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Citrus Leaves Exposed to Citrus Canker Bacterium Produce Different Phenylpropanoids Based on Susceptibility, and Different from the Non-host Orange Jessamine

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Citrus canker (CC) caused by *Xanthomonas citri* subsp. *citri* (*Xcc*) impacts citrus production worldwide by reducing yield and blemishing fruit. The relative tolerance to CC among different citrus genotypes varies from highly susceptible lime and grapefruit to highly tolerant kumquat. A controlled greenhouse investigation was performed using a minimally destructive inoculation method on six *Rutaceae* family genotypes with varying tolerance to *Xcc*, from non-host to highly susceptible, including orange jessamine (*Murraya paniculata*), kumquat (*Citrus japonica*), calamondin (*C. reticulata* x *C. japonica*), sweet orange (*C. sinensis*), grapefruit (*C. paradisi*), and lime (*C. aurantifolia*). This investigation compared the responses of these six genotypes to infection with *Xcc* with respect to phenylpropanoid production. In response to *Xcc* infection all tested citrus genotype hosts, regardless of susceptibility, produced various phenylpropanoids, including umbelliferone, scoparone, herniarin, auraptene, and a polymethoxylated flavone. The character and degree of phenylpropanoid production varied between hosts. The similarly treated non-host orange jessamine did not exhibit altered phenylpropanoid production in response to *Xcc*.

Bacterial citrus disease caused by *Xanthomonas citri* subsp. *citri* (*Xcc*) negatively impacts worldwide citrus production (Das, 2003). Citrus canker (CC) was first identified in the early 19th century and was spread with the global expansion of citrus cultivation (Dopson, 1964; Luthra, 1942; Schubert et al., 2001). *Xcc* is transmitted by wind and rain, and CC manifests as a non-systemic infection on immature peel, stem, and leaf tissue (Bock et al., 2005; Gottwald et al., 2007; Graham et al., 2004). The relative susceptibility to CC among different genotypes of the *Citrus* family varies from highly susceptible lime and grapefruit to highly tolerant kumquat.(Graham et al., 1992a; Graham et al., 1992b; Lee, 1918). Symptom development of CC and *in planta* populations of *Xcc* following the artificial inoculation of the leaf tissue of different species of citrus using a minimally destructive injection method presents a spectrum of host responses that range from faint chlorosis, to small and sparse canker lesions and necrotic tissue, to high concentrations of inoculum-producing lesions (Fig. 1). More susceptible genotypes support higher *Xcc* population levels in leaf tissue, sustain these populations over longer periods of time, and exhibit more severe and widespread disease symptoms.

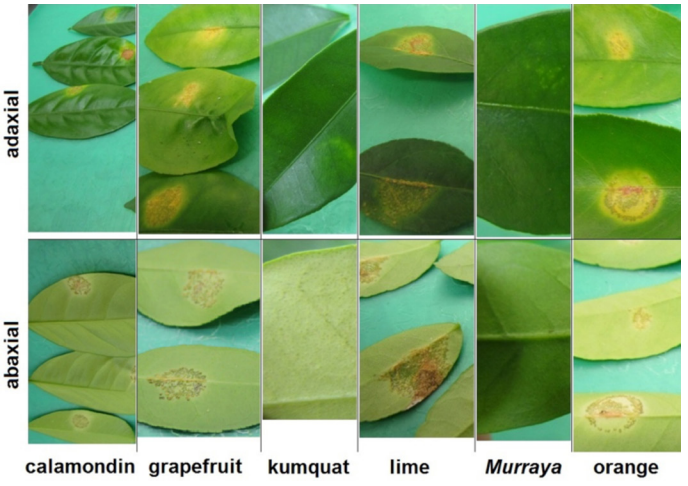

Fig. 1. A collection of pictures of the development of citrus canker on the adaxial and abaxial leaf surfaces of six different *Citrus* genotypes, 21 days after artificial inoculation with *Xcc*, prior to tissue excision for methanol extraction.

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Materials and Methods

A greenhouse experiment at the United States Department of Agriculture, Horticultural Research Laboratory in Ft. Pierce, FL, was conducted using six different genotypes of the *Citrus* family, including orange jessamine (*Murraya paniculata* L. Jack), kumquat (*Citrus japonica* Meiwa) grafted on 'Cleo' rootstock, calamondin (*Citrus reticulata* × *C. japonica*), 'Ridge Pineapple' sweet orange (*C. sinensis* L. Osbeck), 'Duncan' grapefruit (*C. paradisi* Macfadyen), and 'Key' lime (*Citrus* ×*aurantifolia*). Inoculum of bacterial populations of *Xcc* were injected into leaf tissue using a pulse injection apparatus (Pulse NeedleFree Systems, Inc., Lenexa, KS), while leaf tissue of control plants was injected with only sterile water.

At 14 and 21 days past innoculation (DPI) leaf tissue from the point of inoculation was excised and subjected to methanol extraction. Leaf tissue extracts were analyzed by fluorescence and mass spectra. Compound identification was done by matching molecular weights and fluorescence emission spectra and by observing exact peak overlaps with commercial standards.

Results and Discussion

Methanol extracts from leaf tissue of kumquat, calamondin, sweet orange, grapefruit, and lime infected with *Xcc* exhibited multiple fluorescent peaks not present, or present at barely perceptible levels, in control leaf tissue of the same genotype. Compounds found in *Xcc* inoculated leaf tissue of calamondin included scoparone (6,7-dimethoxycoumarin), herniarin (7-methoxycoumarin), umbelliferone (7-hydroxycoumarin), polymethoxyflavone (PMF), and auraptene (7-Geranyloxycoumarin). These peaks increased in intensity from the *Xcc* inoculated 14 DPI samples to the *Xcc* inoculated 21 DPI samples, indicating an increase in their concentration in assayed leaf tissue. Extracts from sweet orange leaf tissue inoculated with *Xcc* contained scoparone and PMF, both of which increased in concentration from 14 DPI to 21 DPI. Extracts from *Xcc* inoculated grapefruit leaf tissue contained umbelliferone and PMF, which increased in concentration from 14 DPI to 21 DPI. Inoculated kumquat leaf tissue contained herniarin and PMF. Relative to the other studied genotypes, fluorescent peaks in *Xcc* inoculated kumquat samples tended to be the least intense, indicating relatively low concentrations of detected compounds. Inoculated lime leaf tissue extracts contained scoparone, PMF, and herniarin. While herniarin was present in both inoculated and non-inoculated lime tissue, the fluorescent peak associated with it was typically higher in non-inoculated tissue. Despite the decreased fluorescence strength of herniarin in lime leaf tissue infected with *Xcc* relative to control samples, it remained the largest detected peak from that genotype. The next largest fluorescent peak in all lime samples was due to limettin (citropen; 5,7-dimethoxycoumarin). The presence and relative strength of the limettin fluorescence peak appeared to be independent of *Xcc* infection. Bergamottin was present in lime leaf tissue extracts and appeared to be independent of *Xcc* infection. Detected compounds in inoculated lime either decreased (herniarin, PMF, bergamottin) or remained unchanged (scoparone, limettin) from 14 DPI to 21 DPI. All orange jessamine leaf tissue extracts, inoculated and control, 14 DPI and 21 DPI, were identical.

Generally speaking, with the exception of orange jessamine, all tested *Citrus* genotypes accumulated phenolic compounds in leaf tissue in response to inoculation with *Xcc*. While orange jessamine, a member of the *Citrus* family, is a non-host of *Xcc*, bacterial populations will persist in its leaf tissue for more than 30 days after artificial inoculation. However, the fluorescent peaks produced from orange jessamine leaf tissue samples exhibit no differences between inoculated and non-inoculated samples. These multiple similarities in response after inoculation between different susceptible genotypes in terms of the accumulation of phenylpropanoids in leaf tissue inoculated with *Xcc,* contrasts directly with the lack of change in the phenolic profile in orange jessamine leaf tissue, a non-host of *Xcc*.

Literature Cited

Bock, C.H., P.E. Parker, and T.R. Gottwald, 2005. Effect of simulated wind-driven rain on duration and distance of dispersal of *Xanthomonas axonopodis* pv. *citri* from canker-infected citrus trees. *Plant Dis*. 89:71-80.

Das, A.K., 2003. Citrus canker—A review. J. Appl. Hort. 5:52–60.

Dopson, R.N. 1964. The eradication of citrus canker. Plant Dis. Rpt. 48:30–31.

Gottwald, T.R., R.B. Bassanezi, L. Amorim, and A. Bergamin-Filho. 2007. Spatial pattern analysis of citrus canker-infected plantings in Sao Paulo, Brazil, and augmentation of infection elicited by the Asian leafminer. Phytopathology 97:674–683.

Graham, J.H., T.R. Gottwald, J. Cubero, and D.S. Achor. 2004. *Xanthomonas axonopodis* pv. *citri*: factors affecting successful eradication of citrus canker. Mol. Plant Pathol. 5:1–15.

Graham, J.H., T.R. Gottwald, T.D. Riley, and D. Achor. 1992a. Penetration through leaf stomata and growth of strains of *Xanthomonas campestris* in citrus cultivars varying in susceptibility to bacterial diseases. Phytopathology 82:1319–1325.

Graham, J.H., T.R. Gottwald, T.D. Riley, and M.A. Bruce. 1992b. Susceptibility of citrus-fruit to bacterial spot and citrus canker. Phytopathology 82:452–457.

Lee, H.A. 1918. Further data on the susceptibility of rutaceous plants to citrus-canker. J. Agr. Res. 15:0661–0666.

Luthra, J.C and A. Sattar. 1942. Citrus canker and its control in Punjab. Punjab Fruit J. 6:179–182.

Schubert, T.S., S.A. Rizvi, X. Sun, T.R. Gottwald, J.H. Graham, and W.N. Dixon. 2001. Meeting the challenge of eradicating citrus canker in Florida—Again. Plant Dis. 85:340–356.