Olfactory responses of the leafminer *Liriomyza sativae* (Dipt., Agromyzidae) to the odours of host and non-host plants

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Abstract: Electroantennogram (EAG) responses of male and female leafminer *Liriomyza sativae* Blanchard to 15 species of host and non-host plant odours were recorded. The most distinct EAG responses of both sexes were obtained in the host plants bean and tomato. EAG responses to other host plants, such as cucumber, celery, chrysanthemum, towel gourd and cotton were reduced. Odours of non-host plants, such as Chinese rose, tobacco and morning glory elicited minimal EAG responses. In a behavioural study, both sexes of *L. sativae* were more attracted by the odours of host plants than of non-host plants. Thus, olfactory cues are important for host plant location by *L. sativae*. In addition, the combination of strong EAG and behavioural responses for both males and females to specific plant odours suggests that host plant volatiles can be important as aggregation cues for both sexes.

1 Introduction

The leafminer *Liriomyza sativae* Blanchard is a polyphagous pest worldwide, causing economic damage to vegetable and ornamental crops. Females of *L. sativae* puncture the upper leaf surface for feeding and oviposition. The larvae feed in the leaf mesophyll tissue and form a serpentine mine, thereby reducing the photosynthetic capacity of leaves (Johnson et al., 1983). Substantial variability occurs in specific biological characteristics of *Liriomyza* spp. on different plants (Parrella et al., 1983; Parrella and Bethke, 1984). As the immature stages of the leafminer are obligatory internal plant feeders, detection of suitable hosts by female leafminers is critical for survival of offsprings (Zehnder and Trumble, 1984). Previous studies demonstrated that many *Liriomyza* spp. exhibit host plant preference (Zehnder and Trumble, 1984; Parkman et al., 1989; Carolina and Johnson, 1992; Zhao and Kang, 2001a). Another field study showed that *L. sativae* infest many species of weeds, but the number of leafminers on weed hosts declined when tomato plantings were present (Schuster et al., 1991). Therefore, the decline in the number of leafminer larvae observed in weeds appeared to be related more to the preference for tomato plantings than to the availability of weeds.

Many factors affect the host plant preference of *Liriomyza* species. The distribution and density of leaf trichomes, as well as the nutritional status of the host plants, were found to be important in host selection (Fagoonee and Toory, 1983; Knodel-Montz et al., 1985; Minkenberg and Ottenheim, 1990). More recently, Abdel and Ismail (1999) determined that some 'secondary plant substances of no nutritive value', such as glucosinolate, affected leafminer acceptance and infestation in crucifers.

Searching for an oviposition site probably involves several sensory systems operating simultaneously and in a sequence; olfaction is generally believed to be the most important sensory system for many phytophagous insects. For example, the medfly *Ceratitis capitata* Wiedemann (Light et al., 1988; Cosse et al., 1995) and carrot fly, *Psila rosae* (Guerin and Visser, 1980), have been shown to respond to the odours of their host plants. Fagoonee and Toory (1983) proved that chemical attraction could contribute to host plant selection of *Liriomyza trifolii*. However, they found no further evidence, and little is known about the effect of olfaction in host plant location by *Liriomyza* species. Therefore, the present study was undertaken to investigate olfactory sensitivity of *L. sativae* to host plants using both the electroantennogram (EAG) technique and behavioural assays in an olfactometer.

2 Materials and methods

2.1 Insect and plants

Liriomyza sativae was reared under conditions described by Zhao and Kang (2000). They were reared on bean (*Phaseolus vulgaris* L.) at $25 \pm 1°C$ with a photoperiod of 14 h light : 10 h dark. Only unmated adults less than 24-h post-emergence were used for EAG analyses and olfactometer bioassays.

All plants (table 1) were grown in an experimental field at the Institute of Zoology, Chinese Academy of Sciences. Plant samples were taken from the leaf petiole so as to minimize the

Table 1. *Plants used in EAG test*

Family	Scientific name	Common name
Leguminosae	*Phaseolus vulgaris* L.	Common bean
	Vigna unquiculate W.ssp (L.) Verd.	Cowpea
Solanaceae	*Lycopersicon esculentum* Mill.	Tomato
	Solanum melongena L.	Eggplant
	Capsicum annuun grossum	Bell pepper
	Nicotiana tobacum	Tobacco
Cucurbitaceae	*Cucumis sativus* L.	Cucumber
	Luffa acutangula Roxb.	Towel gourd
	Cucurbita pepo L.	Squash
Malvaceae	*Gossypium hirsutum*	Cotton
Convolvulaceae	*Pharbitis purpurea* (L.) Voigt	Morning glory
Umbelliferae	*Aouyn graveikebs* L.var.dulce Dc.	Celery
Vitaceae	*Parthenocissus tricuspidata* (Sieb. et Zucc.) Planch	Creeper
Rosaceae	*Rose chinensis*	Chinese rose
Compositae	*Chrysanthemum morifolium* (cv. sinense)	Chrysanthemum

number of cut ends through which volatiles from within the tissues might escape. Cartridges were loaded with a fresh sample of each plant every morning, left at room temperature and used throughout the day.

2.2 EAG recording technique

2.2.1 Antenna preparation

Each antenna was prepared according to the method described by ZHAO and KANG (2001b). Briefly, the test insect's head was moved, and the tip of the arista cut off. EAGs were recorded with electrolyte-filled (Ringer's Solution) glass capillary Ag-AgCl electrodes; the reference electrode was positioned into the haemocoel of the cranial cavity, while the recording electrode was connected with the cut arista. The ventral surface of the antenna was oriented to face the odour stream. Only the left antenna from each insect was used in these experiments.

2.2.2 Odour delivery system

The odour delivery system was similar to that described by CHEN and KANG (2000). The odour source cartridges consisted of glass tubes 70 mm long and 25 mm diameter containing 2 g fresh weight of plant material (WHITE and CHAPMAN, 1990). Healthy and intact leaves, weighting over 2 g, were used for the EAG analyses on undamaged foliage. The leaves were weighed in a balance. The petiole was cut until the leaf weighed 2 g. Wound fluid from the cut end of the petiole was absorbed by filter paper and the cut end would dry after a while. The cartridges were sealed at each end with a rubber stopper wrapped in a piece of foil. A short length of a 7-mm-diameter glass tube was passed through each end of the rubber stopper to allow air to move through the cartridge. A constant flow (1.0 l/min) of charcoal-filtered and humidified compressed air was passed over the antenna through a disposable nozzle positioned *ca.* 1 cm from the antenna. Test substances were introduced into the air stream by inserting the tubing of the cartridge into the hole of the delivery tube and injecting the contents of the cartridge quickly and smoothly. The stimulation duration was 0.2 s. Each stimulus was followed by an interval of *ca.* 90 s of clean air. The signal was amplified 10 times by a Universal AC/DC amplifier UN-06 and viewed on the computer screen. EAGs were recorded with a Syntech EAG recording system for Windows (Syntech UN-06, The Netherlands).

2.2.3 Experimental procedure

For each stimulus, EAGs were recorded from at least 10 antennae of each sex. After every third to fifth test, a 'control'

stimulation (an empty cartridge) and 'standard' stimulation (using fresh cartridges impregnated with 1 μl of 95% hexan-1-ol) were presented. EAGs of test plants were evaluated by measuring the maximum amplitude of negative deflection ($-$mV) elicited by a given stimulus and then subtracting the amplitude of the response to the preceding control. The mV responses to all plants were converted to percentage values of the response to the accompanying standard. EAG data were subjected to ANOVA and mean values were compared by least significant difference LSD.

2.2.4 Olfactometer behavioural bioassays

Behavioural assays on both sexes of *L. sativae* were performed in a perspex olfactometer as described by VET et al. (1983). The humidified air stream (50% relative humidity), drained through a hole in the centre of the device, was set at 20 l/h for each of the four arms. The test stimuli were placed at the distal end of the arms, with 'no stimulus' humidity air as control. As the leafminers were reared on bean leaves through many generations, bean leaves were used as reference odour in the test. Treatments comprised: (i) bean leaves vs. tomato leaves; (ii) bean leaves vs. cucumber leaves; (iii) bean leaves vs. creeper leaves; and (iv) bean leaves vs. Chinese rose leaves. One fly was introduced into the hole in the centre of the chamber. When the fly climbed out of the hole, its position was recorded every 10 s for 3 min. If the fly showed little activity, the experiment was terminated and the fly was replaced. The entire apparatus was rotated 90° after every five to eight insects were tested. Each treatment was replicated more than 25 times and the percentage of time spent in each arm was analysed using ANOVA followed by Duncan's multiple range analysis. All the tests were carried out at room temperature $25 \pm 3^{\circ}$C.

3 Results

3.1 EAGs

Mean responses of the male [-1.13 (± 0.03 SE) mV] and female [-1.07 (± 0.04 SE) mV] *L. sativae* to the hexan-1-ol standard (95%, 1 μl) were not significantly different.

There were differences in the magnitude of the EAG responses of *L. sativae* to different plant odours (P $<$ 0.05) (fig. 1). Both male and female antennae were significantly more responsive to bean and tomato.

The EAG responses to other host plants, such as cucumber, celery, chrysanthemum, towel gourd and cotton were less pronounced. The lowest magnitude of responses were observed for Chinese rose, tobacco and morning glory.

The pattern of responses to different plants was similar in both sexes of the leafminer. With few exceptions, no significant differences were detected in the responses of male and female flies to various plants.

3.2 Olfactometer

The results of bioassays in the olfactometer are shown in fig. 2. In treatments 1 (bean vs. tomato vs. control air) and 2 (bean vs. cucumber vs. control air), the leafminers spent equivalent times searching in the olfactometer arms with plant odours, but significantly more than in the control air ($P < 0.01$) (fig. 2a,b). In

treatments 3 (bean vs. creeper vs. control air) and 4 (bean vs. Chinese rose vs. control air), leafminers spent most of the time in arms with bean odour; the time spent in the arms with odours of Chinese rose and creeper was not different from time spent in the control air (fig. 2c,d). The olfactory response patterns of male and female leafminer were similar in each bioassay.

Because bean was employed as a reference in each treatment, we compared the mean values of the time spent in each odour to determine potential preferences of leafminers for the test plant odours (fig. 3). Both sexes spent significantly more time with the odours of bean, tomato and cucumber than odours of Chinese rose and creeper ($P < 0.01$). There was no significant preference detected between bean, tomato and cucumber. The times associated with Chinese rose and creeper were not significantly different from those of the control air.
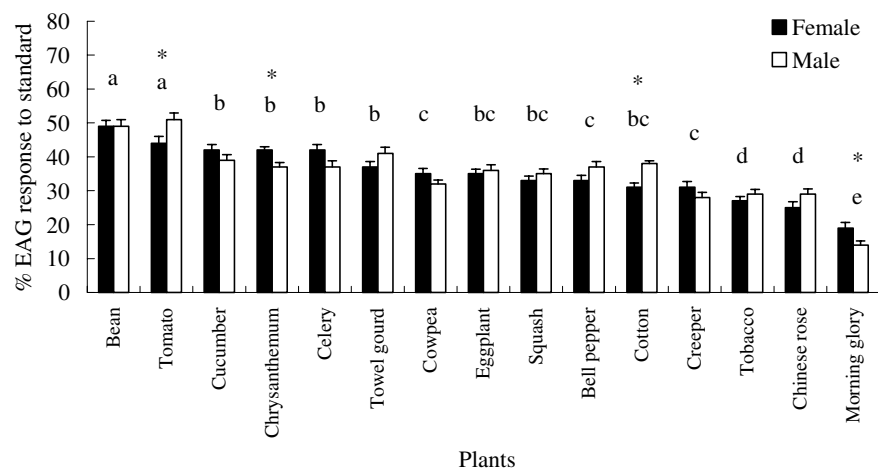

Fig. 1. Mean EAGs of L. sativae in response to the odours of healthy plant leaves. Vertical lines show standard errors ($n > 10$). Mean values with the same letter are not significantly different ($P < 0.05$). The asterisks above the bars show difference between sexes ($P < 0.05$)
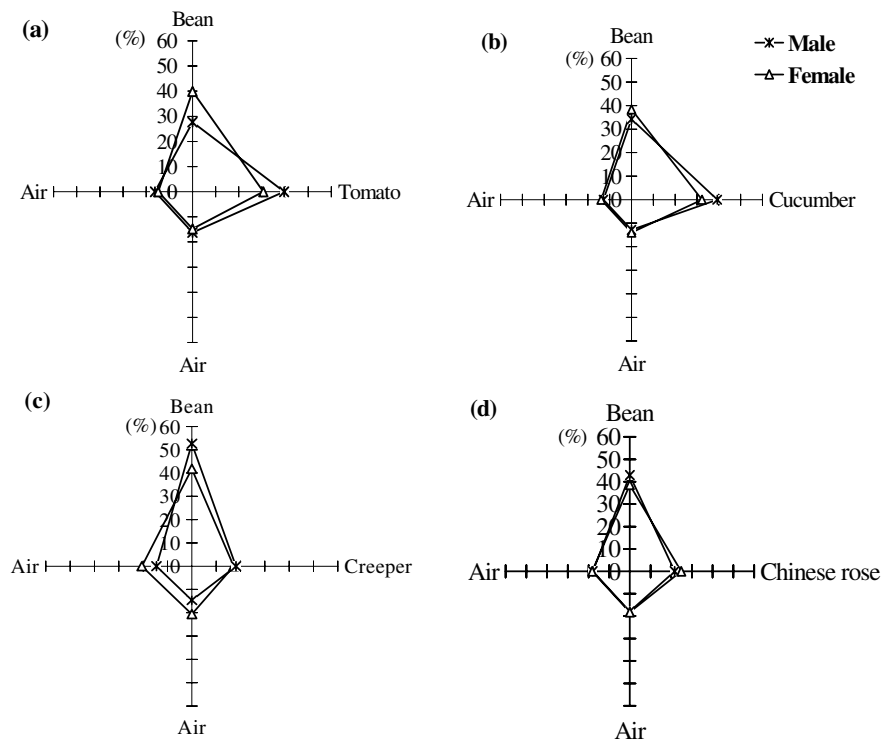

Fig. 2. Time spent by L. sativae in each odour plume within a four-arm olfactometer ($n > 25$)
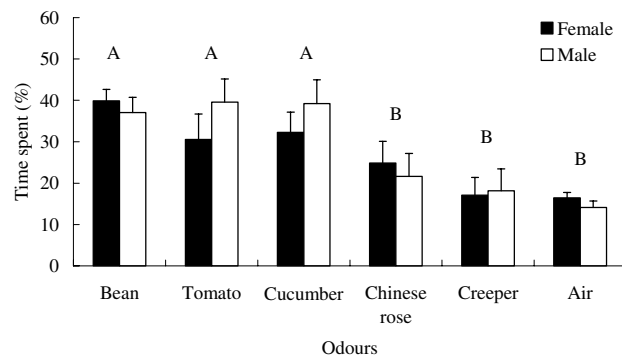
Fig. 3. *Behavioural responses of* L. sativae *in the odours of host (bean, tomato and cucumber) and non-host plants (Chinese rose and creeper). Vertical lines show standard errors (n > 25). Mean values with the same letter are not significantly different (P < 0.01)*

4 Discussion

No significant sexual differences were found in EAG responses to odours of fresh leaves among the 15 plants tested, which may be due to the high similarity in the antennal sensilla of sexes (ZHAO and KANG, 2002). A similarity in antennal responses of male and female insects to plant odours has been previously noted for many other insect species (LIGHT, 1988 and references therein). One potential explanation for this correspondence is that males and females live in the same habitat and may share the same chemical cues to locate host plants on which to feed and mate.

Although the host plant range of *L. sativae* is extremely broad and diverse, our approach of EAG analysis demonstrates that the leafminer antennae selectively respond to host plant odours. The magnitude of the EAG response to different plants can be classified into three categories. The strongest, most distinct responses were elicited by bean and tomato. The leafminers were reared on bean continually for more than 60 generations and larval host experience may affect the preferences of resulting adults (CAROLINA and JOHNSON, 1992). However, tomato is a preferred host plant of this leafminer (ZEHNDER and TRUMBLE, 1984), and this preference was not lost despite an extended period of rearing on bean. An intermediate response category included cucumber, squash, cowpea, bell pepper, eggplant and celery, cotton, chrysanthemum, towel gourd and creeper. All of them are host plants of the leafminer except creeper. Thus, additional cues, probably following contact, are used to avoid oviposition on this plant.

Only a slight response was detected in the third category, which included Chinese rose, tobacco and morning glory. Chinese rose is a non-host plant of *L. sativae*, and it has been demonstrated that among all the polyphagous leafminers, only *L. trifolii* can use plants in Rosaceae as hosts (KANG, 1996). Tobacco and morning glory are seldom attacked by *L. sativae*, possibly because of the presence of nicotine and other defensive chemicals.

Behavioural studies in the olfactometer produced analogous results: plants producing the strongest EAG responses had odours that were most attractive to the leafminer adults. They usually spent more time searching in the odour of host plants, such as bean, tomato and cucumber. Little time was used searching in the non-host plant odours. Thus, *L. sativae* most likely employ olfaction in host plant location.

Host plant finding by insect can be disturbed by non-host plants in their vicinity, which provide the camouflage, diversionary or repellent components found in diverse ecosystems (UVAH and COAKER, 1984). Blending the odour of non-host plant species with the attractive host plant odour blocks the release of upwind responses of Colorado potato beetle (THIERY and VISSER, 1986). Intercropping host plants with non-host plants may reduce attacks by *Liriomyza*.

Acknowledgements

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