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# Fall Armyworm, *Spodoptera frugiperda* (J.E. Smith) (Lepidoptera: Noctuidae), Female Moths Respond to Herbivore-Induced Corn Volatiles

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## Keywords

Behavior, host selection, plant odors, insect–plant interaction

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## Abstract

In response to herbivore attack, plants release herbivore-induced plant volatiles (HIPVs) that represent important chemical cues for herbivore natural enemies. Additionally, HIPVs have been shown to mediate other ecological interactions with herbivores. Differently from natural enemies that are generally attracted to HIPVs, herbivores can be either attracted or repelled depending on several biological and ecological parameters. Our study aimed to assess the olfactory response of fall armyworm-mated female moths toward odors released by mechanically and herbivore-induced corn at different time intervals. Results showed that female moths strongly respond to corn volatiles, although fresh damaged corn odors (0–1 h) are not recognized by moths. Moreover, females preferred volatiles released by undamaged plant over herbivore-induced plants at 5–6 h. This preference for undamaged plants may reflect an adaptive strategy of moths to avoid competitors and natural enemies for their offspring. We discussed our results based on knowledge about corn volatile release pattern and raise possible explanations for fall armyworm moth behavior.

## Introduction

Plant chemical defenses that are produced against insect herbivory only after attack are called induced defenses. There are two types of induced plant defenses: (i) direct defenses—which concern the mechanisms that affect the biology or behavior of herbivores; and (ii) indirect defenses—which consist of mechanisms that recruit herbivore natural enemies, such as parasitoids and predators. A common indirect defense is the emission of plant volatiles released after herbivore attack, called herbivore-induced plant volatiles (HIPVs). They recruit carnivorous arthropods which use them as important chemical cues for foraging mainly at long distances (Dicke & Sabelis 1988, Dicke *et al* 1990, Turlings *et al* 1991, Steinberg *et al* 1993). Nevertheless, HIPVs are not restricted to mediate interactions only with natural enemies, but also with other trophic levels such as herbivores (Halitschke *et al* 2008).

Herbivores present chemosensory receptors that enable them to perceive plant odors in order to find conspecifics or host to feed or oviposit (Bernays & Chapman 1994). Host selection by female moth is crucial for offspring performance as newly hatching larvae are not able to actively search for hosts due to their restricted mobility.

HIPVs have been shown to be either attractive or repellent for herbivores, although most female lepidopterans are repelled by HIPVs (De Moraes *et al* 2001, Kessler & Baldwin 2001, Bruinsma *et al* 2007). Herbivore response towards HIPVs depends on the gender (Rodríguez-Saona *et al* 2006), mating status (Arab *et al* 2007), developmental stage (Yoneya *et al* 2010) and herbivore species (Szendrei & Rodríguez-Saona 2010). Given that HIPVs are released only by damaged plants, they can inform herbivores the presence of competitors as well as natural enemies which are recruited to emitting plants. At the same time, HIPVs can represent important cues to herbivores in host location (Carroll *et al* 2006). By the plant's perspective, if HIPVs are repellents to herbivores, plants

greatly benefit from induced defenses as they attract natural enemies and avoid being more intensively attacked.

The fall armyworm, *Spodoptera frugiperda* (J.E. Smith) (Lepidoptera: Noctuidae), is a cosmopolitan pest of corn crops (Sparks 1979) that has been known to attack other crops in Brazil, such as rice, wheat, and sorghum (Crócomo & Parra 1985, Busato *et al* 2002, 2004). Pesticides are heavily used to control the fall armyworm in corn crops in Brazil, leading to serious side effects, such as environmental impacts and the selection for resistant populations (Diez-Rodríguez & Omoto 2001). Therefore, other strategies should be used in order to avoid that the fall armyworm become even more difficult to control. In this context, behavioral studies can provide important information for developing integrated management tactics, particularly by identifying odors that play a role in host selection by pests. Semiochemicals could be employed for behavior manipulation in field, for example (Khan *et al* 2006, Unsicker *et al* 2009).

Our study aimed at assessing the preference of *S. frugiperda* gravid moths towards volatiles released by mechanical-damaged plants and artificially induced corn volatiles in a Y-tube olfactometer. Given that quality and quantity of volatiles of herbivore-damaged corn plants change in time course (Turlings *et al* 1998), we tested herbivory induction at different time intervals for assessing fall armyworm response towards volatile blends. Our results revealed the importance of HIPVs for host plant selection by fall armyworm moths.

## Material and Methods

### Plants and insects

Commercial corn seeds (*Zea mays*, variety SWB551 Dow Agrosciences) susceptible to the lepidopteran *S. frugiperda* were planted individually in plastic pots containing about 300 g substrate and maintained in the greenhouse under a photoperiod of 12L:12D. The photophase began at 7:00AM and the scotophase at 7:00PM. Only corn plants aging 10–12 days after emergence that had three to four leaves were used in the bioassays, and they were selected because this is stage they are naturally infested by *S. frugiperda* (Cruz & Turpin 1982) and are commonly used for volatile collection (Degen *et al* 2004).

In order to rear *S. frugiperda*, larvae of different ages were initially collected in corn fields in Piracicaba, Sao Paulo and maintained in the laboratory under controlled conditions ( $25 \pm 3^\circ\text{C}$ ;  $70 \pm 10\%$  RH 12-h photophase). Larvae were reared on an artificial diet (Greene *et al* 1976) individualized in glass vials (8.5 cm height and 2.2 cm diameter) up to the pupal stage, when they were separated by sex (Butt & Cantu 1962) and placed in PVC cages (10 cm diameter and 22 cm height) for adult emergence. The inner

walls of PVC cages were covered with office paper to serve as a substrate for oviposition. Adults were fed on 10% honey solution, which was renewed every other day to prevent contamination.

### Olfactometer bioassays

Bioassays were conducted with 3- to 5-day-old adult female of *S. frugiperda* with no plant volatile experience. They had previously spent at least two nights in cages containing *S. frugiperda* males for mating. In order to tentatively select mated moths, we only used females with enlarged abdomen, which were likely gravid.

We evaluated the attractiveness of corn plant volatiles using a glass Y-tube olfactometer (Zhang *et al* 2009) which consisted of a central tube (20.5 cm long and 3 cm diameter) and two arms (20.5 cm long and 3 cm diameter, offset by  $120^\circ$ ). The arms ended with a glass plug connected to the glass chambers containing the odor sources (i.e. plants or blank). The olfactometer was connected to a vacuum pump which pulled air from the room. Air was purified through a charcoal filter and humidified before entering in the treatment chambers and Y-tube olfactometer. Airflow was calibrated to 800 ml/min using a flow meter at the end of each arm. All bioassays were conducted during the scotophase (2000–2400 hours) at controlled conditions ( $25 \pm 3^\circ\text{C}$ ,  $60 \pm 10\%$  RH).

Moths were positioned individually at the beginning of the central arm of the olfactometer and observed for 10 min after they had passed the threshold line located 7 cm from the center of the equipment. Choice was considered after the insect crossed the 10 cm line of the odor arm and spent at least 1 min at that arm. Only insects that made the decision for one arm within the observation period were considered for posterior analysis. Each female moth was used a single time to avoid associative learning. Around ten insects were tested per night depending on their responsiveness and plant odors were changed each hour. Each bioassay had at least 30 replicates. To avoid side bias, the olfactometer was turned  $180^\circ$  to invert the side of odor arms after each replicate.

At the end of bioassays, olfactometer, glass chambers, and connections were washed with neutral washing soap (Extran<sup>®</sup> 10%), distilled water, 90% ethanol (v/v), and 93% acetone (v/v). All glass material was dried at  $160^\circ\text{C}$  for 1 h.

Firstly, in a control experiment, we tested moth choice towards odors from blank vs. blank to verify if there was no side bias in the olfactometer set up. This bioassay was followed by a test of female moth preference between odors from undamaged plant (UP) over the blank (B). This assay was followed by a test to assess if mechanically damaged plants (MDP) produced volatiles that affect herbivore responsiveness against B and UP. Mechanical leaf

damage was produced with a rotary metal cutter. Three parallel lines were traced with the rotary cutter on the third leaf of corn plants. Odors released by MDP were tested at time intervals 0–1, 5–6, and 24–25 h against B, but only MDP 5–6 and 24–25 h odors were tested against UP.

Subsequently, we assessed moth responsiveness to herbivore-induced plants (HIP) at the same intervals as the previous bioassays against B. Only treatments that were attractive over B were tested against UP odors. In order to simulate herbivory in corn plants, 10  $\mu$ l of *S. frugiperda* regurgitate was applied on mechanically damaged leaves. Regurgitate was collected from third instars of *S. frugiperda* fed on corn for at least 24 h using a micropipette.

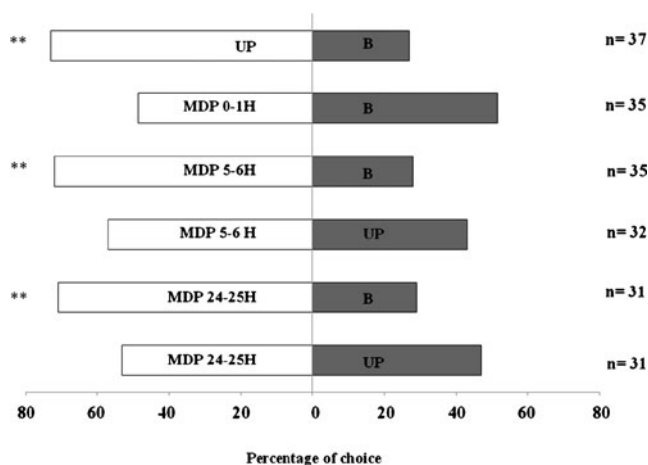
### Statistical analysis

Statistical analysis was performed using the software Statistica 6.0. Differences between behavioral responses of mated female moths under pairs of treatments were analyzed by the Chi-square test ( $\alpha=0.05$  and  $\alpha=0.01$ ). Individuals that did not make a choice were excluded from the statistical analysis.

## Results

Female fall armyworm moths did not show preference between B vs. B what indicated that olfactometer set up was properly clean and calibrated ( $\chi^2=0.273$   $p=0.601$ ).

Fall armyworm females showed a very high responsiveness to UP (Fig 1; UP vs. B:  $\chi^2=7.811$   $p=0.005$ ) as well as to



**Fig 1** Olfactory preference of *Spodoptera frugiperda* gravid females to volatiles emitted by undamaged corn plant (UP) and mechanically damaged corn plants (MDP) at 0–1, 5–6, and 24–25 h. All plant treatments that differed against blank (B) were compared to volatiles from undamaged plant (UP). \*\* $p=1\%$  significance according to chi-square test; (n=number of replicates).

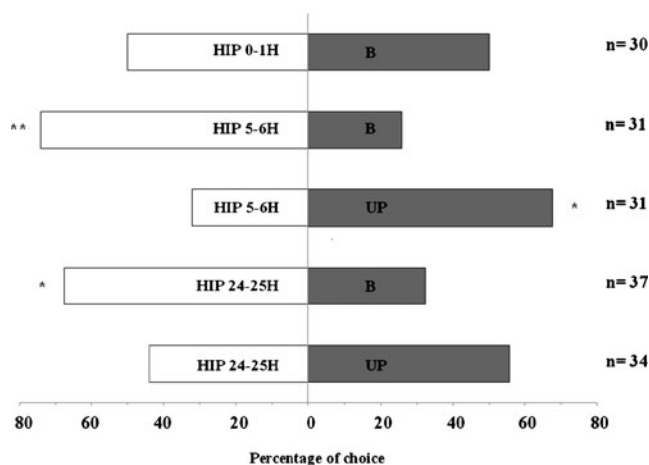
MDP 5–6 and 24–25 h when compared to B (MDP 5–6 h vs. B:  $\chi^2=7.111$   $p=0.007$ ; MDP 24–25 h vs. B:  $\chi^2=5.452$   $p=0.019$ ). Only odors from MDP 0–1 h were not attractive to moths ( $\chi^2=0.029$   $p=0.865$ ). However, moths did not discriminate MDP 5–6 and MDP 24–25 h over UP odors (MDP 5–6 h vs. UP:  $\chi^2=0.500$   $p=0.479$ ; MDP 24–25 h vs. UP:  $\chi^2=0.032$   $p=0.857$ ).

The same pattern in attractiveness was found when the plants were treated with the regurgitate: HIP 0–1 h showed no attractiveness, whereas HIP 5–6 and HIP 24–25 h were attractive to the moths (Fig 2; HIP 0–1 h vs. B:  $\chi^2=0.000$   $p=1.000$ ; HIP 5–6 h vs. B:  $\chi^2=7.258$   $p=0.007$ ; HIP 24–25 h vs. B:  $\chi^2=4.568$   $p=0.032$ ). However, given a choice between HIP 5–6 h and UP, female moths preferred UP ( $\chi^2=3.903$   $p=0.048$ ), but did not discriminate between HIP 24–25 h and UP ( $\chi^2=0.471$   $p=0.493$ ).

## Discussion

Our results showed that mated female moths are strongly attracted to corn plant volatiles, although they exhibit differential responsiveness depending on volatile plant blend. Fall armyworm moths were attracted to undamaged maize and mechanically damaged plants 5–6 and 24–25 h. However, no preference was exhibited between mechanically damaged plants 5–6 h and 24–25 h over undamaged plants (Fig 1).

Undamaged corn plants release linalool, which is likely the compound responsible for female moth attraction (Jönsson & Anderson 1999, D'Alessandro & Turlings 2006). Mechanical damage generally does not elicit differential volatile plant blend later than 2 h unless mechanical



**Fig 2** Olfactory preference of *Spodoptera frugiperda* gravid females to volatiles emitted by undamaged corn plant (UP) and herbivore-induced corn plants (HIP) at 0–1, 5–6, and 24–25 h. All plant treatments that differed against blank (B) were compared to volatiles from undamaged plant (UP). \* $p=5\%$  significance and \*\* $p=1\%$  significance according to chi-square test; (n=number of replicates).

damage is continuous (Mithöfer *et al* 2005). Therefore, moths may have not discriminated odors from undamaged plants and mechanically damaged plants 5–6 and 24–25 h due to their similar volatile blends.

By contrast, odors released by fresh damaged plants (both mechanically damaged and herbivore-induced plants 0–1 h) were not attractive to female moths (Figs 1 and 2). Volatile blend immediately released by mechanically damaged corn, irrespective of regurgitate application, is mainly composed by green leaf volatiles (GLVs) which comprise six-carbon alcohols, aldehydes, and esters (Turlings *et al* 1998, Hoballah & Turlings 2005). Some GLVs have been shown to be electrophysiologically active to female *Spodoptera* moths (Jönsson & Anderson 1999, Malo *et al* 2004), but no studies have shown whether female moths are attracted or repelled by GLVs. In view of that, GLVs may have masked some important volatile compounds for host location by moths, such as linalool, or even repelled.

Application of fall armyworm regurgitate to mechanical damaged corn leaves is known to induce differential volatile blend in terms of quality and quantity (Turlings *et al* 1991). According to several reports (Turlings *et al* 1998, Hoballah *et al* 2002, 2004, Degen *et al* 2004, Köllner *et al* 2004), the total emission peak in corn occurs around 5–7 h after *Spodoptera* regurgitate treatment, and the blend is mainly composed by terpenes.

Our data demonstrate that female moths of *S. frugiperda* were highly attracted to herbivore-damaged plants 5–6 h (Fig 2). Nevertheless, moths preferred undamaged plant odors over an herbivore-damaged blend 5–6 h. This preference may reflect an adaptive behavior of moths of choosing non-infested plants as oviposition sites in order to avoid competition for their offspring as well as the low availability of food when larvae start hatching. Moreover, herbivore-induced plant volatiles (HIPVs) are important attractive chemical signals for natural enemies and, therefore, they represent high risks of parasitism and predation for offspring.

On the other hand, *S. frugiperda* larvae have been shown to prefer odors from herbivore-infested over undamaged plants (Carroll *et al* 2006). This study suggested that larval attraction is dose-dependent of linalool, which is emitted by both undamaged and herbivore-induced plants, but in larger amounts by the last. In this case, given that larval mobility is restricted and that this stage is more susceptible to natural enemies, if larvae prefer odors from infested plants, they can more easily recognize host presence as HIPVs are more abundant and highly detectable. Therefore, they can find suitable hosts as other conspecific are feeding on the plant.

The latest tested time interval 24–25 h did not show any effect in *S. frugiperda* behavior as moths did not exhibit preference between herbivore-induced and undamaged

plants. At this time interval, herbivore-induced blend most likely ceased and volatile blend is not different from undamaged plants (Turlings *et al* 1998).

In conclusion, our study revealed an interesting behavior of *S. frugiperda* female moths toward volatiles from herbivore-induced corn. Mated moths use odors to differentiate infested from non-infested corn plants and they likely use this strategy to avoid competitors and natural enemies that threaten the offspring. The next steps are to identify the plant volatiles responsible for moth attraction which can be a potential source of semiochemicals for behavioral manipulation in the field.

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## References

- Arab A, Trigo JR, Lourenção AL, Peixoto AM, Ramos F, Bento JMS (2007) Differential attractiveness of potato tuber volatiles to *Phthorimaea operculella* (Gelechiidae) and the predator *Orius insidiosus* (Anthracoridae). *J Chem Ecol* 33:1845–1855
- Bernays EA, Chapman RF (1994) Host-plant selection by phytophagous insects. Chapman & Hall, New York, p 312
- Bruinsma M, Van Dam NM, Van Loon JJA, Dicke M (2007) Jasmonic acid-induced changes in *Brassica oleracea* affect oviposition preference of two specialist herbivores. *J Chem Ecol* 33:655–668
- Busato GR, Grützmacher AD, Garcia MS, Giolo FP, Zotti MJ, Martins AF (2002) Consumo e utilização de alimento por *Spodoptera frugiperda* (J.E. Smith) (Lepidoptera: Noctuidae) originária de diversas regiões do Rio Grande do Sul, das culturas de milho e arroz irrigado. *Neotrop Entomol* 31:525–529
- Busato GR, Grützmacher AD, Garcia MS, Giolo FP, Stefanello GJ Jr, Zotti MJ (2004) Preferência para alimentação de biótipos de *Spodoptera frugiperda* (J.E. Smith, 1797) (Lepidoptera: Noctuidae) por milho, sorgo, arroz e capim-arroz. *R Bras Agrociencia* 10:215–218
- Butt BA, Cantu E (1962) Sex determination of lepidopterous pupae. USDA, Washington, p 7p
- Carroll MJ, Schmelz EA, Meagher RL, Teal PEA (2006) Attraction of *Spodoptera frugiperda* larvae to volatiles from herbivore-damaged maize seedlings. *J Chem Ecol* 32:1911–1924
- Crócomo WB, Parra JRP (1985) Consumo e utilização de milho, trigo e sorgo por *Spodoptera frugiperda* (J. E. Smith, 1797) (Lepidoptera: Noctuidae). *Rev Bras Entomol* 29:225–260
- Cruz I, Turpin FT (1982) Efeito da *Spodoptera frugiperda* em diferentes estágios de crescimento da cultura do milho. *Pesq Agropec Bras* 17:355–359
- D'Alessandro M, Turlings TCJ (2006) Advances and challenges in the identification of volatiles that mediate interactions among plants and arthropods. *Analyst* 131:24–32
- De Moraes CM, Mescher MC, Tumlinson JH (2001) Caterpillar-induced nocturnal plant volatiles repel conspecific females. *Nature* 410:577–580
- Degen T, Dillmann C, Marion-Poll F, Turlings TCJ (2004) High genetic variability of herbivore-induced volatile emission within a broad range of maize inbred lines. *Plant Physiol* 135:1928–1938
- Dicke M, Sabelis MW (1988) How plants obtain predatory mites as bodyguards. *Neth J Zool* 38:148–165



- Dicke M, Sabelis MW, Takabayashi J, Bruin J, Posthumus MA (1990) Plant strategies of manipulating predator-prey interactions through allelochemicals: prospects for application in pest control. *J Chem Ecol* 16:3091–3118
- Diez-Rodríguez GI, Omoto C (2001) Herança da resistência de *Spodoptera frugiperda* (J.E. Smith) (Lepidoptera: Noctuidae) a lambda-cialotrina. *Neotrop Entomol* 30:311–316
- Greene GL, Leppla NC, Dickerson WA (1976) Velvetbean caterpillar. A rearing procedure and artificial medium. *J Econ Entomol* 69:447–448
- Halitschke R, Stenberg JA, Kessler D, Kessler A, Baldwin IT (2008) Shared signals—“alarm calls” from plants increase apparency to herbivores and their enemies in nature. *Ecol Letters* 11:24–34
- Hoballah MEF, Turlings TCJ (2005) The role of fresh versus old leaf damage in the attraction of parasitic wasps to herbivore-induced maize volatiles. *J Chem Ecol* 31:2003–2018
- Hoballah MEF, Tamó C, Turlings TCJ (2002) Differential attractiveness of induced odors emitted by eight maize varieties for the parasitoid *Cotesia marginiventris*: is quality or quantity important? *J Chem Ecol* 28:951–968
- Hoballah MEF, Köllner TG, Degenhardt J, Turlings TCJ (2004) Costs of induced volatile production in maize. *Oikos* 105:168–180
- Jönsson M, Anderson P (1999) Electrophysiological response to herbivore-induced host plant volatiles in the moth *Spodoptera littoralis*. *Physiol Entomol* 24:377–385
- Kessler A, Baldwin IT (2001) Defensive function of herbivore-induced plant volatile emissions in nature. *Science* 291:2141–2144
- Khan ZR, Pickett JA, Wadhams LJ, Hassanali A, Midega CAO (2006) Combined control of *Striga* and stemborers by maize–*Desmodium* spp. intercrops. *Crop Prot* 25:989–995
- Köllner TG, Scheene C, Gershenzon J, Degenhardt J (2004) The sesquiterpene hydrocarbons of maize (*Zea mays*) form five groups with distinct developmental and organ-specific distributions. *Phytochemistry* 65:1895–1902
- Malo EA, Castrejón-Gómez VR, Cruz-López L, Rojas JC (2004) Antennal sensilla and electrophysiological response of male and female *Spodoptera frugiperda* (Lepidoptera: Noctuidae) to conspecific sex pheromone and plant odors. *Ann Entomol Soc Am* 97:1273–1284
- Mithöfer A, Wanner G, Boland W (2005) Effects of feeding *Spodoptera littoralis* on lima bean leaves. II. Continuous mechanical wounding resembling insect feeding is sufficient to elicit herbivory-related volatile emission. *Plant Physiol* 137:1160–1168
- Rodríguez-Saona C, Poland TM, Miller JR, Stelinski LL, Grant GG, Groot P, Buchan L, MacDonald L (2006) Behavioral and electrophysiological responses of the emerald ash borer, *Agrilus planipennis*, to induced volatiles of Manchurian ash, *Fraxinus mandshurica*. *Chemoecology* 16:75–86
- Sparks AN (1979) A review of the biology of the fall armyworm. *Florida Entomol* 62:82–87
- Steinberg S, Dicke M, Vet LEM (1993) Relative importance of infochemicals from the first and second trophic level in long-range host location by the larval parasitoid *Cotesia glomerata*. *J Chem Ecol* 21:525–539
- Szendrei Z, Rodríguez-Saona C (2010) A meta-analysis of insect pest behavioral manipulation with plant volatiles. *Entomol Exp Appl* 134:210–210
- Turlings TCJ, Tumlinson JH, Eller FJ, Lewis WJ (1991) Larval-damaged plants: source of volatile synomones that guide the parasitoid *Cotesia marginiventris* to the microhabitat of its hosts. *Entomol Exp Appl* 58:75–82
- Turlings TCJ, Lengwiler UB, Bernasconi ML, Wechsler D (1998) Timing of induced volatile emissions in maize seedlings. *Planta* 207:146–152
- Unsicker SB, Kunert G, Gershenzon J (2009) Protective perfumes: the role of vegetative volatiles in plant defense against herbivores. *Curr Opin Plant Biol* 12:479–485
- Yoneya K, Kugimiya S, Takabayashi J (2010) Specialist leaf beetle larvae use volatiles from willow leaves infested by conspecifics for reaggregation in a tree. *J Chem Ecol* 36:671–679
- Zhang N, Jian-Ying G, Fang-Hao W, Gang W (2009) Oviposition and feeding preferences of *Spodoptera exigua* (Hubner) (Lepidoptera: Noctuidae) to different host plants. *Acta Entomol Sinica* 52:1229–1235