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ORIGINAL RESEARCH

Wind speed and wind-associated leaf injury affect severity of citrus canker on Swingle citrumelo

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Abstract Citrus canker (caused by the bacterial pathogen *Xanthomonas citri* subsp. *citri*, *Xcc*) can cause severe damage to citrus. It is endemic in Florida, and occurs in other citrus growing regions. The bacterium is dispersed predominantly in rain splash. To simulate dispersal in splash, and to investigate the effect of wind speed on infection, young plants of Swingle citrumelo were exposed to sprayed inoculum at different wind speeds. Wind was generated using an axial fan, and a pressurized sprayer delivered the inoculum spray. In the five experiments, higher wind speeds (>10 m s^{-1}) consistently resulted in higher incidence and severity of citrus canker developing. By 15 ms^{-1}, there was a dramatic increase in disease. Visible injury to leaves of Swingle citrumelo due to wind was evident at wind speeds ≥ 13 m s^{-1}. The relationship between wind speed and disease, and wind speed and injury was described by a logistic model. More disease was associated with visible injury as the wind speed increased, and disease not associated with visible injury also increased with wind speed. The petiole-leaflet junction was more often infected at higher wind speeds (≥17 m s^{-1}). The concentration of the *Xcc* inoculum increased the incidence and severity of citrus canker in all experiments. Reducing wind speed in citrus groves with the aid of wind breaks may contribute to a reduction in the severity of an epidemic by reducing dispersal and infection events.

Keywords Epidemiology · Dispersal · Bacteria · Crop loss · Integrated disease management

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Introduction

Citrus canker, caused by the bacterial pathogen (*Xanthomonas citri* subsp. *citri*, *Xcc*) is a serious disease of several citrus species and occurs in many

tropical and sub-tropical citrus producing regions around the world (Schubert et al. 2001), and recently has become endemic in Florida. Infection with citrus canker can directly reduce yield, but also causes blemishes on fruit making them unfit for the fresh trade (Gottwald et al. 2009). The pathogen is spread in rain splash (Danos et al. 1984; Gottwald et al. 1988, 1992a, b; Pruvost et al. 1999, 2002). Higher wind speeds enhance the potential spread (Bock et al. 2005, 2010a, b), and infection resulting from a 50 km dispersal event has been documented (Irey et al. 2006; Gottwald and Irey 2007).

The epidemic of citrus canker continues to expand in Florida. In the mid-1990s the disease was established in a small area of SE Florida (Schubert et al. 2001). It has subsequently become widespread, particularly on account of major storms that crossed the state in 2004 (Hurricanes Charlie, Francis, Ivan and Jeanne), 2005 (Hurricane Wilma) and again in 2008 (Tropical Storm Fay) (Irey et al. 2006; Gottwald and Irey 2007). Numerous rainstorms at local scales contribute to spread of the pathogen in rain splash and in droplets spread by wind over greater distances (Pruvost et al. 2002; Gottwald et al. 2002; Irey et al. 2006; Gottwald and Irey 2007). Bacteria in droplets dispersed by wind in single storm events has lead to disease on susceptible citrus up to 579 m from sources of inoculum (Gottwald et al. 2002), and studies on the effect of wind speed dispersing inoculum in rain splash from diseased trees confirmed a many-fold increase in the quantity of *Xcc* in the splash downwind of infected trees at higher wind speeds (Serizawa et al. 1969; Serizawa 1981; Bock et al. 2005, 2010a, b). The greatest quantity of *Xcc* dispersed occurs in the first few minutes of leaf wetting (Bock et al. 2005), often when the wind speed in severe rainstorms is highest.

Once the rain splash containing inoculum impinges on the leaf surface, various studies have ascertained the role of different factors in supporting infection of citrus leaves with *Xcc*. Temperature and leaf wetness appear to play a role in calm conditions (Christiano et al. 2009), and leaf age has a profound effect, with young, undamaged leaves being particularly susceptible to infection directly through stomata (Lee 1922; Stall et al. 1982; Gottwald and Graham 1992). Apart from entry through stomata, injury due to leaf miner, or mechanical injury from management activities or high wind can also provide a portal for infection (Jesus et al. 2006; Christiano et al. 2007; Gottwald et al. 2007). The amount of injury caused by wind has not been quantified but was noted in respect to infection with citrus canker (Serizawa and Inoue 1974; Serizawa 1981). Past work has demonstrated how rain splash results in dispersal and infection of undamaged leaves by the pathogen (Graham et al. 1992; Pruvost et al. 2002)). Indeed, very few bacteria are needed to cause infection through stomata (Gottwald and Graham 1992). Several studies confirm that splash when combined with increased wind speed promotes infection of grapefruit leaves (*Citrus paradisi*, Bock et al. 2006) and Natsudaidai (*Citrus natsudaidai*, Serizawa et al. 1969; Serizawa and Inoue 1974; Serizawa 1981). Disease on grapefruit leaves was increased at wind speeds of 6.5 and 8.5 m s^{-1} compared to calm conditions or wind at 5 m s^{-1}; and more disease developed at greater concentrations of inoculum (Bock et al. 2006). However, the relative quantity of infection over a range of wind speeds typical of Florida thunderstorm conditions has not been ascertained. Furthermore, the relationship between wind speed and subsequent symptom development remains uncharacterized, and the effects of wind-related injury on disease have not been quantified.

Although rain with moderately calm conditions is commonplace in Florida, thunderstorms during the summer are also frequent, often associated with wind speeds >10 m s^{-1}. Furthermore, tropical storms and hurricanes impact the Florida citrus industry, and invariably have wind speeds greater than 20 m s^{-1}, and are of interest in view of the recent storms criss-crossing the Florida peninsula and spreading *Xcc* (Irey et al. 2006; Gottwald and Irey 2007). Knowing the effect of wind speed can be valuable in aiding plans for optimizing citrus plantings and windbreaks (Gottwald and Timmer 1995; Behlau et al. 2008). Windbreaks can lower wind speeds in the grove and reduce dispersal (Bock et al. 2010a, b), stomatal infection, and tissue injury that provides an additional portal for *Xcc* to infect. The objectives of this study were 1) to quantify increases in disease due to increased wind speed, and 2) to characterize the relationship between wind speed and the subsequent development of disease in citrus, 3) to ascertain the effect of inoculum quantity, and 4) to establish the relationships between wind speed, wind-induced injury, and disease, under simulated wind-blown rain conditions.

Materials and methods

Plants

One to two year-old Swingle citrumelo (*Poncirus trifoliata* X *Citrus paradisi*) plants grown from seed in containers were used to assess effects of wind speed and concentration on infection, injury and subsequent disease development. Plants were chosen for uniformity in size (35–45 cm tall, 20–30 leaves per plant) and growth stage, with the basal leaves of the new flush approximately three-quarter expanded. They were maintained in the greenhouse under disease-free conditions until being transported to a contained USDA field site in Broward Co., Florida.

Inoculum and inoculation

Inoculum was prepared from 4-day old colonies of *Xcc* cultured on petri-plates on KCB nutrient agar (NA). KCB agar is 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 approximated photometrically, and accurate concentrations of the viable, applied inoculum were obtained by dilution plating of the prepared suspension onto nutrient agar. The resulting numbers of colony forming units (CFUs) were counted, and subsequently the applied quantity of CFU ml^{-1} calculated. Depending on the experiment, the suspension was diluted to 0, 10^3, 10^4, 10^5 and 10^6 bacteria ml^{-1}. The inoculum was applied using a pressurized sprayer (30 psi) through a fixed wand with three full cone nozzles (D-4 nozzles, Spraying Systems, Co., Wheaton, IL) positioned 50 cm from the plant, and directed towards the foliage. Each inoculation treatment was applied for 2 min. Following inoculation, plants were maintained in a greenhouse at 27°C for 28 days, then assessed for disease and injury.

Wind generation

Wind was generated using one of two different axial fans, and the wind speed was measured by anemometers. In the first three experiments (14 Feb, 14 Jun and 12 Oct 2006) an electric fan (Model AM22 2HP, Air Max Fans, Florence, SC) was used and produced wind speeds up to approx. 17 m s^{-1} at 1 m distance. Wind speed was adjusted by placing the fan at different distances from the plants. For the experiment on 14 Feb the fan was positioned at 0 (no wind), 1, 2, 3, 4 and 5 m; on 14 Jun 0 (no wind), 1, 2 and 5 m; and on 12 Oct at 0 (no wind), 1, 1.5, 2 and 5 m. In the remaining two experiments (17 Oct and 13 Dec 2006), the fan was powered by an airboat motor (a 350 cubic inch V-8 Chevrolet engine, American Airboats Corp., Orange, TX) on a trailer frame and generated winds with velocities of 0 to >27 m s^{-1} at 2 m distance. With the airboat, wind speed was altered by adjusting the motor rpm. It was operated at 0 (no wind), 1000, 2000, 3000 and 4000 rpm.

Experiment design

In all five experiments, the plants were placed in secured container racks at set distances in front of the air-max fan and positioned to be in the main air flow. The airboat motor did not have to be moved as wind speed was adjusted using the throttle. The sprayer nozzle wand was set on a stand and the plume adjusted to spray onto the foliage. In four of the experiments a factorial design was used (wind speed x concentration). The remaining experiment was a single factor experiment with wind speed only. There were three replicate plants randomly assigned to each wind speed x treatment combination, or to each wind speed in the single factor experiment. The individual treatments were applied arbitrarily to each of the plants in sequence.

Disease assessment and injury rating

All leaves were photographed using a 7-megapixel camera (Canon EOS 10D, Canon USA, Inc., Lake Success, NY) or a flatbed scanner (Epson 1600, Epson America, Inc., Long Beach, CA). Disease severity was subsequently estimated visually from the images on a leaf-by-leaf basis from each plant, counting the number of lesions on each leaf, and estimating the percent area covered by symptoms of citrus canker. The number of canker lesions associated with injury was also enumerated. Also, the number of leaves that developed canker at the leaflet-petiole junction was counted on each plant—infection of the leaflet base leads to premature abscission of the

leaflets. The injury inflicted was diverse and included puncture wounds to the lamina from the axillary thorns, scratches on the leaf surface, tears, and loss of sections of the leaf due to the lamina tearing. To investigate disease associated with injury, an ordinal scale was developed to account for the diverse damage due to wind:

0 = no damage
1 = one superficial scratch
2 = several superficial scratches, and/or some minor leaf penetration and punctures
3 = superficial to major scratches, some leaf penetration (punctures) and minor tears in lamina
4 = superficial scratches, punctures, scratches penetrate through leaf and leaf torn
5 = superficial scratches, punctures, scratches penetrate through leaf, leaf severely torn, with pieces of leaf missing

Meteorological measurements

To take into account different conditions between experiments, actual wind speeds were recorded 20 cm upwind of the canopy. The drop in wind speed generated by these fans over 20 cm is negligible. Wind speed and temperature were monitored using Campbell Scientific weather monitoring equipment (Campbell Scientific, Inc., Logan, UT). Young anemometers (Model 03101-L, R.M Young Company, Traverse City, MI) were used to measure wind speed, and Model 107 temperature probes were used to measure the temperature in the plant canopy. Data were logged to a Campbell Scientific Inc. CR23X datalogger, and wind speed and temperature measurements recorded every 5 s, from which two-minute means and standard deviations were calculated.

Data analysis

All data were analyzed with SAS V9.2 (SAS Systems, Cary, NC). All four factorial experiments were initially analyzed individually using GLM to ascertain main effects and interactions of wind speed and concentration on the incidence and severity of citrus canker symptoms that developed on the leaves, the amount of injury caused, and the disease associated with that injury. A one-way analysis was used to analyze the single-factor experiment on 17 Oct. *F*-values and *P*-values were used to ascertain whether wind speed or concentration of inoculum had a significant effect on the variable. A means separation (P=0.05) of these data was done using Tukeys HSD test. To investigate the relationships between wind speed and incidence and severity of citrus canker, and injury, and the relationship between injury and disease severity a logistic regression model $y = c/1 + Ae^{-Bx}$, where a and b are related to the rate of change and point of inflection of the curve, and c = the upper asymptote, and e is the exponent) was fitted to these data from each date, and to the combined data from all five experiments. The goodness-of-fit for the models was based on the analysis *F*- and *P*-values, a significant *P*-value indicating a good fit. The coefficient of variation (R^2), which provides a measure of how much of the variability in Y is explained by X, was also assessed as a measure of model adequacy. To explore the relationship between severity of injury, disease incidence or severity, and wind speed, a 3-dimensional surface model was fit using Table Curve 3D (Systat Software, Inc., San Jose, CA).

Results

Overview

The highest mean wind speed, measured at 0.5 m from the foliage surface in each experiment on 14 Feb, 14 Jun, 12 Oct, 17 Oct and 13 Dec were 13.6, 15.7, 16.8, 20.9 and 27.7 m s^{-1}, respectively. Highest wind speeds in the first three experiments were lower compared to the experiments on 17 Nov and 13 Dec (Table 1). Various factors affected wind speed including the type of fan used, ambient weather conditions, and exact position of the plants relative to the fan. The disease incidence (percent leaves infected) and severity (number of lesions per infected leaf) in the four factorial experiments (14 Feb, 14 Jun, 12 Oct and 13 Sep) are shown in Figs. 1a–d and 2a–d, respectively. The incidence and severity in the single-factor (wind speed only) experiment on 17 Nov are shown in Fig. 3a and b.

Effect of wind speed on disease and leaf damage

Percent leaves infected due to the main effect of wind speed increased significantly (Table 2). The number of lesions per infected leaf, and the percent infected

Table 1 The concentrations of *Xanthomonas citri* subsp. *citri* inoculum and mean wind speed for each experiment[a] testing the effect of wind speed and inoculum concentration on subsequent disease development

Date (2006)	Inoculum concentration (CFU ml^{-1})	Wind speed, m s^{-1}, (standard deviation of mean wind speed)
14 Feb	$7\times10^{3,4,5}$	0.5 (0.4), 2.6 (2.1), 4.5 (1.7), 5.5 (0.8), 7.7 (0.7), 13.6 (1.1)
14 Jun	$7\times10^{3,4,5,6}$	0.3 (0.3), 4.5 (0.5), 6.1 (1.0), 15.7 (2.3)
12 Oct	$5\times10^{3,4,5}$, 8×10^{5}, 1×10^{6}	0.1 (0.2), 2.8 (1.7), 4.2 (1.0), 5.9 (0.8), 8.9 (0.6), 16.8 (0.6)
17 Oct	8×10^{4}	0.9 (0.4), 6.2 (1.1), 11.3 (0.6), 16.2 (1.5), 20.9 (1.7)
13 Dec	$8\times10^{3,4,5}$	1.5 (0.5), 6.1 (0.7), 13.0 (0.6), 20.7 (1.5), 27.7 (1.7)

[a] In all experiments duration of wind exposure was 2 min

petioles also increase on all dates except the experiments on 14 Feb and 14 Jun.

The incidence of leaves injured increased significantly with wind speed, except on 14 Jun and 12 Oct, and the severity of the injury on injured leaves increased on all dates except on 14 Feb and 12 Oct. A very few leaves on some plants had injury in calm conditions or at very low wind speeds—presumably from transport or handling. Injury was consistently most severe in those two experiments receiving the highest wind speeds (17 Oct and 12 Dec, wind speeds >20 m s^{-1}). The percent of lesions associated with

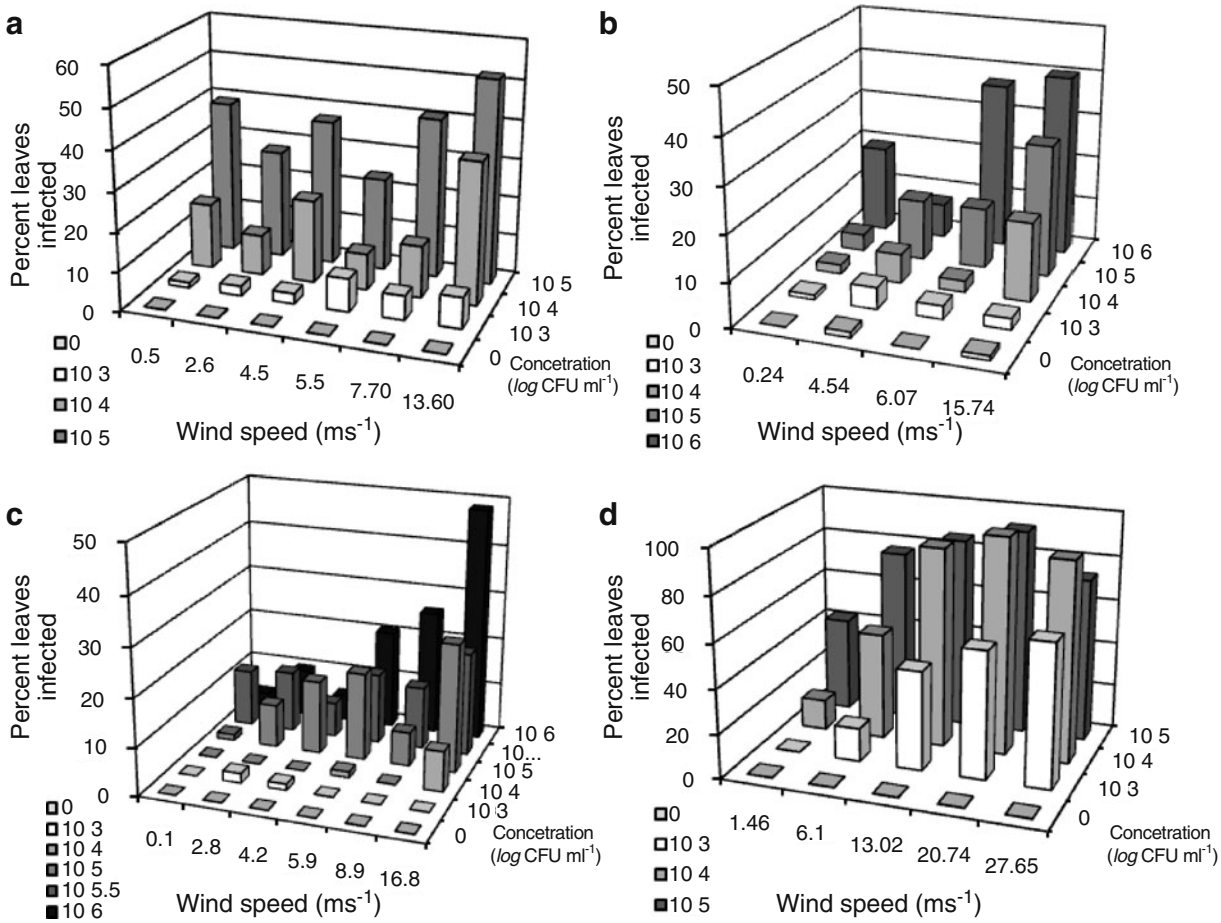

Fig. 1 The effect of wind speed and concentration of inoculum of *Xanthomonas citri* subsp. *citri* on the incidence of symptoms of citrus canker (percent leaves infected) on leaves of Swingle citrumelo in four experiments on **a.** 14 Feb, **b.** 14 Jun, **c.** 12 Oct, and **d.** 13 Dec 2006

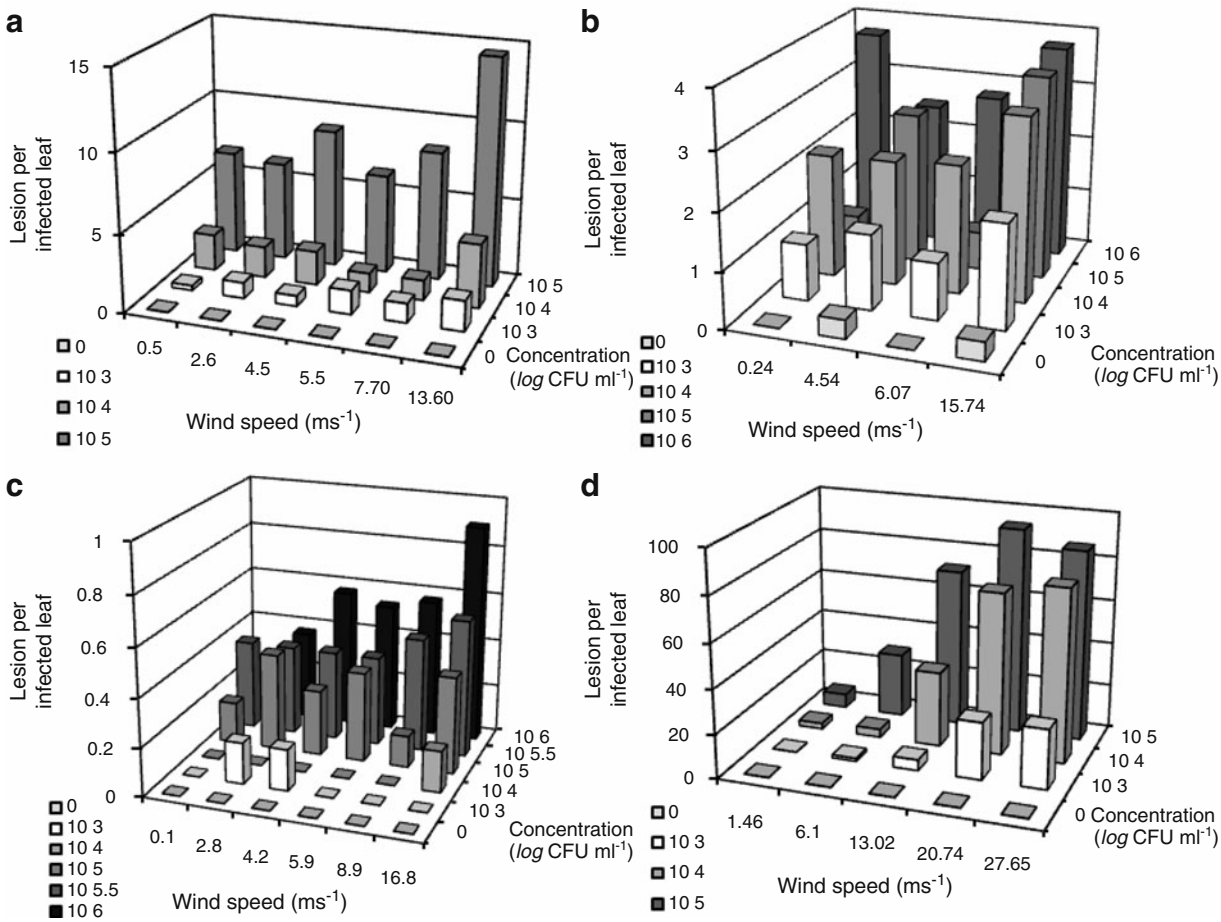

Fig. 2 The effect of wind speed and concentration of inoculum of *Xanthomonas citri* subsp. *citri* on the severity of symptoms of citrus canker (lesions per infected leaf) on leaves of Swingle citrumelo in four experiments on **a.** 14 Feb, **b.** 14 Jun, **c.** 12 Oct, and **d.** 13 Dec 2006

injury increased with wind speed in all experiments except on 14 Jun and 12 Oct. The number of lesions not associated with visible injury also increased in all experiments except those on 14 Feb and 14 Jun.

Among experiments the mean percent leaves infected ranged from 9.5–59.3% (Table 3). Similarly, the number of lesions per infected leaf (1.4–35.9) and percent petioles infected (0.13–21.8%) varied among

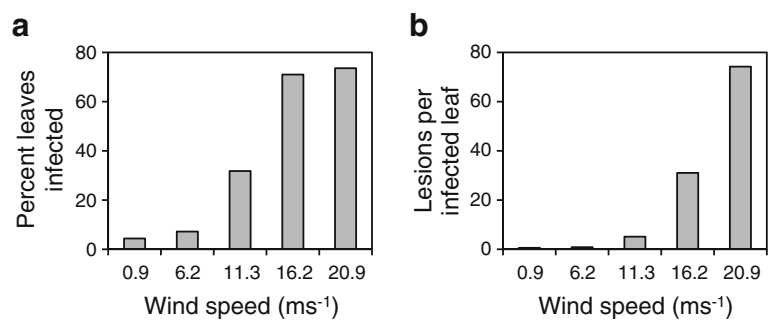

Fig. 3 The effect of wind speed on *Xanthomonas citri* subsp. *citri* on **a.** the incidence of symptoms of citrus canker (percent leaves infected), and **b.** the severity of symptoms of citrus canker (lesions per infected leaf) on leaves of Swingle citrumelo in a single experiment on 17 Oct 2006

Table 2 General linear modeling analysis of the main effect of wind speed on measures of foliar citrus canker and leaf injury on young plants of Swingle citrumelo

Variable	Expt.	MSE (df)[a]	*F*-value (*P*-value)[b]
Percent leaves infected	14 Feb	409 (5,36)	3.11 (0.0195)
	14 Jun	498 (3,40)	7.18 (0.0006)
	12 Oct	583 (5,60)	4.92 (0.0008)
	17 Oct	10059 (4,40)	39.6 (<0.0001)
	13 Dec	6336 (4,30)	28.2 (<0.0001)
Number of lesions per infected leaf	14 Feb	19.4 (5,36)	1.09 (0.38)
	14 Jun	14.6 (3,40)	1.91 (0.14)
	12 Oct	12.4 (5,60)	7.95 (<0.0001)
	17 Oct	9237 (4,40)	41.2 (<0.0001)
	13 Dec	7383 (4,30)	48.3 (<0.0001)
Percent petioles infected	14 Feb	0.39 (5,36)	0.82 (0.54)
	14 Jun	1.14 (3,40)	1.73 (0.18)
	12 Oct	47.9 (5,60)	7.9 (<0.0001)
	17 Oct	5021 (4,40)	29 (<0.0001)
	13 Dec	4828 (4,30)	41.4 (<0.0001)
Percent leaves injured	14 Feb	66.9 (5,36)	2.08 (0.09)
	14 Jun	22.9 (3,40)	1.85 (0.15)
	12 Oct	2.23 (5,60)	1.4 (0.24)
	17 Oct	7678 (4,40)	61.3 (<0.0001)
	13 Dec	4675 (4,30)	26.8 (<0.0001)
Injury severity per injured leaf	14 Feb	0.91 (5,36)	1.31 (0.28)
	14 Jun	3.7 (3,40)	6.5 (0.0011)
	12 Oct	0.28 (5,60)	0.83 (0.54)
	17 Oct	17.2 (4,40)	62.3 (<0.0001)
	13 Dec	18.2 (4,30)	51.7 (<0.0001)
Percent lesions associated with injury	14 Feb	450 (5,36)	6.15 (0.0227)
	14 Jun	21 (3,40)	1.72 (0.17)
	12 Oct	2.2 (5,60)	1.4 (0.24)
	17 Oct	7677 (4,30)	85 (<0.0001)
	13 Dec	950 (4,30)	3.8 (0.0132)
Number lesions not assoc with injury per infected leaf	14 Feb	11 (5,36)	0.65 (<0.6625)
	14 Jun	1.7 (3,40)	1.54 (0.22)
	12 Oct	11 (5,60)	7.0 (<0.0001)
	17 Oct	3887 (4,30)	26 (<0.0001)
	13 Dec	4728 (4,30)	48 (<0.0001)

[a] *MSE* mean square error, and *df* degrees of freedom effect and error

[b] *F* *F*-distribution value, and *P* probability the *F*-value is significant

experiments. The greatest infection was in the experiments with the highest maximum wind speed.

The percent leaves with injury ranged from 0.4–21.3% (Table 4), with those experiments with the highest wind speed having more leaves injured. Among experiments, the severity of injury per leaf (0.16–1.58), the percent lesions associated with injury (0.6–15.2%) and the number of lesions not associated with injury (0.8–21.8%) was variable.

The Tukey's means separation for each date showed that the percent leaves infected was consistently greatest at the highest wind speeds in each experiment (Table 3). The number of lesions per infected leaf, and the percent petioles infected also increased, but the increase was not always significant. Similarly injury, and disease associated with injury (Table 4) generally increased with wind speed but the increase was not always significant. Those experiments

Table 3 Tukey's means separation[a] analysis (P=0.05) on the effect of different wind speeds on measures of citrus canker symptoms on Swingle citrumelo

Variable	Expt no.	Wind speed (m s^{-1})[b]						Grand mean
		1	2	3	4	5	6	
% leaves infected	14 Feb	19.0ab	13.5a	20.3ab	13.8a	20.1ab	32.1b	19.8
	14 Jun	5.2a	6.7a	11.2ab	18.1b			10.3
	12 Oct	0.1a	6.7a	5.8a	10.9ab	9.17a	20.97b	9.5
	17 Oct	4.4a	7.2a	31.8b	71.0c	73.6d		37.6
	13 Dec	18.6a	46.6b	73.3c	82.3c	75.9c		59.3
Number of lesions per infected leaf	14 Feb	3.1a	3.1a	3.9a	3.0a	3.6a	6.8a	3.9
	14 Jun	1.5a	1.8a	1.3a	2.4a			1.8
	12 Oct	0.6a	1.0a	1.2a	1.3a	1.3a	3.2b	1.4
	17 Oct	0.6a	0.8a	5.1a	31.0b	74.2c		22.6
	13 Dec	3.0a	11.4a	36.8b	64.2c	64.1c		35.9
Percent petioles infected	14 Feb	0.5a	0.0a	0.0a	0.0a	0.0a	0.3a	0.13
	14 Jun	0.0a	0.3a	0.0a	0.6a			0.22
	12 Oct	0.0a	0.0a	0.0a	0.0a	8.9a	16.8b	0.82
	17 Oct	0.0a	0.0a	5.9a	45.8b	43.9b		19.1
	13 Dec	0.0a	0.5a	19.7b	34.4b	54.2c		21.8

[a] Numbers with the same letter are not significantly different at P=0.05

[b] Wind speeds (1–6) in each experiment: 14 Feb 06 = 0.5,2.6,4.5,5.5,7.7,13.6 m s^{-1}; 14 Jun 06 = 0.3,4.5,6.1,15.7 m s^{-1}; 12 Oct 06 = 0.1,2.8,4.2,5.9,8.9,16.8 m s^{-1}; 17 Oct 06 = 0.9,6.2,11.3,16.2,20.9 m s^{-1}; 13 Dec 06 = 1.5,6.1,13.0,20.7,27.7 m s^{-1}

with the highest wind speeds (17 Oct and 13 Dec) demonstrated consistent increases in all variables measured (maximum wind speeds in these two experiments was >20 m s^{-1}).

The relationship between wind speed, disease and leaf damage

A logistic model described the relationships between the main effect of wind speed and symptoms of citrus canker on, and severity of injury to, leaves of Swingle citrumelo (Table 5). Various other models were also tested (linear, Gompertz and Weibull) but generally gave less good fits compared to the logistic model, so only the logistic analysis is presented. In four of the five experiments the logistic model provided a good description of the relationship between wind speed and increase in percent leaves infected (F=26–579, $P\geq$0.012), number of lesions per infected leaf (F=66–12052, $P\geq$0.0031), and the percent petioles infected (F=6–8×10^{12}, $P\geq$0.012). The coefficient of determination (R^2) ranged from 0.61 to 1.00.

The relationship between wind speed and injury, disease associated with injury, and disease not associated with injury, were also described with logistic models (Table 6). In four of the five experiments, a logistic model described the relationship between percent leaves injured and wind speed (F=9–31081, $P\geq$0.04), and between the number of lesions per injured leaf not associated with injury and wind speed (F=125–6799, $P\geq$0.0053). In three of the five experiments, a logistic model described the relationship between injury severity per injured leaf and wind speed (F=18–21453, $P\geq$0.02) and between percent lesions associated with injury and wind speed (F=21–1110, $P\geq$0.0161).

The logistic model was also fitted to the combined data from all five experiments for the seven variables (Table 7 and Fig. 4a–g), and the model fit was consistently significant (F=17–66, P<0.0001, R^2=0.43–0.81).

In three of the four experiments, the relationship between the severity of injury per injured leaf and the number of lesions associated with that injury (Table 8)

Table 4 Tukey's means separation[a] analysis (P=0.05) on the effect of different wind speeds on leaf injury and measures of citrus canker symptoms on Swingle citrumelo

Variable	Expt no.	Wind speed[b]						Grand mean
		1	2	3	4	5	6	
Percent leaves with injury	14 Feb	3.7a	2.4a	5.8a	3.0a	2.9a	9.6a	4.6
	14 Jun	1.8a	1.7a	2.6a	4.4a			2.6
	12 Oct	0.0a	0.3a	0.0a	0.9a	0.3a	0.8a	0.4
	17 Oct	0.0a	0.4a	2.4a	39.3b	64.2c		21.3
	13 Dec	0.5a	4.6a	9.2a	36.2b	53.6b		20.8
Injury severity per injured leaf	14 Feb	0.9a	0.7a	0.8a	0.5a	0.7a	1.3a	0.81
	14 Jun	0.03a	0.03a	0.03a	0.09a			0.67
	12 Oct	0a	0.2a	0a	0.3a	0.13a	0.27a	0.16
	17 Oct	0a	0.2a	0.6a	2.0b	3.2c		1.21
	13 Dec	0.2a	0.4ab	1.2b	2.5c	3.5d		1.58
Percent lesions associated with injury	14 Feb	8.1a	4.1a	9.2a	3.4a	5.7a	41.3b	12.0
	14 Jun	12.2a	13.9a	11.9a	22.8a			15.2
	12 Oct	0a	0a	0a	0.3a	0a	3.2a	0.6
	17 Oct	0a	2.8a	9.1ab	26.0bc	35.9c		14.8
	13 Dec	11.1ab	2.6a	2.9a	15.3ab	27.5b		11.9
Number lesions not assoc with injury per infected leaf	14 Feb	2.9a	2.9a	3.5a	2.9a	3.4a	5.7a	3.6
	14 Jun	1.1a	1.3a	1.0a	1.7a			1.3
	12 Oct	0.6a	1.0a	1.2a	1.3a	1.3a	3.1b	0.8
	17 Oct	0.6a	0.8a	4.7a	22.4b	49.0c		15.5
	13 Dec	2.5a	10.4a	36.0b	46.6bc	55.5c		21.8

[a] Numbers with the same letter are not significantly different at P=0.05

[b] Wind speeds (1–6) in each experiment: 14 Feb 06 = 0.5,2.6,4.5,5.5,7.7,13.6 m s^{-1} ; 14 Jun 06 = 0.3,4.5,6.1,15.7 m s^{-1} ; 12 Oct 06 = 0.1,2.8,4.2,5.9,8.9,16.8 m s^{-1} ; 17 Oct 06 = 0.9,6.2,11.3,16.2,20.9 m s^{-1} ; 13 Dec 06 = 1.5,6.1,13.0,20.7,27.7 m s^{-1}

was described by a logistic model (F=163–369, $P \geq$ 0.0061). With the data combined from all five experiments (Table 8, Fig. 4h), the fit was also good (F=369, P<0.0001, R^2=0.97).

With respect to wind speed, these data show that although there might be an initial, gradual increase in disease and injury (and disease associated with injury) with wind speeds of 0–10 ms^{-1}, neither disease nor injury increased dramatically until wind speeds reached 12–15+ m s^{-1}, when there was a greater rate of increase in disease and damage due to wind speed.

The 3-dimensional surface fits of the percent infected leaves, and number of lesions per infected leaf, to severity of injury and wind speed was best described by a polynomial model (Table 9 and Fig. 5). Both injury and infection increase with wind speed, with disease apparently increasing as a result of both direct infection and via visible injury sites.

Disease and concentration of *Xcc*

The GLM analysis confirmed significant effects of inoculum concentration (Table 10) on percent leaves infected, the number of lesions per infected leaf, and the number of lesions not associated with leaf injury, in all four factorial experiments. The effect of concentration on the percent petioles infected was only significant on 12 Oct and 13 Dec (highest maximum wind speeds applied on these two dates). There was no significant effect of concentration on the percent of lesions associated with injury in any of the four experiments. Tukey's means separation (Table 11) shows the trends with concentration and quantity of disease in the different experiments on the four different experiment dates. There was some difference between experiments (Fig. 6a–d), with the experiment on 13 Dec having noticeably greater

Table 5 Logistic regression analysis[a] of the relationship between wind speed and various measures of citrus canker incidence and severity in young plants of Swingle citrumelo

Variable	Date	MSE (df)[b]	*F* (*P*-value)[c]	*a*	*b*	*c*	R^{2d}
Percent leaves infected	14 Feb 06	838 (3,6)	38 (0.007)	4635	−5.84	0.06	0.71
	14 Jun 06	173 (3,4)	37 (0.12)	21	−1.32	0.20	0.95
	12 Oct 06	231 (3,6)	26 (0.012)	27	−2.02	0.19	0.89
	17 Oct 06	3834 (3,5)	205 (0.005)	76	−5.92	0.50	0.99
	13 Dec 06	6800 (3,5)	579 (0.0017)	79	−1.66	0.33	0.99
Number of lesions per infected leaf	14 Feb 06	34 (3,6)	66 (0.0031)	3181	−7.16	0.07	0.86
	14 Jun 06	4 (3,4)	21 (0.16)	567	−6.02	0.03	0.70
	12 Oct 06	5 (3,6)	123 (0.0012)	977	−7.23	0.09	0.97
	17 Oct 06	2164 (3,5)	12052 (<0.0001)	92	−7.83	0.44	1.00
	13 Dec 06	3237 (3,5)	505 (0.002)	67	−3.47	0.29	1.00
Percent petioles infected	14 Feb 06	0.13 (3,6)	6 (0.07)	15875	−7.62	−5.49	0.61
	14 Jun 06	0.13 (3,4)	2 (0.45)	310	−8.64	0.15	0.76
	12 Oct 06	181 (3,6)	$8x10^{12}$ (<0.0001)	17	−43.0	4.85	1.00
	17 Oct 06	2029 (3,5)	3372 (<0.0001)	45	−41.1	3.46	1.00
	13 Dec 06	1486 (3,5)	59 (0.017)	64	−3.67	0.19	0.98

[a] Logistic regression $\left(y = c/1 + Ae^{-Bx}\right)$ was used to explore the relationship between wind speed and disease, where), where *a* and *b* are related to the rate of change and point of inflection of the curve, *c* = the upper asymptote, and *e* the exponent

[b] *MSE* mean square error and degrees of freedom

[c] *F F*-distribution value that tests goodness of fit for the model. *P* Probability the *F*-value is significant

[d] R^2 = coefficient of determination (proportion of variability accounted for by model), with non-linear models is defined as 1-SSE/CSS, where SSE is the variance of the full model and CSS is the variance of the mean model

disease at specific concentrations compared to other dates. Several factors might influence this, including the spectrum of leaf ages, the proportions of leaves at different ages and most importantly wind speed. The wind speeds in the 13 Dec experiment were greater than those in the other three experiments (27 m s^{-1}), and higher wind speed resulted in more infection. Highest wind speeds were more comparable across the remaining three experiments (13.6–16.8 m s^{-1}).

Wind speed x concentration interactions

The GLM analysis showed a significant interaction between wind speed and concentration on percent leaves infected in the 14 Jun experiment only (Table 12). There was a significant interaction between wind speed and concentration affecting lesions per infected leaf and percent petioles infected on 12 Oct and 13 Dec, but not on the other two dates. With the percent lesions associated with injury there was a significant interaction only on 14 Feb, while the number of lesions not associated with injury was significantly affected by the interaction on 12 Oct and 13 Dec, but not on the other two dates. On 14 Feb and 14 Jun maximum wind speeds were <16 m s^{-1}. The interaction between concentration and wind speed was not entirely consistent among experiments, and these data suggest that concentration x wind speed interactions were more common at wind speeds ≥16 m s^{-1}. There were no interactions between wind speed and concentration of inoculum on measures of injury.

Discussion

In all five experiments higher wind speeds resulted in more citrus canker developing on leaves of Swingle citrumelo. Among the first three experiments the maximum wind speed was ≤16.8 m s^{-1}, while the wind source in the third and fourth experiments produced wind speeds in excess of 20 m s^{-1}. Nonetheless, the five experiments showed similar trends in disease over the shared ranges of wind speed, and the

Table 6 Logistic regression analysis[a] of the relationship between wind speed and citrus canker incidence and severity, and leaf injury in young plants of Swingle citrumelo in five experiments (see data in Tables 3 and 4).

Variable	Date	MSE (df)[b]	*F* (*P*-value)[c]	*a*	*b*	*c*	R^{2d}
Percent leaves injured	14 Feb 06	50 (3,6)	12 (0.04)	10387	−8.42	0.10	0.67
	14 Jun 06	11 (3,4)	38 (0.12)	600	−5.93	0.07	0.94
	12 Oct 06	0.7 (3,6)	9 (0.033)	0.67	−300	68	0.60
	17 Oct 06	1891 (3,5)	31081 (<0.0001)	65	−11.9	0.76	1.00
	13 Dec 06	1427 (3,5)	364 (0.0027)	60	−4.86	0.25	1.00
Injury severity per injured leaf	14 Feb 06	1.4 (3,6)	18 (0.02)	847	−7.24	0.05	0.38
	14 Jun 06	0.82 (3,4)	78 (0.08)	374	−7.20	0.10	0.99
	12 Oct 06	0.06 (3,6)	2.9 (0.21)	0.23	−1.90	0.62	0.34
	17 Oct 06	5.0 (3,5)	629 (0.0016)	3.72	−5.81	0.37	1.00
	13 Dec 06	6.9 (3,5)	21453 (<0.0001)	4.27	−3.23	0.17	1.00
Percent lesions associated with injury	14 Feb 06	610 (3,6)	21 (0.0161)	46092	−10.27	0.24	0.92
	14 Jun 06	332 (3,4)	42 (0.11)	3084	−5.67	0.05	0.90
	12 Oct 06	3.4 (3,6)	101 (0.0016)	387	−12.0	0.43	0.99
	17 Oct 06	684 (3,5)	1110 (0.0009)	39	−5.92	0.37	1.00
	13 Dec 06	345 (3,5)	7.35 (0.12)	30169	−9.42	0.09	0.78
Number lesions not assoc with injury per infected leaf	14 Feb 06	26 (3,6)	109 (0.0015)	1789	−6.60	0.06	0.88
	14 Jun 06	2.2 (3,4)	29 (0.14)	338	−5.80	0.03	0.74
	12 Oct 06	5.2 (3,6)	125 (0.0012)	779	−7.00	0.09	0.97
	17 Oct 06	975 (3,5)	6799 (0.0001)	62.5	−7.00	0.40	1.00
	13 Dec 06	2212 (3,5)	187 (0.0053)	53.2	−3.11	0.29	0.99

[a] Logistic regression $\left(y = c/1 + Ae^{-Bx}\right)$ was used to explore the relationship between wind speed and disease, where), where *a* and *b* are related to the rate of change and point of inflection of the curve, *c* = the upper asymptote, and *e* the exponent

[b] *MSE* mean square error and degrees of freedom

[c] *F F*-distribution value that tests goodness of fit for the model. *P* Probability the *F*-value is significant

[d] R^2 = coefficient of determination (proportion of variability accounted for by model), with non-linear models is defined as 1-SSE/CSS, where SSE is the variance of the full model and CSS is the variance of the mean model

Table 7 Logistic regression analysis[a] of the relationship between wind speed and citrus canker incidence and severity, and leaf injury in young plants of Swingle citrumelo, with the analysis based on the combined data from all five dates (see data in Tables 3 and 4).

Variable	MSE (df)[b]	*F* (*P*-value)[c]	*a*	*b*	*c*	R^{2d}
Percent leaves infected	10009 (3,23)	40 (<0.0001)	93.4	0.46	0.16	0.66
Number of lesions per infected leaf	4685 (3,23)	47 (<0.0001)	72	−13.19	0.73	0.81
Percent petioles infected	2537 (3,23)	44 (<0.0001)	56.6	−5.66	0.31	0.81
Percent leaves injured	2867 (3,23)	43 (<0.0001)	57.8	−9.05	0.50	0.80
Injury severity per injured leaf	13 (3,23)	66 (<0.0001)	5.12	−3.43	0.16	0.81
Percent lesions associated with injury	1405 (3,23)	17 (<0.0001)	31.18	−2.44	0.18	0.43
Number lesions not assoc with injury per infected leaf	2702 (3,23)	39 (<0.0001)	63.4	−6.11	0.32	0.77

[a] Logistic regression $\left(y = c/1 + Ae^{-Bx}\right)$ was used to explore the relationship between wind speed and disease, where), where *a* and *b* are related to the rate of change and point of inflection of the curve, *c* = the upper asymptote, and *e* the exponent

[b] *MSE* mean square error and degrees of freedom

[c] *F F*-distribution value that tests goodness of fit for the model. *P* Probability the *F*-value is significant

[d] R^2 = coefficient of determination (proportion of variability accounted for by model), with non-linear models is defined as 1-SSE/CSS, where SSE is the variance of the full model and CSS is the variance of the mean model

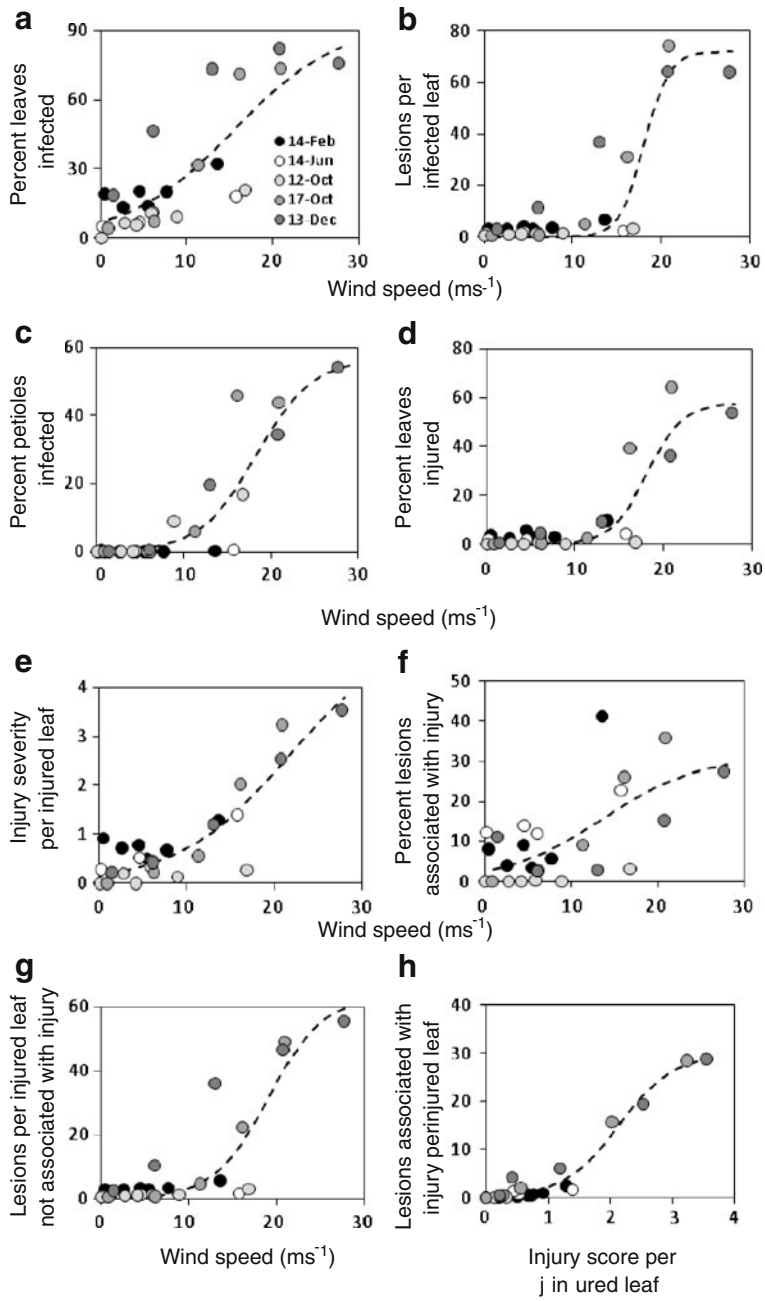

Fig. 4 The logistic relationship between wind speed and development of symptoms of citrus canker subsequent to exposing young plants of Swingle citrumelot to spray inoculum of *Xanthomonas citri* subsp. *citri* at different wind speeds. **a.** percent leaves with symptoms, **b.** number of canker lesions per infected leaf, **c.** percent petioles infected, **d**. percent leaves visibly injured, **e**. injury severity per infected leaf, percent leaves with lesions associated with visible injury, **f**. percent lesions associated with visible injury per injured leaf, **g**. number of lesions per injured leaf not associated with visible injury, and **h.** The logistic relationship between visible injury severity and number of lesions of citrus canker associated with injury per injured leaf. Results from five experiments are shown. Parameters and model fit statistics are shown in Tables 5 (a-c), 6 (d-g) and 8 (h)

combined data showed the relationship between wind speed and increase in incidence and severity of symptoms was described by a logistic model. Increasing wind speed from 0 to 10 m s^{-1} caused a gradual increase, but by 10–15 ms^{-1}, there was a dramatic increase in disease incidence and severity to at least 20 ms^{-1}, at which point the rate appeared to slow for most disease variables measured. In a previous study (Serizawa and Inoue 1974; Serizawa 1981), wind speed appeared to cause an increase in disease at about 6.5–8.5 m s^{-1}, approx 5–10 m s^{-1} lower than observed in the current study. Several factors might explain this, including position of the wind speed sensor relative to the foliage, the cultivar or species of citrus being used, as well as its state of flush and possibly environmental preconditioning of the plant.

Table 8 Logistic regression analysis[a] of the relationship between severity of injury per injured leaf and the number of lesions associated with that injury (see Fig. 4 h)

Date	MSE (df)[b]	*F* (*P*-value)[c]	*a*	*b*	*c*	R^{2d}
14 Feb 06	2.8 (3,3)	229 (0.0005)	1824	−9.79	2.48	0.99
14 Jun 06	1.98 (3,1)	6.4 (0.28)	1.72	−2.08	5.68	0.66
12 Oct 06	0.11 (2,4)	5.4 (0.072)	0.33	−126.4	571	0.65
17 Oct 06	355 (3,2)	917 (0.0011)	30.59	−4.17	2.10	1.00
13 Dec 06	420 (3,2)	163 (0.0061)	35.0	−3.08	1.31	0.99
All dates combined	766 (3,23)	369 (<0.0001)	30.17	−4.80	2.25	0.97

[a] Logistic regression $\left(y = c/1 + Ae^{-Bx}\right)$ was used to explore the relationship between wind speed and disease, where), where *a* and *b* are related to the rate of change and point of inflection of the curve, *c* = the upper asymptote, and *e* the exponent

[b] *MSE* mean square error and degrees of freedom

[c] *F F*-distribution value that tests goodness of fit for the model. *P* Probability the *F*-value is significant

[d] R^2 = coefficient of determination (proportion of variability accounted for by model), with non-linear models is defined as 1-SSE/CSS, where SSE is the variance of the full model and CSS is the variance of the mean model

Host resistance might result in less disease developing at all wind speeds. Serizawa and Inoue (1974) used Natsudaidai (*C. natsudaidai*), which is susceptible to canker, while in this study Swingle citrumelo was used, which, while susceptible to canker, has smaller leaves and is a trifoliate. Smaller, trifoliate leaves might have slightly different aerodynamics and consequently respond differently to wind, and thus affect the relationship between wind and infection, and although the number of stomata on leaves of different cultivars might affect subsequent development of disease, some previous work suggested this was not the case (Graham et al. 1992).

For the first time canker symptoms associated with visible injury due to wind was quantified. Visible injury to leaves of Swingle citrumelo due to wind was evident at wind speeds of 13 m s^{-1} (and two-minutes exposure—the time component of exposure is an important factor), but did not increase dramatically until 15 m s^{-1}, with the relationship between wind speed and injury being described by a logistic model. More disease was also associated with the visible injury as the wind speed increased. Furthermore, disease not associated with visible injury also increased with wind speed—perhaps associated with direct stomatal entry (Serizawa and Inoue 1974; Graham et al. 1992). The importance of leaf injury as access for *Xcc* has been demonstrated with leaf miner and mechanical injury (Jesus et al. 2006; Christiano et al. 2007), and our data confirm that the wind speeds common in thunderstorms in Florida could cause injury, and with inoculum present, the pathogen gained access through the wound. Indeed, injury as a result of wind damage could remain susceptible to infection for many days (Koizumi 1983). Furthermore, only visible injury was scored, not injury at a microscopic level. Cuticular damage, microscopic epidermal scratches and damaged cells might provide access to the pathogen, but remain to be ascertained. The presence of rain, canker lesions and strong or gusty wind provides ideal conditions for dispersal of *Xcc*, and subsequent infection of susceptible citrus. In citrus growing areas where thunderstorms associated with wind speeds >10 m s^{-1} are not

Table 9 A three-dimensional *x-y* polynomial surface model fit to describe the relationship between wind speed injury and disease symptoms for A) numbers of lesions per infected leaf, and B) severity per infected leaf

Variable	MSE (df)	*F* (*P*-value)	*a*	*b*	*c*	*d*	R^2
Percent leaves infected	4286 (3,22)	22.6 (<0.0001)	21.5	1.53	13.9	0.02	0.76
Number of lesions per infected leaf	2953 (3,22)	19.7 (<0.0001)	5.3	1.5	11.1	0.02	0.73

[a] Surface fit described by an *x-y* polynomial where the disease severity score, $z = a + bx + clny + d(lny)^2$

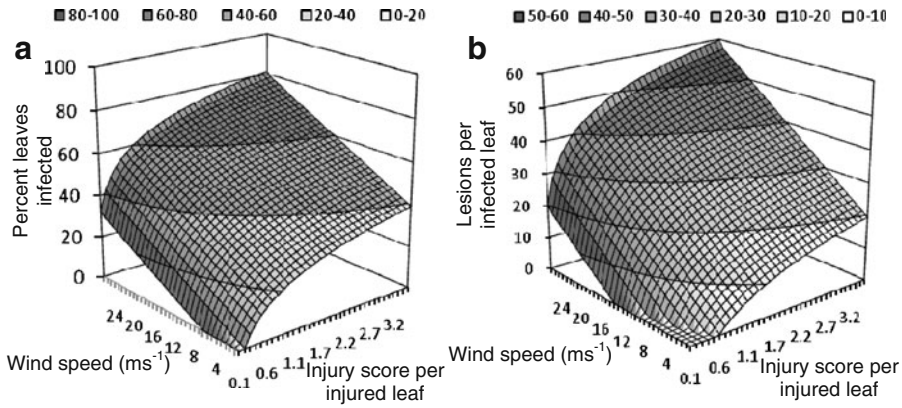

Fig. 5 Three-dimensional surface models fit to describe the relationship between wind speed, injury and disease symptoms for A. percent leaves infected and B. number of lesions per infected leaf. Surface fit described by an *x-y* polynomial where the disease severity score, $z = a + bx + clny + d(lny)^2$. Parameters and model fit statistics in Table 9

common, or where hurricanes and tropical storms do not occur, the effect of wind on dispersal, infection and leaf injury will be much lower.

Leaf injury was diverse and covered various wound types including puncture wounds, scratches, tears and loss of sections of the lamina. These were likely caused by a combination of wind buffeting and the leaf being whipped against the axillary thorn on the stem (Serizawa and Inoue 1974). The petiole-leaflet junction was more often infected at higher wind speeds. Leaflet motion in strong wind could damage the leaflet-petiole connection and allow entry of *Xcc*. Petiole infections were common at wind speeds ≥17 m s^{-1}. Once symptoms progressed (3–5 weeks), the leaflets of the trifoliate were easily dislodged as the petiole infection developed. This

Table 10 General linear modeling analysis of the effect of inoculum concentration on various measures of foliar citrus canker incidence and severity, and leaf injury in four experiments on young plants of Swingle citrumelo

Variable	Expt	MSE (df)[a]	F-value (P-value)[b]
Percent leaves infected	14 Feb	4642 (2,36)	35 (<0.0001)
	14 Jun	1293 (4,40)	18.7 (<0.0001)
	12 Oct	1160 (4,60)	9.8 (<0.0001)
	13 Dec	6183 (2,30)	28 (<0.0001)
Number of lesions per infected leaf	14 Feb	285 (2,36)	16 (<0.0001)
	14 Jun	14.6 (4,40)	8.4 (<0.0001)
	12 Oct	36 (4,60)	23 (<0.0001)
	13 Dec	7908 (2,30)	51.7 (<0.0001)
Percent petioles infected	14 Feb	0.26 (2,36)	0.56 (0.57)
	14 Jun	0.48 (4,40)	0.72 (0.58)
	12 Oct	25 (4,60)	4 (0.0071)
	13 Dec	1488 (2,30)	13 (<0.0001)
Percent lesions associated with injury	14 Feb	799 (2,36)	4.6 (0.17)
	14 Jun	16848 (4,40)	2.47 (0.06)
	12 Oct	18 (4,60)	0.9 (0.47)
	13 Dec	282 (2,30)	1 (0.34)
Number lesions not assoc with injury per infected leaf	14 Feb	283 (2,36)	17 (<0.0001)
	14 Jun	11 (4,40)	10 (<0.0001)
	12 Oct	35 (4,60)	22 (<0.0001)
	13 Dec	6385 (2,30)	65 (<0.0001)

[a] *MSE* mean square error, and *df* degrees of freedom effect and error

[b] *F* *F*-distribution value, and *P* probability the *F*-value is significant

Table 11 Tukey's means separation[a] analysis (P=0.05) on the effect of different inoculum concentrations of *Xanthomonas citri* subsp. *citri* on incidence and severity measures of citrus canker symptoms on Swingle citrumelo

Variable	Expt no.	Concentration (CFU ml^{-1})[b]					
		1	2	3	4	5	6
Percent leaves infected	14 Feb	0a	4.8b	17.8c	36.7d		
	14 Jun	0a	0.6b	2.8b	7.1bc	15.0d	26.0d
	12 Oct	0a	0.5b	1.6b	12.9c	13.4c	18.9c
	13 Dec	0a	36.0b	67.7c	74.1c		
Number of lesions per infected leaf	14 Feb	0a	11.8b	38.4c	58.0d		
	14 Jun	0a	0.2b	1.3bc	1.9 cd	2.5 cd	3.2d
	12 Oct	0a	0.2b	0.2b	1.2bc	1.8b	3.6a
	13 Dec	0a	11.8b	38.4c	57.5d		
Percent petioles infected	14 Feb	0a	0a	0.1a	0.2a		
	14 Jun	0a	0a	0a	0.3a	0.3a	0.3a
	12 Oct	0a	0a	0a	0.2a	1.2ab	2.7b
	13 Dec	0a	10.4b	26.2c	28.8c		
Percent lesions associated with injury	14 Feb	0a	4.4a	14.5ab	17.0b		
	14 Jun	0a	21.9ab	11.6ab	11.3ab	31.3b	
	12 Oct	0a	0a	0a	2.3a	0.6a	
	13 Dec	0a	8.6a	16.8a	10.3a		
Number lesions not assoc with injury per infected leaf	14 Feb	0a	0.8b	1.8b	8.1c		
	14 Jun	0a	0.2b	0.5b	1.4bc	2.2c	2.2c
	12 Oct	0a	0.2b	0.2b	1.2bc	1.7c	3.6d
	13 Dec	0a	9.5b	30.5c	50.7d		

[a] Numbers with the same letter are not significantly different at P=0.05

[b] Concentrations (1–6) of inocula used in each experiment: 14 Feb $7\times10^{0,3,4 \text{ and } 5}$ CFU ml^{-1}; 14 Jun $7\times10^{0,3,4,5,6}$ CFU ml^{-1}; 12 Oct $5\times10^{0,3,4,5}$, 8×10^{5}, 1×10^{6} CFU ml^{-1}; 13 Dec $8\times10^{0,3,4,5}$ CFU ml^{-1}

infection location has potential to contribute to loss of photosynthetic area. Gottwald et al. (1988) observed cyclical defoliation in trees following infection with *Xcc*, and Serizawa and Inoue (1974) noted an increase in infection associated with the petiolar region on Natsudaidai at wind speeds of 6.5 m s^{-1}, which is lower than observed in the current study. Differences between host species are a possible explanation for this difference. Natsudaidai has single, large leaves, while Swingle is a trifoliate, with small, individual leaflets.

Higher wind speed resulted in an overall increase in the incidence and severity of infected leaves, i.e., more resistant, older leaves became infected, and those leaves already infected developed more lesions. Leaf age is an important determinant of susceptibility of citrus (Lee 1922; Serizawa and Inoue 1974; Stall et al. 1982; Gottwald and Graham 1992; Graham et al. 1992). Leaves that are one-half to three-quarters expanded are most vulnerable to direct infection through stomata in the absence of wind or physical injury (Graham et al. 1992; Pruvost et al. 2002; Bock et al. 2010a, b). In this study, some leaves developed disease in the absence of wind, therefore, the incidence and severity of disease will depend, in large part, on the spectrum of leaf ages in the tree canopy. Some of the variability among the five experiments in disease could be due to this variation in leaf age, despite an attempt to choose plants of comparable size, age and flush condition. Furthermore, not all citrus are equally susceptible to citrus canker, and although wind speed resulted in an increase in the incidence and severity of symptoms of citrus canker on Swingle citrumelo, this relationship may not be the same for citrus species and cultivars that vary in leaf type (entire vs. trifoliate) and mesophyll susceptibility (Gottwald et al. 1993). The conditions plants were subject to prior to exposure to inoculum could also influence the likelihood of infection.

Concentration of the *Xcc* inoculum increased the incidence and severity of citrus canker in all experiments, and confirms some previous observations (Serizawa and Inoue 1974; Serizawa 1981). The highest incidence and severity were encountered in the experiment with the highest wind speeds (27 m s^{-1}, Dec 13), presumably because greater wind

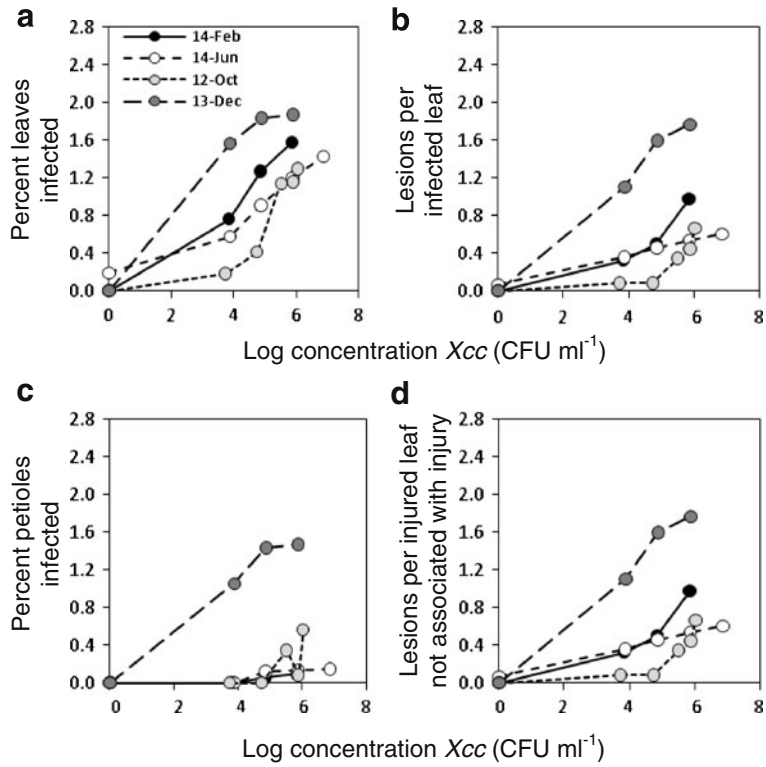

Fig. 6 The effect of concentration on development of symptoms of citrus canker subsequent to exposing young plants of Swingle citrumelo to spray inoculum of *Xanthomonas citri* subsp. *citri* at various concentrations from zero to 10^6 cfu per ml. Log transformed means and concentrations are shown to illustrate data from all four experiments. **a.** percent leaves with symptoms, **b.** number of canker lesions per infected leaf, **c.** percent petioles with symptoms of canker. **d.** number of lesions per injured leaf not associated with visible injury. Results from four experiments are shown. Untransformed means are shown in Table 11. Means are averaged across wind speeds

Table 12 General linear modeling analysis of the interaction between wind speed and inoculum concentration and measures of foliar citrus canker severity on young plants Swingle citrumelo

Variable	Expt.	MSE (df)[a]	*F*-value (*P*-value)[b]
Percent leaves infected	14 Feb	112 (10,36)	0.9 (0.58)
	14 Jun	176 (12,40)	2.5 (0.014)
	12 Oct	156 (20,60)	1.3 (0.21)
	13 Dec	481 (8,30)	2.1 (0.063)
Number of lesions per infected leaf	14 Feb	7 (10,36)	0.4 (0.9)
	14 Jun	1.3 (12,40)	0.8 (0.67)
	12 Oct	6.0 (20,60)	3.8 (<0.0001)
	13 Dec	747 (8,30)	4.9 (0.0006)
Percent petioles infected	14 Feb	0.5 (10,36)	1.1 (0.40)
	14 Jun	0.89 (12,40)	1.4 (0.23)
	12 Oct	22 (20,60)	3.7 (<0.0001)
	13 Dec	329 (8,30)	2.8 (0.019)
Percent lesions associated with injury	14 Feb	1174 (10,36)	6.8 (<0.0001)
	14 Jun	692 (12,40)	1.0 (0.45)
	12 Oct	18.7 (20,60)	0.9 (0.55)
	13 Dec	255 (8,30)	1.0 (0.44)
Number lesions not assoc with injury per infected leaf	14 Feb	8.7 (10,36)	0.5 (0.87)
	14 Jun	1.2 (12,40)	1.1 (0.41)
	12 Oct	5.9 (20,60)	3.7 (<0.0001)
	13 Dec	574 (8,30)	5.9 (0.0002)

[a] *MSE* mean square error, and *df* degrees of freedom effect and error

[b] *F* *F*-distribution value, and *P* probability the *F*-value is significant

speed increases infection at a given concentration. Both the number of lesions not associated with visible injury, and the percent petioles infected were affected by an interaction between concentration and wind speed with wind speeds >16 m s^{-1} (12 Oct, 13 Dec). The reason for this is not known, but perhaps at wind speeds >16 m s^{-1} the force of the wind speed combined with increased concentration results in more frequent and effective infection.

With the citrus canker host-pathogen system, calm conditions would favor autoinfection (defined here as infection occurring in the same tree). Wind speeds that increase dispersal will increase the chance of both autoinfection and alloinfection (defined here as an infection arriving at a new tree). Increased wind speed results in many-fold more bacteria being dispersed greater distances (Gottwald et al. 2002; Bock et al. 2005; Irey et al. 2006; Bock et al. 2010a, b), and also results in greater infection. Thus by reducing wind speed in a grove, both dispersal of *Xcc*, and infection of the host by *Xcc*, can be reduced. Indeed wind breaks have been documented as a management tool that can help reduce the amount citrus canker in susceptible citrus (Gottwald and Timmer 1995; Gottwald et al. 2007; Behlau et al. 2008), and these data (and others) may provide some explanation why windbreaks can be effective at reducing the severity of the epidemic. They also provide a basis for considering a strategy for the choice, orientation and structure of windbreaks to achieve greatest reductions in grove wind speeds. This becomes a real option when critical wind speed effects on dispersal and infection processes have been characterized, providing information that can be applied to help reduce citrus canker in groves.

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