

Correlation of Resistance and Susceptibility of Citrus to *Alternaria alternata* with Sensitivity to Host-Specific Toxins

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ABSTRACT

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The host ranges of the rough lemon pathotype and the tangerine pathotype of *Alternaria alternata*, cause of brown spot disease of citrus, were determined by use of inoculations and by reaction to their host-specific toxins (ACR-toxin I, ACT-toxin Ib). Of 67 species, cultivars, and hybrids examined, only two (rough lemon, *Citrus jambhiri*, and

Rangpur lime, *Citrus limonia*) were susceptible to the rough lemon pathotype and sensitive to its toxin. In contrast, 28 citrus species and cultivars were susceptible to the tangerine pathotype and sensitive to its toxin; these included susceptible genotypes of grapefruit, tangerine (mandarin), tangelo, tangelolo, tangerine-tangelo, and tangor.

Two distinct pathotypes of *Alternaria alternata* (Fr.:Fr.) Keissl. (formerly, *A. citri* Ellis & N. Pierce in N. Pierce) are known to cause a brown spot disease on young leaves and fruits of certain citrus cultivars. One strain, called the tangerine pathotype, was first reported in 1966 from Queensland, Australia (17) and in 1976 from Florida, the United States (19). This strain is pathogenic

to Dancy tangerine and Emperor mandarin but has no effect on rough lemon. The other strain, called the rough lemon pathotype, was also reported in 1976 from the United States (19). This pathotype is pathogenic to rough lemon but not to Dancy tangerine or Emperor mandarin.

Both pathotypes produce host-specific toxins (HSTs) in spore-germination fluids and in liquid culture (9,10, Kohmoto, *unpublished*). These HSTs have the same selectivity for plants as do the pathogens that produce them (10). The structure of the major

HST (ACR-toxin I or ACRL-toxin I) of the rough lemon pathotype was elucidated independently by Gardner et al (4) and Nakatsuka et al (15). Next, a toxin (ACTG-toxin) of the tangerine pathotype was characterized by Kono et al (11). Recently, however, we have found that the tangerine pathotype produces at least two HSTs (ACT-toxin Ib and Ic) that differ in chemical structures from ACTG-toxin (14). ACT-toxin Ib and Ic contain 8-substituted-9,10-epoxy-9-methyl-decatrienoic acid; this compound is found also in AK- and AF-toxins (6,14). The major ACT-toxin Ib is selectively toxic to Dancy tangerine, Emperor mandarin, and several Japanese pear cultivars (8, Kohmoto, unpublished) that are not known to be affected by the pathogen in nature (18) but have been shown to be susceptible to the pathogen in laboratory tests (8, Kohmoto, unpublished).

There are examples for which host range was determined by use of HSTs rather than by inoculation with the pathogen (2,12,13,20). In Japan no brown spot disease of citrus has been recorded to date, even though citrus has been cultivated for a long time.

The host-specific toxins may be useful to test for susceptibility of prevalent citrus cultivars without risking the introduction and spread of the pathogen. This might also be useful in plant breeding. We report here concomitant tests of citrus cultivars by use of toxin sensitivity (ACR-toxin I and ACT-toxin Ib) and inoculations with the fungi to determine host ranges. A preliminary report of this work has appeared (1).

MATERIALS AND METHODS

Plant and pathogens. Young leaves of 67 citrus cultivars were used in the experiments. All leaves were harvested from field-grown trees at the National Fruit Tree Research Station (Okitsu Branch) in May, late August, and early September, 1987. Bioassays were completed immediately after harvest. Highly virulent isolates of *A. alternata* rough lemon pathotype (AC-325) and tangerine pathotype (AC-320) were kindly provided by J. O. Whiteside,

TABLE 1. Susceptibility of citrus to *Alternaria alternata* pathotypes and sensitivity to their respective toxins

Citrus ^a	Fungus from rough lemon		Fungus from Dancy tangerine		Citrus ^a	Fungus from rough lemon		Fungus from Dancy tangerine	
	Inoculation ^b	Toxin ^c	Inoculation ^b	Toxin ^c		Inoculation ^b	Toxin ^c	Inoculation ^b	Toxin ^c
Blood orange					Pummelo				
Ruby	R	—	R	—	Anseikan	R	—	R	—
Citron					Hirado buntan	R	—	R	—
Citron	R	—	R	—	Mato buntan	R	—	R	—
Grapefruit					Sour orange				
Marsh	R	—	S	++	Sour orange	R	—	R	—
Redblush	R	—	S	++	Sweet orange				
Star ruby	R	—	S	++	Fukuhara	R	—	R	—
Wheeny	R	—	S	++	Trovia	R	—	R	—
Kumquat					Valencia	R	—	R	+
Marumi	R	—	R	—	Tangelo				
Lemon					Minneola	R	—	S	++
Lisbon	R	—	R	—	Orlando	R	—	S	++
Meyer	R	—	R	—	Sampson	R	—	S	++
Rough lemon	S	++	R	—	San jacinto	R	—	S	++
Villafranca	R	—	R	—	Seminole	R	—	S	++
Lime					Yalaha	R	—	S	++
Mexican lime	R	—	R	—	Thornton	R	—	S	++
Rangpur lime	MS	++	R	—	Tangelolo				
Sweet lime	R	—	R	—	Wekiwa	R	—	S	++
Mandarin					Tangerine-tangelo				
Calamondin	R	—	S	++	Lee	R	—	S	++
Clementine	R	—	R	—	Osceola	R	—	S	++
Cleopatra	R	+	R	+	Page	R	—	S	++
Dancy tangerine	R	—	S	++	Robinson	R	—	S	++
Emperor	R	—	S	++	Tangor				
King	R	—	R	—	Iyo-kan	R	—	S	++
Kinokuni	R	—	S	++	Kiyomi	R	—	R	—
Mandarini	R	—	R	—	Miyuchi-iyō	R	—	S	++
Cardivo di	R	—	R	—	Otani-iyō	ND	—	ND	++
Ciaculli	R	—	R	—	Umatilla	R	—	S	++
Ponkan	R	—	S	++	Yuzu				
Tachibana	R	—	R	—	Yuzu	R	—	R	—
Unshu	R	—	R	—	Miscellaneous variety in Japan				
Mandarin hybrid					Amanatsu	R	—	MS	++
Encore	R	—	R	—	Hassaku	R	—	R	—
Frua	R	—	S	++	Hyuganatsu	R	—	R	—
Kara	R	—	R	—	Kawachi bankan	R	—	R	—
Pixie	R	—	S	++	Kinkoji	R	—	R	—
Navel orange					Kobayashi mikan	R	—	R	—
Suzuki navel	R	—	R	—	Natsumikan	R	—	MS	++
Washington navel	R	—	R	—	Sanbokan	R	—	R	—
					Yama mikan	R	—	R	—

^aCitrus varieties are horticulturally subdivided.

^bThe fungal pathotypes were a rough lemon strain (AC-325) and a tangerine strain (AC-320). Drops (40 μ l) containing spores (5×10^5 spores/ml) were placed on the lower surfaces of inverted leaves, incubated in a moist chamber at 27 C in the dark, and reactions were determined after 48 h. Reactions: S (fully susceptible), brown necrotic lesions spread over the leaves; MS (moderately or intermediate susceptible), necrosis was observed to be limited only below the drop; R (resistant), no necrotic lesion was observed.

^cThree concentrations (100, 10, and 1 μ g/ml) of toxins were used (ACR-toxin I from rough lemon pathotype and ACT-toxin Ib from tangerine pathotype). Leaf necrosis was recorded after 48 h. Reactions: ++, severe necrosis (more than 50% of leaf area became necrotic) with toxin at 1 μ g/ml; +, slight necrosis on toxin-treated sites at 100 μ g/ml; —, no necrosis (insensitive to toxin).

University of Florida. The isolates were maintained on potato-dextrose agar slants in test tubes.

Toxin preparation and assay. The virulent isolates were grown in still cultures in a modified Richards' solution for 3 wk at 25 C. Toxins were purified from the culture filtrates by previously described methods (9, Kohmoto, unpublished). Partially purified ACR-toxin I and ACT-toxin Ib each were toxic to susceptible leaves at a concentration of 0.01 $\mu\text{g}/\text{ml}$. Toxins were dissolved in methanol and diluted with water to 100, 10, and 1 $\mu\text{g}/\text{ml}$ for the leaf necrosis assay. The final concentration of methanol in all cases was < 1.0%. Young citrus leaves were scratched at the center of the lower surface with a scissors. The leaves were turned lower side up, and solution was placed on the wounded site of each leaf. The leaves were incubated in a moist chamber in the dark for 48 h at 27 C; subsequent development of necrosis was recorded.

Inoculation with the pathogens. Drops (40 μl) of spore suspensions (5×10^5 spores per ml) of *A. alternata* rough lemon or tangerine pathotype were placed on the lower surfaces of cut citrus leaves. The leaves were incubated in a moist chamber in the dark for 48 h at 27 C, and the necrotic area on each leaf was measured.

RESULTS AND DISCUSSION

Sixty-seven citrus types (species and cultivars) were examined for susceptibility to both pathotypes of *A. alternata* and for sensitivity to ACR-toxin I and ACT-toxin Ib (Table 1). Only two kinds of citrus, rough lemon and rangpur lime, were susceptible to the rough lemon pathotype and to its HST (ACR-toxin I) at a concentration of 1 $\mu\text{g}/\text{ml}$. The toxin sensitivities of Rangpur lime and rough lemon were very similar, although Rangpur lime was moderately resistant to the pathogen. Leaves of Cleopatra mandarin, which were resistant to the pathogen, developed slight necrosis around the toxin application sites after application of a high concentration of ACR-toxin I (100 $\mu\text{g}/\text{ml}$). This intermediate response of Cleopatra mandarin to the toxin has been reported previously (3). The other plants examined were resistant to both the toxin and the pathogen. Thus, specificity of the rough lemon pathotype was highly correlated with sensitivity to its toxin. This finding confirms results reported by Gardner (3).

In contrast to the case with the rough lemon pathotype, 28 out of 67 citrus types were susceptible to the tangerine pathotype and were sensitive to its toxin (ACT-toxin Ib) (Table 1). Based on their breeding backgrounds (7), the susceptible cultivars were divided into four groups. The first contains hybrids with Dancy tangerine or its derivatives as one of the parents. These include cvs. Frua, Lee, Minneola, Orlando, Osceola, Page, Pixie, Robinson, Sampson, Seminole, Yalaha, and Wekiwa. All were susceptible to the tangerine pathotype and highly sensitive to its ACT-toxin Ib at a low concentration (1 $\mu\text{g}/\text{ml}$) (Table 1). The second group contains hybrids reported as likely to have had Dancy tangerine or one of its derivatives as a parent. Included are the cultivars Calamondin, Iyo-kan, Miyauchi-iyu, Otani-iyu, San Jacinto, and Thornton. All were very sensitive to the toxin and susceptible to the pathogen. The third group contains the grapefruit cultivars Marsh, Redblush, Star Ruby, and Wheeny; they were very sensitive to the toxin and susceptible to the pathogen. The fourth group contains several citrus types with unknown origin including the cultivars Amanatsu, Cleopatra, Emperor, Kinokuni, Natsumikan, Ponkan, Umatilla, and Valencia. Of these, Emperor, Ponkan, and Umatilla were very sensitive to the toxin and susceptible to the pathogen. Cleopatra, Valencia, Amanatsu, and Natsumikan were affected by the toxin only at a high concentration (100 $\mu\text{g}/\text{ml}$) but were resistant (Cleopatra and Valencia) or slightly susceptible (Amanatsu and Natsumikan) to the pathogen. These intermediates may have sensitive sites for the toxin but modifying factor(s) that alter the toxin sensitivity of the cells. One possible explanation of resistance of the cultivars Cleopatra and Valencia to the pathogen could be that amounts of toxin released during spore germination

(estimated, about 0.01 μg per spore) were not enough to gain entry by the fungus. Overall, there appears to be a correlation between the host range of the tangerine pathotype and sensitivity to its host-selective toxin.

All tested cultivars of tangelo that were derived from intercrosses with Dancy tangerine as one parent were susceptible to the pathogen and sensitive to its toxin. All examined cultivars of tangerine-tangelo that were derived from crosses of progenys (susceptible) of Dancy \times Duncan and Clementine (resistance) also were susceptible/sensitive. Nova, a hybrid of Clementine \times Orlando, was also reported to be susceptible (5).

We can now propose a working hypothesis on the genetic background of susceptibility of citrus to *A. alternata* tangerine pathotype and to its toxin; susceptibility is dominant and is transferred from Dancy tangerine to its progeny. Recently, Hutton et al (5) suggested that brown spot susceptibility is controlled by one dominant gene, based on work with Murcott tangor and Dancy tangerine. However, a careful genetic analysis is necessary to confirm this conclusion, because citrus is a highly heterogenic plant with polyembryony (7).

Prevalent cultivars of citrus in Japan indicate that an outbreak of citrus brown spot is possible. It is of interest that seven popular cultivars in Japan (Amanatsu, Iyo-kan, Kinokuni, Miyauchi-Iyo, Natsumikan, Otani-Iyo, and Ponkan) are susceptible to the pathogen and to its ACT-toxin Ib. To date, these citrus cultivars have not encountered the tangerine pathotype of *A. alternata*. Iyo-kan, Miyauchi-iyu, and Ponkan are as susceptible to the pathogen and as sensitive to the toxin as are Dancy tangerine and Emperor mandarin. Otani-Iyo is also highly sensitive to the toxin, but no spore inoculations were completed. Amanatsu and Natsumikan were sensitive to toxin but were somewhat resistant to the pathogen. They are commercially important cultivars in Japan and are superior genetic resources for citrus breeding.

Fortunately, the brown spot disease has not occurred to date in citrus orchards of Japan, but there is still a danger because the toxin-producing pathogen may be a biochemical mutant of the cosmopolitan saprophytic fungus, *A. alternata* (16). Thus, release of highly sensitive hybrid citrus genotypes is unwise. Elimination of the dominant gene for sensitivity at breeding level is by far the most satisfactory and reliable control measure.

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Serological and Chemical Variability among *Alternaria alternata* Type W Isolates in Florida

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ABSTRACT

BAKER, C. A., JONG, H., and RAVENEL, P. K. 1991. Serological and chemical variability among *Alternaria alternata* Type W isolates in Florida. *Phytopathology* 81:722-726.

The genetic relationships among 40 Type W pathotype isolates of *Alternaria alternata* were determined by serological and chemical methods. The isolates were grouped into six serotypes (PESV, PESC, PESD, PESE, PESH, and PESI) and four chemotypes (PESV, PESD, PESH, and PESI). The serotypes were defined by the presence of specific antigens (PESV, PESD, PESH, and PESI) and the chemotypes were defined by the presence of specific toxins (ACT, AM, AF, and AF-1). The serotypes and chemotypes were related to the geographical origin of the isolates and to the host plant species. The serotypes and chemotypes were also related to the host plant species. The serotypes and chemotypes were also related to the host plant species.

As sources vary for serotypes, where are Type W isolates, and the study was conducted in different isolation units, and Type W isolates. The genetic relationships among isolates among the three isolates of Type W (PESV, PESD, PESH) were defined by the presence of specific antigens (PESV, PESD, PESH, and PESI) and the chemotypes were defined by the presence of specific toxins (ACT, AM, AF, and AF-1). The serotypes and chemotypes were related to the geographical origin of the isolates and to the host plant species. The serotypes and chemotypes were also related to the host plant species.

Three isolates of the strawberry pathotype (PESV) have been identified. The primary strain (PESV-1) was an important pathogen of strawberry in central and western Florida, and it was able to infect mandarins (PESV-1).

serotype (PESV-1) was defined by the presence of specific antigens (PESV, PESD, PESH, and PESI) and the chemotypes were defined by the presence of specific toxins (ACT, AM, AF, and AF-1). The serotypes and chemotypes were related to the geographical origin of the isolates and to the host plant species. The serotypes and chemotypes were also related to the host plant species.