

## Host-Related Olfactory Behavior in a Fruit-Piercing Moth (Lepidoptera: Erebidae) in Far Eastern Russia

Jennifer M. Zaspel,<sup>1</sup> Vladimir S. Kononenko,<sup>2</sup> Rickard Ignell,<sup>3</sup> and Sharon R. Hill<sup>3,4</sup>

<sup>1</sup>Department of Entomology, Purdue University, 901 W State Street, West Lafayette, IN 47907, <sup>2</sup>Laboratory of Entomology, Institute of Biology and Soil Sciences Far Eastern Branch of Russian Academy of Sciences, RF-690022, Vladivostok, Russia, <sup>3</sup>Unit of Chemical Ecology, Department of Plant Protection Biology, Swedish University of Agricultural Sciences, 230 53 Alnarp, Sweden, and <sup>4</sup>Corresponding author, e-mail: sharon.hill@slu.se

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### Abstract

The host preference of the economically important fruit piercing moth, *Calyptra lata* (Butler 1881), was studied when exposed to different fruits and the odors of those fruits in enclosed feeding assays and in a two-choice olfactometer. The fruits consisted of three ripe and locally available types: raspberries, cherries and plums. Moths were released in cages with the ripened fruit and observed for any feeding events, which were then documented. Moths fed on both raspberries and cherries, but not on plums. To test the role of olfactory cues in fruit preference, male moths were released singly in the two choice olfactometer, with one type of fruit odor released in one arm and background control air in the other. The behavior of the moths was recorded on video. Parameters scored were 1) time to take off, 2) flight duration and 3) total time to source contact. The moths showed a significant preference for raspberry odor, exhibited a neutral response to cherry odor and significantly avoided the odor of plums. These results indicate that *Calyptra lata* demonstrates selective polyphagic feeding behavior and uses olfactory cues from both preferred and non-preferred fruits to detect and locate potential food sources. The possible implications for pest control are discussed.

**Key words:** *Calyptra lata*, olfaction, fruit, host preference, polyphagy

The genus *Calyptra* Ochseneimer (Lepidoptera: Erebidae: Calpinae) is unique among other Lepidoptera in that the males of several species have demonstrated the ability to pierce vertebrate skin, drill into the tissues beneath and suck blood (Bänziger 1968, 1975; Zaspel et al. 2007). As a result of this unusual behavior, the majority of research has focused on the ecology and evolution of blood feeding in this lineage (Büttiker 1962; Bänziger 1975; 1979; 1980; 1982; 1983; 1986; 1987; 1989; Zaspel et al. 2007; Zaspel and Branham 2008; Hill et al. 2010; Zaspel et al. 2011; Zaspel et al. 2012; Zaspel et al. 2014). Consequently, the interaction of these moths with their ancestral and obligate hosts, fruit-bearing plants, has been largely overlooked (Bänziger, 2007). Several *Calyptra* species, including *Calyptra lata*, are economically important pests of fruit orchards across Asia (Hattori 1969; Uchida et al. 1978; Togashi 1982; Park et al. 1988; Bänziger 1982; 2007; Bhumannavar and Viraktamath, 2013). It is the ability of the adult moths to locate and pierce ripe fruit that makes these moths such pests (Bänziger 2007; Bhumannavar and Viraktamath 2013).

As nocturnal insects that forage in low light conditions, the vast majority of moths studied have been found to rely heavily on olfaction to detect, locate and discriminate among potential hosts in the context of background environmental odors over a range of

distances (for review see Carrasco et al. 2015). Erebid fruit-piercing moths, including *Calyptra*, travel long distances from their daytime habitats in search of their fruit hosts (Hattori 1969; Uchida et al. 1978; Togashi 1982; Park et al. 1988; Bhumannavar and Viraktamath 2013). Some *Calyptra* species are known to be polyphagic, and are capable of feeding on 10 or more disparate fruit species (Bänziger 2007; Bhumannavar and Viraktamath 2013). As a consequence, the olfactory system of these moths needs to be both sensitive and selective if the moths are to detect and discriminate among host and non-host efficiently at a distance (Carrasco et al. 2015).

Here, we investigated the role that olfaction plays in host finding and recognition in the fruit-piercing moth, *Calyptra lata*. We identified three locally available potential fruit hosts from the collection area in far eastern Russia. These fruit hosts were chosen based on those previously described for other *Calyptra* species (Bänziger 2007), and their availability in the moths' habitat at the time of the experiments. We assessed the suitability of these fruit hosts for the field-collected *C. lata* in feeding experiments carried out under enclosed conditions. We further demonstrated that *C. lata* males respond to odors from different fruits, indicating an olfactory-based host preference. The implications of olfactory-related host seeking

for *Calyptra* ecology and its potential for biological pest control are discussed.

## Materials and Methods

### Animals and specimen collection

All *Calyptra lata* used in this study were wild-collected using standard techniques: suspended white sheets illuminated by a 60 W mercury vapor lamp (Winter 2000). Live specimens were collected into separate plastic vials (37 ml) from the Gornotayeznaya Biological Station (GBS) (N 43° 41.917 E 132° 09.131), Primorye Territory in Russia between 25 July 2014 and 04 August 2014. While both sexes were collected, individuals were sexed and only males were subsequently used in the study to avoid the risk of female sex pheromone contamination confounding the fruit seeking olfactory behavior.

### Behavioral assays

#### Feeding assay

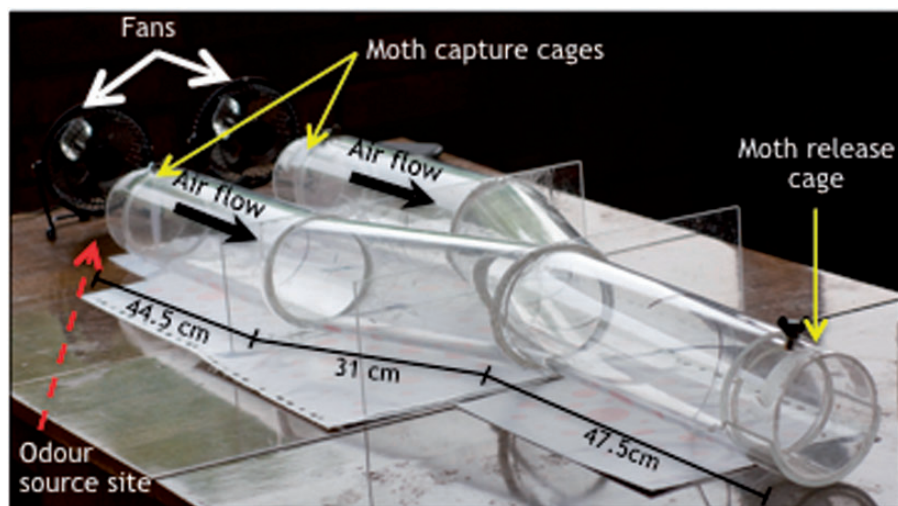
Male *C. lata* were presented with a single fruit-type in a bug dorm cage between 12 and 24 h post-capture (enclosed conditions). The cages were monitored for feeding activity during this period, and feeding events were photographed.

#### Y-tube assay

A Y-tube olfactometer (Fig. 1), consisting of plexiglass tubes (100 mm inner diameter × 120 cm total length), was used for the olfactory bioassays. Two USB powered fans (model nr. IK51167, Plexgear, SE) passed air into each arm of the Y-tube at  $30 \text{ cm s}^{-1}$ , as measured 20 cm downstream of the capture cages in the centre of the arms. As these experiments were conducted on the site of the field collections, the incoming air was redolent with natural background odors, and no charcoal or other air filters were employed. The experiments were conducted on an outdoor wind-sheltered balcony (4 × 5 m) surrounded by brick walls (1 m high) and under an overhang (2.5 m in height). During the experiments, the temperature and relative humidity ranged from 17 to 22°C and 88 to 98% RH. The experiments were conducted between 23:30 and 05:30 under extremely low light conditions. Experiments were visualized using a night vision video camera (Sony Handycam Super Steady Shot,

HDR-SR11, CA). To allow the moths to adapt to the release cages, all moths were placed in the cages >30 min prior to the start of the experiment. To minimize any contamination, the olfactometer was aired daily with the fans running for at least 1 h prior to the experiments. The interior surfaces of the bioassay were never directly handled by the experimenter to minimize potential odor contamination.

The stimuli were chosen from local plants producing ripe fruit at the time of the study which were found within 200 m of the specimen collection point. The stimuli, raspberries (*Rubus crataegifolius* Bunge; ca. 50 g), cherries (*Prunus tomentosa*; ca. 50 g) or one medium sized plum (ca. 3–4 cm diameter; *Prunus cf. domestica*), were placed upwind of one of the arms of the Y-tube, and behind polyester gauze to render them invisible to the moths in the extreme low-light conditions. Volatile emission from the fruit was not directly controlled in this experiment, in the absence of odor collection equipment. However, we did choose amounts of fruit that approximated similar overall volatile release rates ( $\leq 1.5$  fold difference) based on previous studies for plum (Pino and Quijano 2012), cherry (Goliáš et al. 2012; Hayaloglu and Demir 2016) and raspberry (Malowicki et al. 2008). Moths were tested singly in the Y-tube to choose between a stimulus (fruit) embedded in the natural background air stream and the natural background air. The moths were released into the Y-tube downwind and their behavior was observed and recorded for 3 min. A positive response (source contact) was recorded when the moth landed on the gauze in front of the stimulus. Three parameters were measured: 1) time to take off, which is the duration of time between the opening of the release cage and when the moth moved upwind out of the release cage; 2) flight duration, which is the duration of time between leaving the release cage and making source contact; and 3) the total time to source contact, which is the duration between opening the release cage and source contact. Moths tested to air alone in the Y-tube did not enter either arm or make source contact within the 3 min of testing. To control for any directional bias, the stimuli were exchanged between the arms, and the arm to which the moth responded was recorded. There was no bias for either arm ( $\chi^2_1 = 0.8005$ ;  $P = 0.3709$ ). Each night, a randomly selected two of the three stimuli were tested to control for any night-to-night variation.



**Fig. 1.** A photograph of the Y-tube olfactometer set-up. Thick white arrows indicate the source of the air flow (two USB-powered desk fans); thick black arrows indicate the direction of the airflow; the dashed (red) arrow indicates the placement of the odor source (i.e. fruit); and the thin (yellow) arrows indicate the moth release and capture cages.

## Statistical analyses

Behavioral data were analyzed using either a  $\chi^2$  test or else logistic regression was used to perform comparisons between the blank control and the test treatments, as indicated in the text (PROC LOGISTIC, SAS Institute 2002).

## Results

### Host choice in *Calyptra lata* males

#### Feeding preference

*Calyptra lata* males fed on local soft-bodied fruit under enclosed conditions, i.e. wild raspberries and wild cherries (Fig. 2). Five individual moths were tested to each fruit. One individual was found to feed on cherries, while three individuals were observed feeding on raspberries. This soft fruit-piercing behavior was not, however, ubiquitous to all such fruits, as the moths did not feed on the offered plums.

#### Host-seeking and recognition

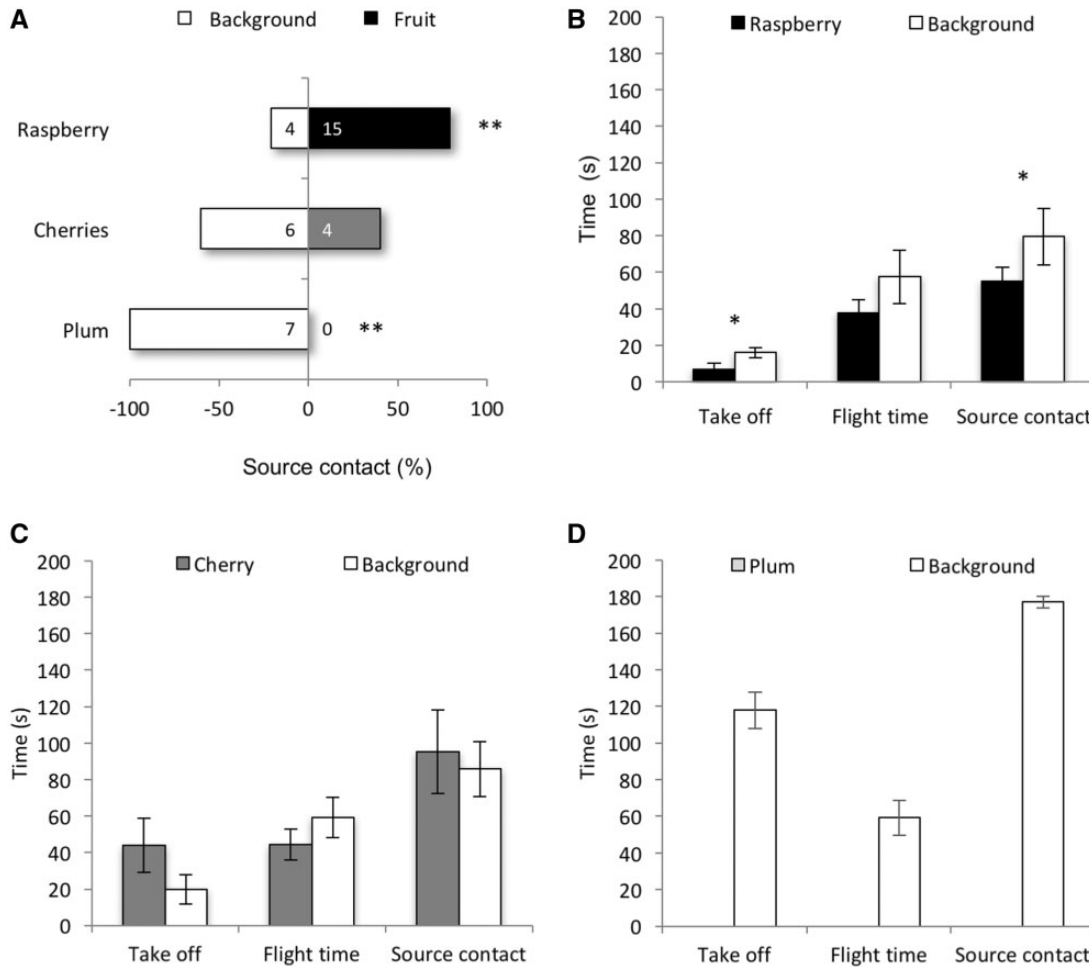
Of the *C. lata* males released into the Y-tube olfactometer with raspberry volatiles embedded into one of the airstreams, 61% were

activated to fly upwind (19 of 31 total) and 79% of these activated males responded positively to the raspberry odor plume by landing on the source ( $\chi^2$  test,  $\chi_1^2 = 6.37$ ,  $P = 0.01$ ; Fig. 3A). When tested against wild cherry volatiles, 91% of males were activated (10 of 11) and 40% of these landed on the source ( $\chi_1^2 = 0.40$ ,  $P = 0.53$ ; Fig. 3A). In contrast, the odor of a ripe medium sized plum did not attract *C. lata* males, as 100% of activated males (i.e. 7 of 9) flew towards the background odor control ( $\chi_1^2 = 7.00$ ,  $P = 0.01$ ; Fig. 3A).

Males that demonstrated a preference for raspberry odor took less time to reach the source than those that flew to the background odor (GLM,  $\chi_1^2 = 4.64$ ,  $P = 0.03$ ; Fig. 3B). The flight time to raspberry odor was not significantly different from background alone ( $\chi_1^2 = 2.78$ ,  $P = 0.10$ ), suggesting that the reduction in time to source is primarily due to the reduction in the time to flight take off. There was no difference in the total time to source landing ( $\chi_1^2 = 0.16$ ,  $P = 0.69$ ), time to take off ( $\chi_1^2 = 2.46$ ,  $P = 0.12$ ) or flight time ( $\chi_1^2 = 0.93$ ,  $P = 0.33$ ) of the male moths which flew to cherry odor or to background odor alone (Fig. 3C). Since all males that flew when exposed to the plum odor, flew to the background odor, there can be no direct comparison among the times taken to take off, fly the length of the Y-tube and make source contact (Fig. 3D).



**Fig. 2.** *Calyptra lata* male (A) feeding under enclosed conditions on raspberry, *Rubus crataegifolius* Bunge (B), and (C) feeding on (D) cherry, *Prunus tomentosa* at the Gornotayeznaya Biological Station, Primorye Territory, Far Eastern Russia. The white arrow indicates the proboscis piercing a raspberry (A).



**Fig. 3.** *Calyptra lata* males responded differentially to various fruit odors when tested in a Y-tube olfactometer. (A) More *C. lata* males made source contact to raspberry odor (black bar) than cherry odor (dark grey bar), and no males made source contact in response to plum odor. The time to take off, flight time to source and the total time to source landing of *C. lata* males responding to raspberry (B), cherry (C) and (D) plum (light grey bar) odor are presented compared to background air (white bars). Error bars represent the standard error of the mean. Significant differences are indicated by asterisks (\* $P \leq 0.05$  and \*\* $P \leq 0.01$ ).

## Discussion

Host preferences were assessed in wild-caught *C. lata* from the Primorye Territory, Russia using three locally available fruits. While *C. lata* has been found in mixed orchard landscapes, including e.g. pear, grape, plum, apple and peach (Hattori 1969; Park et al. 1988; Bänziger 2007), we present what appears to be the first evidence of a preferential polyphagous feeding behavior. *Calyptra lata* demonstrated a selective fruit preference by feeding on raspberries and cherries, but not, under the same conditions, on plums. While these moths were wild caught, and thus we have no knowledge of their prior host-related experiences or how these will affect their choice, the moths had access to all three potential fruit hosts in the local environment during at least that night; within 200 m of where the moths were caught. Such apparent selective detection and recognition of a preferred host relies greatly on olfaction for many fruit moths (Bengtsson et al. 2001; Bengtsson et al. 2006; Tasin et al. 2006; Landolt and Guédot 2008). Here, we demonstrate that olfaction plays a significant role for *C. lata* in host seeking and host preference.

The majority of the moths (61–78%) took flight and oriented upwind in the two-choice olfactometer, suggesting that a moving air column and potentially generic fruit odors may play a role in the activation and attraction of *C. lata* to potential fruit hosts, as

demonstrated in other fruit moths, such as the codling moth (Landolt and Guédot 2008) and the apple fruit moth (Knudsen and Tasin 2015). The time to take off, however, was significantly reduced in the presence of raspberry odor compared to the background control, indicating that these moths can detect potential hosts by their odors. The odor of the fruits on which *C. lata* fed, elicited either attraction (raspberry) or a neutral response (cherry), while odors of the fruit on which no feeding was recorded were avoided (plum). Taken together, this indicates that fruit odors are sufficient to influence host seeking as well as host recognition in *C. lata* males, and are also important in the detection and avoidance of non-preferred hosts. As *C. lata* can migrate up to 500 m each night (Hattori 1969), the ability to accurately identify preferred hosts from a distance directly reduces the energy expended in host finding, which is one of the main fitness costs associated with polyphagy (Jactel et al. 2001; Bengtsson et al. 2006).

A recent review of the management of fruit piercing moths states that none of the historically recommended control methods are effective, including insecticides, arsenical baits, bagging the fruit, flood light treatment and smoking the orchard in the evenings (Bhumannavar and Viraktamath 2013). Thus, an exploration of new control options is to be strongly encouraged. The manipulation of olfactory behavior with fruit and fruit host-derived odors can be

used in the integrated pest management of fruit moths (Bengtsson et al. 2006; Witzgall et al. 2008; Landolt and Guédot 2008; Knudsen and Tasin 2015). Planting orchards with mixed fruit trees may provide some protection from fruit pests. The presence of a preferred but non-commercial host, may act as a 'trap crop', drawing the moths away from the commercial crop (Bengtsson et al. 2006; Bhumannavar and Viraktamath 2013). Alternatively, planting non-host trees among the hosts may prevent the pests from finding and entering the orchards, by reducing the attractiveness of the overall odor plume emanating from the area (Jactel et al. 2001). For established orchards, synthetic odors and odor blends that are based on preferred fruit hosts, have been shown to be effective in trapping both male and female moths as part of orchard monitoring programmes (Knight and Light 2005; Knudsen and Tasin 2015). Traps emitting odors from a preferred fruit host, such as pear for the codling moth and rowanberry for the apple fruit moth, when deployed in an orchard of a less preferred host, such as apple, can accurately identify when the fruit moth is present above the economic threshold and thus indicate when and where to apply control measures (Knight and Light 2005; Bengtsson et al. 2006; Knudsen and Tasin 2015). Continued investigation into these and other fruit hosts and non-hosts of *C. lata*, as well as the further identification of behaviorally active volatiles emitted from these fruits, may lead to a monitoring tool for use in the control of these moths.

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