Botrytis: Biology, Pathology and Control

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Top row: Scanning electron microscopy (SEM) images of conidiophores and attached conidia in *Botrytis cinerea*, top view (*left*, Brian Williamson) and side view (*right*, Yigal Elad); hypothetical cAMP-dependent signalling pathway in *B. cinerea* (*middle*, Bettina Tudzynski).
Second row: Identification of a drug mutation signature on the *B. cinerea* transcriptome through macroarray analysis - cluster analysis of expression of genes selected through GeneAnova (*left*, Muriel Viaud et al., INRA, Versailles, France, reprinted with permission from 'Molecular Microbiology 2003, 50:1451-65, Fig. 5 B1, Blackwell Publishers, Ltd'); portion of Fig. 1 chapter 14, life cycle of *B. cinerea* and disease cycle of grey mould in wine and table grape vineyards (*centre*, Themis Michailides and Philip Elmer); confocal microscopy image of a *B. cinerea* conidium germinated on the outer surface of detached grape berry skin and immunolabelled with the monoclonal antibody BC-12.CA4 and anti-mouse FITC (*right*, Frances M. Dewey (Molly), Chapter 11).
Bottom row: SEM images of *B. cinerea* conidia germinated on a bean leaf (*left*, Y. Elad); on raspberry stigma (*centre*, B. Williamson) and on a rose petal (*right*, Y. Elad).

Contents

Preface

There has been great progress in the science of *Botrytis* spp. and the diverse and complex interactions they make with plants, and the application of this science in agriculture and horticulture throughout the world. Therefore *Botrytis* spp. are of keen interest to scientists, crop consultants, farmers and students of agribusiness and plant protection. It is important to present this knowledge in one comprehensive volume that is a synthesis of this research endeavour. This book is being published on the occasion of the 2004 Thirteenth International *Botrytis* Symposium in Antalya, Turkey in the series following *Botrytis* symposia that took place in Invergowrie, Dundee, Scotland (1966); Siut-Truiden, Belgium (1968); Sweden (1971); Teresin, Skierniewice, Poland (1973); Gradignan, Bordeaux, France (1976); Amersfoort, The Netherlands (1979); Aberdeen, Scotland (1982); Alba, Torino, Italy (1985); Neustadt, Germany (1989); Gouves, Heraklion, Crete, Greece (1992); Wageningen, The Netherlands (1996); and Reims, France (2000).

The book is the result of intensive work of 43 authors, all of whom are leading scientists in the *Botrytis* sciences. Thanks to them the book is a comprehensive update of the subject and to all of them we owe our gratitude. The twenty interconnected chapters of the book are grouped according to three major themes: the fungus and its pathogenicity factors; plant reactions to infection; and epidemiology and management of important *Botrytis*-incited diseases. This book adopts a multidisciplinary approach to integrate the state-of-the-art knowledge in all key areas of common interest in the fungi and their plant interactions. The book includes detailed reviews of *Botrytis* spp. and the diseases they cause in plant systems and provides a comprehensive description of these fungal necrotrophs, including their diversity of response to the environment, their speciation and relatedness, sources of variation for evolution and molecular genetics and genomics. Aspects of *Botrytis*-host interactions, pathogenicity factors, the plant's reactions to infection, morphology and cellular organisation, signalling, key enzymes, reactive oxygen species and oxidative processes in disease on-set, secondary metabolites as plant defence substances and the role of phytohormones in such reactions are emphasized in the book. Several innovative approaches for disease management of this group of destructive pathogens and methods of detection, epidemiological studies and chemical and biological control are also discussed.

The number of publications concerning *Botrytis* spp. in international databases has increased steadily in the last three decades from c. 170 to more than 350 per year. Inevitably only a small selection of these publications is cited. During the compilation of this book the aim was to create a most comprehensive treatise on the rapidly developing science of *Botrytis* and to serve as a stimulus to future research for the benefit of agriculture and horticulture and all those who serve these industries.

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during sabbatical leave taken in the School of Biological and Chemical Sciences, Birkbeck, University of London and Y. Elad is grateful to J. L. Faull and S. Baker for their interest in this endeavour. B. Williamson acknowledges funding from the Scottish Executive Environment and Rural Affairs Department during the preparation of this book and for a 30-year period of work on *Botrytis cinerea* and other soft fruit pathogens at the Scottish Crop Research Institute, Dundee.

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21 April 2004

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CHAPTER 1

BOTRYTIS SPP. AND DISEASES THEY CAUSE IN AGRICULTURAL SYSTEMS – AN INTRODUCTION

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Abstract. Some leading characteristics and historical notes on *Botrytis* spp. are described here. *Botrytis* spp. infect many host plants in all climate areas of the world, infecting mainly upper plant parts at pre- and post-harvest stages. Bulbs, seeds and other propagation material also suffer infection. Infection can occur in high humidity in the presence or absence of water films. Infection may be quiescent, aggressive, restricted or widely developing. The production of high numbers of conidia poses a long lasting threat to susceptible hosts. Genotypic and phenotypic variation is most important in the broad spectrum pathogen *B. cinerea*. Moreover, changes in populations in response to selection by exposure to xenobiotics, especially fungicides, are quite common in the genus and fungicide resistance has been recorded in *Botrytis* populations throughout the history of the modern fungicide era. Detailed studies on the precise conditions that promote infection, disease development and survival of inoculum have provided the essential epidemiological information required for design of control strategies. For example, cultural methods have been developed that increase aeration and drying of the plant canopy to reduce the risk of *Botrytis* epidemics. The increasing requirement for alternative approaches to reduce farmers' dependency on use of fungicides led to the evaluation and exploitation of potential biocontrol agents capable of substantial disease suppression in a commercial context, and within integrated crop management systems.

1. Introduction

It is almost a quarter of a century since a major textbook on *Botrytis* spp. was published (Coley-Smith et al., 1980). That erudite text was a milestone in plant pathology and much of the information it contained is still valid today. However, there have been many important scientific advances in the understanding of these interesting and often destructive fungi since that time and it is appropriate that a new volume is published to describe these findings. This book is a distillation of knowledge obtained about *Botrytis* species during the last 25 years. Each chapter describes a particular aspect of fungal biology and its impact on disease processes and host response. New technologies have arisen that have been most rewarding

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when applied to long-standing problems or to test new hypotheses and many of these are covered in this book. Although the chapters cover specific topics and should stand-alone to some extent, inevitably there is some overlap. The editors have attempted to provide linkage between chapters where possible so that readers can follow associated material to better understand the practical implications of the advances made in fundamental science. In the following introductory text we provide some historical notes to make a bridge with the new information offered in later chapters.

Botrytis cinerea and other *Botrytis* species are important pathogens of nursery plants, vegetables, ornamental, field and orchard crops and stored and transported agricultural products (Chapters 14-17 and 19). Considerable effort is invested in protecting the agricultural produce against *Botrytis* before and after harvest. The market size for anti-*Botrytis* products have been US$ 15-25 million in recent years. The intensity of anti-*Botrytis* measures taken by farmers continued unabated throughout the last 20 years but our understanding of the processes that govern *Botrytis* life cycles, pathogenicity and epidemiology have become comprehensive. MacFarlane (1968, cited in Jarvis, 1977) counted in the Review of Applied Mycology 235 host species belonging to a variety of families affected by *B. cinerea*. Other species of *Botrytis* are specific to certain hosts; they have restricted host range and usually affect single or a limited number of hosts. Interestingly, the more restricted host specificity in *Botrytis* spp. occurs on monocotyledonous plants.

Over the last 125 years, *Botrytis* spp. have been investigated by an increasing number of specialists in diverse fields including chemistry, biochemistry, molecular and cell biology, genetics, morphology and histology, taxonomy, host-parasite interaction, ecology and epidemiology (Jarvis, 1977; Coley-Smith et al., 1980; Verhoeff et al., 1992). They have been the subject of an immense number of published studies.

2. Geographical and ecological occurrence

In the introduction to the book 'The Biology of Botrytis' (1980) Coley-Smith referred to *Botrytis* spp. as temperate area pathogens perhaps because of the vast research that has been carried out in such areas or due to its importance on vineyard grapes. Nevertheless, species of the genus *Botrytis* occur wherever their hosts are grown, ranging from tropical and subtropical to cold areas. For example Anderson (1924) recorded *B. cinerea* in Alaska and Yunis and Elad (1989) dealt with this pathogen in warm and dry areas. A rapid rate of conidial germination, infection, mycelium growth and conidiation occur in many *Botrytis* spp. under a wide range of microclimate conditions and pose severe disease management problems all around the world.

The potent effect of near-ultraviolet light (320-400 nm) on induction of conidiation and the characterisation of potential photoreceptors was discussed fully by Epton and Richmond (1980). However, new research on the importance of light quality for infection with inoculated conidia is cited in Chapter 2. *Botrytis* spp. are regarded as high humidity pathogens (Chapter 2) and their conidia germinate at high

humidity (Snow, 1949). In many patho systems infection occurs in the presence of a film of water on the susceptible plant tissue. The role of water drops (Brown, 1916) and nutrients in germination and infection have been long recognised. However, it is interesting that the pathogen is also able to infect plants when no film of water exists on the plant surfaces (Williamson et al., 1995; Elad, 2000; Chapter 2). A change in spread, importance and range of hosts that are severely affected by *Botrytis* spp. is partly associated with the increasing importance of protected cropping in greenhouses or plastic tunnels (Chapter 17) and partly with change in the intensification and growth practices of open field crops. Although *Botrytis* spp. can be isolated from some soils (Lorbeer and Tichelaar, 1970) and are also present on seeds, bulbs and corms (Chapters 15 and 16), they are more commonly isolated from upper plant parts (leaves, flowers, fruits, buds and stems), and in some cases upper root parts and stem bases. Symptoms range from restricted lesions to dry or spreading soft rots, with or without the appearance of conspicuous sporulating colonies. *Botrytis* spp. are highly active at moderate temperatures, however, the ability of *B. cinerea* to be active at temperature as low as 0^{o}C (Brooks and Cooley, 1917) makes it an important pathogen of stored products and a challenge for disease management during storage and shipment (Chapter 19). Most *Botrytis* spp. sporulate profusely and dry conidia are dispersed through the air making this group of pathogens a constant threat to susceptible crops. The limiting factor for epidemic outbreaks is usually the occurrence of the appropriate microclimatic conditions, rather than the amount of inoculum (Shtienberg and Elad, 1997).

3. Variability and adaptability

Botrytis has been recognized as a genus since Micheli erected it in 1729. In early times it was sometimes confused with *Sclerotinia* spp. but clarifications were made (Smith, 1900) and confusion was dispelled (Whetzel, 1945). The connection between *Botrytis* spp. and their *Botryotinia* teleomorphs was finally established during the 1940s and 1950s (Groves and Loveland, 1953). Four decades later, improved techniques for culture and spermatization (Faretra et al., 1988) allowed the mating of *Botrytis* strains for genetic analysis. Having multinucleate conidia and hyphal compartments, *Botrytis* isolates have a tendency to change constantly during successive generations *in vitro* and under field conditions. Genotypic and phenotypic variation is very common in *B. cinerea* (Chapter 3). Use of DNA population markers and sexual and vegetative compatibility studies have revealed limited evidence of clonality in *B. cinerea*. The roles of sexual reproduction and heterokaryosis in the determination of variation are still under study.

Changes in populations selected by xenobiotics are quite common in this species (Chapter 3). Development of resistance to fungicides has been recorded in *Botrytis* populations throughout the history of the modern fungicide era (Chapter 11). Reavill (1954) noted that *B. cinerea* could tolerate chlorinated nitrobenzene fungicides and when systemic benzimidazole fungicides were first used *Botrytis* spp. rapidly developed resistant isolates (Bollen and Scholten, 1971), later followed by resistance to the dicarboximides (Katan, 1982). A decade later the molecular basis of these

mutations was identified (Yarden and Katan, 1993). The management of *Botrytis* by chemical fungicides still poses a serious challenge to crop advisers (Chapter 11).

Development of complex strategies to cope with *Botrytis*-incited diseases has been necessary since the 1980s (Vincelli and Lorbeer, 1989). Following detailed studies on the precise conditions that promote infection and disease development, cultural methods were developed giving farmers a range of tools to assist in avoiding serious crop damage. Cultural methods that ensure ventilation and drying of plant canopy after rain, whilst maintaining adequate water supply to the roots, are the most effective means developed so far for prevention of *Botrytis* epidemics (Elad and Shtienberg, 1995). Rational warning systems based on conditions highly conducive to spore germination and host penetration for disease development have been developed for some crop systems (Chapter 18). Microorganisms on plant surfaces interact with Botrytis germination conidia (Newhook, 1951, 1957; Wood, 1951; Bhatt and Vaughan, 1962; Blakeman and Fraser, 1971; Blakeman, 1972) or conidiation (Köhl and Fokkema, 1993). Increasing public awareness of some potential drawbacks of chemical fungicides was addressed by the development of alternative control measures making use of microbial antagonists that are capable of disease suppression (Dubos, 1992); some of these agents were developed subsequently into commercial products (Elad and Freeman, 2002), but they are still commercially much less significant than the chemical measures (Chapter 13).

4. Quiescent, restricted and aggressive infection

One intriguing phenomenon associated with *Botrytis* infection is the ability of this pathogen to be quiescent in the host tissue for varying periods (Williamson, 1994; Elad, 1997). Originally this phase of infection was termed ‘non-aggressive’ as opposed to aggressive when lesions are expanding (Beaumont et al., 1936) and later the phenomenon was described as latent or quiescent infection and found to be common in many hosts (Jarvis, 1962; Verhoeff, 1970; Chapter 2). The ultrastructure of *Botrytis*-plant interactions is described in Chapter 5.

Plants possess a range of pre-formed and induced defences for combating an infection. The antifungal activities of the induced phytoalexins, such as wyerone in *Vicia faba*, were described fully by Mansfield (1980). Many of these defences include secondary metabolites: stilbenes including resveratrol, saponins including α-tomatin, cucurbitacins, proanthocyanidins and tulipalin A, structural barriers, cell wall modifications, but also the pathogenesis-related (PR) proteins (Chapter 9). *Botrytis* species have evolved mechanisms to counteract some of these defence responses.

As a pathogen of grape berries, *B. cinerea* is economically extremely important. Its plant-pathogen interactions and epidemiology were thoroughly studied and reviewed (Jarvis, 1980; Ribéreau-Gayon et al., 1980). The pathogen may completely destroy grape berries, inflicting heavy crop losses as grey mould. Alternatively, under certain conditions, it may cause a slow decay permitting the berries to desiccate considerably. Such dry berries affected by 'noble rot' are harvested and processed into valuable sweet wines (e.g. the Sauternes of France, the

Trockenbeerenauslese of Germany, the Aszu of Hungary and Botrytisized wines in other places). Grapes affected by the destructive grey mould are of low value for making wine not only because of the weight loss but also because of interference with fermentation and changing the flavour and colour of the wine. Among all the many *Botrytis* plant hosts, grey mould management in vineyards is therefore the most important target for agrochemical companies and researchers. The noble rots are not described in this book because they were covered extensively by Ribéreau-Guyon et al. (1980)

Scientists were fascinated by *Botrytis* conidial infection of plant tissues very early in plant pathology. Ward (1888) described the infection of lilies by germ tubes of a *Botrytis* spp. In early times penetration was regarded as a purely mechanical process (Blackman and Welsford, 1916). McKeen (1974) described enzymatic dissolution of faba bean cuticles that triggered three decades of research that has given a vast amount of information on secreted hydrolytic enzymes and their involvement in penetration and tissue maceration by *Botrytis* (Chapter 7). *Botrytis* spp. have turned out to be an important model for host cell wall enzymatic degradation, and before the turn of this century valuable molecular biological research uncovered some of the genes responsible for *Botrytis* pathogenicity (Ten Have et al., 2002; Chapters 4 and 7).

Over the last 25 years there have been substantial advances in methodologies for separation, quantification and identification of fungal and plant metabolites and other labile chemical species. Recent work provides evidence that *B. cinerea* exploits the production of active oxygen species (AOS) in colonising plant tissues (Chapter 8). Hydrogen peroxide and other AOS are produced by the fungus and interact with the plant-based antioxidant systems in determining the outcome of the infection process. Biochemical processes appear to be of importance for lesion development, and the perturbation of the free radical chemistry (Muckenschnabel et al., 2003). Transition metal redox processes (particularly those involving iron), the regulation of enzymes (of both plant and fungal origin), the production of toxic metabolites in the host, and host signalling and programmed cell death are all involved in these processes.

5. Molecular basis of host-parasite interactions

The availability of molecular genetic techniques since the late 1980s brought a revolutionary break-through in the understanding of the pathogenic strategies of *Botrytis*. It allowed for the first time the unequivocal identification of pathogenicity/virulence genes and hence an ability to define molecular targets for developing innovative fungicide and resistant host plants in the future (Chapters 4, 7, 8, 20). Since the first successful molecular transformation of *B. squamosa* (Huang et al. 1989), molecular tools and techniques have been rapidly adapted to the special requirements of *Botrytis.* Investigations using these tools have increased exponentially in the last few years, especially making it possible to perform targeted gene inactivation and functional analysis of all the putative pathogenicity factors identified in the wealth of biochemical, physiological and genetic data of the last

decades. As a consequence of these innovations, the science of molecular genetics underpins many of the chapters in this book. A list of 45 genes has so far been functionally identified (Chapter 4). However, only very few of the "classical" candidate genes have survived the rigorous molecular testing, which in some cases was unexpected. Recent work has also established that cyclic AMP (cAMP) and conserved MAP kinase signalling pathways play crucial roles during pathogenesis in *B. cinerea* (Chapter 6).

Molecular tools today offer many more possibilities for testing long-established hypotheses: the availability of "genomics" tools allows unbiased approaches which will give us a complete picture of the factors involved in the complex interaction processes of this potent and variable pathogen and assist the development of specific alternative defence strategies, including modified host resistance (Chapter 20). In combination with high-throughput screens it will be possible to develop new fungicides based on our detailed knowledge of the refined strategy of *Botrytis* to overcome its host's defence. However, due to the high variability of *B. cinerea* the fight probably never will be finally settled!

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CHAPTER 2

THE ECOLOGY OF *BOTRYTIS* ON PLANT SURFACES

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Abstract. The initiation of disease by members of *Botrytis* species depends on a complex sequence of biological events involving host and environment sensing, chemical and physical interactions between the fungal propagules and the host surface and the microbial interactions on the surface of the host. The pathogen's inoculum is central to the understanding of this interaction. This chapter describes the inoculum ecology of *Botrytis* species on plant surfaces and relates this information to an understanding of disease initiation. *Botrytis* species deploy several propagules and survival structures. A knowledge of the precise behaviour of these propagules, especially the hydrophobic conidia, when dispersed and deposited on the host at high relative humidity in the presence or absence of water droplets is important for disease initiation and control. The responsiveness of propagules to the environment, and the diversity shown in attack strategies by these pathogens are discussed with examples of the infection pathways used. Special comment is made about suitable inoculation procedures to study grey mould in leaves and fruits.

1. Introduction

Botrytis species have a necrotrophic life style occurring as pathogens infecting a single specific host or closely related host, or as the broad spectrum pathogen *B. cinerea* infecting numerous host plants: after infection and death of host tissues all these fungi can survive and sporulate as saprophytes on the necrotic tissue, or produce long-term survival structures, such as sclerotia. These survival structures can be associated with living plants or with plant debris lying on or buried in soil. For species more specialized in their parasitism (*B. aclada*, *B. byssoidea*, *B. squamosa*, *B. gladiolorum*, *B. tulipae*, *B. elliptica*, *B. fabae*), the inoculum source will inevitably be within the crop, or debris from a previous crop in the vicinity. For *B. cinerea*, for which host range is extremely wide, the primary inoculum also is most likely generated within the crop (Johnson and Powelson, 1983), but the potential for incoming primary inoculum from a different crop or weed host is greater than for the host-specific pathogens, and will be affected by the phasing of crop growth and harvest within a district or region.

The fungus exists in the different habitats as mycelia, micro- and macro-conidia, chlamydospores, sclerotia, apothecia and ascospores and these are dispersed by

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diverse means (Jarvis, 1980b). Although *B. cinerea* releases its macroconidia mainly in dry air currents, it is surprising that the majority of published work describes infection arising from suspensions of conidia in water droplets. This chapter summarises the new information available about the behaviour of *B. cinerea* and other *Botrytis* spp. and their responsiveness to different micro-environments, especially the effects of relative humidity (RH). It is particularly difficult to measure and maintain the RH of a host when inoculations are made and the host is incubated for periods to determine the outcome of the interaction. Harrison et al. (1994) reviewed these technical difficulties and devised specialised equipment that provides the best regulation of RH known to the authors. Results of work performed with dry-conidial inoculations, as well as the most recent achievements in inoculation with water droplets, are discussed.

2. Survival

The disease cycles of *Botrytis* species and the growth habit and phenologies of their host plants are often inextricably linked. Dormant or metabolically inactive fungal structures play a central role in each of these disease cycles. Each part of the fungus thallus can serve as a survival structure.

2.1. Sclerotia

All species of *Botrytis* form sclerotia which may, depending on isolate and cultural conditions, differ in size and shape. Sclerotia are generally considered to be the most important structures involved in the survival of *Botrytis* species. Sclerotia can survive adverse environmental conditions, can produce apothecia after a sexual process and possess a considerable capacity for producing successive crops of conidia in many *Botrytis* species (Coley-Smith, 1980). Under laboratory conditions, *B. cinerea* sclerotia continue to sporulate for about 12 weeks after the production of the first crop of conidia (Nair and Nadtotchei, 1987). Suppression of sporulation when the conidia were left on sclerotia and resumption of sporulation when the conidia were removed from the surface could extend the period of conidial production. Under natural conditions, rainfall would be expected to dislodge conidia from germinating sclerotia and initiate conidial production by removing the suppression in sporulation.

The internal structure and histochemistry of sclerotia of *B. cinerea* and *B. fabae* are similar; the rind walls contain melanic pigments, the medullary hyphae are surrounded by a continuous matrix of β-glucans, and the intracellular nutrient reserves are protein, glycogen, polyphosphate and lipid (Backhouse and Willets, 1984). The genetic control of the switch from rapid vegetative growth to production of sclerotia is not known. Recent work with the closely related species *Sclerotinia minor* suggests that β-carotene may be important for protection against oxidative stress when sugars and other nutrients decline in presence of light (Zervoudakis et al., 2003).

Formation of sclerotia in the field is generally associated with plant tissue. However, it also occurs in insects. Louis et al. (1996) demonstrated the ability of the vinegar fly, *Drosophila melanogaster,* to serve as vector for *B. cinerea*. Long-term *D. melanogaster*/*B. cinerea* relationships were found during the life of the insect. Conidia germinated in the insect foregut, developed into mycelium, and differentiated into microsclerotia, which can be carried by the flies during their entire life. Since the fly overwinters as an adult, it was concluded that it could play a role in winter conservation of *B. cinerea* inoculum.

2.2. Chlamydospores

Chlamydospores have been found in *B. cinerea*, *B. anthophila* and *B. fabae* (Coley-Smith, 1980). The chlamydospores of *B. cinerea* are hyaline cells of extremely variable form and size (Urbasch, 1983, 1986). They are generally found in ageing cultures and commonly occur in the stromatic sectors of cultures of the fungus which are contaminated by other organisms, and in association with sclerotia. Chlamydospores are formed as terminal or intercalary cells by transformation of vegetative mycelium parts and are liberated by hyphal disintegration. They were observed on and in tissue of naturally and artificially infected tomato and *Fuchsia hybrida* leaves and their numbers increased in older lesions (Urbasch, 1983, 1986). Under moist conditions and without added nutrients, the chlamydospores germinated on the leaves by microconidia which remained dormant. When fresh nutrients were supplied to the chlamydospores, they germinated with hyphae penetrating the host, or they produced a new crop of macroconidia. Histological studies of the infection process by *B. elliptica* show the formation of corresponding structures after conidium germination on oriental lily leaves (Hsieh et al., 2001). On tomato fruit, unsuccessful penetration was often associated with germ tubes which, after attachment to the host, differentiate into several cells (chlamydospores) at the point of attachment (Rijkenberg et al., 1980). On fruit of nectarine, plum and pear, germlings produced from dry airborne *B. cinerea* conidia formed chlamydospores on short germ tubes when fruits were subjected to intermittent dry periods, or were kept for 48 h at 5°C (Holz, 1999). Chlamydospores can therefore serve as short term survival structures which may help the fungus to overcome short unfavourable periods encountered on plant surfaces (Urbasch, 1983, 1986).

2.3. Conidia

Conidia of *Botrytis* are generally regarded as short-lived propagules in the field and their survival will largely be determined by temperature extremes, moisture availability, microbial activity and sunlight exposure. In the soil, *Botrytis* species are not particularly effective competitors and their conidia are subjected to fungistasis (Coley-Smith, 1980). Conidia of *B. cinerea* were able to survive on fruit surfaces of kiwifruit, remaining viable and infectious throughout the growing season (Walter et al., 1999b). Salinas et al. (1989) reported that conidia stored dry were able to survive at room temperature for up to 14 months, when some conidia were capable of

germinating *in vitro* and on ray florets of gerbera flowers to cause lesions. However, on the surface of Anjou pears, the viability of *B. cinerea* conidia after 7 weeks had declined to 10% germination (Spotts, 1985). In Scotland, conidia of *B. fabae* placed out of doors on cobwebs gradually lose their infectivity; only 15% of conidia were infective after 10 days exposure to ambient weather during summer (Harrison, 1983). When *B. cinerea* conidia were exposed to direct sunlight at midday in an Israeli summer, survival was only minutes (Rotem and Aust, 1991). In a New Zealand vineyard, mean percentages of conidia germinating after exposure to 4 h of sunlight ranged between 81 and 91% and between 49 and 50% after 8 h of sunlight exposure. Upon re-exposure on the second day, just 10 min of exposure to sunlight caused germination to drop between 26 and 27% for all isolates tested (Seyb, 2003). The UV spectrum of sunlight appeared to be the most important environmental factor influencing mortality of conidia (Rotem and Aust, 1991; Seyb, 2003).

Microconidia, which occur in all *Botrytis* species, provide an alternative microscopic propagule for these fungi when subjected to adverse conditions. In general they are found in ageing cultures of the fungus or those which are contaminated by other organisms, and in association with sclerotia. Microconidia develop from germ tubes produced by macroconidia, more mature hyphae, inside empty hyphal cells, and from appressoria and sclerotia (Jarvis, 1980a; Lorenz and Eichhorn, 1983). Germlings of *B. cinerea* form microconidia and chlamydospores in a corresponding manner on plant surfaces. On tomato plants, the dedifferentiation of *B. cinerea* appressoria proceeded by production of microconidia directly on appressoria, or by terminally and laterally outgrowing hyphae and their subsequent formation of microconidia (Urbasch, 1985a). The appressoria lost their function and the infection process at the site of interaction was interrupted. A similar process was infrequently observed on fruit surfaces of nectarine and plum that were subjected to intermittent dry periods, or were kept at 5°C after inoculation with dry, airborne *B. cinerea* conidia (Holz, 1999). Although their sole function is believed to be one of spermatization, they may also help the fungus to survive adverse conditions. The unicellular structures are generally produced in chains, but Urbasch (1984a) noted that after prolonged adverse conditions, *B. cinerea* formed clusters of microconidia bearing phialides and then embedded aggregates of these conidia in mucilage, which is then enclosed within a protective covering (hülle). Due to protection by this covering, the enclosed microconidial aggregates survived on dry agar plates without degeneration for up to 6 months and formed new mycelia when placed on fresh media. Urbasch (1984b) described a microcycle induced by nutritional deficiency that leads to production of microconidia and the oxygen concentration determined whether macro- or microconidia resulted, the latter being favoured by low O_2 concentrations. She also provides a good ultrastructural analysis of the differentiation of microconidia and comments on their rather thick outer wall (0.2 μm) suggestive of long-term survival (Urbasch, 1985b).

Macroconidia of *B. fabae* on agar films, buried in moist soil, germinated within a few days to produce short germ tubes which bore phialides and microconidia (Harrison and Hargreaves, 1977). After 29 days in moist soil, the macroconidia were dead and ruptured whereas the microconidia appeared to be quite healthy. Some

germination was observed amongst microconidia which had been left outside for 25-27 days during winter, suggesting that exposure to cold may be a factor in breaking the dormancy of microconidia. The ability of the microconidia to remain dormant under adverse conditions suggests that they may be important in the survival of *B. fabae* from one season to the next.

2.4. Mycelium

The survival of mycelium of *Botrytis* species under natural conditions has hardly been investigated and, unless particular care is taken, it is often difficult in practice to decide whether survival is by mycelium or whether microsclerotia or chlamydospores are involved. There is some evidence that the mycelium of certain *Botrytis* species, and especially those more specialized in their parasitism, can survive for considerable periods in bulbs, seeds and other vegetative plant parts (Coley-Smith, 1980). *B. cinerea* is considered to be a characteristic component of aerial surfaces of some species of plants whilst being absent or infrequently isolated from others. The frequency of isolation of the fungus tends to increase as the season progresses, reflecting an increasing ability to enter plant tissue as a weak parasite or as a saprophyte during senescence (Blakeman, 1980). Kobayashi (1984) observed that *B. cinerea* conidial masses developed throughout the year from mycelium in the fallen petals of 28 plant species belonging to 19 genera of 14 families.

3. Inoculum production and dispersal

It is generally assumed that for *B. cinerea*, inoculum is always present in the field and that production, liberation and dispersal of inoculum is an ongoing process (Jarvis, 1980b). This is clearly not always the case in all crops (Sosa-Alvarez et al., 1995; Seyb, 2003). There are various factors essential for high propagule numbers in the air: a viable, productive inoculum source, conditions favourable for propagule production, and for their dispersal at the source site. Correlations have been found between dispersal and conditions favourable for sporulation (usually surface wetness with moderate temperature) in many *Botrytis* species (Jarvis, 1980b). The frequency and duration of wetness events, and temperature, vary greatly during a growing season. It is anticipated that interrupted wetness periods, and temperature fluctuations, will affect the number of propagules produced (Rotem et al., 1978). A complicated relationship thus exists in the field between environmental conditions and propagule production and dispersal.

3.1. Dispersal and deposition

If it is to infect, the pathogen must conquer space (Zadoks and Schein, 1979), that is to move from the primary source and land on susceptible tissue. Each part of the fungus thallus can serve as a dispersal unit. These propagules are dispersed by wind, rain and insects.