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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 of citrus fruit

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.

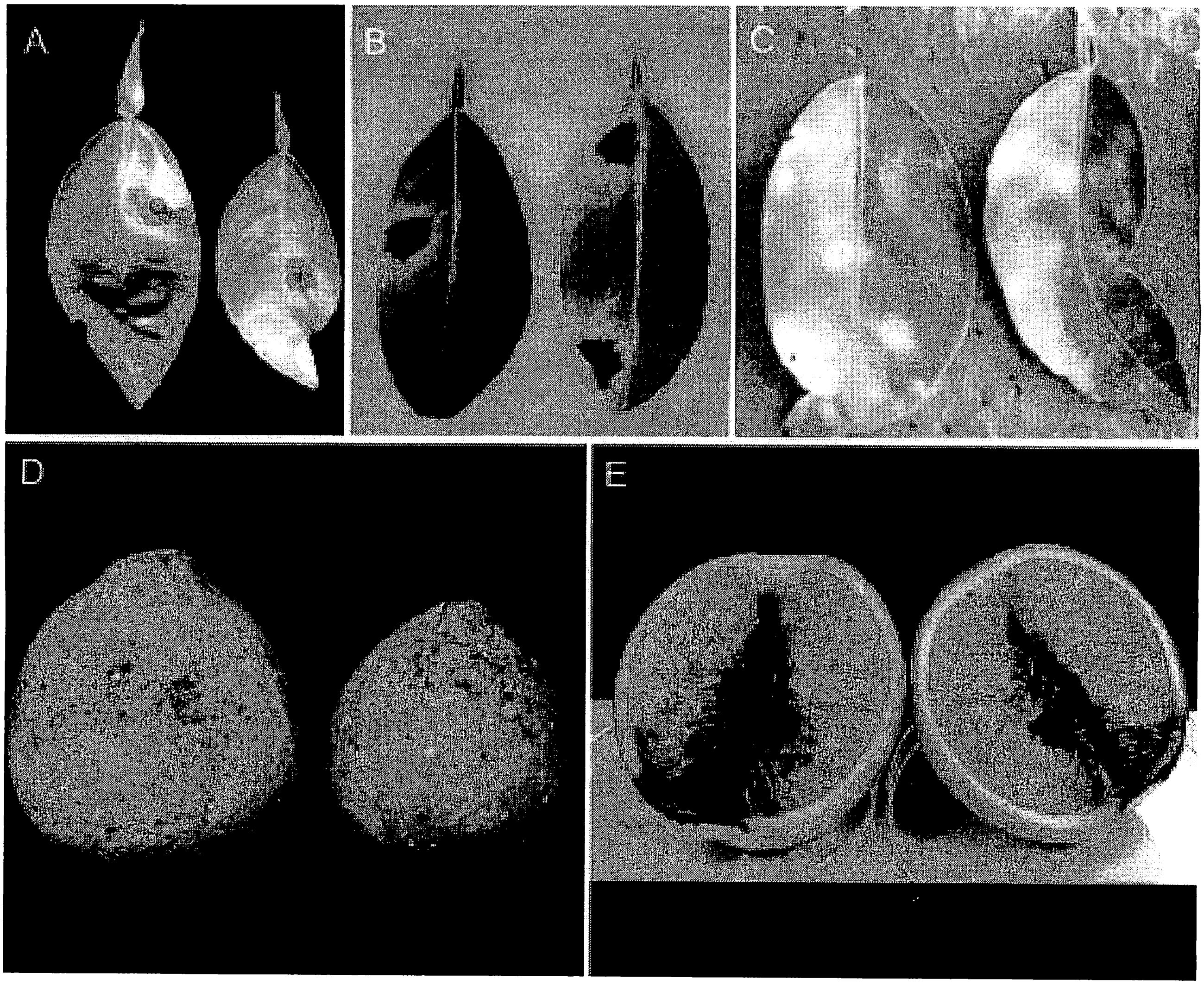

Fig. 1. A, Symptoms of Alternaria brown spot on mature Minneola tangelo leaves. B, Alternaria leaf spot symptoms on rough lemon leaves. C, Symptoms of *Mancha foliar* on Mexican lime leaves (reprinted with permission from the Compendium of Citrus Diseases, 2nd ed., American Phytopathological Society, 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

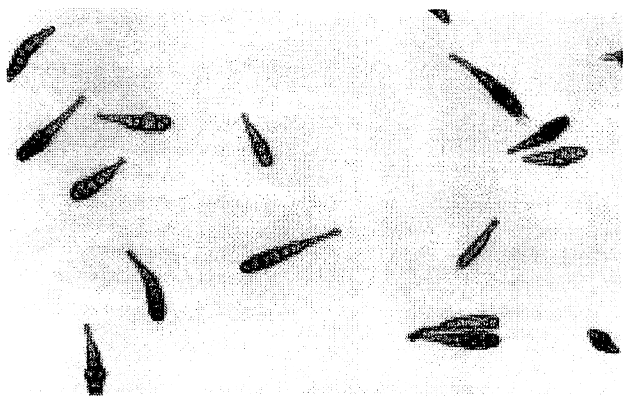

Fig. 2. Scanning electron micrograph of conidiophores of *Alternaria alternata* emerging from a stomata on a mature leaf lesion.

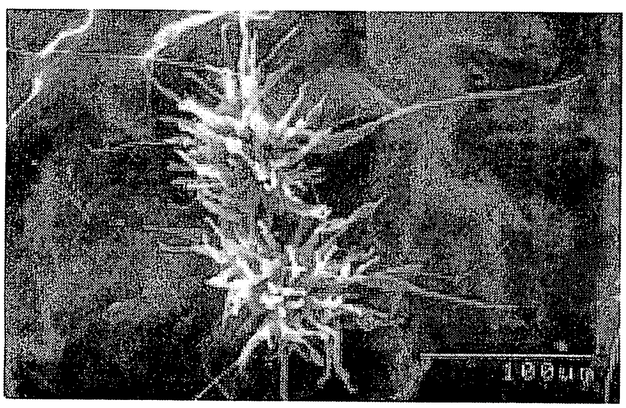

Fig. 3. Conidia of *Alternaria alternata* (×250).

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 examined 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 ∝m) with long beaks (60–90 ∝m). *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 (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-