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The distribution and spread of citrus canker in Emerald, Australia

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Abstract. Citrus canker is a disease of citrus and closely related species, caused by the bacterium *Xanthomonas citri* subsp. *citri*. This disease, previously exotic to Australia, was detected on a single farm [infested premise-1, (IP1). IP is the terminology used in official biosecurity protocols to describe a locality at which an exotic plant pest has been confirmed or is presumed to exist. IP are numbered sequentially as they are detected] in Emerald, Queensland in July 2004. During the following 10 months the disease was subsequently detected on two other farms (IP2 and IP3) within the same area and studies indicated the disease first occurred on IP1 and spread to IP2 and IP3. The oldest, naturally infected plant tissue observed on any of these farms indicated the disease was present on IP1 for several months before detection and established on IP2 and IP3 during the second quarter (i.e. autumn) 2004. Transect studies on some IP1 blocks showed disease incidences ranged between 52 and 100% (trees infected). This contrasted to very low disease incidence, less than 4% of trees within a block, on IP2 and IP3. The mechanisms proposed for disease spread within blocks include weather-assisted dispersal of the bacterium (e.g. wind-driven rain) and movement of contaminated farm equipment, in particular by pivot irrigator towers via mechanical damage in combination with abundant water. Spread between blocks on IP2 was attributed to movement of contaminated farm equipment and/or people. Epidemiology results suggest: (i) successive surveillance rounds increase the likelihood of disease detection; (ii) surveillance sensitivity is affected by tree size; and (iii) individual destruction zones (for the purpose of eradication) could be determined using disease incidence and severity data rather than a predefined set area.

Introduction

Citrus canker is a serious disease of citrus caused by the bacterium *Xanthomonas citri* subsp. *citri*. This disease causes major economic losses in many citrus-growing countries including the USA (Irey *et al*. 2006; Gottwald and Irey 2007), Argentina (Canteros 2004) and Brazil (Leite and Mohan 1990). It also poses a considerable threat to the Australian citrus industry both in direct crop losses and the potential loss of export markets. The Australian Bureau of Agricultural and Resource Economics estimated the net benefit of eradicating the disease to Queensland alone was about $70 million. It was, therefore, imperative that when citrus canker was reported from Emerald, Australia in July 2004, it was rapidly contained and eradicated. A Pest Quarantine Area (PQA) with an approximate radius of 50 km and encompassing all cultivated citrus in the Emerald district and the National Citrus Canker Eradication Program were established to manage the containment and eradication of citrus canker. As the PQA encompassed the township of Emerald, delimiting surveys were commenced on commercial and residential premises and plants of all known and suspected hosts of citrus canker were inspected, including the native species *Citrus glauca*. This is an experimental host of *X. citri* subsp. *citri* and host susceptibility was demonstrated using bacterial isolates from the 1920s (Peltier 1918; Peltier and Frederich 1920, 1924) and from the Emerald incursion (Hailstones *et al*. 2005). Leaves of *C. glauca* plants collected from several geographic locations in Australia, including the Emerald district, were used in the latter study.

During the 10 months following the first report of the disease at a farm coded infested premise-1 (IP1), citrus canker was confirmed on two more farms within the PQA, coded IP2 and IP3. Following the detection of the disease on IP3, the eradication zone was extended beyond the three IPs to encompass the entire PQA. This included destruction of all host plants within the PQA with the exception of *C. glauca*. Due to the abundance of this species in the Emerald area, the detection and destruction of all *C. glauca* plants within the PQA was not feasible and instead only plants near commercial premises and the Emerald township were removed. The eradication campaign was completed in early 2009 and area freedom status for citrus canker was obtained for the Emerald growing district. The total cost of the eradication campaign was estimated at $17.6 million and required in excess of 200 000 staff hours to complete. Despite tracing investigations conducted as part of the emergency

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response and also by the Australian Quarantine and Inspection Service, the mechanism of disease introduction into the Emerald region remains unproven. Previous smaller outbreaks of citrus canker that occurred in Northern Australia (Hill 1918; Broadbent *et al*. 1992) and the Torres Strait (Jones *et al*. 1984; Shivas 1987) were also successfully eradicated.

The taxonomic nomenclature of *X. citri* subsp. *citri* has undergone several changes and previous synonyms include *X. smithii* subsp. *citri* (Schaad *et al*. 2005, 2006) and *X. axonopodis* pv. *citri* (Dunger *et al*. 2005). In addition, *X. fuscans* subsp. *aurantifolii* and *X. alfalfae* subsp. *citrumelonis* can infect and cause diseases on citrus which can superficially appear similar to citrus canker (Schaad *et al*. 2006). Using a DNA fingerprint technique based on that described by Koeuth *et al*. (1995), representative bacterial isolates collected from three infested premises in the PQA were compared with a range of known strains and identified as *X. citri* subsp. *citri* (D. Hailstones, pers. comm.). Representative isolates from the three premises were also shown to be pathogenic to citrus in detached leaf assays (M. Weinert, unpubl. data).

Typical symptoms of citrus canker were reviewed by Goto (1992) and Schubert *et al*. (2001) and are described as tan, brown or grey lesions which protrude from both surfaces of leaf tissue and are surrounded by a water-soaked margin and a yellow halo. The lesions on fruit and stems are similar in appearance but often lack a definable halo. Successful establishment and spread of citrus canker occurs when the presence of free water coincides with the presence of susceptible citrus tissues and is enhanced by wind of at least 8 m/s. Bacterial cells ooze from lesions in the presence of free moisture (Serizawa 1981; Timmer *et al*. 1991) and disperse to form new infections via stomata (Civerolo 1984; Goto 1992; Graham *et al*. 1992) or wounds caused by insects, equipment or wind (Serizawa 1981; Schubert *et al*. 2001). Citrus leaf tissue is susceptible to stomatal infection for a short time, typically between 50 and 80% of leaf expansion (Koizumi 1981; Goto 1992). Similarly, green stem tissue needs a period of expansion before it becomes susceptible to stomatal infection (Koizumi 1981), but precisely when it is no longer susceptible is less clear. However, some indication of the time of stomatal infection can be determined by the age of the infected vegetative flush where stem material remains relatively immature.

Dispersal of the bacterium has been shown to occur through wind-driven rain (Kuhara 1978; Stall *et al*. 1980; Serizawa 1981; Bock *et al*. 2005) and circumstantially up to at least 10 km during severe storm events such as tornadoes (Gottwald *et al*. 1997). Disease spread has been further investigated and a model based on wind direction data and threshold parameters of 8 m/s wind speed and 0.32 cm/h rainfall has been proposed to explain most of the long-distance secondary disease spread observed in Florida (Irey *et al*. 2006). Disease spread is also believed to occur on contaminated equipment and persons, but long-distance spread between geographical regions is most likely to occur via diseased plant material (Civerolo 1984; Schubert *et al*. 2001).

This paper outlines the distribution of citrus canker within the Emerald region and explores the potential mechanisms of its spread between and within premises. It also details the severity and incidence of the disease in different farms, provides an estimate of when the epidemic first established in the region and the rates of spread of the disease in the Emerald climate. A complicating factor in the evaluation of the mechanisms of disease spread within the PQA during this study was the lack of data recorded for IP1. Due to the pace of the tree destruction for the purpose of disease eradication, there was insufficient time to collect details of potential source(s) of inoculum, estimate the overall inoculum available for long distance spread and identify the number of disease foci present on the farm. The implications of our observations for future management of citrus canker incursions in Australia are discussed.

Methods

Pathogen identification

Field identification of plants affected by citrus canker was based on visual examination for symptoms as described by Goto (1992) and Schubert *et al*. (2001), with most infections confirmed by PCR-indexing using a modified method based on those described by Cubero and Graham (2002) and Hartung *et al*. (1993). For PCR-indexing, one-half of a lesion was excised and soaked overnight in ~200 μL of sterile water. The exudate was diluted 1 : 10 in water and a 2-μL aliquot used in a 25-μL reaction. The remaining PCR reagents included 1× buffer (Invitrogen, Carlsbad, CA, USA), 3 mM $MgCl_2$, 400 μM of each dNTP, 500 nM of forward and reverse primers, 10 g/L Triton X-100, 1 g/L gelatin and 1 U *Taq* DNA polymerase (Invitrogen) made up to volume with purified water. Each sample was tested using the primer pairs, 2 and 3 (Hartung *et al*. 1993), and J-pth1 and J-pth2 (Cubero and Graham 2002), in separate reactions. Reactions were incubated at 94°C for 1 min then through 35 cycles of 94°C for 30 s, 55°C for 30 s and 72°C for 45 s, followed by a final incubation of 72°C for 5 min. PCR products were separated in a 20 g/L agarose gel in 0.5× Tris-borate-EDTA and visualised by staining with ethidium bromide (Sambrook *et al*. 1989).

Epidemiology study blocks

The severity and incidence of citrus canker were evaluated in orchard blocks on all three IPs. Figure 1 shows the relative locations of each IP and the study blocks. There was insufficient time to study all affected blocks on IP1 before they were destroyed, so from observations made of the disease throughout IP1 during delimiting surveys, the three most severely infested blocks (1D, 2D and 5D in Stage 1) were chosen. All blocks which contained citrus canker on IP2 (1, 7, 8, 24, 36 and 41) and IP3 (one block only) were studied. The number of hectares of each citrus variety and the approximate tree age for each study block is shown in Table 1. The trees on all three IPs were irrigated using water sourced from the Nogoa River either directly (IP2) or indirectly from on-farm holding dams (IP1 and IP3). The trees on all premises were drip-irrigated with the exception of Blocks 7 and 8 (IP2) which were watered using overhead centre pivot irrigators.

Estimating the age of infected tissue

Plant tissues were assumed to be infected via stomata if lesions were circular in morphology and lacked obvious signs of a wound. Where possible the age of infected tissues was

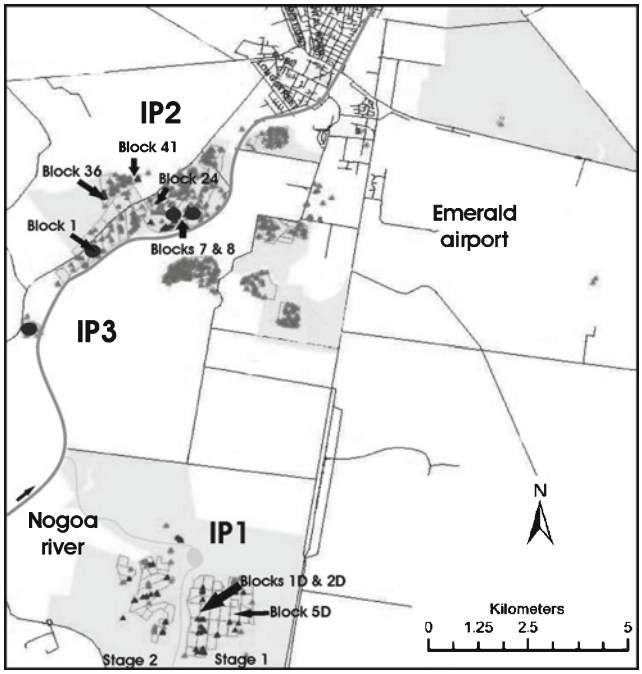

Fig. 1. Schematic representation of the spatial relationship between infested premise-1 (IP1), IP2 and IP3 within the Pest Quarantine Area and the blocks used for epidemiology studies. The four citrus canker infestations which occurred during autumn 2004 are indicated by large black dots, the location of samples collected during surveillance are indicated by grey triangles. The locations of all commercial citrus properties in the Emerald growing district are indicated by light grey shading and production blocks delimited by lines. The direction of flow of the Nogoa River is shown by a black arrow. Weather data were from the station at the Emerald airport.

estimated by counting back through the vegetative growth flush events as suggested by Graham *et al*. (2004). The time when citrus canker established on a tree was inferred from the age of the oldest infected plant tissues, typically stem.

Field evaluations of infested blocks

Studies on IP1 were done by inspecting trees along arbitrarily selected transects in each block (Fig. 2*a*, *b*). All trees were inspected by surveillance staff on the IP2 and IP3 study blocks, with the exception of IP2 Blocks 7 and 8, where only trees adjacent to the overhead irrigator wheel paths were inspected (Fig. 3*a*, *b*). Following disease detection on these blocks, a detailed examination was made by pathology staff and included rating each tree for disease severity and estimating the flush age of infected plant tissues. A focal point of disease was confirmed where possible for each block by identifying those trees having the highest disease severity rating and oldest infected flush. Disease severity was rated as an estimation of the percentage of total foliage (total number of leaves and stems) displaying symptoms. Trees were rated as very low for up to 5%, low for between 5 and 10%, moderate for between 10 and 25% and severe for greater than 25% of total foliage affected. For IP2 Blocks 1 and 36, disease severity was assessed in different canopy zones by height (upper, middle and lower) and/or aspect (north, south, east and west). The incidence of citrus canker for each block was calculated by expressing the number of diseased trees as a percentage of the total number of trees inspected. For any symptomatic stems considered to be more than five flushes old, the diameter, length, and number of growth rings were measured. The percent of bark development was estimated as a measure of maturity, and the diameter the citrus canker lesions measured.

Table 1. An overview of the citrus blocks investigated on infested premise-1 (IP1), IP2 and IP3 and the results of epidemiology studies conducted on each block

The total number of trees inspected is listed for each block and the disease incidence for each block calculated as a proportion of these inspected trees. For blocks 1, 36 and 41 on IP2 and IP3 all trees within the block were inspected. Where possible the season and year of disease establishment was estimated from the oldest naturally infected plant flushes detected on each block

Farm	Block code	Citrus host[A]	Size of block (ha)	Age of trees since planting (yr)	Severity[B]	Infected trees	Total trees inspected	Incidence (%)	Detection	Establishment
IP1	1D	Cara Cara, Murcott, Imperial	8	3–4	M	136	145	94	July 2004	Unknown
	2D	Cara Cara	8	3–4	S	162	162	100	July 2004	Unknown
	5D	Imperial	8	2–3	M	124	238	52	July 2004	Unknown
IP2	7	Imperial	27	16	S	92	2357	3.9	October 2004	Autumn 2004
		Murcott	28	16						
		Hickson	4	16						
	8	Murcott	36	15	S	21	1318	1.6	November 2004	Autumn 2004
	1	Minneola	13	6	S	62	6142	1	March 2005	Autumn 2004
	36	Taylor Lee	3	7	L	119	3652	3.3	April 2005	Spring 2004
	24	Unknown	Unknown	Unknown	VL	1	1	100	November 2004	Unknown
	41	Minneola			VL	5	1171	0.4	February 2005	Unknown
IP3		Lemon, Grapefruit, Hickson	3–4	3	S	52	1340	3.9	May 2005	Autumn 2004

[A]The citrus hosts listed are Cara Cara, a sweet orange selection; Imperial, Hickson and Taylor Lee, mandarin varieties; Murcott a tangerine hybrid and the tangelo variety Minneola.

[B]Maximum disease severity observed on the block, VL = very low, L = low, M = moderate, S = severe.

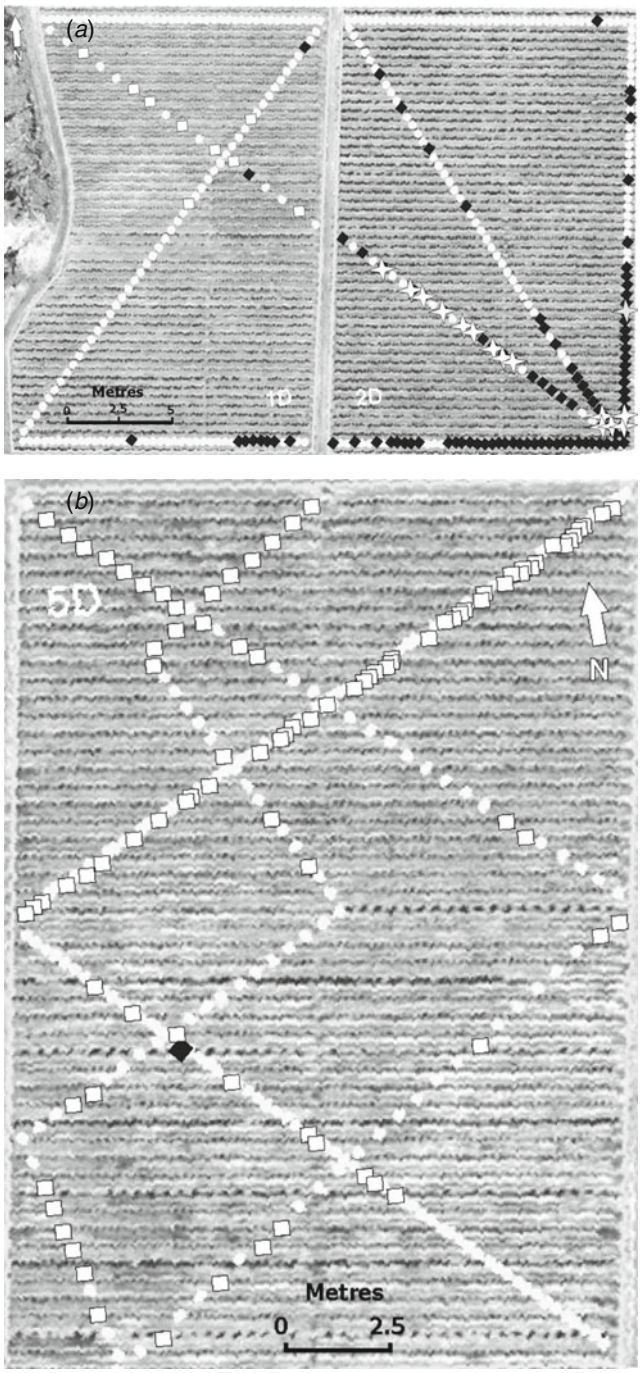

Fig. 2. The distribution of citrus canker on infested premise-1 blocks (*a*) 1D and 2D, and (*b*) 5D. The trees inspected along each transect and their respective disease severity (white dot = no symptoms, white square = very low, black diamond = moderate, white star = severe) are shown.

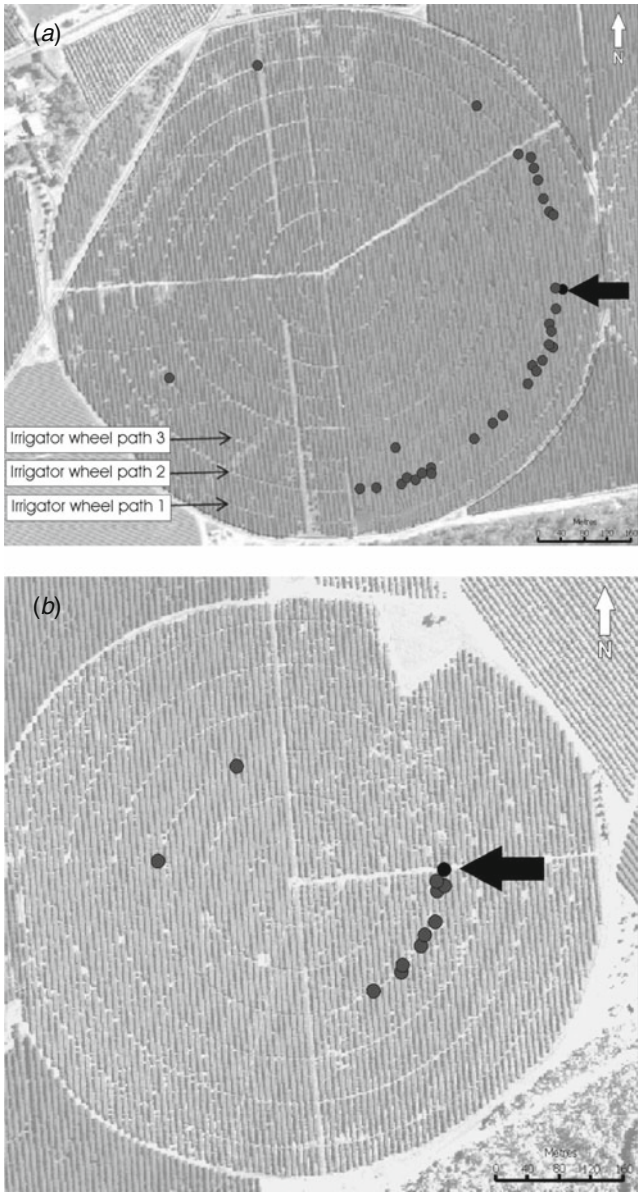

Fig. 3. The distribution of citrus canker on infested premise-2 blocks (*a*) 7 and (*b*) 8. Grey dots represent infected trees and primary disease foci are indicated with a black dot and arrow. Selected irrigator wheel paths and row numbers on Block 7 (*a*) are indicated by arrows. Pivot irrigators operated in both a clockwise and anticlockwise direction.

Evaluation of the sensitivity of surveillance for citrus canker

Inspection of trees for citrus canker by surveillance staff typically involved two people, one on either side, for large trees (>2 m high), whereas smaller trees (<2 m high) were inspected on all sides by a single person. To evaluate the sensitivity of detection of citrus canker using these strategies, a surveillance sensitivity study was conducted in a block of 306 large mature Taylor Lee mandarin trees (IP2 Block 36), and a block of 200 small immature grapefruit and lemon trees (IP3). The trees on these blocks were inspected once by surveillance officers, then a second time, more intensively, as part of the field evaluations conducted by pathologists. The sensitivity of detection of each surveillance strategy was then calculated as a percentage of the detections recorded by the pathologist.

C. glauca *surveillance*

C. glauca has been shown to be an experimental host of *X. citri* subsp. *citri* (Peltier 1918; Peltier and Frederich 1920, 1924; Hailstones *et al*. 2005). Inspection of all *C. glauca* plants within a 600–1800-m zone adjacent to commercial citrus properties was done by surveillance staff using a similar method to that described above. Suspect citrus canker lesions were sampled and sent for diagnostic testing.

Statistical analyses

All analyses were performed using GENSTAT 9th edition data analysis software (Lawes Agricultural Trust, Rothamsted Experimental Station, UK). The proportions of infected trees for the different canopy zones were compared using a chi-square test. If this overall test was significant ($P < 0.05$), further chi-square tests were applied using the method described by Fleiss (1981) to identify the zones that contributed to the significant difference. Disease spread in association with the pivot irrigation equipment on IP2 Blocks 7 and 8 was investigated using a two-sample binomial test to compare the disease incidence on trees adjacent to, and one position away from, the irrigator wheel path.

Weather data

Weather data collected from the nearest weather station located at the Emerald airport (Fig. 1), ~5–10 km from the IP, were sourced from the Bureau of Meteorology (http://www.bom.gov.au, accessed 23 July 2009). To identify the direction of wind-driven rain experienced in Emerald between January 2003 and July 2004, the direction of wind gusts of at least 8 m/s experienced during rainfall of at least 0.32 cm/h were plotted using the wind rose software WRPLOT View, available through Lakes Environmental Software (http://www.weblakes.com), Ontario, Canada. The parameters for wind speed and rainfall were described by Irey *et al*. (2006) in their development of the WRIV model to explain long distance spread of citrus canker in Florida.

Results

Pathogen identification

Typical symptoms of citrus canker were observed in the PQA on leaf, stem and fruit tissues (Fig. 4*a–e*) and included several varieties such as orange, mandarin, lemon, grapefruit, tangor, tangelo and citrange. Lesions were either from natural infections of stomata or from physical wounding associated with movement of equipment, wind damage, leaf miner feeding or other insect-feeding damage. The field visual identifications of infected trees were confirmed by sampling and indexing the samples for the presence of *X. citri* subsp. *citri* by PCR. Representative presumptive infected trees on IP2 Blocks 7 and 8 and from IP1 study blocks and all presumptive infected trees on the remaining IP2 study blocks and IP3 were confirmed infected by PCR.

Disease establishment

On IP1, the oldest infected stem was observed on a Cara Cara orange tree from Block 2D. This stem was also the oldest present on the tree. As the tree was at least 3 years old, it was not possible to estimate the age of the infected stem by counting back through flush events and instead phenological measurements were taken. The stem measured ~36 mm in diameter, was 80% covered with bark and had two to four growth rings. The stem contained several citrus canker lesions of 1.6–5.4 mm in diameter that were assumed to be derived from natural infections. The presence of *X. citri* subsp. *citri* within this stem material was confirmed by PCR as described above. The age of the oldest infected stem material on IP2 and IP3 was estimated by counting back through recent flushes and indicated the disease established on these premises during autumn 2004 (Table 1). The disease established in three IP2 blocks during this time.

Epidemiology studies

Transects studied in IP1 Blocks 1D and 2D showed the disease incidence was high and occurred mostly in the south-eastern corner of Block 2D (Table 1; Fig. 2*a*) but did not identify the foci of disease on these blocks. Of the 289 trees inspected, 15 were severely affected by citrus canker, but most of the remaining trees had very low disease severity. By contrast, on Block 5D the disease incidence and severity was lower, with moderately affected trees the maximum severity detected from the 238 trees inspected and this was limited to only a few trees (Table 1; Fig. 2*b*). Citrus canker was detected in all but 1 of the 18 blocks in total surveyed on IP1 and included the oldest blocks on the farm, established in 2000 (Stage 1) and also the newer plantings, established in 2003–04 (Stage 2) less than 1 km away. The incidence and severity of the disease was higher in Stage 1. The disease was also detected in the screenhouse nursery present on the farm.

The incidence of citrus canker on IP2 and IP3 was much lower than that on IP1 and with the exception of a few trees the disease severity on IP2 and IP3 was also low (Table 1). On each pivot-irrigated block on IP2 (Blocks 7 and 8) single trees were identified as the disease foci and statistical analyses supported the conclusion that disease was spread from these points by the pivot irrigator machinery (Block 7, $P < 0.001$ and Block 8, $P = 0.005$). This spread was probably via a mechanical abrasion in the presence of abundant water, as damage to trees by the irrigation equipment was observed on both blocks. On Block 7, of the 234 trees inspected next to the second wheel path, 87 were infected, compared with only 3 of the 229 adjacent trees (Fig. 3*a*). Similarly, on Block 8, 16 of the 144 trees next to the fourth wheel path were infected, compared with only 4 of the 144 next adjacent trees (Fig. 3*b*). Two further infected trees were detected on the third irrigator wheel path of Block 7 but were neither close to each other nor to any other infected tree. Although most of the infections detected on Block 7 were of Imperial mandarin trees, infected Murcott tangerine and Hickson mandarin trees were also detected.

On Block 1 (IP2), two Minneola tangelo trees both of which had been overgrown by their Troyer citrange rootstock, which is a citrus variety highly susceptible to citrus canker (Peltier 1918; Peltier and Frederich 1920, 1924) were identified as the disease foci. From this point the disease spread to five nearby trees during spring 2004, then further and mostly in a north-westerly direction to another 43 trees during summer 2004–05

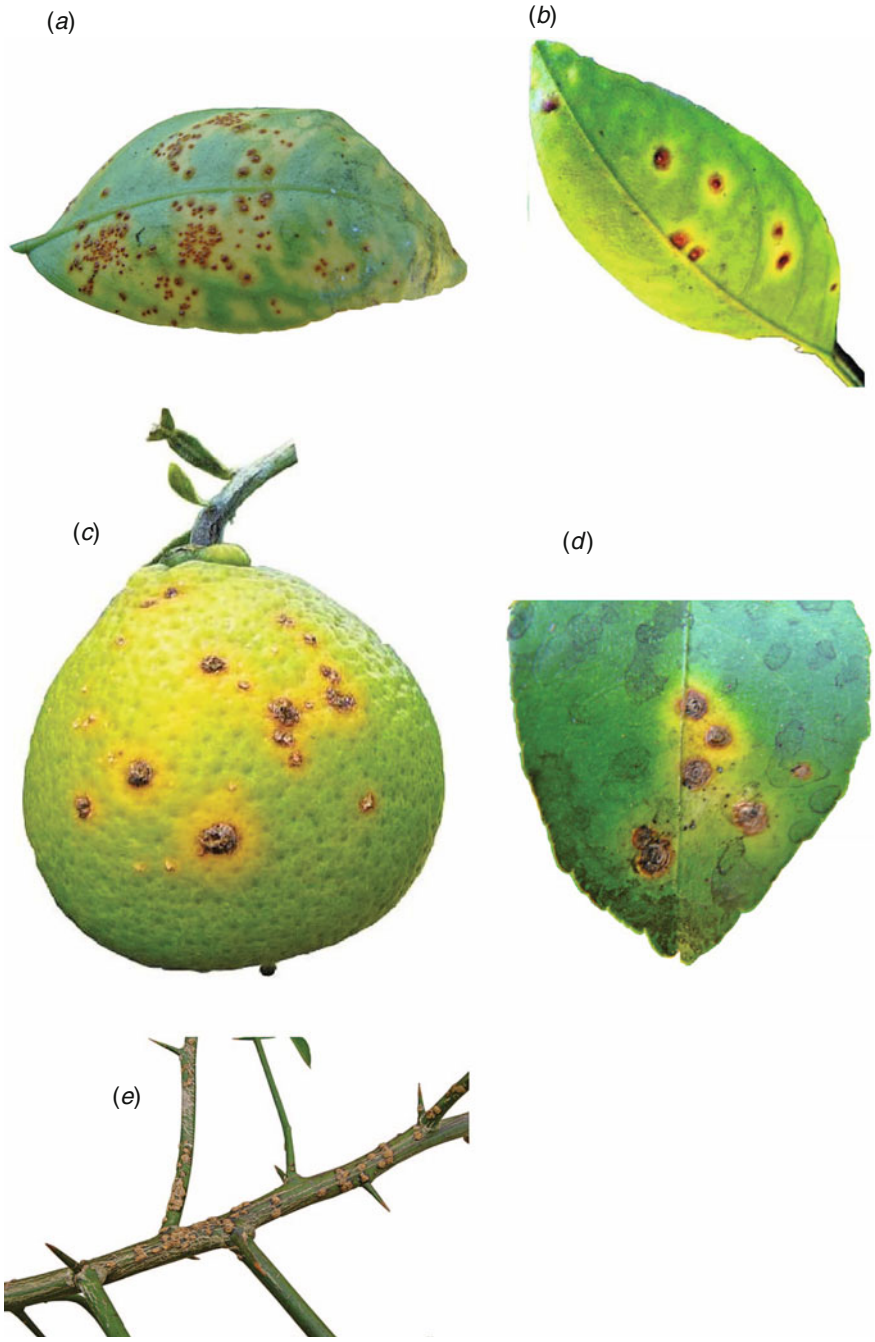

Fig. 4. Photographs of typical citrus canker symptoms observed on samples collected from (*a*, *b* and *c*) the citrus tangelo variety Minneola and (*d* and *e*) the citrange rootstock Troyer grown on infested premise-2, Block 1. Lesions present on leaf tissue developed during (*a*) a summer flush and photographed within 1–3 months after infection and that on (*d*) an autumn flush and photographed 12 months after infection.

(Fig. 5*a*). The disease was significantly associated with the eastern side and the middle-upper sections of the canopy (Tables 2 and 3; $P < 0.001$ and $P < 0.01$, respectively).

On Block 36 (IP2), seven Taylor Lee mandarin trees, widely dispersed, all had symptoms present on spring 2004 tissues (Fig. 5*b*). One of these seven trees was overgrown by its Troyer citrange rootstock and was rated the most severely affected tree on the block. Although it was the most severely affected tree detected, its location relative to the remaining six trees meant it was unlikely to be the disease focus. Instead the disease appears to have spread from all seven trees mostly along the rows (Fig. 5*b*). The presence of the disease was not strongly associated with either side of the canopy ($P = 0.053$) but was predominantly found in the middle canopy section ($P < 0.001$)

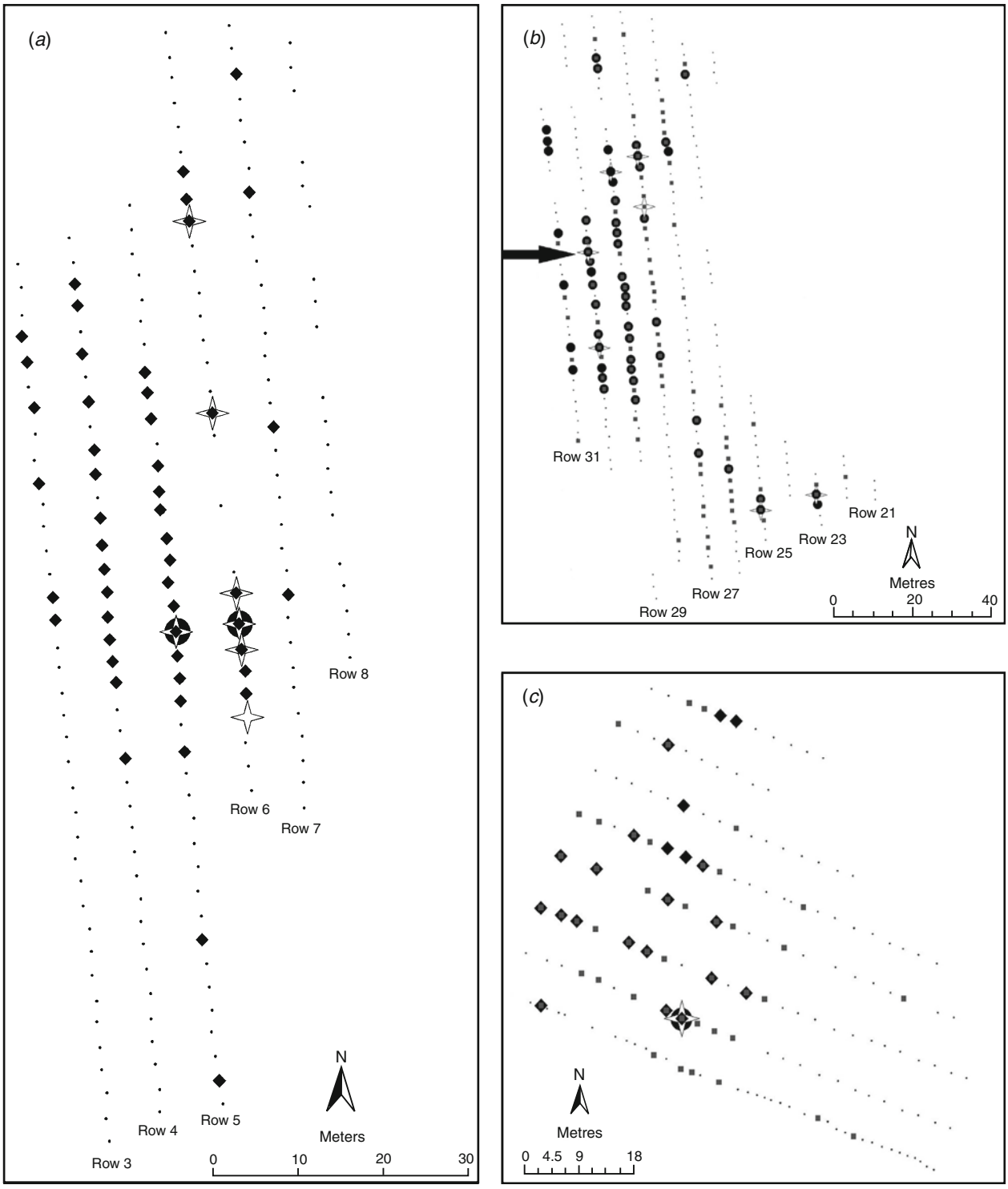

Fig. 5. The distribution of citrus canker on (*a*) infested premise-2 (IP2) Block 1, (*b*) IP2 Block 36 and (*c*) infested premise-3 (IP3). In (*a*) and (*c*) the primary disease foci are equivalent to the oldest infected flush (autumn 2004) and indicated with large black dots. White stars represent infected spring 2004, black diamonds summer 2005, grey squares autumn 2005 flush and uninfected trees are represented by small black dots. Selected row numbers are indicated and row 3 represents the edge of Block 1. No citrus trees were grown to the west of this row. Similarly, the northern and western ends of this IP3 block are shown. In (*b*) the most severely affected tree is indicated with a black arrow and the seven trees with the oldest infected flush (spring 2004) are indicated with white stars. Two distinct summer 2005 infected flushes were observed and these are indicated by black dots and grey squares, with the black dots representing the older of the two summer flushes. Uninfected trees are represented by small black dots. Selected row numbers are shown and row 31 represents the edge of the block. No citrus trees were grown to the west of this row, but the block continued further in the remaining three directions than is shown.

(Tables 2 and 3). Furthermore, the trees were large and protruded into or across the inter-row space. Citrus canker was also detected on five trees on Block 41 (IP2) all of which had low disease severity and on a single tree on Block 24 (IP2).

On IP3, the disease spread from a single grapefruit tree to 21 grapefruit and lemon trees to the north and north-west during summer 2004–05, then in various directions to a further 30 trees in autumn 2005 (Fig. 5*c*).

Sensitivity of surveillance for citrus canker

The sensitivity of detection of citrus canker on large trees by surveillance staff (Block 36) was calculated to be 42%. Closer inspection by pathologists revealed that approximately two-thirds of the infected trees on this block had a disease severity of fewer than 10 infected leaves per tree. Although disease severity was similar at the second site which had much smaller trees, the sensitivity was greater, with 72% of infected trees detected during surveillance.

Establishment and spread of citrus canker in the Emerald climate

The Emerald region has a dry winter (~30 mm of rainfall/month) and a wet summer (90–100 mm of rainfall/month), with average minimum and maximum temperatures of 8 and 23°C in winter and 21 and 34°C in summer. Disease symptoms were observed on plant flushes which developed during autumn, summer and spring and using data for disease spread within Block 1 (IP2) and IP3, the rate of natural spread under Emerald climatic conditions was estimated. For each autumn-established disease focus tree, a further 0–3 trees were infected during spring, and then 6–21 trees, for each previously infected tree, became infected with citrus canker during summer. During the following autumn disease spread decreased with only 1–2 new trees developing disease per previously infected tree.

To evaluate spread from IP1 to IP2 and IP3 from storm activity, the WRIV model used by Irey *et al*. (2006) was applied. Using this model, weather conditions were observed which potentially could have resulted in spread of the disease over long distances within the PQA, but the direction of spread was in a south-south-westerly direction. There were no commercial citrus plantations present in this direction.

Table 2. The proportion of infected trees with citrus canker symptoms on different sides of the canopy for those trees on infested premise-2 Blocks 1 and 36

Disease symptoms were observed at more than one location on some trees

Block	Total infected trees	Canopy side	Infected trees per side	Proportion per side
1	62	West	21	0.339
		East	36	0.581
		North	15	0.242
		South	12	0.194
36	119	West	86	0.723
		East	72	0.605

Table 3. The proportion of infected trees with citrus canker symptoms on different height zones of the canopy for those trees on infected premise-2 Blocks 1 and 36

Disease symptoms were observed at more than one height on some trees

Block	Total infected trees	Canopy height	Infected trees per zone	Proportion per zone
1	62	Low	13	0.210
		Middle	26	0.419
		High	34	0.548
36	119	Low	50	0.420
		Middle	92	0.773
		High	54	0.454

C. glauca *surveillance*

C. glauca plants growing in the Emerald district were typically stunted and had low amounts of foliage compared with plants growing in subtropical or tropical environments. Approximately 350 000 *C. glauca* plants were inspected by surveillance staff and 52 suspect samples were taken. All samples tested negative either by visual examination or by PCR indexing.

Discussion

The severity, incidence and wide distribution of citrus canker present on IP1 compared with IP2 and IP3 suggest that the disease established on this farm and subsequently spread to the other two farms during autumn 2004, though the mechanism for this spread remains unknown. There was no evidence to suggest that the disease was introduced into IP1, IP2 and IP3 each as unique incursions into Australia. Determining when the disease established on IP1 was difficult as it depended on estimating the age of old infections of stems or trunks, which relies on interpretation of growth rings, stem diameters and bark development, all of which can vary enormously with variety, climate and cultural practices (Schubert *et al*. 2001; Abrams and Hock 2006). Additionally, the susceptibility of stem tissue to infection via natural openings as it ages is unclear. Some researchers have reported that stems lose susceptibility as they approach maturity (Goto 1962, 1972) whereas others indicate infection can occur on mature stems (Peltier and Frederich 1926). Given the imprecise nature of estimating the age of old infections, an accurate estimate of when citrus canker was established on IP1 was not possible, but the mature age of the infected stem and the large lesions present on it, indicate the disease was present for several months before its detection in July 2004.

In comparison, determining the three primary disease establishment points on IP2 and the single point on IP3 was possible by estimating the age of the plant growth, as infected tissues were all on recent vegetative flushes. All four disease establishment points appear to have developed during autumn 2004 and are all 9–11 km north-west from IP1. Irey *et al*. (2006) developed the WRIV model to explain that storm activity was the mechanism responsible for most long distance spread of citrus canker in Florida. Applying this model to the Emerald outbreak, the only potential long distance spread of the disease from IP1 was to the south-south-west, the opposite direction to IP2 and IP3.

There is no substantive evidence to identify the mechanism of spread of citrus canker from IP1 to the other properties. However, considering the disease foci on IP2 and IP3 all appear to have developed at the same time, spread by a single event is considered more probable than the simultaneous spread by more than one mechanism of dispersal. Tracing investigations have not confirmed any movement of equipment or persons between IP1 and the other infested premises, but the possibility of such movements cannot be entirely discounted. IP2 and IP3 are downstream from IP1 along the Nogoa River and the possibility of runoff water from IP1 contaminating this irrigation source with the bacterium was considered but thought unlikely for several reasons. First, only one rainfall event was identified which could have resulted in floodwater overflowing the IP1 on-farm dam and entering the river. This rainfall was in mid summer and as the primary infection foci on IP2 and IP3 are dated as autumn, this was too early to explain disease transfer. Second, a more uniform pattern of disease incidence would be expected from bacterial cells dispersed in irrigation water. Third, *X. citri* subsp. *citri* does not survive well when highly diluted in water (Goto 1992), and finally the irrigation systems used on the farms, with the exception of those used on the IP2 pivot blocks, were filtered thus preventing uptake of any small pieces of infected tissue if present in the river water. Furthermore, most of the trees on IP2 and IP3 were drip-irrigated, thus there would have been spatial separation between susceptible foliage and the delivery point of any potential inoculum in the irrigation water. The IP2 pivot blocks were not filtered and infected leaves or fragments thereof could have passed through and come into contact with wet leaves that were damaged by the irrigation travelling tower. However, if this had occurred from the mid-summer rainfall event, a higher disease incidence and severity on these blocks might have been expected, especially considering the frequent use of the pivot irrigators. Other mechanisms of spread have been considered, such as the movement of citrus debris by wildlife (birds, pigs etc.), in windstorms and through deliberate acts. However, in the absence of any substantive evidence or confirmatory studies, supporting arguments for any particular mechanism/s of inter-property spread are highly speculative and inconclusive.

Several different mechanisms of intra-block disease spread were observed during this study. The distances and directional nature of disease spread within Block 1 (IP2) and IP3 are similar to that observed in studies conducted in grove plantations in Argentina (Gottwald *et al*. 1988) in which the spread was attributed to wind-driven rain. Furthermore, on Block 1, the presence of the disease was associated with one side and the middle-upper sections of the tree canopies, which also shows the spread was directional. A possible alternative mechanism for this observed disease spread is via dispersal of bacteria or infected plant tissues during routine crop sprays which are applied at high pressure. However, in this case, a more even distribution of disease around the focal point would be expected, rather than the biased pattern of disease spread that was observed. Therefore, it is concluded that wind-driven rain was responsible for the spread of citrus canker on Block 1 and IP3. In contrast, disease spread on Block 36 (IP2) was mostly along the rows, predominantly to one section of the canopy and relatively evenly distributed on both sides of the rows. This, in combination with the protrusion of tree canopies into the inter-row spaces, suggests that disease transfer was probably from farm equipment moving along the rows. Spread of citrus canker within Blocks 7 and 8 was clearly in association with movement of the pivot irrigators. The age of infected tissues, low amount of disease incidence and severity on IP2 suggests it is unlikely that spread between blocks occurred as a result of storm activity. It appears three separate, simultaneous, introductions of the pathogen into IP2 (Blocks 1, 7 and 8) occurred and subsequent spread to the remaining three blocks (Blocks 36, 24 and 41) was probably via equipment or people involved with routine crop maintenance.

Climatic conditions affect both the development and rate of spread of citrus canker (Peltier 1920; Peltier and Frederich 1926; Kuhara 1978; Serizawa 1981; Irey *et al*. 2006; Pria *et al*. 2006). In the Emerald region, although infections occurred during autumn, the disease spread was very limited and in contrast to that observed during summer. Thus, it was important to complete several successive rounds of surveillance to allow both the incidence and severity to increase to detectable levels. This was done and disease detections on IP2 and IP3 occurred in survey rounds which followed periods of climatic conditions optimal for disease development and spread. The results from this study also show that the sensitivity of citrus canker detection is affected by tree size and maturity.

The limited distance of intra-block spread during wet weather from recently established disease foci supports the eradication strategy first implemented in the Emerald PQA. The strategy was based on that used in eradication campaigns in Florida (Schubert *et al*. 2001) and involved the destruction of all host plants within a 600-m radius of the most distally infected plants. However, citrus canker was also spread between blocks on IP2 to distances greater than 600 m, most likely as a result of movement of contaminated people or equipment from a disease-affected block to an unaffected block. As the initial eradication strategy would not have encompassed these outlying infestations, and in consideration of all possible modes of disease transfer, the eradication strategy was subsequently changed to encompass the whole PQA as a destruction zone. Furthermore, the results from this study suggest that eradication strategies should be developed and modified according to individual disease outbreaks, based on epidemiological information and risk analysis. For very recent disease outbreaks where intra-block spread is minimal a small circular radius or preferably a block-based eradication zone may be applicable, but for longer established outbreaks where the risk of inter-block or inter-premise spread is greater, a more expansive eradication strategy may be necessary.

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