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The biology of *Colletotrichum acutatum*

by

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Abstract

Colletotrichum acutatum is major pathogen of fruit crops, causing economically important losses of temperate, subtropical and tropical fruits worldwide. However, few studies have been carried out on key aspects of its biology. This is mainly because traditionally isolates of *C. acutatum* were often wrongly identified as *C. gloeosporioides*. Effective separation of the two species was not possible until the introduction of molecular tools for taxonomy. The life cycle of *C. acutatum* comprises a sexual and an asexual stage and much remains to be resolved regarding the genetics of sexuality and the effects of the sexual stage on population structure. *Colletotrichum acutatum* exhibits both infection strategies described for *Colletotrichum* species, i.e. intracellular hemibiotrophy and subcuticular-intramural necrotrophy, and may also undergo a period of quiescence in order to overcome resistance mechanisms in immature fruit such as pre-formed toxic compounds and phytoalexins, or due to the unsuitability of unripe fruit to fulfill the nutritional and energy requirements of the pathogen. *Colletotrichum acutatum* may overwinter as mycelium and/or appressoria in or on different parts of the host. Conidia are water-born and spread by rain episodes so infections are usually highest during the wettest periods of the growing season. Current management strategies for this fungus comprise the exploitation of cultivar resistance, cultural, chemical, and biological control methods, and preventive strategies such as disease-forecasting models. This review focuses on the current knowledge of biological aspects of *C. acutatum* and related *Colletotrichum* species and includes a discussion of the progress towards their control.

Key words: Anthracnose, Ascomycete taxonomy, fungal diseases, infection, appressorium, host pathogen interactions, postharvest, fungicide, fruit, and disease control.

Resumen

Colletotrichum acutatum es uno de los principales hongos patógenos en agricultura y responsable de importantes pérdidas económicas en frutales en áreas tanto de climas templados como subtropicales y tropicales. Sin embargo, existen pocos estudios sobre aspectos clave de su biología. Esto es debido a que, tradicionalmente, muchos aislamientos de *C. acutatum* se han identificado como *C. gloeosporioides*. El uso de técnicas de biología molecular ha posibilitado la distinción entre ambas. El ciclo vital de *C. acutatum* comprende una fase sexual y otra asexual, y todavía quedan por conocer muchos aspectos genéticos de su fase sexual y de su relevancia en la estructura de la población. *Colletotrichum acutatum* posee los dos tipos de estrategias de infección descritas en el género *Colletotrichum*, intracelular hemibiotrófica y subcuticular-intramural necrotrófica, y puede incluso establecer un periodo de latencia con la finalidad de hacer frente a los mecanismos de defensa del hospedante tales como: existencia de compuestos tóxicos preformados y fitoalexinas, la escasez de nutrientes del propio tejido del hospedante para hacer frente a los requerimientos energéticos del patógeno. *Colletotrichum acutatum* generalmente inverna como micelio y/o apresorios en distintas partes del hospedante. Los conidios requieren la presencia de agua para ser producidos y su dispersión se produce con la lluvia. Las actuales medidas de manejo de este hongo comprenden el aprovechamiento de la distinta resistencia de cultivares, formas y manejo de los cultivos, métodos químicos y de control biológico, así como estrategias preventivas tales como modelos de predicción de las enfermedades. Así pues, el objetivo de este trabajo es presentar los conocimientos actuales sobre distintos aspectos de la biología de *C. acutatum* y otras especies relacionadas e incluye una discusión sobre los adelantos para el control de este hongo.

Palabras clave: antracnosis, taxonomía de ascomicetes, enfermedades fúngicas, infección, apresorio, interacciones patógeno-hospedante, postcosecha, fungicida, fruta, control de enfermedades.

Introduction

Colletotrichum Corda is a large genus of Ascomycete fungi, containing species that are amongst the most successful plant pathogenic fungi, causing significant economic damage to crops in tropical, subtropical, and temperate regions (Bailey & Jeger, 1992). Their economic impact has lead to extensive studies on diverse aspects of its biology such as, host specificity (Correl & al., 2000; Freeman, 2000), cell biology of infection processes (Bailey & al., 1992; O'Connell & al., 2000), fungal-host interaction (Prusky & Plumbley, 1992; Prusky, 1996; Prusky & al., 2000), genetic diversity (Freeman, 2000), and epidemiology (Förster & Adaskaveg, 1999; Timmer & Brown, 2000). Species of this genus have been used as models for studying infection strategies and host-parasite interactions (Perfect & al., 1999), defining the genetic basis of fungal symbiotic life styles (Rodriguez & al., 2000), and for developing infection (Fitzell & al., 1984) and disease forecasting systems (Dannenberger & al., 1984; Timmer & Zitko, 1993, 1996; Monroe & al., 1997; Adaskaveg & al., 2001, 2002; Uddin & al., 2002).

One of the most pathogenic species of this genus is *Colletotrichum acutatum* J.H. Simmonds, which causes anthracnose and blight in agriculturally important hosts such as almond (*Prunus dulcis* (Mill.) D.A. Web.) (Ogawa & English, 1991; Adaskaveg & Hartin, 1997; Förster & Adaskaveg, 1999) (Fig. 1), avocado (*Persea* spp.) (Freeman, 2000), peach (*Prunus persica* L.) (Adaskaveg & Hartin, 1997; Zaitlin & al., 2000), blueberries (*Vaccinium* spp.) (Smith & al., 1996; Schilder & al., 2001; Yoshida & Tsukiboshi, 2002) (Fig. 2), citrus (*Citrus* spp.) (Zulfiqar & al., 1996; Timmer & Brown, 2000), mango (*Magnifera indica* L.) (Fitzell, 1979; Arauz, 2000), olive (*Olea europaea* L.) (Martín & García-Figueres, 1999), and strawberry (*Fragaria* × *ananassa* Duch.) (Smith & Black, 1990; Howard & al., 1992; Curry & al., 2002).

Colletotrichum acutatum can affect most parts of the plant, from the roots to the leaves, blossoms, twigs, and fruit, causing diseases such crown root rot, defoliation, blossom blight, and fruit rot (Figs. 1 and 2). However, as for most *Colletotrichum* species, the most significant losses due to infection by *C. acutatum* are incurred when fruit is attacked (Bailey & Jeger,

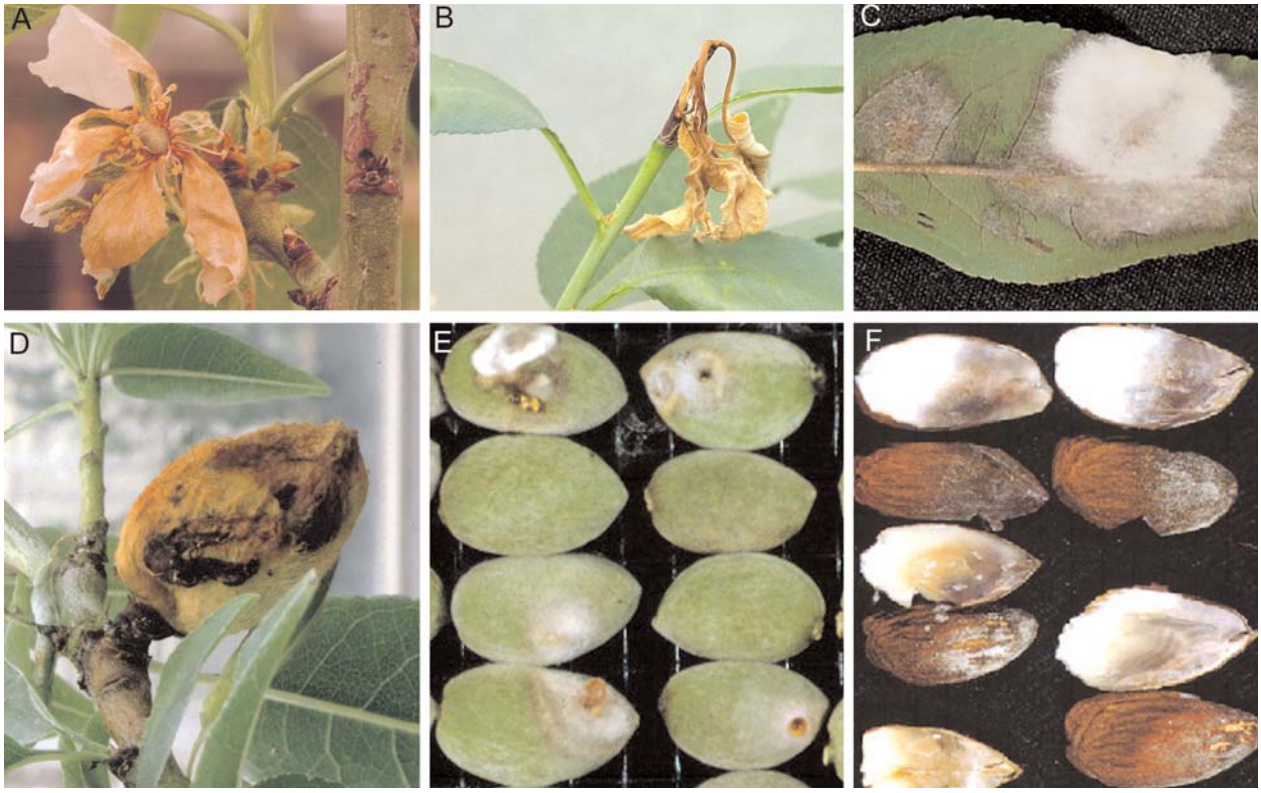

Fig. 1. Symptoms of infection of *Colletotrichum acutatum* in almond tissues. *Colletotrichum acutatum* causes pre- and post-harvest losses by affecting most parts of the tree. **A,** Blossom blight. Disease symptoms first become apparent on almond blossoms. **B,** The infection may continue into the spurs and shoots resulting in shoot dieback. **C,** Hyphae of the fungus growing in a senescent leaf. **(D-F),** Fruit can be infected at all stages of development. **D,** Infected almond fruit. **E**, Quiescent infection manifesting after incubation under high humidity. **F,** Quiescent infection in almond kernels. Infected kernels may show internal bluish staining after the nuts are harvested.

1992). There are two distinct types of *Colletotrichum* diseases affecting fruit, those causing disease on immature and developing fruit in the field (pre-harvest) and those damaging mature fruit at harvest and during storage (post-harvest). Fruit affected by post-harvest *Colletotrichum* species often appear completely healthy at the time of harvest, with disease symptoms only manifesting themselves during storage (Figs. 1E-F and 2B). This is due the ability of many *Colletotrichum* species to cause latent or quiescent infections in which the fungus infects immature fruit in the field and then becomes dormant until the fruit ripens, at which time it resumes its growth causing disease on the fruit (Prusky & Plumbey, 1992; Prusky, 1996).

In spite of its economic impact, few studies have been carried out on key aspects of the biology of *C. acutatum*. An improved understanding of its developmental biology, infection processes, host pathogen interactions and epidemiology may lead to the development of more efficient control and management strategies. Thus, the purpose of this review is to compile the current knowledge on the biology of *C. acutatum* and related pathogenic species in relation to anthracnose of economically important fruit crops such as almond, blueberry and citrus. A discussion of the recent progress towards control of this fungus is also included.

The taxonomic status

Fungi classified in the ascomycete (telomorphic) genus *Glomerella* Spauld. & H. Schrenk and the coelomycete (anamorphic) genus *Colletotrichum* have

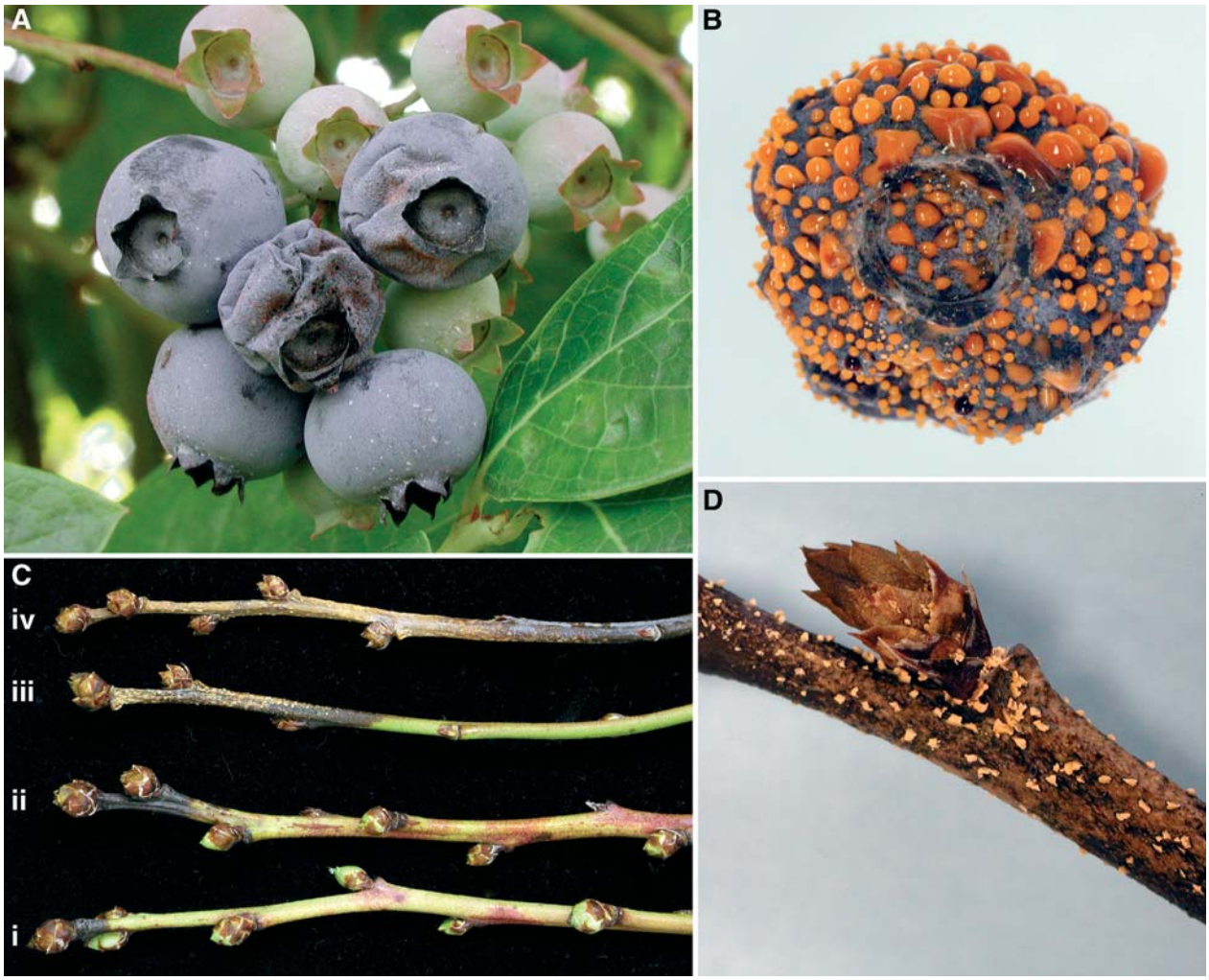

Fig. 2. Symptoms of infection of *Colletotrichum acutatum* on blueberry tissues. **A,** Anthracnose fruit-rot. Fruit do not develop symptoms until they are mature (blue). Bright orange spore masses are produced within shriveled, sunken areas on the fruit surface. The sticky spore masses spread to other fruit by splashing water and contact. **B,** Under optimal conditions in storage, the fungus may sporulate covering the entire fruit surface with bright orange spore masses. **C** (i-iv), Growth and colonization of blueberry twigs by *C. acutatum.* This fungus overwinters in flower buds. In the spring the fungus grows out of the buds (i) and into surrounding tissue. The fungus then grows down the twig killing the tissue and eventually sporulates (ii-iv). **D,** Bright orange *C. acutatum* spore masses on a dead blueberry twig.

proved some of the most challenging to taxonomists. While the generic limits are today relatively well defined, the concept of a species in the genus *Colletotrichum* is not well established nor universally accepted (Sutton, 1992). The present taxonomic concepts of the group largely follow von Arx (1957, 1970) and Sutton (1980). Morphological characteristics and host range have traditionally been used to define the species, although excessive reliance on the latter has led to a proliferation of unnecessary names. This may be partly due to the wide host range of a number of *Colletotrichum* species and the fact that several *Colletotrichum* species may be associated with a single host (Freeman & al., 1998). The problem is exemplified by the confusion that exists with regard to the *Colletotrichum* species that infect fruit. *Colletotrichum acutatum* and *C. gloeosporioides* (Penz.) Penz. & Sacc. are the two members of the genus that are most commonly associated with fruit rots in the literature. *Colletotrichum gloeosporioides* is considered a cumulative species and is found on a wide variety of fruits, including almond, apple, avocado, citrus, mango olive, and strawberry (Fitzell, 1979; Sutton, 1992; Freeman & Shabi, 1996; Freeman & al., 1998; Martín & García-Figueres, 1999; Arauz, 2000; Timmer & Brown, 2000). Likewise, *C. acutatum* has also been reported to infect a large number of fruit crops (Freeman & al., 1998; Martín & García-Figueres, 1999; Adaskaveg & Förster, 2000; Yoshida & Tsukiboshi, 2002).

Colletotrichum acutatum and *C. gloeosporioides* are morphologically very similar and because of their overlapping host ranges and the extensive variability that their isolates show in culture, it has been very difficult to separate them by traditional taxonomical methods. Nonetheless, these two species have been successfully separated based on a number of characteristics including culture morphology, conidium shape and size, and host-range (Smith & Black, 1990; Sutton, 1992; Förster & Adaskaveg, 1999). However, these techniques have to be used with caution, as they are prone to error. For example, Förster & Adaskaveg (1999) and Adaskaveg & Förster (2000) included culture morphology, and conidium size and shape in a comparison of strains isolated from almond assigned to *C. acutatum,* and strains isolated from citrus identified as *C. gloeosporioides*. Isolates of each species could be distinguished by conidium shape when cultures were grown on potato dextrose agar (conidia with rounded ends were identified as *C. gloeosporioides* and conidia with pointed ends as *C. acutatum*). However, on pea straw agar the conidial size of the two species overlapped, showing that this character is not reliable for distinguishing between the two species. Another character often used for isolate description is colony morphology. Colonies of *C. gloeosporioides* are usually gray in appearance while *C. acutatum* colonies had a pink or orange phenotype (Zulfiqar & al., 1999; Martín & García-Figueres, 1999). Förster & Adaskaveg (1999) found that almond isolates of *C. acutatum* had two different phenotypes, one gray and one pink, and therefore much precaution needed to be taken when using this character for species segregation. Other characters, however, have been helpful for separation of isolates of *C. acutatum* from *C. gloeosporioides*, e.g. growth rates (slow, in *C. acutatum* and fast in *C. gloeosporioides*), optimum growth temperature (25 ºC in *C. acutatum* vs. 30 ºC in *C. gloeosporioides*), and sensitivity to benomyl (Adaskaveg & Förster, 2000).

Isozyme electrophoresis has also been used to discriminate between similar *Colletotrichum* species such as *C. fragariae* Brooks and *C. gloeosporioides* (Bonde & al., 1991). Recent studies have also shown that *C. acutatum* and *C. gloeosporioides* isolates from olive also differ in their enzymatic properties, i.e. their ability to hydrolyze casein (Martín & García-Figueres, 1999). These properties could be used to distinguish between isolates of *C. acutatum* and *C. gloeosporioides* in other pathosystems, and may represent a new and useful property to be included for differentiating between these two species.

Many problems still remain in providing a workable taxonomy of the genus *Colletotrichum*. However, molecular biology has provided new insights into systematics, particularly in the delimitation of species and defining inter- and intraspecific relationships. In recent years, the use of molecular biological techniques has led to the reclassification of a number of *C. gloeosporioides* isolates as *C. acutatum* (Smith & al., 1996; Jayasinghe, & al., 1997; Martín & García-Figueres, 1999; Peres, & al., 2002). Several laboratories have also now begun to decipher the relationships among *Colletotrichum* isolates from fruit-rots (Freeman & Shabi, 1996; Shi & al., 1996; Johnston & Jones, 1997; Kuramae-Izioka & al., 1997; Lardner & al., 1999; Freeman & al., 2001). A detailed study on a diverse population of *C. acutatum* from fruit rots, lupin, and pine in New Zealand, reported that this species can be considered as a "group species", *C. acutatum sensu lato* (broad sense) (Lardner & al., 1999). Within this collective group, four distinct *C. acutatum sensu stricto* (narrow sense) groups, including the original one first described by Simmonds were distinguished, based on sequence analysis of the D2 domain of the rDNA large subunit (Johnston & Jones, 1997). Recently, Freeman & al. (2001) characterized isolates of *C. acutatum sensu* Simmonds from several diverse hosts and different

geographical regions using various molecular methods. They showed that there was considerable diversity among *C. acutatum* isolates, and identified four subgroups within *C. acutatum*. Group I included U.S. isolates from almond, apple, peach, and pecan, group II isolates from anemone, olive and strawberry, group III isolates from almond in Israel and strawberry in Spain, and group IV contained a single isolate from anemone in the Netherlands.

Future molecular studies on *C. acutatum* and other fruit-rotting *Colletotrichum* species should be oriented towards defining these species more accurately in genetic terms. Current methods of obtaining genomic data for use in systematics can be laborious, expensive and potentially error prone (Camacho & al., 1997; Zhang & al., 1997). Therefore, the current species concept should also take into account morphological criteria, considering the overall biology and ecology of the organism in question. Without such an approach we could encounter similar problems with molecular-based methodologies that were encountered when species classification was solely based on morphological criteria.

Reproduction and Genetics

The life cycle of *Colletotrichum* species comprises a sexual and an asexual stage. In general terms, the sexual stage accounts for the genetic variability and the asexual stage is responsible for the dispersal of the fungus. Sexual recombination in most *Colletotrichum* species is rare in nature and to date only 11 out of about 20 *Colletotrichum* species have *Glomerella* teleomorphs. Furthermore, sexual reproduction in *Glomerella* is more complex than is usual for most ascomycete fungi. Fungal species that reproduce sexually can usually be classified as either self-fertile (homothallic), or self-sterile (heterothallic). However, *Glomerella* is unusual because within a single species some strains are both self-fertile and cross-fertile, while others are cross-fertile but self-sterile (Chilton & Wheeler, 1949; Wheeler, 1954). Based on extensive studies on the genetics of mating in *G. cingulata*, it was concluded that heterothallism in this species is derived from homothallism via mutations in genes controlling steps in the morphogenetic pathway necessary for self-fertility (Wheeler, 1954).

The sexual stage of *Colletotrichum acutatum* has never been found in nature. However, studies have shown that there is extensive genetic diversity and heterogeneity within this species (Johnston & Jones, 1997; Lardner & al., 1999; Freeman & al., 2001). One hypothesis for this diversity is the occurrence of sexual recombination between strains of the fungus. Further evidence for the existence of sexual recombination in *C. acutatum* has come from recent studies in which the teleomorph, *Glomerella acutata,* was generated in artificial culture (Guerber & Correll 1997, 2001). Moreover, it was found that *Colletotrichum acutatum* isolates from the same host were in general, self-sterile but crosses between *C. acutatum* isolates from different hosts readily produced the teleomorph, *G. acutata*.

Another mechanism by which genetic diversity may be generated in *Colletotrichum acutatum* populations is through vegetative compatibility. The term vegetative compatibility refers to the ability of individual fungal strains to undergo mutual hyphal anastomosis, resulting in viable fused cells containing nuclei of both parental strains in a common cytoplasm (Katan, 2000). Hyphal anastomosis is a common phenomenon in many fungi (e.g. *Neurospora* Shear & B.O Dodge and *Aspergillus* Link), and the genetic status of the anastomosed cell reflects the genetic relatedness of the component nuclei. When the nuclei are genetically identical (e.g. due to fusion between two hyphae of the same monoconidial culture), the anastomosed cell is a *homokaryon*. On the other hand, when the anastomosing hyphae belong to genetically different strains, the resultant anastomosed cell is a *heterokaryon*. Since reproduction in many *Colletotrichum* populations is mainly or exclusively vegetative, the only means of exchanging genetic material between two strains would be anastomosis and heterokaryosis. These processes occur between some *Colletotrichum* isolates but not others and, in some cases, seem to be restricted by the existence of vegetative incompatibility (Brooker & al., 1991; Chacko & al., 1994). Isolates that cannot form a viable heterokaryon with each other are, in effect, genetically isolated. Isolates that can anastomose with one another and form viable heterokaryons are placed in the same vegetative-compatibility group (VCG) to indicate this fact. They may potentially share a common gene pool, and are isolated from other strains or VCGs within the species by the incompatibility mechanism (Katan, 2000). Vegetative compatibility groups have been used quite widely to study the population structures of a number of *Colletotrichum* species including *C. gloeosporioides* and *C. acutatum* (Chacko & al., 1994; Correll & al., 1994). These studies indicate that the genetics of sexual and vegetative compatibility in *C. gloeosporioides* and *C. acutatum* are quite similar (Correll & al., 2000). However, much remains to be resolved regarding the genetics of sexual and vegetative compatibility in *C. acutatum* and the effects of these mechanisms on population structure.

Microscopical events in the Host-Pathogen Interaction

Pre-penetration events and conditions affecting early development. The early stages of fungal development during the infection process (Figs. 3 and 4) are essentially the same for all *Colletotrichum* species and can be separated into stages including: 1) the deposition of conidia on plant surfaces, 2) attachment of conidia to those surfaces, 3) germination of conidia, 4) production of appressoria, 5) penetration of the plant epidermis, 6) growth and colonization of plant tissues, and 7) production of acervuli and sporulation (Jeffries & al., 1990; Prusky & al., 2000).

In *C. acutatum,* some conidia (Fig. 3A) do not follow the usual stages of development. These conidia undergo microcyclic conidiation in which the conidium germinates and produces a secondary conidium directly from the first without producing a germ-tube (Fig. 3B) or undergoing vegetative growth (Leandro & al., 2001; Diéguez-Uribeondo & al., 2003a) (Fig. 3C). In others, the conidia germinate and produce a germ-tube that grows along the plant surface until it comes into contact with other *C. acutatum* hyphae or conidia. Upon contact, the germ-tubes undergo hyphal anastomosis (Fig. 3D) (Diéguez-Uribeondo, 2003a; Wharton & Schilder, 2003).

The occurrence and relevance of each stage in the infection process may vary depending on the conditions of growth, the host tissue, the particular species, and or the fungal isolate (Bailey & al., 1992; Zulfiqar & al., 1996; Diéguez-Uribeondo & al., 2003a). Assuming that the fungal conidia encounter the right host, the most important microclimatic parameters influencing the timing of fungal development are wetness and temperature (Duthie, 1997). The chronology of infection by *C. acutatum* has been established on several hosts including citrus, almond, strawberry, and blueberry (Zulfiqar & al., 1996; Leandro & al., 2001; Curry & al., 2002; Diéguez-Uribeondo & al., 2003a, 2003b; Wharton & Schilder, 2003). These studies have shown that germination and germ tube differentiation (i.e. appressorium formation or microcyclic conidiation), occur within a few hours (ca. 3 to 48 h), and consequently infections by this fungus can occur rapidly under favorable conditions.

In spite of the extensive studies on the influence of environmental conditions in the development of *Colletotrichum* diseases (Dannenberger & al., 1984; Timmer & Zitko, 1993, 1996; Monroe & al., 1997; Uddin & al., 2002), only a few studies have focused on the influence of microclimatic parameters on early development and differentiation during pre-penetration events (Fitzell & al., 1984; Dodd & al., 1991). Studies of this kind are important for determining the exact conditions and timings required for infection by *Colletotrichum* species, and have been successfully used to

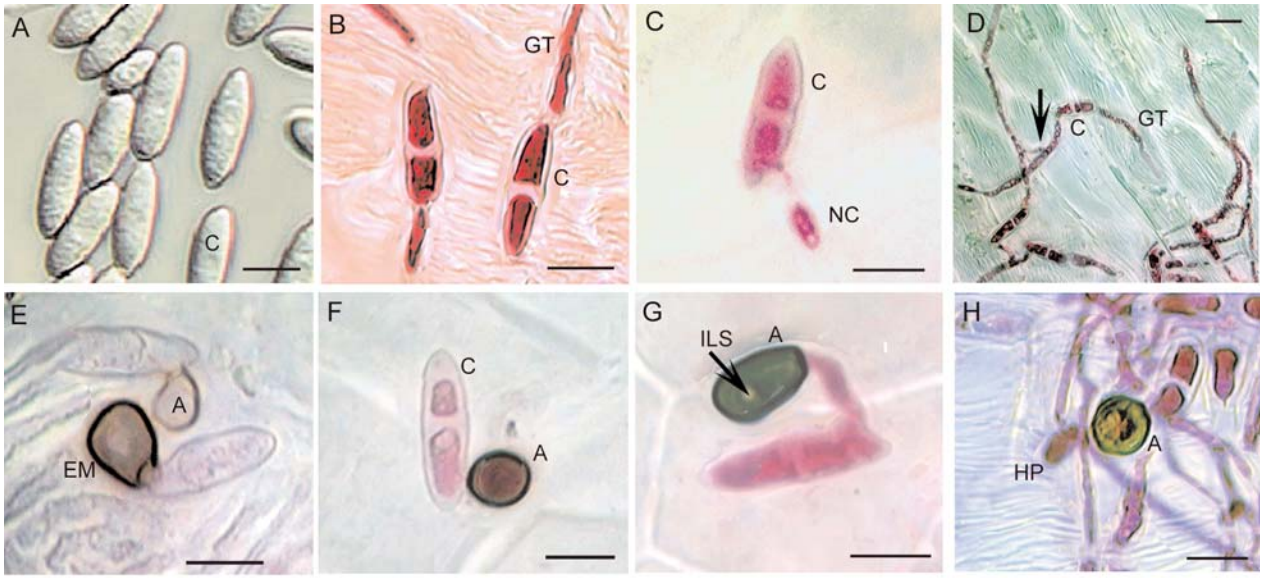

Fig. 3. Light micrographs of the early development stages of *Colletotrichum acutatum* on almond leaves. **A,** Conidia (C). Bar = 5 µm. **B,** Septated conidia (C) with germ tubes (GT). Bar = 5 µm. **C,** Secondary conidiation. A conidium (C) is forming a new conidium (NC). Bar = 5 µm. **D,** On almond petal, conidia (C) produce long germ tubes (GT) that anastomosed (arrow) 24 h after inoculation. Bar = 10 µm. **E,** Early stages of appressoria formation. Unmelanized appressorium (A) and early melanization (EM). Bar = 5 µm. **F,** A conidium (C) forming an appressorium (A). No infection peg has been formed yet. Bar = 5 µm. **G,** An appressorium (A) with an internal light spot (ILS), i.e. penetration peg. Bar = 5 µm. **H**, hypha penetrating (HP) petal tissue. The penetrating hypha has been developed from an appressorium (A). Bar = 5 µm.

develop models to estimate infection levels of mango anthracnose caused by *C. acutatum.* However, they can be very tedious and prone to error because of the large numbers of conidia that have to be assessed using a light microscope. Recent studies have used digital image analysis to study the influence of microclimatic parameters on early conidial development and infection (Diéguez-Uribeondo & al., 2003a, 2003b). Using this technique the effect of environmental conditions and host factors on the initial stages in the infection process can be more easily quantified in a large sample size (Fig. 5). The use of larger sample sizes and the automated assessment of fungal development should enable the development of more accurate disease forecasting models (Adaskaveg & al., 2001, 2002; Diéguez-Uribeondo & al., 2002).

Penetration and tissue colonization events. The penetration process is a critical stage in any plant-pathogen interaction (Howard & Valent, 1996). In *Colletotrichum*, the penetration of host tissues generally relies on formation of specialized infection structures called appressoria (Perfect & al., 1999) (Figs. 3E-H and 4A). Appressoria allow the fungus to penetrate the host cuticle and epidermal cell wall directly by means of a narrow penetration peg that emerges from the base of the appressorium (Figs. 3H and 5). Although rare in *Colletotrichum,* there are reports of indirect penetration of tissue through stomata (Fig. 4B) (Latunde-Dada & al., 1999; Diéguez-Uribeondo & al., unpublished), or wounds without the formation of appressoria (Sénéchal & al., 1987; Van der Bruggen & Maraite, 1987; Zulfiqar & al., 1996). The importance of appressoria in the infection process has been illustrated in several studies with melanin deficient mutants and the inhibitors of melanin biosynthesis (Kubo & Furusawa, 1991; Mendgen & Deising, 1993).

A number of detailed light and electron microscopy studies have been carried out on the infection process in *Colletotrichum* and reviewed in detail by Bailey & al. (1992) and O'Connell & al. (2000). Although pre-penetration events are basically the same for all *Colletotrichum* species, major differences become apparent after penetration, when two types of infection strategy can be distinguished: intracellular hemibiotrophy (Fig. 6A) and subcuticular, intramural necrotrophy (Fig. 6B) (Bailey & al., 1992; Skipp & al., 1995).

Many *Colletotrichum* species initially establish infection by means of a brief biotrophic phase, associated with large intracellular primary hyphae (Fig. 7A and 8A). They later switch to a destructive, necrotrophic phase, associated with narrower secondary hyphae, which ramify throughout the host tissue. In species, which adopt this strategy, the initial biotrophic phase can vary in duration from less than 24 h to over 72 h (O'Connell & al., 1985; Latunde-Dada & al., 1996; Wharton & Julian, 1996). Primary hyphae formed by these species can also vary greatly in morphology. In some species (e.g. *C. destructivum* O'Gara on cowpea, *Vigna unguiculata* (L.) Walp.), the primary hyphae are entirely confined to the initially infected epidermal cell (Latunde-Dada & al. 1996). In *C. malvarum* (A. Braun & Casp.) Southw. on *Sida* spp., *C. lindemuthianum* (Saccardo & Magnus) Scribner on bean (*Phaseolus vulgaris* L.), and *C. orbiculare* (Berk & Mont.) on cucumber (*Cucumis sativus* (L.), initial penetration of epidermal cells is immediately followed by the formation of a large, spherical infection vesicle. One or more intracellular primary hyphae then grow out from the infection vesicle and go on to colonize many other host cells (Bailey & al., 1996; Mould & al., 1991; O'Connell & al., 1985). In other *Colletotrichum*

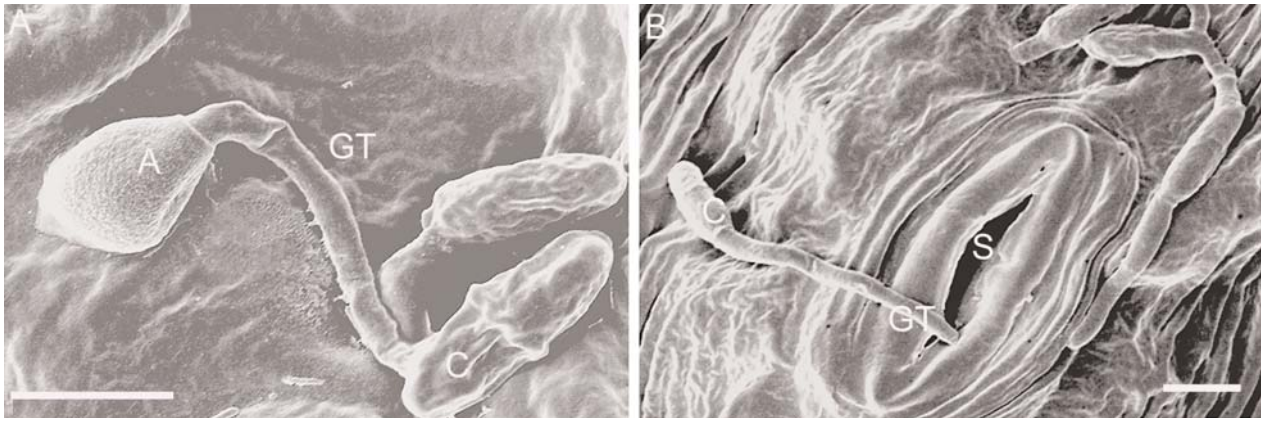

Fig. 4. Scanning electron micrographs of germinated conidia of *Colletotrichum acutatum* on almond leaf tissue. **A,** A conidium (C) with a germ tube (GT) has differentiated into a globose appressorium (A). **B,** A germ tube is penetrating the host through a stoma (S). Bars = 5 µm.

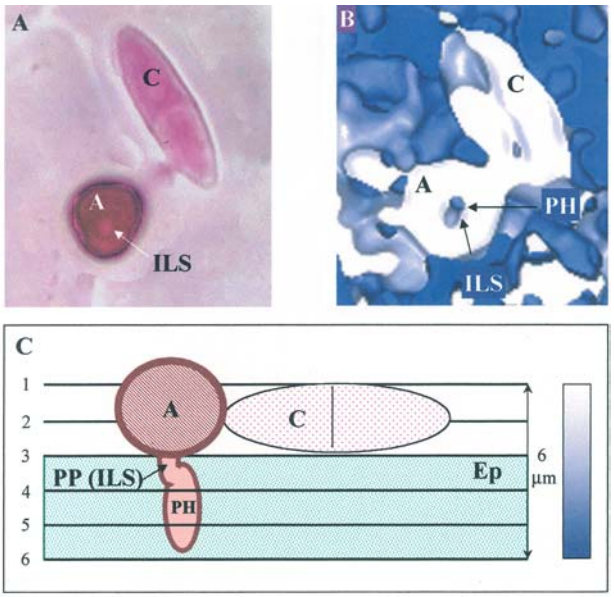

Fig. 5. Digital image analysis of the initial infection process by *Colletotrichum acutatum* on almond leaves. **A,** A montage image from a sequential series of 6 partially focused images taken at 1 µm intervals, of an conidium (C) that has developed an appressorium (A) with an internal light spot (ILS) on the epidermis (Ep) of the plant host **B,** Color depth map relief of the montage image. Dark blue indicates areas in focus originated from the bottom of the sample while white indicates areas in focus from the top of the sample. Depth relief can be observed at the ILS region, which corresponds with the formation of the penetration peg (PP) and penetration hypha (PH). **C,** A scheme representing a transversal view of the infection process based on data generated by digital image analysis with color-depth relief scale bar.

species (e.g. *C. sublineolum* (Ces.) Wils. on *Sorghum bicolor* (L.) Moench, and *C. acutatum* on *Pinus radiata* D. Don), the morphological distinction between infection vesicles and primary hyphae is less clear (Nair & Corbin, 1981; Wharton & Julian, 1996).

The initial benign biotrophic phase in hemibiotrophic *Colletotrichum* species is followed by a destructive necrotrophic phase, which commences with the appearance of narrow secondary hyphae. These hyphae ramify throughout host tissues both inter- and intracellularly (O'Connell & al., 1985; Wharton & al., 2001). At this stage in some *Colletotrichum* species e.g. *C. lindemuthianum*, host cells are killed rapidly in advance of infection and host cell walls are extensively degraded by fungal depolymerizing enzymes (Wijesundera & al., 1984). However, in others e.g. *C. sublineolum,* host cells are not killed in advance of the infection (Wharton & Julian, 1996; Wharton & al., 2001).

The second type of infection strategy employed by *Colletotrichum* species, subcuticular, intramural necrotrophy, is illustrated by the infection of cowpea (*Vigna unguiculata*) and cotton (*Gossypium hirsutum* L.) by *C. capsici* (Syd.) EJ Butler & Bisby, and onion (*Allium cepa* L.) by *C. circinans* Syd. & P. Syd. (Walker, 1921; Roberts & Snow, 1984; Pring & al., 1995). Following formation of appressoria and penetration of the cuticle, the pathogen does not immediately penetrate the underlying epidermal cell wall and enter into the cell lumen, but instead develops beneath the cuticle, within the periclinal and anticlinal walls of epidermal cells (Fig. 7 B). Intramural development is associated with extensive swelling and dissolution of host cell walls. However, it is unclear whether the underlying host cells remain alive. No visible symptoms appear until 24 h after penetration, so it is possible that a brief biotrophic phase or a benign necrotrophic phase may occur. After the brief symptomless period, the fungus starts to spread rapidly throughout the host tissue both intra- and intercellularly, killing host cells and dissolving cell walls ahead of the infection. This is similar to the necrotrophic phase in the intracellular hemibiotrophs. However, this type of infection strategy is not associated with the production of morphologically distinct primary and secondary mycelia.

There have only been a few detailed studies on the penetration and colonization of host tissue by *C. acutatum* (Nair & Corbin, 1981; Curry & al., 2002; Diéguez-Uribeondo & al., 2003a; Wharton & Schilder, 2003). These studies indicate that the infection strategy adopted by *C. acutatum* depends on the host being colonized. Thus, on stolons and leaves of strawberry, *C. acutatum* acts as a subcuticular, intramural necrotroph with no detectable biotrophic stage, while on blueberry and almond the fungus appears to adopt both infection strategies (Curry & al., 2002; Diéguez-Uribeondo & al., 2003a; Wharton & Schilder, 2003). This fungus may also change its infection strategy when colonizing different host tissues or cultivars. For example, in almond it was observed to behave differently when infecting petal and leaf tissues (Diéguez-Uribeondo & al., 2003a). In blueberry, *C. acutatum* acted as an intracellular hemibiotroph when infecting ripe fruit from susceptible cultivars (Wharton & Schilder, 2003). However, when infecting ripe fruit from the resistant cultivar "Elliott" it was observed to act as a subcuticular, intramural necrotroph (Wharton & Schilder, unpublished). Furthermore, in susceptible cultivars, the hemibiotrophic mode of colonization of the fruit tissue leads to the formation of pulvinate acervuli by 108 hours post inoculation (Fig. 9). In the resistant cultivar "Elliott" where the subcuticular, intramural necrotrophy mode of growth occurred (Fig. 8B), acervuli were not observed until 192 hours post inoculation and then they appeared stunted and did not contain many conidia (Wharton & Schilder, unpublished).

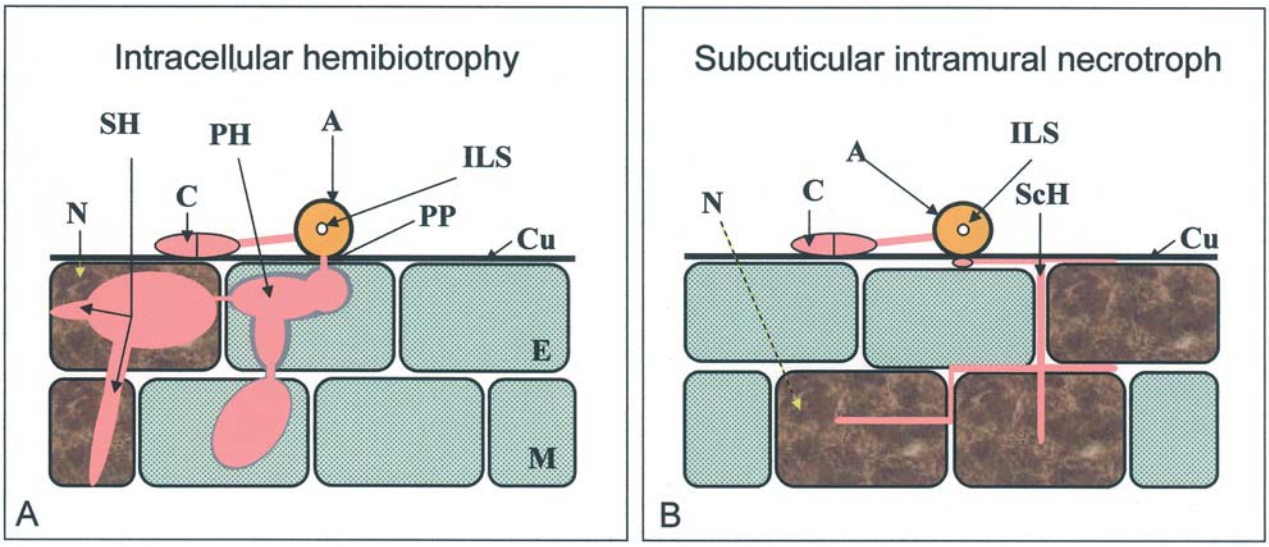

Fig. 6. Infection strategies adopted by C*olletotrichum* species as described by Bailey & al. (1992) and O'Connell & al. (2000). The initial stages of early differentiation are the same for both strategies. A conidium (C) germinates and forms and appressorium (A). The appressorium produces a penetration pore and peg (PP) which penetrates the cuticle (Cu) of the host and as a result an internal light spot (ILS) can be seen in the appressorium. **A,** In intracellular hemibiotrophs the penetration peg penetrates the epidermal cell and swells to produce an infection vesicle and broad hyphae, named primary hyphae (PH), which may colonize adjacent epidermal (E) and mesophyll cells (M). During the early stages of this type of colonization, the interaction between the host and the pathogen is biotrophic (living cell represented in green). The subsequent necrotrophic (N) (represented in brown) interaction is characterized by the formation of thin secondary hyphae (SH). These secondary hyphae grow intracellularly and intercellularly while secreting cell wall degrading enzymes and killing the host cells. **B,** In subcuticular intramural necrotrophs, host colonization is initially by subcuticular (ScH), and intramural hyphae the biotrophic phase is very short or does not occur. The fungus quickly spreads throughout the tissue and grows both inter- and intracellularly.

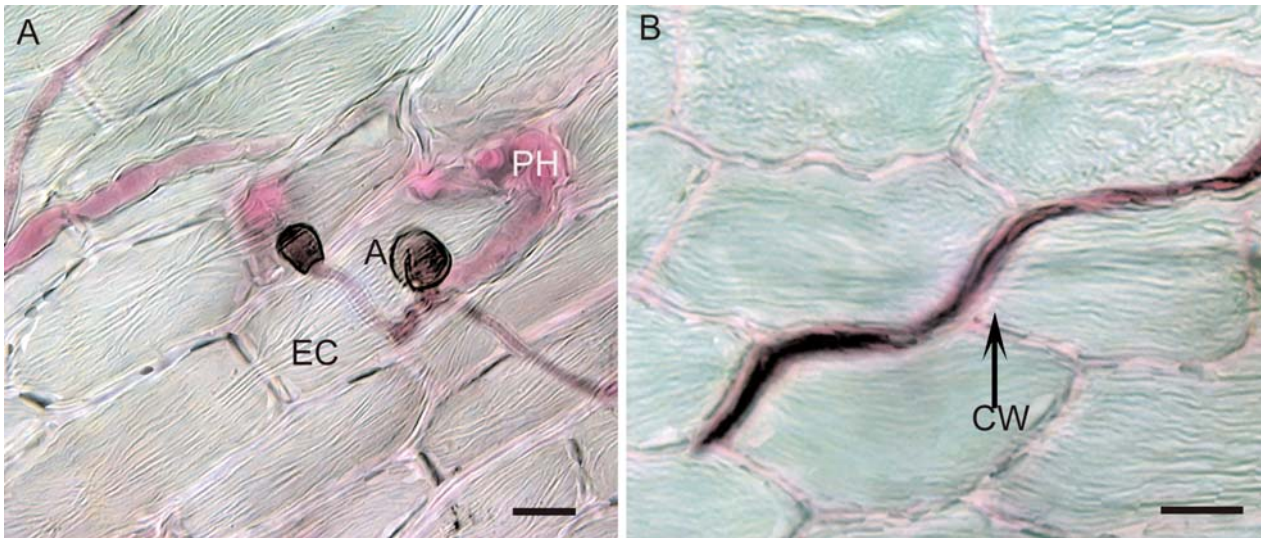

Fig. 7. Light micrographs of almond petal tissue inoculated with *Colletotrichum acutatum*. **A,** Intracellular colonization of petal tissue. The fungus penetrates the epidermal cells (EC) of the petal from an appressorium (A) and forms thick primary hyphae (PH) characteristic of biotrophic stage. **B,** Subcuticular and intramural colonization of almond petal tissue. The fungus does not immediately enter into the cell lumen and instead grows beneath the cuticle within the epidermal cell walls (CW). Bars = 5 µm.

Latency (quiescent infections). The term quiescent infection describes a "quiescent or dormant parasitic relationship, which after a time, changes to an active one" (Verhoeff, 1974; Swinburne, 1983). The infection process and the phenomenon of quiescence has been extensively studied in other *Colletotrichum* species, e.g. *C. gloeosporioides* on avocado, and *C. musae* (Berkeley & Curtis) von Arx on bananas (*Musae* spp.) (Prusky & al., 1982; Jeffries & al., 1990; Prusky & al., 1991a; Prusky, 1996). This allows us to speculate

as to the events that may occur during the infection and colonization of host tissue by *C. acutatum.* From the limited studies on post-penetration events that lead to successful colonization of host tissue by *C. acutatum*, it has been determined that this fungus can undergo a period of quiescence in almond (Adaskaveg & Förster, 2000), apple (Biggs, 1995), blueberry (Daykin & Milholland, 1984), and strawberry (Howard & al., 1992) similar to other fruit-rotting fungi.

In most *Colletotrichum* species exhibiting a quiescent period, quiescence occurs after the formation of appressoria and/or initial penetration of the host cuticle (Muirhead & Deverall, 1984; Rappel & al., 1989; Prusky & al., 1991a; Prusky & Plumbey, 1992). There have been conflicting reports as to whether dormancy actually occurs in ungerminated melanized appressoria or after the formation of infection pegs. Chakravaty (1957) found subcuticular hyphae of *C. musae* in immature bananas soon after infection. Studying the same system, Muirhead & Deverall (1984) found both melanized and unmelanized appressoria on the unripe fruit surface. The latter produced penetrating hyphae on unripe fruits, which resulted in necrosis of epidermal cells and the initiation of a host defense response. Melanized appressoria remained quiescent and ungerminated until the fruit ripened. Thus, they suggested that ungerminated melanized appressoria were responsible for the majority of quiescent infections observed in ripe bananas. Anatomical studies of *C. gloeosporioides* infection of avocado revealed that appressoria persisted on unripe fruit (Binyamini & Schiffmann-Nadel, 1972). In a re-examination of the infection process, Prusky & al. (1991a) and Rappel & al. (1989), found that the appressoria produced a short infection peg in the peel of unripe fruit. In blueberry, light microscopy studies by Daykin & Milholland (1984), of the early stages of infection by *C. acutatum* on unripe fruit showed that some appressoria germinated and produced subcuticular hyphae. However, it has yet to be determined whether or not these infections result in: i) cell necrosis and the initiation of a host defense response as in the *C. musae*-banana pathosystem, ii) as a quiescent ungerminated melanized appressoria, or iii) as a quiescent germinated appressoria.

Biochemical Basis of Host-Pathogen Interactions

The successful colonization of host tissues by a pathogen depends on its ability to overcome the host defenses. The resistance of immature fruits to colonization by *Colletotrichum* species may be related to one of four mechanisms within the host: 1) Pre-formed toxic compounds that inhibit pathogen growth are present in unripe but not ripe fruit; 2) Unripe fruit does not provide a suitable substrate to fulfill the nutritional and energy requirements of the pathogen; 3) The enzyme 'potential' of the fungus is inadequate to colonize unripe fruit; and 4) Phytoalexin production in unripe fruit (Jeffries & al., 1990).

Preformed fungitoxic compounds. The role of preformed compounds in quiescence and resistance have described in detailed in the *C. gloeosporioides*-avocado pathosystem (Prusky & al., 1982, 1985, 1988). Initial studies identified a preformed antifungal diene, (*cis*, *cis*-1-acetoxy-2-hydroxy-4-oxo-heneicosa-12, 15-diene) in the peel of unripe avocado fruit and showed that it was present at concentrations above that required to inhibit growth of *C. gloeosporioides in vitro*, and subsequently decreased in concentration as the fruits ripened. Further studies revealed that fungal lipoxygenase activity degraded the diene and was inhibited by epicatechin present in the peel of unripe fruit. The concentration of epicatechin also declined during ripening. Thus, it was concluded that a decline in epicatechin levels lead to increased lipoxygenase activity, which in turn reduced the quantity of antifungal diene and consequently allowed colonization of the host tissue by the pathogen.

Pathogen nutrition. Ripening fruit undergo a vast array of biochemical changes, the most obvious of which are the conversion of storage carbohydrates to soluble sugars. In a number of fungal diseases, resistance has been related to sugar content (Horsfall & Dimond, 1957). In guava (*Psidium guajava* L.), Sing & Sharma (1981) observed that fruit from varieties that were resistant to *Glomerella cingulata* had high levels of soluble solids and higher levels of ascorbic acid than fruit from susceptible varieties. In blueberry, studies carried out on the antifungal properties of extracts from ripe fruit from wild highbush blueberry plants (Cipollini & Stiles, 1992a, 1992b, 1993) indicated that the main antifungal compounds present in ripe fruit were water-soluble, non-alkaloidal chemicals such as phenolics and acids. Furthermore, the authors noted an increase in the antifungal activity of extracts in the presence of added organic acids (1% citric acid) and suggested that this was due to an interaction between the phenolic constituents of the extracts and the acids. Thus, they proposed that resistance in ripe blueberries might be due to a combination of factors including acid levels in the fruit and an interaction between simple phenolic compounds and organic acids, and not necessarily individual fungitoxic compounds. However, recent studies on the infection of resistant blueberry cultivars by *C. acutatum* have shown that phenolic compounds accumulate in in-

fected cells and surround fungal hyphae in a process similar to that observed in other resistant host-*Colletotrichum* interactions (Wharton & Julian, 1996; Wharton & Schilder, unpublished).

Enzyme potential of the fungus. A consistent feature of *Colletotrichum* diseases is the production of sunken lesions, which typically involve the death and maceration of infected tissues (Bailey & al., 1992). *Colletotrichum* species are known to produce a wide range of enzymes capable of destroying structural components of plant tissues and some that kill plant cells. The two most frequently encountered enzymes are those that degrade carbohydrates and thus dissolve cell walls, and those that hydrolyze cuticles. Cell wall degrading enzymes such as polygalacturonases, pectin lyases and proteases, are considered to have a role in establishing infection and macerating tissues (Bailey & al., 1992). Polygalacturonases have been extensively studied and some *Colletotrichum* species, e.g. *C. gloeosporioides* on avocado, have been shown to produce several different forms (Prusky & al., 1989). *Colletotrichum acutatum* has been shown to secrete polyglacturonase and pectin lyase in culture and pectin lyase activity was detected in rubber (*Hevea brasiliensis* Muell.) leaf tissue infected with *C. acutatum* (Fernando & al., 2001). Furthermore, many fruit tissue starts to show extensive degradation and loss of cohesion about 120h after inoculation with *C. acutatum* isolates (Wharton, personal observation), which is characteristic of enzymatic tissue degradation (Wijesundera & al., 1989).

Phytoalexin production. There is no clear evidence to link phytoalexins with quiescence in *Colletotrichum* infections of fruit (Prusky & Plumbley, 1992). However, phytoalexins have been identified in several anthracnose fruit-rot pathosystems. When unripe banana fruit were inoculated with *C. musae* conidia, necrotic spots developed in the tissues beneath the inoculation droplets (Brown & Swinburne, 1980). Thin layer chromatography of solvent extracts from necrotic tissue yielded five fungitoxic compounds that were not present in healthy tissue. These compounds declined and could not be detected when the lesions began to expand. Phytoalexins have also been identified in the anthracnose of *Capsicum annum* L. caused by *Colletotrichum capsici* and *Glomerella cingulata* (Adikaram, 1981; Adikaram & al., 1982). When unripe fruit were inoculated with these two species the phytoalexin capsicannol was produced (Adikaram & al., 1983). In ripening fruit capsidiol was also produced in addition to capsicannol, but both were absent at the onset of lesion expansion. There are no published accounts of phytoalexins occurring in mango or avocado fruits infected with *Colletotrichum* species. Extensive biochemical studies of extracts from inoculated ripe blueberry fruit, carried out by Cipollini & Stiles (1992a, 1992b, 1993) also failed to identify any phytoalexins. However, biochemical studies on unripe blueberries and other fruits inoculated with *C. acutatum* have yet to be been carried out, and thus it is possible that phytoalexins may occur in unripe blueberry fruit but diminish at the onset of ripening, as in banana.

Disease cycle and epidemiology

The epidemiology of several anthracnose diseases of tropical fruits has been studied at various stages of

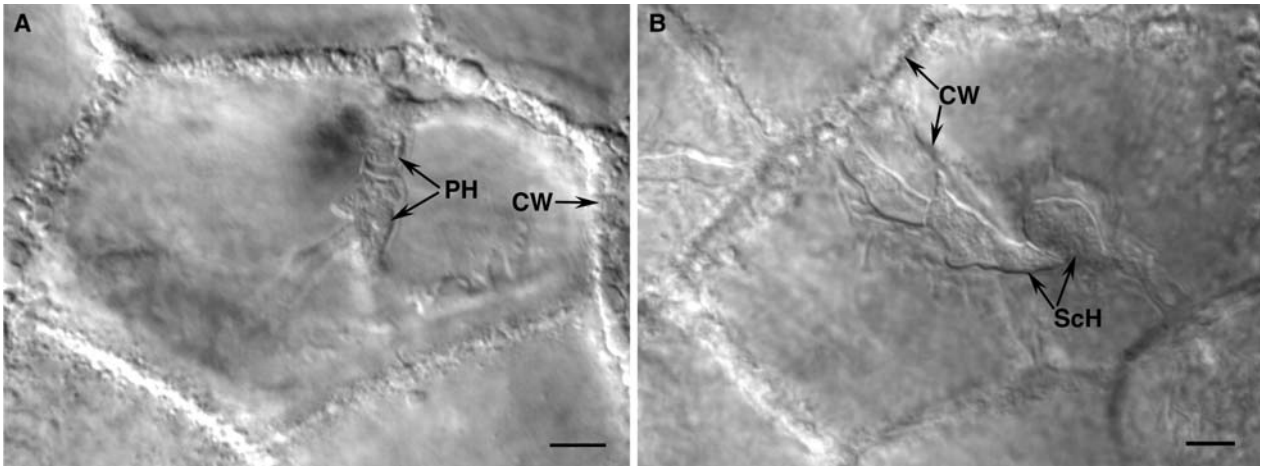

Fig. 8. Light micrographs of ripe blueberry fruit inoculated with *Colletotrichum acutatum*. **A,** Intracellular hemibiotrophic-like infection structures are seen in epidermal cells of susceptible cultivar "Jersey", 48 hours after inoculation. **B,** Subcuticular intramural-like infection structures are seen growing beneath the fruit cuticle in the resistant cultivar "Elliott" 48 hours post inoculation. CW = host epidermal cell wall; PH = primary hyphae; ScH = subcuticular hyphae. Bars = 5 µm.

crop development. In most *Colletotrichum* diseases conidia are water-borne with the occurrence of quiescent infections being highest during the wettest periods of the growing season (Denham & Waller, 1981; Fitzell & Peak, 1984; Darvas & Kotze, 1987). In avocado, citrus and mango, it was shown that infected leaves in the tree canopy were the main source of inoculum, with conidia being rain–splash dispersed to unripe fruit (Denham & Waller, 1981; Fitzell & Peak, 1984; Fitzell, 1987). However, in mango and citrus infected flowers also contributed to inoculum levels (Fitzell & Peak, 1984; Zulfiqar & al., 1996). In almond, mummified fruit represent the main source of conidia for infections (Adaskaveg & al., 2000).

Infection by *Colletotrichum* can take place at all stages of fruit development (Hartung, & al., 1981; Daykin & Milholland, 1984, Adaskaveg & al., 2000). In blueberry, the fungus is thought to overwinter as mycelium in and on blighted twigs, which act as the main source of inoculum in the spring (Milholland, 1995). However, recent data suggest that the primary source of overwintering inoculum may be from dormant flower buds (Fig. 2C) (DeMarsay & Oudemans, 2002; Wharton & Schilder, unpublished). In studies carried out on the cultivar 'Bluecrop' in New Jersey, flower buds accounted for 72% of overwintering infections (DeMarsay & Oudemans, 2002). In screening experiments carried out on the susceptible cultivar 'Jersey' in Michigan, 57% of healthy looking flower buds were found to be infected, and of those infected, 82% of the infections were caused by *C. acutatum* (Wharton & Schilder, unpublished). It was observed that as the flower buds broke dormancy, the fungus grew out of the buds and colonized the surrounding stem tissue, causing black lesions around the infected buds (Fig. 2C). These lesions grew up to 2 cm in length, and resulted in the death of the flower bud and any tissue above the lesion (Fig. 2C). After about 7 days, sporulating masses were observed in the dead tissue (Fig. 2C-D).

In the field, the fungus sporulates on infected tissues during periods of extended wetness in the spring, and conidia of *C. acutatum* are dispersed by rain splash (Caruso & Ramsdell, 1995). As in citrus and strawberry, secondary conidiation, may play a role in early-season dispersal of *C. acutatum* conidia on blueberries (Zulfiqar & al., 1996; Timmer & Brown, 2000; Leandro & al., 2001; P. Oudemans, personal communication). In citrus, the conidia densities decline with time in the absence of bloom, and through normal leaf drop and mortality of buttons and twigs (Timmer & Brown, 2000). In blueberry, peak spore dispersal coincides with flowering and early fruit development stages (Hartung & al., 1981; Wharton & al., 2002). A second peak occurs at fruit maturity, apparently coinciding with sporulation of the fungus on ripe fruit (Wharton & al., 2002). As described above, on immature blueberry fruit, the fungus initiates quiescent infections, and disease symptoms are not observed until the fruit begins to ripen. A proposed disease cycle for the growth and sporulation of *Colletotrichum* on blueberry fruit is shown in Fig. 10.

Fungal control

Effective control of *Colletotrichum* diseases usually involves the use of one or a combination of the following: 1) resistant cultivars, 2) cultural control, 3) chemical control, and 4) biological control using antagonistic organisms. The applicability of control strategies depends as much on the characteristics of the crop on which they are being used as on the disease at which they are targeted.

Resistant Cultivars. Resistance to disease is perhaps the most significant aspect of disease control in agricultural crops, but has been exploited to a lesser extent in fruit crops mainly due to the longer time frame required for breeding and selecting for resistance and the shorter-term advantages of chemical control. Cultivar resistance in fruit crops is complicated by the ability of most *Colletotrichum* fruit pathogens to form quiescent infections. In most host-pathogen interactions, resistance involves the triggering of host defense responses that prevent or retard pathogen growth and may be conditioned by a single gene pair, a host resistance gene and a pathogen avirulence gene (Flor, 1971). However, such gene-for-gene interactions are not usually involved in the resistance of fruits to postharvest diseases caused by *Colletotrichum* (Prusky & al., 2000). Instead, resistance is usually the result of several genes interacting in a way that is not well understood. Resistance in fruit to postharvest pathogens has been described as a "dynamic incompatibility" (Prusky & al., 2000). The response of the host's resistance genes to products of a pathogen's avirulence genes prevents or retards pathogen growth under specific host physiological conditions, as described above. However, the physiological status of the host changes as it matures, ripens, and senesces. Storage, mechanical injury, temperature extremes, and anoxia also alter host physiology, and when physiological changes in the host inhibit defense responses to pathogen activities, the interaction becomes compatible, leading to host colonization and disease. Thus, postharvest differences in resistance among cultivars may be due as much to the conditions under which the fruit is stored

as to the occurrence of defense compounds (Prusky & al., 1991b). Therefore it is probably more useful to define resistance and susceptibility of fruit cultivars to postharvest disease in terms of the incubation period after fruit ripening, with resistant cultivars having a longer storability and shelf life than susceptible ones.

Differences in susceptibility of almond cultivars of California have been noted for blossoms, leaves and fruits both in the field and laboratory (Adaskaveg & al., 2002; Diéguez-Uribeondo & al., 2002). Cultivar 'Nonpareil' appeared to be most tolerant to *C. acutatum* inoculations and 'NePlus Ultra' the most susceptible, while cultivars 'Carmel' and 'Wood Colony' had intermediate susceptibility. In blueberry, there are a few commercially available cultivars (e.g. 'Elliott', 'Brigitta Blue') that are considered "resistant" to anthracnose (Ehlenfeldt & Stretch, 1997). The most widely grown resistant cultivar in Michigan is 'Elliott', which has become popular in the last 25 years due to its very late harvest and long storage life (Jim Hancock, personal communication). It is interesting to note that most blueberry cultivars in which resistance has been found are late season cultivars, with fairly high acid contents. However, as mentioned above few biochemical studies have been carried out into the biochemical basis of resistance in blueberry, and screening trials for resistance to anthracnose have not been able to correlate high acid content to resistance (Ballinger & al., 1978).

Host plant resistance would seem to be a logical and efficient way to control anthracnose disease. However,

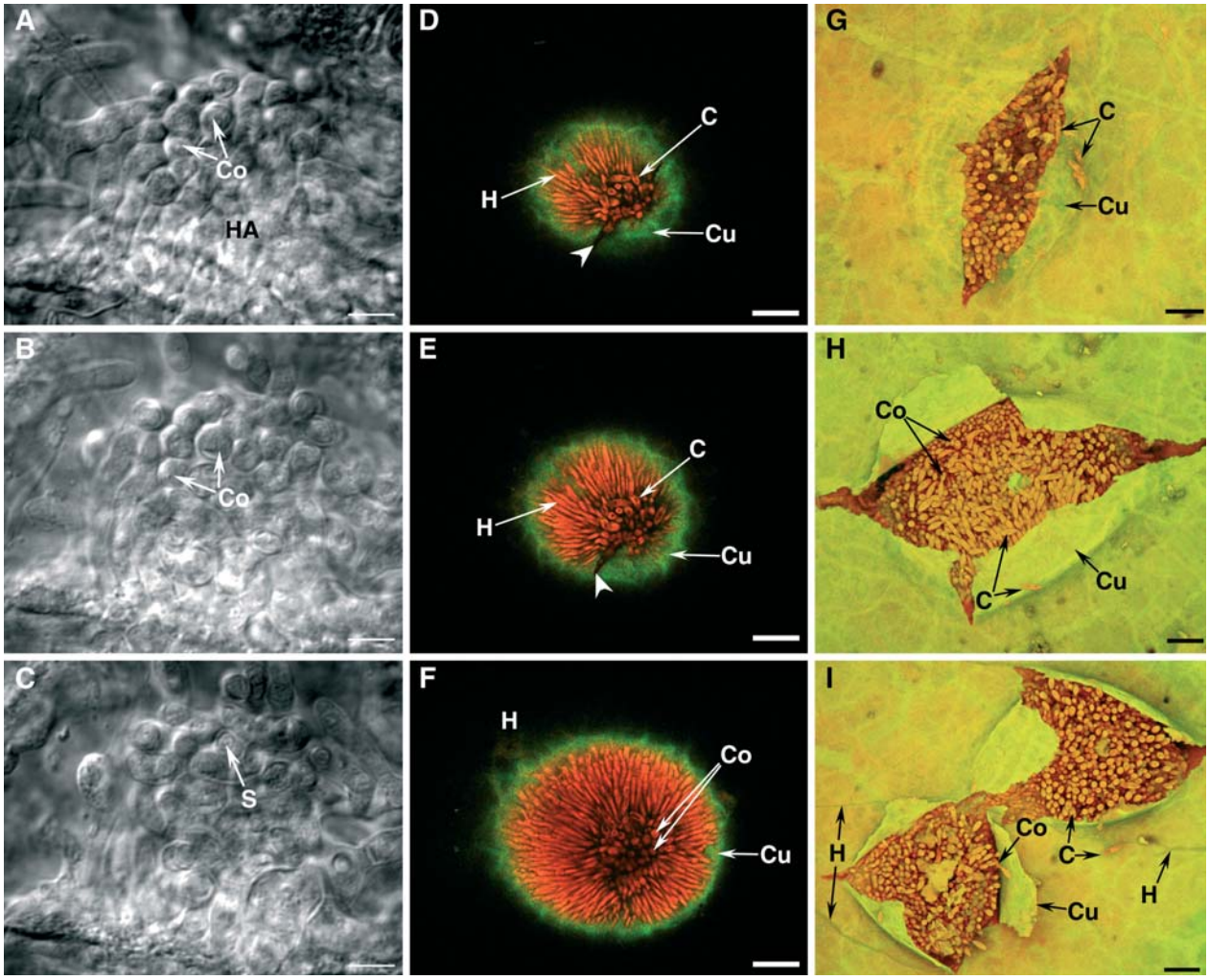

Fig. 9. Confocal scanning laser microscopy micrographs of acervulus formation by *Colletotrichum acutatum* in ripe fruit of the susceptible blueberry cultivar "Jersey". **A-C,** Conidiophores (Co) develop from hyphae in hyphal aggregates (HA) in epidermal cells. A septum (S) usually formed between conidiophores and hyphae in the aggregates; 96 h after inoculation (images **A-C** are optical sections taken using differential interference contrast optics at 0.16 µm *z* intervals). **D-F,** Acervuli are fully developed by 108 h after inoculation. Hyphae (H) and conidia (C) had ruptured the epidermal cell wall (arrow head) and cuticle (Cu) (images **D-F** are confocal micrographs taken at 1 µm *z* intervals). **G-I**, Acervuli are pulvinate, do not contain setae, and were observed in abundance by 120 h after inoculation (images **G-I** are projections of 90 optical sections taken at 1, 0.61 and 0.81 µm *z* intervals respectively). Bars = 5 µm (A-C), 20 µm (D-F).

aside from the costs associated with replacing an established crop with a resistant or more tolerant cultivar, most growers tend to select cultivars based on criteria other than disease resistance.

Cultural control. This usually refers to the range of methods used to control diseases, mostly using tactics aimed at disease avoidance through phytosanitation, manipulation of cropping patterns or by enhancing resistance and avoiding predisposition. However, in relation to fruit crops it also involves the use of proper sanitation techniques during processing of the harvested fruit, transportation, packaging and storage, to avoid exposure of fruit to the pathogen. It also involves proper handling to avoid abiotic factors such as mechanical injury, temperature extremes, and anoxia, which can predispose the fruit to infection by the pathogen. The ubiquitous nature of inoculum sources of *Colletotrichum* diseases and their often-rapid epidemic development under suitable conditions reduces the effectiveness of many pre-harvest general phytosanitary practices. However, general orchard hygiene has a place in integrated disease control, as removal of obvious inoculum sources such as diseased leaves and fruit can increase the efficiency of chemical control (Waller, 1972, 1988).

The prerequisite for wet conditions to coincide with susceptible crop stages for development of *Colletotrichum* epidemics also offers an opportunity for disease control through the manipulation of cropping patterns and or irrigation (Fitzell & Peak, 1984; Fitzell & al., 1984). In blueberry, this can be achieved to some extent by pruning techniques such as the fine pruning of old, fruited and dead twigs that remain in the bush from year to year. As described above, such twigs are thought to act as one of the main sources of *Colletotrichum* inoculum for the infection of susceptible young fruit in the spring. Overhead irrigation is often used in the spring to mediate frosts since blueberry soils are often found in topographically low areas that are prone to frosts. Although the use of overhead irrigation may mediate frost damage it provides ideal conditions for the infection of young fruit.

Chemical control. Chemical control methods are widely used on fruit crops, partly because the value of the produce gained usually offsets the relatively expensive inputs, in terms of machinery, materials and labor, and transportation and storage, which are required, and partly because the availability and efficiency of chemical control is relatively greater than that of other control methods (Jeger & Plumbley, 1988). Generally, *Colletotrichum* diseases can be controlled by a wide range of chemical such as copper compounds, dithiocarbamates, benzimidazole and triazole compounds, and other fungicides such as chlorothalonil, imazalil and prochloraz (Waller, 1992). Newer classes of fungicides such as the strobilurins (e.g. azoxystrobin and pyraclostrobin) are also proving highly effective against *Colletotrichum* species that infect fruits (Schilder & al., 2001). However, the problem of fungicide tolerance may arise quickly if a single compound is relied upon too heavily.

For successful chemical control of many *Colletotrichum* diseases timing and placement are of critical significance. In general, fungicides must be applied to protect the young expanding crop tissues, whether leaves, blossoms or fruit, against infection during periods of wetness (Fitzell & al., 1984). Both rapid expansion of the fruit surfaces and the natural erosion of the fungicide by rainfall make adequate fungicide protection often difficult to achieve, and repeated applications are often necessary to maintain protection in diseases such as mango anthracnose (Fitzell & Peak, 1984). However, poorly timed fungicide applications may actually lead to an increase in the severity of disease due to the disturbance of natural biocontrol mechanisms and increased crop susceptibility (Griffiths, 1981).

Currently, control of blueberry and almond anthracnose is primarily accomplished by chemical means. The following fungicides are reported to have activity against *C. acutatum*: fosetyl-AL (Alliette), captan (Captan), benomyl (Benlate), chlorothalonil (Bravo), ziram (Ziram), fenbuconazole (Indar 75 WP), microbutanil (Rally 40WP), thiophanate methyl (Topsin 75WP), azoxystrobin (Abound) and pyraclostrobin (Cabrio) (Adasakaveg & Förster, 2000; Schilder, 2002). However, the use and effectiveness of these fungicides may be limited by various factors. For example, benomyl has recently been withdrawn by the manufacturer and only limited stocks remain. The use of fosetyl-Al tends to be costly, and chlorothalonil cannot be used after petal fall in blueberry because of phytotoxicity to the fruit. In addition, Captan is currently considered a B-2 carcinogen and its use is restricted by some fruit processors. Ziram has a minimum14-day pre-harvest interval, whereas the number or applications of azoxystrobin and pyraclostrobin allowed per season is limited as part of a fungicide resistance management protocol. This means that growers have to utilize their fungicide options wisely to attain effective control. Although treatment with fungicides can significantly reduce the incidence and severity of disease, eradication cannot normally be achieved (Adasakaveg & Förster, 2000). Thus, if treatments are stopped and conditions favorable for disease re-occur, the disease in the crop may subsequently increase. Applications prior conducive conditions are thus required and rota-

tion programs between fungicides of different classes are highly recommended (Adaskaveg & Förster, 2000). Development of models to predict anthracnose risk due to environmental conditions can efficiently reduce the timing of fungicide applications. Such models are currently used for citrus anthracnose (Timmer & Brown, 2000). In almond, models to predict infection periods by *C. acutatum* and to improve timing of fungicide applications are currently being developed based on the biology of the fungus, disease progress curves in the field and development under defined growth chamber conditions (Adaskaveg & al., 2002, Diéguez-Uribeondo & al., 2002).

Biological control. Biological control methods for *Colletotrichum* diseases have not received much attention until recently even though the potential of biological control through the effect of phyllosphere antagonists has been realized for some time (Lenné & Parbery, 1976). The possibilities for biological control of post harvest fruit diseases have been reviewed by Jeger & Jeffries (1988) and Korsten & Jeffries (2000), and the effects of surface microflora on the incidence of anthracnose diseases such as coffee berry disease, avocado anthracnose, and mango anthracnose, are now being clarified with a view to enhancing naturally occurring biocontrol mechanisms (Masaba & Waller, 1992; Korsten & Jeffries, 2000). Although most of the technology is still at the research stage, recent progress has resulted in a number of new commercial products, including Aspire™, BioSave™, Trichodex™, AQ10™, and Avogreen™ (Janisiewicz, 1998; Korsten & al., 1998; Wilson, 1997). However, although not all the aforementioned products were developed specifically for use against anthracnose (e.g. Apsire™ is marketed to control *Botrytis* and *Penicillium* spp), they have been evaluated for the control of anthracnose (Korsten & al., 1998). Most of these products have been developed for post harvest applications as this situation offers more advantages for biocontrol strategies (Ko-

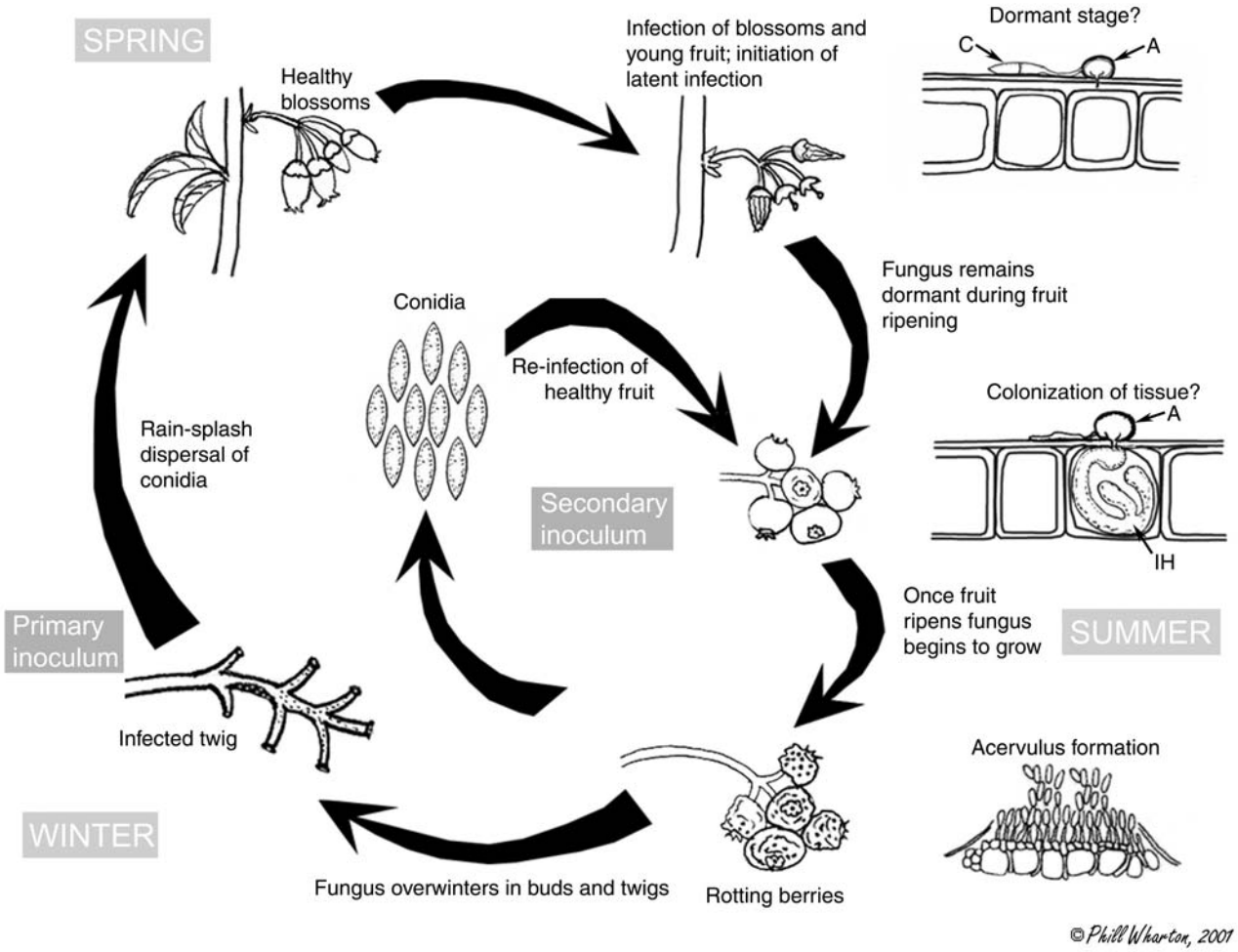

Fig. 10. Hypothetical disease cycle for blueberry anthracnose caused by *Colletotrichum acutatum*. Stages in the lifecycle which are poorly understood are followed by a question mark. C = conidium; A = appressorium; IH = infection hyphae.

rsten & al., 1998). For example, environmental conditions during fruit transportation and storage are generally more uniform than in the field and can often be manipulated. The biomass of the harvested fruit is also much less than that of the standing crop, easier to treat in a uniform manner, and more suited to directly target the pathogen with an appropriate biocontrol formulation.

Currently there are no commercial products registered in the USA for use against *C. acutatum* on blueberry or almond. However, bio-fungicides containing the antagonistic bacteria *Bacillus subtilis* (e.g. SerenadeTM, RhapsodyTM) and *Candida oleophila* (e.g. AspireTM) are in the testing phase to determine their efficacy against *C. acutatum*.

Conclusions

Studies on mango anthracnose and *Colletotrichum* diseases of citrus have shown that development of cost-effective and efficient control strategies for the management of these diseases was facilitated by an accurate identification of the pathogen and gaining an understanding of the host-pathogen interactions that determine successful pathogenesis (Fitzell & Peak, 1984; Fitzell, & al., 1984; Timmer & Brown, 2000). A knowledge of factors such as the conditions required for germination and appressorium formation on the host surface, infection and colonization of host tissues and spore production, enabled disease prediction models to be developed which significantly reduced the number of fungicide sprays required to control these diseases (Fitzell & Peak, 1984; Fitzell & al., 1984; Timmer & Brown, 2000). However, the precise determination of the etiology of *Colletotrichum* diseases requires more than just the isolation and identification of a *Colletotrichum* species from plant tissue. *Colletotrichum* species can be easily and readily isolated from both diseased and apparently healthy tissue of many crops and several ecological studies have shown that *Colletotrichum* species can co-inhabit lesions formed by other pathogens and exist as epiphytes and asymptomatic endophytes in a large variety of plant species (Rodriguez & al., 2000; Waller & Bridge, 2000; Leandro & al., 2001). Thus, a prerequisite to understanding the lifecycles of these fungi is a thorough knowledge of the infection processes and colonization of tissues.

There have been only a few studies on the infection process of *C. acutatum* and no detailed studies on the pre- and post-infection events (including the quiescent phase) that lead to successful colonization of fruit (Daykin & Milholland, 1984; Curry & al., 2002; Diéguez-Uribeondo & al., 2003a; Wharton & Schilder, 2003). As described above, the infection of fruit by *Colletotrichum* species can be separated into stages including the deposition of conidia, germination, appressoria formation, growth and colonization of plant tissues, and production of acervuli and sporulation. The proposed disease cycle of blueberry anthracnose in Fig. 10 indicates several stages (followed by a question mark) that are currently not well understood. The nature of the dormant stage, for instance, is still unclear. The dormant stage is very important in the survival of the fungus from the initial infection of the young, green fruit, until the fruit ripens and colonization takes place. Understanding in what form, and where exactly the fungus survives this period, which can last for more than a month, could help elucidate this crucial component of the lifecycle. It could also indicate a very vulnerable stage in the fungal lifecycle that could be used in developing specific control strategies. Next is an understanding of the timing and nature of the colonization process. Again, it is not clear when and to what extent the fungus colonizes fruit tissues before it reemerges and sporulates. This is also a stage that we believe could yield some clues as to possible resistance mechanisms.

Host resistance in fruit is often described in terms of dynamic incompatibility and host barriers can occur at different stages of the infection and colonization process. Dynamic incompatibility could occur at the stage of inhibition of appressorium development, inhibition of fungal penetration, or inhibition of fungal colonization. In blueberry, the question remains as to what potential defense responses are triggered, or suppressed in fruits at different physiological stages? And what the roles of pre-formed or inducible barriers are to pathogen attack? As described above, several studies have been carried out into the antifungal properties of extracts from ripe blueberry fruit from wild highbush blueberry plants as they relate to fruit decay and herbivore preference (Cipollini & Stiles, 1992a, 1992b, 1993). These studies indicated that the main antifungal compounds present in ripe blueberry fruit were water-soluble, non-alkaloidal chemicals such as phenolics and acids. They proposed that resistance in ripe blueberries may be due to a combination of factors including acid levels in the fruit and an interaction between simple phenolic compounds and organic acids, and not necessarily individual fungitoxic compounds. To date, no studies have investigated the biochemical composition of unripe blueberry fruit. This is important as studies have shown that there are differences in the chemical composition of blueberries both at different stages of maturity and between cultivars (Connor & al., 2002; Hakkinen & Torronen, 2000;

Kalt & McDonald, 1996). Furthermore, an improved understanding of the physiology and underlying biochemical processes involved in the induction and maintenance of quiescence could lead to new disease control measures, such as have been developed in avocado (Prusky & al., 1991b).

Given the epidemiological versatility of *Colletotrichum* diseases, the production of high-quality fruit, should follow an integrated approach in order to achieve maximum yields. Where possible, resistant cultivars can be combined with judicious pruning and proper irrigation timing to reduce inoculum levels and disease pressure. More detailed information on the biology of the pathogen will be needed to help optimize use and timing of the available fungicides. As more reduced-risk and biological control products become available, they may be substituted for older chemistries to lessen negative impacts on users, consumers, and the environment.

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References

Adaskaveg, J.E. & Förster, H. 2000. Occurrence and management of anthracnose epidemics caused by Colletotrichum species on tree fruit crops in California. In: Prusky, D., Freeman, S. & Dickman, M.B. (eds.), Colletotrichum*: Host Specificity, Pathology, and Host-Pathogen Interaction*, pp. 317-336. The American Phytopathological Society. St. Paul MN.

Adaskaveg, J.E. & Hartin, R.J. 1997. Characterization of Colletotrichum acutatum isolates causing anthracnose of almond and peach in California. *Phytopathology* 87: 979-987.

Adaskaveg, J.E., Diéguez-Uribeondo, J., Förster, H., Thompson, D., Teviotdale, B.L., Connell, J., Edstrom, J., Duncan, R., Freeman, M. & Verdegaal, P. 2001. Epidemiology and management of almond anthracnose and brown rot in California. In: *Proc. of the 29th Annual Almond Conference,* pages 45-53. The Almond Board of California. Modesto, CA.

Adaskaveg, J.E., Diéguez-Uribeondo, J., Förster, H., Erickson, E., Teviotdale, B.L., Connell, J., Edstrom, J., Duncan, R., Hendricks, L., Freeman, M. & Verdegaal, P. 2002. Epidemiology and management of almond anthracnose and brown rot in California. In: *Proc. of the 30th Annual Almond Conference*, pages 79-487. The Almond Board of California. Modesto, CA.

Adikaram, N.K.B. 1981. A study of the latent infection of fruit of Capsicum spp. by Colletotrichum capsici and Glomerella cingulata. Ph D Thesis, Queen's University of Belfast.

Adikaram, N.K.B., Brown, A.E. & Swinburne, T.R. 1982. Phytoalexin involvement in latent infection of Capsicum annum L. fruit by Glomerella cingulata (Stonem.). *Physiological Plant Pathology* 21: 161-170.

Adikaram, N.K.B., Brown, A.E. & Swinburne, T.R. 1983. Observations on infection of Capsicum annum fruit by Glomerella cingulata and Colletotrichum capsici. *Transactions of the British Mycological Society* 80: 395-401.

Arauz, L.F. 2000. Mango anthracnose: Economic impact and current options from integrated management. *Plant Disease* 84: 600-611.

Bailey, J.A. & Jeger, M.J. (eds). 1992. *Colletotrichum: Biology, Pathology and Control*. CAB International. Wallingford UK.

Bailey, J.A., O'Connell, R.J., Pring, R.J. & Nash, C. 1992. Infection strategies of Colletotrichum species. In: Bailey, J.A. & Jeger, M.J. (eds.), *Colletotrichum: Biology, Pathology and Control,* pp. 88-120. CAB International. Wallingford UK.

Bailey, J.A., Nash, C., Morgan, L.W. & O'Connell, R.J. 1996. Molecular taxonomy of Colletotrichum species causing anthracnose on the Malvaceae. *Phytopathology* 86: 1076-1083.

Ballinger, W.E., Maness, E.P. & McClure, W.F. 1978. Relationship of stage of ripeness and holding temperature to decay development of blueberries. *Journal of the American Horticultural Societ*y 103: 130-134.

Biggs, A.R. 1995. Detection of latent infections in apple fruit with paraquat. *Plant Disease* 79: 1062-1067.

Binyamini, N. & Schiffmann-Nadel, M. 1972. Latent infection in avocado due to Colletotrichum gloeosporioides. *Phytopathology* 62: 592-594.

Bonde, M.R., Peterson, G.L. & Maas, J.L. 1991. Isozyme comparisons for identification of Colletotrichum species pathogenic to strawberry. *Phytopathology* 81: 1523-1528.

Brooker, N.L., Leslie, J.F. & Dickman, M.B. 1991. Nitrate nonutilizing mutants of Colletotrichum and their use in studies of vegetative compatibility and genetic relatedness. *Phytopathology* 81: 672-677.

Brown, A.E. & Swinburne, T.R. 1980. The resistance of immature banana fruits to anthracnose (Colletotrichum musae [Berk. & Curt.] Arx.). *Journal of Phytopathology - Phytopathologische Zeitschrift* 99: 70-80.

Camacho, F.J., Gernandt, D.S., Liston, A., Stone, J.K. & Klein, A.S. 1997. Endophytic fungal DNA, the source of contamination in spruce needle DNA. *Molecular Ecology* 6: 983-987.

Caruso, F.L. & Ramsdell, D.C. 1995. In: F.L. Caruso & D.C. Ramsdell (eds.). *Compendium of Blueberry and Cranberry Diseases*, 87 pp. APS Press. St. Paul MN.

Chacko, R.J., Weidemann, G.J., TeBeest, D.O. & Correll, J.C. 1994. The use of vegetative compatibility and heterokaryosis to determine potential asexual gene exchange in Colletotrichum gloeosporioides. *Biological Control* 4: 382-389.

Chakravaty, T. 1957. Anthracnose on banana (Gloesporium musarum Cke & Massae) with special reference to latent infection in storage. *Transactions of the British Mycological Society* 40: 337-345.

Chilton, S.J.P. & Wheeler, H.E. 1949. Genetics of Glomerella. VII. Mutation and segregation in plus cultures. *American Journal of Botany* 36: 717-721

Cipollini, M.L. & Stiles, E.W. 1992a. Antifungal activity of ripe ericaceous fruits-phenolic-acid interactions and palatability for dispersers. *Biochemical Systematics and Ecology* 20: 501-514.

Cipollini, M.L. & Stiles, E.W. 1992b. Relative risks of fungal rot for temperate ericaceous fruits-effects of seasonal-variation on selection for chemical defense. *Canadian Journal of Botany* 70: 1868-1877.

Cipollini, M.L. & Stiles, E.W. 1993. Fruit rot, antifungal defense, and palatability of fleshy fruits for frugivorous birds. *Ecology* 74: 751-762.

Connor, A.M., Luby, J.J. & Tong, C.B.S. 2002. Variation and heritability estimates for antioxidant activity, total phenolic content, and anthocyanin content in blueberry progenies. *Journal of the American Society for Horticultural Scienc*e 127: 82-88.

Correll, J.C., Guerber, J.C. & Rhodes, D.D. 1994. Genetic and molecular diversity of populations of Glomerella cingulata, Colletotrichum gloeosporioides and Colletotrichum acutatum on apple fruit. In: *Proceedings of Fifth International Mycological Conference*. Vancouver B. C. Canada.

Correll, J.C., Guerber, J.C., Walsiwa, L.A., Sherril, J.F. & Morelock, T.E. 2000. Inter and intra species variation in Colletotrichum and mechanism which affect population structure. In: Prusky, D., Freeman, S. & Dickman, M.B. (eds.), Colletotrichum*: Host Specificity, Pathology, and Host-Pathogen Interaction*, pp. 145-179. The American Phytopathological Society. St. Paul MN.

Curry, K.J., Abril, M., Avant, J.B. & Smith, B.J. 2002. Strawberry anthracnose: Histopathology of Colletotrichum acutatum and C. fragariae. *Phytopathology* 92: 1055-1063.

Danneberger, T.K., Vargas, J.M. & Jones, A.L. 1984. A model for weather-based forecasting of anthracnose on annual bluegrass. *Phytopathology* 74: 448-451.

Darvas, J.M. & Kotze, J.M. 1987. Avocado fruit diseases and their control in South Africa. *South African Avocado Grower's Association Yearbook* 10: 117-119.

Daykin, M.E. & Milholland, R.D. 1984. Infection of blueberry fruit by Colletotrichum gloeosporioides. *Plant Disease* 68: 948-950.

DeMarsay, A. & Oudemans, P.V. 2002. Reservoirs of Colletotrichum acutatum in dormant and growing highbush blueberry. *Phytopathology* 92: S143.

Denham, T.G. & Waller, J.M. 1981. Some epidemiological aspects of post-bloom fruit drop disease (Colletotrichum gloeosporioides) in citrus. *Annals of Applied Biology* 98: 65-77.

Diéguez-Uribeondo, J., Förster, H. & Adaskaveg, J.E. 2002. Temperature and wetness periods requirements for almond anthracnose development on leaves and blossoms. *Phytopathology* 92: S19.

Diéguez-Uribeondo, J., Förster H. & Adaskaveg, J.E. 2003a. Subcuticular and intracellular hemibiotrophic development of Colletotrichum acutatum on almond. *Phytopathology* 93: S21.

Diéguez-Uribeondo, J., Förster, H. & Adaskaveg, J.E. 2003b. Digital image analysis of the internal light spots of appressoria of Colletotrichum acutatum. *Phytopathology* 92: 923-930.

Dodd, J.C., Estrada, A.B., Matcham, J., Jeffries, P. & Jeger, M.J. 1991. The effect of climatic factors on Colletotrichum gloeosporioides, causal agent of mango anthracnose, in the Philippines. *Plant Pathology* 40: 568-575.

Duthie, J.A. 1997. Models of the response of foliar parasites to the combined effects of temperature and duration of wetness. *Phytopathology* 87: 1088-1095.

Ehlenfeldt, M.K. & Stretch, A.W. 1997. Identifying sources of resistance to mummy berry and anthracnose in highbush blueberry. *Acta Horticulturae* 446: 281-286.

Fernando, T.H.P.S., Jayasinghe, C.K. & Wijesundera, R.L.C. 2001. Cell wall degrading enzyme secretion by Colletotrichum acutatum, the causative fungus of secondary leaf fall of Hevea basiliensis (rubber). *Mycological Research* 105: 195-201.

Fitzell, R.D. 1979. Colletotrichum acutatum as a cause of anthracnose of mango in New-South-Wales. *Plant Disease Reporter* 63: 1067-1070.

Fitzell, R.D. 1987. Epidemiology of anthracnose disease of avocados. *South African Avocado Grower's Association Yearbook* 10: 113-116.

Fitzell, R.D. & Peak, C.M. 1984. The epidemiology of anthracnose disease of mango - Inoculum sources, spore production and dispersal. *Annals of Applied Biology* 104: 53-59.

Fitzell, R.D., Peak, C.M. & Parnell, R.E. 1984. A model for estimating infection levels of anthracnose disease of mango. *Annals of Applied Biology* 104: 451-458.

Flor, H.H. 1971. The current status of the gene-for-gene concept. *Annual Review of Phytopathology* 9: 275-296.

Förster, H. & Adaskaveg, J.E. 1999. Identification of subpopulations of Colletotrichum acutatum and epidemiology of almond anthracnose in California. *Phytopathology* 89: 1056-1065.

Freeman, S. 2000. Genetic diversity and host specificity of *Colletotrichum* species on various fruits. In: Prusky, D., Freeman, S. & Dickman, M.B. (eds.), Colletotrichum: *Host Specificity, Pathology, and Host-Pathogen Interaction*, pages 131-144. The American Phytopathological Society. St. Paul MN.

Freeman, S. & Shabi, E. 1996. Cross-infection of subtropical and temperate fruits by Colletotrichum species from various hosts. *Physiological and Molecular Plant Pathology* 49: 395-404.

Freeman, S., Katan, T. & Shabi, E. 1998. Characterization of Colletotrichum species responsible for anthracnose diseases of various fruits. *Plant Disease* 82: 596-605.

Freeman, S., Minz, D., Maymon, M. & Veibil, A. 2001. Genetic diversity within Colletotrichum acutatum sensu Simmonds. *Phytopathology* 91: 586-592.

Griffiths, E. 1981. Pathogenic plant diseases. *Annual Review Phytopathology* 19: 69-82.

Guerber, J.C. & Correll, J.C. 1997. The first report of the teleomorph of Colletotrichum acutatum. *Plant Disease* 81: 1334

Guerber, J.C. & Correll, J.C. 2001. Characterization of Glomerella acutata, the teleomorph of Colletotrichum acutatum. *Mycologia* 93: 216-229

Hakkinen, S.H. & Torronen, A.R. 2000. Content of flavonols and selected phenolic acids in strawberries and Vaccinium species: influence of cultivar, cultivation site and technique. *Food Research Internationa*l 33: 517-524.

Hartung, J.S., Burton, C.C. & Ramsdell, D.C. 1981. Epidemiological studies of blueberry anthracnose disease caused by Colletotrichum gloeosporioides. *Phytopathology* 71: 449-453.

Horsfall, J.G. & Dimond, A.E. 1957. Interactions of tissue sugar, growth substances and disease susceptibility. *Zietschrift fur Pflanzenkrankheiten und Pflanzenschutz* 64: 415-419.

Howard, C.M., Maas, J.L., Chandler, C.K. & Albregts, E.E. 1992. Anthracnose of strawberry caused by Colletotrichum complex in Florida. *Plant Disease* 76: 796-981.

Howard, R.J. & Valent, B. 1996. Breaking and entering: Host penetration by the fungal rice blast pathogen Magnaporthe grisea. *Annual Review of Microbiology* 50: 491-512.

Janisiewicz, W.J. 1998. Biocontrol of postharvest diseases of temperate fruits. In: Boland, G.J. & Kuykendall, L.D. (eds.), *Plant-Microbe Interactions and Biological Control*, pages 171-198. Marcel Dekker NY.

Jayasinghe, C.K., Fernando, T.H.P.S. & Priyanka, U.M.S. 1997. Colletotrichum acutatum is the main cause of Colletotrichum leaf disease of rubber in Sri Lanka. *Mycopathologia* 137: 53-56.

Jeffries, P., Dodd, J.C., Jeger, M.J. & Plumbey, R.A. 1990. The biology and control of Colletotrichum species on tropical fruit crops. *Plant Pathology* 39: 343-366.

Jeger, M.J. & Jeffries, P. 1988. Alternatives to chemical usage for disease management in the post-harvest environment. *Aspects of Applied Biology* 17: 47-57.

Jeger, M.J. & Plumbley, R.A. 1988. Post-harvest losses caused by anthracnose (Colletotrichum gloeosporioides) of tropical fruit and vegetables. *Biodeterioration* 7: 642-646.

Johnston, P.R. & Jones, D. 1997. Relationships among Colletotrichum isolates from fruit-rots assessed using rDNA sequences. *Mycologia* 89: 420-430.

Kalt, W. & McDonald, J. E. 1996. Chemical composition of lowbush blueberry cultivars. *Journal of the American Society for Horticultural Science* 121: 142-146.

Katan, T. 2000. Vegetative compatibility in Colletotrichum. In: Prusky, D., Freeman, S. & M.B. Dickman (eds.), Colletotrichum*: Host Specificity, Pathology, and Host-Pathogen Interaction*, pp. 145-179. The American Phytopathological Society, St. Paul MN.

Korsten, L. & Jeffries, P. 2000. Potential of biological control of diseases caused by Colletotrichum. In: Bailey, J.A. & Jeger, M.J. (eds.), Colletotrichum: *Biology, Pathology and Control,* pp. 266-291. CAB International. Wallingford UK.

Korsten, L., Towsen, E. & Claasens, V. 1998. Evaluation of avogreen as a postharvest treatment for controlling anthracnose and stem-end rot on avocado fruit. *South African Avocado Grower's Association Yearbook* 21 (in press).

Kubo, Y. & Furusawa, I. 1991. Melanin biosynthesis: prerequisite for successful invasion of the plant host by appressoria of Colletotrichum and Pyricularia. In: Cole, G.T. & Hoch, H.C. (eds.), *The Fungal Spore and Disease Initiation in Plants and Animals,* pp. 205-218. Plenum Press NY.

Kuramazae-Izioka, E.E., Lopes, C.R., Souza, N.L. & Machado, M.A. 1997. Morphological and molecular characterization of Colletotrichum spp. from citrus orchards affected by postbloom fruit drop in Brazil. *European Journal of Plant Pathology* 103: 323-329.

Lardner, R., Johnston, P.R., Plummer, K.M. & Pearson, M.N. 1999. Morphological and molecular analysis of Colletotrichum acutatum sensu lato. *Mycological Research* 103: 275-285.

Latunde-Dada, A.O., O'Connell, R.J., Nash, C. & Lucas, J.A. 1999. Stomatal penetration of cowpea (Vigna unguiculata) leaves by a Colletotrichum species causing latent anthracnose. *Plant Pathology* 48: 777-784.

Latunde-Dada, A.O., O'Connell, R.J., Nash, C., Pring, R.J., Lucas, J.A. & Bailey, J.A. 1996. Infection process and identity of the hemibiotrophic anthracnose fungus (Colletotrichum destructivum) from cowpea (Vigna unguiculata). *Mycological Research* 100: 1133-1141.

Leandro, L.F.S., Gleason, M.L., Nutter, F.W., Wegulo, S.N. & Dixon, P.M. 2001. Germination and sporulation of Colletotrichum acutatum on symptomless strawberry leaves. *Phytopathology* 91: 659-664.

Lenné, J.M. & Parbery, D.G. 1976. Phyllosphere antagonists and appressoria formation in Colletotrichum gloeosporioides. *Transactions of the British Mycological Society* 66: 334-336.

Masaba, D. & Waller, J.M. 1992. Coffee Berry Disease: The current status. In: Bailey, J.A. & Jeger, M.J. (eds.), Colletotrichum: *Biology, Pathology and Control*, pages 237-249. CAB International. Wallingford UK.

Martín, M.P. & García-Figueres, F. 1999. Colletotrichum acutatum and C. gloeosporioides caused anthracnose on olives. *European Journal of Plant Pathology* 105: 733-745.

Mendgen, K. & Deising, H. 1993. Infection structures of fungal plant pathogens - a cytological and physiological evaluation. *New Phytologist* 124: 193-213.

Milholland, R.D. 1995. Anthracnose Fruit Rot (Ripe Rot). In: Caruso, F.L. & Ramsdell, D.C. (eds.). *Compendium of Blueberry and Cranberry Diseases*, 17 pp. APS Press. St Paul, MN.

Monroe, J.S., Santini, J.B. & Latin, R. 1997. A model defining the relationship between temperature and leaf wetness duration, and infection of watermelon by Colletotrichum orbiculare. *Plant Disease* 81: 739-742.

Mould, M.J.R., Boland, G.J. & Robb, J. 1991. Ultrastructure of the Colletotrichum trifolii-Medicago sativa pathosystem. II. Post-penetration events. *Physiological and Molecular Plant Pathology* 38: 195-210.

Muirhead, I.F. & Deverall, B.J. 1984. Role of appressoria in latent infections of banana fruits by Colletotrichum musae. *Physiological Plant Pathology* 19: 77-84.

Nair, J. & Corbin, J.B. 1981. Histopathology of Pinus radiata seedlings infected with Colletotrichum acutatum f. sp. pinea. *Phytopathology* 71: 777-783.

O'Connell, R.J., Bailey, J.A. & Richmond, D.V. 1985. Cytology and physiology of infection of Phaseolus vulgaris by Colletotrichum lindemuthianum. *Physiological and Molecular Plant Pathology* 27: 75-98.

O'Connell, R.J., Perfect, S.E., Hughes, H.B., Carazaniga, R. & Bailey, J.A. 2000. Dissecting the cell biology of Colletotrichum infection processes. In: Prusky, D., Freeman, S. & Dickman, M.B. (eds.), Colletotrichum: *Host Specificity, Pathology, and Host-Pathogen Interaction*, pages 57-76. The American Phytopathological Society. St. Paul MN.

Ogawa, J.M. & English, H. 1991. Diseases of Temperate Zone Tree Fruit and Nut Crops. In: *University of California, Division of Agriculture and Natural Resources*, pp. 345- 461. Oakland CA.

Peres, N.A.R., Kuramae, E.E., Dias, M.S.C. & de Souza, N.L. 2002. Identification and characterization of Colletotrichum spp. affecting fruit after harvest in Brazil. *Journal of Phytopathology - Phytopathologische Zeitschrift* 150: 128-134.

Perfect, S.E., Hughes, H.B., O'Connell, R.J. & Green, J. 1999. Colletotrichum: A model genus for studies on pathology and fungal-plant interactions. *Fungal Genetics Biology* 27: 186-198.

Pring, R.J., Nash, C., Zakaria, M. & Bailey, J.A. 1995. Infection process and host range of Colletotrichum capsici. *Physiological and Molecular Plant Pathology* 46: 137-152.

Prusky, D. 1996. Pathogen quiescence in postharvest diseases. *Annual Review of Phytopathology* 34: 413-434.

Prusky, D. & Plumbley, R. A. 1992. Quiescent infections of Colletotrichum in tropical and subtropical fruit. In: Bailey, J.A. & Jeger, M.J. (eds.), Colletotrichum: *Biology, Pathology and Control*, pages 337-357. CAB International. Wallingford UK.

Prusky, D., Gold, S. & Keen, N.T. 1989. Purification and characterization of an endopolygalacturonase produced by Colletotrichum gloeosporioides. *Physiological* and *Molecular Plant Pathology* 35: 121-133.

Prusky, D., Kobiler, I. & Jacoby, B. 1988. Involvement of epicatechin in cultivar susceptibility of avocado fruits to Colletotrichum gloeosporioides after harvest. *Journal of Phytopathology - Phytopathologische Zeitschrift* 123: 140-146.

Prusky, D., Plumbley, R. A. & Kobiler, I. 1991b. Effect of CO_2 treatment on the induction of antifungal diene in unripe avocado fruits. *Physiological and Molecular Plant Pathology* 39: 325-334.

Prusky, D., Keen, N.T., Sims, J.J., & Midland, S.L. 1982. Possible involvement of an anti-fungal diene in the latency of Colletotrichum gloeosporioides on unripe avocado fruits. *Phytopathology* 72: 1578-1582.

Prusky, D., Kobiler, I., Ardi, R., Beno-Moalem, D., Yakoby, N. & Keen, N.T. 2000. Resistance mechanisms of subtropical fruits to Colletotrichum gloeosporioides. In: Prusky, D., Freeman, S. & Dickman, M.B. (eds.), Colletotrichum*: Host Specificity, Pathology, and Host-Pathogen Interaction*, pp. 232-244. The American Phytopathological Society. St. Paul MN.

Prusky, D., Kobiler, I., Fishman, Y., Sims, J.J., Midland, S.L. & Keen, N.T. 1991a. Identification of an antifungal compound in unripe avocado fruits and its possible involvement in the quiescent infections of Colletotrichum gloeosporioides. *Journal of Phytopathology - Phytopathologische Zeitschrift* 132: 319-327.

Prusky, D., Kobiler, I., Jacoby, B., Sims, J.J. & Midland, S.L. 1985. Effect of inhibitors of lipoxygenase activity and its possible relation to latency of Colletotrichum gloeosporioides on avocado fruits. *Physiological and Molecular Plant Pathology* 27: 269-279.

Rappel, L., Irwin, J.A.G. & Muirhead, I.F. 1989. The secret life of avocado anthracnose. *Queensland Agricultural Journal* 115: 31-32.

Roberts, R.G. & Snow, J.P. 1984. Histopathology of cotton boll rot caused by Colletotrichum capsici. *Phytopathology* 74: 390-397.

Rodriguez, R.J., US Geological Survey, WA & Redman, R.S. 2000. Colletotrichum as model system for defining the genetic basis of fungal symbiotic life styles. In: Prusky, D., Freeman, S. & Dickman, M.B. (eds.), Colletotrichum: *Host Specificity, Pathology, and Host-Pathogen Interaction*, pp. 232-244. The American Phytopathological Society. St. Paul MN.

Schilder, A.M.C. 2002. Small fruit fungicides. In: *Fruit Spraying Calendar of Michigan State University Extension Bulletin E-154*, pp. 95-105. Michigan State University. East Lansing, MI.

Schilder, A.M.C., Gillett, J.M. & Sysak, R.W. 2001. Evaluation of fungicides for control of anthracnose fruit rot of blueberries. *Fungicide and Infanticide Tests* 2001: SMF5, [http://www.scisoc.org/online/FNtests/2001/top.htm].

Sénéchal, Y., Sanier, C., Gohet, E. & D'Auzac, J. 1987. Différents modes de pénétration du Colletotrichum gloeosporioides dans les feuilles d'Hevea brasiliensis. *Comptes Rendus De L' Academie Des Sciences Serie Iii-Sciences De La Vie-Life Sciences* 305: 537-542.

Shi, Y.J.C. Correll, J.C. Guerber J.C. & Rom, C.R. 1996. Frequency of Colletotrichum species causing bitter rot of apple in the southeastern United States. *Plant Disease* 80: 692-696.

Singh, J.P. & Sharma, S.K. 1981. Screening and chemical basis of resistance in guava varieties to anthracnose (Glomerella cingulata). *Haryana Journal of Horticultural Sciences* 10:155-157.

Skipp, R.A., Beever, R.E., Sharrock, K.R., Rikkerink, E.H.A. & Templeton, M.D. 1995. Histopathological, genetic, Biochemical and molecular basis in Colletotrichum. In: Singh, U.S., Kohmoto, K. & Singh, R.P. (eds.), *Pathogens and Host Parasite Specificity in Plant Disease*, pp. 119-143. Elsevier Science Ltd. Oxford UK.

Smith, B.J. & Black, L.L. 1990. Morphological, cultural, and pathogenic variation among Colletotrichum species isolated from strawberry. *Plant Disease* 74: 69-76.

Smith, B.J., Magee, J.B. & Gupton, C.L. 1996. Susceptibility of rabbiteye blueberry cultivars to postharvest diseases. *Plant Disease* 80: 215-218.

Sutton, B.C. 1980. *The Coelomycetes: Fungi Imperfecti with Pycnidia, Acervuli and Stromata.* Commonwealth Mycological Institute. London.

Sutton, B.L. 1992. The genus Glomerella and its Colletotrichum anamorph. In: Bailey, J.A. & Jeger, M.J. (eds.), Colletotrichum: *Biology, Pathology and Control*, pp. 1-28. CAB Internationa. Wallingford UK.

Swinburne, T.R. 1983. Quiescent infections in post-harvest diseases. In: Dennis, C. (ed.), *Post-harvest Pathology of Fruits and Vegetables*, pp. 1-21. Academic Press. London UK.

Timmer, L.W. & Brown, G.E. 2000. Biology and control of anthracnose diseases of citrus. In: Prusky, D., Freeman, S. & Dickman, M.B. (eds.), *Colletotrichum*: *Host Specificity, Pathology, and Host-Pathogen Interaction*, pp. 300-316. The American Phytopathological Society. St. Paul MN.

Timmer, L.W. & Zitko, S.E. 1993. Relationships of environmental factors and inoculum levels to the incidence of postbloom fruit drop of citrus. *Plant Disease* 77: 501-503.

Timmer, L.W. & Zitko, S.E. 1996. Evaluation of a model for prediction of postbloom fruit drop of citrus. *Plant Disease* 80: 380-383.

Uddin, W., Serlemitsos, K. & Viji, G. 2002. A temperature and leaf wetness duration-based model for prediction of gray leaf spot of perennial ryegrass turf. *Phytopathology* 93: 336-343.

Van der Bruggen, P. & Maraite, H. 1987. Histopathology of cassava anthracnose disease caused by Colletotrichum gloeosporioides f. sp. manihotis. *Parasitica* 43: 3-21.

Verhoeff, K. 1974. Latent infection by fungi. *Annual Review of Phytopathology* 12: 99-107.

von Arx, J.A. 1957. Die Arten der Gattung Colletotrichum Corda. *Journal of Phytopathology - Phytopathologische Zeitschrift* 29: 413.

von Arx, J.A. 1970. A revision of the fungi classified as Gloeosporium. *Bibliotheca Mycologica* 24:1.

Walker, J.C. 1921. Onion smudge. *Journal of Agricultural Research* 20: 685-721.

Waller, J.M. 1972. Water-borne spore dispersal in coffee berry disease and its relation to control. *Annals of Applied Biology* 71: 1-18.

Waller, J.M. 1988. New developments in integrated disease management of tropical plantation crops. In: *Proceedings of the Brighton Crop Protection Conference - Pests and Diseases*, pp. 297-305.

Waller, J.M. 1992. Colletotrichum diseases of perennial and other cash crops. In: Prusky, D., Freeman, S. & Dickman, M.B. (eds.), Colletotrichum: *Host Specificity, Pathology, and Host-Pathogen Interaction*, pp. 167-185. The American Phytopathological Society. St. Paul MN.

Waller, J.M. & Bridge, P.D. 2000. Recent advances in understanding Colletotrichum diseases of some tropical perennial crops. In: Bailey, J.A., & Jeger, M.J. (eds.), Colletotrichum: *Biology, Pathology and Control*, pp. 337-345. CAB International. Wallingford UK.

Wharton, P.S. & Julian, A.M. 1996. A cytological study of compatible and incompatible interactions between Sorghum bicolor and Colletotrichum sublineolum. *New Phytologist* 134: 25-34.

Wharton, P.S. & Schilder, A.M.C. 2003. Infection and colonization of blueberry fruit by Colletotrichum acutatum. *Phytopathology* 93: S90.

Wharton, P.S., Dickman J. & Schilder, A.M.C. 2002. Timing of spore release by Colletotrichum acutatum in Michigan blueberry fields. *Phytopathology* 92: S86.

Wharton, P.S., Julian A.M. & O'Connell, R.J. 2001. Ultrastructure of the infection of Sorghum bicolor by Colletotrichum sublineolum. *Phytopathology* 91: 149-158.

Wheeler, H.E.1954. Genetics and evolution of heterothallism in Glomerella. *Phytopathology* 44: 342-353.

Wijesundera, R.L.C., Bailey, J.A., & Byrde, R.J.W. 1984. Production of pectin lyase by Colletotrichum lindemuthianum in culture and in infected bean (Phaseolus vulgaris) tissue. *Journal of General Microbiology* 130: 285-290.

Wijesundera, R.L.C., Bailey, J.A., Byrde, R.J.W. & Fielding, A.H. 1989. Cell wall degrading enzymes of Colletotrichum lindemuthianum - Their role in the development of bean anthracnose. *Physiological and Molecular Plant Pathology* 34: 403-413.

Wilson, C.L. 1997. Biocontrol of aerial plant diseases in agriculture and horticulture: Current approaches and future prospects. *Journal of Industrial Microbiology and Biotechnology* 19: 188-191.

Yoshida, S. & Tsukiboshi, T. 2002. Shoot blight and leaf spot of blueberry anthracnose by Colletotrichum acutatum. *Journal of General Plant Pathology* 68: 246-248

Zaitlin, B., Zehr, E.I. & Dean, R.A. 2000. Latent infection on peach caused by Colletotrichum gloeosporioides and by Colletotrichum acutatum. *Canadian Journal of Botany* 22: 224-228.

Zhang, W., Wendel, J.F. & Clark, L.G. 1997. Bamboozled again! Inadvertent isolation of fungal rDNA sequences from bamboos (Poaceae: Bambusoideae). *Molecular Phylogenetics and Evolution* 8: 205-217.

Zulfiqar, M., Brlansky, R.H. & Timmer, L.W. 1996. Infection of flower and vegetative tissues of citrus by Colletotrichum acutatum and C. gloeosporioides. *Mycologia* 88: 121-128.

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