

ECOLOGY, BEHAVIOR AND BIONOMICS

Phyllocnistis citrella Stainton (Lepidoptera: Gracillariidae) and Its Relationship with the Citrus Canker Bacterium *Xanthomonas axonopodis* pv *citri* in Brazil

MARCONE C.M. CHAGAS¹, JOSÉ R.P. PARRA², TAKAO NAMEKATA³, JOHN S. HARTUNG⁴
AND PEDRO T. YAMAMOTO⁵

¹EMBRAPA/EMPARN, Caixa postal 188, 59020-390, Natal, RN. E. mail: marcone@rn.gov.br

²ESALQ/USP, Depto. de Entomologia, Fitopatologia e Zoologia Agrícola, Caixa postal 09, 13418-900, Piracicaba, SP.

³Instituto Biológico, Caixa postal 70, 13001-970, Campinas, SP

⁴USDA, Beltsville Agricultural Research Center, BLDG 010 A, Beltsville, MD 20705-2350, USA.

⁵Fundecitrus, Caixa postal, 391, 14.807-040, Araraquara, SP.

Neotropical Entomology 30(1): 55-59 (2001)

Phyllocnistis citrella Stainton (Lepidoptera: Gracillariidae) e Sua Relação com a Bactéria do Cancro Cítrico *Xanthomonas axonopodis* pv *citri* no Brasil

RESUMO – Estudou-se em laboratório (28±2°C, 70±10% UR e fotofase de 14h) e em casa-de-vegetação a relação entre as lesões provocadas pelo ataque do minador-dos-citros, *Phyllocnistis citrella* Stainton, e a infecção causada pela bactéria do cancro cítrico *Xanthomonas axonopodis* pv *citri*. Utilizaram-se, como hospedeiro do minador, plantas de laranja caipira cultivadas em tubetes. Folhas inoculadas com lagartas de segundo e terceiro ínstaes ou pupas apresentaram índices de infecção bacteriana de 94,3, 98,3 e 100%, respectivamente. A taxa de infecção foliar por *X. axonopodis* pv *citri* em folhas lesionadas pelo inseto foi semelhante àquela observada em folhas danificadas mecanicamente e inoculadas posteriormente com bactéria (94,1 a 97% de pústulas bacterianas). A bactéria penetra também através dos estômatos. Aos sete dias após a inoculação, constatou-se uma taxa de infecção 11 vezes menor nas folhas que não foram lesionadas pelo inseto. O percentual de folhas com cancro aumentou aos 14 dias para 41,2%; esse valor é cerca de 50% menor se comparado às folhas que foram atacadas pelo inseto. Ficou demonstrada a importância dos danos provocados pelo minador-dos-citros no aumento do cancro cítrico, uma vez que as condições favoráveis de temperatura e umidade relativa no interior das minas construídas pelas lagartas, contribuem para um melhor desenvolvimento da bactéria.

PALAVRAS-CHAVE: Insecta, praga, minador-dos-citros, doença bacteriana, vetor.

ABSTRACT – The relationship of the citrus canker bacterium *Xanthomonas axonopodis* pv *citri* with the citrus leafminer (CLM), *Phyllocnistis citrella* Stainton was investigated. The experiment was conducted under laboratory conditions at 28±2°C, 70±10% RH and 14h photophase and in a greenhouse. Sweet orange (*Citrus sinensis* L. Osbeck) "Caipira" cv was used to rear CLM. Plants inoculated with 2nd and 3rd instar larvae or pupae showed high percentages (94.3, 98.3 and 100%, respectively) of bacterium-infected leaves. The damage caused by this insect was responsible for the increase in citrus canker infestation. The leaf infection rate by *X. axonopodis* pv *citri* on pre-injured leaves was similar to that observed on mechanically damaged leaves inoculated with the bacterium, with 94.1% to 97.0% of the leaves presenting bacterial pustules. The bacterium can also penetrate through the stomata. An 11-fold lower infection rate was observed as compared to the leaves injured by the insect seven days after inoculation. Under such conditions the percentage of cankered leaves increased to 41.2% at 14 days, a value corresponding to about 50% of the leaves attacked by the insect. In this paper it is also pointed out the significance of the damages caused by CLM in terms of the increase of citrus canker, since the favorable microclimatic conditions of temperature and relative humidity inside the mines built by the larvae account for an improved development of the bacterium.

KEY WORDS: Insecta, pest, disease, citrus, leafminer, vector.

Since the citrus leafminer (CLM), *Phyllocnistis citrella* Stainton was reported in Brazil, in 1996 (Prates *et al.* 1996), citrus growers have been deeply concerned with the insect, not only for the damage caused by the larvae to the plants (Clausen 1931, Badawy 1967, Heppner 1993, Prates *et al.* 1996, Gravena 1994, Lourenção & Muller 1994) but also because the insect is likely to be associated with the dissemination of the citrus canker bacterium *Xanthomonas axonopodis* pv *citri*. After the CLM was reported in the State of São Paulo, a rapid dispersion of the pest into other citrus-producing areas was reported and it is now believed to occur in every Brazilian States.

The direct damage caused by the insect can contribute to the penetration and development of the bacterium through injured tissues since, according to Graham *et al.* (1996), bacterial cells are conveyed along the galleries while the larvae mine through the leaves. Surveys conducted under field conditions in other countries have shown rates of citrus canker infestation of up to 75% in citrus leaves when CLM is present (Sohi & Sandhu 1968, Heppner 1993). The disease known worldwide as the Asiatic citrus canker is very harmful to several citrus species and is more often found where abundant rain and high temperatures occur simultaneously (Pruvost *et al.* 1997).

Few studies relating CLM to the dissemination of the citrus canker bacterium are available. Nevertheless, a marked increase of the inoculum source in the areas injured by the miner has taken place in some of the producing areas of the State of São Paulo, Brazil, in the past three years. Surveys conducted by Fundecitrus (Citriculture Defense Fund) have pointed to a preoccupying scenario ever since the CLM was reported in the country. The number of citrus canker foci in the State of São Paulo has increased approximately 10 times (from 45 foci in 1996 to 433 in 1998) (C. A. Massari – unpublished) and the disease clearly tends to increase since the pest was introduced into Brazil. Thus, the present study was conducted to determine the relationship between different levels of the lesion caused by the CLM and the degree of infection by *X. axonopodis* pv *citri* in citrus plants and to obtain information for the understanding of the insect/disease relationship.

Material and Methods

The experiment was conducted in laboratory at $28 \pm 2^\circ\text{C}$, $70 \pm 10\%$ RH and 14h photophase, and greenhouse conditions at the Department of Entomology, Plant Pathology and Agricultural Zoology of “Escola Superior de Agricultura Luiz de Queiroz”, University of São Paulo, (ESALQ/USP) and at the Experimental Station of the Instituto Biológico, at Piracicaba and Presidente Prudente counties, respectively, in the State of São Paulo (SP).

“Caipira sweet orange” seedlings (*Citrus sinensis* L. Osbeck) 20-25 cm tall were cultivated in tubes containing vermiculite and plant organic substrate medium (1:1) and used to rear CLM. The plants were pruned and kept under natural conditions in a screened area and/or in incubators set at $25 \pm 1^\circ\text{C}$, $60 \pm 10\%$ RH and 14h photophase. Fifteen to 20 days after pruning the plants with flushing were offered for

egg-laying inside cages with CLM pairs for no longer than two days (Chagas & Parra 2000).

After egg laying, the plants were transferred to an incubator set at temperatures ranging from 18°C to 32°C (Chagas 1999). Due to the differential rate of insect development as a function of temperature, insects of different development stages were simultaneously obtained. Thus, plant flushes with different-aged leafminer larvae or pupae were sprayed (inoculated) with a suspension of *X. axonopodis* pv *citri* cells concentrated to 10^5 - 10^6 colony forming units (cfu)/ml derived from the maceration of infected citrus plant leaves in the grove of the Experiment Station of the Instituto Biológico, Presidente Prudente, SP.

Six treatments, each consisting of 80 replicates, with 20 plants with 4 bacterium-inoculated leaves in a completely randomized design, were evaluated as follow: A - Leaves without miner sprayed with distilled water; B - Leaves without miner inoculated with bacteria; C - Leaves infested with 2nd instar larvae (2 days of age) and inoculated with bacteria; D - Leaves infested with 3rd instar larvae (4 days of age) and inoculated with bacteria; E - Leaves infested with miner pupae and inoculated with bacteria, and F - Fully expanded leaves injured with an abrasive (carborundum) and inoculated with bacteria.

The plants were transferred to plastic boxes and kept in a rearing room at $28 \pm 2^\circ\text{C}$, $70 \pm 10\%$ RH and 14h photophase. The sets made up of plants and cages were separated for each treatment, disposed on metal racks placed inside plastic trays (35 x 40 x 8 cm) containing a nutritive solution at 2 g of Miracle-Gro® fertilizer (15-30-15 NPK, copper, iron, manganese, molybdenum, and zinc) in two liters of water in order to keep them turgescient and to keep environment moisture close to 80%.

The evaluations were performed at the 7th and 14th day after the experiment was set up and the presence or absence of *X. axonopodis* pv *citri* was recorded from the four first leaves of the upper third part of each plant. The results obtained were submitted to ANOVA and the means were compared by the Tukey test at the 5% level of probability.

Results and Discussion

The damage caused by the citrus leafminer was responsible for the increase in citrus canker since the plants inoculated with 2nd and 3rd instar larvae or pupae showed high percentages (94.3, 98.3 and 100%, respectively) of bacterium-infected leaves 14 days after inoculation, with no significant differences between treatments. Although a numerical trend to increased damage according to insect age was observed (Fig. 1). This observation is probably related to the different dimensions of the injuries produced by the insects associated with the time required by the bacterium to develop inside the mines. These rates exceed by as much as 33% those observed by Sohi & Sandhu (1968) under field conditions. This result can be attributed to differences in experimental conditions and/or to the citrus species used. The rate of infection with *X. axonopodis* pv *citri* on pre-injured leaves by CLM was similar to that observed on mechanically damaged leaves inoculated with the bacterium since 94.1% to 97.0% of the

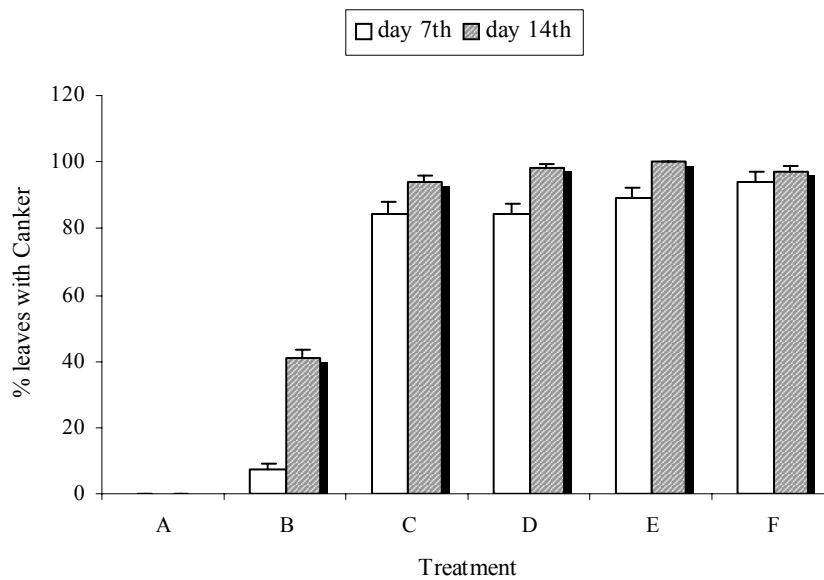


Figure 1. Effect of injuries caused by different instars of *P. citrella* on the infection with the citrus canker bacterium *X. axonopodis* pv *citri* in *C. sinensis*. A (without bacterium inoculation), B (inoculation via stomata, without damage), C (inoculated with 2nd instar larvae), D (inoculated with 3rd instar larvae), E (inoculated with pupae), and F (inoculated with mechanical damage).

leaves presented bacterial pustules (Fig. 1).

Even though a close relationship between the citrus leafminer and increased bacterial pustules on leaves has been demonstrated, it remains unclear whether the leafminer larvae are responsible for the spreading of bacterial cells along the

mines as reported by Graham *et al.* (1996). In the present study the canker rates observed when the plants were inoculated after the insects had completed the larval period (pupal treatment) were found to be similar to those observed in treatments with plants containing larvae only (Fig 2).

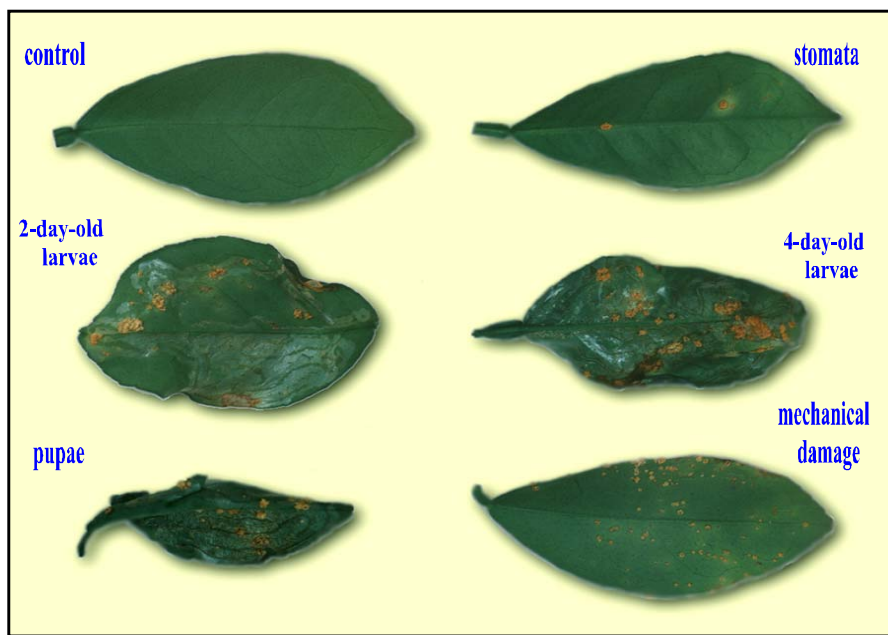


Figure 2. Leaves of “caipira sweet orange” (*C. sinensis*) with different levels of lesions of the citrus leafminer, associated with the pustules of the citrus canker bacterium, *X. axonopodis* pv *citri*, and bacterium penetration via stomata and after mechanical damage in relation control plants.

Therefore, further study on the inter-relationships with other areas are needed since investigations on various insect groups have shown that some species can be considered as secondary disseminating agents (mechanical transmission) of bacteriosis in flowers, shoots and/or leaves of other plant species (Johnson 1934, Amante *et al.* 1971, Emmett & Baker 1971).

On the other hand, it is known that the bacterium can also penetrate through the stomata (see treatment B, Fig. 1); in this case, however, the infection rate was 11 times lower compared to the leaves injured by the insect seven days following inoculation. Under such conditions the percent of cankered leaves increased to 41.2% at 14th day. This value corresponds to an insect attack of about 50% of the leaves. Thus, differences in this type of infection can occur depending on citrus variety since the amount, size and kind of stomata along with other morphological characteristics of the host such as thickness of the epidermal layer, amount of fatty glands and palisade tissue and/or intracellular spaces may differ among citrus varieties and/or species (Latif & Yunus 1951, Ba-Angood 1977, Graham *et al.* 1992).

It is clear that, regardless of the conditions under which the studies are carried out, there is a close correlation between CLM infestation and bacterium infestation, as previously reported by Gottwald *et al.* (1997) in the USA.

The present study also indicates the significance of the damage caused by the CLM in terms of the increase in citrus canker since the favorable microclimatic conditions of temperature and relative humidity inside the mines built by the larvae favor improved development of the bacterium. This is shown by the increase in bacterial pustules (higher coalescence) on attacked leaves as compared to those infected via the stomata or even by mechanical inoculation, thus resulting in an increase of the inoculum source (Fig. 2).

Therefore we can infer that the increased area with citrus canker in the State of São Paulo is directly associated with the attack by CLM, since the problem increased in this State after the pest was introduced in March 1996. In addition, the problem clearly tends to become worse in the presence of abundant rain and simultaneous high temperatures (Pruvost *et al.* 1997), since in the State of São Paulo this condition – occurring from September through April – is characterized by intense plant flushing (a condition required for the occurrence of CLM). Even considering the increased damage potential of this insect in warmer regions of the State of São Paulo during this period and its close association with the citrus canker bacterium, this relationship and the resulting levels of infection may vary from region to region according to the variety and/or citrus species crop, microclimatic conditions, miner population and/or amount of inoculum found in the agroecosystem.

Acknowledgements

The authors wish to thank Fundecitrus (Citriculture Defense Fund) for financial support, Patrícia Milano, Alexandre de S. Pinto and Heraldo Negri de Oliveira from Department of Entomology, Plant Pathology and Agricultural Zoology, ESALQ/USP and the research team from the Instituto Biológico de São Paulo (Presidente Prudente, SP),

for the valuable assistance with the insect rearing system and experiments.

Literature Cited

- Amante, E., M.A. Vulcano & J. Abrahão. 1971.** Observações preliminares sobre a influência da entomofauna na dispersão dos uredosporos da ferrugem do cafeeiro (*Hemileia vastatrix*). *Biológico* 37: 102-105.
- Ba-Angood, S.A.S. 1977.** A contribution to the biology and occurrence of the citrus leaf miner, *Phyllocnistis citrella* Stainton (Lepidoptera: Gracillariidae) in the Sudan. *Z. Angew. Entomol.* 83: 106-111.
- Badawy, A. 1967.** The morphology and biology of *Phyllocnistis citrella* Stainton, a citrus leaf-miner in the Sudan. *Bull. Soc. Entomol. Egypte* 51: 95-103.
- Chagas, M.C.M. 1999.** *Phyllocnistis citrella* Stainton, 1856 (Lepidoptera: Gracillariidae): bioecologia e relação com o cancro cítrico. Tese de Doutorado, Escola Superior de Agricultura “Luiz de Queiroz”, Universidade de São Paulo. Piracicaba, 67p.
- Chagas, M.C.M. & J.R.P. Parra. 2000.** *Phyllocnistis citrella* Stainton (Lepidoptera: Gracillariidae): técnica de criação e biologia em diferentes temperaturas. *An. Soc. Entomol. Bras.* 29: 227-235.
- Clausen, C.P. 1931.** Two citrus leafminers of the far east. Washington: USDA, 1931. (USDA. Technical Bulletin, 252). *Rev. Appl. Entomol.* 19: 649.
- Emmett, B.J. & L.A.E. Baker. 1971.** Insect transmission of fireblight. *Plant Pathol.* 20: 41-45.
- Gottwald, T.R., J.H. Graham & T.S. Schubert. 1997.** An epidemiological analysis of the spread of citrus canker in urban Miami, Florida, and synergistic interaction with the Asian citrus leafminer. *Fruits* 52: 383-390.
- Graham, J.H., T.R. Gottwald, H.W. Browning & D.S. Achor. 1996.** Citrus leafminer exacerbated the outbreak of Asiatic citrus canker in South Florida. In: International Conference on *Citrus leafminer*, Orlando, 1996. Proceedings. Orlando, University of Florida, 1996. 83p.
- Graham, J.H., T.R. Gottwald, H.W. Browning, T.D. Riley & D.S. Achor. 1992.** Penetration through leaf stomata and growth of strains of *Xanthomonas campestris* in citrus cultivars varying in susceptibility to bacterial diseases. *Amer. Phytopathol. Soc.* 82: 1319-1325.
- Gravena, S. 1994.** “Minadora das folhas dos citros”: a mais nova ameaça da citricultura brasileira. *Laranja* 15: 397-404.
- Heppner, J.B. 1993.** Citrus leafminer, *Phyllocnistis citrella*,

in Florida (Lepidoptera: Gracillariidae: Phyllocnistinae). Trop. Lepid. 4: 49-64.

Campinas: CATI, 1996. 3p. (CATI. Comunicado Técnico, 129).

Johnson, E.M. 1934. Dissemination of angular leaf spot of tobacco by the southern tobacco worm. Phytopathology 24: 1381-1382.

Pruvost, O., C. Vernière, J. Hartung, T.R. Gottwald & H. Quetelard. 1997. Towards an improvement of citrus canker control in Reunion island. Fruits 52: 375-382.

Latif, A. & C.M. Yunus. 1951. Food plants of citrus leafminers in Punjab. Bull. Entomol. Res. 42: 311-316.

Sohi, G.S. & M.S Sandhu. 1968. Relationship between Citrus leaf-miner (*Phyllocnistis citrella* Stainton) injury and Citrus canker [*Xanthomonas citri* (Hasse) Dowson] incidence on *Citrus* leaves. J. Res. Punjab Agric. Univ. 5: 66-69.

Lourenção, A.L. & G.W. Müller. 1994. "Minador das folhas dos citros": praga exótica potencialmente importante para a citricultura brasileira. Laranja 15: 405-412.

Prates, H.S., O. Nakano & S. Gravena. 1996. "Minadora das folhas de citros" *Phyllocnistis citrella*, Stainton, 1856.

Received 11/V/2000. Accepted 22/II/2001.
