REVIEW

Alternaria diseases of citrus – Novel pathosystems

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Summary. Citrus is affected by four diseases caused by *Alternaria* spp. Brown spot of tangerines, leaf spot of rough lemon, postharvest black rot of fruit occur widely in citrus areas of the world and are caused by different pathotypes of *A. alternata*. Mancha foliar occurs only on Mexican lime in western Mexico and is caused by *A. limicola*. Tangerine and rough lemon pathotypes produce host-specific toxins that affect membranes and respiration, respectively. Black rot is always associated with wounds and is caused by most citrus-associated isolates of *A. alternata* that produce endopolygalacturonase. Alternaria brown spot is a serious disease of susceptible tangerines and their hybrids in semi-arid Mediterranean climates as well as in more humid areas. Conidia, produced on lesions on mature and senescent leaves and stems under humid conditions, are dispersed by wind, and infect all juvenile tissues of susceptible cultivars when temperature and leaf wetness conditions are favorable. Commercially acceptable cultivars resistant to brown spot are being developed. Disease severity can be reduced by planting disease-free nursery stock on wider spacings, pruning tree skirts, and reducing irrigation and nitrogen fertilization. However, fungicides such as dithiocarbamates, triazoles, strobilurins, iprodione, or copper fungicides are used in most areas for disease control. A disease-forecasting model, the Alter-Rater, has been developed in Florida to assist in timing fungicide sprays.

Key words: *Alternaria alternata,* Alter-Rater, disease models, toxins.

Introduction

Alternaria species cause four distinct diseases of citrus, namely, Alternaria brown spot of tangerines and their hybrids, Alternaria leaf spot of rough lemon, Alternaria black rot of fruit, and Mancha foliar on Mexican lime. The Alternaria brown spot pathogen "tangerine pathotype" affects many tangerines and their hybrids and produces lesions on immature fruit and leaves, induces leaf and fruit drop, and produces spots and corky lesions on mature fruit. The Alternaria leaf spot pathogen "rough lemon pathotype" produces similar symptoms on rough lemon leaves and pinpoint lesions on fruit and affects only rough lemon and Rangpur lime. The latter two pathogens produce host-specific toxins (HSTs) and the host range of

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each is very restricted (Kohmoto *et al.*, 1979; 1991). Alternaria black rot is a post-harvest disease that occurs worldwide and produces internal decay of all commercial citrus. The *Alternaria* fungi causing the above diseases are small-spored, are morphologically similar, and all are considered intra-specific variants of *A. alternata* (Peever *et al.*, 2003). Pathogenicity tests, toxin assays, detection of toxin biosynthesis genes, or other genetic markers are required to distinguish these fungi. Mancha foliar is characterized by the production of small lesions on the leaves of Mexican lime and a few other citrus varieties in western Mexico. This disease is caused by a large-spored species, *Alternaria limicola* (Palm and Civerolo, 1994) and is not associated with the production of an HST.

The diseases

Alternaria brown spot of tangerines

The tangerine pathotype of *A. alternata* affects many tangerines and hybrids (Timmer *et al.*, 2000a) and affects leaves, twigs and fruit. On young leaves, the disease produces minute brown to black spots. Symptoms can appear in as little as 24 h after infection. Lesions usually continue to expand and large areas of the leaf may be killed by the host-selective ACT-toxin (Kohmoto *et al.*, 1993) even without tissue colonization. Chlorosis and necrosis can extend along the veins as toxin is translocated acropetally. On mature leaves, the disease appears as distinct brown lesions surrounded by a yellow halo (Fig. 1A). Affected leaves often abscise. Young shoots are also infected producing brown lesions 1 to 10 mm in diameter. Infected twigs die back especially if the leaves have fallen. On fruit, brown to black lesions can vary from minute spots to large crater-like lesions (Fig. 1D). Corky eruptions sometimes form and can be dislodged forming a pockmark on the surface. Severely affected fruit abscise reducing yield, and blemishes on the remaining fruit greatly diminishing marketability.

Alternaria leaf spot of rough lemon

This disease affects only rough lemon and Rangpur lime, which are common rootstocks in some citrus-growing areas. Thus, this disease is only commercially important in nurseries and seed production blocks. Symptoms on leaves are very similar to those produced on tangerines (Fig. 1B) (Timmer *et al.*, 2000a). The toxin produced by this pathotype is distinct from the tangerine pathotype (Kohmoto *et al.*, 1979), and is called ACR-toxin or ACRL-toxin (Gardner *et al.*, 1985; Nakatsuka *et al.*, 1986a). Symptoms do not appear on rough lemon leaves for about 3 days following inoculation compared to 24 h for the brown spot disease. Symptoms on fruit are merely small brown specks (Timmer *et al.*, 2000a) and are quite reduced relative to the tangerine pathotype.

Mancha foliar

Mancha foliar is a disease that primarily affects Mexican lime and occurs only in Western Mexico (Becerra *et al.*, 1988; Timmer *et al.*, 2000a). On Mexican lime, Mancha foliar produces small, reddish brown lesions on leaves that are surrounded by chlorotic halos (Fig. 1C). Affected leaves often abscise and twigs may die back. Small raised lesions are produced on fruit, but the symptoms disappear as the fruit develops. Mancha foliar also occurs on grapefruit, navel oranges, and Tahiti lime, but seldom causes significant damage. Most other citrus is resistant to the disease.

Black rot

Black rot affects the central columella of the fruit and can affect all species of citrus (Brown and McCornack, 1972). External symptoms are not often apparent and, if present, appear as a small brown to black spot on the stylar end of the fruit (Brown and Eckert, 2000) (Fig. 1E). Affected fruit are more brightly colored than normal fruit due to ethylene generated in response to infection. It appears that most small-spored isolates of *Alternaria* are capable of causing black rot. These include saprophytic isolates colonizing dead or senescent tissues, epiphytes from healthy leaves, as well as the tangerine and rough lemon pathotypes (Bhatia, Peever, and Timmer, unpublished). A wound or a natural crack is required for penetration of the fungus. The ability to produce endopolygalacturonase appears essential for isolates to cause black rot (Isshiki *et al.*, 2001). None of the black rot strains tested to date produce HST.
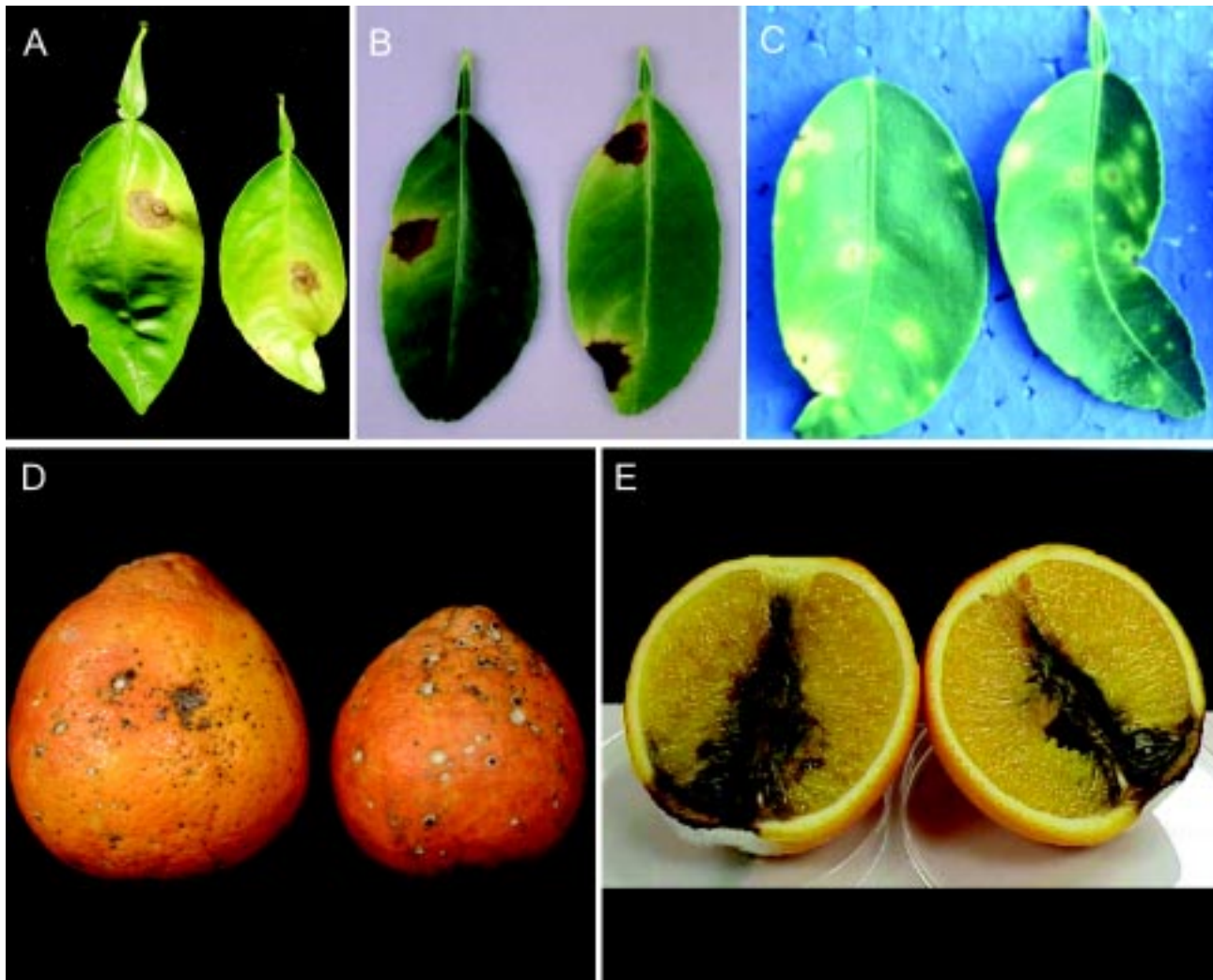
Fig. 1. A, Symptoms of Alternaria brown spot on mature Minneola tangelo leaves. B, Alternaria leaf spot symptoms on rough lemon leaves. C, Symptoms of Mancha foliar on Mexican lime leaves (reprinted with permission from the *Compendium of Citrus Diseases*, 2nd ed., American Phytopathological Society, St. Paul, MN, USA). D, Symptoms of Alternaria brown spot on Minneola tangelo fruit. E, Black rot symptoms on sweet orange fruit.

The pathogens

Morphology, taxonomy, and classification

The first citrus-associated *Alternaria* species to be formally described was *A. citri*, the causal agent of citrus black rot (Pierce, 1902). Brown spot and rough lemon leaf spot pathogens were subsequently identified as *A. citri* based on their morphological similarity to the black rot fungus (Doidge, 1929; Ruehle, 1937; Kiely, 1964; Pegg, 1966). However, the brown spot and leaf spot fungi are known to be biologically and pathologically distinct from the black rot fungi because the former are able to infect young leaves and fruit and produce host-specific toxins (Kiely, 1964; Whiteside, 1976). The brown spot and leaf spot pathogens were considered *A. alternata* by Kohmoto *et al.* (1979) based on a published description of conidial morphology and measurements
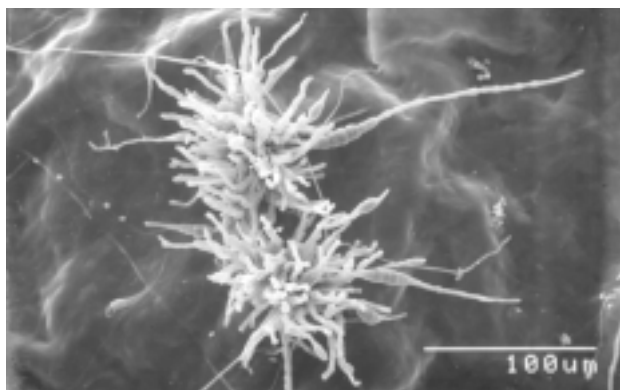
Fig. 2. Scanning electron micrograph of conidiophores of *Alternaria alternata* emerging from a stomata on a mature leaf lesion.

Fig. 3. Conidia of *Alternaria alternata* (×250).

of *A. alternata* (Simmons, 1967). Solel (1991) designated the tangerine pathogen as *A. alternata* pv. *citri*. However this nomenclature does not address the status of the rough lemon pathogen. The terms "tangerine pathotype" and "rough lemon pathotype" have been applied to denote the unique pathological attributes of the brown spot pathogen and rough lemon leaf spot pathogens, respectively, and we prefer these designations. Recent morphotaxonomic research has attempted to clarify the identity of small-spored isolates of *Alternaria* associated with brown spot and leaf spot of citrus. One hundred and thirty-five isolates from the worldwide collection of L.W. Timmer and T.L. Peever, including isolates from rough lemon leaf lesions and brown spot lesions on tangerines and tangerine hybrids, were ex-

amined and ten morphological species were described (Simmons, 1999), none of which was considered representative of *A. alternata* or *A. citri*.

A phylogenetic analysis of small-spored, citrus-associated *Alternaria* isolates was recently completed and included the ten morphological species recently described by Simmons (1999), several black rot isolates, and small-spored reference species from other hosts (Peever *et al.*, 2003). Using the ex-type isolates of Simmons (1999), it was possible to directly map the morphological species onto a phylogeny estimated from a combined dataset consisting of a partial sequence of the coding region of an endopolygalacturonase (endoPG) gene (Isshiki *et al.*, 2001; Peever *et al.*, 2002) and two anonymous regions of the genome. The analysis revealed eight well-supported clades which could be interpreted as eight phylogenetic species. The clades were broadly congruent with the morphological species; however, three clades contained more than one morphological species and one morphological species (*A. citrimacularis*) was polyphyletic.

Black rot isolates were distributed throughout the combined phylogeny in three clades. One black rot isolate was found in the same phylogenetic lineage as two saprophytic *A. alternata* isolates, another was found in a phylogenetic lineage with several brown spot and leaf spot isolates and a third was found in a lineage with *A. arborescens*, a host-specific toxin-producing pathogen of tomato (Peever *et al.*, 2003). These results clearly demonstrate that phylogenetically distinct small-spored *Alternaria* taxa can be associated with black rot and raise questions about the validity of *A. citri* as a phylogenetic taxon. We find that many small-spored *Alternaria* species are able to cause black rot. Phylogenetically diverse isolates from black rot, brown spot and rough lemon leaf spot from citrus and additional small-spored isolates from non-citrus hosts were all able to induce black rot when inoculated into wounded citrus fruit (Bhatia, Peever and Timmer, unpublished). The lack of correlation between phylogenetic lineage and unique phenotypic, ecological or pathological characters among the small-spored citrus-associated *Alternaria* raises questions about the practical utility of both the morphological species and species defined using only phylogenetic criteria. The occurrence

of multiple morphological species in several clades and the polyphyly of at least one morphological species clearly indicate that the morphological species do not reflect evolutionary relationships among these fungi. Until it can be demonstrated that unique ecological, biological or biochemical characters can be associated with a specific phylogenetic lineage, we advocate collapsing all small-spored, citrus-associated *Alternaria* isolates, including brown spot, rough lemon leaf spot and black rot isolates, into a single species, *A. alternata*.

Mancha foliar is caused by *A. limicola* Simmons and Palm, the only large-spored species of *Alternaria* known to affect citrus (Palm and Civerolo, 1994). In contrast to the non-pathogenic isolates of *Alternaria* and those that cause brown spot, leaf spot, or black rot, *A. limicola* is clearly distinguishable morphologically and through molecular methods (Peever *et al.*, 2003). The conidia of this species are large (16–22×140–190 µm) with long beaks (60–90 µm). *A. limicola* produces various toxins in culture, but they are not host specific as are the ACT and ACR-toxins (Becerra *et al.*, 1988; Timmer *et al.*, 2000a).

Host specificity

There is a high degree of host specificity among isolates from tangerine and those from rough lemon (Kohmoto *et al.*, 1979, 1991; Peever *et al.*, 1999). In Florida, the vast majority of the isolates collected from Minneola tangelo were pathogenic to that host and only 3% were non-pathogenic and none was pathogenic to rough lemon (Peever *et al.*, 1999). Most isolates from rough lemon were pathogenic on the host of origin, but a few were pathogenic on Minneola tangelo and not rough lemon. A substantial portion, 44%, were not pathogenic to either host.

When disease symptoms were first found on grapefruit and on Sunburst tangerine in Florida, the possibility of host specificity within the tangerine pathotype was raised (Timmer and Peever, 1997). Using random amplified polymorphic DNA, isolates from grapefruit and the tangerine hybrid Nova could be distinguished from those from Robinson, Sunburst, Minneola, Orlando, and Murcotts (Peever *et al.,* 2000). However, cross inoculation studies on the different hosts did not support the host specificity seen with molecular markers. In the inoculations, Minneola was consistently the most susceptible followed by Orlando, Sunburst, Nova, and grapefruit in decreasing order of susceptibility regardless of the source host of the isolates.

Disease cycle and epidemiology

Alternaria brown spot of tangerine is the only Alternaria disease of citrus for which there is an appreciable amount of information regarding epidemiology. Since leaf spot of rough lemon is relatively unimportant commercially, the ecology and epidemiology of this pathogen have been little studied. The disease cycle is simple since there is no teleomorph known for *A. alternata* (Timmer, 1999). Conidia are produced primarily on the surface of lesions on mature or senescent leaves (Fig. 2,3) and on blighted twigs. Relatively few, if any, are produced on young lesions on leaves or mature lesions on fruit. Conidium production is greatest when leaves are lightly moistened or held at high humidity, with fewer produced where leaves are very wet (Timmer *et al.*, 1998). Almost no conidia are formed at low or moderate relative humidities if leaves are free of moisture. Conidium production, dispersal, and infection are presumed to be similar to those of the tangerine pathotype. Likewise control measures have not been established for this disease.

Release of conidia from sporulating brown spot lesions is triggered by rainfall or by sudden changes in relative humidity (Timmer *et al.,* 1998). Rainfall is probably most important for spore release in humid areas such as Florida (US) or Brazil. However, in Mediterranean areas where little rain falls during the susceptible period, spore release may be triggered by a sharp drop in relative humidity (RH) when the dew dries. Spores are dispersed by wind currents and are eventually deposited on the surface of susceptible tissues. With dew the following night, the conidia germinate and eventually infect the leaves or fruit. Penetration of the leaf can occur directly or through stomata and in studies in Israel is consistently associated with appressoria formation (Solel and Kimchi, 1998). Preliminary observations in Florida indicate that penetration occurs through stomata on the undersurface of the leaf without appressorium formation (Bha-

tia *et al*., 2002).

The optimum temperature for infection is 27°C (Canihos *et al., 1999*). As temperatures decline, longer wetting periods are needed for infection to occur. At 32°C, little infection occurs even with ample moisture. Small amounts of infection can occur with leaf wetness durations of 4–8 h, but usually 10–12 h of wetness are needed for substantial infection (Canihos *et al.,* 1999; Timmer *et al.,* 2000b). In many semi-arid areas, temperatures are cool at night when dew occurs and may be below the optimum. Thus longer wetting periods may be required for infection. As with *Alternaria* spp. from other crops, germinated conidia may survive the heat of the day and continue development the following night (Rotem, 1994). In humid areas such as Florida, Colombia, or Brazil, temperatures following rainfall may be near the optimum during the period of susceptibility.

In Mexico*,* Mancha foliar occurs primarily in the dry, cool winters and diminishes with warm, wet weather in summer (Stapleton and Garcia-Lopez, 1988). Conidia germinate in 4 h and infect leaves in about 12 h. In the field, infection levels are highest when temperatures drop below 24°C. Sporulation begins about one week after the symptoms appear.

Dithiocarbamate fungicides are effective for disease control. Applications are needed one week after shoot emergence and again two weeks later (Timmer *et al*., 2000a).

In the field, black rot occurrence is sporadic. The disease is more common in semi-arid areas because *Alternaria* is more prevalent as an epiphyte, endophyte, and saprophyte there than in humid areas. It occurs commonly in navel oranges because of natural openings created by growth of the navel (Brown and Eckert, 2000). It is also frequently observed in lemons, especially where fruit is held in cold storage for long periods. Pre- and post-harvest fungicide applications provide very little control of the disease. If black rot occurs in the grove, harvest must be delayed until most of the affected fruit has fallen. Hormones, such as 2, 4-D applied postharvest, can delay senescence and reduce the incidence of the disease.

Geographical distribution and diversity

Alternaria brown spot disease was first reported on Emperor mandarin in Australia in 1903 (Cobb, 1903) and the causal agent was identified as a species of *Alternaria* in 1959 after a number of organisms were investigated as possible pathogens (Kiely, 1964; Pegg, 1966). The disease later appeared in the USA (Whiteside, 1976), and now occurs in Israel (Solel, 1991), South Africa (Schutte *et al*., 1992), Turkey (Canihos *et al*., 1997), Spain (Vicent, 2000), and Brazil and Argentina (Goes *et al*., 2001; Peres *et al*., 2003). Due to the morphological similarity between the brown spot and black rot pathogens, the former was originally identified as *A. citri* Ellis and Pierce (Pegg, 1966; Whiteside, 1976), a fungus that had been first described as the cause of citrus black rot. Alternaria leaf spot of rough lemon was first reported from South Africa (Doidge, 1929). Alternaria black rot, also known as stem-end rot, was reported as early as the 1900s in California (Pierce, 1902; Roger and Earle, 1917; Bartholomew, 1926; Bliss and Fawcett, 1944).

Isolates of *A. alternata* were collected from Minneola tangelo in the United States, Colombia, Australia, Israel, Turkey and South Africa to infer the worldwide population structure and phylogeography of the pathogen (Peever *et al*., 2002). Both RAPD markers and sequence data from an endoPG revealed that isolates from Florida and Colombia were distinct from isolates sampled in the other parts of the world. The endoPG data separated isolates into three phylogenetic lineages which correlated to country of origin. Isolates from the United States, Australia, South Africa and Israel were found in a second clade whereas isolates from Australia, Israel, South Africa, and Turkey were found in a third clade. Peever *et al*. (2003) speculated that these three phylogenetic lineages were introduced independently into each citrus-growing region from a common source population on plant material. This source population may have been Southeast Asia, the center of origin of citrus, although little is known about the disease or the pathogen in this region. Isolates were tested for pathogenicity on detached leaves of Minneola tangelo and the Florida strains were significantly more virulent than strains from other locations. Isolates from Florida may be more virulent because they have more copies of genes controlling toxin

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biosynthesis (*ACTT*) and produce more ACT-toxin than isolates from other locations. A small percentage of the worldwide isolates were identified as being non-pathogenic or having greatly reduced virulence and these isolates are currently being screened for toxin production and the presence of *ACTT* sequences.

Pathogenesis

Brown spot and leaf spot diseases

The host specificity of the tangerine and rough lemon pathotypes of *A. alternata* depends upon the production of HSTs that possess the same selectivities as the pathogens (Kohmoto *et al*., 1979, 1991). The toxin from the rough lemon pathotype was named ACR or ACRL-toxin, and that from the tangerine pathotype was named ACT-toxin. The structure of the major form of ACR-toxin, ACRL-toxin I (MW 496), was characterized as a dihydropyrone ring with a polyalcohol side chain (Gardner *et al*., 1985; Nakatsuka *et al*., 1986a). ACR-toxin causes water congestion, veinal necrosis (Kohmoto *et al*., 1979; Akimitsu *et al*., 1994), inhibition of ^{14}C-proline incorporation (Gardner *et al*., 1985), and induces a rapid increase of electrolyte leakage (Kohmoto *et al*., 1979; Akimitsu *et al*., 1994) only on the toxin-sensitive citrus species, rough lemon and Rangpur lime. Interestingly, light irradiation can suppress toxin-induced necrosis as well as electrolyte leakage (Akimitsu *et al*., 1994), and a dark period of more than 3 h during the light irradiation overcomes the suppressive effect of light (Akimitsu *et al*., 1994).

The major form of HST produced by the tangerine pathotype was designated as ACT-toxin I (Kohmoto *et al*., 1993). The structure of ACT-toxin is closely related to AK- and AF-toxins, which are the HSTs produced by the Japanese pear and strawberry pathotypes of *A. alternata*, respectively (Nakashima *et al.,* 1985; Nakatsuka *et al*., 1986b, 1990; Kohmoto *et al*., 1993). These toxins share a common 9,10-epoxy-8-hydroxy-9-methyl-decatrienoic acid moiety (Nakashima *et al.,* 1985; Nakatsuka *et al*., 1986b; Kohmoto *et al*., 1993). ACT-toxin causes veinal necrosis and a rapid increase in electrolyte loss from susceptible leaves, but the toxin has no effect on resistant leaves (Kohmoto *et al*., 1993). The mode of action of ACT-toxin is still uncertain, but a rapid loss of electrolytes from leaf tissues and ultrastructural changes of cells treated with the toxin indicated that the primary action site of ACT-toxin was likely the plasma-membrane (Kohmoto *et al*., 1993).

A cluster of genes controlling biosynthesis of ACT-toxin was identified using heterologous probes of *AKT* sequences which control biosynthesis of a 9,10-epoxy-8-hydroxy-9-methyl-decatrienoic acid moiety in AK-toxin from the Japanese pear pathotype of *A. alternata* (Tanaka *et al*., 1999). Portions of these genes used as heterologous probes detected homologous in several isolates of *A. alternata* tangerine pathotype but not from isolates that do not produce ACT-toxin such as the rough lemon pathotype, saprophytes, or black rot isolates (Masunaka *et al*., 2000).

The target site of ACR-toxin was identified as the mitochondrion. Electron microscopic examination of the toxin-treated cells showed that 10 to 18% of the mitochondria were disrupted within one hour after treatment with the toxin. Crista were swollen, fewer in number and vesiculated with a lower matrix density. Disruption of mitochondrial functions by ACR-toxin was also examined by monitoring an oxidative-phosphorylation and mitochondrial membrane potential using isolated physiologically active citrus mitochondria (Akimitsu *et al*., 1989). ACR-toxin caused uncoupling similar to classic protonophores, such as 2,4-dinitrophenol or carbonyl cyanide m-chlorophenyl hydrazone, with a loss of membrane potential, but the effects differed slightly from other uncouplers because the toxin also causes leakage of co-factor, NAD^+ from the tricarboxylic acid cycle (Akimitsu *et al*., 1989). These effects of ACR-toxin are specific to mitochondria isolated from susceptible cultivars. Dancy tangerine, Emperor mandarin, and grapefruit, which are susceptible to ACT-toxin, are completely insensitive to ACR-toxin I (Akimitsu *et al*., 1989).

Defense responses of citrus inoculated with Alternaria leaf spot pathogens have not been examined in detail to date. The expression patterns of several defense-related genes including lipoxygenase (*RlemLOX*) (Gomi *et al*., 2002a), hyperperoxide lyase (Gomi *et al*., 2003), chalcone synthase (Gotoh *et al*., 2002; Nalumpang *et al*.,

2002a), polygalacturonase-inhibiting protein (Gotoh *et al.*, 2002; Nalumpang *et al.*, 2002a, 2002b), chitinases (Gomi *et al.*, 2002b) genes have been characterized in rough lemon in response to these pathogens. All these genes are highly inducible in rough lemon leaves by infection with nonpathogenic *A. alternata*, and expression of these genes was detected within 2 h after wounding or inoculation of rough lemon leaves with conidia of the *A. alternata* tangerine pathotype (isolate SH20), which is pathogenic to tangerines and mandarins but not to rough lemon (Gomi *et al.*, 2002a, 2002b; Gotoh *et al.*, 2002; Nalumpang *et al.*, 2002a, 2002b). Another nonpathogenic strain of *A. alternata* (isolate O-94), which is not pathogenic to any citrus tested (Akimitsu *et al.*, 1989; Kohmoto *et al.*, 1991), also induced expression of these genes within 2 h, but the intensity of the bands was not as strong as those induced by SH20. Because there are no apparent morphological or biochemical differences between SH20 and O-94 except that SH20 produces ACT-toxin (Kohmoto *et al.*, 1979, 1991, 1993; Masunaka *et al.*, 2000), the toxin might have a role as elicitor in the greater accumulation of the transcripts. In contrast, induction of these defense-related gene expression was delayed or suppressed when pathogenic *A. alternata* rough lemon pathotype (AC325) was inoculated on rough lemon leaves (Gomi *et al.*, 2002a, 2002b; Gotoh *et al.*, 2002; Nalumpang *et al.*, 2002a, 2002b). This system, using either an HST-producing or a non-producing *A. alternata* strain, which led to a clear susceptible or resistant response in rough lemon leaves, may be a good model for further evaluation of the role of other defense-related genes including PR-proteins.

Alternaria black rot disease

Although the black rot pathogen has been known to produce several toxins such as tenuazonic acid, alternariol methyl ether, and alternariol (Logrieco *et al.*, 1990), these toxins have no known role in pathogenesis. The pathogenicity of this fungus depends upon production of an extracellular enzyme that can degrade pectic polymers in cell walls during infection. The possible role of cell wall-degrading enzymes in pathogenicity, including penetration, maceration, nutrient acquisition, plant defense induction, and symptom expression have been investigated

(Cooper, 1983, 1984; Walton, 1994). The endoPGs have been purified and the genes cloned from both a black rot pathogen and a rough lemon pathotype isolate (Isshiki *et al.*, 1997, 2001). The sequences of these genes and biochemical characteristics of the enzymes they encode are highly similar. However, these genes in the respective pathogens were disrupted by gene targeting, the phenotypes of the mutants were completely different (Isshiki *et al.*, 1997, 2001). An endoPG mutant of the black rot pathogen was significantly reduced in its ability to cause black rot symptoms and penetration into citrus fruits as well as in the maceration of potato tissue and could not colonize citrus peel segments (Isshiki *et al.*, 2001). In contrast, an endoPG mutant of the rough lemon pathotype was unchanged in pathogenicity on rough lemon leaves (Isshiki *et al.*, 2001). Thus, an endoPG was not required for pathogenicity of the rough lemon pathotype and it played different roles in the pathogenicity of these two closely related fungi.

Disease control

Host resistance

Relatively few citrus species and cultivars are susceptible to Alternaria brown spot and most of the susceptibility occurs in the progeny of Dancy tangerine. Many of the susceptible cultivars such as Minneola, Orlando, Nova, Lee, and Sunburst have Dancy tangerine in their parentage. The disease occurs on other varieties not known to have Dancy in their background such as Murcott, Emperor, and Ponkan. Alternaria brown spot has been observed on Ellendale and Idith mandarins, calamondin, and red grapefruit in Israel (Solel and Kimchi, 1997) and on grapefruit in Florida (Timmer and Peever, 1997). Brown spot in grapefruit is associated usually with severe disease in nearby plantings of susceptible tangerines and their hybrids. Many other citrus species may be infected by artificial inoculation or are affected by the toxin but are not affected in the field (Kohmoto *et al.*, 1979; Solel and Kimchi, 1997).

Based on the pattern of susceptibility of various citrus cultivars and hybrids, Kohmoto *et al.* (1991) concluded that the susceptibility was inherited from the Dancy parent as a dominant

trait. Dalkilic (1999) found that segregation from a cross of Clementine tangerine, a resistant species, and a Clementine × Minneola hybrid produced a 1:1 ratio of susceptible:resistant progeny. The reciprocal cross, however, yielded a 3:1 ratio of resistant to susceptible offspring. He concluded that resistance was recessive but the phenotype may have been affected by cytoplasmically inherited paternal traits. Dalkilic (1999) identified RAPD markers that were consistently associated with resistance.

Dancy tangerine is the source of much of the susceptibility to Alternaria brown spot and was selected in Florida and used as a parent in much of the breeding program for new tangelos and more complex hybrids. Interestingly, Alternaria brown spot appeared in Australia on Emperor mandarin (Cobb, 1903) long before it was described in Florida (Whiteside, 1976). Brown spot has also appeared on Ponkan mandarin in Brazil (Goes *et al*., 2001) but little is known about the susceptibility of this cultivar. Molecular phylogenetic studies of the brown spot pathogens collected from around the world indicate that the Florida population is ancestral to the other populations (Peever *et al*., 2002). The authors speculate that the pathogen originated with citrus in southeast Asia and the most ancestral population was introduced into Florida. However, the possibility remains that the citrus pathogen may have originated elsewhere from other toxin-producing *Alternaria* populations on other hosts.

Since relatively few cultivars are susceptible to brown spot, selection or production of commercially acceptable, brown-spot resistant cultivars seems feasible. This could be achieved by classical breeding or induced in various existing cultivars by molecular techniques, irradiation, or induced mutagenesis. An Alternaria-tolerant cultivar similar to Minneola will be released soon from the University of Florida (F.G. Gmitter, personal communication).

Cultural practices

There is little research on the effects of cultural practices on the severity of brown spot infection. However, studies of the effect of environmental factors that affect disease severity (Canihos *et al*., 1997; Timmer *et al*., 1998, 2000b) provide some information to suggest certain cultur-al practices which might be helpful. Avoidance of overhead irrigation and use of under-tree irrigation systems in Florida has reduced disease severity in some groves. Wider spacing and skirting of trees allow better ventilation and seem to reduce disease severity. Avoidance of excess irrigation and nitrogen fertilization have been recommended for some time to avoid production of large amounts of susceptible tissue (Whiteside, 1976; Timmer *et al*., 2001). While perhaps helpful, it is difficult to reduce irrigation and fertilization enough without jeopardizing fruit production. Scheduling of hedging and topping just prior to the dry season in Florida allows development of the consequent flush of growth with a minimum of infection and inoculum production.

Planting of new groves with Alternaria-free nursery stock has been helpful in reducing brown spot in the early years (Timmer, 2003). Groves initiated using healthy stock in Florida have remained relatively disease-free for a surprisingly long time, sometimes up to 6–8 years, even when inoculum is present in vicinity. This may indicate that long distance dispersal of conidia occurs rarely. Healthy nursery stock can be produced by selecting budwood from healthy mother plants and then growing trees in greenhouses with sub-irrigation (Timmer, 2003). While cultural measures alone are seldom sufficient for commercial control, they can greatly reduce inoculum levels and disease severity and enhance the efficiency of fungicide control programs.

Chemical control

Foliar fungicide applications are usually necessary to produce fruit with good external quality in areas where Alternaria brown spot is common. Depending on the climate in different areas, from 3 to 15 applications may be needed. In the early years after discovery of the disease in Florida, captafol was widely used and highly effective for disease control (Whiteside, 1976; Timmer and Zitko, 1997). Since captafol has a long residual and is redistributed, few applications were needed for good disease control. However, this product is no longer registered in most areas due to health concerns. Iprodione is also very effective for disease control (Timmer and Zitko, 1992, 1994, 1997; Solel *et al*., 1997) but resistance developed in Israel (Solel *et al*., 1996) limits

its usefulness in some groves. Copper fungicides are widely used for Alternaria control in Florida (Timmer *et al.*, 2003) and when applied on a timely basis provide good control of the disease. However, copper products cause stippling of the fruit when applied at high temperatures and must be used with caution. Other fungicides that are effective and registered in Israel are the dithiocarbamates, triazoles, and famoxadon (Sadowsky *et al.*, 2002a). Recently the strobilurin fungicides have been evaluated and proven effective for control of brown spot (Bhatia *et al.*, 2002a, 2002b; Sadowsky *et al.*, 2002a; Timmer, 2002). Azoxystrobin and pyraclostrobin are generally more effective than trifloxystrobin. Strobilurins are single site of action fungicides (Sierotski *et al.*, 2000a, 2002b) and thus, prone to development of resistance and must be alternated or mixed with other products.

Numerous products that induce resistance in the host have been evaluated for control of Alternaria brown spot (Agostini *et al.*, 2003). Products containing or producing phosphites or salicylic acid significantly reduce disease severity. These materials have some promise in a program alone or perhaps in combination with standard fungicides (Sadowsky *et al.*, 2002b).

Fruit must be protected from petal fall until about mid-summer, but some infection may occur after that time. Disease levels are generally low and lesions usually small, but some large black lesions can occur as a result of late infection in Israel and may induce fruit drop (Sadowsky *et al.*, 2002a). In addition it is often necessary to protect spring flush foliage to prevent build-up of high levels of inoculum prior to fruit set. In high rainfall areas, it may not be possible to control disease adequately. In Colombia, a small, but highly profitable production of Minneola tangelos was eliminated when *Alternaria* was introduced on nursery stock from Florida. Production of high quality fruit is also difficult in Florida and Brazil now that *A. alternata* is well-established in those areas.

The Alter-Rater, a system for timing of fungicide application was developed in Florida (Timmer *et al.,* 2000b). The factors used in the model are: 1) the occurrence of rainfall over 2.5 mm; disease severity is related to the occurrence but not the amount of rainfall; 2) the total hours of

Table 1. The daily scores assigned by the Alter-Rater model to various combinations of rainfall, leaf wetness, and temperature (reprinted with permission from Bhatia *et al.*, 2003).

Rainfall >25 mm	Leaf wetness >10 h	Average daily temperature (°C)	Assigned score[a]
+	+	20–28	11
+	+	>28	8
+	+	<20	6
+	-	20–28	6
+	-	>28	4
+	-	<20	3
-	+	20–28	6
-	+	>28	6
-	+	<20	4
-	-	20–28	3
-	-	>28	0
-	-	<20	0

[a] Assigned scores are accumulated daily and fungicide applications are made when the point total reaches 50 for groves of highly susceptible varieties with a history of severe disease; 100 for groves of less susceptible varieties and histories of less severe disease; and 150 for tolerant varieties and/or histories of low disease severity.

leaf wetness; about 10 h are needed for significant infection, and 3) average daily temperature; temperature between 20 and 32°C are optimal. Points are assigned to each day based on the weather and are accumulated until a preassigned threshold is reached and then an application of fungicide is made (Table 1). Thresholds are assigned based on the disease history in the grove and the susceptibility of the cultivar. The system has proven successful in Florida as long as the threshold is properly selected (Bhatia *et al*., 2003). The system also appears to be functioning well in Brazil (N.A.R. Peres and L.W. Timmer, unpublished). In semi-arid areas where no rainfall occurs after bloom, the system is likely to be less useful. Since temperatures and dew periods are often uniform from day to day, timing of spray would probably not differ substantially from a calendar spray program.

Concluding remarks

Alternaria diseases of citrus represent some interesting pathosystems. The tangerine pathotype produces a host-specific toxin and is closely related to similar fungi that affect Japanese pear, apple, strawberry, and other hosts. The rough lemon pathotype produces another host-specific toxin which differs chemically as well as in its mode of action from the tangerine pathotype. The genes for toxin production are located on a very short chromosome in the pathogen. The evolutionary history of these pathogens could be fascinating if it can be elucidated. *Alternaria alternata* is a common saprophyte on citrus leaves in the grove, but these isolates are able to cause black rot of fruit after harvest. Such saprophytes are commonly isolated from leaf lesions of rough lemon, but rarely from Minneola tangelo. The ecological relationship of the various isolates on the leaf surface needs further investigation to elucidate the roles of different isolates of the same species. In contrast to many citrus diseases, the development of resistant cultivars by classical breeding or genetic manipulation appears feasible. Resistance of citrus to Alternaria brown spot is inherited as a recessive trait. Further investigation of the epidemiology, fungicide activity, and timing should improve disease control on the short-term.

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