienberg and Elad (1997) found that over three years, a rain forecasting system did not enable BOTMAN to perform significantly better than a weekly fungicide insurance program in unheated tomato and cucumber crops. However, a 4-day weather forecast proved more useful than immediate past records of weekly averages of surface wetness (calculated from dewpoint) of 7 h/d and 9.5 h/d at night temperatures between 9 and 21°C. By the time the requisite data have been collected and analysed, infection has already begun, and is an irreversible action even with the use of fungicides, which act mostly on germinating spores and thus too late to stop infection. Surface wetness is the key factor in all infections, and so its prediction from rates of change in surface and ambient air temperatures combined, by data processor, with simultaneous rates of change in VPD would be more timely in the immediate application of environmental control measures (Section 8.4.2).

Powdery mildew epidemics have a somewhat less complicated sequence of events prior to infection than grey mould epidemics but they, too, are ultimately dependent on the deposition of dew (Cobb *et al.*, 1978; Quinn and Powell, 1982; Powell, 1990; Jewett and Cerkauskas, unpublished results).

Control of any fungus-incited disease is achieved by breaking any of the pathways in life cycles similar to those of Fig. 8.1 (Jarvis, 1992) but the denial of water to germinable spores is the most important.

Computer models can be used to optimize greenhouse climate for both crop production and pest and disease control. For example, in The Netherlands a climate management program was developed for optimal production of tomatoes and is linked to a model for biological control of greenhouse whitefly by *Encarsia formosa* Gahan (van Roermund *et al.*, 1997). Further, the model can be extended with a humidity management module which prevents the development of fungal diseases.

Integration of pest and disease control primarily by manipulating the environment is a highly complex problem (Shipp *et al.*, 1991). Clarke *et al.* (1994), in describing a computer-managed system, considered the holistic production system as a six-hierarchy complex of factors in which any change at one level affected the other five levels. Thus, any change in greenhouse climate, whether engineered or not, effects changes in pesticide

efficacy, biological control agents, pests and disease vectors, diseases, and ultimately productivity and profit.

There are a number of electronic decision support systems for various facets of greenhouse pest and disease control and production strategy (Papadopoulos *et al.*, 1997). Jones *et al.* (1986, 1988) described an expert system with grower selection of climate set points based on his experience; Jacobson (1987) further developed an expert system with pre-set points for tomato production; and Dayan *et al.* (1983) developed TOMGRO that modelled physiological processes in tomato. Only Martin-Clouaire *et al.* (1993) considered disease escape in their model for tomato. Van Roermund *et al.* (1997), however, described the apposition of a whitefly control model to a production model, to which can be added a disease-avoidance model. Clarke *et al.* (1994) and Jewett *et al.* (1996) described a holistic Harrow Greenhouse Crop Management System (HGCMS) for both greenhouse tomato and cucumber. In addition to providing blueprints for production in which the grower has his own input, HGCMS provides user-friendly diagnoses for diseases, pests, biological controls and physiological disorders. It accepts climate monitoring. In addition, HGCMS allows the grower to enter economic data, and will analyse it for him. Conflict resolution, as far as can be agreed among experts, is a feature of HGCMS but ultimately the grower can accept or reject the advice of HGCMS.

The use and analysis of computer models and controls depends, of course, on a reasonable degree of computer literacy among growers, together with a basic understanding of plant growth and pest and disease biology. Otherwise reliance on expert advisory services is obligatory.

#### 8.4.8. ENVIRONMENTS FOR MICROBIAL CONTROLS

In general, the microclimates for the successful deployment of fungal antagonists and parasites are close to those that promote plant infection by pathogens. Ideally, then, pre-emptive colonization of the phylloplane, as it is for rhizosphere, is the preferred strategy (Andrews, 1992). Adaptation to that microenvironment is a prerequisite (Dickinson, 1986). This colonization can also be achieved by enhancing indigenous populations of phylloplane antagonists (Jarvis and Atkey, unpublished results, in Jarvis, 1992). Similarly, the use of green manures and composts can achieve control in the rhizosphere without the necessity of isolating, registering and redeploying specific antagonists (Jarvis and Thorpe, 1981; Hoitink and Fahy, 1986; Ebben, 1987). McPherson and Harriman (1994) have suggested that in recirculating hydroponic systems, antagonist populations build up naturally in a disease-suppressive system that is reminiscent of take-all decline in wheat.

#### 8.4.9. CONCLUSIONS

The primary objective of the commercial greenhouse grower is to obtain maximum yield per unit area of space with the least financial input. However, in order to achieve this, certain minimum standards in environment management have to be maintained in such matters as crop spacing, pruning and training, irrigation, fertilization, **CO<sub>2</sub>** supplies, and temperature and humidity regimes. While much is known about disease epidemiology and insect behaviour, scant attention, however, has been paid to the manipulation of greenhouse environments expressly to avoid disease epidemics and insect infestations,

which together can easily account for 30% crop losses (Pimentel, 1991). This is a significant factor in a grower's balance sheet which is often overlooked, and usually dealt with simplistically by indiscriminate pesticide applications (Regev, 1984).

Careful analyses of epidemiological and epizootic data can indicate environments to be avoided or encouraged in greenhouse operations but integrating the desired environments into those wanted by the grower solely to maximize yields by physiological means is extremely difficult. The solution of these problems requires the consensus of several specialized experts, experienced crop advisors and, not least, good growers, whose experience and intuition are not to be ignored. The construction of predictive models can provide valuable insight into how environments affect diseases and insects, but experts can differ widely on which environment is best to escape, for example, lettuce downy mildew, or grey mould, or whiteflies or thrips. Resolution of these apparent conflicts can now be attained, or at least reasonable compromises achieved, by the inference engine in a computer expert system (see Chapter 12). One developed by Clarke *et al.* (1994) and Jewett *et al.* (1996) is a decision support system for greenhouse tomatoes and cucumbers that collates expert opinions on all aspects of crop production, including disease and pest management, the grower's own input, and internal and external environmental parameters. It can also provide the financial consequences of various actions, as well as of no action. Ultimately, the grower, whose brain no-one can replace, has the final decision.

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## HOST-PLANT RESISTANCE TO PATHOGENS AND ARTHROPOD PESTS

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### 9.1. Introduction

The aim of searching for host-plant resistance or tolerance is to develop cultivars that show little or no reduction in their normal yields when they are exposed to pests and diseases. Growers profit from better yields from resistant crops that need much less use of expensive pesticides and consumers benefit from vegetables with smaller amounts of chemical residues.

The capacity of plants to adapt to abiotic and biotic factors was known even to growers in ancient times. When they selected those plants that gave the highest yields and lowest levels of pests and diseases they were unknowingly exploiting genetic resistance. Scientific plant selection started around 1900, and in the following thirty years new varieties with more and more genes of resistance were released. However, subsequent experience revealed that genetic resistance has limits and that sometimes it only serves to combat low pest populations or to delay pest infection; sometimes, resistant cultivars stimulate the selection of pest populations able to live and reproduce on previously resistant cultivars. Consequently, host-plant resistance is best exploited in combination with other techniques like crop rotation, control of weeds within the crops and surrounding areas, biological control of animal pests, etc. Host-plant resistance is then one but important link in the chain of Integrated Pest Management.

### 9.2. Terminology

A host plant is a species in which or on which another organism lives. An organism that obtains some advantage from a host plant without benefiting the plant is usually termed a parasite. However, because parasite is used in other chapters of this book for the arthropod species used in biological control, we shall employ the term pest from FAO terminology to denote those weeds, animal species and microorganisms that damage crops. The term pathogen applies to specific microorganisms like bacteria, fungi, mycoplasmas and viruses, that parasitize plants. Plant disorders caused by pathogens are diseases. An animal pest is any animal that usually damages crops (nematodes, insects, mites, etc.). Aggressive strains of a pest are those strains that cause severe symptoms of disease in the plant genotypes attacked. A physiological race of a fungus, bacteria or virus with genes that enable it to attack a specific host-plant genotype is a virulent race; conversely, an avirulent race cannot attack this specific host-plant genotype.

Painter (1951) defines host-plant resistance as the relative amounts of heritable

characteristics of a plant that influence the degree of damage produced by a pest. Host-plant resistance is then: (i) heritable and controlled by one or more genes; (ii) measurable because its magnitude can be determined; (iii) relative because measurements are comparative with those of a susceptible plant of the same species that is damaged severely by the pest; and (iv) variable because it may be modified by biotic or abiotic factors. If the particularly sensitive phases of plant development do not coincide with the optimum conditions for pest development one speaks about escape.

Against the enormous numbers of pests and plant species in the world, host-plant resistance is common and host-plant susceptibility is exceptional. The combinations of the many types of barriers to infection (resistance characteristics) in a plant species and their collective effectiveness give rise to a series of genotypes that range from highly susceptible to highly resistant. When a pest cannot establish a compatible relationship under any condition with a certain plant genotype, then the genotype is said to be immune or absolute resistant to the pest. Resistance shown by non-host plants is termed non-host resistance, basic resistance, or basic incompatibility. Non-host resistant plants can exhibit resistance to their specific pests. If a plant expresses some resistance to all isolates or races of a pest it has non-race-specific resistance. If it expresses resistance to only one isolate or pest race it has race-specific resistance.

A tolerant plant may be colonized by a pest to the same extent as susceptible plants, but there is no reduction in yield quantity and quality. The converse of tolerance is sensitivity. Tomato yellow leaf curl virus (TYLCV), for example, produces very mild or no symptoms in both *Lycopersicon chilense* Dun. LA-1969 and *Lycopersicon pimpinellifolium* (Jusl.) Mill. LA-1478, but the concentration of virus antigen in the resistant cultivar LA-1969 is less than 1% of that in the susceptible 'Moneymaker' cultivar, while the concentration in the tolerant cultivar LA-1478 is similar to that in 'Moneymaker' (Fargette *et al.*, 1996). Rapid recovery of the plant after animal-pest attack is also considered as tolerance.

### 9.3. Resistance Mechanisms

Defence mechanisms present in the plant before pest attack are constitutive mechanisms and those induced by the infection process are induced mechanisms. Plants do induce responses instead of only constitutive and permanently present resistance because of: (i) chemicals produced by the plant as a result of interactions with pests may be toxic not only for the pest but also for the plant itself leading to a lower plant fitness when no pests attack the plant; and (ii) to produce defence chemicals may be costly, so that plants should allocate resources to defence only when and where interaction with pest occurs.

Constitutive and induced mechanisms may be either morphological or chemical. Examples of morphological constitutive defence mechanisms are the waxes of the cuticle that form a hydrophobic surface preventing water retention and pathogen deposition and germination. Thicker cuticles impede or make difficult penetration of insects, mites and pathogens, particularly when the latter penetrate by appressorium pressure. Thick and tough epidermal cell walls make difficult or impossible direct

insect and fungal penetration; lignification or suberization give additional effective protection. The size and distribution of stomata and lenticels are associated with resistance to those insects, bacteria and fungi that make their entries through these structures. Internal barriers to movement through plant tissues like leaf-vein bundle sheaths and sclerenchyma cells may limit the spread of some pathogens and may prevent penetration of the phloem by aphids and whiteflies.

Chemical constitutive defence compounds interfere with the growth and reproduction of pests. The germination of some conidia is inhibited by compounds excreted by the plant. There are also internal secretions of inhibitors like phenolic acids in coloured onions and tomatine in tomato (Isaac, 1992). Plant tissues may contain antifungal agents produced by normal plant metabolism and, because the concentration of these compounds do not increase in response to infection, they are termed phytoanticipins to distinguish them from the phytoalexins, other chemical defence compounds produced only as a response to infection and that rapidly reach effective antimicrobial levels around the site of infection (van Etten *et al.*, 1994). Different plant families produce their characteristically different types of phytoalexins. For example, Fabaceae produce isoflavonoids, and Solanaceae, sesquiterpenes. Furthermore, pest damage can also induce an indirect defence, i.e. a defence that improves the effectiveness of natural enemies of the pest. Plants respond to damage by herbivorous mites or insects with the production of volatile chemicals that attract enemies of the herbivore, such as predators or parasitoids. This plant response occurs both locally and systemically (Dicke, 1994).

The morphological and chemical induced defence mechanisms of plants to pests are sometimes associated with the hypersensitive response, a process that leads to the rapid necrosis of infected cells. The pathogen can survive for some time in the necrosed cells around the site of original infection (Milne, 1966), but the rest of the plant remains healthy. The hypersensitive response is induced by specific elicitors of the pest that interact with specific receptors of the plant (elicitor-receptor model) and, in a number of plant species, it is commonly activated by viruses, bacteria, fungi, insects or mites. The elicitor-receptor model is confirmed in the pathosystem tomato *Cf-9-Fulvia fulva* (Cooke) Cif. (= *Cladosporium fulvum* Cooke) race 9 (De Wit, 1992). However, in the pathosystem tomato-*Pseudomonas syringae* van Hall pv. *tomato* (Okabe) Young *et al.*, the hypersensitive response is initiated when the serine-threonine kinase encoded by the resistance gene of the plant interacts physically with the avirulence gene of *Pseudomonas* (Tang *et al.*, 1996).

When a virus triggers a hypersensitivity response in a resistant plant, the tissues that surround the necrotic patches develop some localized acquired resistance to further attack by the same or other viruses (Ross, 1961a). The acquired resistance can be shown also by leaves not directly infected by the inductor virus (leaves without hypersensitive necrotic patches) and Ross (1961b) called this phenomenon systemic acquired resistance. Systemic acquired resistance is not common and even, if present, it does not always protect against a second systemic virus (Roggero and Pennazio, 1988). Pathogen-related proteins and salicylic acid appear to be involved in the mechanism of systemic acquired resistance.

Changes in plants after damage by pests or stresses can either decrease or increase

plant resistance. The increase in resistance is called induced resistance that is usually systemic and increases with the degree of injury to the plant and reflects complex cytological, histological and physiological changes in the plant. For example, animal pest feeding activities produce short-term responses that affect animal pest feeding behaviour (Karban and Myers, 1989), but also long-term responses that can vary from premature leaf abscission to altered morphology, like increased hair density. Induced resistance elicited by pathogens is also termed cross protection and usually occurs when a plant has been inoculated by a mild strain of the infecting pathogen sometime before the attack of an aggressive strain. Concurrent protection is a special case of virus cross protection in which the protector virus does not replicate to detectable levels (the plant seems to be immune to that virus), however, the protector virus can induce protection against the second virus (Ponz and Bruening, 1986).

In plants, the two major resistance mechanisms against herbivorous insects are antixenosis (interference with insect behaviour) and antibiosis (interference with insect physiology). The usual patterns of insect approach, landing, probing, feeding and egg-laying on a susceptible plant can be disturbed by resistance and induce non-preference or non-acceptance. These disturbances modify the behaviour of the insect and so protect a plant in the initial phase of an attack. Many examples of plant substances with repellent, deterrent or antifeedant properties are known. Several groups of toxic, secondary plant compounds like alkaloids, flavonoids and terpenoids may adversely affect the growth, development, generation-time and fertility of the insects. Some plant morphological characteristics that can interfere with or modify the behaviour of the insect are colour, shape, type of cuticle wax and the hairiness of plant stalks and leaves.

#### **9.4. Genetics of Host-Plant Resistance**

The fact that in nature host plants and their pests coexist, even though the pests may sometimes severely damage the plants indicates that they have evolved together and have established a dynamic equilibrium of resistance-virulence. Should either pest virulence or host-plant resistance increase without opposition, then the particular plant or the pest will be eliminated. Consequently, genetic studies of host-plant resistance should include studies of pest virulence genetics.

##### **9.4.1. INHERITANCE OF RESISTANCE**

In a segregating plant population, variations of expressed resistance to a particular pest may be either continuous or discontinuous depending on the number of resistance genes involved. Continuous variation from susceptible to resistant plants indicates that the resistance is polygenic which means that it is the sum of the small, individual expressions of many genes. Discontinuous variation indicates that the resistance is monogenic or oligogenic (controlled by one or a few genes) that may be either dominant or recessive major genes: individual plants fall into relatively well-defined classes of resistance or susceptibility. Genes of resistance are frequently clustered in

linkage groups or complex loci and sometimes comprise genes involved in the recognition of more than one taxonomically unrelated pest (Crute, 1994). The first reported genetic study of resistance was published in 1905. Since then, the enormous amount of work in this field shows that resistance in many cases is inherited in a simple way. Dominance is very common, especially in hypersensitive responses, and recessive resistance occurs much less frequently. Inter-allelic gene interaction (epistasis) is only reported in a few cases (Niks *et al.*, 1993).

9.4.2. THE GENE-FOR-GENE CONCEPT

In gene-for-gene relationships, the host-plant resistance expression to a particular pest depends on the pest genotype, and the observed virulence of the pest depends on the host genotype. Flor (1956) demonstrates that for each gene that governs resistance in the plant there is a specific gene that governs virulence in the pest. This relationship became known as the gene-for-gene concept and was first shown for a number of fungal diseases and later for viruses, bacteria, nematodes, insects and parasitic plants (e.g. *Orobancha*). Today, it is generally accepted that the interaction takes place between the, usually dominant, alleles of the resistance and the, usually dominant, alleles of the avirulence. The gene-for-gene concept might then be reworded as: any resistance gene can act only if a locus in the pest carries a matching gene for avirulence (Niks *et al.*, 1993).

Table 9.1 displays the 16 possible combinations when two genes of resistance in a homozygous diploid plant are matched by two genes of avirulence in the haploid pest. Susceptible plants without no genes of resistance, ( $r_1r_1r_2r_2$ ) are attacked by all races of the pest, even those without genes of virulence ( $A_1A_2$ ). Pests that carry two genes of virulence ( $a_1a_2$ ) attack all plants independently of their combinations of genes of resistance. The two pest/plant combinations  $A_1R_1$  or  $A_2R_2$  trigger the often hypersensitive resistance response (plant and pest are incompatible). The combination  $a_1A_2R_1r_2r_2$  is compatible because the avirulence gene  $A_2$  is not matched by the corresponding  $R_2$  host allele. The four possible combinations given by,  $A_1a_2$  and  $a_1A_2$ , with  $R_1r_2$  and  $r_1R_2$ , illustrate the differential interaction that reveals the occurrence of a gene-for-gene relationship. The differential interaction is used to classify pathotypes and to differentiate genes of resistance.

TABLE 9.1. Interaction of two plant genes of resistance in a plant with two pest genes of virulence according to the gene-for-gene concept

|   |          | Resistance (R) or susceptibility (r) alleles in the plant |          |          |          |
|---|----------|---|----------|----------|----------|
|   |          | $R_1R_1$  | $R_1R_2$ | $r_1r_1$ | $r_1r_2$ |
| Avirulence (A) or virulence (a) genes in the pest | $A_1A_2$ | -   | -        | -        | +        |
|   | $A_1a_2$ | -   | -        | +        | +        |
|   | $a_1A_2$ | -   | +        | -        | +        |
|   | $a_1a_2$ | +   | +        | +        | +        |

-: Incompatibility, +: Compatibility (infection)



The gene-for-gene interaction produces absolute resistance, or absolute susceptibility, of the host plant against a race of the pest. This race-specific response is termed vertical resistance and is very effective, but only against certain genotypes of a particular pest species. If the resistance is effective against all genotypes of the pest species without differential interaction, the resistance would be race-non-specific or horizontal resistance. The gene-for-gene concept presumably also applies to horizontal (usually polygenic) resistance, although this lacks proof until now.

### 9.5. Durability of Resistance

Johnson and Law (1975) proposed the term durable to describe long-lasting resistance. Durability does not imply that resistance is effective against all variants of a pest, but that the resistance has merely given effective control for many years in environmental conditions favourable to the pest (Russell, 1978).

Where susceptible cultivars are grown, the pest population comprises a set of races in dynamic equilibrium, but one or two of the races will tend to predominate. If a resistant cultivar is introduced, the predominant races either will not propagate, or their propagation rate will be substantially less than normal. In both cases, if one or some races can propagate effectively in the resistant cultivar, their proportions in the pest population will increase because they no longer have competition from the other races. A new outbreak of the pest will occur because the resistance will have been effectively "broken". It is difficult to determine whether a pest population is composed of a mixture of races, some present in very small proportions, or whether the pest produces virulent mutants that disappear from the pest population unless there is a compatible resistant host plant in which they can propagate.

In theory, when the introduced resistance is complete, the predominant races will disappear and more virulent races will spread. The spread will be faster than when the introduced resistance is only partial because the virulent and dominant races will compete. Before the introduction of the first resistant tomato cultivars, the predominant if not the only tobacco mosaic virus (TMV) race was race 0. When *Tm-1* resistant cultivars were introduced, the pathogen population changed and very soon TMV race 1 progressively predominated. *Tm-2* cultivars resistant to TMV races 0 and 1 were not much better because TMV race 2 quickly spread. *Tm-1* proved to be resistant to race 2 and cultivars with *Tm-1* and *Tm-2* were released. Again, the resistance of these new cultivars was quickly broken down because TMV race 1-2 predominated. These case histories of *Tm-1*, *Tm-2* and *Tm-1-Tm-2* cultivars support the "lack of durability hypothesis" of complete resistance. However, the subsequent release of cultivars with the ***Tm-2*<sup>2</sup>** allele resistant to TMV races 0, 1, 2 and 1-2 effectively controlled TMV for 20 years. Why the *Tm-1* and *Tm-2* resistances were so ephemeral, and that of ***Tm-2*<sup>2</sup>** has lasted more than 20 years, we do not know. Other examples of durable resistances governed by major genes are resistance to *Stemphylium* in tomato and to *Cladosporium* in cucumber. Examples of low durability resistances are those to *F. fulva* in tomato and to *Bremia* in lettuce.

Resistance to insects tends often to be partial and polygenic. It appears then unlikely that more virulent populations (biotypes) adapted to partial resistant cultivars might be

selected. However, transgenic cultivars that carry the *Bt* gene from *Bacillus thuringiensis* Berliner rely on a monogenic factor that has a very high expression and, as McGaughey (1988) reports, several species of insects like *Helicoverpa* (= *Heliothis*) *zea* (Boddie), quickly adapt to tolerate the *Bt*-gene toxin. The use of partially resistant cultivars reduces the selection pressures on insect populations and this effectively delays the development of virulent biotypes.

The type of reproduction of a pest greatly influences the durability of host-plant resistance. Aphids, for example, exploit their capacity to reproduce parthenogenetically to colonize resistant cultivars and large populations quickly develop from a few individuals able to overcome host-plant resistance. Soilborne pests, on the other hand, spread more slowly than airborne pests and thus virulent biotypes or races of pathogens take long times to colonize the area in which a resistant cultivar is grown.

Some kinds of host-plant resistance are more durable than others. For example, those which involve changes in plant morphology (growth of hairs or trichomes that interfere with insect movements or feeding activities, or water repellent, waxy surfaces and thickened epidermis of leaves that prevent fungal spores from sticking to the leaf or resist the penetration of some fungi, etc.) require complex changes in the pest to successfully adapt to and overcome the defensive strategy of modified plant structure, and complex changes take very long time.

## 9.6. Breeding to Improve Host-Plant Resistance

Resistant plant varieties are produced by breeding programs that involve: (i) search for sources of resistance; (ii) evaluation of the resistance found; and (iii) selection in segregating generations. To growers, the pest resistance of a new variety is only one characteristic out of many, and it is not the most important. Therefore, plant breeders have to bear in mind that the agronomic characteristics of a new resistant variety must be as good as, or better than, previous non-resistant varieties when the pest to which it is resistant is not present. If not, no matter how good its resistance to a particular pest is, the variety is most unlikely to be grown on a large scale.

### 9.6.1. SOURCES OF RESISTANCE

If the resistance to a particular pest is already present in commercial cultivars (either hybrids or open pollinated cultivars) the source of resistance for our breeding programme would be the resistant commercial cultivar most similar to our ideotype. Commercial cultivars have genes for high yield and quality, for resistances to some pests, for adaptation to specific environments like greenhouses, etc., that must be exploited. Should resistance to the target pest not be present in a commercial cultivar, the first step the plant breeder must take is to search the literature for plants described as sources of resistance, obtain seeds of those source plants, and then evaluate the level of their resistance to help decide whether the source plants might serve as the starting point of the breeding programme. If the desired resistance is not yet described, it can be searched for in accessions from germplasm banks. The usual search sequence is: landraces, wild forms, related species and related genera.

Should it be impossible to find a source of high-level resistance in germplasm collections, the breeding material might still be manipulated by mutation, tissue culture and molecular genetic techniques to produce new variability. Artificially induced mutations have produced a small number of commercial cultivars and, except in those resistances that involve recessive characters in vegetatively propagated ornamental crops, the method is not to be recommended. When cell or tissue cultures are grown for extended periods, genetic variation, termed somaclonal variation, usually takes place. Examples of useful variation from tissue culture are resistance to *Bipolaris oryzae* (Breda de Haan) Shoemaker (= *Helminthosporium oryzae* Breda de Haan) and resistance to the herbicide glyphosate. However, in spite of these examples, there are serious doubts about using somaclonal variation as a source of variability, mainly because of the unstability of the variation. To increase the variability of a species by genetic manipulation is limited principally because it is difficult to identify and clone genes. As the number of cloned genes increases, more variability will be generated by plant transformation. The expression of viral DNA sequences in transgenic plants may produce virus-resistant plants that introduce new variability into the gene pool of the plant species.

#### 9.6.2. EVALUATION OF RESISTANCE

Plant populations must be exposed to the pest in such a way that resistant and susceptible plants can be differentiated as quickly and clearly as possible. Field screening has the advantage that the cost per plant tested is low and, more importantly, that the test conditions simulate those under which commercial crops grow. However, field screening has disadvantages, it is dependent on the weather, whether or not the pest will develop is always uncertain, and other pests may interfere with the tests. Screening under controlled conditions like glasshouses or climatized rooms gives standardized environmental conditions, and the amount of pest present and its distribution can be controlled, but the conditions of growth are not representative of those under which commercial crops grow.

The expression of resistance in a host-parasite system is not constant but it depends largely on the composition and amount of the inoculum, on the stage of development of the plant and on the conditions under which the resistance is evaluated. Small amounts of inoculum produce little or no symptoms in susceptible plants and so resistance may be overestimated. For example, in the pepper-*Phytophthora capsici* Leonian system, concentrations of  $10^2$  zoospores/cm<sup>3</sup> of some isolates produce no mortality on 'Morrón' cultivar, but concentrations of  $10^4$  produce 100% mortality (Gil Ortega *et al.*, 1995). Breeders prefer to test plants as early as possible because seedlings need less space and time to develop and, in general, are less resistant than mature plants. The expression of resistance is greatly influenced by environmental variables (like light, temperature, soil fertility) and the distribution pattern of plant genotypes in the field. To measure resistance properly, the values of those environmental variables should all be within the range of values of the conditions under which commercial crops grow. The expression of resistance shows no constant relationship with light parameters. Host-plant resistance to *Manduca sexta* (Johannsen) in the wild tomato *Lycopersicon hirsutum* Humb. & Bonpl. f.

*glabratum* Mull. increases when plants grow under long-day-light conditions (Kennedy *et al.*, 1981), but low-intensity light like that of cloudy days tends to reduce the expression of resistance to insects (Smith, 1989). Temperatures outside the range of conditions under which commercial crops are grown reduce the expression of resistance in a number of host-pest systems (Smith, 1989). However, Gómez-Guillamón and Torés (1992) report that three lines of melon, when grown at normal temperatures for commercial crops, show resistance to *Sphaerotheca fusca* (Fr.) Blumer. [= *Sphaerotheca fuliginea* (Schechtend.:Fr.) Pollacci] at 26°C but are susceptible below 21°C. High doses of nitrogenous fertilizers generally increase the susceptibility while additional applications of potassium and phosphorus fertilizers increase resistance. When resistance of different genotypes is assessed in small plots, resistant genotypes will export small levels of inoculum, but will receive high levels of inoculum from susceptible genotypes that, in turn, export more inoculum than they receive and, consequently, the resistance of resistant genotypes will tend to be underestimated in comparison with that of the same genotypes measured in trials carried out in large plots or in separate plots. This phenomenon is termed interplot interference and can be mitigated by including control cultivars with different levels of resistance as references. In any case, small differences found in the level of infection in small plots should be most carefully noted (Parlevliet and van Ommeren, 1984). Pests that generally display a vertical dispersion show smaller interplot interference than pests that display a horizontal dispersion.

The principal application of *in vitro* resistance screening is to select those cells, calli, or somatic embryos that show resistance to the toxin of a pathogen. Advantages of this technique are: (i) large numbers of individuals can be processed; (ii) haploid cells reveal concealed recessive traits; (iii) it can exploit somaclonal variation; and (iv) the uniformity of the experimental environmental conditions helps discriminate slight quantitative differences of plant resistances. Disadvantages are: (i) it is limited to tests for pathogens that produce toxins; (ii) cells that survive infection may be physiologically adapted and not genetic variants; (iii) resistance at cellular level is not necessarily expressed in the whole plant; and (iv) *in-vitro* resistance screening does not detect defence mechanisms that are based on differentiated tissues.

### 9.6.3. SELECTION METHODS

After a source of resistance has been identified and an appropriate evaluation procedure has been set up, the next step is to integrate the resistance into the set of agronomic characters that a cultivar needs for success on the market. The donor of resistance should be selected taken into account that the closer the genotype of the donor to that of the cultivar to be improved, the shorter will be the process of introduction of resistance. Complete resistance is frequently easier to manage than partial resistances. Complete resistance is essential for pests damaging the end-product of the crop because the greenhouse-grown produce must be of prime quality without cosmetic damage like spots or scars that reduce consumer acceptability.

Most cultivars of the greenhouse-grown species are hybrids. To produce a resistant hybrid the resistance has to be introduced into one of the parents (dominant resistance), or in both parents (recessive resistance). The appropriate selection procedure for monogenic resistances is backcross and for polygenic resistances is recurrent selection.

Marker-assisted selection recovers genes linked to markers. The markers are more easily scored than the genes of resistance. To ensure that only a minor fraction of the individuals selected are recombinants, the linkage between the marker and the target gene in coupling phase should be <5 cM. A repulsion-phase marker linked at <10 cM provides higher efficiency than that of a 1 cM coupling-phase linkage (Kelly, 1995). Marker-assisted selection do not need inoculation of pests, so that it avoids the errors caused by failed infection, incomplete penetrance of the resistance and variability of aggressiveness. In addition, breeding for resistance can be carried out where inoculations of healthy plants in the field are not allowed for safety reasons. The susceptibility to Fenthion insecticide shown by tomato seedlings on detached leaves that carry the *Pto* gene of resistance to *P. syringae* pv. *tomato* is used as an indirect indicator to select for resistance to this bacteria (Laterrot, 1985). The isozyme marker *Aps-I* has been used commercially for many years as a substitute for screening with nematodes to select for the *Mi* resistance gene in tomato. *Mi* genotypes can now be selected by a PCR-based marker that is more tightly linked to *Mi* than *Aps-I* (Williamson *et al.*, 1994).

Screening tests for resistance to multiple pests are sometimes of doubtful validity because infection by one pest may interfere with the infections by other pests. Marker-assisted techniques avoid infection and can help to introduce several genes each resistant to a different pest. Marker-assisted selection also offers considerable potential to transfer polygenic (quantitative) resistance because markers have high heritability ( $h=1$  for molecular markers) and direct selection of resistance genes is masked by environmental effects. In tomato, molecular markers have been discovered for oligogenic (Danesh *et al.*, 1994) and for polygenic resistances (Neinhuis *et al.*, 1987).

A solution to control pathogens that infect roots is to use resistant rootstocks. They are used for several greenhouse crops such as tomatoes, eggplants, melons, watermelons, cucumbers, carnations and roses. However, for roses, rootstock grafting is done to improve disease resistance and to change the vigour and longevity of the crop.

### 9.7. Strategies to Improve Durability

The vast majority of the resistant cultivars rely on the use of single, major genes and these have proved remarkably successful, even though severe breakdown of resistance occurs from time to time. Several strategies are proposed to reduce the risk of resistance breakdown when major genes of resistance are used.

Multilines or cultivar mixtures are formed either by phenotypically similar lines, or cultivars that each contain a different single, race-specific gene of resistance. No examples of multilines or cultivar mixtures occur among the species usually grown in greenhouses.

Gene deployment uses several cultivars each with a different gene of resistance and grown within a clearly defined area. If the pest produces a virulent race on the cultivar grown, another cultivar that carries another gene of resistance will be grown in the area from next year until a new virulent race breaks its resistance. The next cultivar grown will either be the first cultivar or a new one with resistance to the last virulent race.

Gene deployment, as multilines, exploits the diversity of the host-plant population to stabilize the pest population and avoid the appearance of virulent races. To effectively use gene deployment all the growers of the area must use cultivars with the same resistance gene.

Pyramiding resistance genes involves the introduction into the same cultivar, of all, or as many as possible, of the genes of resistance for a pest. The rationale behind pyramiding is that the pest will need several mutations from avirulence to virulence to overcome the resistance and that the probability of two or more successive mutations is extremely low because it is the product of the probability of each mutation. The gene *Pto* protects tomato against *P. syringae* pv. *tomato* race 0 and some resistant cultivars *Pto/+* have been released. Stockinger and Walling (1994) found the novel genes of resistance *Pto-3* and *Pto-4* that can withstand races 0 and 1. According to Buonaurio *et al.* (1996), pyramiding *Pto*, *Pto-3*, and *Pto-4*, in one cultivar may provide the optimum solution for this disease control.

Integrated pest management aims to keep the pest population continuously at a low level. Because the probability that new races of the pest will emerge is proportional to the population level of the pest, integrated pest management will reduce the possibility that a new virulent race will develop, and, consequently, the durability of race-specific resistance may increase.

## 9.8. Advantages and Disadvantages of Host-Plant Resistance

Some of the many advantages of pest control by resistant cultivars over control by pesticides are: (i) the technique is easy to apply because the grower only has to buy resistant cultivars; (ii) it is relatively inexpensive, seed of resistant cultivars is no more expensive than seed of non-resistant cultivars; (iii) completely resistant cultivars need no chemicals for pest control and even partially resistant cultivars need much less to control pests; (iv) resistant cultivars can be incorporated into integrated pest management programmes and when combined with biological control give a cumulative effect; (v) adverse environmental effects are minimal or nil, pesticide pollution is much reduced; and (vi) resistant cultivars, except transgenic cultivars, are acceptable to the public. Some of the disadvantages of resistant cultivars are: (i) it takes a long time to develop a resistant cultivar; (ii) resistant cultivars control only one pest, while pesticides are often effective against several pests; (iii) resistance must be introduced in each new cultivar; and (iv) the pest may adapt to the resistance and this limits the durability of resistant cultivars.

## 9.9. Present Situation of Host-Plant Resistance in Commercial Cultivars Adapted for Greenhouse Cultivation

Control of pests by resistant cultivars has been a generally successful approach and new resistant cultivars appear regularly on the seed market. Greenhouse crops are particularly suitable candidates for the introduction of resistance because the high income of greenhouse crops permits the cost.

Tomato is the most important vegetable world-wide and is the focus of attention of many seed companies. Commercial tomato cultivars can be crossed with wild species that offer the main source for genes of resistance. Resistance for almost any tomato pest is now known, but only some of them have been introduced into tomato cultivars (Table 9.2). Commercially available cultivars contain multiple resistances to several diseases, but almost all their resistances are monogenic and complete.

**TABLE 9.2. Host-plant resistance in commercial greenhouse cultivars and their level of utilization (\*low, \*\*medium, \*\*\*high)**

|  |     |   |     |
|--|-----|---|-----|
| <u>Tomato</u>  |     | <u>Cucumber</u>                                     |     |
| <i>Fusarium oxysporum</i>                                      | *** | <i>Cladosporium cucumerinum</i>                     | *** |
| <i>f. sp. lycopersici</i>                                      |     | <i>Corynespora cassiicola</i>                       | *** |
| <i>Fusarium oxysporum f. sp. radialis-lycopersici</i>          | **  | <i>Erysiphe cichoracearum</i>                       | *   |
| <i>Verticillium albo-atrum</i> and <i>Verticillium dahliae</i> | *** | <i>Sphaerotheca fusca</i>                           | *   |
| <i>Pyrenochaeta lycopersici</i>                                | *   | <i>Pseudoperonospora cubensis</i>                   | *   |
| <i>Fulvia fulva</i>  | *** | Cucumber mosaic virus (CMV)                         | *   |
| <i>Phytophthora infestans</i>                                  | *   | <u>Melon</u>  |     |
| <i>Stemphylium</i> spp.  | *** | <i>Fusarium oxysporum f. sp. melonis</i>            | *** |
| <i>Leveillula taurica</i>                                      | *   | <i>Erysiphe cichoracearum</i>                       | **  |
| <i>Pseudomonas syringae</i> pv. <i>tomato</i>                  | **  | <i>Sphaerotheca fusca</i>                           | **  |
| <i>Ralstonia solanacearum</i>                                  | *   | <i>Pseudoperonospora cubensis</i>                   | *   |
| Tobacco mosaic virus (TMV)                                     | *** | Zucchini yellow mosaic virus (ZYMV)                 | *   |
| Tomato yellow leaf curl virus (TYLCV)                          | *   | Papaya ring spot virus (PRSV)                       | *   |
| Tomato spotted wilt virus (TSWV)                               | *   | Melon necrotic spot virus (MNSV)                    | *   |
| <i>Meloidogyne</i> spp.  | *** | <i>Aphis gossypii</i>                               | **  |
| <u>Pepper</u>  |     | <u>Bean</u>   |     |
| <i>Phytophthora capsici</i>                                    | *   | <i>Colletotrichum lindemuthianum</i>                | *** |
| <i>Xanthomonas vesicatoria</i>                                 | *   | <i>Pseudomonas syringae</i> pv. <i>phaseolicola</i> | *   |
| Tobacco mosaic virus (TMV)                                     | *** | Bean common mosaic virus (BCMV)                     | *** |
| Cucumber mosaic virus (CMV)                                    | *   | <u>Lettuce</u>                                      |     |
| Potato virus Y (PVY)   | **  | <i>Bremia lactucae</i>                              | **  |
|  |     | Lettuce mosaic virus (LMV)                          | **  |

In sweet pepper, resistant sources are widely available in wild relatives. Currently, the resistance in cultivars is principally for viruses (Table 9.2). Most insect pests are under good biological control and so breeding for resistance is not pursued.

Cucumber, in contrast with tomato and sweet pepper, has a narrow genetic base. No wild relatives are available to provide genes of resistance. Nevertheless, some important successes have been achieved against cucumber mosaic virus (CMV), *Corynespora cassiicola* (Berk. & M.A. Curtis) C.T. Wei and *Cladosporium cucumerinum* Ellis & Arth. (Table 9.2). Downy-mildew [*Pseudoperonospora cubensis* (Berk. & M.A. Curtis) Rostovzev] is a serious problem in cucumber. Although genes of resistance are present, commercial cultivars only have partial resistance. A combination of partial resistance, biological control and other acceptable control measures of this disease seems to offer the best solution.

Melon has a number of botanical varieties that have provided the resistances introduced in commercial cultivars (Table 9.2). Powdery-mildew is the main fungal disease in greenhouse cultivation and almost completely resistant cultivars for the races 1 and 2 are available in the market. Resistance to papaya ring spot virus (PRSV) has been bred in melons for tropical and subtropical conditions where the virus assumes more importance. Resistance to zucchini yellow mosaic virus (ZYMV) is race specific and not effective against a second pathotype of the virus. Partial resistance to *Aphis gossypii* Glover prevents colony formation and may reduce the incidence of aphid-borne viruses.

Lettuce shows wide genetic variation, and wild species are available to carry out crosses with commercial material. Biological control is more difficult in leaf vegetables than in fruit vegetables because very short cropping cycles and, therefore, resistance breeding is more needed here. Complete monogenic resistance is present against *Bremia lactucae* Regel, based on a gene-for-gene system, but resistance is not durable (Table 9.2).

In floriculture, resistance breeding is a recent development. There are less incentives to breed resistant cultivars due to zero-tolerance, high cosmetic demands, fashion products with a short commercial life-span (a few years), many species and cultivars mostly grown on a small acreage and fewer restrictions on use of pesticides in floriculture than for food crops. In chrysanthemum, complete monogenic resistance against *Puccinia horiana* Henn. is known and commercially exploited; in addition, partial resistances against leafminers and thrips have been found. *Fusarium oxysporum* Schlechtend.:Fr. f. sp. *dianthi* (Prill. & Delacr.) W.C. Snyder & H.N. Hans. severely affects carnations mainly during the hot season and two races are known. Host-plant resistance to race 1 due to a single gene is now introduced into most commercial cultivars. Host-plant resistance to race 2 is polygenic and it is expressed when all the resistance loci are heterozygous or homozygous for the dominant alleles that confer the resistance; susceptibility would occur when there are one or more homozygous recessive alleles (Arús *et al.*, 1992). However, in spite of the complexity of the genetic basis of this resistance, resistant cultivars with good field resistance have been released.

### 9.10. Perspectives

The durability of a resistance increases when as many as possible genes of resistance are introduced into a cultivar. However, most of the resistances introduced in commercial cultivars to date are only monogenic, mainly because to pyramid several resistance genes for one pest in the same cultivar is difficult and costly. Appropriate molecular markers would make this task easier. Future improvement of screening techniques and indirect selection will make it easier to breed host plants with polygenic resistances.

Partial resistance is controlled by many genes with small individual effects and, although it is potentially more durable than monogenic complete resistance, it is rarely used because it is difficult: (i) to distinguish and to select the individual effect of each gene in segregating generations; (ii) to evaluate commercially the advantage of the



partial resistance; (iii) to convince the growers about the benefits of resistant cultivars that show some disease symptoms. Partial resistance, in combination with biological control, can lead to sufficient control.

Public concern about the effects of pesticides have resulted in governments to make laws to reduce the use of pesticides. The best way to avoid or reduce the use of pesticides in greenhouse crops is to introduce integrated pest management techniques that include the use of resistant cultivars. The disadvantages of resistant cultivars are much less than their advantages (as explained in Section 9.8), consequently the prospects for the future development of many more resistant cultivars appear excellent.

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## DISINFESTATION OF SOIL AND GROWTH MEDIA

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### 10.1. Introduction

Soilborne plant pathogens constitute a major problem of plant protection in greenhouses. This is basically due to the pathogens' ability to survive for several years in the soil (or in used container media) as dormant resting structures (sclerotia or microsclerotia, chlamydospores and resting mycelia) until a susceptible crop is introduced again into the same plot. These structures are able to withstand adverse environmental conditions and chemical applications, thus creating major control problems in the world agriculture. The same holds true for other soilborne pests such as arthropods, nematodes, parasitic plants and weeds, although different mechanisms of persistence are involved. To date, fumigation (or steaming) is the most effective approach to control soilborne pests. Soil solarization (SSOL), applied to soil or growth media alone, or in combination with reduced doses of soil fumigants or other amendments, can also control most soilborne plant pathogens effectively.

This chapter reviews the management of soilborne pathogens in glass or plastic greenhouses through a wide range of chemical and physical treatments as well as SSOL, taking into consideration the forthcoming ban (scheduled now to 2005 for most of the world) on the use of methyl bromide (MBr), and the current lack of alternatives for some of its current uses. Specific chemicals such as herbicides and other pesticides are beyond the scope of this review, although some combinations of those chemicals [e.g. Ethyl dipropil thiolcarbamate (EPTC)] with SSOL have been found to be highly effective.

### 10.2. Steaming

Steaming, aerated steam (Dawson and Johnson, 1965), overheated and hot water treatments are used in greenhouses, especially when container (growth) media are used. Steam has been applied for soil disinfestation for almost a century. Plant pathogens (as well as other pests) are eliminated by steaming due to heating to lethal levels or to physical damages incurred to their resting structures, even in cases of heavy soil contamination. Moreover, steaming usually shows a growth stimulation effect on the following crop.

The "classic" steaming by Hoddesdon pipes, dug into the soil, is no longer used. This holds true also for heating the soil to 80–100°C. As this treatment results, in many cases, in a biological vacuum in the treated soil, heating the soil or growth substrate to 70°C – mainly by aerated steam – is now favoured; this treatment leaves part of the saprophytic population uncontrolled (Bollen, 1985).

Careful soil preparation is essential for good steam penetration. The soil should be tilled as deep as possible, preferably by a shovel-plough, and then left for complete drying before steaming. It is important to reduce amount of plant debris, especially when steaming growth medium. Good preparation permits good steam penetration and enables pest control in heavy soils, but might still result in only partial control in very light sandy soils. Steaming of aerated growth substrates, such as tuff stones, vermiculite, etc., is usually good, but peat soils pose difficulties due to their high water content.

Soil steaming is done either by “passive” or “active” techniques. In passive steaming, steam is blown to the surface, under a covering sheet, and left to heat the upper layer. Lower layers are then heated by heat transmission. This process continues until 100°C is reached at a depth of 10 cm (Runia, 1983). Disinfestation of deep layers, especially in sandy soil, might be only partial.

Active steaming can be done by either positive or negative pressure. Both techniques employ drainage systems, based on pipes laid at a 50–70 cm depth, and approximately 80 cm apart. With the “positive pressure” technique the steam is blown through holes located along the pipes. The “negative pressure” involves an improved technique, utilizing the advantages of the two above-mentioned application methods. The steam is released over the treated area under plastic sheeting, as for passive steaming, assuring rapid and even distribution throughout the plot surface, followed by active suction to the deeper layers of the soil, achieved by negative pressure applied through the drainage system. This technique, widely used in The Netherlands, is much cheaper than the two others, due to energy saving caused by the faster heat transfer (Runia, 1983). Despite this, steaming treatments are expensive, and are feasible mainly in places where there are heating systems (used mainly for heating the greenhouse during the cold season) or if applied by contractors (Anonymous, 1994). Steaming, however, can be useful and economical for disinfestation of shallow layers of growth media placed on tables, as is usually done in nurseries.

### 10.3. Soil Fumigation

Soil fumigation is done by applying toxic pesticides to the soil by various means, and these fumigants move down and across the soil profile and reach the target organisms directly, or by a very efficient secondary distribution due to their relatively high vapour pressure. MBr is by far the most effective fumigant (Klein, 1996). However, current concerns regarding the possible role of MBr in ozone depletion and its forthcoming phase out have triggered research efforts to develop optional methods for soil disinfestation. Other soil fumigants used for greenhouses include methyl isothiocyanate (MIT), **CS<sub>2</sub>-releasing** compounds, formaldehyde, dichloropropene, etc. (Anonymous, 1994; Ristaino and Thomas, 1997).

#### 10.3.1. FUMIGATION WITH MBr

MBr is the most powerful soil fumigant with a very broad spectrum of activity. Many

soilborne fungi (e.g. *Rhizoctonia* spp., *Pythium* spp., *Phytophthora* spp., *Sclerotinia sclerotiorum* (Lib.) de Bary, *Sclerotinia minor* Jagger, *Sclerotium rolfsii* Sacc., *Verticillium* spp. and many *Fusarium* spp.) are sensitive to MBr. In contrast, some soilborne bacteria, such as *Clavibacter michiganensis* (Smith) Davis *et al.* ssp. *michiganensis* (Smith) Davis *et al.* [= *Corynebacterium michiganensis* (Smith) Jensen ssp. *michiganensis* (Smith) Jensen], are not satisfactorily controlled at regular (commercial) rates of application (Antoniou *et al.*, 1995a). The effectiveness of MBr fumigation also depends on proper soil preparation, irrigation reaching approximately 60% of “field capacity” and a tight covering of the fumigated soil with plastic (mostly polyethylene) sheeting. MBr is applied to the soil at a rate of 50 to **110 g/m<sup>2</sup>**, either by injection as a cold liquid just before covering, or by distribution as a cold or hot gas under the mulch released through a manifold of perforated pipes or from 0.3–1 disposable containers which are opened under the mulch. The duration of the application depends on soil temperature (1–2 days at 15°C, 3 days at 10–15°C at the 0–20 cm-deep soil layer, but more than 4 days at 8–10°C at the same depth) (Klein, 1996). Possible problems due to the toxicological hazards of MBr are related mainly to the health danger for applicators and to the increase in inorganic bromine residues in edible plant products. MBr was found in a few cases in water near greenhouses in The Netherlands, where PVC water pipes were improperly placed only 10 cm deep in the ground.

In 1992, MBr was listed by the Montreal Protocol as an ozone depleting material, and a procedure for banning its use was initiated (Gamliel *et al.*, 1997b). According to this decision, MBr will not be available in developed countries after 2005, and its consumption will be gradually reduced during the period remaining until the ban goes into effect (Anonymous, 1997).

There are some MBr uses without any known substitute yet (Anonymous, 1994). Continuous efforts are now underway, to reduce MBr dosages and minimize its emission and negative side-effects on the environment. Most solutions are based on using improved, virtually impermeable mulching films. Common low- and high-density polyethylene films are poor barriers, and allow the escape of MBr at very high rates, especially where the film temperature is higher than 40°C (as is the case in most greenhouses when the film is exposed to solar irradiation). The permeability of MBr through impermeable film (normally co-extruded with a barrier layer protected by polyethylene coating from both sides), is only **0.001–0.0001 g/m<sup>2</sup>/h**, depending on the barrier formula, compared with emission of **5 g/m<sup>2</sup>/h** for regular low density polyethylene. Control of a pest is a factor of pesticide concentration (C) and exposure time (T). Thus, extending MBr retention in soil under impermeable films for a longer period allows the use of reduced MBr dosages with the same CT values, without reducing control efficacy. Fungal pathogens such as *Fusarium oxysporum* Schlechtend.:Fr. f. sp. *dianthi* (Prill. & Delacr.) W.C. Snyder & H.N. Hans., *Fusarium oxysporum* Schlechtend.:Fr. f. sp. *radicis-lycopersici* W.R. Jarvis & Shoemaker, *Fusarium oxysporum* Schlechtend.:Fr. f. sp. *cucumerinum* J.H. Owen, etc., were controlled by reduced dosage of MBr at 25–50% of the recommended dose under impermeable films (Antoniou *et al.*, 1997; Gamliel *et al.*, 1997b,c). Further reduction is possible by deeper burying of the film edges into the soil and by continuous mulching,

or by combination with SSOL (Grinstein *et al.*, 1995; Antoniou *et al.*, 1996; Gamliel *et al.*, 1997b).

### 10.3.2. FUMIGANTS WITH MIT

*Dazomet (3,5, dimethyl-tetrahydro-1,3,5,(2H) thiodiazino-thione)*

Dazomet is a product formulated either as a powder (85% a.i.) or as granules (98% a.i.). The chemical is gradually hydrolyzed to at least four subproducts, MIT being the main one. Dazomet is effective against *Verticillium dahliae* Kleb., *Verticillium albo-atrum* Reinke & Berthier, *Rhizoctonia solani* Kühn, *S. sclerotiorum*, *Phytophthora* spp. and *Pythium* spp. at a rate of 400–600 kg a.i./ha. The fumigant can be used for the control of several diseases in seed beds, greenhouses, or in field grown vegetables, cotton, tobacco and ornamentals. It is applied to the soil by spreading or irrigating followed by mechanical mixing (such as rotovator cultivation or shovel plough) into the soil. The chemical, which is not applicable at temperatures lower than 8°C, is also partially effective against insects, various nematodes and weed seeds. One of the disadvantages of dazomet is the long period (three weeks) needed after application of the chemical before planting or sowing is permissible (Anonymous, 1994; Middleton and Lawrence, 1995).

*Metham sodium (sodium methylthiocarbamate) (MES)*

MES is effective against several soilborne pathogens in both in covered and open outdoor cultivation. In water solutions MES rapidly changes to methyl isothiocyanate (MIT). The broad spectrum of controlled pathogens includes Pythiaceus fungi, races of *Fusarium oxysporum* Schlechtend.rFr., *S. sclerotiorum*, *S. rolfsii*, *V. dahliae* and species of *Phialophora*, *Phoma*, *Botrytis*, etc. Since resting structures are present mainly in the upper 40 cm of the soil profile, and since MES is 100% water soluble, it is most effective when applied via the sprinkler irrigation system. The chemical is used at various doses according to the target pathogen and/or the soil type to be disinfected. The recommended dosages for sandy, heavy, and very heavy soils are 490–650, 800 and 1000 l/ha, respectively. Soil temperature is also a critical factor in the effective application of the chemical: fluctuating between 10 and 30°C at a soil depth of 10 cm is best. Use of MES for chemigation is an effective procedure against soilborne pathogens. However, side effects may arise under certain conditions, such as when the irrigation water is contaminated with urban sewage. *Fusarium oxysporum* Schlechtend.:Fr. f. sp. *cepae* (H.N. Hans.) W.C. Snyder & H.N. Hans, on onion has been controlled by MES application, but fumigation resulted in the eradication of endomycorrhizal fungi, reduced onion growth and increased the population of another bacterial pathogen of onions, *Pseudomonas gladioli* Severini pv. *allicola* Young *et al.*, which replaced Fusaria and caused very serious damage (Kritzman and Ben-Yephet, 1990).

### 10.3.3. SOIL FUMIGATION AND PROBLEMS OF ALTERNATIVES TO MBr

Fumigants other than MBr, having a much narrower range, are registered and used in

various cropping systems. These include nematicides (dichloropropene), fungicides ( $\text{SO}_2$  releasing pesticides) and other. These are used on relatively small scale and will not be dealt in this paragraph (Anonymous, 1994). It is clear that with the currently available fumigants, there is no satisfactory replacement to MBr. The use of other fumigants involves identification of the casual agent, and in many cases the use of a mixture of two or more chemicals, to control a wider range of disease agents, pest and weeds in the treated plot (Anonymous, 1994). Di-Trapex (methyl isothiocyanate 20 + dichloropropane-dichloropropene 80), may serve as an example to this tendency, as this pesticide was formulated to control both pest controlled by MES and the root-rot nematode. Furthermore, data regarding residual effect of the above mentioned fumigants before planting is needed while their environmental impact is not yet fully clear.

#### 10.4. Soil solarization (SSOL)

SSOL represents one of the very few cases where a new non-chemical control procedure has been adopted by greenhouse growers in several parts of the world, within a relatively short period of time (Katan *et al.*, 1976, 1987). SSOL is based on trapping solar irradiation by tightly covering the wet soil, usually with transparent polyethylene or other plastic sheets (Grinstein and Hetzroni, 1991). This results in a significant elevation (10–15°C above normal, depending on the soil depth) of soil temperatures up to the point where most pathogens are vulnerable to heat when applied for 4–6 weeks and controlled either directly by the heat, or by chemical and biological processes generated in the heated soil (DeVay and Katan, 1991).

##### 10.4.1. EFFECT OF SSOL ON FUNGAL DISEASES

Ecological observations and quantitative measurements carried out after the application of the technique have differentiated the pathogens into two main categories. It should be pointed out that a pathogen could be effectively controlled by solarization in one region but less effectively in another depending on environmental, and cultural parameters. A partial list of soilborne pathogens and pests which are controlled by solarization as reported for greenhouses and open fields is listed in Table 10.1. It is important to mention that application of SSOL in a close greenhouse, or by employing two layers mulch further improves its effects (Kodama and Fukui, 1982; Garibaldi and Tamietti, 1984; Garibaldi and Gullino, 1991).

##### 10.4.2. BACTERIAL DISEASES CONTROLLED BY SSOL

Relatively, only few reports about SSOL and bacterial diseases were published. Application of SSOL (1–2 months soil mulching with transparent polyethylene films) in tomato plastic houses drastically reduced symptoms caused by *C. michiganensis* ssp. *michiganensis* (Antonioni *et al.*, 1995b) while MBr (**68 g/m<sup>2</sup>**) was ineffective in controlling the disease. Populations of Gram-positive bacteria were reduced by 64–99%

by SSOL (Stapleton and Garza-Lopez, 1988). Bacterial populations of cultures of *C. michiganensis* ssp. *michiganensis* infiltrated into tomato stem segments were embedded at various soil depths prior to the application of SSOL. A sharp decrease or elimination of the pathogen in solarized compared to MBr-treated plots was observed. *Streptomyces* spp., causing deep pitted scab of potatoes and pod-wart disease of peanut, were successfully controlled (Grinstein *et al.*, 1995). Negative effects, due to control of beneficial Rhizobia were also reported (Abdel-Rahim, 1987).

**TABLE 10.1. Fungal soilborne pathogens controlled by SSOL (a partial list)**

| Pest   | Crop   | Selected references   |
|--|--|---|
| <i>Fusarium</i> spp.                                       | Basil, cucumber, melon, strawberry, tomato, watermelon | Kodama <i>et al.</i> , 1980; Kodama and Fukui, 1982; Martyn and Hartz, 1986; Tjamos and Makrynakis, 1990; Grinstein and Ausher, 1991; Oliveira, 1992; Antoniou <i>et al.</i> , 1997; Garibaldi <i>et al.</i> , 1997 |
| <i>Phoma lycopersici</i> (= <i>Diplodina lycopersici</i> ) | Tomato   | Cartia, 1989  |
| <i>Phytophthora</i> spp.                                   | Avocado, tomato  | Cartia, 1989; Grinstein and Ausher, 1991  |
| <i>Pyrenochaeta</i> spp.                                   | Onion, tomato  | Malathrakis <i>et al.</i> , 1983; Garibaldi and Tamietti, 1984; Tjamos, 1984; Cartia, 1989; Grinstein and Ausher, 1991  |
| <i>Pythium</i> spp.  | Various  | Hilderbrand, 1985; Stapleton and Garza-Lopez, 1988  |
| <i>Rhizoctonia solani</i>                                  | Various  | Tamietti and Garibaldi, 1989; Grinstein and Ausher, 1991  |
| <i>Sclerotinia</i> spp.                                    | Lettuce  | Porter and Merriman, 1985; Materrazzi <i>et al.</i> , 1987; Vannacci <i>et al.</i> , 1988; Phillips, 1990   |
| <i>Sclerotium cepivorum</i>                                | Onion  | Abdel-Rahim <i>et al.</i> , 1983; Porter and Merriman, 1985   |
| <i>Verticillium</i> spp.                                   | Eggplant, tomato, strawberry                           | Katan <i>et al.</i> , 1976; Cartia, 1989; Tjamos <i>et al.</i> , 1989; Grinstein and Ausher, 1991   |

#### 10.4.3 PARTIAL CONTROL OF FUNGAL DISEASES BY SSOL

The heat tolerant *Monosporascus* sp. and *Macrophomina phaseolina* (Tassi) Goidanich, root-knot nematode *Meloidogyne* spp. and some weeds, e.g. *Cyperus rotundus* L. and the annual weed *Melilotus sulcatus* Desf. are only partially controlled by SSOL. *Fusarium oxysporum* f. sp. *dianthi* is also considered as one of the wilt pathogens not easily controlled by SSOL (Rubin and Benjamin, 1983; Gamliel and Stapleton, 1997).

#### 10.4.4. BIOLOGICAL CONTROL ASPECTS OF SSOL

Disturbances in the biological equilibrium of the soil microflora, following soil fumigation or steaming, are known to be drastic and undesirable. Application of SSOL,



however, favours the survival and increase of several heat-tolerant micro-organisms able to act as antagonists against soilborne pathogens, such as *Talaromyces flavus* (Klöcker) A.C. Stolk & R.A. Samson, *Aspergillus terreus* Thom in Thom & Church, fluorescent pseudomonades and others (Greenberger *et al.*, 1987; Tjamos and Paplomatas, 1987; Tjamos *et al.*, 1991). Solarization favours establishment of added antagonists such as *Trichoderma* spp. and *A. terreus*, saprophytic Fusaria and other (Martyn and Hartz, 1986; Triolo *et al.*, 1988).

The survival of thermophilic genera of *Bacillus*, *Actinomyces*, as well as the build-up of fluorescent pseudomonads and other populations of rhizosphere bacteria were reported (Stapleton and DeVay, 1982, 1984; Kaewruang *et al.*, 1989; Gamliel and Katan, 1991; Antoniou *et al.*, 1995a). The effect of SSOL can be improved also by combination with no-pesticide organic amendments incorporated into the soil before mulching. This can be related both to the release of toxic materials by combination of heating and biological activity, and to positive changes in soil microflora. Gamliel and Stapleton (1997) reported control of root rot nematodes by mixing chicken manure or dry cabbage leaves in the plot before mulching (see also Chapter 23).

### 10.5. Combining Disinfestation Methods

One of the major limitations of SSOL is its climate dependence. Another problem diverts from the need to keep the treated area for 35–60 days without any crop. Partial control of some pests, as well as reduced efficacy in marginal seasons limit solarization use in many places. These constraints can be reduced, or solved, by combining solarization with other control measures at reduced dosages. The control efficacy may be increased due to additive effect. More likely it is due to synergistic effect caused by the hotter environment which increases vapour pressure and chemical activity of the added pesticide. Another reason for the improved activity of the pesticide is the weakening of the resting structure by the heat (Freeman and Katan, 1988).

Reduced doses of MBr, impermeable plastics and solarization were applied against a variety of diseases, e.g. *F. oxysporum* f. sp. *cucumerinum* of cucumbers, *C. michiganensis* ssp. *michiganensis* of tomatoes (Antoniou *et al.*, 1996, 1997), the melon sudden wilt (Gamliel *et al.*, 1997b), Verticillium of potatoes (Grinstein *et al.*, 1979), deep pitted scab of potatoes, Fusarium crown rot in tomatoes, soil sickness of *Gypsophila* sp. Reduced rates of MBr (**34 g/m<sup>2</sup>**) combined with simultaneous solarization effectively controlled corky root rot disease of tomatoes (Tjamos, 1984) and Verticillium wilt of globe artichoke (Tjamos and Paplomatas, 1987).

Reduced doses of chemicals are recommended as an alternative approach to the acute toxicity of full fumigation. However, their effectiveness is dependent on combinations with other pesticides or with non-chemical procedures. Sublethal fumigation is considered here in combination with SSOL (Gamliel *et al.*, 1997b). Combining sublethal fumigation with solarization could be focused on the following: (i) MBr fumigation followed immediately by solarization; (ii) simultaneous application of solarization with reduced doses of various fumigants; and (iii) solarization followed by fumigant for pathogens that are heat tolerant.

Recent studies show that the control efficacy of reduced dose of MBr combined with solarization was highly increased when applied after a short heating period, 2–3 days after the mulching (Gamliel *et al.*, 1997a). Application of MBr after the termination of the SSOL, however, can control some of the beneficial micro-organism populations which remain in the solarized plot, and has to be considered carefully.

Current reports mainly referring to field crops with applicability to covered crops deal with combinations of chemicals with SSOL. They include MES for the control of *V. dahliae* and *Fusarium oxysporum* Schlechtend.:Fr. f. sp. *vasinfectum* (Atk.) W.C. Snyder & H.N. Hans. (Ben-Yephet, 1988), dazomet either alone or in combination with SSOL to control *Phoma terrestris* E.M. Hans. on onions (Porter and Merriman, 1985), and MBr and SSOL for the control of *Pyrenochaeta lycopersici* R. Schneider & Gerlach on tomatoes (Tjamos, 1984). Reduced doses of MES (12.5 or **25 ml/m<sup>2</sup>**) applied singly or in combination with SSOL have destroyed propagules of *V. dahliae* and *F. oxysporum* f. sp. *vasinfectum* in a naturally infested cotton field (Ben-Yephet, 1988). The combination also reduced the time needed to kill sclerotia of *V. dahliae* by one week (Ben-Yephet, 1988). Dazomet (750 kg/ha) either alone or in combination with solarization has reduced disease incidence and severity of pink root rot (caused by *P. terrestris*) and of white rot (caused by *Sclerotium cepivorum* Berk.) of onions and increased yield by at least 100% (Abdel-Rahim *et al.*, 1983). Reduced rates of MBr (**34 g/m<sup>2</sup>**) combined with simultaneous solarization effectively controlled corky root rot disease of tomatoes (Tjamos, 1984) and Verticillium wilt of globe artichokes (Tjamos and Paplomatas, 1987).

Synergism in reducing disease incidence can be observed between fumigants and fungal antagonists of soilborne pathogens. Solarization in combination with *Gliocladium virens* J.H. Miller, J.E. Giddens & A.A. Foster proved to be a potential control strategy against southern blight of tomatoes (Ristaino *etal.*, 1991).

## 10.6. Prospects and Difficulties of Soil Disinfection

Soil fumigation with chemicals may have negative effects on the environment, could be extremely dangerous to humans, and may leave toxic residues in plant products. Thus, innovative approaches are desperately needed by the farmers and are under great demand by the consumers. Research towards exploiting SSOL by combining reduced doses of allowed fumigants, or various antagonists, could be one of the most promising approaches. This could also result in reducing duration of solarization thus making the method more acceptable by the farmers. Furthermore, sublethal fumigation in combination with solarization could solve many problems, since the combination is suitable for areas marginal for the application of solarization, and is able to reduce the duration of solarization to one half. SSOL in combination with biocontrol agents could exploit the weakening effect imposed by solar heating and could prolong its effectiveness.

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## **PESTICIDES IN IPM: SELECTIVITY, SIDE-EFFECTS, APPLICATION AND RESISTANCE PROBLEMS**

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### **11.1. Importance of Selective Pesticides in IPM Programmes**

The success of released or naturally occurring biological control agents in preventing pest outbreaks in protected crops has led the greenhouse industry to be particularly conscious of the necessity of applying selective pesticides. The activity of a selective pesticide is confined to a narrow range of specific pests (Heitefuß, 1975). In IPM, the process of developing the selectivity of a pesticide aims to maximize its specific effect against pests and diseases and minimize its effect on non-target organisms (Hull and Beers, 1985). Thus the selectivity of a pesticide is often used to express its harmlessness for beneficial organisms. The selectivity of the action and of the toxicity of a pesticide is dependent on its physiological selectivity and/or on the application procedures (Poehling, 1989). Physiological selectivity is expressed by reduced sensitivity of an organism to the pesticide due to pesticide metabolism and to the availability of the appropriate enzymes in the target organisms (Hassall, 1982). Application procedures comprise the dose rate, mode of action, method and timing.

The use of chemical pesticides that cause undesired side effects on non-target beneficial organisms may lead to pest outbreaks. In tomatoes, multiple application of the broad-spectrum carbamate methomyl for the control of leafminer infestation (*Liriomyza sativae* Blanchard) eliminated the naturally occurring beneficial parasitoid complex, which, without chemical treatment, reduced the pest population to 50% of the level found in pesticide-treated plots (Oatman and Kennedy, 1976). To avoid these consequences the harmful effects of pesticides on the natural enemies of target pests must be avoided or minimized for successful implementation of biological control agents within IPM strategies. Some pests and pathogens have developed resistance towards certain chemical pesticides, and this must also be considered in order to prevent misuse of pesticides.

In this chapter we will deal with the selectivity of pesticides in relation to effects on beneficial organisms that can be used in greenhouses, the potential for improving applications for better performance and selectivity, and the problems of resistance of the pests or diseases to the chemicals used in greenhouses.

### **11.2. Types of Side-Effects on Beneficial Organisms**

Pesticides can exhibit primary or secondary effects on predators, parasitoids and pathogens of target pests. Primary effects are direct or indirect, depending on their

exposure and on the biological parameter influenced. Direct mortality of beneficial organisms may be caused by direct contact during application, pesticide residues, taking up contaminated prey, intoxication by fumigants, and contact or contamination with soil disinfectants.

Indirect or sublethal effects on beneficial arthropods include decreases in reproduction, oviposition, parasitization, predation, longevity and egg viability, and a delay in development and shifting of the sex-ratio. Morphological and behavioural changes may also occur (Elzen, 1989).

Secondary effects due to pesticides include killing the prey/host of a beneficial organism or of species which produce alternative food like honeydew (Huffaker, 1990), taking up contaminated food (Sell, 1984; Celli *et al.*, 1997), and directly stimulating the pest; for example, some pyrethroids enhance reproduction in *Tetranychus urticae* Koch.

Pesticides directly affect entomopathogenic fungal biocontrol agents by inhibition of spore germination and vegetative development (mycelial growth), and they also reduce the viability of conidia (McCoy *et al.*, 1988) and their survival and activity on plant surfaces. Viability and infectivity of the infective juveniles (J3) of entomopathogenic nematodes are also adversely affected (Rovesti *et al.*, 1988).

Side-effects of pesticides on natural enemies may vary between and within taxonomic groups. From their comprehensive data on the side-effects of pesticides, Theiling and Croft (1988) concluded that predators were more tolerant to pesticide treatment than parasitoids, except for fungicides, towards which susceptibility was not greatly affected. The tolerance of aphid natural enemies decreases from Coccinellids > Chrysopids > Syrphids > Hemiptera > Hymenoptera (Hodek, 1973). Evaluation of effects within taxonomic groups revealed that the classification of the effects of 74 compounds tested against the parasitoids *Encarsia formosa* Gahan, *Aphidius matricariae* Haliday and *Leptomastix dactylopii* Howard corresponded by more than 78% (Hassan *et al.*, 1983, 1987, 1988, 1991, 1994). In a comparison of trial results with 81 test compounds for predatory mite species occurring in orchards and vineyards with *Phytoseiulus persimilis* Athias-Henriot, the same level was reached in 64% of the test compounds.

Differences in susceptibility have been recorded between taxonomically close species, and even between strains within the same species. *Eretmocerus mundus* Mercet adults were less susceptible to residues of amitraz, thiodicarb and cypermethrin than *E. formosa* or *Encarsia pergandiella* Howard (Jones *et al.*, 1995). Among *Aphidius* species, *A. matricariae* was more tolerant to dimethoate than *Aphidius rhopalosiphi* de Stefani Perez or *Aphidius colemani* Viereck (Maise *et al.*, 1997). The response of several species of entomopathogenic fungi to copper incorporated in agar differed. *Paecilomyces farinosus* (Holmsk.) A.H.S. Brown & G. Sm. was more tolerant than *Verticillium lecanii* (A. Zimmerm.) Viégas, *Beauveria bassiana* (Balsamo) Vuillemin and *Metarhizium anisopliae* (Metschnikoff) Sorokin (Baath, 1991). The entomopathogenic nematodes *Steinemema carpocapsae* (Weiser), *Steinemema feltiae* (Filipjev) and *Heterorhabditis* HP88 exhibited different tolerance levels to 9 tested pesticides (Zimmerman and Cranshaw, 1990). Repeated exposure of local strains to chemicals may cause natural enemies to develop tolerance to pesticides. This is the case of *P. persimilis* and organophosphorus compounds (OPs) (Goodwin and Welham, 1992) and of *Aphidoletes aphidimyza* (Rondani) and azinphos-methyl (Warner and Croft, 1982). Developmental stage may

greatly influence the response of natural enemies to pesticides. The susceptibility of *A. aphidimyza* and *Chrysoperla cornea* (Stephens) to pesticides with contact mode of action increased from the egg stage to the adults (Bartlett, 1964). In contrast, pesticide susceptibility was lowest in treated adults of *Coccinella septempunctata* L. (Zeleny *et al.*, 1988) and in eggs of *P. persimilis*, while in the coccinellid the egg stage and in the predatory mite the larvae or protonymph stage were the least tolerant (van Zon and Wysoki, 1978; Blümel and Stolz, 1993). However, compounds with modes of action that regulate or inhibit insect growth resulted in high mortality of *C. carnica* larvae, but not of the adults, whose fertility was only slightly affected (Vogt, 1992).

The host may offer parasitoids different degrees of protection against pesticides; unprotected stages of parasitoids (e.g. adult hymenoptera) and protected stages (e.g. different developmental stages in aphid mummies) show different levels of mortality after the same pesticide treatment. Avermectin B killed 50% of *E. formosa* protected in the whitefly scales in a direct contact test, but 79% of the adult wasps after contact with the dried residue (Zchori-Fein *et al.*, 1994). *Leptomastix dactylopii* protected in *Planococcus citri* (Risso) were barely affected by topical treatment of endosulfan, while the adults were severely damaged in residual tests (Reddy and Bhat, 1993). Even sexes of the same species may present different susceptibility against pesticides. In 5 different populations of *Diglyphus begini* (Ashmead) (Rathman *et al.*, 1992) and in predatory mites, males are less tolerant than females.

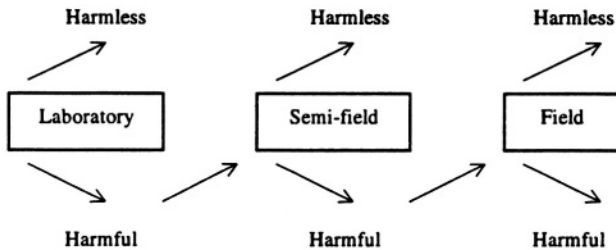
### 11.3. Tests and Approaches to Detect Side-Effects of Pesticides

One of the most comprehensive programmes to test side-effects of pesticides on beneficial organisms was set up by the IOBC/WPRS working group "Pesticides and Beneficial Organisms" (Hassan, 1989). In the first step, arthropod species and microorganisms that were regarded as the most important natural enemies in the different crops were identified. For these species test methods at different levels were developed. Pesticide screening is based on a sequence of three steps in laboratory, semi-field and field conditions, as shown in Fig. 11.1. The sequential programme assumes that pesticides that are harmless in the laboratory will also be safe in semi-field and field conditions, and do not need to be evaluated in further steps. When a chemical, however, is categorized as harmful in one step, its effect at the next step cannot be inferred, and the sequence must be continued until it finishes at field conditions or displays no negative effects.

The pesticides are usually tested at the highest recommended field rate as commercial formulations. The laboratory methods aim to evaluate the direct, initial toxicity of pesticide residues on susceptible and protected developmental stages of the test arthropods and are thus classified as lab-a- and lab-b-tests. The aim of the first test is the detection of pesticides which are harmless to the test organism after worst case exposure to dried pesticide residue on a defined test surface (glass or sand) after a single application of the test compound. The results of the tests should include the mortality (direct effect) and the reproduction (sublethal effect) of the test organism. Information about the duration of the effect of a pesticide is provided by the persistence test. Plant material (e.g. leaves) is sprayed with the test pesticide and left on the plant under greenhouse conditions for



residue aging. Leaf samples undergo a further test, similar to the lab-a-test. The next test is the semi-field test which is carried out on pesticide residues or as a direct application on the plants with the test arthropods, and is kept under more natural conditions. Sublethal effects, behavioural changes, and the effect of more than one application of the test product are thus evaluated. The range of tests developed for a selection of organisms important in greenhouse crops is presented in Table 11.1. Most of the information that follows in this section may be found in the IOBC/WPRS Bulletin 1988,11(4); 1992,15(3); 1994,17(3).



**Figure 11.1. Sequential IOBC/WPRS procedure for testing effects of pesticides on natural enemies (after Hassan, 1989).**

The lab-a-test for parasitoids (*E. formosa*, *A. matricariae*) and for *A. aphidimyza* adults (on leaf material) is carried out as a residual contact test with adult wasps or gall midges. Mortality and reproduction (parasitization of the host or number of eggs deposited) are evaluated. In the lab-b-test, the protected stages of the parasitoids in their hosts (aphid mummies; whitefly scales) are directly sprayed with the pesticide solution and the emergence rate from the hosts is assessed. The lab-a-test for predatory mites is a residual contact test starting with predatory larvae or protonymphs. During the test the mortality rate, escaping rate and reproduction per female are evaluated.

The same testing procedure is used as in the lab-a-test for *Orius niger* (Wolff), and the emergence from the deposited eggs is also assessed. The lab-b-test for *O. niger* is the same as the lab-a-test, but uses predatory bug adults. The lab-b-test for *A. aphidimyza* is carried out with larvae as a residual contact test on leaves and is also appropriate for a persistence test. Laboratory tests for *C. carnea* and *Syrphus corollae* Fabricius follow the same principles. Larvae are tested in a residual contact test to assess the mortality and reproduction of the test organisms. A laboratory test for Coccinellids has also been described in detail for *Hippodamia oculata* (Thunberg). A residual contact test with larval stages is carried out to evaluate mortality and duration of development. The adults deriving from this first testing phase are used to check reproduction, duration of sexual maturation of females, and emergence from the deposited eggs.

Persistence tests or tests to detect the duration of harmful effects of the pesticide residue are very similar for nearly all test organisms. Suitable plants are sprayed and kept under greenhouse conditions for different periods. Leaf samples are collected at regular intervals and are used as test surfaces, as in the lab-a-test or the lab-b-test. Mortality and

reproduction are again assessed. In this case persistence must be considered as an extended laboratory test. For *C. carnea* and *Episyrphus balteatus* (DeGeer) the test is carried out on treated plants and, in addition to the above mentioned parameters, changes in the behaviour of *E. balteatus* can be examined.

**TABLE 11.1. Test methods on different test levels of the sequential testing scheme developed within the IOBC/WPRS working group "Pesticides and Beneficial Organisms"**

| Test organism   | Lab-a-test     | Lab-b-test | Extended laboratory test/<br>persistence | Semi-field test<br>initial tox. | Semi-field test<br>persistence/<br>greenhouse |
|---|----------------|------------|--|---------------------------------|---|
| <b>Parasitoids</b>  |                |            |  |                                 |   |
| <i>Encarsia formosa</i>   | X <sup>1</sup> | X          | X  | X                               |   |
| <i>Aphidius matricariae</i>   | X              | X          | X  |                                 |   |
| <i>Leptomastix dactylopii</i>   | X              |            |  |                                 |   |
| <i>Aphytis melinus</i>  | X              |            |  |                                 |   |
| <b>Predatory mites</b>  |                |            |  |                                 |   |
| <i>Phytoseiulus persimilis</i>  | X              |            | X  | X                               |   |
| Others  | X              |            | X  |                                 |   |
| <b>Predators</b>  |                |            |  |                                 |   |
| <i>Chrysoperla carnea</i>   | X              |            | X  | X                               | X   |
| <i>Syrphus corollae</i>   | X              |            |  |                                 |   |
| <i>Episyrphus balteatus</i>   |                |            |  | X                               | X   |
| Coccinellids ( <i>Semiadalia</i> ,<br><i>Harmonia</i> , <i>Coccinella</i> ) | X              |            |  |                                 |   |
| <i>Orius niger</i>  | X              | X          | X  | X                               |   |
| <i>Aphidoletes aphidimyza</i>   | X              | X          | X  |                                 |   |
| <b>Entomopathogenic fungi</b>   |                |            |  |                                 |   |
| <i>Beauveria bassiana</i> ,   | X              | X          | X  | X                               |   |
| <i>Beauveria brongiarthii</i>   |                |            |  |                                 |   |
| <i>Metarhizium anisopliae</i>   | X              | X          | X  | X                               |   |
| <i>Verticillium lecanii</i>   | X              |            |  |                                 |   |
| <b>Entomopathogenic nematodes</b>   |                |            |  |                                 |   |
| <i>Steinernema</i> sp.  | X              |            |  |                                 |   |

<sup>1</sup>X: existing test methods

The sequential IOBC testing scheme for *B. bassiana* and *M. anisopliae* comprises all three test levels. In the lab-tests the mycelial growth on agar containing pesticides is measured. The production and viability of conidia is assessed with a bioassay to check virulence. It has been proposed to switch from tests on solid medium to a worst case test for growth inhibition in liquid medium, where the mycelium, as the most sensitive stage of the fungus, is immersed into the pesticide solution. In the semi-field test conidia are mixed with standard soil and treated with the test pesticide. The soil is then incubated and the number of spores per unit of soil is determined. To check the virulence of the tested fungus at each step of the sequential scheme the *Galleria*-bait-method may be used. The results of an *in vivo* assay, in which leaf discs are sprayed with the conidial suspension of the beneficial fungus on a dried residue of the test pesticide have been described. Side-

effect testing at the infective juvenile J3 stage of entomopathogenic nematodes is carried out in a 2-step scheme. First, the viability and the behaviour *in vitro* in pesticide solutions is checked. In the next step, mobility and infectivity are examined in a bioassay in soil.

Compatibility of pesticides with bumble-bees used as natural pollinators in greenhouses is classified in four categories, which allow or exclude the use of bumble-bees or recommend a certain period after pesticide application during which the hives should be removed from the greenhouses.

Comprehensive data collections about side-effects of pesticides on natural enemies are available from commercial suppliers of beneficial organisms (Biobest, 1998) and also in the tables published by the IOBC/WPRS working group.

#### 11.4. Effects of Chemical Pesticides on Beneficial Organisms Used in Greenhouses

Information on specific pesticide effects on natural enemies and pathogens may be found in the published results of the Joint Testing Programmes by the IOBC/WPRS Working Group "Pesticides and Beneficial Organisms" (Hassan *et al.*, 1983, 1987, 1988, 1991, 1994; Croft, 1990; Sterk *et al.*, 1998) and in many other references. Some examples selected from the literature are included in Table 11.2.

Generally herbicides, acaricides and fungicides have less effect than insecticides, although mycopesticides are highly susceptible to fungicides.

(i) Effect on beneficial predators. For predatory mites most pyrethroids and carbamates were harmful, both in initial toxicity and in reproduction and persistence trials with the susceptible juvenile predators. *Aphidoletes aphidimyza* showed a similar susceptibility to insecticide/acaricide treatments, and was also affected by OPs. OPs caused varying levels of mortality in predatory mites (see Section 11.3). In coccinellids, high mortality rates were caused by nearly all tested compound groups, except the microorganisms and soap. Chrysopids were not harmed by acaricides, most pyrethroids, soap or microorganisms, but were affected by most of the insect growth regulators (IGRs) and most of the OPs. For predatory bugs, pyrethroids, carbamates, most OPs and few of the IGRs proved to be harmful. Fungicides and herbicides were relatively harmless for coccinellids, chrysopids and predatory bugs, but partly harmful to predatory mites.

(ii) Effects on beneficial parasitoids. Synthetic pyrethroids and pyrethrin were very harmful to adults, regardless of the test species. In tests with the protected stages, several pyrethroids were only slightly harmful, but in combination with a persistence of more than one week this advantage was neutralized. OPs were very harmful to the unprotected stages and with few exceptions also to the protected life stages, and showed high persistence as residues. Carbamates were harmful in both types of laboratory tests, but some had a persistence shorter than three days. IGRs and most of the acaricides were harmless to both the susceptible and the protected developmental stage of the parasitoids. Plant extracts (except pyrethrin), soap and microorganisms were harmless. Fungicides belonging mainly to the group with a broad-spectrum and protective mode of action were harmful to adult parasitoids and revealed detrimental effects which persisted over one week. In tests with the protected life stage, however, all fungicides

were considered harmless. Very few herbicides were harmful to adult wasps, but not for other developmental stages.

TABLE 11.2. Effects of pesticides on natural enemies. The type of tests are indicated in column headings: 1, lab-a-test; 2, lab-b-test; 3, persistence test; 4, semi-field or greenhouse test (see Section 11.4 in this chapter for further explanations on these types of tests). Effects have been categorized according to the following criteria. In laboratory and semi-field tests: -, <50% total effect; o, total effect between 51 and 99%; +, >99% total effect. In persistence tests: -, effect on <50% for lesser than one week; o, effect on 51-99% for lesser than one week; +, effect on >99% for more than one week. More than one effect classification indicates different test results. Compiled from published results of the Joint Testing Programmes by the IOBC/WPRS Working Group "Pesticides and Beneficial Organisms" (Hassan *et al.*, 1983, 1987, 1988, 1991, 1994; Croft, 1990; Sterk *et al.*, 1998) and also from many other references

| Pesticide common name                                 | Groups of natural enemies and types of test |   |    |   |                         |    |    |    |                |    |   |   |             |   |    |   |                 |   |   |   |    |
|---|---|---|----|---|-------------------------|----|----|----|----------------|----|---|---|-------------|---|----|---|-----------------|---|---|---|----|
|   | Predatory mites                             |   |    |   | Coccinellids/Chrysopids |    |    |    | Predatory bugs |    |   |   | Parasitoids |   |    |   | Entomopathogens |   |   |   |    |
|   |   |   |    |   |                         |    |    |    |                |    |   |   |             |   |    |   | Nemat. Fungi    |   |   |   |    |
|   | 1   | 2 | 3  | 4 | 1                       | 2  | 3  | 4  | 1              | 2  | 3 | 4 | 1           | 2 | 3  | 4 | 1               | 2 | 1 | 2 | 4  |
| <b>Organophosphorus</b>                               |   |   |    |   |                         |    |    |    |                |    |   |   |             |   |    |   |                 |   |   |   |    |
| Chlorpyrifos  | o+  |   |    |   | +                       | +  | +  |    | o+             | +  | + |   |             | + | o  | + |                 |   | - | o | -  |
| Diazinon  | -+  |   |    |   | +                       | -  | o+ |    | -o             | -  | o |   |             | + | o  | + |                 |   | o | o | -  |
| Heptenophos   | -+  | - |    |   | o+                      | o  | -  |    | -o             | -o | o |   |             | + | o  | - |                 |   | o | - | +  |
| Phosmet   | o+  |   |    |   | o+                      | +  | o+ |    | -              | o  |   |   |             | + | o  |   |                 |   | - |   | o  |
| Triazophos  | -+  |   |    |   | +                       | +  | +  |    | o+             | +  | + |   |             | + | o+ | + |                 |   |   |   | o  |
| <b>Organochlorines</b>                                |   |   |    |   |                         |    |    |    |                |    |   |   |             |   |    |   |                 |   |   |   |    |
| Endosulfan  | o   |   | o  |   | o+                      | -o |    |    |                |    |   |   |             | + | o  | - |                 |   | - |   |    |
| Lindane   | -   |   |    |   | o                       |    |    |    |                |    |   |   |             | + | -  | + |                 |   | - |   |    |
| <b>Carbamates</b>                                     |   |   |    |   |                         |    |    |    |                |    |   |   |             |   |    |   |                 |   |   |   |    |
| Methomyl  | +   |   | o+ | + | o+                      | +  |    |    | +              | +  |   |   |             | + | o  |   |                 | o | o | o |    |
| Oxamyl  | +   | o |    |   | +                       | +  | o  |    | o              | +  |   |   |             | + | o  |   |                 | + | - |   | o- |
| Pirimicarb  | -o  |   | -  | o | -o                      | -  |    |    |                | o  |   |   |             | + | -  | - |                 |   | - |   |    |
| Propoxur  | o+  |   |    | o | +                       |    |    |    | +              |    |   |   |             | + |    |   |                 |   | - |   |    |
| <b>Pyrethroids</b>                                    |   |   |    |   |                         |    |    |    |                |    |   |   |             |   |    |   |                 |   |   |   |    |
| Deltamethrine   | +   |   |    | + | o+                      |    |    | o  | +              |    |   |   |             | + | +  | + | +               |   | - |   | -  |
| Fenpropathrin   | +   |   | +  | + | +                       | o  |    | o  | +              |    |   |   |             | + | +  | o | +               |   | - | o | -  |
| <b>IGR's</b>  |   |   |    |   |                         |    |    |    |                |    |   |   |             |   |    |   |                 |   |   |   |    |
| Diflubenzuron   | -   |   |    | - | -                       | o  |    | o+ | o              | -  | + |   |             | - |    |   |                 |   | - |   | -  |
| Fenoxycarb  | -   |   |    | - | -                       | -  |    | -o | +              |    |   |   |             | - |    |   |                 |   | - |   | o  |
| Teflubenzuron   | -   |   |    | - | o+                      | +  |    | +  | o+             |    |   |   |             | - | -  | - |                 |   | - | - | -  |
| <b>Natural origin</b>                                 |   |   |    |   |                         |    |    |    |                |    |   |   |             |   |    |   |                 |   |   |   |    |
| Azaridachtin  | -   | - |    |   |                         |    |    |    |                |    |   |   |             | - | -  |   |                 |   |   |   |    |
| <i>Bacillus thuringiensis</i> ssp. <i>kurstaki</i> ,  | -   |   |    |   | -                       | -  | -  |    | -              | -  |   |   |             | - |    |   |                 |   | - | - | -  |
| <i>Bacillus thuringiensis</i> ssp. <i>tenebrionis</i> |   |   |    |   |                         |    |    |    |                |    |   |   |             |   |    |   |                 |   |   |   |    |
| Nicotine  |   |   |    |   | -                       | o  | -  |    |                |    |   |   |             |   |    |   |                 |   |   |   |    |
| Pyrethrum + rotenone                                  | +   | o |    |   | o                       |    |    |    |                |    |   |   |             |   |    |   |                 |   |   |   |    |
| <i>Verticillium lecanii</i>                           | -   |   |    |   | -                       | -  |    |    |                |    |   |   |             | - |    |   |                 |   | - | - |    |

TABLE 11.2. Effects of pesticides on natural enemies. The type of tests are indicated in column headings: 1, lab-a-test; 2, lab-b-test; 3, persistence test; 4, semi-field or greenhouse test (see Section 11.4 in this chapter for further explanations on these types of tests). Effects have been categorized according to the following criteria. In laboratory and semi-field tests: -, <50% total effect; o, total effect between 51 and 99%; +, >99% total effect. In persistence tests: -, effect on <50% for lesser than one week; o, effect on 51-99% for lesser than one week; +, effect on >99% for more than one week. More than one effect classification indicates different test results. Compiled from published results of the Joint Testing Programmes by the IOBC/WPRS Working Group "Pesticides and Beneficial Organisms" (Hassan *et al.*, 1983, 1987, 1988, 1991, 1994; Croft, 1990; Sterk *et al.*, 1998) and also from many other references (cont.)

| Pesticide common name     | Groups of natural enemies and types of test |   |   |   |                         |    |   |   |                |    |   |   |             |    |   |   |                 |   |   |    |   |
|---------------------------|---|---|---|---|-------------------------|----|---|---|----------------|----|---|---|-------------|----|---|---|-----------------|---|---|----|---|
|                           | Predatory mites                             |   |   |   | Coccinellids/Chrysopids |    |   |   | Predatory bugs |    |   |   | Parasitoids |    |   |   | Entomopathogens |   |   |    |   |
|                           |   |   |   |   |                         |    |   |   |                |    |   |   |             |    |   |   | Nemat. Fungi    |   |   |    |   |
|                           | 1   | 2 | 3 | 4 | 1                       | 2  | 3 | 4 | 1              | 2  | 3 | 4 | 1           | 2  | 3 | 4 | 1               | 2 | 1 | 2  | 4 |
| <b>Other insecticides</b> |   |   |   |   |                         |    |   |   |                |    |   |   |             |    |   |   |                 |   |   |    |   |
| Abamectin                 | o   | + |   | o |                         |    |   |   | -              | o  |   |   | o           | -o |   |   | -               |   |   |    |   |
| Amitraz                   | +   |   |   |   | -o                      |    |   | o | -              | -  |   |   | +           | o  | o | + | -               |   |   | o  |   |
| Buprofezin                | -o  |   |   |   | -o                      | -o |   | o | -              | -  |   |   | -           | -  |   |   | -               | - |   |    |   |
| Cyromazine                | +   |   |   |   | +                       | +  | o | o | o              | o  | - |   | o           | -  |   |   | -               | - |   |    |   |
| Imidacloprid              | +   |   |   |   | -                       |    |   |   | +              |    |   |   |             |    |   |   | -               |   |   |    |   |
| Pyriproxifen              | -   | - |   |   | -                       |    |   |   | -              | -  |   |   | -           | o  |   |   |                 |   |   |    |   |
| <b>Acaricides</b>         |   |   |   |   |                         |    |   |   |                |    |   |   |             |    |   |   |                 |   |   |    |   |
| Dicofol                   | o+  |   | o | + | o                       | -  |   |   |                |    |   | o | +           | o  | + | - | -               |   |   |    |   |
| Fenbutatin oxide          | -   |   |   |   | -                       | -  |   |   |                |    |   |   | -           |    |   |   | -               |   |   |    |   |
| <b>Fungicides</b>         |   |   |   |   |                         |    |   |   |                |    |   |   |             |    |   |   |                 |   |   |    |   |
| Bitertanol                | -   |   |   |   | -                       | -  | - |   | -              | -  |   |   | -           |    |   |   | -               |   |   | +  | o |
| Chlorothalonil            | -o  |   |   |   | -                       | -  | - |   | -              | -  |   |   | -           |    |   |   | -               |   |   | o+ | + |
| Copper oxychloride        | -   |   |   |   | -                       | -o | - |   | -              | -  |   |   | +           | -  | - |   | -               |   |   | +  |   |
| Iprodione                 | -   |   | - |   | -                       | -  | - |   | -              | -  |   |   | -           | -  | - |   | -               |   |   | o- |   |
| Quinomethionate           | o   |   |   |   | o                       | o  |   |   |                |    |   |   | +           | -  | - |   | -               |   |   |    |   |
| Sulphur                   | +   | o |   |   | +                       | +  | o |   | -              | -o |   |   | +           | -  | + |   | -o              |   |   |    |   |
| Triforine                 | -   |   |   |   | -                       | o- | - |   | -              |    |   |   | -           |    |   |   | -               |   |   | o  | o |
| <b>Herbicides</b>         |   |   |   |   |                         |    |   |   |                |    |   |   |             |    |   |   |                 |   |   |    |   |
| Glyphosate                | -o  |   |   |   | o-                      |    |   |   | -              |    |   |   | -           |    |   |   | -               |   |   |    |   |
| 2,4 D                     | -   |   |   |   | -                       | -  |   |   | -              | -  |   |   | -           |    |   |   | -               |   |   |    |   |

(iii) Entomopathogens. Only a small number of carbamates out of the tested insecticides/acaricides affected entomopathogenic nematodes, while fungicides proved to be mainly harmless. Insecticides, acaricides and herbicides in most cases did not adversely influence the mycelial growth or the sporulation of the fungal species *V. lecanii*, *B. bassiana*, and *M. anisopliae* in laboratory tests or during infectivity tests in the greenhouse. Half of the fungicides examined in all types of tests affected at least one of the three test fungi, whereas one fourth of the fungicides were harmless for all of them. Effects could not generally be attributed to the mode of action of the fungicides. *Verticillium lecanii* was slightly more affected than *B. bassiana*.

(iv) Sublethal effects on natural enemies. Besides direct toxicity caused by a number of "classical" insecticides, sublethal effects were also demonstrated in several investigations. Among sublethal effects of pesticide application on natural enemies

which are reported in the literature are: development prolongation, reduced egg production or its total inhibition, decrease in prey consumption, changes in searching or foraging behaviour, alteration of pathogenicity in entomopathogens, and increased tendency to escape from treated surfaces. The importance of repellence of pesticide compounds for beneficials is difficult to classify. On the one hand, repellence may negatively influence natural enemies by expelling them from their host or prey which they need for further population development; on the other, beneficials can be protected from possibly hazardous contact with contaminated plant surfaces or prey/hosts. Both effects are undesirable, especially in greenhouses, where mass reared arthropods are intentionally introduced as biological control agents, and because the natural enemies would cease to be effective as control agents, particularly when untreated refuges are scarce.

Insect growth regulators, like diflubenzuron, chlorfluazuron, fenoxycarb, flufenoxuron and teflubenzuron, which are incorrectly considered as harmless to many beneficials, in fact interfere with the viability of eggs, the moulting process, and the reproduction of several predators.

The influence of different formulations of pesticides on their effects on natural enemies was shown for endosulfan, which as an emulsifiable concentrate (EC) formulation resulted in up to 17% less mortality of *P. persimilis* than the wettable powder (WP) formulation in a residual laboratory test (Blümel *et al.*, 1993). For *E. formosa* the EC formulation of tebufenpyrad was more toxic than the WP formulation (van de Veire, 1995).

### 11.5. Influence of Pesticide Application on the Selectivity of a Pesticide

The relatively small areas in greenhouses - compared to arable agriculture - and high plant density dictate in many cases the use of manually operated spraying equipment. In an enclosed structure, good ambient conditions can exist for applying very small particles and using artificial air movement to improve pesticide distribution and pest control. Conversely, improved chemical control can adversely affect bio-agents such as bumble-bees, antagonistic fungi and beneficial arthropods, factor which has to be considered when choosing a pesticide. Pesticide application in enclosed areas also imposes the risk of breathing air that contains small particles of pesticides. Personal protective clothing is often hot and uncomfortable, and farmers tend to spray unprotected.

Unfortunately, many growers continue to use high volume (HV) spraying (>1000 l/ha of spraying solution). HV spraying to run off leads to wastage to the order of 70–90% of the chemical dripping to the ground (Matthews, 1992). The low concentration of a.i. with HV applications reduces the hazard to the operator, who is often heavily contaminated by the pesticide, but may not give adequate control, and growers are thus forced to repeat sprays at frequent intervals. The whole area becomes contaminated with pesticides, making it impossible to integrate biological control with chemicals. The volume of spray and wastage due to runoff can be reduced significantly by changing nozzles to produce small droplets which do not coalesce on the target (Matthews, 1992). A widely used piece of equipment is the knapsack mistblower.

As an alternative to HV spraying, the use of thermal or cold foggers gives the grower clear savings in time and labour, although they are only suitable in totally enclosed greenhouses. Deposition is improved with cold fogging, but persistence is less. The shorter persistence obtained with cold foggers allows the introduction of natural enemies quicker after treatment than when a thermal fogger is used, and a greenhouse can be treated when parasitoids are protected inside the infested host stages (Lingappa *et al.*, 1972). Additionally, cold fogging allows the use of a wider range of pesticides, e.g. insecticides perhaps with higher selectivity, such as *Bacillus thuringiensis* Berliner which has been used successfully by cold fogging.

Another technique, vaporization, is suitable for small areas (approximately  $100 \text{ m}^2$ ). The pesticide (e.g. sulfur) is placed on a small heater installed inside a wide pipe. After evaporation or sublimation, the pesticide condenses to small particles (e.g.  $2\text{--}8 \text{ }\mu\text{m}$ ) and is carried up by the heated air directed by the pipe. The dispersion and settling of particles of this size is influenced by the inside air circulation systems and they fall mainly on the upper side of the leaves, rendering minimal residual effect.

Alternatives to spray treatments include application of granules or drenches and chemigation by drip irrigation to the soil, when systemic pesticides can be used. Specific treatments can be combined with a pesticide or other types of lure, e.g. yellow cards in a “lure and kill” method. Thrips have been controlled with a polybutene sticky surface combined with an insecticide (Thripstick). Specific baits cause only minimal damage to non-target organisms, as their chance of exposure is very low.

The timing of the pesticide treatment is crucial in order to avoid the susceptible life stage of the non-target organism. Where chemical pesticides adversely affect the entomopathogenic fungus *V. lecanii*, they should not be applied at the same time, but after a delay (Schuler, 1991). Similarly, the alternation of chemical fungicides with the fungal biocontrol agent *Trichoderma harzianum* Rifai T39 is preferred to the use of a tank mix of this biocontrol agent with chemicals for the control of foliar pathogens (Shtienberg and Elad, 1997). Selective application can also be carried out by considering spatial factors and using the systemic pesticides as granules or seed treatment to preserve plant-inhabiting beneficials. Limited areas can be treated with hand-held air-assisted spinning disc sprayers. Multiple applications of a pesticide may cause a severe reduction in the number of natural enemies, without achieving a satisfactory control of the target pest. In contrast, a single, better timed application of the same pesticide can control the pest to the same extent, without seriously damaging the natural enemies, thus improving overall control. Keeping the pest below the economic threshold has been achieved with different use of oxamyl and methamidophos against *L. sativae* and its parasitoid complex in tomatoes (Schuster *et al.*, 1979).

Systemic fungicides, which were harmful to *V. lecanii* when applied as sprays, did not affect the fungus pathogenicity against *Aphis gossypii* Glover on cucumber when applied as a soil drench (Wilding, 1972). Another possibility for the partial preservation of natural enemies is the treatment of selected strata of the plants, e.g. flowers, and leaving the lower part of the canopy untreated, thus maintaining a significant population of natural enemies (Scopes and Biggerstaff, 1973). These localized treatments are gaining acceptance where insects are used to pollinate crops and growers release natural

enemies such as *E. formosa*. In one study, the application of pyriproxifen to the upper parts of tomato plants infested with greenhouse whitefly effectively reduced the pest, but did not damage the parasitoid *E. formosa*, which, though susceptible to this compound, was situated in the whitefly pupae on the lower parts of the plants (van de Veire, 1995).

### 11.6. Pesticide Resistance and Anti-Resistance Strategies in IPM

Pests and pathogens may overcome the toxic effect of pesticides by metabolizing the active ingredient into less toxic components, developing a change in the target site, reducing the absorption of the chemical or by avoiding exposure to the compound. Resistance development is the most severe challenge to pesticide. In greenhouses, pesticide-resistant strains of fungi and pests have appeared frequently. This phenomenon occurs because the greenhouse is a closed system in which the population of selected strains is not diluted by the outdoor wild population. Usually, the existence of epidemic conditions in greenhouses is a prerequisite for the development of resistant populations of pathogens and pests. Moreover, the optimal conditions for their development in greenhouses prevail for long periods. The number of life cycles is increased due to the optimal conditions or the extended time they prevail, and control necessitates frequent pesticide applications. The latter result in high selection pressure towards resistance to pesticides. The main pathogens which are known to develop resistance to fungicides in greenhouses are *Botrytis cinerea* Pers.:Fr., *Pseudoperonospora cubensis* (Berk. & M.A. Curtis) Rostovzev (downy mildew of cucurbits), *Didymella bryoniae* (Auersw.) Rehm (gummy stem blight of cucurbits), *Sphaerotheca fusca* (Fr.) Blumer. [= *Sphaerotheca fuliginea* (Schlechtend.:Fr.) Pollacci] (powdery mildew of cucurbits), *Puccinia horiana* Henn., *Uromyces dianthi* (Pers.:Pers.) Niessl (= *Uromyces caryophyllinus* G. Wint.) and *Fusarium oxysporum* Schlechtend.:Fr. f. sp. *gladioli* (L. Massey) W.C. Snyder & H.N. Hans.

The benzimidazole fungicides (benomyl, carbendazim, thiophanates) have a high resistance potential against pathogens because they have a specific mode of action. The resistance is usually not associated with a significant loss of fitness of the pathogen. It occurs in populations of *B. cinerea*, *D. bryoniae*, *Fusarium* and powdery mildews. Mixtures and alternations with multi-site contact fungicides may delay this selection, before resistance becomes apparent.

Acute problems of resistance to dicarboximide fungicides (e.g. iprodione, procymidone, vinclozolin) have arisen when fungicides are used intensively and exclusively over many seasons (Gullino *et al.*, 1989). Isolates are moderately resistant and tend to be almost as fit as sensitive strains in the absence of fungicides. It is recommended to restrict the number of dicarboximide treatments to no more than three per crop in greenhouses where resistance is found, and even in the absence of detectable resistant strains. When infection pressure is high, it is usually recommended to alternate or mix these fungicides with protectants such as chlorothalonil, captan, TMTD, or with biocontrol which do not usually select for resistance. However, TMTD may interfere with natural enemies (Section 11.4).



Ergosterol biosynthesis inhibitors (EBIs) are a group of fungicides which include triazole, imidazole and pyrimidine fungicides which inhibit C14 demethylation and morpholines. Unlike the sharp, significant nature of resistance towards benzimidazoles and dicarboximides mentioned above, the resistance towards EBIs develops in the form of slow shifts in the pathogen population. For instance, powdery mildews in greenhouses were controlled for several years by benzimidazoles, hydroxypyrimidines, pyrazophos, and EBIs. Resistance is known in populations of *S. fusca* but the alternation of fungicides, which is practised in many countries, is helping to deal with the problem. It is generally recommended to rotate or mix EBI fungicides with fungicides from other groups as well as with biocontrol.

The failure of disease control in greenhouses is exemplified by the history of gray mold epidemics. Multiple resistant isolates occur in greenhouses that bear the resistance towards benzimidazole, diethofencarb, dicarboximides and ergosterol biosynthesis inhibitors (Pommer and Lorenz, 1982; Elad *et al.*, 1992). The extreme summer conditions do not interfere with the survival of fungicide-resistant isolates (Yunis and Elad, 1989). Table 11.3 illustrates the situation for Israeli vegetable greenhouses sampled in 1997 by exposing plates of *Botrytis* selective medium containing test fungicides from various groups (for method, see Elad and Shtienberg, 1995).

**TABLE 11.3. Resistance towards fungicides in greenhouse populations of *B. cinerea* (number of colonies grown on exposed plates containing *Botrytis* selective medium indicating comparable levels)**

| Crop     | Place      | Group of fungicides, test fungicide in plate and concentration ( $\mu\text{g/ml}$ ) |                |                |                   |
|----------|------------|---|----------------|----------------|-------------------|
|          |            | None  | Benzimidazoles | Dicarboximides | EBIs              |
|          |            |   | Benomyl (5)    | Iprodione (5)  | Fenbuconazole (1) |
| Cucumber | Ahituv     | 100   | 98             | 29             | 21                |
|          | Yama A     | 23  | 18             | 3              | 22                |
|          | Yama B     | 59  | 7              | 4              | 72                |
|          | Tzafit     | 6   | 7              | 1              | 6                 |
| Tomato   | Bet Shikma | 77  | 100            | 10             | 100               |
|          | Sde Moshe  | 18  | 16             | 1              | 14                |
|          | Yama       | 46  | 48             | 1              | 47                |
|          | Ibtan      | 10  | 3              | 2              | 1                 |

Phenylamide fungicides that inhibit RNA synthesis were introduced in the late 70s for Phycomycetes control. During the 70s *P. cubensis* was controlled mainly with protective applications of dithiocarbamates and chlorothalonil. In the early 80s the phenylamide metalaxyl was released and soon afterwards resistant strains were selected. Metalaxyl-resistant strains seem to be more competitive than wild-type strains (Cohen *et al.*, 1983). Resistance was found also in *Phytophthora infestans* (Mont.) de Bary on tomato and *Bremia lactucae* Regel on lettuce. Anti-resistance mixtures of metalaxyl with protectant fungicides were developed to cope with phenylamide resistance.

In order to reduce the pressure towards development of resistance in pathogen

populations, it is usually better to limit the exposure of the pathogen to a group of fungicides. The number of applications of fungicides of the same mode of action has to be limited, especially against fungi with many cycles during the growing season. Moreover, the application of non-chemical methods is also recommended.

Insecticide and acaricide resistance of nearly all important arthropod greenhouse pests is well documented (Georghiou and Mellon, 1983). Besides genetic and operational factors that influence the selection of resistant individuals, biotic reasons such as generation turn-over, number of offspring per generation and type of reproduction have a major impact on resistance development. Most of the pest species on greenhouse crops favour resistance selection with regard to these biological parameters.

Recently *Bemisia tabaci* (Gennadius) and *Bemisia argentifolii* Bellows & Perring have developed resistance against a range of conventional insecticides as well as against IGRs and juvenile hormone analogs (Cahill *et al.*, 1994; Horowitz *et al.*, 1994), and *Frankliniella occidentalis* (Pergande) developed resistance against most pesticide groups (Anonymous, 1988), resulting in severe economic losses in the affected crops. Pesticide resistance can also develop in natural enemies and has been found in all taxonomic groups (Croft and Strickler, 1983). The differences in the occurrence and the level of pesticide resistance in predators and parasitoids can be explained by the influence of the factors such as food limitation and differential susceptibility to the chemical.

Chemical resistance management strategies for pests comprise different approaches classified as management by moderation (low dosages, reduced number of applications), management by saturation (suppressing detoxification) and management by multiple attack (application of mixtures) (Georghiou, 1983). For IPM programmes additionally non-target effects on natural enemies have to be considered, which might not always correspond with the aforementioned strategies.

### 11.7. Future Aspects

Modern techniques used in greenhouses for pesticide application allow a low input of chemicals while achieving good coverage of the right part of the plant. Selective application can also direct the active ingredient to the right target, with lowered effect on beneficial organisms. However, it is important to know the undesired side effects of chemical use in greenhouses. The use of side effect data by advisory services or growers may lead to problems due to contradictory information about the effects of the same pesticides resulting from differences in test methods, different test laboratories carrying out the tests and the formulation of the pesticide used in different countries. Therefore, uniform labelling of the non-target effects of plant protection products already during the process of authorization as proposed in the European Plant Protection Legislation (EU-Directive 414/91, including all annexes) is desirable. The basic requirements to fulfil the legislative demands were formulated during the "Workshop of European Standard Characteristics of Beneficial Arthropod Testing" (Barrett *et al.*, 1994). Resulting from this workshop 11 different ring test groups for the

standardization and harmonization of existing test methods and for the development of new test methods were formed. As an outcome of this joint initiative by governmental research centres, industry, commercial test laboratories and contributions from the European and Mediterranean Plant Protection Organization (EPPO), a harmonized labelling of plant protection products concerning the non-target effects is expected.

Other topics for the implementation of side-effect data into IPM practice still need to be addressed. Most of the data about side-effects of pesticides on beneficials is derived from laboratory tests or even higher test levels with only one application of the product. However, in practice, even when natural enemies are used against arthropod pests, chemical treatment can be necessary against fungal diseases. Often these fungicides have to be applied not once, but several times at certain intervals. These applications can lead to an accumulation of the product on the plants, affecting the beneficial organisms. This situation becomes more complicated when mixtures of different active ingredients are used.

Very few chemical pesticides are selective for natural enemies. Improvements in the compatibility of beneficial organisms with pesticide application by selecting beneficials with some resistance towards chemical pesticides have been attempted, but this is often a cumbersome procedure as the pesticides used may change quickly. Besides the degree of resistance, its stability and its possible influence on the fitness of the tolerant organisms are features that must be assessed before the selected organisms can be used in pest or disease control. For phytoseiids development of pesticide resistance against several insecticide groups, acaricides and fungicides, and even against sulphur has been extensively described (Fournier *et al.*, 1985; Croft and van den Baan, 1988). Alternatively, pesticides are applied spatially to selected areas or in frequencies which reduce the target pest to a sufficient extent, but minimize harm to natural enemies and thus allow a combination or synergized effect of both the chemical and the biological controls (Theiling and Croft, 1988; Zhang and Sanderson, 1990).

Another important topic in the assessment of side-effects is examining whether natural pesticides or natural enemies themselves affect beneficial organisms, as reported in studies of the impact of entomopathogenic nematodes on non-target organisms (Bathon, 1996). Fransen and van Lenteren (1993) could not find detrimental effects of the entomopathogenic fungi *Aschersonia aleyrodis* Webber on the parasitoid *E. formosa*, while Sterk *et al.* (1995) observed no effect of a commercial strain of *Paecilomyces fumosoroseus* (Wize) Brown & Smith on *P. persimilis*, *E. formosa* and *Onus insidiosus* (Say). However, Pavlyushin (1996) detected direct and sublethal effects of entomopathogenic fungi on Chrysopids in the laboratory.

The present status of resistance of pests or pathogens in greenhouses is often unknown; growers tend to apply excess amounts of chemical, and control is not achieved. The development of tools for monitoring resistance should facilitate the assessment of different management options.

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**DECISION TOOLS FOR INTEGRATED PEST MANAGEMENT**

J. Leslie Shipp and Norman D. Clarke

**12.1. Introduction**

Greenhouse pest and disease problems are often the result of complex interactions among many variables such as greenhouse environment, nutrition, production practices, growing media, other pest and disease outbreaks, economics and environmental and social concerns. As a result, managing or preventing pest and disease outbreaks requires an interdisciplinary approach, which will vary according to the problem. Greenhouse industry is a very technologically-advanced agro-food industry with computerized climate control and fertigation systems in widespread commercial use. These systems offer precise and versatile tools for controlling and manipulating the greenhouse and plant environment, but also affect pest and disease outbreak dynamics. Biological control agents are commercially-available for most of the major insect and mite pests and cultural control measures are also viable management strategies to chemical control, especially for disease prevention (Clarke *et al.*, 1994a). With all these management strategies and other variables that can impact upon IPM, the grower can use as much assistance as possible to collect, collate, understand and integrate, where necessary, the information needed to choose the most viable solution for the problem at that point in time. The purpose of this chapter is to provide an overview of the decision-making process and decision tools as they apply to IPM of greenhouse crops.

**12.2. Decision-Making Process**

Decision-making is the process of selecting and implementing an action with the intention of producing a favourable outcome. The quality of decisions can be enhanced by using a structured, analytic methodology to decision-making. Analytic decision-making is based on logic and considers all available data and alternatives. The structured decision-making process consists of five basic steps: problem recognition and definition, alternative generation, alternative evaluation, alternative selection and decision implementation (Souder, 1980; Tregoe and Kepner, 1981). These steps do not necessarily follow one another sequentially without deviation, but often decision-makers must backtrack and repeat some steps.

Problem recognition and definition begins with recognition of a deviation between actual conditions and established standards or desired conditions. A clear, concise problem statement, defining what the variance is and is not, when and where the variance occurs, etc., should be developed. The problem statement must go beyond the symptoms and identify the true cause of the problem. For example, if *Botrytis cinerea*



Pers.:Fr. infects your crop and you only apply fungicides, you are treating the symptom (*Botrytis*) and are doing nothing to alleviate the cause of the infection. The cause of the infection may be poor sanitation, inadequate climate control or excessive plant stress.

Alternative generation is a creative process whereby alternative solutions are identified. Brainstorming at this time can result in some very novel ideas and also some non-feasible suggestions. Not all suggestions may be used, but discussion of them may help improve upon the more feasible solutions.

Alternative evaluation involves setting goals to be achieved by solving the problem and quantifying each alternative in terms of its value, cost, risk and other decision criteria. Establishing specific and measurable goals assists the decision-maker in quantifying a problem. Most greenhouse growers have many goals, including maximizing profit. Other goals may include increased productivity, increased product quality and employee safety. Decision criteria are attributes of a solution that can be measured or estimated. These attributes are used to evaluate the different alternatives that are generated in step two. Decision criteria for selecting a pesticide may include cost, efficacy, compatibility with biological control agents, safety and days to harvest. Decision aids or tools, such as decision matrix, decision tree, linear programming, simulation models, expert systems and decision support systems, can be used to more fully understand the scope of the problem, the differences among alternatives, and the relative worth of each. [This is only a partial list of the many tools that are available for decision-making. For more information on other decision tools, such as game theory, linear regression, forecasting and network models, the reader is referred to an operations management book by Heizer and Render (1991).]

Based upon the evaluation, the alternative that best satisfies the goal(s) is selected. Numerous methods or decision rules have been suggested for selecting among alternatives (Souder, 1980; Montgomery, 1983) such as the dominance rule (choose A1 over A2 if A1 is better than A2 on at least one attribute and not worse than A2 on all other attributes), lexicographic rule (choose A1 over A2 if it is better than A2 on the most important attribute; if this requirement is not met, base the choice on the next important attribute) and addition of utilities rule (choose the alternative with the greatest sum of weighted values across all attributes). Further analysis of the selected alternative may be conducted to verify the decision and identify possible adverse consequences.

Sometimes the most challenging phase of decision-making is trying to implement the selected alternative. An implementation plan that specifies the barriers and obstacles to acceptance of the decision, and ways that these can be overcome, is as important as the decision itself.

### **12.3. Sources of Information for Decision-Making in IPM**

When making IPM decisions, it is vital that the decision-maker search for information that will help solve the problem. The search for information can help in all steps of the decision process. It may reveal facts about the situation that will result in redefinition of the problem. Valuable insight into the different alternatives and data by which they can be evaluated can be provided. The information search can also reveal how the selected alternative may be implemented.

For greenhouse growers, we only found one survey (van Lenteren, 1990) that was related to sources of information for decision-making. This survey listed growers' journals and study groups as the most important sources for Dutch growers. Surveys of other types of agricultural producers found that significant sources of information include a grower's own experience and records, extension publications and bulletins, extension specialists, grower magazines, universities, colleges and research institutions, other growers, private industry salesmen (chemical, equipment, etc.) and independent consultants (Blackburn *et al.*, 1983; Carlson and Guenther, 1989; Ortmann *et al.*, 1993; Buchner *et al.*, 1996). Greenhouse growers can and do also obtain their decision-making information from similar sources (van Lenteren, 1990).

A grower's own experience and records can be one of the most important sources of decision-making information. If a pest problem reoccurs, a grower can use their records to see how well previously implemented alternatives performed. Records can also be used to obtain evaluation data such as cost and effectiveness of chemicals and biologicals.

Extension publications can provide general recommendations for IPM in greenhouse crops (Anonymous, 1996), while detailed information on specific pests and diseases can be obtained from books (Gerling, 1990; Jarvis, 1992) or other publications (Jarvis and McKeen, 1991; Malais and Ravensburg, 1992). In addition, every grower is advised to own a good pest and disease identification reference (Hussey and Scopes, 1985; Powell and Lindquist, 1992; Howard *et al.*, 1994) and a nutritional disorder identification reference (Winsor and Adams, 1987). These references can assist growers in quickly identifying crop disorders. Commercially produced grower magazines are widely used by growers and often report on new ideas and techniques for IPM.

Government extension advisors have traditionally been the main source of pest and disease management information for growers in many countries. Recently, government cutbacks in several countries have severely reduced the number and availability of the extension advisors. As a result, there has been an increase in the number of private consultants in the greenhouse industry. Sales representatives can also be a valuable source of information, providing advice on the use of their products. Other greenhouse growers, especially study groups, are also an important source of information. Association with other growers allows one the opportunity to obtain, discuss and compare information on new IPM practices and innovations.

### 12.3.1. THE INTERNET

A new source of IPM information is the Internet. The Internet has many features that can be used to assist in the management of greenhouse crops. One of the most widely used features is electronic mail (e-mail). Provided one knows the address, one can send and receive messages from anyone connected to the Internet including other growers, extension advisors and researchers. Another useful tool is the browser. A browser is an application that knows how to interpret and display documents that it finds on the Internet. Most browsers can access other Internet services including Anonymous FTP (File Transfer Protocol for downloading files), e-mail and news groups.

One way for growers to use the Internet is to find information relating to pest and

disease management. Many useful sites can be found on the Internet that are related to horticulture and greenhouse management. Extension/research sites provide many extension documents and information on current research projects for growers. In addition to product information and pricing, commercial sites also provide lots of related information. All sites contain good links to other related sites.

When using information from the Internet, however, one should exercise caution. Anyone can put up a web site and publish anything on the site. Therefore, be aware of the source and quality of the information. Unlike books and journal articles, web documents are not peer reviewed so there is no guarantee that the information is accurate. As well, the main purpose of commercial sites is to advertise their products.

Another consideration when using the Internet for decision support is finding the relevant information that one requires. Searching the Internet using a search engine such as Yahoo <<http://www.yahoo.com/>> can generate thousands of matches. For example, a search for IPM generated 3714 matching sites. Determining which sites are truly helpful can take a considerable amount of time. This problem can be alleviated somewhat by carefully choosing keywords to search. Searching for IPM and greenhouse reduced the number of matching sites to 81. Another option is to find and search topic specific databases. The web site <<http://ag.arizona.edu/Ext/MASTER-GARDENER/>> is a searchable database comprising over 1000 horticultural and agricultural web sites.

## **12.4. Application of Decision Tools for IPM**

Decision tools are techniques for modelling actual systems and are thus simplifications of actual conditions. They have become widely accepted for several reasons. Decision tools or models are less expensive and disruptive than experimenting with the actual systems and can force the decision-maker to analyse the problem in a logical and systematic manner. Decision tools allow managers to ask “what if” questions and evaluate different scenarios. They can also reduce the time needed to make a decision. On the other hand, models can be expensive and time consuming to develop. The results may be misused and misunderstood because of the complexity of models and because models may use assumptions that oversimplify actual systems.

As stated earlier, many tools are available to assist growers in making IPM decisions in the greenhouse. Practical applications of many decision tools in IPM are reviewed in Norton and Mumford (1993). Although none of the many examples presented are specific to greenhouse IPM, the techniques presented can be applied to many greenhouse IPM problems. The following sections discuss the application of decision-making tools to IPM in greenhouses.

### **12.4.1. DECISION TABLES AND TREES**

Decision tables and trees are simple yet powerful tools to assist in the decision-making process. These tools can be used to logically and systematically select among alternatives and the structure provided by these tools can give a valuable framework for further investigations.

### Decision Matrix

The decision matrix is used to select among alternatives using the addition of utilities rule. Consider a hypothetical situation where one must select a fungicide from three different alternatives (Fig. 12.1). In this example, four evaluation attributes or criteria are established and weights of importance are assigned to each. These weights reflect the beliefs, concerns and experiences of the decision-maker. Each alternative is evaluated and graded on a 0 to 10 scale on how well it satisfies the criterion. The grade is multiplied by the weight and recorded. The alternative with the greatest sum of weighted values across all criteria (chemical 2) is selected. If the lexicographic rule had been used, then chemical 1 would be selected.

| Criteria                       | Weight | Chemical 1 | Chemical 2 | Chemical 3 |
|--------------------------------|--------|------------|------------|------------|
| Cost                           | 40     | 10<br>400  | 10<br>400  | 7<br>280   |
| Effectiveness                  | 25     | 10<br>250  | 9<br>225   | 10<br>250  |
| Compatibility with biologicals | 20     | 5<br>100   | 9<br>180   | 8<br>160   |
| Days to harvest                | 15     | 5<br>75    | 8<br>120   | 5<br>75    |
| Total                          | 100    | 825        | 925        | 765        |

#### Rating scale, R

|                |        |
|----------------|--------|
| Excellent      | = 9–10 |
| Good           | = 7–8  |
| Fair           | = 5–6  |
| Poor           | = 3–4  |
| Unsatisfactory | = 0–2  |

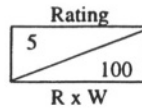


Figure 12.1. Decision matrix to select among fungicides.

### Pay-off Matrix

A pay-off matrix helps the decision-maker economically evaluate alternatives. Pay-off matrices can be used both for decision-making under risk (where the decision-maker knows the probability of occurrence of the outcomes for each alternative) and decision-making under uncertainty (whether probabilities are unknown).

A possible pay-off matrix is presented for thrips control on sweet pepper in Ontario greenhouses under uncertainty (Table 12.1). The pay-off for each combination of alternative and state of nature (an occurrence or situation over which the decision-maker has little or no control) is included in the matrix. In this example, the states of nature are low, medium and high levels of thrips attack. The do nothing alternative

shows the cost of damage caused by the three levels of thrips attack. The other alternatives include both the cost of the control strategy and its ability to reduce thrips levels and damage. The chemical alternative also includes an estimate of yield reduction resulting from crop injury.

**TABLE 12.1. Pay-off matrix for decision-making under uncertainty**

| Strategy           | Level of thrips infestation |         |         | Maximum outcome in row    | Minimum outcome in row | Row average      |
|--------------------|-----------------------------|---------|---------|---------------------------|------------------------|------------------|
|                    | Low                         | Medium  | High    |                           |                        |                  |
| Do nothing         | -6,000 <sup>1</sup>         | -18,000 | -60,000 | <b>-6,000<sup>2</sup></b> | -60,000                | -28,000          |
| Chemical control   | -32,500                     | -36,200 | -49,500 | -32,500                   | -49,500                | -39,400          |
| Biological control | -7,000                      | -16,000 | -32,000 | -7,000                    | <b>-32,000</b>         | <b>-18,333</b>   |
|                    |                             |         |         | Maximax ↑                 | Maximin ↑              | Equally likely ↑ |

<sup>1</sup>Expected loss (US\$/ha) for the different control strategies (control cost + damage loss)

<sup>2</sup>Numbers in bold indicate the preferred strategies

With decision-making under uncertainty, the decision-maker can use three different rules for selecting among the strategies. The maximax (optimistic) rule selects the alternative (do nothing) that maximizes the maximum outcome for every alternative. The maximin (pessimistic) rule selects the alternative (biological control) that maximizes the minimum outcome for every alternative. The equally likely rule finds the alternative (biological control) with the highest average outcome and assumes that each state of nature is equally likely to occur.

With a situation where a grower has kept detailed records of thrips levels in the greenhouse, the probabilities of thrips attacks can be calculated. A pay-off matrix (Table 12.2) can be developed for this situation where the decision is being made under risk. The expected monetary value (EMV) for alternative *i* is:

$$EMV(i) = \sum_{j=1}^n \$ij * p_j$$

where *n* is the total number of outcomes, **\$ij** is the payoff of alternative *i* for outcome *j*, and **pj** is the probability of outcome *j*.

A risk-neutral grower, who is unconcerned with year to year variations in outcomes, would choose biological control, which has the highest EMV (in our example, the lowest crop loss). Most growers are more likely to be risk-adverse and choose a strategy that gives acceptable outcomes at high pest levels. In this case, an extremely risk-adverse grower would also choose biological control, which has the best outcome under

the worst conditions. Similarly, a risk taker may choose to do nothing, which has the best outcome under the best conditions. Although pay-off matrices help to economically select among alternatives, they do not allow for non-economical criteria to be considered (such as compatibility with *Bombus* spp. pollinators). If a cost can be determined for these criteria, then they should be included in the analysis.

**TABLE 12.2. Pay-off matrix for decision-making under risk**

| Strategy           | Probability of thrips levels |              |            | EMV                        |
|--------------------|------------------------------|--------------|------------|----------------------------|
|                    | Low (0.2)                    | Medium (0.5) | High (0.3) |                            |
| Do nothing         | -6,000 <sup>1</sup>          | -18,000      | -60,000    | -28,200                    |
| Chemical control   | -32,500                      | -36,200      | -49,500    | -39,450                    |
| Biological control | -7,000                       | -16,000      | -32,000    | <b>-19,000<sup>2</sup></b> |

<sup>1</sup>Expected loss (US\$/ha) for the different control strategies

<sup>2</sup>Number in bold indicates the preferred strategy

### *Decision Trees*

Many problems consist of sequential decisions over time. When more than one set of decisions are necessary, a decision tree is appropriate. A decision tree is a graphic display of the decision process which indicates decision alternatives, states of nature and their respective probabilities, and pay-offs for each combination of alternative and state of nature (Heizer and Render, 1991).

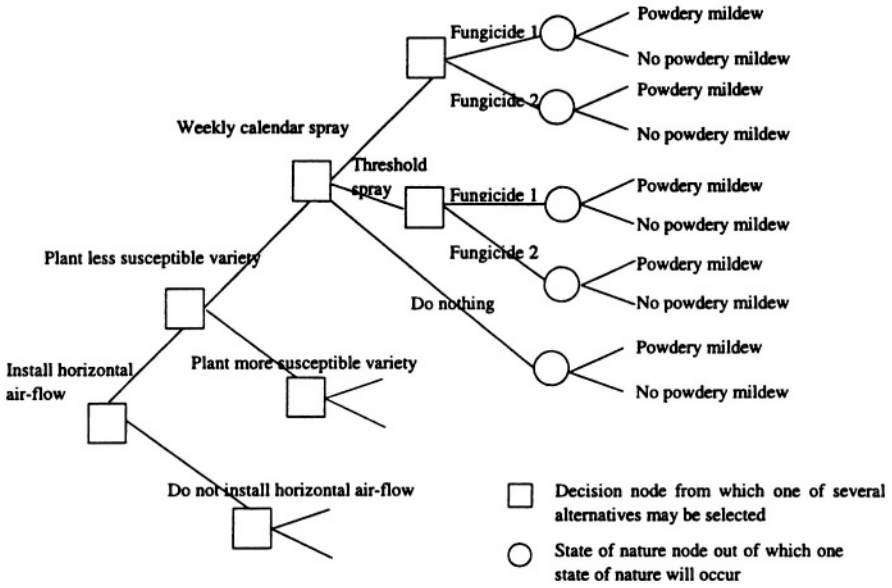
A decision tree is shown for powdery mildew management in Fig. 12.2 (state of nature probabilities and pay-offs are not included). Note that as the crop season progresses, the number of options decreases. If the probabilities of powdery mildew occurrence and the pay-offs are added to the tree, the EMV can be calculated for each branch and the best decision determined (Heizer and Render, 1991). Even if probabilities and pay-offs are not known, decision trees are still very useful by laying out all possible options and providing a framework for deciding which options and strategies need further investigation.

#### 12.4.2. DATABASE SYSTEMS

Database systems (DBS) consist of a collection of interrelated data and a set of application programs to access and manipulate the data. The different data items are stored in related files or tables. The application programs usually provide functions to enter, edit, browse, query and analysis the data.

DBS can help solve pest and disease management problems in several ways. First, the development of a DBS can help to better organize and understand the problem. One of the first steps in developing a DBS is to develop a data model. The data model identifies the paths of information flow, specific data items and relationships among data items. DBS can also assist decision-making by storing detailed records of past pest

and disease management strategies, along with the outcomes and costs of these strategies. These records can help the decision-maker select among different strategies based upon past results.



**Figure 12.2. Decision tree for powdery mildew management.**

There are many commercial greenhouse cost accounting and financial management DBS available. These packages usually provide facilities to record costs (including pest control) and sales throughout the cropping season (Brumfield, 1992). These DBS can assist in tracking pest management costs in the greenhouse.

A DBS for greenhouse pest surveillance, Emerald ICM, is commercially available (Van Vliet Automation Ltd, 1996). Pest survey data is collected with a hand-held computer in the greenhouse and uploaded to a personal computer. The data is used to generate colour maps of pests and their severity over time. Applications of pesticides, fungicides and biologicals are also recorded. Emerald ICM allows the grower to monitor the progress of pest and predator movement and analyse the effectiveness of different control strategies.

12.4.3. SIMULATION MODELS

A model is a description of a system. Models may be scaled physical objects, mathematical equations and relations, or graphical representations of actual systems. For purposes of this discussion, a simulation model is a mathematical-logical representation of a system which can be exercised in an experimental fashion on a

digital computer (Pritsker, 1984). In terms of decision-making, simulation models allow us to examine different alternatives and how these alternatives perform under different conditions.

Simulation models have been used to study various field pests (Rabbinge *et al.*, 1989; Goodenough and McKinion, 1992). Pest population growth, fungal passive dispersal, insect active dispersal and predator-prey interactions have been simulated (Rabbinge *et al.*, 1989). Pest systems have also been modelled in the greenhouse environment.

Nachman (1991) simulated the dispersal of two-spotted spider mite (*Tetranychus urticae* Koch) and its predator *Phytoseiulus persimilis* Athias-Henriot in a greenhouse cucumber crop. Spider mite oviposition rate, death rate and emigration rate to other plants are dependent on the health of plants. The birth, death and emigration rates of the predator are linked to the predation rate. The simulation model was used to study fluctuations in overall population densities within the greenhouse.

Biological control of the leafminer species *Liriomyza trifolii* (Burgess) in greenhouse-grown chrysanthemums (Heinz *et al.*, 1993) and *Liriomyza bryoniae* (Kaltenbach) in greenhouse tomato (Boot *et al.*, 1992) by the parasitoid *Diglyphus isaea* (Walker) have been simulated. Heinz *et al.* (1993) assumed a constant greenhouse temperature of 27°C and that the population dynamics of the leafminer are independent of the quality of host plant. Simulation results indicated that successful biological control was unlikely when parasitoid releases are initiated later than 14 d after planting regardless of the release rate. Using a different approach, Boot *et al.* (1992) used ambient temperature and tomato leaf nitrogen content to determine the population dynamics of *L. bryoniae*. The timing and growth of leafminer generations were simulated and the results validated with greenhouse experiments, although no practical strategies for parasitoid were given. These two models could be used to explore different strategies for biological control of leafminers.

Disease infection and progression (Jarvis, 1992) and arthropod pest populations (Minkenberg and Ottenheim, 1990) are dependant upon plant nutrition. Crop growth simulation models, such as those developed for greenhouse tomato (Dayan *et al.*, 1993) and cucumber (Marcelis, 1994) could supply input parameters to pest population models. A feedback loop could potentially predict crop yield reductions due to the pest. Greenhouse microclimate also affects disease development (Jarvis, 1992) and insect population dynamics (Minkenberg and Helderma, 1990; Shipp and Gillespie, 1993). Microclimate models that simulate the climate within the crop canopy (Goudriaan, 1989; Yang, 1995) could be combined with disease and pest models to investigate climate control strategies for pest and disease management.

Currently, pest management simulation models are being used at the research level to better understand interactions between pests and their control agents. An example is the simulation model developed by van Roermund *et al.* (1997) to evaluate release strategies for the parasitoid, *Encarsia formosa* Gahan, for control of greenhouse whitefly, *Trialeurodes vaporariorum* (Westwood) on greenhouse crops. As personal computers become more powerful, we envision simulation models being used by growers to evaluate pest management strategies. However, before this happens more research needs to be done to develop models for other pests and diseases. As well, these



models must be validated and in a format that non-modellers can understand how to operate the models and interpret the outputs. Model validation can be difficult due to inadequate experimental data, inappropriate assumptions and lack of knowledge regarding some of the physical processes being modelled. Despite these problems, simulation models certainly have a lot of potential for analysing various pest management strategies and understanding interactions between the pest, its biological control agents, the crop and the crop microclimate.

#### 12.4.4. EXPERT SYSTEMS

Expert systems (ES) are computer programs that emulate the decision-making ability of a human expert. ES contain knowledge in one specific problem area or domain as opposed to knowledge about general problem-solving techniques. ES usually consist of a set of rules that were obtained from an expert to solve a particular problem, and an inference engine that decides which rules to execute. In terms of decision-making, ES can be used as tools for summarizing information and knowledge, selecting among alternative solutions, exploring and evaluating alternative scenarios, assessing risks, diagnosing problems, outlining approaches to problem solving and teaching non-experts the problem-solving approaches of experts (Holt, 1989).

ES technology has been applied to greenhouse pest management. The most common type of application is the diagnosis of crop diseases and pests. Several ES have been developed to identify greenhouse tomato disorders and recommend possible control actions for the identified disorder (Blancard *et al.*, 1985; Gauy and Gauthier, 1991). With these ES, the user is prompted to enter information about the symptoms displayed by the tomato plant. The ES then uses expert rules to match the observed symptoms with a disorder.

Boulard *et al.* (1991) developed an ES to determine the climatic setpoints to control the climate for greenhouse tomato. In addition to information on the outside weather conditions and the current greenhouse climate, the ES also incorporated expert rules on climate and tomato diseases and physiological aspects. This system attempted to optimize conditions for energy use, crop growth and disease prevention and control. In a separate research effort and using a different approach, Manera *et al.* (1991) developed an ES with similar objectives for greenhouse production and pest management under Mediterranean conditions.

Although not specific to greenhouses, other pest management-related ES have been published. Logan (1988) developed an expert system to automatically assemble a model describing insect population phenology. The program offers time savings and compares well with a human expert. Messing *et al.* (1989) describe NERISK, an expert system that assesses the impact of pesticides on beneficial arthropod predators and parasitoids in agricultural systems. Similar ES could be developed and be useful for greenhouse pest management.

Although ES are very good at assisting in the decision-making process, certain issues should be considered before undertaking the development of one. First, it takes considerable time and resources to complete an ES. The time and commitment of an expert(s) is required as well as a knowledge engineer (an individual who extracts,

organizes and programs the knowledge of an expert). The intended user of the ES must be consulted and involved during the development. Ongoing maintenance is required (McClure, 1993) as the knowledge contained in the ES may become outdated. These and other issues relating to ES development are reviewed by Clarke *et al.* (1994b).

#### 12.4.5. DECISION SUPPORT SYSTEMS

Technically, any aid that assists a decision-maker could be defined as a decision support system (DSS). However, for this discussion we will consider DSS as computer programs that help decision-makers solve problems through direct interaction with data and models (Sprague and Watson, 1989). DSS usually use a combination of decision tools including ES, database systems, simulation and other computer models.

DSS have been used to solve pest management problems for field crops such as cotton (Goodell *et al.*, 1990) and apples (Travis *et al.*, 1992). The cotton DSS uses a cotton crop simulation model and expert advice on pests, diseases and weeds to provide recommendations on the timing of irrigation, fertilizer and pesticides. In the greenhouse, Jones *et al.* (1989) described a DSS where crop models were combined with an ES to choose optimal environmental setpoints for greenhouse tomato. The ES contained a knowledge base for variables that have not been well modelled, such as the length of time that humidity may remain high without a disease outbreak.

BOTMAN (Shtienberg and Elad, 1997) makes decisions concerning whether to spray the biological control agent Trichodex (developed from an isolate of *Trichoderma harzianum* Rifai T39) or fungicides for integrated biological and chemical control of *B. cinerea* in non-heated greenhouse vegetable production. BOTMAN uses weather forecasts, past weather and a *B. cinerea* risk index to predict the severity of outbreaks of grey mould. Based upon the expected severity, application of the biological control agent or a fungicide is recommended. Results show that BOTMAN controlled *B. cinerea* as well as weekly fungicide applications while significantly reducing the number of fungicide applications. Compared to a strategy of weekly Trichodex applications, BOTMAN was also significantly better. Another system, GREENMAN, was developed to deal with other greenhouse diseases. It is based on criteria that are similar to BOTMAN and, likewise, controls diseases such as leaf mould [*Fulvia fulva* (Cooke) Cif.] and white mould [*Sclerotinia sclerotiorum* (Lib.) de Bary] (Elad and Shtienberg, 1997).

A DSS for integrated management of greenhouse vegetables [Harrow Greenhouse Manager (HGM)] has been developed at the Greenhouse and Processing Crops Research Centre, Harrow, Ontario, Canada. The HGM contains modules for the following: (i) expert diagnosis of insect and mite pests of the crop; (ii) expert diagnosis of crop diseases and physiological disorders; (iii) IPM recommendations for pest, disease and physiological disorder control, and identification of conflicting recommendations; (iv) cost allocation, including pest control expenses, to crops; (v) record-keeping capabilities including crop production, labour, insect counts, disease occurrence and control measures that were implemented; (vi) tools to determine tank mixes for fertigation systems; (vii) analysis section to analyse relationships between any recorded entity (such as insect counts and crop yield); and (viii) climate data retrieval

from climate control systems that are BACnet compatible. Currently, HGM contains the knowledge for greenhouse cucumber and tomato crops.

The approach in the development of the HGM was to provide a framework for integrated crop management (ICM) (Clarke *et al.*, 1994a). ICM is a multidisciplinary approach that integrates pest and disease protection strategies with routine cultural practices and environmental and fertigation regimes into a common decision-making process. It is not acceptable to manage one component of the greenhouse in isolation since the component can potentially affect all other aspects of the greenhouse crop.

DSS have a lot of potential in greenhouse pest management, particularly if greenhouse climate is integrated with control strategies. To utilize climate in controlling diseases and insects, DSS will need to control the microclimate at the leaf surface. DSS will need to integrate models that predict the microclimate at the plant surface from spatially averaged climate data with crop and pest simulation models. Computerized DSS will be required at the grower level to enable growers to interpret all the information necessary for ICM.

## 12.5. Conclusions

Decision-making is a very important part of greenhouse pest management. However, it is becoming more complicated and demanding in an industry that rapidly changes yearly. The grower can no longer rely on the old tools for information gathering and decision-making. Greenhouse operations are often 2–10 ha in size and must be operated more like a business corporation rather than a family-owned operation.

The Internet can provide a readily accessible and up-to-date source of information that can link a grower to current technology that is being used throughout the world. In the future, databases, DSS and other decision tools are going to become the main method for assisting the grower in making decisions as these tools can handle large data sets in an organized fashion and quickly form conclusions or solve problems.

Interpreting and managing technical information for decision-making without computers will be beyond the means of individual growers. First, the quantity of data required is large and the management of this data is impossible without computer systems. Computerized climate-control systems can quickly generate megabytes of data. Add crop production data, fertigation records, pest count records, etc. and the data quickly becomes unmanageable without computer technology. Second, the relationships between the various crop production factors are complex. For example, greenhouse climate can affect the effectiveness of entomopathogens, but, at the same time, provide conditions that are conducive to a disease outbreak, such as *B. cinerea*. In this case, climate directly affects the crop, a biological control agent and the epidemiology of a plant pathogen. Expert knowledge or simulation models can help improve our understanding of how all these factors interact.

Although the systems developed to date are useful for providing solutions to greenhouse crop management problems, the technology is still a long way from a DSS that provides true ICM strategies. To meet this goal, an improved understanding of the response of crops, pests, pathogens and biological controls to climate is required. Also

those responses need to be mathematically or heuristically modelled and incorporated into DSS. Finally, DSS have to be validated in commercial greenhouses under actual production conditions.

AH of the issues relating to database systems, simulation models and expert systems apply to DSS. In addition, we have found that the user interface and data entry procedures play a much bigger role with DSS. With pest control, future control actions are dependent upon previously implemented controls. For example, using *Encarsia* for whitefly control may limit the chemicals available for control of grey mould. However, the HGM does not know *Encarsia* is in the greenhouse unless the grower has recorded it in the database. Therefore, user interface and data entry procedures must be structured to streamline the time that it takes for the grower to enter the required data. Growers are showing a strong interest in databases and DSS and are beginning to incorporate them into their daily operational decision-making processes.

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