Eur J Plant Pathol (2009) 124:1–7
DOI 10.1007/s10658-008-9389-8

Modelling the progress of Asiatic citrus canker on Tahiti lime in relation to temperature and leaf wetness

R. S. C. Christiano · M. Dalla Pria · W. C. Jesus Junior · L. Amorim · A. Bergamin Filho

Received: 25 March 2008 / Accepted: 13 October 2008 / Published online: 28 October 2008

Abstract The combined effect of temperature (15°C, 20°C, 25°C, 30°C, 35°C, 40°C and 42°C) and leaf wetness duration (0, 4, 8 12, 16, 20 and 24 h) on infection and development of Asiatic citrus canker (*Xanthomonas citri* subsp. *citri*) on Tahiti lime plant was examined in growth chambers. No disease developed at 42°C and zero hours of leaf wetness. Periods of leaf wetness as short as 4 h were sufficient for citrus canker infection. However, a longer leaf duration wetness (24 h) did not result in much increase in the incidence of citrus canker, but led to twice the number of lesions and four times the disease severity. Temperature was the greatest factor influencing disease development. At optimum temperatures (25–35°C), there was 100% disease incidence. Maximum disease development was observed at 30–35°C, with up to a 12-fold increase in lesion density, a 10-fold increase in lesion size and a 60-fold increase in disease severity.

Keywords *Xanthomonas* · *Citrus latifolia* · Monomolecular · Generalised beta · Moisture · Epidemiology

R. S. C. Christiano (✉) · M. Dalla Pria · W. C. Jesus Junior · L. Amorim · A. Bergamin Filho
Department of Entomology, Phytopathology and Zoology, ESALQ - University of São Paulo, Piracicaba, SP, Brazil 13418-900
e-mail: rockchristiano@gmail.com

Introduction

Tahiti lime (*Citrus latifolia*), also known as Persian lime, is highly sensitive to cold weather and is grown exclusively in tropical climates. Major producers of Tahiti lime are Mexico and Brazil. In Brazil, São Paulo State has ~80% of the national production of Tahiti lime fruit (Anonymous 2007a). These fruits are primarily destined for domestic and foreign fresh fruit markets.

The Asiatic citrus canker, caused by the bacterium *Xanthomonas citri* subsp. *citri* (Gabriel et al. 1989), subsp. nov. (*Xcc*), formerly *X. axonopodis* pv. *citri*, is one of the most severe diseases in São Paulo citriculture. Present in the state since 1957 (Rossetti 1977), citrus canker has been quarantined and subject to a continuous eradication effort. *Xcc* infects through stomata and wounds on the leaves, stems and fruit. Infection via stomata occurs only in immature leaves, causing a raised corky lesion, visible on both sides (McLean 1921). Severe disease causes fruit drop, defoliation, dieback and overall decline of trees. Citrus canker has increased with the introduction of the Asian citrus leafminer (*Phyllocnistis citrella*). This insect wounds leaves and exposes mesophyll tissues to *Xcc* infection, increasing the threat of *Xcc* to citrus production (Gottwald et al. 1997; Bergamin Filho et al. 2000; Christiano et al. 2006).

In addition, quarantine restrictions of citrus canker on national and international trade of seedlings and fresh fruit have caused great economic impact. For

example, the US no longer has commercial lime production, reflecting the loss of trees due to the citrus canker eradication programme in Florida (Anonymous 2007b). Lime production fell from 44 million pounds in 2000 to zero in 2003. Consequently, the US now imports limes from Mexico and other countries to compensate for the estimated 2.15 pounds per capita per year demand.

Environmental conditions, mainly temperature and moisture, play a fundamental role in the development of disease epidemics. Early studies suggest a temperature range of 20–30°C is necessary for citrus canker development (Peltier 1920; Koizumi 1977). However, a recent study showed citrus canker develops best at 30–35°C (Dalla Pria et al. 2006). In addition, temperature influences the symptom expression and length of incubation period, reducing or increasing it (Koizumi 1976; Dalla Pria et al. 2006). In each citrus species, the bacterium can express unique behaviour under the same environmental conditions (Peltier 1920). Free moisture is essential for bacterial dissemination and initiation of an infection process. Only a few minutes of free moisture are required for exudation of bacteria from citrus canker lesions (Timmer et al. 1991; Pruvost et al. 2002). However, the duration of free moisture necessary for bacterial infection through stomatal openings is still unclear (Goto 1992; Gottwald and Graham 1992; Graham et al. 1992). Understanding the conditions that cause citrus canker epidemics is fundamental to developing effective disease control strategies.

The objectives of this study were to quantify the effects of temperature and moisture on infection and the colonisation processes of *Xcc* in growth chambers so as to explain the development of epidemics under field conditions (Krans and Hau 1980; Rotem 1988). The influence of temperature and leaf wetness duration on disease incidence, length of incubation period, lesion density, lesion size, and disease severity of Asiatic citrus canker was quantified on Tahiti lime leaves, under controlled conditions.

Materials and methods

The *Xcc* (isolate IBSBF 1421) was cultivated on nutrient agar medium for 48 h at ~28°C. The bacterial culture was diluted in sterile distilled water and the suspension was calibrated using a colorimeter. Tahiti lime plants were obtained by budding onto *Poncirus trifoliata* in plastic tubes (20×1.5 cm). Plants 25 cm tall with fresh flush (immature leaves) were spray-inoculated with a suspension of *Xcc* at 10^6 CFU ml^{-1}. Spray inoculation was accomplished by atomising the inoculum at a distance of 25 cm from the leaves until run-off.

Inoculated plants were wrapped in wet plastic bags (moist chamber conditions) and transferred into growth chambers (Conviron E-7) at temperatures of 15°C, 20°C, 25°C, 30°C, 35°C, 40°C and 42°C, and photoperiod of 12 h. Effect of leaf wetness duration was studied by maintaining the plants in moist chamber conditions for 0, 4, 8, 12, 16, 20 and 24 h. Forty-eight h after inoculation, the growth chambers of treatments at 40°C and 42°C were set to 35°C during the dark period to avoid excessive heat stress on the plants. The experiment consisted of 49 treatments (seven temperatures × seven leaf wetness durations), arranged in a split-plot design with temperature as main plots and wetness as subplots, with eight plants per subplot. The experiment was conducted three times.

Disease variables of citrus canker evaluated were: disease incidence, length of incubation period, disease severity, lesion density, and lesion size. Disease incidence of plants was assessed every 2 days. Incubation period was defined as time from inoculation to appearance of visible symptoms on 50% of the final infected plant units (Vanderplank 1963). Two symptomatic leaves per plant were collected and digitalised at 30, 40, and 60 days after inoculation for treatments at 25–42°C, 20°C, and 15°C, respectively. Lesion size and disease severity were measured by software, QUANT v. 1.0 (Vale et al. 2001). Disease severity was percentage of diseased area of leaves. The lesion size was calculated by the total lesion area, divided by the total number of lesions. The lesion density was obtained by dividing the number of lesions by the foliar area.

Disease incidence data were used to plot disease progress curves. The standardised area under the disease progress curve (AUDPC) for each treatment was calculated by trapezoidal integration of the respective observation period. Using non-linear regression of STATISTICA software (StatSoft, Inc. 2001), the generalised beta function (Hau and Kranz 1990) and the monomolecular model (Campbell and Madden 1990) were respectively fitted to the data to

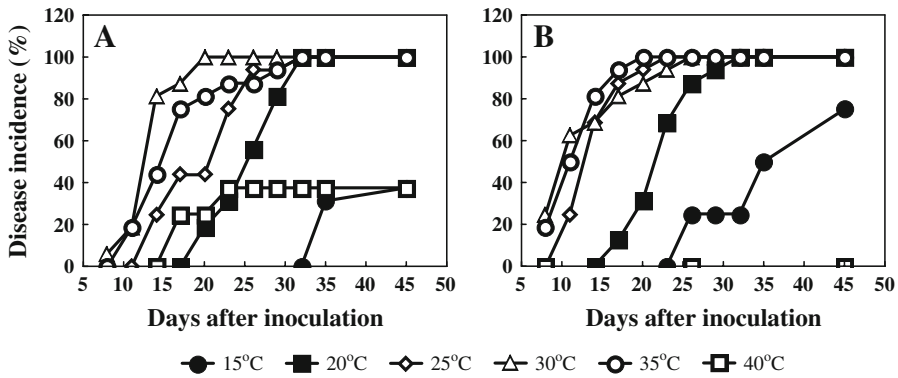

Fig. 1 Progress of citrus canker (disease incidence) on Tahiti lime under different temperatures with leaf wetness duration of 4 h (**a**) and 24 h (**b**). Each point represents the mean of three experiments

describe effects of temperature and leaf wetness duration on AUDPC, lesion density, lesion size, and disease severity. The generalised beta function is described as $Y = b_1(T\text{-}b_2)^{b3}(b_4\text{-}T)^{b5}$, and the monomolecular model is $Y = b_6(1\text{-}b_7\exp(\text{-}b_8M))$. Estimated parameters are b_1–b_8; minimum and maximum temperatures are b_2 and b_4; T is temperature (°C); M is leaf wetness duration (h); and Y is one of the specified disease variables (AUDPC, lesion density, lesion size, or disease severity). Combining the effect of temperature and leaf wetness duration allowed the fitting of a response surface of the disease variables, namely a monomolecular-beta function: $Y = b_1(T\text{-}b_2)^{b3}(b_4\text{-}T)^{b5}b_6(1\text{-}b_7\exp(\text{-}b_8M))$ (Hau and Kranz 1990).

Results

Citrus canker symptoms on Tahiti lime plants were observed at all temperatures except 42°C, where leaves fell off the plants due to excessive heat. For all treatments at 0 h wetness, no disease symptoms were observed. Disease incidence progress under each tested temperature at 4 and 24 h leaf wetness duration are shown in Fig. 1. All plants at 20–35°C showed symptoms of citrus canker (100% of disease incidence). At 15°C, a minimum final disease incidence of 40% was obtained with 4 h leaf wetness, and a maximum incidence of 75% with 24 h leaf wetness. At 40°C, disease appeared with 4–12 h leaf wetness, with the final incidence <50%.

The mean incubation period was 24, 14, 11, 10 and 12 days at 20°C, 25°C, 30°C, 35°C and 40°C, respectively. At 15°C, lesions appeared >26 days post-inoculation. The effect of leaf wetness duration on incubation period was minimal: at most the difference in the length of incubation period was 3 days between different wetness periods, except at 15°C when it was >10 days.

Disease severity and lesion density were weakly affected by leaf wetness duration as shown by the monomolecular model (data shown only for disease severity at 30°C, Fig. 2a and Table 1). There was a slight increase with prolonged leaf wetness at each

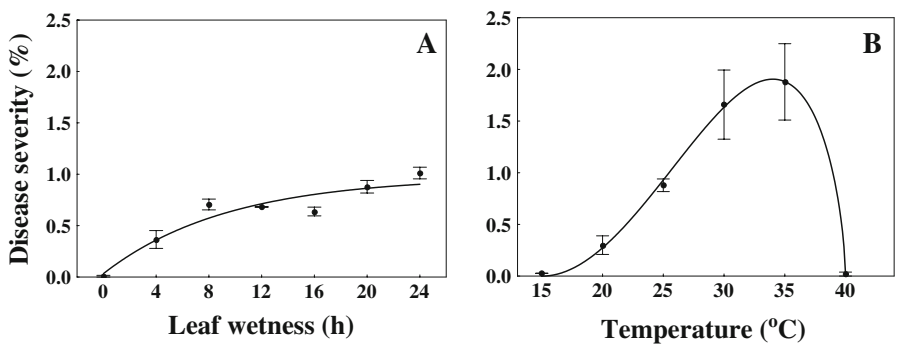

Fig. 2 Effect of leaf wetness duration at 30°C **a** and effect of temperature with 24 h leaf wetness duration **b** on disease severity of citrus canker on Tahiti lime. Each point represents the mean of three experiments. Vertical bars represent standard errors. Lines represent the values predicted by the monomolecular model (**a**) and the generalised beta function (**b**). Estimated parameters are shown in Table 1

Table 1 Estimated parameters and coefficients of determination (R^2) of non-linear models fitted to describe the relationship of AUDPC (Fig. 3a), lesion density (Fig. 3b), lesion size (Fig. 3c), and disease severity (Fig. 2a,b and d) of citrus canker on Tahiti lime with temperature and leaf wetness duration

	Parameters								R^2
	b_1 (SE)[a]	b_2 (SE)	b_3 (SE)	b_4 (SE)	b_5 (SE)	b_6 (SE)	b_7 (SE)	b_8 (SE)	
AUDPC*	1.58[b] (0.71)	14.0 (0.61)	0.87 (0.01)	40.0 (0.01)	0.077 (0.16)	1.69 (5.26)	0.003 (0.10)	0.67 (0.04)	0,89
Lesion density	0.12[b] (0.14)	13.0 (3.41)	1.17 (0.56)	40.0 (6.59)	0.202 (0.01)	0.30 (0.01)	0.005 (0.02)	0.14 (0.80)	0.72
Lesion size	0.18[b] (0.41)	14.4 (2.48)	1.12 (0.01)	40.6 (0.19)	0.695 (0.89)	0.06 (0.01)	-3.5E-3 (0.09)	2.54 (24.47)	0.87
Disease severity	–	–	–	–	–	0.97[c] (0.09)	0.03 (0.01)	0.11 (0.31)	0,89
	0.001[d] (0.36)	15.0 (5.55)	2.02 (0.01)	40.0 (0.70)	0.63 (0.01)	–	–	–	0.99
	0.001[b] (0.01)	13.0 (11.75)	2.21 (1.48)	40.0 (2.50)	0.75 (0.01)	1.44 (0.01)	0.028 (0.01)	0.02 (0.06)	0.94

[a] Standard errors of the estimated parameters are given in parentheses.

[b] Estimated parameters of the monomolecular-beta function: $Y = b_1(T\text{-}b_2)^{b3}(b_4\text{-}T)^{b5}\, b_6(1\text{-}b_7\exp(-\ B_8M))$.

[c] Estimated parameters of the monomolecular model: $Y = b_6(1\text{-}b_7\exp(\text{-}b_8M))$ fitted to disease severity under different leaf wetness durations at 30°C.

[d] Estimated parameters of the generalised beta function: $Y = b_1(T\text{-}b_2)^{b3}(b_4\text{-}T)^{b5}$ fitted to disease severity under different temperatures at 24 h leaf wetness duration

temperature. Lesion size was not influenced by leaf wetness (data not shown). Lesion density, lesion size, and disease severity increased progressively with increasing temperature to ~35°C, after which they decreased rapidly up to 40°C as shown by the generalised beta function (data shown only for disease severity at 24 h leaf wetness, Fig. 2b and Table 1).

The monomolecular-beta function allowed the fit of a response surface for data of AUDPC, lesion density, lesion size, and disease severity under combinations of temperature and leaf wetness duration (Fig. 3, Table 1). AUDPC value increased with rising temperature up to 38°C (Fig. 3a). Leaf wetness duration >4 h had no effect on AUDPC.

Lesion density increased drammatically with rising temperature, from 0.15 lesion cm^{-2} at 15°C to 1.83 lesion cm^{-2} at 35°C with 24 h leaf wetness (Fig. 3b). Leaf wetness duration had less effect, for example, at 35°C, lesion density was about twice as great with 24 h as with 4 h leaf wetness (0.81 vs. 1.82 lesion cm^{-2}). Maximum estimated lesion density was 1.84 lesion cm^{-2} at 36°C with 24 h leaf wetness. Lesion size did not increase between 4 and 24 h leaf wetness (Fig. 3c). However, temperature was a crucial factor affecting lesion size. Lesion size was 10 times greater at 30°C than at 15°C. The maximum estimated lesion size was 1.31 mm^2 at 30.5°C.

The response surface of disease severity (Fig. 3d) shows disease severity increased considerably with increasing temperature and leaf wetness duration, with a maximum disease severity of 2.23% at 33.2°C and with 24 h leaf wetness. The minimum and maximum temperatures, estimated by the monomolecular-beta function, were 13°C and 40°C, respectively (Table 1). At 33.2°C and with 24 h wetness duration, disease severity was four times higher than with 4 h (0.58%) and 60 times higher than at 15°C (0.04%).

Discussion

Citrus canker infection on Tahiti lime leaves was able to occur in periods of free moisture as short as 4 h. Gottwald and Graham (1992) showed that only two cells of *Xcc* placed in a stomatal chamber are sufficient for infection and subsequent disease development. In addition, *Xcc* can modify the environments on citrus tissue surfaces, producing extracellular polysaccharides to aid in bacterial adhesion, survival, and infection (Goto and Hyodo 1985). Comparing

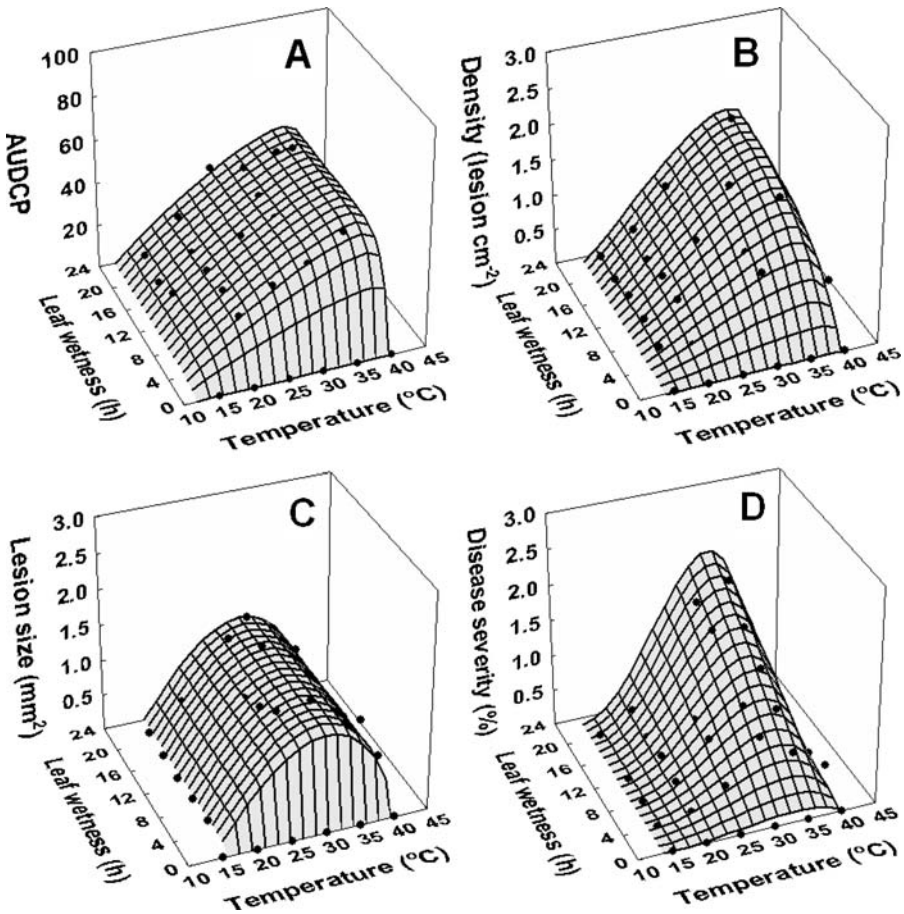

Fig. 3 Combined effect of temperature and leaf wetness duration on the AUDPC **a**, lesion density **b**, lesion size **c**, and disease severity **d** of citrus canker on Tahiti lime. Each point represents the mean of three repetitions. Lines represent the values predicted by the monomolecular-beta function. Estimated parameters are given in Table 1

leaf wetness duration of 4–24 h, a longer period of free moisture had a modest effect on citrus canker development, with lesion density increasing twice and disease severity by four times; however lesion size was not significantly affected by wetness duration.

Temperature had the greatest effect on citrus canker development. Temperatures in the range of 20–35°C resulted in disease on 100% of Tahiti plants. The optimum temperature range was 25–35°C. Maximum disease development was observed at 30–35°C, resulting in maximum disease severity with a minimum incubation period (10–11 days). At a low temperature (<15°C), citrus canker disease might be able to infect and develop; however, the disease took longer (>26 days) to become visible. In this study, the minimum temperature tested was 15°C, but citrus canker on Tahiti lime probably does not occur at or below 10°C, as demonstrated on other citrus species by Koizumi (1977) and Dalla Pria et al. (2006), who found that minimum temperature for infection is ~12°C. However, in greenhouse conditions, Peltier (1920) showed *Xcc* infection may take place at 5°C and remain latent until temperature increases. At high temperatures (>35°C), citrus canker was negatively affected, with decreasing disease severity. Maximum temperature for disease development was 40°C. At this temperature, disease only develops with short periods of leaf wetness (<12 h) probably due to the combination of high temperature and high humidity being a limiting factor for viability of *Xcc* (Peltier 1920).

Incubation period of citrus canker symptom was shortest (10 days) at the optimum temperature and longest (>26 days) at lower temperatures. Leaf wetness only slightly affected the incubation period by shortening it by 1–3 days under long periods of free moisture. These results support previous studies showing incubation period is primarily a function of temperature (Koizumi 1977; Verniere et al. 2003).

The size of citrus canker lesions is linked to the development of *Xcc* populations produced in the

citrus tissue (Graham et al. 1992). Therefore, temperature affects lesion size by influencing the rate of bacterial growth. In addition, Bock et al. (2005) found a positive relationship between temperature and number of bacteria dispersed. As expected, leaf wetness duration does not affect bacterial growth in tissues and may only be necessary for a short period to allow initial bacterial infection.

The effect of temperature and leaf wetness duration on citrus canker progress on Tahiti lime was similar on sweet orange varieties (Valencia, Pera, Natal, and Hamlin) under the same environmental conditions (Dalla Pria et al. 2006). Citrus canker disease on sweet orange varieties showed the same optimum temperature range of 25–35°C, with the incubation period varying between 10–14 days, a minimum leaf wetness duration of 4 h, and a maximum disease development at 30–35°C and with 24 h leaf wetness. Maximum disease severity on Tahiti lime plants was higher (2.23%) than Hamlin (1.86%), the highest disease severity observed on sweet oranges. Apparently, the Tahiti lime plant is as or more susceptible than Hamlin for citrus canker, supported by data from Gottwald et al. (2002) showing the two citrus species in the same resistance category.

Environmental conditions affect both the pathogen and the host plant. In the study of Reuther (1977), citrus growth rate increased progressively from 13°C to a maximum at 30°C, with growth halting above 40°C. The effect of temperature on citrus canker development is similar to the effect of temperature on citrus growth. In both organisms, maximum growth rate occurs at ~30°C. Thus, the conditions that support a high rate of growth in citrus are also responsible for the most rapid development of citrus canker caused by *Xcc*.

In tropical areas, the average yearly temperature range is 25–35°C, rarely higher than 40°C or lower than 10°C. Also, moisture is usually present in the early morning and this free moisture is sufficient for *Xcc* infection and survival, as shown in this study and others (Timmer et al. 1996; Verniere et al. 2003). Thus, if temperature and free moisture are satisfactory for citrus canker development, the main factors influencing occurrence of citrus canker in tropical areas are wind and rain, both of which influence dispersal of *Xcc* (Stall et al. 1980; Serizawa 1981; Bergamin Filho and Hughes 2000; Gottwald et al. 2002)

Unfortunately there is no chemical control for bacterial citrus canker. The only way to eradicate the disease is to destroy trees that are infected or exposed (Bergamin Filho and Hughes 2000; Gottwald et al. 2001, 2002). One infected tree near a citrus grove is capable of spreading bacteria for several miles in a tropical storm (Danos et al. 1984; Gottwald et al. 1997; Bergamin Filho et al. 2001). Citrus canker is extremely contagious; it only takes one infected tree or piece of fruit to start an outbreak, and a low inoculum level such as 10^1 cfu $ml^{-1}$is enough to infect Tahiti lime leaves injured by the Asian citrus leafminer, 10^2 cfu ml^{-1} to infect through wounds, and 10^4 cfu ml^{-1} to infect through stomata (Christiano et al. 2006). Inspection of citrus and use of preventive chemicals such as copper should be intensified in the summer, when high temperatures, wind, and rain are combined so as to minimise disease spread. Even in winter, in areas with intense dew, preventive action should be maintained when the temperature is >15°C. In addition, windbreaks (Gottwald and Timmer 1995) and disease eradication programmes (Gottwald et al. 2001) are important tools to consider in the management of citrus canker.

Acknowledgements This work was supported by FAPESP (Brazil). We thank Dr. Júlio Rodrigues Neto (Instituto Biologico, Campinas, SP, Brazil) for supplying the isolate IBSBF 1421.

References

Anonymous (2007a). *Brazil Agrianual 2007*. São Paulo: AgraFNP.

Anonymous (2007b). *Fruit and tree nuts: situation and outlook yearbook*. Economic Research Service, USDA. Retrieved January, 29 2007, http://www.ers.usda.gov/publications/FTS/2007/Yearbook/FTS2007.pdf

Bergamin Filho, A., & Hughes, G. (2000). *Citrus canker epidemiology — methodologies and approaches: a moderated discussion session. Proceedings, International Citrus Canker Research Workshop* (pp. 24–25). Ft. Pierce, Florida.

Bergamin Filho, A., Amorim, L., Gottwald, T. R., & Laranjeira, F. F. (2001). *Spatial distribution of citrus canker in São Paulo - Brazil. Proceedings of the 8th International Workshop on Plant Disease Epidemiology*. (pp. 28–29). Ouro Preto, Brazil.

Bergamin Filho, A., Amorim, L., Laranjeira, F., & Gottwald, T. R. (2000). *Epidemiology of citrus canker in Brazil with and without the Asian citrus leafminer. Proceedings, International Citrus Canker Research Workshop* (pp. 6). Ft. Pierce, Florida.

Bock, C. H., Parker, P. E., & Gottwald, T. R. (2005). Effect of simulated wind-driven rain on duration and distance of dispersal of *Xanthomonas axonopodis* pv. *citri* from canker-infected citrus trees. *Plant Disease*, *89*, 71–80. doi:10.1094/PD-89-0071.

Campbell, C. L., & Madden, L. V. (1990). *Introduction to plant disease epidemiology*. New York: Wiley.

Christiano, R. S. C., Dalla Pria, M., Jesus Junior, W. C., Amorim, L., & Bergamin Filho, A. (2006). Effect of citrus leaf-miner damage, mechanical damage and inoculum concentration on severity of symptoms of Asiatic citrus canker in Tahiti lime. *Crop Protection (Guildford, Surrey)*, *26*, 59–65. doi:10.1016/j.cropro.2006.03.016.

Dalla Pria, M., Christiano, R. C. S., Furtado, E. L., Amorim, L., & Bergamin Filho, A. (2006). Effect of temperature and leaf wetness duration on infection of sweet oranges by Asiatic citrus canker. *Plant Pathology*, *55*, 657–663. doi:10.1111/j.1365-3059.2006.01393.x.

Danos, E., Berger, R. D., & Stall, R. E. (1984). Temporal and spatial spread of citrus canker within groves. *Phytopathology*, *74*, 904–908.

Gabriel, D. W., Kingsley, M. T., Hunter, J. E., & Gottwald, T. (1989). Reinstatement of *Xanthomonas citri* (ex Hasse) and *X. phaseoli* (ex Smith) to species and reclassification of all *X. campestris* pv. *citri* strains. *International Journal of Systematic Bacteriology*, *39*, 14–22.

Goto, M. (1992). Citrus canker. In J. Kumar, H. S. Chaube, U. S. Singh, & A. N. Mukhopadhyay (Eds.), *Plant diseases of international importance* (pp. 250–269). Englewood Cliff: Prentice-Hall.

Goto, M., & Hyodo, H. (1985). Role of extracellular polysaccharides of *Xanthomonas campestris* pv. *citri* in the early stage of infection. *Annals of the Phytopathological Society of Japan*, *51*, 22–31.

Gottwald, T. R., & Graham, J. H. (1992). A device for precise and non disruptive stomatal inoculation of leaf tissue with bacterial pathogens. *Phytopathology*, *82*, 930–935. doi:10.1094/Phyto-82-930.

Gottwald, T. R., & Timmer, L. W. (1995). The efficacy of windbreaks in reducing the spread of citrus canker caused by *Xanthomonas campestris* pv. *citri*. *Tropical agriculturist*, *72*, 194–201.

Gottwald, T. R., Graham, J. H., & Schubert, T. S. (1997). Citrus canker in urban Miami: an analysis of spread and prognosis for the future. *Citrus Industry*, *78*, 72–78.

Gottwald, T. R., Graham, J. H., & Schubert, T. S. (2002). Citrus canker: The pathogen and its impact. *Plant Health Progress*, published online. doi:10.1094/PHP-2002-0812-01-RV.

Gottwald, T. R., Hughes, G., Graham, J. H., Sun, X., & Riley, T. (2001). The citrus canker epidemic in Florida: the scientific basis of regulatory policy for an invasive species. *Phytopathology*, *91*, 30–34. doi:10.1094/PHYTO.2001.91.1.30.

Graham, J. H., Gottwald, T. R., Riley, T. D., & Achor, D. (1992). Penetration through leaf stomata and strains of *Xanthomonas campestris* in citrus cultivars varying in susceptibility to bacterial diseases. *Phytopathology*, *82*, 1319–1325. doi:10.1094/Phyto-82-1319.

Hau, B., & Kranz, J. (1990). Mathematics and statistics for analysis in epidemiology. In J. Kranz (Ed.), *Epidemics of plant diseases. Mathematical analysis and modeling* (pp. 12–52). Berlin: Springer.

Koizumi, M. (1976). Incubation period of citrus canker in relation to temperature. *Bulletin of Fruit Tree Research*, *3*, 33–46.

Koizumi, M. (1977). Relation of temperature to the development of citrus canker in the spring. *Proceedings of the International Society of Citriculture*, *3*, 924–928.

Kranz, J., & Hau, B. (1980). Systems analysis in epidemiology. *Annual Review of Phytopathology*, *18*, 67–83. doi:10.1146/annurev.py.18.090180.000435.

McLean, F. T. (1921). A study of the structure of stomata of two species of citrus in relation to citrus canker. *Bulletin of the Torrey Botanical Club*, *48*, 101–106. doi:10.2307/2480340.

Peltier, G. L. (1920). Influence of temperature and humidity on the growth of *Pseudomonas citri* and its host plants and on infection and development of the disease. *Journal of Agricultural Research*, *20*, 447–505.

Pruvost, O., Boher, B., Brocherieux, C., Nicole, M., & Chiroleu, F. (2002). Survival of *Xanthomonas axonopodis* pv. *citri* in leaf lesions under tropical environmental conditions and simulated splash dispersal of inoculum. *Phytopathology*, *92*, 336–346. doi:10.1094/PHYTO.2002.92.4.336.

Reuther, W. (1977). Citrus. In P. T. Alvim, & T. T. Kozlowski (Eds.), *Ecophysiology of Tropical Crops* (pp. 409–439). Londres: Academic.

Rossetti, V. (1977). Citrus canker in Latin America: a review. *Proceedings of the International Society of Citriculture*, *3*, 918–924.

Rotem, J. (1988). Techniques of controlled-condition experiments. In J. Kranz, & J. Rotem (Eds.), *Experimental Techniques in Plant Disease Epidemiology* (pp. 19–31). Berlin: Springer.

Serizawa, S. (1981). Recent studies on the behavior of the causal bacterium of the citrus canker. *Proceedings of the International Society of Citriculture*, *1*, 395–397.

Stall, R. E., Miller, J. W., Marco, G. M., & DeEchenique, B. I. C. (1980). Population dynamics of *Xanthomonas citri* causing cancrosis of citrus in Argentina. *Proceedings of the Florida State Horticultural Societies*, *93*, 10–14.

Timmer, L. W., Gottwald, T. R., & Zitko, S. E. (1991). Bacterial exudation from lesions of Asiatic citrus canker and citrus bacterial spot. *Plant Disease*, *75*, 192–195.

Timmer, L. W., Zitko, S. E., & Gottwald, T. R. (1996). Population dynamics of *Xanthomonas campestris* pv. *citri* on symptomatic and asymptomatic citrus leaves under various environmental conditions. *Proceedings of the International Society of Citriculture*, *1*, 448–451.

Vale, F. X. R., Fernandes Filho, E. I., Liberato, J. R., & Zambolim, L. (2001). Quant — a software to quantify plant disease severity. *Proceedings of the 8th International Workshop on Plant Disease Epidemiology* (pp. 160). Ouro Preto, Brazil.

Vanderplank, J. E. (Ed.). (1963). *Plant Diseases: Epidemics and Control*. New York: Academic.

Verniere, C. J., Gottwald, T. R., & Pruvost, O. (2003). Disease development and symptom expression of *Xanthomonas axonopodis* pv. *citri* in various citrus plant tissues. *Phytopathology*, *93*, 832–843. doi:10.1094/PHYTO.2003.93.7.832.