Comparison of Resistance to Asiatic Citrus Canker Among Different Genotypes of *Citrus* in a Long-Term Canker-Resistance Field Screening Experiment in Brazil

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Abstract

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Asiatic citrus canker (ACC) is caused by *Xanthomonas citri* subsp. *citri*. The disease results in yield loss and renders fruit unfit for the fresh market. A 6-year study in Paraná State, Brazil, was conducted to compare the susceptibility of 186 genotypes of citrus representing sweet orange (*Citrus sinensis*), mandarin (*C. reticulata*), Mediterranean mandarin (*C. deliciosa*), Clementine mandarin (*C. clementina*), Satsuma mandarin (*C. unshiu*), sour orange (*C. aurantium*), lemon (*C. limon*), sweet lime (*C. aurantifolia*), grapefruit (*C. paradisi*), and four hybrids (*C. reticulata* × *Citrus* sp., *C. reticulata* × *C. paradisi*, *C. reticulata* × *C. sinensis*, and *C. unshiu* × *C. sinensis*). Sweet orange (*C. sinensis*) was represented by the most genotypes ($n = 141$). The number of lesions per leaf was assessed 18 times from 2005 to 2010 (up to 4 times per year). The data were analyzed using mixed-model analysis of fixed and random effects, which showed a total of six resistance-susceptibility groupings of species and hybrids. Based on species, the most resistant genotypes, on average, included Satsuma and lemon

(mean lesions per leaf = 4.32 and 4.26, respectively), and the most susceptible genotypes were grapefruit and sweet lime, with 14.84 and 10.96 lesions per leaf, respectively. Genotypes of mandarin, sour orange, Mediterranean mandarin, and sweet orange had intermediate severity (5.48 to 9.56 lesions per leaf). The hybrids also showed a range of ACC severity but all were in the more resistant groupings (5.26 to 7.35 lesions per leaf). No genotype was immune to ACC. The most resistant genotype was ‘Muscia’ (*C. reticulata*) and the most susceptible was ‘Valencia Frost’ (*C. sinensis*) (1.86 and 14.78 lesions per leaf, respectively). Approximately one-sixth of the genotypes showed a negative relationship of mean lesions per leaf with time, suggesting increasing resistance as they aged, due to a reduction in either new flush or plant size and structure. These results of the relative susceptibility of different citrus genotypes can be used in future research and to assist in varietal selection or for breeding purposes both within Brazil and other regions where ACC is an issue.

Asiatic citrus canker (ACC; caused by *Xanthomonas citri* subsp. *citri* (ex Hasse)) (50) occurs in several tropical and subtropical citrus growing regions (16,51). It is widespread in southern Brazil, where it has substantial effect on the yield and marketability of fresh citrus and reduced citrus yield (3). Reports from other parts of the world confirmed that it is one of the most important diseases of citrus, as witnessed by the costly and prolonged eradication efforts in Florida (20,31,51) and Brazil (5). Subsequent to cessation of eradication in Florida (21,31), ACC has continued to spread in citrus orchards. *X. citri* subsp. *citri* infects the foliage, fruit, and shoots of the citrus plants, initially causing small water-soaked lesions to develop that enlarge and become erumpent and necrotic. The pathogen is naturally spread in rain splash but wind can dramatically increase the effectiveness of dispersal and infection (8); it can also be spread by farm equipment and by personnel (20). Injury, both that by wind (8) and due to insect feeding, particularly by the Asiatic citrus leafminer (CLM; *Phyllocnistis citrella* Stain-

ton), can increase the incidence and severity of the disease (7,14,29). Severe ACC can result in premature leaf and fruit drop (2,3).

São Paulo State, Brazil, is the largest orange juice producer in the world, and has been fighting an epidemic of ACC throughout the state since 2009. Today, ACC control efforts are almost exclusively the citrus grower’s responsibility and, as a consequence, disease foci detection and eradication are less intensive than in previous decades. In 2009, the São Paulo state government altered its eradication policy, making it less effective in suppressing epidemic development (5,6). Furthermore, the newest state law (approved in October 2013) determines the eradication of canker-symptomatic plants only. Thus, for the first time since detection of ACC in Brazil in 1957, neither an eradication zone nor eradication of potentially exposed plants is mandatory. The state survey of ACC conducted in 2012 showed 1.39% of sweet orange orchards had at least one symptomatic tree, which was the highest disease incidence ever observed in São Paulo state. In comparison, from 1999 to 2009, incidence of ACC ranged from 0.08 to 0.70% (6). These data suggest that ACC will probably continue to spread in São Paulo state and, as has occurred in other regions and countries in the Americas, ACC management by growers will be the main disease control strategy.

Some methods are available to manage ACC. These include the use of copper bactericidal sprays (2,3), windbreaks (which might provide some protection; 2,22), systemically acquired resistance (SAR) inducers (25,26), orchard hygiene (8), and, where pathogen arrival is recent or where ACC is not widely distributed, eradication—including in Brazil, where it has been practiced since 1957

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(5). Using insecticidal sprays to reduce CLM populations is also important in areas where the insect occurs and ACC is a problem (14,29). Although copper bactericidal sprays reduce the disease, the pathogen has shown an ability to develop resistance to copper, which could become a major problem if this were to become widespread (4). Although some promising developments appeared with SAR inducers, they might be restricted for use on young trees (25,26).

Host resistance is probably the most effective and environmentally sound way to manage ACC. Host resistance occurs within citrus and its close relatives (1,19,36,38,46), and various field- and greenhouse-based methods have undertaken to screen for resistance (1,10,18,19,36,43). Both conventional breeding methods and, more recently, transgenic approaches have been used in attempts to develop ACC-resistant citrus (11,27,28,42). These methods hope to offer long-term solutions to ACC, although the latter has various technical and consumer acceptability issues. Although conventional breeding approaches work, they can be challenging in citrus (52). Regardless of the methods used to develop new ACC-resistant germplasm, screening of the germplasm must eventually be done in the field, and this will remain an important component of germplasm testing for the foreseeable future.

X. citri subsp. *citri* has a broad host range but only within the genus *Citrus*, and the vast majority of species are reported to be susceptible to the disease (19). Various methods have been used to assess susceptibility to ACC, including pinprick inoculation and injection infiltration (19,40) or spray inoculation (1) but this can result in different results to screening germplasm in the field due to loss of “field resistance” (19,24,38,54,55). To provide natural and epidemiologically realistic methods to challenge the resistance and test susceptibility, field screening in areas where the pathogen naturally reaches epidemic proportions is preferable. Screening for resistance in citrus germplasm and related species has been done in many different countries (19,36–38,41,46). The most resistant species of citrus identified include *Citrus reticulata* Blanco (mandarin) and among the most susceptible are *C. paradisi* Macfad. (grapefruit), and *C. aurantifolia* (Christm.) Swingle (key lime and sweet lime) (19,52). There are few reports of comprehensive screenings of germplasm from different genetic backgrounds under the same field conditions but such information would be valuable to breeding programs and, potentially, for growers wishing to make varietal choices in ACC-prone areas.

Considering the economic importance of citrus and the impact of ACC in many regions of the world, particularly in Brazil and the United States, there is compelling reason to seek sources of resistance within the genus *Citrus* that might be deployed using conventional or genetic modified breeding to ensure sustainable production of this crop. We describe an experiment in Brazil testing 186 genotypes of citrus, including nine species, commercially important varieties, and hybrids, over a period of six consecutive years under field conditions to obtain robust, long-term information on susceptibility to this harmful disease.

Materials and Methods

Species and hybrids of *Citrus*. In total, 186 genotypes were used in the study (Table 1). Of these, there was 1 genotype of *C. limon* (L.) Burm. f., *C. aurantium* L., *C. aurantifolia* (Christm.) Swingle, and *C. paradisi* Macfad.; 3 of *C. unshiu* (Swingle) Marcow. and *C. deliciosa* Ten.; 9 of *C. clementina* Blanco, 17 of *C. reticulata* Blanco; and 141 of *C. sinensis* (L.) Osbeck; the remaining 9 genotypes were hybrids of the other species (1 of *C. unshiu* × *C. sinensis* and *C. reticulata* × *Citrus* sp.; 2 of *C. reticulata* × *C. paradisi*; and 5 of *C. reticulata* × *C. sinensis*). All citrus genotype budwoods were derived from the active germplasm bank of the Centro de Citricultura “Sylvio Moreira” in Cordeirópolis, São Paulo, Brazil. Part of the material had been recently introduced from Italy, Portugal, and Spain through the Citrus Experimental Station of Bebedouro (EECB, Bebedouro, São Paulo, Brazil). Nursery trees were produced under screen-protected conditions and grafted using Rangpur lime ‘Limeira’ (*C. limonia* Osbeck) as rootstock.

Experiment location and management. The field experiment was located in Maringá, in Northwestern Paraná state (latitude 23°25' S and longitude 52°10' W) at an elevation of 554.9 m. According to Koeppen's climate, it is a tropical climate modified by elevation, with hot summer temperatures and with the warmest month >22°C. The average annual temperature is 16.7°C, the average minimum is 10.3°C, and the average maximum 33.6°C, with an average relative humidity of 66%. The average annual rainfall is 1,500 mm. Frosts are infrequent and concentrated in the winter period. Prevailing winds are from the northeast and the soil of the experimental area classified as typic Haplodox.

The citrus trees were planted in April 2003. Plants were spaced 3.5 m within the row and 5.0 m between rows in a randomized block design with two replications (= blocks), and four plants of each genotype per replicate (plot). As a source of inoculum for natural disease spread to the 186 genotypes tested, every four plants were interspersed by one plant of ‘Baia’ (*C. sinensis*), which had been inoculated in November 2004 using a bacterial suspension of a highly virulent strain of *X. citri* subsp. *citri* (1) at 10⁸ CFU/ml.

Soil preparation and liming before planting, fertilization, weed control, and cultural management were carried out following standard protocol for commercial citrus production in Brazil (39). Chemical control was used to manage mites and fruit flies but no specific insecticidal treatments were made for CLM. No bactericidal products or copper-based agro-chemicals were applied to the plants in the experiment.

Disease assessment. Disease was assessed in two ways. First, severity was assessed by counting the number of lesions on a sample of 10 symptomatic leaves sampled randomly from each plant (eight plants per cultivar sampled per assessment date). Second, incidence was estimated as the proportion of leaves with lesions in the canopy. Each plant was scored using a 0-to-5 linear category scale (0 = no leaves diseased and a maximum of 5 = 75 to 100% leaves diseased) adapted from Namekata et al. (43). Assessments of severity and incidence were made a total of 18 times from 2005 to 2010, with up to 4 assessments per year. Assessments were made approximately every 4 to 6 weeks during the growing season from December to May.

Data analysis. All analyses were calculated using SAS (V9.3; SAS Institute, Cary NC). The data met the requirements of normality and were analyzed using a mixed model (proc mixed). Both the incidence data based on the linear category scale and the lesion count data were amenable to parametric analysis, although lesion count data had to be transformed using a square root transformation to stabilize the variance and maintain the normality assumption. Initially, a full model was used with fixed effects of citrus genotype, time as a linear trend, time as a nonlinear quadratic trend, and the interactions of citrus genotype, time as a linear trend, and nonlinear time as a quadratic trend. Block and the interactions of block with citrus genotype, with time as a linear trend, and with time as a nonlinear quadratic trend were all treated as random effects as follows: $\sqrt{Y_{ijkl}} = \mu + \alpha_i + \gamma_{j(i)} + \beta_1 T + \beta_2 T^2 + \beta_{1j(i)} T + \beta_{2j(i)} T^2$ (fixed effects) + $\delta_k + \alpha\delta_{ik} + \gamma\delta_{jk(i)} + \beta_{1k} T + \beta_{2k} T^2 + \varepsilon_{l(ijk)}$ (random effects) (1), where α_i = hybrid species *i*, fixed-effect dummy with the sole purpose of comparing the hybrid species to one another and doesn't contribute to the fitness of the model; $\gamma_{j(i)}$ = genotype *j* nested within hybrid *i*; $\beta_1 T$ = coefficient of the linear trend over time *T*; $\beta_2 T^2$ = coefficient of the quadratic trend over time *T*²; $\beta_{1j(i)} T$ = measure of linear trend over time *T* per genotype within each hybrid species; $\beta_{2j(i)} T^2$ = measure of linear trend over time *T*² per genotype within each hybrid species; δ_k = block effect, a random variable assumed normally distributed with variance σ_{α}^2 , $N(0, \sigma_{\alpha}^2)$; $\alpha\delta_{ik}$ = block-hybrid species interaction effect, a random variable assumed normally distributed with variance $\sigma_{\alpha\delta}^2$, $N(0, \sigma_{\alpha\delta}^2)$; $\gamma\delta_{jk(i)}$ = block-genotype interaction effect, a random variable assumed normally distributed with variance $\sigma_{\gamma\delta}^2$, $N(0, \sigma_{\gamma\delta}^2)$; β_{1k} = block-squared time interaction effect, a random variable assumed normally distributed with variance $\sigma_{\beta_1}^2$, $N(0, \sigma_{\beta_1}^2)$; β_{2k} = block-time interaction effect, a random variable assumed normally

Table 1. Least square means (lsmeans) ranking of citrus genotypes for severity of Asiatic citrus canker (number of lesions per leaf)^{x,y}

Genotype	Species, hybrid	Mean canker lesion count ^z		Grouping
		Square root	Actual	
Muscia	Tangerine mandarin (<i>Citrus reticulata</i>)	1.2	1.86	(3)G
Ponkan	Tangerine mandarin (<i>C. reticulata</i>)	1.22	1.93	(3)G
Harris	Tangerine mandarin (<i>C. reticulata</i>)	1.28	1.96	(3)FG
Rosehaugh Nartijee	Tangerine mandarin (<i>C. reticulata</i>)	1.32	2.33	(3)EFG
De Wildt	Tangerine mandarin (<i>C. reticulata</i>)	1.48	2.88	(3)DEFG
Africa do Sul	Tangerine mandarin (<i>C. reticulata</i>)	1.66	3.73	(3)CDEFG
Dekopon	Tangor (<i>C. reticulata</i> × <i>C. sinensis</i>)	1.71	3.86	(3)BCDEFG
Shamouti	Sweet orange (<i>C. sinensis</i>)	1.79	3.67	(3)ABCDEFG
Okitsu	Satsuma mandarin (<i>C. unshiu</i>)	1.89	4.45	(2)Z(3)ABCDEFG
Okitsu SPA 29	Satsuma mandarin (<i>C. unshiu</i>)	1.93	4.21	(2)YZ(3)ABCDEFG
Szwinkon × Szinkon-Tizon	Tangerine mandarin (<i>C. reticulata</i>)	1.94	4.59	(2)YZ(3)ABCDEFG
Vanilla (SB)	Lemon (<i>C. limon</i>)	1.94	4.26	(2)YZ(3)ABCDEFG
Miyakawawase	Satsuma mandarin (<i>C. unshiu</i>)	1.95	4.28	(2)YZ(3)ABCDEFG
Cristalina	Sweet orange (<i>C. sinensis</i>)	2.01	4.97	(2)XYZ(3)ABCDEFG
Vermelha	Tangerine mandarin (<i>C. reticulata</i>)	2.04	4.73	(2)WXYZ(3)ABCDEF
Loose Jacket	Tangerine mandarin (<i>C. reticulata</i>)	2.07	5.37	(2)VWXYZ(3)ABCDEF
Solid Scarlet	Sweet orange (<i>C. sinensis</i>)	2.09	4.85	(2)UVWXYZ(3)ABCDEF
Nova SPA 3	Tangelo (<i>C. reticulata</i> × <i>C. paradisi</i>)	2.1	5.08	(2)TUVWXYZ(3)ABCDE
Diva	Sweet orange (<i>C. sinensis</i>)	2.14	5.13	(2)STUVWXYZ(3)ABCD
Orange Barlie SRA 559	Sweet orange (<i>C. sinensis</i>)	2.16	5.26	(2)RSTUVWXYZ(3)ABCD
Orange Clanor SRA 391	Tangerine hybrid (<i>C. reticulata</i> × <i>Citrus</i> sp.)	2.17	5.26	(2)QRSTUVWXYZ(3)ABCD
Orange Barlerin SRA 568	Sweet orange (<i>C. sinensis</i>)	2.18	5.18	(2)PQRSTUVWXYZ(3)ABCD
Mex. 114412	Willow-leaf mandarin (<i>C. deliciosa</i>)	2.2	5.54	(2)OPQRSTUVWXYZ(3)ABCD
Tarocco 23	Sweet orange (<i>C. sinensis</i>)	2.22	5.36	(2)NOPQRSTUVWXYZ(3)ABCD
Tarocco 12	Sweet orange (<i>C. sinensis</i>)	2.23	5.35	(2)NOPQRSTUVWXYZ(3)ABCD
Pera Ovo	Sweet orange (<i>C. sinensis</i>)	2.24	5.6	(2)MNOPQRSTUVWXYZ(3)ABCD
Emperor	Tangerine mandarin (<i>C. reticulata</i>)	2.26	5.56	(2)LMNOPQRSTUVWXYZ(3)ABCD
Kyomi	Tangor (<i>C. unshiu</i> × <i>C. sinensis</i>)	2.31	6.22	(2)KLMNOPQRSTUVWXYZ(3)ABC
Sanguino	Sweet orange (<i>C. sinensis</i>)	2.31	5.83	(2)KLMNOPQRSTUVWXYZ(3)ABC
Valencia Mutação	Sweet orange (<i>C. sinensis</i>)	2.35	6.13	(2)JKLMNOPQRSTUVWXYZ(3)ABC
Navelina	Sweet orange (<i>C. sinensis</i>)	2.35	5.92	(2)IJKLMNOPQRSTUVWXYZ(3)ABC
Nova	Tangelo (<i>C. reticulata</i> × <i>C. paradisi</i>)	2.35	6.18	(2)IJKLMNOPQRSTUVWXYZ(3)ABC
Khailily White	Sweet orange (<i>C. sinensis</i>)	2.37	6.57	(2)HIJKLMNOPQRSTUVWXYZ(3)ABC
Valencia Campbell	Sweet orange (<i>C. sinensis</i>)	2.39	6.2	(2)GHIJKLMNOPQRSTUVWXYZ(3)ABC
Do Céu	Sweet orange (<i>C. sinensis</i>)	2.39	6.42	(2)FGHIJKLMNOPQRSTUVWXYZ(3)ABC
Sunburst	Tangerine mandarin (<i>C. reticulata</i>)	2.41	6.73	(2)EFGHIJKLMNOPQRSTUVWXYZ(3)ABC
Mapo	Tangor (<i>C. reticulata</i> × <i>C. sinensis</i>)	2.43	6.71	(2)DEFGHIJKLMNOPQRSTUVWXYZ(3)ABC
Pera Perão	Sweet orange (<i>C. sinensis</i>)	2.43	6.59	(2)DEFGHIJKLMNOPQRSTUVWXYZ(3)ABC
Doble Fina Blood	Sweet orange (<i>C. sinensis</i>)	2.45	6.63	(2)CDEFGHIJKLMNOPQRSTUVWXYZ(3)ABC
Bema IVIA 43	Sweet orange (<i>C. sinensis</i>)	2.45	6.58	(2)BCDEFGHIJKLMNOPQRSTUVWXYZ(3)ABC
Pera Mel	Sweet orange (<i>C. sinensis</i>)	2.46	6.78	(2)BCDEFGHIJKLMNOPQRSTUVWXYZ(3)ABC
Moro 30	Sweet orange (<i>C. sinensis</i>)	2.46	6.5	(2)ABCDEFGHIJKLMNOPQRSTUVWXYZ(3)ABC
Valencia Precoce 1480	Sweet orange (<i>C. sinensis</i>)	2.47	6.99	Z(2)ABCDEFGHIJKLMNOPQRSTUVWXYZ(3)ABC
Moro 31	Sweet orange (<i>C. sinensis</i>)	2.48	6.71	Z(2)ABCDEFGHIJKLMNOPQRSTUVWXYZ(3)AB
Pera Dibbern	Sweet orange (<i>C. sinensis</i>)	2.48	6.64	Z(2)ABCDEFGHIJKLMNOPQRSTUVWXYZ(3)AB
Natal Murcha 2	Sweet orange (<i>C. sinensis</i>)	2.49	6.93	YZ(2)ABCDEFGHIJKLMNOPQRSTUVWXYZ(3)AB
Paulista	Sweet orange (<i>C. sinensis</i>)	2.5	6.6	XYZ(2)ABCDEFGHIJKLMNOPQRSTUVWXYZ(3)AB

(continued on next page)

^x Citrus genotypes were compared by mixed-model analysis. Each mean corresponds to the average of 18 field evaluations of one plot (average of four plants) in each of two blocks in a split-plot design. The Tukey-Kramer adjusted pairwise probability matrix produced by the pdiff option in SAS was converted to letter groupings using a SASmacro (PDMIX800), which takes the probability values for differences among means and converts the information to letter groups, where means with a common letter are not statistically different at $\alpha = 0.05$ (49).

^y Mean of the square root and mean of the actual canker lesion count; lsmean for each genotype was based on all citrus plants evaluated on 18 occasions over 6 years (two blocks, $n = 36$ for each citrus genotype). Standard error of the means = 0.1254. Lesion counts were made on 10 leaves per plant in four plants per block for each genotype in all 18 occasions over 6 years.

^z Based on pairwise comparisons of square root transformed data.

Table 1. (continued from preceding page)

Genotype	Species, hybrid	Mean canker lesion count ^z		Grouping
		Square root	Actual	
Moro 15	Sweet orange (<i>C. sinensis</i>)	2.51	6.74	WXYZ(2)ABCDEFGHJKLMNOPQRSTUVW XYZ(3)AB
Osaceola	Tangerine mandarin (<i>C. reticulata</i>)	2.51	6.98	VWXYZ(2)ABCDEFGHJKLMNOPQRSTU VWXYZ(3)AB
Pera Pirangi	Sweet orange (<i>C. sinensis</i>)	2.51	7.07	VWXYZ(2)ABCDEFGHJKLMNOPQRSTU VWXYZ(3)AB
Azeda Beja	Sour Orange (<i>Citrus aurantium</i>)	2.52	6.94	VWXYZ(2)ABCDEFGHJKLMNOPQRSTU VWXYZ(3)AB
Pera Ovale	Sweet orange (<i>C. sinensis</i>)	2.53	6.79	UVWXYZ(2)ABCDEFGHJKLMNOPQRST UVWXYZ(3)A
Mex. Mogi das Cruzes	Willow-leaf mandarin (<i>C. deliciosa</i>)	2.54	6.9	TUVWXYZ(2)ABCDEFGHJKLMNOPQRST UVWXYZ(3)A
Berna IVIA-43-I (SB)	Sweet orange (<i>C. sinensis</i>)	2.54	6.9	TUVWXYZ(2)ABCDEFGHJKLMNOPQRST UVWXYZ(3)A
Mex. Tardia da Sicilia	Tangerine mandarin (<i>C. reticulata</i>)	2.55	7.09	STUVWXYZ(2)ABCDEFGHJKLMNOPQRST UVWXYZ(3)A
Lima verde	Sweet orange (<i>C. sinensis</i>)	2.56	6.89	STUVWXYZ(2)ABCDEFGHJKLMNOPQRST UVWXYZ(3)A
Valencia Temprana IVIA 25	Sweet orange (<i>C. sinensis</i>)	2.57	7.2	STUVWXYZ(2)ABCDEFGHJKLMNOPQRST UVWXYZ(3)A
Moro 13	Sweet orange (<i>C. sinensis</i>)	2.58	7.48	STUVWXYZ(2)ABCDEFGHJKLMNOPQRST UVWXYZ(3)A
Hamlin Jaraguá	Sweet orange (<i>C. sinensis</i>)	2.59	7.31	RSTUVWXYZ(2)ABCDEFGHJKLMNOPQRST UVWXYZ(3)A
Nules	Clementine mandarin (<i>C. clementina</i>)	2.59	7.19	QRSTUVWXYZ(2)ABCDEFGHJKLMNOPQRST UVWXYZ(3)A
Ouro	Sweet orange (<i>C. sinensis</i>)	2.59	7.51	QRSTUVWXYZ(2)ABCDEFGHJKLMNOPQRST UVWXYZ(3)A
Iapar 73	Sweet orange (<i>C. sinensis</i>)	2.6	7.15	PQRSTUVWXYZ(2)ABCDEFGHJKLMNOPQRST UVWXYZ(3)A
Blood Red	Sweet orange (<i>C. sinensis</i>)	2.61	7.8	OPQRSTUVWXYZ(2)ABCDEFGHJKLMNOPQR STUVWXYZ
Biondo de Caccia	Sweet orange (<i>C. sinensis</i>)	2.61	7.13	OPQRSTUVWXYZ(2)ABCDEFGHJKLMNOPQR STUVWXYZ
Ladu	Tangerine mandarin (<i>C. reticulata</i>)	2.63	7.51	NOPQRSTUVWXYZ(2)ABCDEFGHJKLMNOPQR STUVWXYZ
Pera Comprida	Sweet orange (<i>C. sinensis</i>)	2.65	7.52	MNOPQRSTUVWXYZ(2)ABCDEFGHJKLMNO PQRSTUVWXYZ
Clemengold	Clementine mandarin (<i>C. clementina</i>)	2.68	7.62	LMNOPQRSTUVWXYZ(2)ABCDEFGHJKLMNO PQRSTUVWXYZ
Orange Fukuhara SRA 561	Sweet orange (<i>C. sinensis</i>)	2.7	7.65	KLMNOPQRSTUVWXYZ(2)ABCDEFGHJKLMNO PQRSTUVWXYZ
Cravo	Tangerine mandarin (<i>C. reticulata</i>)	2.7	7.71	JKLMNOPQRSTUVWXYZ(2)ABCDEFGHJKLMNO PQRSTUVWXYZ
Mex. Miúda proc. 49/97	Willow-leaf mandarin (<i>C. deliciosa</i>)	2.72	7.98	JKLMNOPQRSTUVWXYZ(2)ABCDEFGHJKLMNO PQRSTUVWXYZ
Berna Feret IVIA 337	Sweet orange (<i>C. sinensis</i>)	2.73	8.26	IJKLMNOPQRSTUVWXYZ(2)ABCDEFGHJKLMNO PQRSTUVWXYZ
Baia Caracara	Sweet orange (<i>C. sinensis</i>)	2.73	8.31	IJKLMNOPQRSTUVWXYZ(2)ABCDEFGHJKLMNO PQRSTUVWXYZ
Lima	Sweet orange (<i>C. sinensis</i>)	2.74	8.31	HIJKLMNOPQRSTUVWXYZ(2)ABCDEFGHIJKL MNOPQRSTUVWXYZ
Navelina ISA 315	Sweet orange (<i>C. sinensis</i>)	2.74	8.19	HIJKLMNOPQRSTUVWXYZ(2)ABCDEFGHIJKL MNOPQRSTUVWXYZ
Pera Olimpia	Sweet orange (<i>C. sinensis</i>)	2.74	8.03	HIJKLMNOPQRSTUVWXYZ(2)ABCDEFGHIJKL MNOPQRSTUVWXYZ
Valencia	Sweet orange (<i>C. sinensis</i>)	2.77	8.38	HIJKLMNOPQRSTUVWXYZ(2)ABCDEFGHIJKL MNOPQRSTUVWXYZ
Caçula 4	Clementine mandarin (<i>C. clementina</i>)	2.78	8.68	HIJKLMNOPQRSTUVWXYZ(2)ABCDEFGHIJKL MNOPQRSTUVWXYZ
Pera Bianchi	Sweet orange (<i>C. sinensis</i>)	2.79	8.64	HIJKLMNOPQRSTUVWXYZ(2)ABCDEFGHIJKL MNOPQRSTUVWXYZ
Murcote	Tangor (<i>C. reticulata</i> × <i>C. sinensis</i>)	2.79	8.34	HIJKLMNOPQRSTUVWXYZ(2)ABCDEFGHIJKL MNOPQRSTUVWXYZ
Jaffa	Sweet orange (<i>C. sinensis</i>)	2.82	8.49	GHIJKLMNOPQRSTUVWXYZ(2)ABCDEFGHIJKL MNOPQRSTUVWXYZ
Tarocco Blood	Sweet orange (<i>C. sinensis</i>)	2.83	8.77	FGHIJKLMNOPQRSTUVWXYZ(2)ABCDEFGHIJKL MNOPQRSTUVWXYZ
Baianinha	Sweet orange (<i>C. sinensis</i>)	2.83	8.54	FGHIJKLMNOPQRSTUVWXYZ(2)ABCDEFGHIJKL MNOPQRSTUVWXYZ

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Table 1. (continued from preceding page)

Genotype	Species, hybrid	Mean canker lesion count ^z		Grouping
		Square root	Actual	
Telde	Sweet orange (<i>C. sinensis</i>)	2.84	8.61	FGHIJKLMNOPQRSTUVWXYZ(2)ABCDEFGH IJKLMNOPQRSTUVWXYZ
Ortanique	Tangor (<i>C. reticulata</i> × <i>C. sinensis</i>)	2.84	8.62	FGHIJKLMNOPQRSTUVWXYZ(2)ABCDEFGH IJKLMNOPQRSTUVWXYZ
Fullamenuda IVIA 92	Sweet orange (<i>C. sinensis</i>)	2.84	8.85	FGHIJKLMNOPQRSTUVWXYZ(2)ABCDEFGH IJKLMNOPQRSTUVWXYZ
Olivelands	Sweet orange (<i>C. sinensis</i>)	2.85	8.83	FGHIJKLMNOPQRSTUVWXYZ(2)ABCDEFGH IJKLMNOPQRSTUVWXYZ
Finike	Sweet orange (<i>C. sinensis</i>)	2.85	8.59	FGHIJKLMNOPQRSTUVWXYZ(2)ABCDEFGH IJKLMNOPQRSTUVWXYZ
Valencia Taquari	Sweet orange (<i>C. sinensis</i>)	2.85	8.61	FGHIJKLMNOPQRSTUVWXYZ(2)ABCDEFGH IJKLMNOPQRSTUVWXYZ
Ciaculi 60/22a/2 proc. 435196	Sweet orange (<i>C. sinensis</i>)	2.85	8.82	FGHIJKLMNOPQRSTUVWXYZ(2)ABCDEFGH IJKLMNOPQRSTUVWXYZ
Valencia Berry	Sweet orange (<i>C. sinensis</i>)	2.86	8.66	EFGHIJKLMNOPQRSTUVWXYZ(2)ABCDEFGH IJKLMNOPQRSTUVWXYZ
Pera GS	Sweet orange (<i>C. sinensis</i>)	2.86	8.66	EFGHIJKLMNOPQRSTUVWXYZ(2)ABCDEFGH IJKLMNOPQRSTUVWXYZ
Pera IAC	Sweet orange (<i>C. sinensis</i>)	2.86	8.74	EFGHIJKLMNOPQRSTUVWXYZ(2)ABCDEFGH IJKLMNOPQRSTUVWXYZ
Natal	Sweet orange (<i>C. sinensis</i>)	2.87	8.75	EFGHIJKLMNOPQRSTUVWXYZ(2)ABCDEFGH IJKLMNOPQRSTUVWXYZ
Berna	Sweet orange (<i>C. sinensis</i>)	2.88	8.65	EFGHIJKLMNOPQRSTUVWXYZ(2)ABCDEFGH IJKLMNOPQRSTUVWXYZ
Bibwells Bar	Sweet orange (<i>C. sinensis</i>)	2.88	8.9	EFGHIJKLMNOPQRSTUVWXYZ(2)ABCDEFGH IJKLMNOPQRSTUVWXYZ
Caçula 2	Clementine mandarin (<i>C. clementina</i>)	2.88	8.8	EFGHIJKLMNOPQRSTUVWXYZ(2)ABCDEFGH IJKLMNOPQRSTUVWXYZ
Salustiana SPA 11	Sweet orange (<i>C. sinensis</i>)	2.89	9.04	EFGHIJKLMNOPQRSTUVWXYZ(2)ABCDEFGH IJKLMNOPQRSTUVWXYZ
Gardner CV	Sweet orange (<i>C. sinensis</i>)	2.91	8.89	DEFGHIJKLMNOPQRSTUVWXYZ(2)ABCDE FGHIJKLMNOPQRST
Moro 16	Sweet orange (<i>C. sinensis</i>)	2.91	8.95	DEFGHIJKLMNOPQRSTUVWXYZ(2)ABCDE FGHIJKLMNOPQRST
Valencia Late	Sweet orange (<i>C. sinensis</i>)	2.91	9.17	CDEFGHIJKLMNOPQRSTUVWXYZ(2)ABCDE FGHIJKLMNOPQRST
Pera Ipiguá	Sweet orange (<i>C. sinensis</i>)	2.95	9.31	BCDEFGHIJKLMNOPQRSTUVWXYZ(2)ABCDE FGHIJKLMNOPQRS
Ortanique SPA 16	Tangor (<i>C. reticulata</i> × <i>C. sinensis</i>)	2.96	9.24	BCDEFGHIJKLMNOPQRSTUVWXYZ(2)ABCDE FGHIJKLMNOPQRS
Nova Caledona	Sweet orange (<i>C. sinensis</i>)	2.96	9.69	BCDEFGHIJKLMNOPQRSTUVWXYZ(2)ABCDE FGHIJKLMNOPQRS
Orogrande	Clementine mandarin (<i>C. clementina</i>)	2.97	9.44	ABCDEFHIJKLMNOPQRSTUVWXYZ(2)ABC DEFGHIJKLMNOPQR
<i>C. sinensis</i> 1343	Sweet orange (<i>C. sinensis</i>)	2.97	9.38	ABCDEFHIJKLMNOPQRSTUVWXYZ(2)ABC DEFGHIJKLMNOPQR
Hamlin	Sweet orange (<i>C. sinensis</i>)	2.98	9.63	ABCDEFHIJKLMNOPQRSTUVWXYZ(2)ABC DEFGHIJKLMNOPQ
Baia Lane Late	Sweet orange (<i>C. sinensis</i>)	2.98	9.59	ABCDEFHIJKLMNOPQRSTUVWXYZ(2)ABC DEFGHIJKLMNOPQ
Natal Africa do Sul	Sweet orange (<i>C. sinensis</i>)	2.99	9.53	ABCDEFHIJKLMNOPQRSTUVWXYZ(2)ABC DEFGHIJKLMNOP
Baia Leng	Sweet orange (<i>C. sinensis</i>)	3.01	10	ABCDEFHIJKLMNOPQRSTUVWXYZ(2)ABC DEFGHIJKLMNO
Midsweet	Sweet orange (<i>C. sinensis</i>)	3.01	9.67	ABCDEFHIJKLMNOPQRSTUVWXYZ(2)ABC DEFGHIJKLMNO
Baia Cabula	Sweet orange (<i>C. sinensis</i>)	3.01	9.57	ABCDEFHIJKLMNOPQRSTUVWXYZ(2)ABC DEFGHIJKLMN
Sunstar	Sweet orange (<i>C. sinensis</i>)	3.05	9.69	ABCDEFHIJKLMNOPQRSTUVWXYZ(2)ABC DEFGHIJKLM
Clemenules SPA 17	Clementine mandarin (<i>C. clementina</i>)	3.06	9.85	ABCDEFHIJKLMNOPQRSTUVWXYZ(2)ABC DEFGHIJKL
Valencia Withs	Sweet orange (<i>C. sinensis</i>)	3.09	9.96	ABCDEFHIJKLMNOPQRSTUVWXYZ(2)ABC DEFGHIJK
Orange Navelina SRA 332	Sweet orange (<i>C. sinensis</i>)	3.1	10.27	ABCDEFHIJKLMNOPQRSTUVWXYZ(2)ABC DEFGHIJK
Valencia Campbell	Sweet orange (<i>C. sinensis</i>)	3.11	10.29	ABCDEFHIJKLMNOPQRSTUVWXYZ(2)ABC DEFGHIJK
Orange Yoshida Navel SRA 558	Sweet orange (<i>C. sinensis</i>)	3.13	10.47	ABCDEFHIJKLMNOPQRSTUVWXYZ(2)ABC DEFGHIJ
Westin	Sweet orange (<i>C. sinensis</i>)	3.15	10.32	ABCDEFHIJKLMNOPQRSTUVWXYZ(2)ABC DEFGHIJ

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Table 1. (continued from preceding page)

Genotype	Species, hybrid	Mean canker lesion count ^z		Grouping
		Square root	Actual	
Verde de Espanha	Sweet orange (<i>C. sinensis</i>)	3.15	10.35	ABCDEFGHIIJKLMNOPQRSTUVWXYZ(2)ABC DEFGHIJ
China	Sweet orange (<i>C. sinensis</i>)	3.16	10.65	ABCDEFGHIIJKLMNOPQRSTUVWXYZ(2)ABC DEFGHIJ
Wetumpka	Sweet orange (<i>C. sinensis</i>)	3.16	10.59	ABCDEFGHIIJKLMNOPQRSTUVWXYZ(2)ABC DEFGHIJ
Span Americana	Tangerine mandarin (<i>C. reticulata</i>)	3.17	10.67	ABCDEFGHIIJKLMNOPQRSTUVWXYZ(2)ABC DEFGHI
Vascaro Blood	Sweet orange (<i>C. sinensis</i>)	3.18	10.29	ABCDEFGHIIJKLMNOPQRSTUVWXYZ(2)ABC DEFGH
Pardilhó	Sweet orange (<i>C. sinensis</i>)	3.18	10.51	ABCDEFGHIIJKLMNOPQRSTUVWXYZ(2)ABC DEFGH
Clemenules	Clementine mandarin (<i>C. clementina</i>)	3.18	10.68	ABCDEFGHIIJKLMNOPQRSTUVWXYZ(2)ABC DEFGH
Valencia 1231	Sweet orange (<i>C. sinensis</i>)	3.18	10.73	ABCDEFGHIIJKLMNOPQRSTUVWXYZ(2)ABC DEFGH
Kawata Blood	Sweet orange (<i>C. sinensis</i>)	3.19	10.54	ABCDEFGHIIJKLMNOPQRSTUVWXYZ(2)ABC DEFGH
Orange Newhall Navel SRA 343	Sweet orange (<i>C. sinensis</i>)	3.2	10.66	ABCDEFGHIIJKLMNOPQRSTUVWXYZ (2)ABCDEFG
Ovale mut proc. 43 5/96	Sweet orange (<i>C. sinensis</i>)	3.2	10.73	ABCDEFGHIIJKLMNOPQRSTUVWXYZ (2)ABCDEFG
Tarocco 17	Sweet orange (<i>C. sinensis</i>)	3.2	10.88	ABCDEFGHIIJKLMNOPQRSTUVWXYZ (2)ABCDEF
Monica	Sweet lime (<i>C. aurantifolia</i>)	3.21	10.96	ABCDEFGHIIJKLMNOPQRSTUVWXYZ (2)ABCDE
Clementina Comune	Clementine mandarin (<i>C. clementina</i>)	3.22	10.78	ABCDEFGHIIJKLMNOPQRSTUVWXYZ (2)ABCDE
Majorca	Sweet orange (<i>C. sinensis</i>)	3.22	10.86	ABCDEFGHIIJKLMNOPQRSTUVWXYZ (2)ABCDE
Washington Navel	Sweet orange (<i>C. sinensis</i>)	3.22	10.86	ABCDEFGHIIJKLMNOPQRSTUVWXYZ (2)ABCDE
Early Oblong	Sweet orange (<i>C. sinensis</i>)	3.23	10.85	ABCDEFGHIIJKLMNOPQRSTUVWXYZ (2)ABCDE
Kona	Sweet orange (<i>C. sinensis</i>)	3.23	10.94	ABCDEFGHIIJKLMNOPQRSTUVWXYZ (2)ABCD
Stone	Sweet orange (<i>C. sinensis</i>)	3.23	10.99	ABCDEFGHIIJKLMNOPQRSTUVWXYZ (2)ABCD
Valencia 21	Sweet orange (<i>C. sinensis</i>)	3.24	11.24	ABCDEFGHIIJKLMNOPQRSTUVWXYZ (2)ABCD
Baia Navelina	Sweet orange (<i>C. sinensis</i>)	3.24	11.57	ABCDEFGHIIJKLMNOPQRSTUVWXYZ (2)ABCD
Bey Navel	Sweet orange (<i>C. sinensis</i>)	3.24	11.03	ABCDEFGHIIJKLMNOPQRSTUVWXYZ (2)ABCD
Orange Sanford SRA 404	Sweet orange (<i>C. sinensis</i>)	3.24	10.74	ABCDEFGHIIJKLMNOPQRSTUVWXYZ (2)ABCD
Szwinkon	Tangerine mandarin (<i>C. reticulata</i>)	3.24	11.62	ABCDEFGHIIJKLMNOPQRSTUVWXYZ (2)ABCD
Torregrosso	Sweet orange (<i>C. sinensis</i>)	3.25	11.05	ABCDEFGHIIJKLMNOPQRSTUVWXYZ(2)ABC
Moro 28	Sweet orange (<i>C. sinensis</i>)	3.26	11.25	ABCDEFGHIIJKLMNOPQRSTUVWXYZ(2)ABC
Aziza	Sweet orange (<i>C. sinensis</i>)	3.26	11.09	ABCDEFGHIIJKLMNOPQRSTUVWXYZ(2)AB
Valencia Precoce 1435	Sweet orange (<i>C. sinensis</i>)	3.26	11.28	ABCDEFGHIIJKLMNOPQRSTUVWXYZ(2)AB
Orange Skaggs Bonanza				
Navel SRA 202	Sweet orange (<i>C. sinensis</i>)	3.28	11.43	ABCDEFGHIIJKLMNOPQRSTUVWXYZ(2)A
Valencia chaffei	Sweet orange (<i>C. sinensis</i>)	3.28	11.29	ABCDEFGHIIJKLMNOPQRSTUVWXYZ
Tobias	Sweet orange (<i>C. sinensis</i>)	3.3	11.34	ABCDEFGHIIJKLMNOPQRSTUVWXYZ
Rico	Sweet orange (<i>C. sinensis</i>)	3.31	11.45	ABCDEFGHIIJKLMNOPQRSTUVWXYZ
Old bud line	Sweet orange (<i>C. sinensis</i>)	3.31	11.35	ABCDEFGHIIJKLMNOPQRSTUVWXYZ
D. João proc. 49/97	Sweet orange (<i>C. sinensis</i>)	3.31	11.29	ABCDEFGHIIJKLMNOPQRSTUVWXYZ
Laranja rah (SB)	Sweet orange (<i>C. sinensis</i>)	3.32	11.38	ABCDEFGHIIJKLMNOPQRSTUVWXYZ
Rotuma blood	Sweet orange (<i>C. sinensis</i>)	3.32	11.34	ABCDEFGHIIJKLMNOPQRSTUVWXYZ
Setubal	Sweet orange (<i>C. sinensis</i>)	3.33	11.77	ABCDEFGHIIJKLMNOPQRSTUVWXYZ
Strand	Sweet orange (<i>C. sinensis</i>)	3.34	11.53	ABCDEFGHIIJKLMNOPQRSTUVWXYZ
Orama	Sweet orange (<i>C. sinensis</i>)	3.34	11.73	ABCDEFGHIIJKLMNOPQRSTUVWXYZ
Espanole	Sweet orange (<i>C. sinensis</i>)	3.35	11.57	ABCDEFGHIIJKLMNOPQRSTUVWXYZ
Lane late SPA 23	Sweet orange (<i>C. sinensis</i>)	3.35	12.02	ABCDEFGHIIJKLMNOPQRSTUVWXYZ
Castellana ivia-64-3	Sweet orange (<i>C. sinensis</i>)	3.36	11.67	ABCDEFGHIIJKLMNOPQRSTUVWXYZ
Werley	Sweet orange (<i>C. sinensis</i>)	3.4	12.03	ABCDEFGHIIJKLMNOPQRSTUVWXYZ
Arrufatina	Clementine mandarin (<i>C. clementina</i>)	3.4	12.26	ABCDEFGHIIJKLMNOPQRSTUVWXYZ
Grada	Sweet orange (<i>C. sinensis</i>)	3.42	12.18	ABCDEFGHIIJKLMNOPQRSTUVWXYZ
<i>C. sinensis</i>	Sweet orange (<i>C. sinensis</i>)	3.43	12.24	ABCDEFGHIIJKLMNOPQRSTUVWXYZ

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Table 1. (continued from preceding page)

Genotype	Species, hybrid	Mean canker lesion count ^z		Grouping
		Square root	Actual	
Harvard	Sweet orange (<i>C. sinensis</i>)	3.44	12.23	ABCDEFGHJKLMN
Orange Rotuna SRA 511	Sweet orange (<i>C. sinensis</i>)	3.45	12.52	ABCDEFGHJKLM
Vasconcellos	Sweet orange (<i>C. sinensis</i>)	3.45	12.61	ABCDEFGHJKLM
Valencia Late Fla.	Sweet orange (<i>C. sinensis</i>)	3.45	12.55	ABCDEFGHJKLM
Navelina SPA 12	Sweet orange (<i>C. sinensis</i>)	3.46	12.64	ABCDEFGHJKLM
Isle of Pines	Sweet orange (<i>C. sinensis</i>)	3.47	12.68	ABCDEFGHJKLM
Biondo	Sweet orange (<i>C. sinensis</i>)	3.48	12.53	ABCDEFGHJKLM
Valencia Olinda	Sweet orange (<i>C. sinensis</i>)	3.49	12.93	ABCDEFGHJKLM
Sanguinea 66/ssa/12 proc. 435/96	Sweet orange (<i>C. sinensis</i>)	3.49	12.63	ABCDEFGHJKLM
Valencia Cutter	Sweet orange (<i>C. sinensis</i>)	3.49	12.97	ABCDEFGHIJK
Comuna IVIA-105	Sweet orange (<i>C. sinensis</i>)	3.51	12.69	ABCDEFGHIJ
Vale dos Besteiros	Sweet orange (<i>C. sinensis</i>)	3.51	12.83	ABCDEFGHIJ
Fraga proc. 49/97	Sweet orange (<i>C. sinensis</i>)	3.54	13.15	ABCDEFGHI
Convento	Sweet orange (<i>C. sinensis</i>)	3.54	13.04	ABCDEFGH
Seleta do Rio	Sweet orange (<i>C. sinensis</i>)	3.62	13.58	ABCDEFG
Prata proc. 49/97	Sweet orange (<i>C. sinensis</i>)	3.64	13.67	ABCDEF
Valencia 1230	Sweet orange (<i>C. sinensis</i>)	3.68	14.4	ABCDE
Seleta Tardia	Sweet orange (<i>C. sinensis</i>)	3.71	14.25	ABCD
Marrs	Grapefruit (<i>C. paradisi</i>)	3.72	14.84	ABCD
Setubalense proc. 49/97	Sweet orange (<i>C. sinensis</i>)	3.73	14.48	ABC
Tarocco 14	Sweet orange (<i>C. sinensis</i>)	3.75	14.94	AB
Valencia Frost	Sweet orange (<i>C. sinensis</i>)	3.78	14.78	A

Fig. 1. Relationship between the Least square means (Lsmeans) for the incidence of leaves showing symptoms of Asiatic citrus canker and the mean lesions per leaf for 186 genotypes of citrus ($y = -0.48 + 0.31x$; $R^2 = 0.85$; $F = 1079$, $P < 0.0001$).

distributed with variance $\sigma_{\beta_2}^2$, $N(0, \sigma_{\beta_2}^2)$; and $\epsilon_{(ijk)}$ = residual, a random variable assumed normally distributed with variance σ_e^2 , $N(0, \sigma_e^2)$.

Random effects were sequentially removed from the model and observation of the variance components of the resulting analysis was used to eliminate those that did not improve the fit of the model (i.e., those random effects with variance components that were either missing, zero, or close to zero). The maximum likelihood approach was used and the appropriateness of models compared using the Akaike information criterion, the corrected Akaike's information criterion, and the Bayesian information criterion. Thus, a reduced model that included random effects of block and the interactions block-genotype, block-linear time, and block-quadratic time was used in the final analysis. Changes in disease on each genotype with time as a linear trend and time as a nonlinear quadratic trend were calculated at the median time value so that the intercepts and all pairwise comparisons were at 9.5 weeks and were compared with zero (and significant if different from zero). Both a conservative Bonferroni correction and a less conservative false discovery rate (FDR) correction was incorporated in the anal-

ysis of multiple testing for time (so as to maintain the familywise error rate when testing multiple hypotheses) with the α level set at 0.05.

Least square means (Lsmeans) were generated for each citrus genotype. A pairwise comparison of genotypes was made using Tukey-Kramer adjusted pairwise comparisons (pdiff). The pairwise probability matrix produced by the pdiff option in SAS was converted to letter groupings using an SAS macro (PDMIX800) which takes the probability values for differences among means and converts the information to letter groups, where means with a common letter are not statistically different at $\alpha = 0.05$ (49).

The species or hybrid status of each genotype was added to the model to provide a basis to compare species and hybrids as groups (addition of species or hybrid as a fixed effect did not change the model but allowed calculation of the Lsmeans for each species or hybrids as a group in the analysis). Lsmeans were calculated for species and hybrids as described above, with pairwise comparisons and letter groupings based on P values.

Results

There was a very close relationship between the Lsmeans of the canker severity score (mean lesion count per leaf) and the canker incidence data (percent leaves diseased) for individual genotypes (Fig. 1, $R^2 = 0.85$, $P < 0.0001$). The results of the mixed analysis were also very similar for the two disease variables; thus, only the results of the analysis for the canker mean lesion count (severity) are presented.

The results of the mixed-model analysis for canker severity using the reduced model showed that there were significant fixed effects of genotype, linear time, and the quadratic time function (Table 2). None of the genotypes were immune to ACC. The differences between genotypes in the number of lesions ranged from the most resistant, with a mean lesion count per leaf of 1.86 for 'Muscia' (*C. reticulata*), to the most susceptible, with a mean lesion count per leaf of 14.78 for 'Valencia Frost' (*C. sinensis*, based on the square root transformed data although, in Table 1, 'Tarocco 14' [*C. sinensis*] had 14.94 lesions per leaf). The most resistant genotypes were grouped together (group [3]G) and included 14 cultivars from 'Muscia' to 'Cristalina' (with 4.97 lesions per leaf) (Muscia, 'Ponkan', 'Harris', 'Rosehaugh Nartjee', 'De Wildt', 'África do Sul', 'Dekopon', 'Shamouti', 'Satsuma Okitsu', 'Satsuma Okitsu SPA 29', 'Szuwinkon' $\times$ 'Szinon-Tizon', 'Vanilla', 'Satsuma Miyakawase', and 'Cristalina'). These genotypes were mostly members of the species *C. reticulata*, *C. unshiu*, and

C. limon (only one genotype represented). According to the mixed-model analysis, the most susceptible group (A) included 83 genotypes from ‘Orogrande’ Clementine (*C. clementina*, with 9.44 lesions per leaf) to ‘Valencia Frost’ (*C. sinensis*, with 14.78 lesions per leaf). Genotypes of the species *C. sinensis*, *C. clementina*, and *C. paradisi* (only one genotype represented) were most common in this group. The remaining 89 genotypes all had intermediate susceptibility (4.73 to 9.69 lesions per leaf), although there were a total of 59 overlapping ACC severity groups according to the pairwise probability groupings. A heat map showing these relationships is included in the supplementary data online which accompany this article (Supplementary Figure 1).

There were significant differences between genotypes of the different *Citrus* spp. and the hybrids of these species based on mean lesions per leaf (Table 3). There were six groupings of species and hybrids according to the pairwise probability groupings, although some of the species or hybrids were represented by only a single genotype (*C. limon*, *C. aurantium*, *C. aurantifolia*, *C. paradisi*, *C. unshiu* × *C. sinensis*, and *C. reticulata* × *Citrus* sp.). The most resistant species or hybrid grouping that were screened included *C. unshiu* and *C. limon* (mean lesions per leaf of 4.32 and 4.26, respectively), and the most susceptible species were *C. paradisi* (only one genotype represented) and *C. aurantifolia* (only one genotype represented), with 14.84 and 10.96 lesions per leaf, respectively. The other species (*C. reticulata*, *C. aurantium* [only one genotype represented], *C. deliciosa* [only one genotype represented], *C. sinensis*, and *C. clementina*) had intermediate severity (5.48 to 9.56 lesions per leaf). The hybrids also showed a range of ACC severity but all were in the more resistant groupings (5.26 to 7.35 lesions per leaf).

The frequency of severity among these different *Citrus* spp. and the hybrids showed that, where there were several different genotypes of a particular species or hybrid, there was a range in ACC

susceptibility (Fig. 2; Table 1). Among those *Citrus* spp. or hybrids represented by multiple genotypes, *C. clementina* had a range of lesions per leaf from 7.19 (‘Clementina Nules’) to 12.26 (‘Clementina Arrufatina’). *C. unshiu*, represented by three genotypes, had a range of 4.21 (Satsuma Okitsu SPA 29) to 4.45 (Satsuma Okitsu) lesions per leaf, and *C. sinensis*, which was represented by the most genotypes, had 3.67 (‘Shamouti’) to 14.94 (‘Tarocco 14’) lesions per leaf, with ‘Tarocco 14’ being the most susceptible genotype assessed. *C. reticulata* had among the most resistant genotypes, with the number of lesions per leaf ranging from 1.86 (‘Muscia’) to 11.62 (Szuwinkon). Genotypes of *C. deliciosa* had moderate resistance, with 5.54 (‘Mex. 114412’) to 7.98 (‘Mex. Miúda proc. 49/97’) lesions per leaf. The hybrid *C. reticulata* × *C. paradisi* had a mean lesion count of 5.08 (‘Hibrido nova SPA 3’) and 6.18 (‘Nova’), and the genotypes of the hybrid *C. reticulata* × *C. sinensis* a range from 3.86 (Dekopon) to 9.24 (‘Ortanique’).

As noted above, among those species or hybrids represented by only a single genotype, *C. paradisi* was one of the most susceptible to ACC, with 14.84 (‘Marrs’ grapefruit) lesions per leaf, followed by *C. aurantifolia*, with 10.96 lesions per leaf (‘Monica’ sweet lime). *C. aurantium* had 6.94 (‘Azeda Beja’) and *C. limon* had 4.26 (‘Vanilla’) lesions per leaf. A hybrid of unknown origin (*C. reticulata* × *Citrus* sp.) had 5.26 (‘Clanor SRA 391’) lesions per leaf, and the genotype of the hybrid *C. unshiu* × *C. sinensis* (‘Kyomi’) had 6.22 lesions per leaf.

After applying a Bonferroni correction, there were effects of time on ACC severity for some genotypes of citrus (Table 4). Of the 186 genotypes, 33 showed a negative linear trend with time and only two genotypes (‘Ouro’ and ‘Valencia 1230’) showed a significant effect of quadratic time (‘Ouro’ had a negative relationship and ‘Valencia 1230’ a positive relationship). The negative linear relationship suggests that these genotypes became increasingly resistant as they aged due to either genetic resistance, reduced

Table 2. Type III fixed effects for the reduced mixed model analysis of the severity of Asiatic citrus canker (number of lesions per leaf) on leaves of different citrus genotypes over a 6-year period^z

Effect	Num DF	Den DF	F value	Pr > F
Citrus genotype	186	185	81.51	<0.0001
Linear time × Citrus genotype	186	5,950	2.74	<0.0001
Quadratic time × Citrus genotype	186	5,950	1.61	<0.0001

^z Covariance parameter estimates of the random effects: block = 0.00085, Citrus genotype versus block = 0.0055, linear time versus block = 0.000061, quadratic time versus block = 0.000. Fit statistics for the mixed model: Aikake information criterion (smaller is better) 14,888.9, corrected Akaike’s information criterion (smaller is better) 14,992.1, and Bayesian information criterion (smaller is better) 14,154.5.

Table 3. Least square means (lsmeans) of the severity of Asiatic citrus canker (mean lesions per leaf) for different species and hybrids of citrus^v

Species, hybrid	N ^x	Mean of the canker lesion count ^w		SE ^y	Grouping ^z
		Square root	Actual		
Satsuma mandarin (<i>Citrus unshiu</i>)	3	1.92	4.32	0.074	F
Lemon (<i>C. limon</i>)	1	1.94	4.26	0.125	DEF
Tangerine mandarin (<i>C. reticulata</i>)	17	2.10	5.48	0.036	DF
Tangerine hybrid (<i>C. reticulata</i> × <i>Citrus</i> sp.)	1	2.17	5.26	0.125	CDF
Tangelo (<i>C. reticulata</i> × <i>C. paradisi</i>)	2	2.23	5.63	0.090	CDF
Tangor (<i>C. unshiu</i> × <i>C. sinensis</i>)	1	2.31	6.22	0.125	CDF
Willow-leaf mandarin (<i>C. deliciosa</i>)	3	2.48	6.81	0.074	CE
Sour orange (<i>C. aurantium</i>)	1	2.52	6.94	0.125	BCD
Tangor (<i>C. reticulata</i> × <i>C. sinensis</i>)	5	2.54	7.35	0.059	C
Sweet orange (<i>C. sinensis</i>)	141	2.97	9.56	0.023	B
Clementine mandarin (<i>C. clementina</i>)	9	2.97	9.48	0.046	B
Sweet lime (<i>C. aurantifolia</i>)	1	3.21	10.96	0.125	AB
Grapefruit (<i>C. paradisi</i>)	1	3.72	14.84	0.125	A

^v Citrus genotypes were compared by mixed-model analysis. Each mean corresponds to the average of 18 field evaluations of one plot (four plants) in each of two blocks in a split-plot design. The Tukey-Kramer adjusted pairwise probability matrix produced by the pdiff option in SAS was converted to letter groupings using a SASmacro (PDMIX800) which takes the probability values for differences among means and converts the information to letter groups, where means with a common letter are not statistically different at $\alpha = 0.05$ (49).

^w The lsmean for each genotype was based on all citrus plants evaluated on 18 occasions over 6 years ($n = 36$ for each citrus genotype). Lesion counts were made on 10 leaves per plant in four plants per block for each genotype in all 18 occasions over 6 years.

^x Number of genotypes.

^y Standard error (square root transformed data).

^z Based on pairwise comparisons of square root transformed data.

flush with plant age, or plant size or structure. Of these 33 genotypes, the number of lesions per leaf ranged from 3.73 ('África do Sul') to 14.40 ('Valencia 1230'). These 33 genotypes belonged to six *Citrus* spp. and three hybrids (Table 4). The positive nonlinear relationship suggests more complex relationships of time with disease trends. None of the other genotypes showed a significant effect of time over the 6-year duration of this study.

Discussion

No genotype of *Citrus* spp. was completely resistant to ACC but the study provided an analysis of the relative severity of ACC on

the 186 citrus genotypes over a 6-year assessment period in an area prone to epidemics of ACC in Brazil, thus making a very robust comparison. Although some species or hybrids were represented by only a single genotype, there was a contrasting susceptibility to ACC among the different *Citrus* spp. screened over the 6-year period of the experiment. On average, the most susceptible *Citrus* spp. were grapefruit (*C. paradisi*), sweet orange (*C. sinensis*), Clementine mandarin (*C. clementina*), and sweet Lime (*C. aurantifolia*), which are acknowledged as having greater susceptibility compared with other species, as shown in previous studies (1,19). In contrast, among the most resistant species of citrus were manda-

Fig. 2. Genotypes of all species and hybrids of *Citrus* combined, and of each of the species and hybrids of *Citrus* individually, showing the frequency of mean lesion counts of Asiatic citrus canker.

rin (*C. reticulata*), Satsuma mandarin (*C. unshiu*), and lemon (*C. limon*), which also confirms previous reports (1,19,43,47). However, it should also be pointed out that a few genotypes of *C. sinensis* were more resistant than some genotypes of *C. reticulata*, *C. unshiu*, and *C. limon*, and some genotypes of *C. sinensis* were as susceptible as *C. paradisi*, *C. clementina*, and *C. aurantifolia* (Table 1). The remaining species and hybrids were of intermediate susceptibility. As noted, there was a range of susceptibility to ACC within each species or hybrid. Thus, sweet orange (*C. sinensis*), which was represented by the most genotypes, had 3.67 ('Shamouti') to 14.94 ('Tarocco 14') lesions per leaf, although, overall, the genotypes tended to be among the more susceptible. *C. sinensis* is near isogenic (17) but, despite this, members of the species display a wide range of reaction to *X. citri* subsp. *citri*, from moderately resistant to susceptible. *C. sinensis* has previously been shown to have a range of genotype susceptibility to ACC (1,10,19). The genotype 'Muscia' of *C. reticulata* was the most resistant, with as few as 1.86 lesions per leaf, followed by the genotypes 'Satsuma Okitsu SPA 29' and 'Satsuma Okitsu' of *C. unshiu*, with 4.21 to 4.45 lesions per leaf, respectively. 'Marrs' grapefruit, the only representative of *C. paradisi*, was very susceptible (14.84 lesions per leaf). However, due to some species and hybrids being represented by only a single genotype, some caution should be taken when making assumptions about the relative susceptibilities of different species, although the results are in agreement with those previously reported for *Citrus* spp. (1,10,19,43,47).

Many of the species and hybrid genotypes included in this study were included in an unrelated but previously published greenhouse study (1). In that study, the genotypes were separated based on the percent leaf area diseased using four classes: resistant (0 to 5%

area with lesions), moderately resistant (5 to 10%), susceptible (10 to 15%), and highly susceptible (>15%). In general, there was broad agreement with that study but some differences in genotype classification: for example, Amaral et al. (1) found the sweet orange 'Kyomi' to be "resistant" but it was found to get 6.22 lesions per leaf in the current study, where it would likely be classed "moderately resistant". The grapefruit 'Marrs' was classified as "susceptible" in their study but, in the current study, had 14.84 lesions per leaf and, considering the range of disease experienced, would thus be classified as "highly susceptible". Amaral et al. (1) found sweet orange 'Wetumpkai' to be "moderately resistant" but it was found to get 10.59 lesions per leaf in the current study, where it would likely be classed "susceptible". In that study, the sweet orange × mandarin hybrid 'Dekopon' was classified "moderately resistant" but, in the current study, was in the most resistant grouping based on the mixed-model analysis, with 3.86 lesions per leaf. Other genotypes showed differences between the two studies. These contrasts might highlight the different outcome of a greenhouse-based spray-inoculation study compared with a field experiment, where the field resistance is challenged by epidemiologically realistic inoculum and natural conditions without damaging the host tissue to provide entry for the pathogen (19,24,38,40,54,55).

São Paulo state in Brazil and Florida in the United States are the world's largest producers of orange juice and are also major producers of sweet orange. In São Paulo state, more than 450,000 ha of sweet orange are grown, comprising predominantly four genotypes of *C. sinensis* ('Pera', 'Valencia', 'Natal', and 'Hamlin'). Growers in Florida, with less than half of the area of São Paulo state, concentrate their production on 'Hamlin', 'Valencia', and grapefruit. Both regions are facing spread and intensification of the

Table 4. Effect of time on the severity (lesions per leaf) of Asiatic citrus canker (ACC) on different genotypes of citrus

Time, species, hybrid	Citrus genotype	Estimate ^a	t Value	Pr > t	Pr > t (after Bonferroni correction)
Linear					
<i>C. reticulata</i>	Africa do Sul	-0.1159	-5.20	0.00009	0.0167
<i>C. aurantium</i>	Azeda Beja	-0.1031	-4.63	0.00009	0.0167
<i>C. sinensis</i>	Baia Caracara	-0.0905	-4.06	0.00009	0.0167
<i>C. sinensis</i>	Ciaculi 60/22a/2 proc. 435196	-0.0972	-4.36	0.00009	0.0167
<i>C. clementina</i>	Clementina Caçula 4	-0.1406	-6.31	0.00009	0.0167
<i>C. clementina</i>	Clemenules	-0.0872	-3.91	0.00009	0.0167
<i>C. sinensis</i>	Doble Fina Blood	-0.0907	-4.07	0.00009	0.0167
<i>C. reticulata</i> × <i>C. paradisi</i>	Nova SPA 3	-0.1227	-5.51	0.00009	0.0167
<i>C. unshiu</i> × <i>C. sinensis</i>	Kyomi	-0.1102	-4.94	0.00009	0.0167
<i>C. sinensis</i>	Lane Late SPA 23	-0.0998	-4.48	0.00009	0.0167
<i>C. sinensis</i>	China	-0.1013	-4.54	0.00009	0.0167
<i>C. reticulata</i>	Loose Jacket	-0.1291	-5.79	0.00009	0.0167
<i>C. reticulata</i> × <i>C. sinensis</i>	Mapo	-0.1169	-5.25	0.00009	0.0167
<i>C. reticulata</i>	Mex. Tardia da Sicilia	-0.1142	-5.13	0.00009	0.0167
<i>C. sinensis</i>	Midsweet	-0.0955	-4.28	0.00009	0.0167
<i>C. aurantifolia</i>	Monica	-0.1078	-4.84	0.00009	0.0167
<i>C. sinensis</i>	Natal	-0.09	-4.04	0.00009	0.0167
<i>C. sinensis</i>	Navelina SPA 12	-0.0914	-4.10	0.00009	0.0167
<i>C. reticulata</i> × <i>C. paradisi</i>	Nova	-0.0994	-4.46	0.00009	0.0167
<i>C. sinensis</i>	Nova Caledona	-0.1197	-5.37	0.00009	0.0167
<i>C. sinensis</i>	Orama	-0.0947	-4.25	0.00009	0.0167
<i>C. unshiu</i>	Satsuma Okitsu	-0.0979	-4.39	0.00009	0.0167
<i>C. sinensis</i>	Setubal	-0.1167	-5.24	0.00009	0.0167
<i>C. reticulata</i>	Ladu	-0.0948	-4.25	0.00009	0.0167
<i>C. sinensis</i>	Tarocco 14	-0.0999	-4.48	0.00009	0.0167
<i>C. sinensis</i>	Valencia Campbell B	-0.0879	-3.94	0.00009	0.0167
<i>C. sinensis</i>	Moro13	-0.0867	-3.89	0.0001	0.0186
<i>C. sinensis</i>	Skaggs Bonanza SRA 202	-0.0854	-3.83	0.0001	0.0186
<i>C. sinensis</i>	Torregrosso	-0.0856	-3.84	0.0001	0.0186
<i>C. sinensis</i>	Vasconcellos	-0.0846	-3.80	0.0001	0.0186
<i>C. sinensis</i>	Moro 31	-0.0833	-3.74	0.0002	0.0372
<i>C. sinensis</i>	Peraipiguá	-0.0823	-3.69	0.0002	0.0372
<i>C. sinensis</i>	Valencia 1230	-0.0831	-3.73	0.0002	0.0372
Quadratic					
<i>C. sinensis</i>	Ouro	-0.0192	-4.11	0.00009	0.0167
<i>C. sinensis</i>	Valencia 1230	0.0181	3.87	0.0001	0.0186

^a Linear standard error = 0.023, quadratic standard error = 0.005; degrees of freedom = 5950. A negative estimate indicates a negative relationship with time (i.e., a decline in lesions of ACC) whereas a positive estimate indicates an increase in ACC.

ACC epidemic, due to reduced (or no, in the case of Florida) detection and eradication efforts. However, ACC management has been adopted since the 1980s in some regions of Argentina and southern Brazil (Paraná state). No immune genotypes of citrus are grown in these regions but less-susceptible cultivars are used together with wind-break barriers and frequent copper sprays to manage ACC. These management approaches reduce the incidence of ACC and, consequently, increase yield and decrease economic losses resulting from defoliation, fruit drop, and stem dieback. One of the most widely grown *C. sinensis* genotypes in Paraná state was evaluated in the present study: 'Iapar 73', which had 7.15 lesions per leaf. Other widely grown genotypes assessed included 'Valencia' (8.38 lesions per leaf), 'Pera IAC' (8.74 lesions per leaf), 'Natal' (8.75 lesions per leaf), and 'Hamlin' (9.63 lesions per leaf). Sweet orange 'Hamlin' is not permitted for cultivation in Paraná due to its susceptibility to ACC. Some sweet orange genotypes included in the present study have promising potential to be used for citrus production in areas where ACC is now considered endemic or spreading. Almost 40 Sweet orange genotypes had <8 lesions per leaf, similar to or less susceptible when compared with 'Iapar 73'. However, the agronomic and commercial characteristics of most of the citrus genotypes included in the present study have not been evaluated.

The first reports of resistance among Rutaceous germplasm to *X. citri* subsp. *citri* were almost 100 years ago (32,33,37,41,46). In some of these studies, genotypes belonging to the tribe Citreae were described as immune to *X. citri* subsp. *citri* but all *Citrus* spp. tested were found to be more or less susceptible. The most common citrus genotypes used in production in the Americas have been evaluated under field conditions by various authors (19,38,43,54) but all these studies were done before the appearance of CLM on the American continent. The presence of CLM increased the rate of the ACC epidemic and, although not previously demonstrated, the partial resistance shown by some genotypes could, at least in part, be lost. CLM can explain, in part, the increased susceptibility observed among *C. sinensis* cultivars commonly grown in Brazil and Florida, including 'Pera' and 'Valencia', although they were previously considered as less susceptible compared with other cultivars, including 'Hamlin'; however, in this study, they demonstrated similar susceptibility.

In addition to the susceptibility to *X. citri* subsp. *citri* of commercially important citrus genotypes being characterized, it is only recently that the mechanisms involved in resistance to ACC have begun to be elucidated. Apart from field resistance that might be lost using some screening approaches (19,24,38,54,55), McLean and Lee (41) hypothesized that structural characteristics of stomata could be related to the differences between grapefruit (*C. paradisi*) and mandarin (*C. reticulata*). However, Graham et al. (24) did not find differences between number, aperture, and density of stomata in citrus genotypes that could explain the differences in susceptibility to *X. citri* subsp. *citri*. The authors hypothesized that leaf characteristics such as cuticle development and waxes that influence the infiltration of water in leaves, and the greater frequency, size, and duration of growth flushes, are more important for resistance to *X. citri* subsp. *citri* than structure of stomata. More recently, results have suggested that some aspects of host resistance are due to induced defense mechanisms at the cellular and biochemical level (12,34) and, perhaps, differences in stomatal size and density with some genotypes (53). Although none of the 186 genotypes tested in the present study showed immunity to *X. citri* subsp. *citri*, all these mechanisms (field resistance and inducible and preexisting resistance mechanisms) are likely to exist among them.

During the 6 years of the experiment, no specific management for chemical control of CLM was applied and no copper sprays were used to manage *X. citri* subsp. *citri*. Application of chemicals to control both CLM and ACC would reduce the severity of ACC, which might adversely bias results. The relatively severe ACC observed for all genotypes in this study (from 1.86 to 14.40 lesions per leaf) is the true response in the absence of any chemical control for either CLM or ACC. Also, during the assessment, only symp-

tomatic leaves were sampled for the lesion counts. Genotypes ranked as less susceptible (those with ≤ 6 lesions per leaf) had an ACC incidence of 1.5 (i.e., 0 to 20% leaves with ACC; *data not shown*). The relationship between the incidence of leaves with ACC and the mean lesion count per infected leaf for the 186 genotypes had a linear relationship and, thus, were directly comparable. The major reason for choosing the count data is that counts are an accurate (9), continuous measure of the severity. Although linear-interval category scales of the type used in this study are a better basis for hypothesis testing compared with nonlinear scales (8,13,30,45), category scales with intervals >5% are less accurate and precise compared with the 0-to-100% scale (13,44) or lesion counts, both of which provide a continuous scale which has superior accuracy and, thus, is an excellent basis for comparing the severity of disease using parametric statistics. Nonlinear scales or scales based on nonlinear intervals of the 0 to 100% ratio scale are generally analyzed using nonparametric statistics.

Of the 186 different genotypes, only relatively few (35) showed an effect of time over the 6-year period of the experiment. All but one genotype ('Valencia 1230') had a negative relationship with time based on either the linear or quadratic time function. Most likely, the reduction in disease severity with time is due to the change in the tree size and structure as it matures. The proportion of susceptible foliage relative to the whole canopy becomes less in an older tree and, thus, spread of the disease might be hindered by distance; furthermore, because the plants in a mature orchard are tall, they can act as their own wind break, reducing an opportunity for splash dispersed spread of *X. citri* subsp. *citri* (8,22,23,48). The genotypes with a negative relationship between ACC symptoms included 21 *C. sinensis* genotypes and 8 other species or hybrids. No others showed a relationship between severity of ACC and time.

It is worth noting that there are some reports of variation in the reactions of genotypes to different strains of *X. citri* subsp. *citri* (15,35,47), although it has not been explored previously where CLM is a problem; the experiments reported compared genotypes in the presence of CLM. These data provide an assessment of susceptibility of different *Citrus* spp. and hybrids that can be used as a basis for further research, to aid varietal selection, and for a spectrum of breeding purposes within both Brazil and other tropical and subtropical locations where ACC is an issue, including citrus-growing regions throughout the American continent.

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