

Physical and chemical cues affect oviposition by *Neoleucinodes elegantalis*

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Abstract. Recognition and acceptance of a suitable host plant by phytophagous insects requires the integration of visual, physical and chemical cues. The present study investigates the host cues that a specialist insect integrates to optimize oviposition decisions and whether these cues are weighted in a specific way. The study also determines whether the tomato fruit borer *Neoleucinodes elegantalis* (Guenée) (Lepidoptera: Crambidae), an important pest on Solanaceae in Brazil, shows a preference for oviposition sites that differ in physical and/or chemical cues. When styrofoam balls are provided as artificial fruits, *N. elegantalis* deposit significantly more eggs on rough artificial fruits than on smooth ones. Hexane fruit extracts applied to the artificial fruits stimulate female oviposition strongly. Physical and chemical cues also affect the oviposition of females when offered together. Furthermore, certain parts of the artificial fruits are preferred, irrespective of the presence of chemical cues. Both physical and chemical cues affect oviposition decisions; hence, the fruit borer relies on cues of different sensory modalities.

Key words. Artificial fruits, chemical stimuli, egg-laying behaviour, physical stimuli, tomato fruit borer.

Introduction

Finding a suitable host plant is one of the critical steps for reproduction of all phytophagous insects (Thompson & Pellmyr, 1991). Because newly-hatched larvae are relatively immobile, they are unable to find a better host when their mother has oviposited on a host that is unsuitable for larval development (Renwick & Chew, 1994).

Recognition of a suitable host plant requires integration of inputs from physical and chemical senses. Olfaction and vision may be used for long-distance orientation to the host plant. After landing, short-distance judgment requires senses to detect chemical and physical cues, using contact chemoreceptors on antennae, mouthparts and ovipositors. Usually, recognition of

chemical compounds on the plant surface results in a decision to accept or reject the oviposition site (Ramaswamy, 1988; Renwick & Chew, 1994).

Physical characteristics of the oviposition site such as surface texture play a critical role in the final decision to lay eggs (Renwick & Chew, 1994). Some moths prefer to oviposit on hairy or rough surfaces (Ramaswamy, 1988; Rojas *et al.*, 2003; Nava *et al.*, 2005), whereas other species prefer smooth surfaces (Foster *et al.*, 1997; Calatayud *et al.*, 2008).

Chemical compounds present on plant surfaces can be volatiles that contribute toward long-distance orientation to the host plant, or nonvolatile compounds that play a role as oviposition stimulants or deterrents (Renwick & Chew, 1994). It is well established that these chemical cues are very important with respect to the orientation and acceptance of oviposition sites in several species of Lepidoptera (Ramaswamy, 1988; McNeil & Delisle, 1989; Honda, 1995; Peterson & Eelsey, 1995; Hora & Roessingh, 1999; Reddy *et al.*, 2004; Gouinguéné *et al.*, 2005; Lombarkia & Derridj, 2008; Sidney *et al.*, 2008).

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For both physical and chemical cues, preferences among phytophagous insects depend on host suitability because host plants differ in the physical and chemical cues that insects use to discriminate between hosts. Oligophagous insects might concentrate on one or a few cues that are specific to their host plant, and have a high ability to evaluate these cues and discriminate between oviposition sites (Janz & Nylin, 1997). The recognition of these cues depends on the insect sensory system for detecting and decoding the signals available in the host (Dethier, 1982). However, the rejection and acceptance of oviposition sites depends on the analysis of sensory input by the insect's central nervous system and the physiological state of the insect.

The tomato fruit borer *Neoleucinodes elegantalis* (Guenée) (Lepidoptera: Crambidae) is an important oligophagous pest on Solanaceae. Adults are active at night only. First-instar larvae have low mobility and bore inside the fruits within a few minutes of hatching (Eiras & Blackmer, 2003). When the fruits are unavailable, larvae are moribund (W. Pontes, unpublished data). This suggests that mated females are under a strong selection pressure to find the correct site to lay eggs. Hence, mated females should have the ability to detect cues indicating hosts and host quality. *Neoleucinodes elegantalis* also shows a preference for laying eggs on specific places of fruits (i.e. under the calyx) (Blackmer *et al.*, 2001). However, it is not known what kind of physical and chemical cues determine oviposition choice by *N. elegantalis*, or whether they are sensitive to luminescence as members of the closest family Pyralidae appear to be (Briscoe & Chittka, 2001). The present study therefore tests which physical cues (i.e. surface types) and which chemical cues affect oviposition in *N. elegantalis* females, and how these cues of different sensory modality interact.

Materials and methods

Neoleucinodes elegantalis were collected from a commercial tomato crop in Minas Gerais State, Brazil, and reared in the laboratory for 2 years. Both the rearing and experimental conditions were performed under an LD 12 : 12 h photoperiod at 25 ± 1 °C and $71.2 \pm 10\%$ relative humidity. The larvae were reared on *Solanum gilo* (Solanaceae) fruits until pupation. Pupae were sexed and each gender was incubated in separate experimental wooden cages (50 × 50 × 50 cm). On emergence, males and females were kept together during 3 days for copulation in another cage with a cotton piece soaked in a honey solution (10%). After approximately 3 days, the females were used in the experiments, and thereafter dissected to assess the presence of spermatophores in the bursa copulatrix (Burns, 1968). To assess oviposition, Styrofoam® balls (diameter 3.5 cm) were used as artificial fruits. All of the balls were dipped in green melted paraffin to produce a thin wax layer that covered the surface. Nine replicates were studied for each treatment in each experiment, and each replicate involved the oviposition response of a group of ten females.

Physical cues

To test the influence of physical cues on *N. elegantalis*, ovipositing females were offered a choice between two different surfaces. Ten 48-h-old gravid females were used. Longitudinal furrows were made with a razor blade on the surface of the artificial fruits to obtain a rough surface. Intact artificial fruits were considered as smooth. A rough and a smooth artificial fruit were suspended, approximately 25 cm apart, from the top of the cage using wire. Between replicates, and after 24 h within one experiment, the positions of the artificial fruits were interchanged to avoid environmental bias. The females were allowed to oviposit for 48 h. Thereafter, eggs on each artificial fruit were counted. On rough artificial fruits, eggs on the smooth surface and in the furrows were counted separately.

Chemical cues

Tomatoes fruits, *Lycopersicon esculentum* L. (Solanaceae, variety Sensação, Agrocinco, São Paulo), with a diameter of approximately 1.5 cm, were collected in a commercial tomato crop in Minas Gerais State, Brazil, for the extraction of chemical cues. The fruits were weighed and immersed in hexane for 30 min. Subsequently, the solution was reduced to dryness by evaporation of the solvent. The fruit extract was then expressed as gram fruit equivalent per millilitre solvent and kept in a freezer (−18 °C) until used in the tests.

To determine whether these fruit extracts affected the oviposition decision of females, a choice test was performed with artificial fruits containing extracts and controls without extract. The fruit extract was applied on a 5-mm wide strip of filter paper, which was wrapped horizontally around the artificial fruit, like a ring, and glued on the ends. For each treatment, 30 µL of the hexane extract was applied to the filter paper; the same amount of pure hexane was applied to the controls. Groups of ten gravid females were offered a choice between these two artificial fruits for 24 h, after which the number eggs on each artificial fruit was counted. The number of eggs deposited on the waxy surface on the upper face of the strip of paper, and also under the strip of paper, were counted separately and compared between treatments.

Visual and chemical cues

To evaluate the effect of chemical and visual cues on oviposition, four treatments were offered to females: both chemical and visual cues; only visual cues; only chemical cues; and neither of the two cues.

To manipulate visual cues, experimental cages were placed under low light intensity (0.11 lux, measured with a luxometer) providing the minimum light intensity for insect activity and used to rear *N. elegantalis* during the scotophase. The absence of visual cues was mimicked by placing cages in absolute darkness. Chemical cues were offered on smooth artificial fruits to which a hexane extract of tomato fruits was applied,

as described above. Females were allowed to oviposit for 48 h. In this experiment, all treatments were repeated five times with six females per replicate. Eggs were counted after 24 h.

Statistical analysis

A chi-square test was employed to analyse the choice between each treatment in both the physical and chemical tests. The mean percentage of eggs on each of the two artificial fruits was calculated.

To test the combined effects of visual and chemical cues on oviposition of females, eggs were counted on each artificial fruit in all treatments and analyzed with generalized linear model with a quasi-poisson error distribution. All analyses were performed with R statistical system, version 2.4.1. (R Development Core Team, 2006).

Results

Physical cues

Neoleucinodes elegantalis deposited significantly more eggs on rough artificial fruits than on smooth ones ($\chi^2 = 22.65$, $P < 0.0001$) (Fig. 1A). More eggs on rough artificial fruits were found inside the furrows ($\chi^2 = 51.12$, $P < 0.0001$) than on the waxy smooth surface (Fig. 1A).

Chemical cues

More eggs were found in treatments with hexane extracts than in the control ($\chi^2 = 3.92$, $P = 0.0475$) (Fig. 1B). More eggs were laid on the filter paper strips with hexane extracts than on strips with only hexane ($\chi^2 = 8.20$, $P = 0.0041$) (Fig. 2A) and more eggs were laid on the wax layer of the

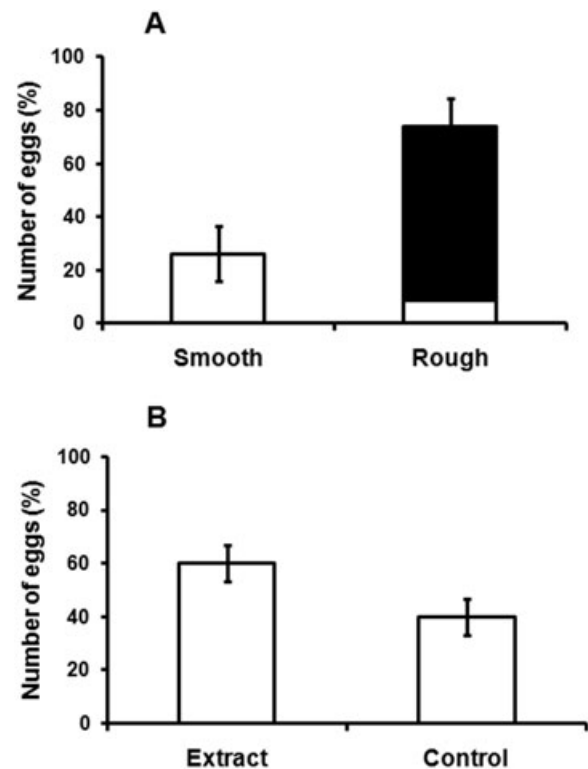


Fig. 1. (A) Mean percent of eggs of *Neoleucinodes elegantalis* deposited on smooth and rough artificial fruits when they were offered simultaneously in a two-choice test. The right bar shows the amount of eggs layed inside furrows (black) on rough artificial fruits. (B) Eggs deposited on artificial fruits treated with hexane extracts of tomato fruits compared with artificial fruits treated with hexane only (control). Error bars indicate standard errors.

artificial fruit with tomato hexane extracts than on that with hexane only ($\chi^2 = 8.26$, $P = 0.0040$) (Fig. 2B). Similarly, more eggs were deposited under of the filter paper strip on the

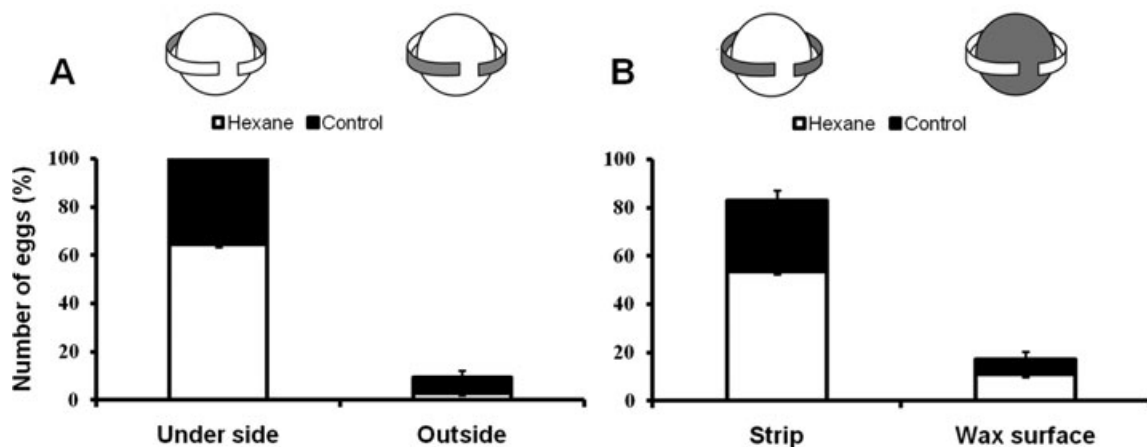


Fig. 2. Number of eggs deposited by *Neoleucinodes elegantalis* on artificial fruits with hexane extract (open bars) and only hexane (black bars). (A) Eggs underside of the strip and the strip (outside) and (B) eggs on the whole strip and the wax surface. Egg deposition areas are highlighted in grey.

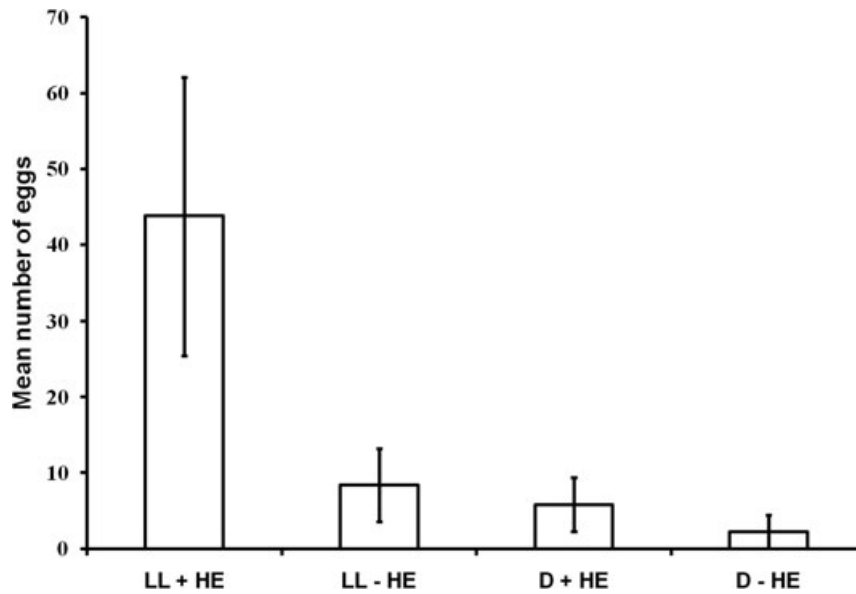


Fig. 3. Mean number of *Neoleucinodes elegantalis* eggs on artificial fruits in visual and chemical conditions (LL, low light intensity; HE, hexane extract; D, absolute darkness). Only the values recorded for the LL + HE treatment are significantly different from the others. Error bars indicate standard errors.

artificial fruits treated with tomato hexane extracts ($\chi^2 = 8.27$, $P = 0.0040$) than under the strip on the artificial fruits treated with solvent only (Fig. 2A, B). Most of the eggs were laid under the paper strip, followed by eggs on the strips, and in smaller quantities on the wax layer of the artificial fruits. This pattern was observed both in treatments (i.e. with hexane extracts and with hexane as control) (Fig. 2A, B).

Visual and chemical cues

Significantly more eggs were laid when both visual and chemical cues were available at the same time ($\chi^2 = 321.3$, $P < 0.0001$, d.f. = 3) (Fig. 3). However, no differences in egg number were found when at least one of the cues (or both of them) was removed.

Discussion

The results of the present study show that physical and chemical cues affect the oviposition of *N. elegantalis*. The effect of physical factors on oviposition in Lepidoptera is well known. Some species prefer smooth surfaces (Foster *et al.*, 1997; Calatayud *et al.*, 2008), whereas other species prefer rough substrates (Ramaswamy, 1988; Rojas *et al.*, 2003; Nava *et al.*, 2005). A preference for smooth surfaces may help the females to sweep their ovipositor over the surface to taste and thereby find a suitable site for oviposition (Calatayud *et al.*, 2008). However, a preference for rough surfaces (Ramaswamy, 1988; Fenimore, 1988) appears to be more general (Rojas *et al.*, 2003).

The large number of eggs found inside the furrows of roughened artificial fruits suggests a preference to oviposit in places that are less exposed to environmental conditions and natural enemies (Janz, 2002). For example, in *Pieris rapae crucivora*, not only surface texture is preferred for egg laying, but also plant parts are preferred that can provide some protection to eggs (Tagawa *et al.*, 2008).

The oviposition behaviour of *N. elegantalis* is observed in some cases: females walk on the surface, thereby contacting the surface of the artificial fruit with their antennae and the tip of the proboscis and dragging the ovipositor over the substrate when walking (W. Pontes, unpublished data). This behaviour suggests that sensory organs for tactile and chemical cues are located on the antennae, proboscis and ovipositor. Many butterflies and moths are known to antennate and drum the plant surface with their ovipositor prior to egg laying, and thus probably are using contact chemoreceptors to taste the suitability of chemical compounds and to explore the surface texture of their host plant (Visser, 1986; Renwick & Chew, 1994; Maher & Thiery, 2004). The antennal receptors of other Lepidoptera species show differential sensitivity to host and nonhost plants (Mercader *et al.*, 2008).

Hexane extracts of fruits stimulate oviposition by *N. elegantalis* significantly. All artificial fruits on which tomato extracts are applied are preferred for oviposition. The application of host plant chemical compounds to artificial substrates makes these sites usually more acceptable for oviposition (Foster & Howard, 1998; Hora & Roessingh, 1999; Heinz & Feeny, 2005; Heinz, 2008).

The fact that a hexane extract of tomato stimulates oviposition in *N. elegantalis* does not rule out the influence of visual cues on oviposition behaviour; light is required to locate a source of attractive chemicals. Most females lay more

eggs in experimental cages when both cues (in this case, visual and chemical) are available. The few eggs that are found on artificial fruits in absolute darkness and without extract (no chemical cue) are perhaps the result of some females that randomly encounter the artificial fruits, land on it, and oviposit.

More eggs are laid when chemical stimuli are combined with rough surfaces; when a filter paper treated with extracts is combined with the artificial fruit, the rough surface of the filter paper receives the highest number of eggs. This shows an interaction of chemical and physical cues on oviposition decisions. Volatiles and nonvolatile compounds are known to stimulate attraction of gravid females (Piñero & Dorn, 2007) and to increase the number of eggs laid on the source (Honda, 1995; Spencer *et al.*, 1999). The present study shows that *N. elegantalis* females also use different sensory modalities to assess oviposition sites.

In both treatments, with and without hexane extract, the insects prefer to lay eggs under the strip, near the edge, which is probably similar to their preference to lay eggs in a furrow. In the field, eggs of *N. elegantalis* are found mainly on the underside of the calyx, and this preference is suggested to be a result of physical rather than chemical cues (Blackmer *et al.*, 2001). In the present study, *N. elegantalis* prefer furrow-like places on the artificial fruits, irrespective of whether they are offered chemical stimulants or not. However, the presence of hexane extracts increases the total number of eggs laid by females significantly, in a combined effect of physical and chemical stimuli presented together.

Acknowledgements

We thank Maurice W. Sabelis and Izabela Lesna for their valuable comments on the manuscript; J. D. Matiello and S.A.S. Souza for assistance with the experiments and their analysis. This research was supported by CNPq-INCT Semiochemicals in Agriculture, PROCAD/CAPES 0083054; and a CNPq scholarship to E.R.L., E.G.C., P.M.T.A. and R.B.

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Accepted 6 January 2010

First published online 25 February 2010