

ATTRACTION OF *Bucephalagonia xanthophis* (HEMIPTERA: CICADELLIDAE) TO VOLATILES OF ITS NATURAL HOST *Vernonia condensata* (ASTERACEAE)

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ABSTRACT: Plant volatiles are important cues for the orientation of herbivorous insects. It is possible that these compounds indicate whether the plant is suitable for feeding and larval development, or for mating aggregation. *Vernonia condensata* (Asteraceae) is known to attract species of leafhoppers, most of them important vectors of the citrus variegated chlorosis (CVC). In this study, we evaluated the role of volatiles of *V. condensata* on the orientation of *Bucephalagonia xanthophis* (Hemiptera: Cicadellidae). Four-arm olfactometer bioassays showed that only males were attracted to the volatiles of the host-plants *Citrus* sp. and *V. condensata*. Furthermore, fresh leaves of *V. condensata* induced a stronger response than volatiles from hexane-extracted leaves. This study opens the possibility to utilize *V. condensata* volatiles for pest management programs of *B. xanthophis*.

Key words: plant-herbivore interaction, leafhopper, trichomes

ATRATIVIDADE DE *Bucephalagonia xanthophis* (CICADELLIDAE) POR VOLÁTEIS DE SEU HOSPEDEIRO NATURAL *Vernonia condensata* (ASTERACEAE)

RESUMO: Os voláteis de plantas são sinais importantes para a orientação de insetos herbívoros. É possível que esses compostos indiquem que a planta é apropriada para alimentação e desenvolvimento larval, ou para agrupamento reprodutivo. *Vernonia condensata* (Asteraceae) atrai espécies de cigarrinhas, muitas delas vetores da clorose variegada dos citros (CVC). Neste trabalho, foi avaliado o papel dos voláteis de *V. condensata* na orientação de *Bucephalagonia xanthophis* (Hemiptera: Cicadellidae). Bioensaios em olfatômetro de quatro braços mostraram que somente os machos foram atraídos pelos voláteis das plantas hospedeiras *Citrus* sp. e *V. condensata*, sendo que os voláteis das folhas frescas de *V. condensata* induziram uma maior resposta quando comparada aos extratos hexânicos da planta. A possibilidade de usar os voláteis de *V. condensata* abre novas perspectivas para programas e alternativas para o manejo de pragas.

Palavras-chave: interação planta-herbívoro, cigarrinha, tricomas

INTRODUCTION

In response to herbivore damage, plants synthesize and release complex blends of volatile compounds that serve as indirect defenses mediating the attraction of predators and/or parasitoids of the herbivores responsible for the damage (Turlings & Wackers, 2004; De Boer et al., 2004; Kessler & Baldwin, 2001; De Moraes et al., 1998; Vet & Dicke, 1992). Herbivory is not the only factor inducing the release of plant volatiles, even deposition of eggs by herbivorous arthropods has also been shown to induce volatile attractants (Hilker & Meiners, 2006; Colazza et al., 2004). However, plant volatiles represent a complex

message that is interpreted according to the biological context of other insects and these compounds may also attract other herbivores and thereby incur ecological costs for plants (Bengtsson et al., 2006; Hori et al., 2006; Srinivasan et al., 2006; Heil, 2004; Kessler & Baldwin, 2002; Visser, 1986).

The leafhopper *Bucephalagonia xanthophis* (Berg) (Hemiptera: Cicadellidae) is the vector of the bacterium *Xylella fastidiosa*, the causal agent of citrus variegated chlorosis (CVC), a disease that in southeastern Brazil affects 40% of the citrus crops (Milanez et al., 2001). Although the genome of *X. fastidiosa* has been sequenced, the mechanisms that control the process of infection and the development of the disease

remain unknown (Simpson et al., 2000), and several aspects of the interaction with the host plants need to be clarified in order to improve management techniques for this important disease.

Based on the knowledge on the tritrophic interactions increases, one of the major challenges in Brazil is how to exploit plant volatile information to develop novel strategies for pest control. The possibility of using plant volatiles for the manipulation of parasitoids appears to be a powerful tool for the control of regional pest crops, as demonstrated by the results obtained in experiments using the parasitoids *T. podisi* in soybean (Moraes et al., 2005) and *Tamarixia radiata* (Waterston) (Hymenoptera: Eulophidae) in citrus (Arab et al., unpublished).

Vernonia condensata Becker (Asteraceae) is a natural host of various Cicadellidae vectors of the CVC disease (Milanez et al., 2001). Insect vectors are strongly attracted to this plant, for instance, the leafhopper *B. xanthophis* is found infecting both citrus and *V. condensata* plants in greenhouses and orchards in the São Paulo State, Brazil. The objective of this preliminary study was to evaluate the role of the volatiles released by *V. condensata* on the response of *B. xanthophis*, with the aim of understanding the biology of this citrus pest in order to improve further control and integrated management programs for this insect.

MATERIAL AND METHODS

Insects and Plants - Adults of the leafhopper *B. xanthophis* were collected from *Duranta repens* (Verbenaceae) plants in Piracicaba, State of São Paulo (22°42' S; 47°37' W), Brazil, transferred to the laboratory, and caged with the plant *D. repens*, to avoid conditioning to volatiles from plants used in the subsequent experiments (Milanez et al., 2003). Stem fragments of one individual of *V. condensata* were collected and rooted in 300 mL plastic tubes and used after the development of the first ten leaves. Insects and plants were kept inside a greenhouse at $25 \pm 5^\circ\text{C}$, $70 \pm 10\%$ RH and natural photoperiod. Fresh leaves from *Citrus* sp. (Rutaceae) and the non-host plant *Paspalum notatum* (Poaceae) were also collected one-hour prior to the beginning of the bioassays.

Leaf Morphology - Scanning Electron Microscopy (SEM) was carried out on leaves of *V. condensata* fixed in Karnovsky (Karnovsky, 1965) and performed in whole mounts. These samples were prepared using critical point drying and gold sputtering of fixed leaves and examined with a Zeiss Mev DSM940A scanning electron microscope.

Four-arm Olfactometer Bioassays - Preference of adults of *B. xanthophis* by host and non-host plant volatile compounds was evaluated in a 30×30 cm four-arm olfactometer, as described by Vet et al. (1983). Plants tested in this bioassay included excised healthy leaves of the host plants *V. condensata* and *Citrus* sp. (Rutaceae), and leaves of the non-host plant *P. notatum* (Poaceae). A wetted cotton was placed at the distal end of the excised leaves to avoid desiccation during the experiment. Bioassays were conducted using males and females of *B. xanthophis*. Each trial consisted of a combination of three odor sources offered to the insects (leaves of *V. condensata*, *Citrus* sp., and *P. notatum*), while one chamber was kept empty (humidified air) (Vet et al., 1983). Plant material was randomly assigned to the arms and replaced after testing five insects to avoid induction of other compounds. Insects were introduced individually at the center of the olfactometer and observed for 10 min. Preference for one of the samples was considered positive if the insect moved towards one field of the olfactometer. The insect response to volatiles was measured using the time spent walking in each odor field. After each trial, the olfactometer was disassembled and its central arena, tubes, and connections were washed with neutral dishwashing soap (5%) and 90% ethanol (Vet et al., 1983). All the bioassays were conducted with fluorescent light inside a controlled environmental room at $25 \pm 3^\circ\text{C}$ and $70 \pm 10\%$ RH. Twenty replicates were conducted per sex in this experiment.

Volatile Extraction of *V. condensata* Leaves - Leaf volatiles of *V. condensata* were collected using a non-invasive extraction method (Duke et al., 2000). Extraction was performed using 20 g of fresh leaves washed with 5 mL of hexane for 30 seconds. The hexane-extract was tested in the four-arm olfactometer, as described above, and compared with the activity of fresh leaves. Hexane was used as solvent control. Twenty replicates were conducted in this experiment.

Statistics - Differences between the means of the time spent by the insects in each field of the olfactometer were evaluated by an analysis of variance (One-Way ANOVA) ($p < 0.05$). Multiple comparisons were conducted with a Tukey test ($p < 0.05$) (Sheskin, 2000).

RESULTS AND DISCUSSION

Some herbivorous arthropods can detect their hosts using volatile compounds released by healthy plants, which can also stimulate oviposition and courtship behaviors in these organisms (Rojas et al., 2003). Only males of the leafhopper *B. xanthophis* responded

to volatiles released for their host plants (Table 1). The time spent by males in the olfactometer also showed differences among the samples tested ($F = 2.96$; $df = 3$; $p = 0.037$; ANOVA). Individuals spent more time for *Citrus* sp. than for *P. notatum* and humidified air ($p = 0.049$ and $p = 0.004$, respectively; Tukey test). Moreover, no differences between the host plants were detected ($p = 0.162$; Tukey test). Differences between the behavioral responses of the insects exposed to plant volatiles and their correlation with sex and physiological state have been reported for many insect-plant interactions (Dickens, 2000). The internal state of an animal, such as reproductive stage or sex, also influences its foraging behavior (see Vet & Dicke, 1992, for review).

Variation in the sensibility of the antennal receptors and specificity between males and females may explain sex biased plant-volatile attraction in some insects (Fraser et al., 2003). Plant volatiles can also influence sex pheromone communication by enhancing both pheromone production and response (Landolt & Phillips, 1997). The effect of the sex pheromone of *Cydia pomonella* L. (Lepidoptera: Tortricidae) on males is greatly enhanced by volatiles released by the host plants, thus increasing communication distances by amplifying weak pheromone signals (Yang et al., 2004). Moreover, the synergism between host plant volatiles and sex pheromones also plays an important role in reproductive isolation (Witzgall et al., 1991). It is possible that volatile compounds of *V. condensata* indicate that the plant is suitable for mating aggregation (Fraser et al., 2003; Meiners & Hilker, 2000); however, further studies are necessary to determine the role of other semiochemicals, such as sex pheromones, on the response of *B. xanthopis* males to host plants.

To determine if volatile compounds can be isolated from leaves of *V. condensata* using a non-invasive extraction protocol, males were tested by comparing the preference for volatiles of hexane leaf extracts against fresh leaves. Volatiles of the host plant elicited orientation on the insects ($F = 12.69$; $df = 1$; $p = 0.006$; ANOVA). There were no differences on the preference of the males of *B. xanthopis* between hexane-extracted-volatiles and fresh leaves treatments ($p = 0.107$; Tukey test). However, fresh leaves of *V. condensata* appear to elicit a stronger orientation on the males of *B. xanthopis* over the solvent control and humidified air ($p = 0.005$ and $p = 0.0003$, respectively; Tukey test), than hexane-extracted-volatiles ($p = 0.210$ and $p = 0.031$, respectively; Tukey test) (Table 2). Although both fresh leaves and hexane-extracted-volatiles elicited orientation behavior on the insects, the variation seen for the activity of both treatments indicate that not all the volatile compounds were recovered using the non-invasive method with hexane. Thus, a direct extraction of the headspace volatiles (Fraser et al., 2003) will be more useful for behavioral bioassays with *V. condensata*.

Other herbivorous insects are attracted to host plant volatiles. The Colorado potato beetle *Leptinotarsa decemlineata* Say (Coleoptera: Chrysomelidae) is attracted to damaged *Solanum tuberosum* L. (Solanaceae) plants (Dickens, 2002). Volatile compounds released by glandular trichomes of *Cordia curassavica* Jaq. R.&S. (Boraginaceae) are strong attractants for the weevil *Cratosomus flavofasciatus* Guerin (Coleoptera: Curculionidae), which is also an important pest of some Brazilian fruit cultivars, including citrus species (Arab & Bento, 2006). Consequently, for herbivores, plant volatiles represent a complex message that is interpreted according to their biological context.

Table 1 - Mean spent time (\pm SE) of adults of *Bucephalagonia xanthopis* to volatiles released by host and non-host plants (N = 20).

Response	Sex	Odour field			
		<i>Vernonia condensata</i>	<i>Citrus</i> sp.	<i>Paspalum notatum</i>	Air
Time/field (seg)*	M	158.10 \pm 45.77 ab	238.75 \pm 46.51 b	132.40 \pm 39.29 a	70.75 \pm 27.21 a
	F	172.80 \pm 49.89 a	147.7 \pm 48.00 a	145.70 \pm 45.79 a	133.80 \pm 45.79 a

*One-way ANOVA ($p < 0.05$). Different letters in the same row indicate differences of time spent between samples (Tukey test). (M = Male; F = Female).

Table 2 - Mean spent time (\pm SE) of males of *Bucephalagonia xanthopis* to fresh leaves and hexane-extracted-volatiles of *Vernonia condensata* (N = 20).

Response	Odour field			
	Hexane	Fresh Leaves	Air	Hexane Extract
Time/field (seg)*	100.0 \pm 40.46 bc	279.05 \pm 55.83 a	42.60 \pm 18.39 c	178.30 \pm 50.62 ab

*One-way ANOVA ($p < 0.05$). Different letters in the same row indicate differences of time spent between samples (Tukey test).

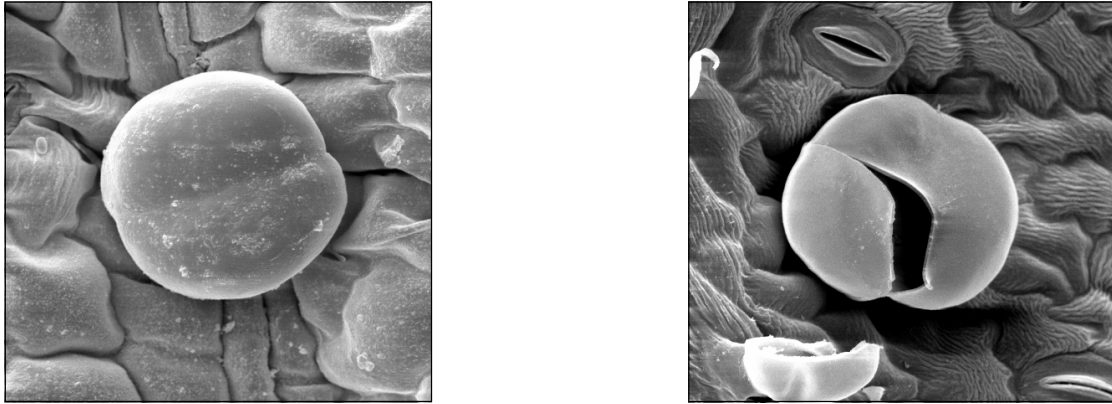


Figure 1 - Capitate-sessile trichomes of *Vernonia condensata*. General view of a trichome filled with secretion (left), and after its content was released (right).

SEM of *V. condensata* leaves showed a high number of capitate-sessile glandular trichomes (Figure 1). The mean number of these structures was 132 trichomes cm^{-2} and 1299 trichomes cm^{-2} on the adaxial and abaxial surfaces, respectively. This result indicates that the higher number of these structures occurs together with stomata. The diameter of the glandular trichomes was $49.1 \pm 4.4 \mu\text{m}$. The presence of glandular trichomes in plants is an important constitutive defense against herbivorous arthropods (Harborne, 1991); however, the presence of these structures could be related to the release of plant volatile compounds that are attractive to the leafhoppers. This possibility should be further investigated.

Field evaluations of plant volatiles suggest that some synthetic blends are attractive to certain insects (James, 2005; James, 2003), which opens new perspectives for the introduction of intercrops or trap crops technologies and traps with synthetic blends of volatiles (Khan et al., 2006; Gohole et al., 2003; James, 2003; Khan et al., 1997). This study involves a mechanistic approach, focusing on the response of the leafhopper *B. xanthopis* to a host plant and opens the possibility to identify and synthesize *V. condensata* volatiles for pest management programs of this insect.

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