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## FEEDING AND SURVIVAL OF CITRUS SHARPSHOOTERS (HEMIPTERA: CICADELLIDAE) ON HOST PLANTS

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### ABSTRACT

The liquid excretion and survival of the sharpshooters *Dilobopterus costalimai* Young and *Oncometopia facialis* (Signoret), vectors of *Xylella fastidiosa* in citrus, were measured on various host plants as an indirect approach to assess their feeding and performance on these hosts and determine suitable plants for laboratory rearing. Adult females of *D. costalimai* showed the highest excretion rate on *Vernonia condensata* (Asteraceae). *O. facialis* excreted larger volumes on three species of *Vernonia* and on *Lantana camara* (Verbenaceae). On average, single *D. costalimai* females excreted a liquid volume equivalent to 292 times its body volume per day when feeding on *V. condensata*, whereas *O. facialis* females excreted 430 times their body volume on the same host. In contrast, the excretion rates of *D. costalimai* and *O. facialis* females on *Citrus sinensis* did not exceed 248 and 140 times their body volume per day, respectively. The mortality of adults after 96 h was lower on hosts upon which higher liquid volumes were excreted; therefore, there is a positive relationship between the excretion rate by the sharpshooters and their nutritional adequacy to hosts. *V. condensata* is a suitable host to maintain adult populations of both sharpshooters in the laboratory.

Key Words: leafhopper vectors, host plant suitability, honeydew excretion, citrus variegated chlorosis

### RESUMO

A taxa de excreção de líquidos e a sobrevivência das cigarrinhas *Dilobopterus costalimai* Young e *Oncometopia facialis* (Signoret) (Hemiptera: Cicadellidae), vetoras de *Xylella fastidiosa* em citros, foram quantificadas em diferentes espécies vegetais, como forma indireta de se avaliar a adequação dessas plantas como hospedeiras das cigarrinhas para estudos ecológicos e de criação em laboratório. O maior volume de excreção líquida por fêmeas de *D. costalimai* foi observado em *Vernonia condensata* (Asteraceae). *O. facialis* excretou maiores volumes em três espécies de *Vernonia* e em *Lantana camara* (Verbenaceae). Alimentando-se em *V. condensata*, uma única fêmea de *D. costalimai* excretou, em média, o equivalente a 292 vezes seu volume corpóreo por dia, enquanto que as fêmeas de *O. facialis* excretaram 430 vezes seu volume corpóreo no mesmo hospedeiro. Em *Citrus sinensis*, as taxas de excreção de *D. costalimai* e *O. facialis* não excederam em 248 e 140 vezes o volume corpóreo por dia, respectivamente. A mortalidade dos adultos após 96 h foi menor naqueles hospedeiros onde houve maior excreção, havendo, portanto, uma relação direta entre a taxa de excreção pelas cigarrinhas e sua adequação nutricional aos hospedeiros. *V. condensata* é um hospedeiro adequado para manter populações de adultos de ambas as cigarrinhas em laboratório.

Translation provided by author

The leafhoppers *Dilobopterus costalimai* Young and *Oncometopia facialis* (Signoret) (Hemiptera: Cicadellidae) are vectors of the bacterium *Xylella fastidiosa* (Roberto et al. 1996), the causal agent of citrus variegated chlorosis (CVC), a disease reported in Brazil in the late 1980s (Rossetti & De Negri 1990), which currently affects 38% of citrus trees in the state of São Paulo, Brazil (68 million plants) (Anonymous 2002).

Cicadellinae leafhoppers, commonly named sharpshooters, are usually found on plant branches, feeding in the xylem vessels of young

shoots. They have well developed suction chambers that allow fluid intake even under strong negative pressure of the xylem (Purcell 1989). They extract most of the nutrients present in the ingested sap, mainly amino acids and organic acids (Andersen et al. 1989), and excrete the liquid excess through the anus. To make up for the low concentration of amino acids in the xylem sap of the plants, these insects usually ingest a large amount of liquids (Raven 1983; Purcell 1989).

Studies on insects feeding directly from the xylem fluid, e.g., the sharpshooters, rarely provide

direct results of the assimilated nutrients due to the fact that this liquid has a low chemical diversity in comparison with other plant tissues and is little likely to contain compounds of secondary metabolism (Raven 1983). Previous studies with the glassy-winged sharpshooter, *Homalodisca coagulata* Say revealed that its adaptation to the host includes high rates of assimilation of organic compounds (above 98%) and excretion of ammonia as a primary product (Andersen et al. 1989; Andersen et al. 1992). The requirement of plant nutrients varies according to the development stage of *H. coagulata*, which rarely completes its development on a single host (Andersen et al. 1989; Brodbeck et al. 1993; Brodbeck et al. 1995). According to Paiva et al. (1996) and Gravena et al. (1998), there is a clear difference between leafhopper species occurring on citrus trees and those on invasive vegetation of orchards; nevertheless, some sharpshooters that occur predominantly on the weeds are eventually trapped in the citrus canopy. Likewise, citrus sharpshooters have been found on a wide range of trees and shrubs in woody habitats adjacent to citrus orchards (J. R. S. Lopes et al., unpublished data).

The goal of this work was to develop a method to collect and measure the liquid excretion of sharpshooters, in order to evaluate feeding and survival rates of *O. facialis* and *D. costalimai* on various host plants, as an indirect approach to determine host suitability and understand the nutritional ecology of these important vectors.

#### MATERIALS AND METHODS

The experiment was performed in a greenhouse at the Dept. of Entomology, Plant Pathology and Agricultural Zoology, University of São Paulo, Brazil. The liquid excretion of *D. costalimai* and *O. facialis* was measured on three plant species of the family Asteraceae (*Vernonia* sp., *V. condensata*, *V. polyanthes*), two of Verbenaceae (*Lantana camara* and *Aloysia virgata*), and one of Rutaceae (*Citrus sinensis*; sweet orange), which are field hosts of these sharpshooters (J.R.S. Lopes et al., unpublished data). Six-month old potted citrus trees were used. The other host plants were 3-4 months old.

Sharpshooters used in the experiment were reared on plants of *V. condensata* in a greenhouse. For collecting the liquid excretion, 1-week old adults of *D. costalimai* and *O. facialis* males and females were individually placed inside 100-ml plastic cages with lids containing ventilation holes covered by a fine fabric (Fig. 1A). The cages were attached with adhesive tape to the young branches of the plants. Feeding was allowed for 96 h; the liquid excretion accumulated in the bottom of the cages was collected daily by a 1-ml syringe, and the volume was measured (Fig. 1B). The data were transformed into liquid excretion

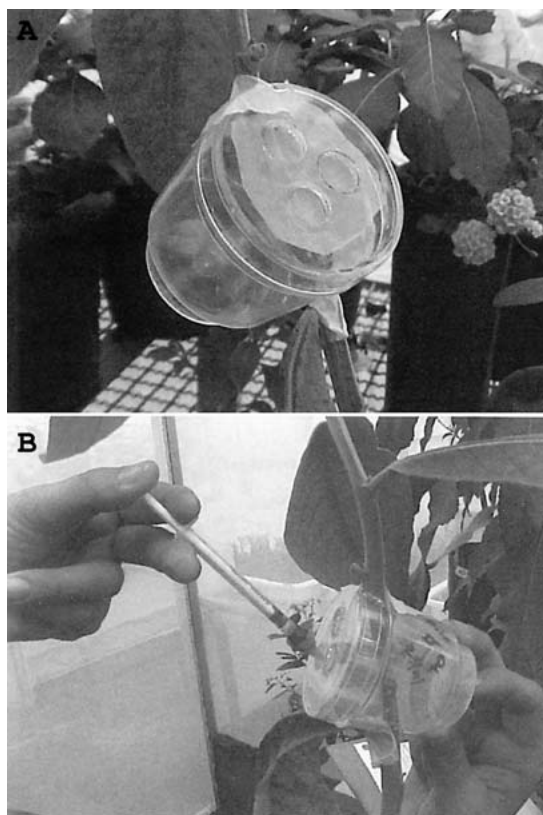


Fig. 1. A) Transparent plastic cage (100 ml) used for confinement of sharpshooters on plant stems. Lid with ventilation holes covered by a fine fabric. B) Collection and measurement of liquid volume excreted in the bottom of the cage by a 1-ml syringe.

volume produced in relation to the body volume of the insects. The body volume was determined by plunging sharpshooter adults into a known volume of liquid excretion and measuring the volume of liquid displaced. Feeding trials were run until 12 replicates were completed. Only replicates in which the insect was alive throughout the 96-h feeding period were considered for the analyses of excretion rates.

The experimental design was in blocks completely randomized with six treatments and 12 replications. The data were analyzed using analysis of variance (ANOVA) followed by the Tukey test ( $P < 0.05$ ).

#### RESULTS AND DISCUSSION

The method developed was efficient to estimate the excretion rates of the sharpshooters. *D. costalimai* adults excreted a higher liquid volume when fed on *V. condensata* (male and female) *Vernonia* sp.(male) and *V. polyanthes*, which are plants of the family Asteraceae. On *V. condensata*,

a single *D. costalimai* female excreted up to 620 times its own body volume in a 24-h period. *D. costalimai* males and females nearly did not feed on *L. camara*, and the liquid excretion of males was null (Table 1). The same trend of higher liquid excretion on Asteraceae was verified for *O. facialis*, except for *L. camara* (Verbenaceae), upon which the excretion was equivalent to that observed on *Vernonia* sp. and *V. condensata*. In 24 h, *O. facialis* females excreted up to 900 and 990 times their body volume when fed on *V. condensata* and *L. camara*, respectively (Table 1). It should be pointed out that under field conditions *L. camara* is frequently visited by adults of *O. facialis* (Gravena et al. 1998).

The mortality of *D. costalimai* adults after 96 h was higher on *A. virgata*, *L. camara* and *C. sinensis*, and null for *V. condensata* and *Vernonia* sp. For *O. facialis* the mortality was also null when fed on *V. condensata*, *Vernonia* sp. and *V. polyanthes* (Table 2). Therefore, the host plants of the family Asteraceae appear to be nutritionally more adequate for both sharpshooters because a lower mortality and a higher liquid excretion occurred on those plants, even though the xylem sap nutrients (amino acids and sugars) considered important to the adults were not measured in this research. Milanez et al. (2001) showed that *V. condensata* is more adequate than *Citrus limonia* for the nymphal development of *D. costalimai* and *O. facialis*, because it shortens the nymphal period and increases the viability of these sharpshoot-

ers. In the present study, male and female adults of *D. costalimai* and *O. facialis* excreted much less on *C. sinensis* than on *V. condensata*, which seems to be an optimum feeding host (Table 1).

The feeding preferences of the sharpshooters might influence their competency as vectors of *X. fastidiosa*. The low rates of sap ingestion and survival on citrus by sharpshooters may theoretically reduce the chances of acquisition of *X. fastidiosa* from or inoculation to the xylem of citrus plants. This might explain in part the low transmission efficiency of *X. fastidiosa* by sharpshooters reported in citrus (Lopes 1999; Yamamoto et al. 2002); other possible factors are related to pathogen-plant or pathogen-vector interactions.

Overall, this study shows a relationship between the liquid volume excreted by *D. costalimai* and *O. facialis* adults and the nutritional adequacy of host plants. It was observed that some plants promote higher feeding and survival rates than the others. Among these hosts, *V. condensata* appears to be the most suitable to maintain adult populations of both sharpshooters in the laboratory. Further studies on oviposition and development of these sharpshooters on various host plants are necessary to understand their nutritional ecology and improve the rearing system. Previous studies showed that *H. coagulata*, a sharpshooter with similar habits, requires different hosts to complete its development (Andersen et al. 1989; Brodbeck et al. 1993; Brodbeck et al. 1995).

TABLE 1. DAILY AVERAGE AND VARIATION INTERVAL (IN PARENTHESIS) OF THE RATIO OF LIQUID VOLUME EXCRETED PER BODY VOLUME OF *DILOPTERUS COSTALIMAI* AND *ONCOMETOPIA FACIALIS*, WHEN FED ON DIFFERENT HOST PLANTS.

Host plant	<i>D. costalimai</i>		<i>O. facialis</i>	
	Male	Female	Male	Female
<i>Vernonia condensata</i>	196 ± 42 a <sup>1</sup> (80 – 520)	292 ± 60 a (160 – 620)	128 ± 51 ab (10 – 630)	430 ± 99 a (40 – 900)
<i>V. polyanthes</i>	134 ± 20 a (42 – 246)	84 ± 38 bc (4 – 380)	164 ± 70 ab (4 – 340)	70 ± 10 b (10 – 530)
<i>Vernonia</i> sp.	98 ± 40 bc (32 – 366)	48 ± 20 bc (40 – 184)	337 ± 58 a (50 – 630)	255 ± 81 a (170 – 680)
<i>Aloysia virgata</i>	24 ± 3 d (20 – 180)	56 ± 20 bc (20 – 520)	31 ± 12 c (2 – 140)	44 ± 24 b (1 – 260)
<i>Lantana camara</i>	0 d	8 ± 2 c (0 – 12)	291 ± 84 a (50 – 810)	411 ± 67 a (80 – 990)
<i>Citrus sinensis</i>	46 ± 2 cd (18 – 128)	120 ± 30 b (20 – 248)	36 ± 20 c (2 – 230)	32 ± 18 b (16 – 140)
ANOVA <sup>2</sup>				
F	4.94	9.87	8.10	11.11
df	5, 66	5, 66	5, 66	5, 66
P	<0.05	<0.05	<0.05	<0.05

<sup>1</sup>Means (±SEM) within columns followed by the same letter do not differ by Tukey test (P > 0.05).

<sup>2</sup>Analysis of variance (ANOVA) statistics; df = degrees of freedom (treatment, residue).

TABLE 2. MORTALITY (%) OF *DILOPTERUS COSTALIMAI* AND *ONCOMETOPIA FACIALIS* ADULTS WHEN CONFINED ON DIFFERENT HOST PLANTS FOR 96 H.

Host plant	<i>D. costalimai</i>		<i>O. facialis</i>	
	Male	Female	Male	Female
<i>Vernonia condensata</i>	0	0	0	0
<i>V. polyanthes</i>	25	40	0	0
<i>Vernonia</i> sp.	0	0	0	0
<i>Aloysia virgata</i>	77	50	57	40
<i>Lantana camara</i>	55	50	25	25
<i>Citrus sinensis</i>	50	57	62	40

The information obtained in this work should be useful for development and application of new vector control strategies involving trap plants or vegetation management in citrus groves.

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