

INFLUENCE OF HOST PLANT ON REPRODUCTIVE BEHAVIOR OF *Leucoptera coffeella* (Guérin-Mèneville) (LEPIDOPTERA: Lyonetiidae)

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ABSTRACT: The objective of this study was to evaluate the effect of the host plant, *Coffea arabica*, the reproductive behavior of *Leucoptera coffeella*. We evaluated: i) the effect of reproductive condition of females in response to the volatile coffee leaves, ii) the proportion of mating, start time and duration of copulation are affected by the presence of volatile coffee and iii) if female *L. coffeella* can lay eggs on another host. The assessment of olfactory response of virgin females and mated to the volatile coffee leaves was done using olfactometer "Y". To evaluate the proportion of copulation, the start time and duration of copulation, couples were used in plastic cages with the presence or absence of volatiles of coffee leaves. And, to assess the oviposition females were offered to them leaves of the host plant and not host. The volatiles emitted by host plant appeared to be not relevant to track their location by females, but these volatiles were important during mating. The proportion of mating, the onset and duration of copulation were significantly altered in the presence of volatile coffee. When a host plant was not offered to females as oviposition site, there was a considerable reduction in the number of eggs deposited, indicating that females use cues of the host plant to lay eggs. Thus, there was the host plant plays an important role in the reproductive behavior of *L. coffeella*.

Index terms: Volatile host plant, coffee, olfactometer, mating, oviposition.

INFLUÊNCIA DA PLANTA HOSPEDEIRA NO COMPORTAMENTO REPRODUTIVO DE *Leucoptera coffeella* (Guérin-Mèneville) (LEPIDOPTERA: Lyonetiidae)

RESUMO: Objetivou-se, neste estudo, avaliar o efeito da planta hospedeira, *Coffea arabica*, no comportamento reprodutivo de *Leucoptera coffeella*. Avaliou-se: i) o efeito da condição reprodutiva das fêmeas em resposta aos voláteis de folhas do café; ii) se a proporção de acasalamento, tempo de início e a duração da cópula são afetados pela presença dos voláteis do café; e iii) se fêmeas de *L. coffeella* podem ovipositar em outro hospedeiro. A avaliação da resposta olfativa de fêmeas virgens e acasaladas aos voláteis de folhas do café foi feita utilizando-se olfatômetro em "Y". Para avaliar a proporção de cópula, o tempo de início e a duração da cópula, foram utilizados casais em gaiolas plásticas com a presença ou ausência de voláteis das folhas do café. E, para avaliar a oviposição das fêmeas foram oferecidas a elas folhas da planta hospedeira e não hospedeira. Os voláteis emitidos pela planta hospedeira pareceram não constituir pista relevante para sua localização pelas fêmeas, mas esses voláteis mostraram-se importantes durante o acasalamento. A proporção de acasalamento, o início e a duração da cópula foram significativamente alterados na presença dos voláteis do café. Quando uma planta não hospedeira foi oferecida às fêmeas como sítio de oviposição, houve uma redução considerável no número de ovos depositados, indicando que fêmeas usam pistas da planta hospedeira para ovipositar. Assim, registrou-se que a planta hospedeira tem papel relevante no comportamento reprodutivo de *L. coffeella*.

Termos para indexação: Voláteis da planta hospedeira, café, olfatômetro, acasalamento, oviposição.

1 INTRODUCTION

The coffee leaf miner, *Leucoptera coffeella* (Guérin-Mèneville) (Lepidoptera: Lyonetiidae) is the main coffee pest in Brazil, due to its generalised occurrence and the economic damage it causes (SOUZA; REIS, 1992). Despite its importance and high level of specialization, little is known about the effect of the host plant, *Coffea arabica* L., over the reproductive behavior of adults of this species. Due to the high level of specialization of

L. coffeella as related to its host plant, a hypothesis is that visual, chemical or tactile signs improve the encounter and acceptance of the plant by the females. In this case, it is expected that activities related to mating and egg laying are altered in the presence of such signs.

Insects are living beings that most use odors to perform their vital functions (finding prey, defense and aggression, host plant selection, choice of oviposition sites, courtship and mating) (TEGONI; CAMPANACCI; CAMBILLAU, 2004).

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In many species of lepidopteran, host plant stimulates the biosynthesis and release of sex pheromone. As for the moth *Helicoverpa zea* (Boddie) (Lepidoptera: Noctuidae), sex pheromone production is induced by the presence of volatiles emitted by cob of corn during the mating period (RAINA; KINGAN; MATTOO, 1992), generating a synchronization between oviposition and availability of the host. Females of many insects can modify the period of sexual attraction, courtship, copulation and oviposition to synchronize with the appropriate host (LANDOLT; PHILLIPS, 1997).

There are several examples in which the volatiles emitted by the host plant are used as stimuli for insects for mating and oviposition (ESTRADA; GILBERT, 2010; LANDOLT; PHILLIPS, 1997; YAN; BENGTSSON; WITZGALL, 1999). The attraction of mated females of *Cydia pomonella* (L.) (Lepidoptera: Tortricidae) to apple odors is well documented (WITZGALL et al., 2005; YAN; BENGTSSON; WITZGALL, 1999). In olfactometer tests, virgin and mated females of *C. pomonella* were more active in the presence of apple volatile than in the open air. However, the mated females were more attracted than virgin females (YAN; BENGTSSON; WITZGALL, 1999), therefore indicating the importance of host plant volatiles for *C. pomonella* locate its oviposition site.

The finding and the correct choice of the host plant by females are vital to the neonate larvae that have little mobility. Thus, females choose, precisely, the food source and location of development of their offspring. Since they are not able to directly evaluate the nutritional quality due to the difference of dietary habits between larvae and adults, females assess the host plant by other means (RENWICK; CHEW, 1994). Location of host plant can involve many sensory modalities, being the final discrimination complex and frequently involving many senses, plants signs and the insect's physiological state. Vision and smell can be used for long range orientation and depend on the insect's way of life. However, when in contact with the plant, the final behavior sequence for it to accept or reject the oviposition spot depends mainly of contact signs and usually of chemicals present on the surface of the plant (RENWICK; CHEW, 1994).

The objective of this study was to evaluate (i) the effect of the reproductive condition of the female *L. coffeella* (virgin and mated) in

response to volatiles of the coffee plant; (ii) if the proportions of mating, time of start and duration of mating would be affected by the presence or absence of volatiles from the host plant; (iii) and if females *L. coffeella*, in non-choice conditions can lay eggs on other hosts.

2 MATERIALS AND METHODS

Collection and maintenance of insects

Coffee leaves mined by *L. coffeella* were collected, weekly, in commercial crops in the region of Viçosa - MG and set in Gerbox® type acrylic boxes (11,0 x 11,0 x 3,5 cm), containing a sponge cut in parallel slits, where the plants' petioles were fixed. Such sponges were immersed in growth hormone solution with benzyl adenine, in the concentration of 10⁻⁶ g/litre (REIS JÚNIOR et al., 2000), keeping the leaves turgid for over three weeks. The recipients containing the leaves were kept in laboratory, under a regime of 12L:12E, at 23 ± 1°C and 70 ± 2% UR. The leaves were examined daily and from them were collected the pupae of *L. coffeella* that were placed, individually, in plastic tubes (2,5 cm Ø x 6,0 cm high) until the emergence of the adults. After the emergence, the individuals were separated by sex and placed in a climatized room in the same raising conditions, to be used in the tests.

Plants collection

The coffee trees (*Coffea arabica* cv. Catuai) (Rubiaceae) used in the experiments were from crops from the region of Viçosa - MG. twenty-four hours before the experiment branches were collected with ± 10 leaves from the medium third of the plant. Only the leaves were used in the experiment, which were randomly plucked from the branch 10 minutes before starting the experiments.

The other plant used was the jenipapo, *Genipa americana* (Rubiaceae), collected at the Botanical Garden (Horto Botânico da UFV em Viçosa - MG). during the performing of the experiments, these plants' leaves were also plucked from the branch 10 minutes before beginning the experiment.

Biotrials

Response of the females to the coffee leaf volatiles

To test the olfactive response of females *L. coffeella* to its host plant, *C. arabica*, a biotrial

was performed in a Y type olfactometer (internal diameter 0,5 cm and arms length 5 cm) the device was constituted by a glass tube shaped as a “Y”, each arm connected through a rubber hose to a 50ml glass erlenmayer, where the odor sources were placed. The airflow was produced by a vacuum pump connected to the base of the glass tube, for a uniform airstream in both arms of the device. The airstream speed inside the olfactometer was 0,05 m/s in each arm, measured by digital flow measurers and calibrated by manual record. Lighting was kept low during the experiments, simulating dawn conditions.

Virgin and mated females were used. Each female was tested individually in the olfactometer, using 4 repetitions of 20 females per treatment, in a total of 80 virgin females and 80 mated females. For each treatment (virgin or mated) the female's response to coffee leaf odor was tested opposed to the air (control). All of the virgin and mated females were assessed when 2 and 3 days old, respectively. Two days old females were mated during the period between 2 and 7h after beginning of photophase. Thus, all the females were tested in the olfactometer during the final five hours of photophase, which consists of the more active egg-laying period. All of the females were used only once and at each test the olfactometer was duely cleaned with alcohol.

For the performing of the tests, the females (mated or virgin) were placed at the lower extremity of the olfactometer, against the airstream formed inside the device. It was considered as a positive response the arrival of the female to the extremity of one of the olfactometer's arms. For every 5 tested females, the odor source positions were inverted. At the end of each repetition, the coffee leaves were changed. Were only included in the statistic analyses virgin and mated females that answered in a period of up to 5 minutes after the beginning of the experiment.

Influence of host plant volatiles in mating proportion on the onset and duration of copulation

Fifty virgin couples of *L. coffeella*, two days old were individualized in plastic cages (2,5 cm Ø x 6,0 cm high). The couples were evaluated in two treatments, cages with the presence (n = 25) or absence of coffee leaf volatiles (n = 25). In all of the situations two cages were used, set together by the openings and separated with an organza type fabric. This was done so that, during

leaf volatiles treatments, the couples could not have direct contact with the coffee leaf.

The tests took place in a room, under the regime 12L:12E, at 23±1°C and 70 ± 2% UR. The mating observations were performed between 2 and 7 hours after photophase beginning. Time measures were assessed using a chronometer and the beginning and the end of copulation were recorded (in minutes). Couples were fed with cotton soaked in a 10% solution of honey and water.

Egg-laying at host plant leaf and non-host

The hypothesis of this experiment was to verify if females of *L. coffeella*, under non choice conditions, can lay eggs at another host from the same family as the coffee tree. Eighty couples two days old mated during the period between 2 and 7hours after photophase began. After that period, the males were discarded from the experiment. The mated females were placed in plastic cages (2,5 cm Ø x 6,0 cm height) individualized. For the eighty mated females fragments of coffee leaves were offered (*C. arabica*) (n = 40) or jenipapo (*Genipa americana L.*) (n = 40). The experiment took place in a room under the regime 12L:12E, at 23±1°C and 70 ± 2% UR. The females were fed with cotton soaked in a 10% solution of honey and water during the entire experiment, being the food changed every two days.

The number of eggs was assessed at the end of photophase each day, and the leaf fragments were changed. The egg count took place until the death of the females. For data analysis was considered the number of eggs, per female, during their entire lifespan.

Statistic analyses

The response data for the females of *L. coffeella* (virgins and mated) for host plant volatiles or open air were analysed using G test (SOKAL; ROHLF, 1995) using software Excel.

The other analyses were processed by the software R (R DEVELOPMENT CORE TEAM, 2005) , using generalized linear models (CRAWLEY, 2002). To test if the host plant volatiles increase in the proportion of mating and alter copulation start, a survival analysis was performed, using Weibull distribution (CRAWLEY, 2002). To evaluate if host plant volatiles alter copulation duration time and if females of *L. coffeella*, under non choice conditions, can lay eggs at another alternative host, an analysis was performed using Poison error distribution, corrected for overdispersion.

3 RESULTS AND DISCUSSION

Females response to the coffee leaf volatiles

All the mated females remained still at the olfactometer's releasing spot, during the time granted for choosing. Permanent antennae movement in an opposite direction to the airstream was a frequently observed behavior in all of the females. As for the female virgins, there was movement, however they were not capable to differentiate between coffee leaves and fresh air (Gp = 2,46; g.l. = 1; p = 0,1165; Fig. 1).

Host plant volatiles' influence in the mating proportion, starting time and copulation duration

The coffee leaf miner's mating behavior was altered when the couples remained in the environment with the presence of coffee leaf volatiles. Mating proportion was significantly higher in the presence of volatiles than in control (air). While around 90% of the couples mated in the presence of volatiles, only 65% did so in the presence of fresh air. In the presence of volatiles, 160 minutes after the experiment started, 50% of the couples had already mated, while in the absence of volatiles such frequency was only achieved after 240 minutes (F (2,72) = 700,67 and $\chi^2 = 0,003$; Fig. 2).

Besides that, it was observed that the couples, in the presence of coffee leaf volatiles, lasted longer in copulation (3,1 mim) than in control (1,4 mim) (F_{2,47} = 4,9472 and P = 0,012; Fig. 3).

Oviposition behavior on host plant and non host plant

A significant difference was noticed in the total number of eggs/female laid in coffee and jenipapo leaves. The number of laid eggs was 24 and 2 on coffee and jenipapo leaves, respectively (F_(1,78) = 137,10 and P < 0,001; Fig. 4).

Females *L. coffeella* did not have any type of preference for the volatiles emanated by the host plan leaves, *C. arabica* when tested in "Y" olfactometer. Although the virgin females have not been attracted by the odors produced by the leaves and fresh air, they had great mobility; on the other hand, the mated females remained still the entire time. Virgin females must search for a sex partner and, therefore, present certain mobility, while mated females must concentrate on oviposition behavior, which probably induces them to remain still.

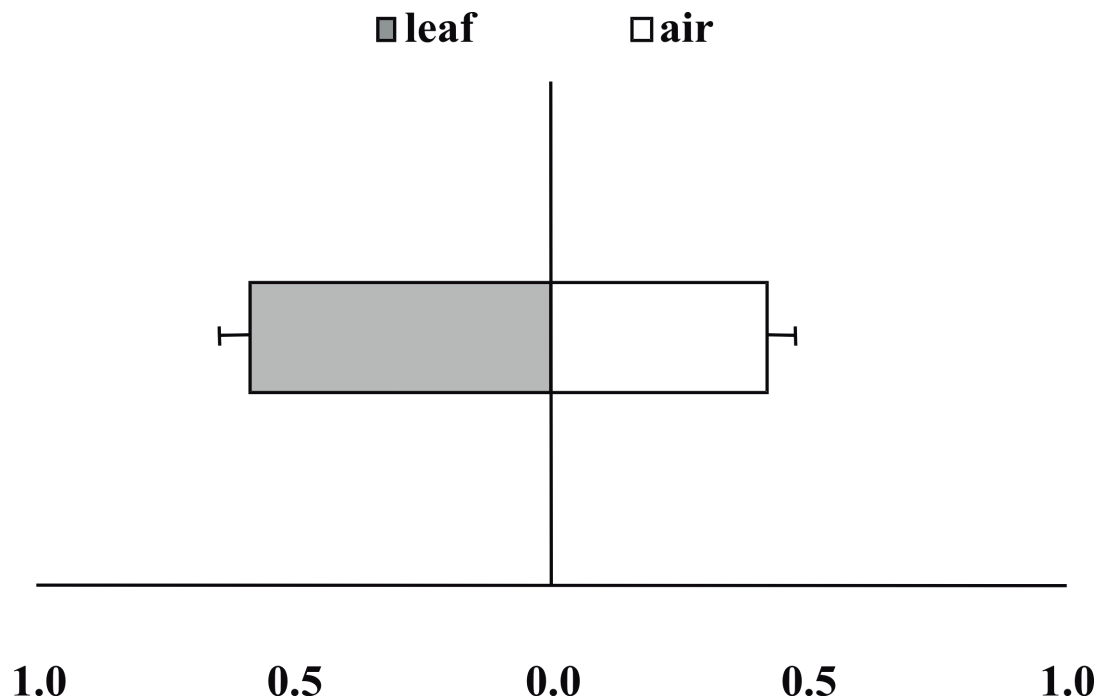


FIGURE 1 - Olfactory response of virgin females of *Leucoptera coffeella* to coffee leaf odors versus air, in a "Y" type olfactometer. The corresponds to the average of four repetitions (repetition – 20 virgin females). (G Test, Gp = 2,4627; g.l. = 1; p = 0,1165).

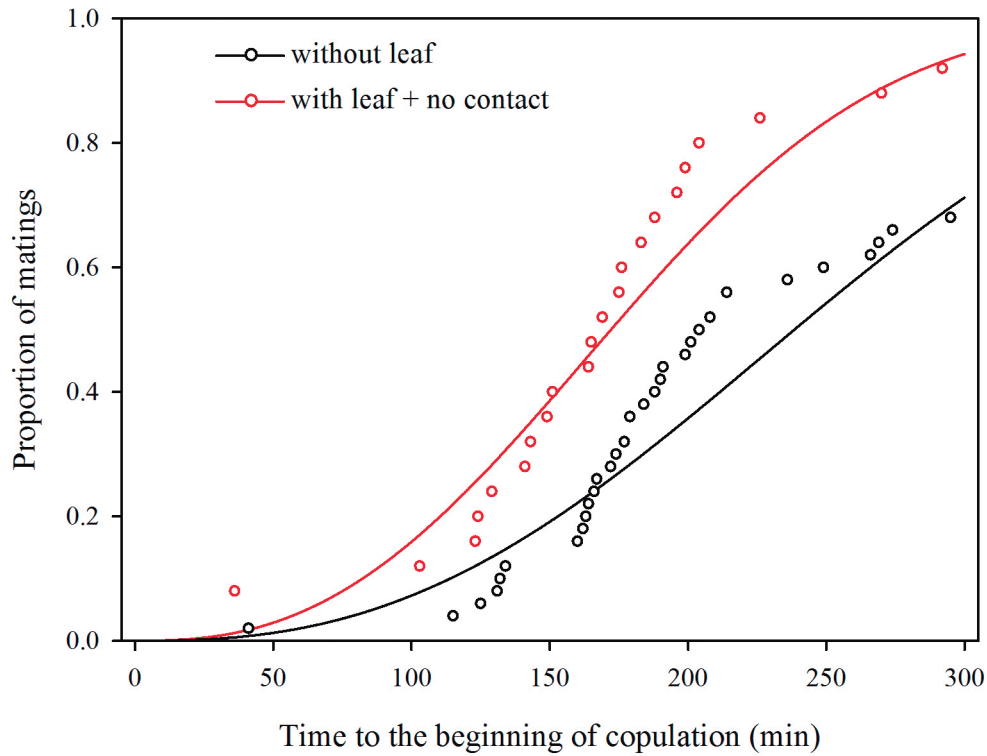


FIGURE 2 - mating proportion and starting copulation time for couples of *Leucoptera coffeella*, in the presence or absence of host plant volatiles ($F_{2,72} = 700,67$ and $\chi^2 = 0,003$).

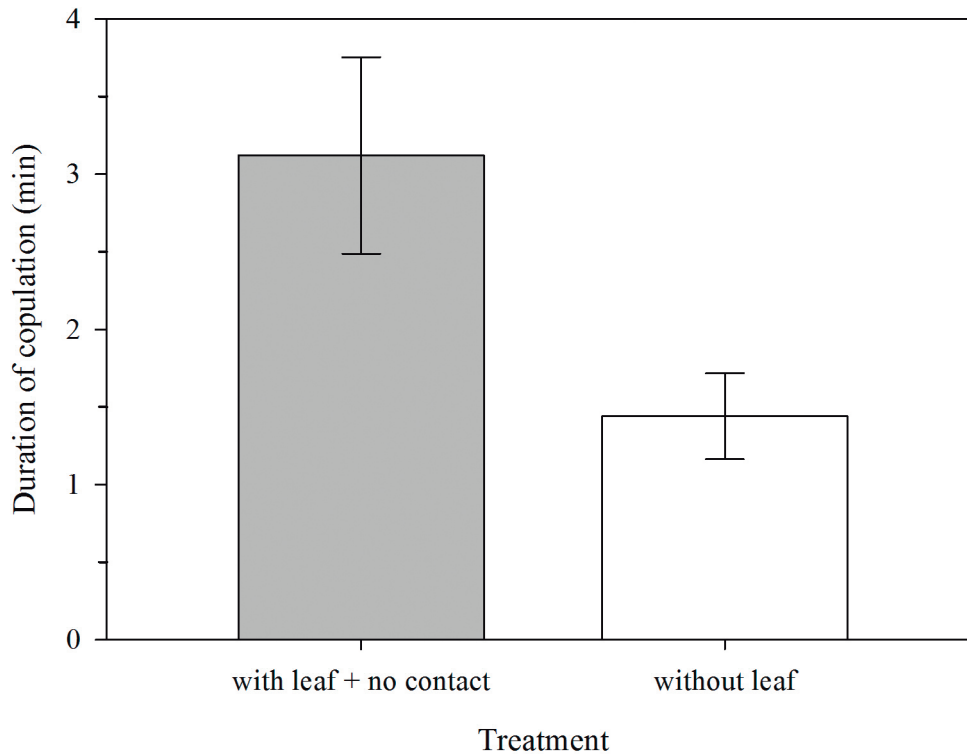


FIGURE 3 - copulation duration for couples of *Leucoptera coffeella*, in the presence and absence of host plant volatiles ($F_{2,47} = 4,9472$ and $P = 0,012$).

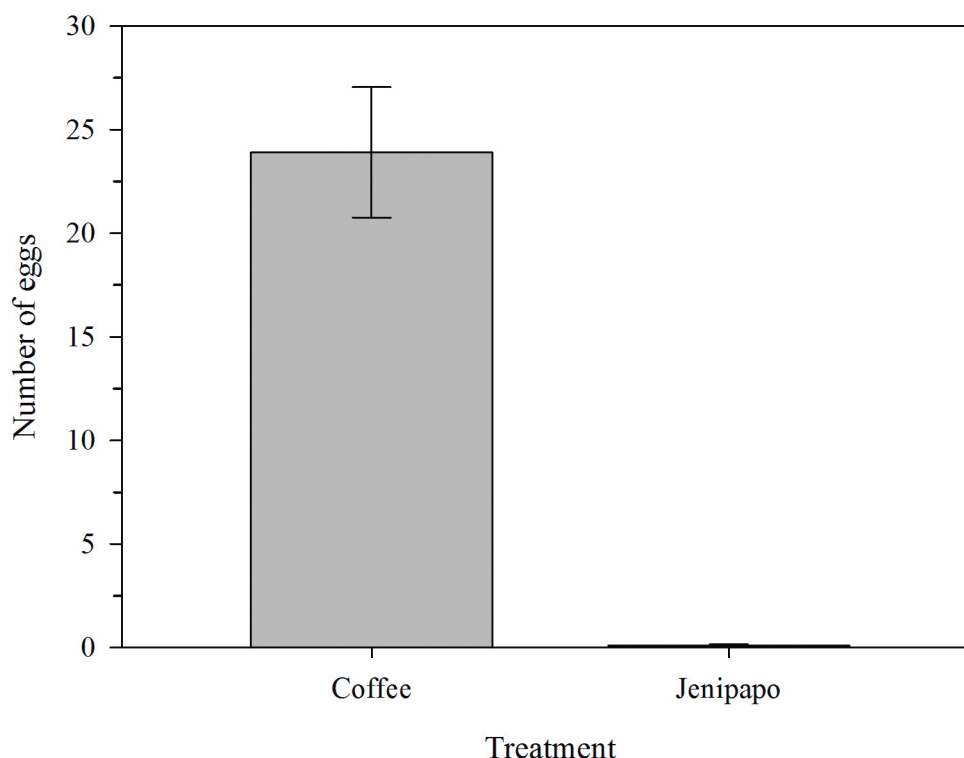


FIGURE 4 - number of accumulated eggs laid per female *Leucoptera coffeella* in the presence of coffee leaf and jenipapo leaf ($F_{1,78} = 137,10$ and $P < 0,001$).

Females of some Lepidopterans such as *Spodoptera frugiperda* (Smith) (Lepidoptera: Noctuidae) (ROJAS; VIRGEN; CRUZ-LÓPEZ, 2003), *Helicoverpa virescens* (Fabricius) (Lepidoptera: Noctuidae) (RAMASWAMY; WA; BAKER, 1987) and *Ethiella zinckenella* (Treit.) (Lepidoptera: Pyralidae) (HATTORI, 1988) do not depend of volatiles to locate host plant. However, such volatiles are determinant for the orientation of many other Lepidoptera females towards their host plants (PHILLIPS; STRAND, 1994; PINERO; DORN, 2009; SOLE et al., 2010).

Leucoptera coffeella has a development cycle in which the crysalids are formed on the host plant or the leaves that fall next to the plant, and, due to that, emergence of adults happens on the plant or around it. Considering the absence of mated female response to the plants' volatiles in this study, one could suppose that, despite the proximity of adult females and their oviposition sites, the semiochemical does not seem to be determinant for the females to locate their host.

It is highlighted that this was the first study using "Y" type olfactometer in order to evaluate the attractiveness of the female *L. coffeella* to the coffee leaf volatiles.

Other studies are needed, with the use of other types of olfactometers, for example, the wind tunnel in order to confirm the non-attractiveness of females to the volatiles emanated from the coffee leaves.

On the other hand, host plant volatiles played a relevant role during the mating activities of *L. coffeella*. Results indicated a strong evidence that host plant volatiles influenced in communication between partners of *L. coffeella*. The couples, when in the presence of plant's volatiles, started copulation sooner and faster than couples in the absence of the plant and succeeded more in copulation. One explanation for such fact is that the host plant may have influenced the female to produce and secrete pheromones earlier and become more attractive, resulting in greater success in mating. Host plant stimulus in pheromone production by the females is well documented in moths from the genus *Helicoverpa* (LANDOLT; PHILLIPS, 1997). Pheromone production by the females of *H. zea* is induced by the presence of host plant (RAINA; KINGAN; MATTOO, 1992). A similar phenomenon was demonstrated also in females of *Helicoverpa*

phloxiphaga (Grote & Robinson) (Lepidoptera: Noctuidae), which require the presence of host plant for the production of sexual pheromone (RAINA, 1988).

Studies with *C. pomonella* (ANSEBO et al., 2005; CORACINI et al., 2004; LIGHT et al., 2001; YANG; BENGTTSSON; WITZGALL, 2004) proved that males and females were attracted by the same chemicals present on the host, and that such chemicals stimulated the male to find the female. In field tests, using septa traps, containing host plant chemicals, males of *C. pomonella* were captured in larger quantities. Such attraction was confirmed in wind tunnel studies (ANSEBO et al., 2004). Possibly, in this case, males of *C. pomonella* use plant's volatiles to distinguish the environment most likely to find females (ANSEBO et al., 2004). In the present study it is not possible to determine if it is the male, the female, or both that respond to the coffee plant's volatiles to optimize mating success.

The high level of specialization of *L. coffeella* in *C. arabica* was highlighted in the oviposition behavior of the females. Rarely the oviposition took place on leaves of *G. americana*, in a non choice situation. As for another moth, *Yponomeuta cagnagellus* (Lepidoptera: Yponomeutidae), which is also highly specialized, females are only able to distinguish host plant by contact signs present on the leaves' surface (HORA; ROESSINGH, 1999). It is most likely that the contact signs present of the surface of the coffee leaf have been responsible for the oviposition behavior difference of the females of *L. coffeella*.

4 CONCLUSIONS

The volatiles emanated by the host plant do not constitute a relevant sign for females of *L. coffeella* to locate their host, when tested in "Y" olfactometer.

The mating proportion, start and duration of copulation of *L. coffeella* were altered in the presence of host plant.

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