EXACERBATION OF CITRUS CANKER BY CITRUS LEAFMINER *PHYLLOCNISTIS CITRELLA* IN FLORIDA

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ABSTRACT

Citrus canker (caused by *Xanthomonas citri* subsp. *citri, Xcc*) is an important bacterial disease of citrus that is spread naturally by rain and wind. Feeding damage to citrus leaves by the citrus leafminer (CLM), *Phyllocnistis citrella* Stainton (Lepidoptera: Gracillariidae), has been shown to promote infection levels of citrus canker in a number of citrus-growing regions around the world. We conducted 2 studies to document that CLM damage exacerbates canker in Florida citrus. In 1 study, young citrus trees of 5 cultivars commonly grown in Florida were inoculated with a culture of *Xcc*. Two groups of trees were studied, 1 group with leaves damaged by CLM and 1 group that was treated with a pesticide to prevent CLM damage. Over all 5 cultivars, comparisons between the 2 groups of trees indicated that CLM damage resulted in 6-fold increase in the number of lesions. No difference was found between the 2 groups with respect to numbers of canker lesions on leaves without CLM damage. In the second study, a survey of commercial citrus groves was conducted to investigate incidence of canker on leaves with and without CLM injury. Low percentages of leaves infected by citrus canker were observed during the survey, with a maximum of 15% of leaves infected in 1 grove. However, during late Jul and Aug in some grapefruit and lemon groves, an average of 79% of leaves with canker had lesions directly associated with CLM damage, and an average of 36 more lesions per leaf was present on leaves with CLM damage. Exacerbation of canker by CLM during Jul and Aug coincided with the time of year when environmental conditions are usually optimal for canker in Florida and when population levels of CLM usually are most abundant. Citrus growers managing citrus canker should benefit from controlling CLM during the summer when conditions are favorable for canker infections, particularly in lemons and grapefruits.

Key Words: *Xanthomonas citri* subsp. *citri*, citrus leafminer, *Phyllocnistis citrella*

RESUMEN

El cancro de los cítricos (causado por *Xanthomonas citri* subsp. *citri, Xcc*) es una enfermedad bacterial importante de los cítricos que es esparcida naturalmente por la lluvia y el viento. Se ha mostrado que el daño a las hojas de cítricos hecho por la alimentación del minador de la hoja de los cítricos (MHC), *Phyllocnistis citrella* Stainton (Lepidoptera: Gracillariidae), promueve los niveles de infección del cancro de los cítricos en un numero de regiones alrededor del mundo donde siembran cítricos. Realizamos 2 estudios para documentar que el daño hecho por MHC agrava el cancro en los cítricos en la Florida. En el primer estudio, árboles jovenes de cítricos de 5 cultivares sembrados comúnmente en la Florida fueron inoculados con un cultivo de *Xcc*. Dos grupos de árboles fueron estudiados, 1 grupo con las hojas dañadas por MHC y 1 grupo que fue tratado con insecticida para prevenir daño hecho por MHC. En todos los 5 cultivares, la comparisión entre los 2 grupos de árboles indico que el daño hecho por MHC resulto en un aumento de 6 veces en el número de lesiones. No diferencia fue encontrada entre los 2 grupos con respecto al número de lesiones de cancro en las hojas no dañadas por MHC. En el segundo estudio, un muestreo de los huertos comerciales de cítricos fue realizado para investigar la incidencia de cancro en las hojas dañadas y no dañadas por MHC. Bajos porcentajes de hojas infectadas con cancro de los citricos fueron observados durante el muestreo, con un maximo de 15% de las hojas infectadas en 1 de los huertos. Sin embargo, durante la última parte de julio y agosto en algunos de los huertos de toronja y limón, un promedio de 79% de las hojas con cancro tenian lesiones directamente asociadas con daño hecho por MHC, y un promedio de 36 mas lesiones por hoja fueron presentes en hojas dañadas por MHC. El incremento de cancro por MHC durante julio y agosto coincidió con la epoca del año cuando las condiciones ambientales usualmente son óptimas para el cancro en la Florida y cuando los niveles de la población de MHC usualmente son mas abundantes. Los agricultores de cítricos que manejan el cancro de cítricos deberian beneficiarse con el control de MHC durante el verano cuando las condiciones son favorables para infecciones de cancro, particularmente en limones y toronjas.

Asiatic citrus canker is an important disease caused by the bacterium *Xanthomonas citri subsp. citri* (*Xcc*) (Hasse) Vauterin et al. (Gabriel et al. 1989). Trees infected by the bacterium develop lesions on leaves, stems and fruit, and severe infections result in defoliation, fruit drop, twig dieback, general tree decline, and badly blemished fruit (Gottwald et al. 2002a; Stall & Seymour 1983; Schoulties et al. 1987). The disease spreads naturally within infected trees, and from infected to non-infected trees, as a result of rain-splash of inoculum or wind-blown rain laden with inoculum (Gottwald et al. 1997, 2001, 2002b; Pruvost et al. 2002). The bacterium normally infects a leaf through penetration of leaf stomata. Healthy citrus leaves are most susceptible to infection by canker when they are young, i.e., flush stage leaves that are 50 to 75% expanded.

Damage by the Asian citrus leafminer (CLM), *Phyllocnistis citrella* Stainton (Lepidoptera: Gracillariidae), has been reported to exacerbate citrus canker (Gottwald et al. 1997) in Australia, Brazil, India, and Yemen (Chagas et al. 2001; Christiano et al. 2007; Cook 1988; Sinha et al. 1972; Sohi & Sandhu 1968). Adult females usually oviposit single eggs on the underside of newly developing leaves. After egg hatch, larvae enter a leaf and begin feeding, making serpentine mines beneath the leaf cuticle. CLM damage thereby exposes the mesophyll layer, and may provide easier access to and infection by *Xcc* than through the usual stomatal path. In Brazil, Jesus et al. (2006) reported that (1) injuries caused by CLM increased the incidence of canker, (2) the disease progressed more rapidly on leaves with CLM damage, (3) CLM injury increased the severity of the disease, and (4) temporal vulnerability of leaves to *Xcc* was increased by CLM damage. Although CLM damage increases the susceptibility of a citrus leaf to citrus canker, the insect itself is not an efficient vector of the disease (Belasque et al. 2005).

The objective of our research was to determine whether damage by CLM can exacerbate citrus canker under Florida conditions. This was accomplished by 2 experiments, one in which potted citrus trees with and without CLM injury were inoculated with *Xcc* and one in which the incidence of lesions/leaf of canker relative to damage by CLM was assessed in commercial citrus groves.

Methods and Materials

Inoculation Experiment

The insects for this study were obtained from a colony established during early 2009 at the USDA-ARS U. S. Horticultural Research Laboratory, Fort Pierce, FL. CLM have since been continuously reared in a greenhouse in BugDorm-2 cages (MegaView Science Education Services Co., Ltd., Taichung, Taiwan) containing young potted citrus trees (*Citrus macrophylla* L.).

Five different citrus cultivars with and without CLM injury were compared for canker incidence: 'Sunburst' mandarin (*Citrus reticulata* Blanco) × (*C. paradisi* Macf. × *C. reticulata*); two sweet orange cultivars (*C. sinensis* (L) Osbeck) varieties, 'Hamlin' and 'Midsweet'; 'Ruby Red' grapefruit (*C. paradisi* L.); and 'Murcott' tangor *C. reticulata* × *C. sinensis*) (8 plants of each cultivar). The experiment followed a completely randomized, 2 × 5 factorial design (factor A = leafminer injury present or absent, factor B = cultivar) with 4 replications per factor A-B combination. The 40 plants were randomly placed outdoors in a 3 by 6 m rack. Half of the plants of each cultivar were treated with imidacloprid, a systemic insecticide absorbed by the roots and transported throughout the plant (Admire Pro, Bayer CropScience, Monheim am Rhein, Germany) (1 mL per 3.8 L on 24 Jul 2009 as a soil drench) to prevent CLM infestations, and half of the plants were not treated with imidacloprid. The plants were trimmed to a uniform height of 51 cm and defoliated (leaving 3 leaves) to encourage new flush shoots. On 18 Aug 2009, a sleeve cage was placed over each tree not treated with imidacloprid, and 5 adult CLM from the lab colony were released into each cage and allowed to oviposit. The cages were removed 2 d later, and an additional 43 adult CLM were released among the 40 trees.

All 40 plants were inoculated on 28 Aug 2009 with a suspension of *Xcc* containing approximately 10^6 colony-forming units per milliliter (CFU/mL). A single batch of inoculum was used to inoculate all 40 plants. Inoculum was prepared from 5-day old colonies of *Xcc* cultured on petriplates on KCB nutrient agar (NA). KCB NA was amended with kasugamycin (16 mg L^{-1}), cephalexin (35 mg L^{-1}) and chlorothalanil (12 mg L^{-1} tetrachloroisophthalonitrile). An inoculum suspension of *Xcc* was prepared by washing plates with several mL of sterile distilled water and pooling the plate washes. The inoculum concentration was adjusted photometrically to 10^6 bacteria mL^{-1}. Accurate estimates of the viable, applied inoculum concentration were obtained by dilution plating of the prepared suspension onto nutrient agar. The resulting numbers of colony forming units (CFUs) were counted. The inoculum was applied to the foliage until run-off with a pressurized sprayer. In addition to artificial inoculations, natural inoculum may have contributed to infections during the course of the experiment because canker was abundant on trees in close proximity to the study area.

Approximately 6 weeks after inoculation (8 Oct 2009), each flush shoot (shoots with fully expanded, tender leaves) on each tree was excised, placed into a bag, and returned to the laboratory to count numbers of leaves with CLM injury, numbers of leaves with canker associated with CLM injury, and numbers of leaves with canker not associated with CLM injury. In addition, numbers of canker lesions associated with CLM mines and numbers not associated with mines were counted. The percentage of leaves infected by canker and mean number of canker lesions per leaf were calculated for each tree.

Field Survey in Commercial Groves

Commercial groves in east-central Florida were surveyed to assess whether citrus canker was exacerbated by CLM damage. An area of trees (4 to 8 ha) was sampled in each grove. Incidence of canker associated and not associated with CLM damage on leaves was assessed in multiple groves at different times of the year during 2008 and 2009 from May through Aug (1 grove was sampled during Oct 2008 and Nov 2009). A total of 14 different groves were surveyed including 2 in Highlands County, 3 in Martin County, 2 in Palm Beach County, and 7 in Saint Lucie County. Some groves were sampled once during 2008 and/or 2009 and some were sampled multiple times over the course of the study. All of the 14 groves surveyed contained mature trees, but the groves varied with respect to cultivar and tree management program although all of the groves were under minimal disease control programs. The intent of sampling was to estimate the incidence of citrus canker symptoms among leaves with and without CLM damage in each grove—not to compare groves or cultivars or to ascertain canker incidence levels that may be common in Florida citrus.

Trees in the groves surveyed were monitored for early stages of a flush. Six weeks after initiation of a flush, 25 flush shoots were excised (1 per tree) along a transect (row) and placed together in a bag. Four such transects were sampled, for a total of 200 flush shoots per grove per sample date. The bagged flush shoots were transported to the laboratory, and each individual leaf associated with a flush shoot was examined to enumerate leafmines, canker lesions associated with leafmines, and canker lesions not associated with leafmines. A dissecting microscope was used to locate and count canker lesions on the leaves. In cases of uncertainty that a lesion on a leaf was citrus canker, an immunological test was performed on the lesion to confirm its identity (*Xcc* ImmunoStrip Test, Agdia Inc., Elkhart, IN 46514) (Gottwald et al. 2009).

Percentages of leaves with CLM damage, canker, and canker associated with CLM damage were calculated for each transect sampled on each date in each grove. For leaves with canker, the following data were collected for each transect in each grove on each sample date: mean number of (1) CLM mines per leaf; (2) canker lesions per leaf; (3) lesions per leaf not associated with CLM (included lesion counts for leaves with and without CLM injury, in the former case lesions were some distance away from a CLM mine); and (4) lesions per leaf directly associated with CLM mines.

Statistical Analyses

Means and standard errors (SEM) were computed with PROC MEANS (SAS Institute 2008) for percentages of leaves infected by canker and numbers of canker lesions per leaf for each cultivar in the inoculation experiment and for each grove in the field survey. Analyses of variance were conducted with PROC GLM (SAS Institute 2008) on arcsine-transformed percentages and on log-transformed lesion counts. Significant differences among cultivars in the inoculation experiment were determined with the Ryan-Einot-Gabriel-Welsch Multiple Range Test (Einot & Gabriel 1975) option within PROC GLM, and between CLM treatments for each cultivar with a paired *t*-test, PROC TTEST (SAS Institute 2008). For the field survey, a paired *t*-test (PROC TTEST) was used to determine statistical differences in mean number of canker lesions per leaf among leaves with and without CLM damage. For each study, the quantitative relationship between mean numbers of canker lesions and CLM mines per leaf was investigated using linear regression, PROC GLM. All evaluations of statistical significance were conducted at $P = 0.05$.

Results and Discussion

Inoculation Experiment

Among the trees infested by adult CLM, 1 grapefruit and 2 'Midsweet' trees died before the end of the study due to root rot. All other trees including those not infested by adult CLM survived the duration of the experiment. A mean (SEM) of 4.9 (0.2) flush shoots per tree, and of 5.5 (0.1) leaves per flush shoot, was examined for canker in this experiment. Some leaves developed CLM injury in trees not intentionally infested by adult CLM, but only a mean (SEM) of 1.4 (1.2)% leaves per tree had leaf mines, few mines were observed on these leaves (Table 1), the mines were incomplete and abandoned (no live larvae), and there were no canker lesions associated with the mines. Not all leaves from the trees we infested with adult CLM developed larval infestations, a mean (SEM) of 29.3 (5.4)% leaves developed CLM injury in these trees (range among cultivars of from 17 to 46%). There was a mean (SEM) of 1.1 (0.2) mines per leaf over all leaves examined from trees we infested with adult CLM (Table 1).

TABLE 1. CANKER LESIONS PER LEAF ON DIFFERENT CITRUS CULTIVARS—LEAVES FROM TREES INFESTED BY ASIAN CITRUS LEAFMINER AND TREES PROTECTED FROM LEAFMINER INJURY.

	Mean (SEM) number of leaf mines per leaf per tree[a]		Mean (SEM) number of canker lesions per leaf per tree[b]	
Cultivar	Protected from leafminers	Infested by leafminers	Protected from leafminers	Infested by leafminers
Hamlin	0.0 (0.0) a	0.6 (0.5) a	1.2 (0.3) a B	4.4 (1.9) b A
Midsweet	0.0 (0.0) a	0.7 (0.1) a	3.5 (1.3) a B	14.9 (7.9) ab A
Murcott	0.1 (0.1) a	1.2 (0.4) a	0.9 (0.5) a B	7.5 (3.1) b A
Sunburst	0.0 (0.0) a	1.1 (0.3) a	2.7 (0.3) a B	9.2 (4.9) b A
Grapefruit	0.0 (0.0) a	2.1 (0.1) a	3.0 (0.9) a B	31.6 (4.2) a A

[a]For data on leaf mines, means in the same column followed by the same letter are not significantly different (P = 0.05), Ryan-Einot-Gabriel-Welsch Multiple Range Test.

[b]For data on canker lesions, means in the same column followed by the same lowercase letter are not significantly different (P = 0.05), Ryan-Einot-Gabriel-Welsch Multiple Range Test. Means in the same row followed by the same upper case letter are not significantly different (P = 0.05), paired t-test. Analyses on log-transformed data, raw data means presented.

There were significantly more leaves with canker on trees with CLM damage than on trees without CLM damage, but there were no significant differences among cultivars (Table 2). However, both CLM injury and cultivar had a significant effect on the incidence of canker (number of lesions per leaf). No significant interaction was found between the main model effects in the analysis of variance on canker incidence (Table 2). A grand mean of 12.3 ± 2.8 lesions per leaf per tree was observed among trees infested by CLM compared to 2.3 ± 0.4 lesions per leaf per tree among trees protected from CLM (Table 1). There were no significant differences among the 5 cultivars in numbers of *Xcc* lesions on leaves from trees protected from CLM, but significantly greater numbers of lesions per leaf were present in grapefruit trees with CLM damage compared to 'Murcott', 'Hamlin' or 'Sunburst' trees with CLM damage. For each cultivar, significantly more canker lesions were present on leaves with CLM damage. On leaves that developed canker in the trees infested by CLM, over all cultivars a mean (SEM) of 17.6 (3.2) lesions per leaf per tree was observed on leaves with CLM injury compared to 4.4 (0.4) lesions on leaves without injury. Total number of canker lesions per leaf per tree increased linearly with the number of mines per leaf per tree (Fig. 1). Regression slopes (SEM) for the individual cultivars were 13.7 (2.0) for grapefruit (r^2 = 0.90); 4.2 (0.5) for 'Hamlin' (r^2 = 0.93); 7.8 (0.7) for 'Murcott' (r^2 = 0.95); and 9.9 (2.3) for 'Sunburst' (r^2

TABLE 2. ANALYSES OF VARIANCE ON PERCENTAGES OF CANKER-INFECTED LEAVES AND NUMBERS OF CANKER LESIONS PER LEAF ON LEAVES FROM TREES PROTECTED AND NOT PROTECTED FROM INJURY BY ASIAN CITRUS LEAFMINER (CLM).

Source	*df*	Mean Square	*F* Value	Pr > *F*
		Percent leaves with canker associated with CLM injury[a]		
Leafminer injury	1	5,630	34.1	<0.0001
Cultivar	4	106	0.7	0.63
Interaction	4	106	0.7	0.63
		Mean number of canker lesions per leaf per tree[b]		
Leafminer injury	1	6.10	30.4	<0.0001
Cultivar	4	1.41	7.0	0.0005
Interaction	4	0.50	2.5	0.07
		Mean number of canker lesions per leaf on leaves without CLM injury[b]		
Insecticide	1	0.02	0.03	0.86
Cultivar	4	1.76	3.61	0.01
Interaction	4	1.55	3.19	0.01

[a]Analyses on arcsine-transformed percentages.

[b]Analyses on log-transformed counts.

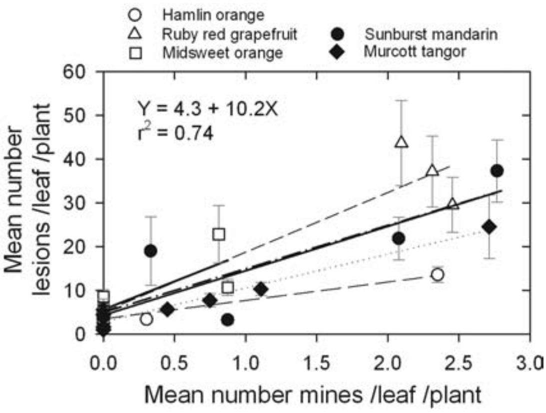

Fig. 1. Quantitative relationship between number of citrus leafminer mines per leaf and number of citrus canker lesions per leaf per plant (F = 97.8, Pr > F = <0.0001, 36 *df*) (vertical error margins represent standard error of the mean number of lesions per leaf per plant). The data are from young potted plants artificially inoculated with *Xanthomonas citri* subsp. *citri* including a set of plants with leafminer damage and a set protected from leafminer damage.

= 0.76) (details of regression analyses not presented). Lack of data for 'Midsweet' precluded declaring a significant relationship. Over all 5 cultivars, the presence of CLM damage caused a 6-fold increase in the number of canker lesions on leaves compared to numbers of lesions on leaves in trees protected from CLM, and more than a 10-fold increase occurred in grapefruit trees (Table 1). These results were similar to those documented in Brazil where, following inoculation with *Xcc*, canker was up to 5 times more severe on leaves with leafminer damage as compared to intact leaves (Christiano et al. 2007).

'Ruby Red' grapefruit is considered highly susceptible to canker while 'Hamlin' oranges are regarded as susceptible; 'Midsweet' oranges, 'Murcott' tangors and 'Sunburst' mandarin are each considered less susceptible to citrus canker (Gottwald et al. 2002; Schubert et al. 2001). Differences among the 5 cultivars were not found in this study with respect to percentages of leaves infected by canker or, in the absence of CLM injury, numbers of lesions per leaf. It was probable that the high inoculation rate used in conjunction with high levels of inoculum in the test area masked the differences among the cultivars in their susceptibility to citrus canker. Although grapefruit is considered more susceptible to canker than 'Midsweet' orange, in this study canker severity among leaves damaged by CLM was similar for these cultivars. CLM damage therefore may increase the susceptibility of a cultivar to citrus canker.

Large numbers of leaves were examined during this experiment that were infected by *Xcc* but that had no CLM injury, over all 5 cultivars, 370 leaves from trees treated with imidacloprid and 160 leaves from trees not treated with the pesticide. Among these leaves, means (SEM) of 4.7 (0.3) and 4.4 (0.4) canker lesions per leaf were observed on leaves from trees treated and not treated with pesticide, respectively. There was no significant difference between these means, indicating that imidacloprid did not influence incidence of canker (Table 2).

Field Survey in Commercial Groves in 2008 and 2009

Appreciable percentages of leaves damaged by CLM were observed in most of the groves surveyed during the months of Jul and Aug, with many averaging more than 45% leaves damaged (Table 3). Over the duration of the study, relatively low percentages of leaves infected by *Xcc* were observed in the groves surveyed. The highest percentage was 15% leaves infected in a lemon grove sampled during 2009. Relatively low percentages of leaves with both CLM and canker reflected the overall low percentages of leaves with canker. However, among leaves with canker, canker was found to be in direct association with CLM damage for a majority of the leaves in lemon and grapefruit groves surveyed during late Jul and Aug (up to 100% in one grove surveyed during Aug 2009) (Table 3). CLM damage promoted little or no increase in percentages of canker-infected leaves during late Jul and Aug in the groves of 'Murcott' or orange trees, or in any of the groves sampled at other times of the year.

Significantly greater numbers of canker lesions were found on leaves with CLM injury in grapefruit and lemon groves during late Jul and Aug (Table 4). However, there were no significant differences between numbers of lesions between leaves with and without CLM damage in 2 groves of sweet orange trees sampled during this time period. Over all samples of canker-infected leaves, mean numbers of canker lesions per leaf per grove increased linearly with mean numbers of CLM mines per leaf per grove (Fig. 2, regression based on data in Table 4). Three of 21 data points were excluded from this analysis because they fell well outside the general data trend. All 3 were associated grapefruit trees. For 2 of these groves (groves 'one' sampled 1 May 2008 and 'three' sampled 21 Jul 2008), few leaves were infected by canker (for grove 'one'- 23 of 1,343 leaves; for grove 'three' - 4 of 1,396 leaves) and little CLM damage was present (none on the canker-infected leaves). However, on the few leaves that were infected by canker, relatively large numbers of lesions were present (more than 50 lesions per leaf on a few leaves), thus inflating the magnitude of the mean number of lesions. For the

TABLE 3. INCIDENCE OF CITRUS CANKER AND DAMAGE BY ASIAN CITRUS LEAFMINER (CLM) IN COMMERCIAL CITRUS GROVES SURVEYED DURING 2008 AND 2009.

Grove	Citrus cultivar[a]	Sample date[b]	Mean (SEM) percent leaves with CLM	Mean (SEM) percent leaves with canker	Mean (SEM) percent leaves with canker associated with CLM	Mean (SEM) percent canker-infected leaves with CLM damage
			2008			
One	GF	1 May	3.9 (0.6)	1.2 (1.0)	0.1 (0.1)	33.3 (33.3)
Two	OR	27 Jun	23.1 (2.2)	0.0 (0.0)	0.0 (0.0)	—
Three	GF	21 Jul	18.4 (2.2)	0.2 (0.1)	0.0 (0.0)	0.0 (0.0)
Four	MIN	21 Jul	0.8 (0.2)	0.0 (0.0)	0.0 (0.0)	—
Five	GF	21 Jul	63.8 (1.4)	0.1 (0.1)	0.0 (0.0)	0.0 (0.0)
Six	OR	21 Jul	47.3 (3.4)	0.0 (0.0)	0.0 (0.0)	—
Seven	LEM	29 Jul	49.3 (4.2)	0.9 (0.3)	0.5 (0.3)	45.2 (24.9)
One	GF	7 Aug	58.1 (5.3)	2.1 (1.7)	1.7 (1.4)	83.1 (0.2)
Eight	GF	12 Aug	60.0 (2.2)	1.8 (0.6)	1.7 (0.6)	88.6 (6.2)
One	GF	7 Oct	12.2 (1.9)	4.6 (3.1)	1.4 (1.2)	22.3 (9.3)
			2009			
Two	OR	5 May	0.4 (0.2)	0.4 (0.3)	0.1 (0.1)	7.1 (7.1)
Seven	LEM	12 May	3.2 (1.5)	2.6 (1.4)	0.0 (0.0)	0.0 (0.0)
Nine	GF	20 May	5.3 (0.6)	6.4 (1.7)	0.0 (0.0)	0.0 (0.0)
Ten	GF	26 May	1.2 (0.8)	0.7 (0.6)	0.0 (0.0)	0.0 (0.0)
Eleven	OR	3 Jun	11.5 (1.7)	0.1 (0.1)	0.0 (0.0)	0.0 (0.0)
Eight	GF	10 Jun	4.1 (0.7)	0.1 (0.1)	0.0 (0.0)	0.0 (0.0)
Twelve	MUR	26 Jun	5.9 (1.2)	0.1 (0.1)	0.0 (0.0)	0.0 (0.0)
Five	GF	26 Jun	9.7 (3.2)	0.1 (0.1)	0.0 (0.0)	0.0 (0.0)
Nine	GF	3 Aug	32.5 (2.6)	0.6 (0.3)	0.5 (0.3)	92.9 (7.1)
Eleven	OR	3 Aug	27.0 (2.6)	10.0 (2.4)	3.9 (1.2)	39.9 (7.4)
Seven	LEM	6 Aug	58.1 (4.8)	15.4 (0.5)	14.8 (0.7)	96.0 (1.6)
Two	OR	6 Aug	37.5 (2.8)	0.3 (0.1)	0.0 (0.0)	0.0 (0.0)
Nine	GF	12 Aug	57.7 (1.5)	0.9 (0.9)	0.9 (0.9)	100.0 (—)
Five	GF	19 Aug	48.8 (2.8)	0.6 (0.5)	0.6 (0.6)	50.0 (50.0)
Twelve	MUR	19 Aug	53.5 (2.3)	0.0 (0.0)	0.0 (0.0)	—
One	GF	2 Nov	48.4 (3.1)	0.0 (0.0)	0.0 (0.0)	—

[a]Citrus cultivar: GF = grapefruit, OR = sweet orange, MIN = 'Mineola' tangerine, MUR = 'Murcott' tangor, LEM = lemon.
[b]Flush was approximately 6 weeks old when the samples were taken.

TABLE 4. INCIDENCE OF CITRUS CANKER ON LEAVES WITH AND WITHOUT DAMAGE BY CITRUS LEAFMINER (CLM) IN COMMERCIAL CITRUS GROVES SURVEYED DURING 2008 AND 2009. DATA EXCLUSIVELY FOR LEAVES WITH CANKER.

Grove	Citrus cultivar[a]	Date	Mean number of CLM mines per leaf	Mean number canker lesions per leaf	Mean number canker lesions per leaf not associated with CLM[b]	Mean number canker lesions per leaf associated with CLM[b]
				2008		
One[c]	GF	1 May	0.1 (0.1)	15.7 (4.2)	15.7 (4.2) a	0.1 (0.1) b
Five	GF	21 Jul	0.0 (—)	1.0 (—)	1.0 (—)	0.0 (—)
Three[c]	GF	21 Jul	0.3 (0.3)	24.8 (12.6)	24.8 (12.6) a	0.0 (0.0) b
Seven	LEM	29 Jul	1.6 (0.3)	12.5 (3.8)	0.9 (0.4) b	11.5 (3.9) a
One	GF	7 Aug	2.4 (0.2)	12.9 (2.8)	1.1 (0.7) b	11.8 (2.9) a
Two	GF	12 Aug	2.0 (0.1)	6.3 (0.8)	0.1 (0.1) b	6.2 (0.8) a
One	GF	7 Oct	0.4 (0.1)	7.0 (1.2)	3.5 (0.5) a	3.5 (1.1) a
				2009		
Two	OR	5 May	0.1 (0.1)	1.3 (0.2)	1.1 (0.2) a	0.1 (0.1) b
Seven	LEM	12 May	0.0 (0.0)	2.1 (0.2)	2.1 (0.2) a	0.0 (0.0) b
Nine	GF	20 May	0.0 (0.0)	3.3 (0.3)	3.3 (0.3)	0.0 (0.0) b
Ten	GF	26 May	0.0 (0.0)	3.7 (1.1)	3.7 (1.1) a	0.0 (0.0) b
Eleven	OR	3 Jun	0.5 (0.5)	1.0 (0.0)	1.0 (0.0) a	0.0 (0.0) b
Eight	GF	10 Jun	0.0 (0.0)	3.0 (2.0)	3.0 (2.0) a	0.0 (0.0) b
Twelve	MUR	26 Jun	0.0 (0.0)	1.0 (0.0)	1.0 (0.0) a	0.0 (0.0) b
Five[c]	GF	26 Jun	2.0 (—)	1.0 (—)	1.0 (—)	0.0 (—)
Nine	GF	3 Aug	1.2 (0.2)	4.8 (1.3)	0.1 (0.1) b	4.7 (1.3) a
Eleven	OR	3 Aug	0.5 (0.1)	3.2 (0.2)	1.5 (0.2) a	1.7 (0.2) a
Seven	LEM	6 Aug	2.3 (0.1)	13.0 (0.8)	0.4 (0.1) b	12.6 (0.8) a
Two	OR	6 Aug	0.4 (0.2)	1.0 (0.0)	1.0 (0.0) a	0.0 (0.0) b
Nine	GF	12 Aug	2.7 (0.2)	8.6 (2.1)	0.0 (0.0) b	8.6 (0.0) a
Five	GF	19 Aug	2.4 (0.3)	11.3 (3.0)	0.2 (0.2) b	11.1 (3.1) a

[a]Citrus cultivar: GF = grapefruit, OR = sweet orange, MIN = 'Mineola' tangerine, MUR = 'Murcott' tangor, LEM = lemon.
[b]In the last 2 columns to the right, for each year pairs of means in the same row followed by the same letter are not significantly different ($P = 0.5$), paired t-test (for pairs of means not followed by a letter, an insufficient number of leaves were available for analysis). Analyses on log-transformed data, raw means and standard errors presented. [c]Considered outliers in the regression for Fig. 2.

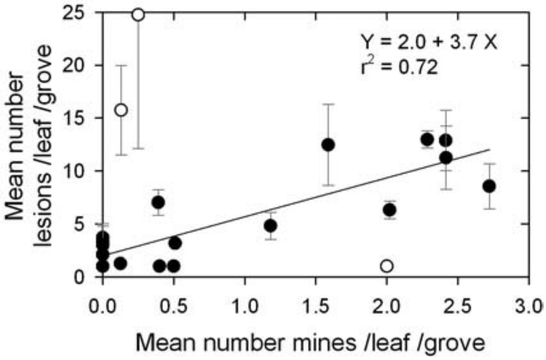

Fig. 2. Quantitative relationship between mean number of citrus leafminer mines per leaf and mean number of citrus canker lesions per leaf (F = 41.8, Pr > F = <0.0001, 17 *df*) (vertical error margins represent standard error of the mean number of lesions per leaf per block). The data were from commercial citrus groves sampled during 2008 and 2009. The 3 white data points were considered outliers and excluded from the analysis.

third grove (grove '5' sampled 26 Jun 2009), a large number of leaves were damaged by CLM but only 1 of 1,443 leaves examined had canker lesions. Environmental conditions may have been unfavorable for canker during Jun 2009, although by Aug appreciable levels of canker were observed in this grove.

With respect to temporal aspects of citrus canker associated with leafminer injury, the results of the study indicated that damage by the citrus leafminer exacerbates citrus canker during periods when environmental conditions are conducive to dispersal of the pathogen in Florida, which is generally during the rainy season from mid to late summer (Gottwald et al. 2002a). Coincidentally, this is the time of year that citrus leafminer populations are usually most abundant in Florida (Lapointe & Leal 2007). Damage by CLM may occur at other times of the year (Table 3) with little or no influence on incidence and severity of citrus canker in Florida.

Our study documents that damage by the citrus leafminer can result in an increase in the incidence and severity of citrus canker in Florida citrus. Compared to reports from other countries documenting increases in citrus canker associated with leafminer injury (e.g., Sohi & Sandu 1968; Venkateswarlu & Ramapandu 1992), the incidence of canker in the Florida groves we surveyed was relatively low. A higher incidence in Florida of canker infection might occur during summers when conditions are more conducive for *Xcc* than during the 2 seasons of our study. Although incidence of canker in the Florida groves we surveyed was relatively low, increases in numbers of lesions on infected leaves with leafminer damage were sometimes much larger than reported in India (Sohi & Sandu 1968; Venkateswarlu & Ramapandu 1992) and Brazil (Christiano et al. 2007). These data suggest that reducing both leafminer populations and sources of *Xcc* inoculum through integrated pest management, particularly during the summer months, would help reduce the incidence of the citrus canker epidemic in Florida.

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