

**University of São Paulo
“Luiz de Queiroz” College of Agriculture**

**Trophic interactions on *Zea* spp. plants involving the herbivores
Spodoptera frugiperda (J.E. Smith), *Diatraea saccharalis* (Fabricius)
and the predator *Doru luteipes* Scudder**

Natalia Naranjo Guevara

Thesis presented to obtain the degree of Doctor in
Science: Area: Entomology

**Piracicaba
2017**

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Biologist

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the predator *Doru luteipes* Scudder**
versão revisada de acordo com a resolução CoPGr 6018 de 2011

Advisor:
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Guevara por todo o amor e carinho, por ser a minha força criadora
para alcançar os nossos sonhos*

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*“Caminante, son tus huellas
el camino y nada más;
Caminante, no hay camino,
se hace camino al andar.
Al andar se hace el camino,
y al volver la vista atrás
se ve la senda que nunca
se ha de volver a pisar.
Caminante no hay camino
sino estrellas en la mar”*

Antonio Machado

SUMMARY

RESUMO.....	9
ABSTRACT	10
1 GENERAL INTRODUCTION.....	11
1.1 Thesis outline.....	17
References	18
2 TRAIL-FOLLOWING BEHAVIOR IN THE EARWIG <i>Doru luteipes</i> SCUDDER	29
Abstract.....	29
2.1 Introduction	30
2.2 Methodology.....	31
2.3 Results	33
2.4 Discussion.....	34
Acknowledgements	36
References.....	37
Tables	41
Figures	42
Abstract.....	43
Introduction	44
Materials and methods.....	47
Results	50
Discussion.....	51
Acknowledgements	54
References.....	54
Tables	63
Figures	64
4 LIFE-HISTORY EVOLUTION OF <i>Zea</i> spp. DETERMINE THE ECOLOGICAL CONTEXT OF PLANTS DEFENSE STRATEGIES IN TROPHIC INTERACTIONS..	69
Abstract.....	69
4.1 Introduction	70
4.2 Methodology.....	73
4.3 Results	77
4.4 Discussion.....	79
Acknowledgements	83

References 84

Tables..... 91

Figures..... 93

RESUMO

Interações tróficas em plantas *Zea* spp. envolvendo os herbívoros *Spodoptera frugiperda* (J. E. Smith), *Diatraea saccharalis* (Fabricius) e o predador *Doru luteipes* Scudder

As tesourinhas (Dermaptera) são em sua maioria insetos noturnos considerados sub-sociais devido ao cuidado maternal e à formação de grupos familiares agregados. Espécies de Dermaptera possuem um papel importante como predadores generalistas em diferentes culturas em todo mundo. Apesar disso, o conhecimento sobre seu comportamento e as interações inseto-planta permanecem pouco estudadas. A tesourinha *Doru luteipes* Scudder (Dermaptera: Forficulidae) é um dos predadores mais frequentes em diferentes culturas, especialmente no milho (*Zea mays* ssp. *mays*), devido ao voraz consumo de ovos e lagartas de *Spodoptera frugiperda* e *Diatraea saccharalis*, pragas chave nesta cultura. O milho é um dos cereais mais produzidos no mundo, embora grandes perdas sejam registradas anualmente devido ao ataque de pragas, apesar do uso de agroquímicos. Estudos recentes demonstraram que plantas domesticadas tais como o milho têm reduzido suas defesas contra os herbívoros em detrimento à intensa seleção de cultivares de rápido crescimento e alta produtividade, quando comparados com seus ancestrais selvagens conhecidos como teosintos (*Zea* spp.). Esta tese foca no estudo das interações tróficas que ocorrem no sistema 'plantas *Zea* – lagartas herbívoras – tesourinhas predadoras' e foi dividida em quatro capítulos. No **Capítulo 1**, apresenta-se uma introdução geral e o esboço da tese. No **Capítulo 2**, a partir da coleta de voláteis de adultos de *D. luteipes* e ensaios de múltipla escolha, foi estudada a possibilidade de uso de algum tipo de comunicação química por trilha entre estes indivíduos que influencia o comportamento de predação. Os resultados indicaram que as tesourinhas produziram e utilizaram estas pistas químicas dos coespecíficos para se orientar em direção aos recursos alimentares. No **Capítulo 3** foi investigado o comportamento de predação das tesourinhas durante a foto e escotofase, e a preferência delas pelos voláteis do milho induzidos por *S. frugiperda* ou *D. saccharalis*, em diferentes intervalos de tempo (dano recente ou dano tardio). As tesourinhas mostraram atividade predatória apenas durante a noite, e foram atraídas pelos odores do milho atacados pelas duas lagartas. O dano recente (voláteis de folhas verdes-VFVs) contém os compostos chaves para a atração de *D. luteipes*. No **Capítulo 4** foi explorado como a domesticação das plantas *Zea* influenciam as interações com insetos por meio das defesas de plantas. Assim como no capítulo anterior, os VFVs apresentaram um importante papel na atração das tesourinhas. Os resultados indicaram que a história de vida do gênero *Zea* influenciou as defesas das plantas e consequentemente, as interações inseto-planta. Em conclusão, o presente trabalho traz novas contribuições ao conhecimento sobre defesas de plantas num contexto tritrófico, bem como às estratégias de predação em *D. luteipes*, o qual pode gerar ferramentas para otimizar a conservação e controle biológico de pragas por predadores em campo.

Palavras-chave: Comportamento de rastreamento; Defesas de plantas; Estratégias de predação; Milho; Teosinto; Voláteis induzidos por herbivoria

ABSTRACT

Trophic interactions on *Zea* spp. plants involving the herbivores *Spodoptera frugiperda* (J. E. Smith), *Diatraea saccharalis* (Fabricius) and the predator *Doru luteipes* Scudder

The earwigs (Dermaptera) contains mostly nocturnal insects, which are considered subsocial due to formation of aggregated family groups and maternal care. Species of Dermaptera have been shown to play an important role as generalist predators in different crop systems worldwide. Despite that, the knowledge about their behavior and insect-plant interactions remains poorly understood. The earwig *Doru luteipes* (Dermaptera: Forficulidae) is one of the most important generalist predators in different crops, especially in maize (*Zea mays* ssp. *mays*) by voraciously consuming eggs and larvae of *Spodoptera frugiperda* (Lepidoptera: Noctuidae) and *Diatraea saccharalis* (Lepidoptera: Crambidae), key pests in this crop. Maize is one of most produced cereals in the world. However, great losses are registered annually due to pest attacks, despite the use of pesticides. Studies have demonstrated that domesticated plants such as maize have reduced defenses against herbivores in detriment to intensely selecting for rapid growth and high yield, when compared with their wild ancestors' species known as teosintes (*Zea* spp.). This thesis focuses on the study of the trophic interactions which occur in the system 'Zea plants – herbivores caterpillars – predator earwig' and is divided in four chapters. In the **Chapter 1** is present a general introduction and the thesis outline. In the **Chapter 2**, we studied through scent collection and multiple-choice bioassays, whether there exists some kind of trail communication between *D. luteipes* individuals which influences their predatory behavior. Our results indicate that earwigs can produce and use chemical cues by conspecifics to orientate them towards foraging resources. In the **Chapter 3**, we investigated the predation behavior of earwigs during photo- and scotophase and their preference for maize volatiles induced by *S. frugiperda* or *D. saccharalis* at different time intervals (early and old damage). Behavior, olfactometry bioassays and plant volatile collections were conducted. As was hypothesized, earwigs showed a predation activity only during night. Besides that, they were attracted by maize odors produced by both caterpillars and showed significant preference to early-damage odors over old-damage. Therefore, it is considered that early-damage volatiles (Green Leaf Volatiles-GLVs) could be the key compounds of *D. luteipes* attraction. In the last part (**Chapter 4**) was explored how domestication of *Zea* plants influences insect-plants interactions through plant defense. Experiments about food utilization by caterpillars, olfactometry bioassays and plant volatile collections were conducted. Interestingly, as in the previous chapter, we suggest that GLVs have an important role in earwig attraction. The results show that life-history of *Zea* plants influences plant defense and consequently the plant-insect interactions. In conclusion, our findings bring a new contribution to the knowledge about plant defenses in a tritrophic context and predatory strategies in *D. luteipes*, which would promote alternatives to optimize the conservation and biological control of pest by predators in the field.

Keywords: Trail-following behavior; Plant defenses; Predatory strategies; Maize; Teosinte; Herbivore induced plant volatiles

1 GENERAL INTRODUCTION

Over the course of coevolution with herbivorous insects, plants have developed a broad spectrum of defense and tolerance mechanisms to fend off and cope with the different attacks. These defenses can be constitutive, like thorns and trichomes, lignified cell walls and secondary metabolites (KARBAN; BALDWIN, 1997; WITTSTOCK; GERSHENZON, 2002) that exist for use against insect attack. Induced responses include defensive mechanisms that become activated upon insect attack, caused by elicitors present in the oral secretions, and/or oviposition fluid of herbivores (TURLINGS et al., 1993; ALBORN et al., 1997; HILKER et al., 2005; LOUIS et al., 2013). Induced responses can be direct or indirect. Elicitors activate signaling cascades, which leads to physiological changes and formation of specific products that interfere with the herbivore development or behavior (PARE; TUMLINSON, 1997).

Direct defenses cause negative impact on physiology or behavior of the attacker through the production of toxins, digestibility reducers, secondary compounds and proteinase inhibitors production (SCHOONHOVEN; VAN LOON; DICKE, 2005). Direct plant defenses can be classified as anti-nutritional and toxic. Anti-nutritional defenses can take place to limit food supply or to reduce nutrients to the attacking insect. Toxic defenses include physical damages and chemical disruptions to attacking insects by specific plant traits (CHEN, 2008). Examples of this kind of defenses include the production of terpenoids, alkaloids, phenylpropanoids, glucosinolates, benzoxazinoids, and nonprotein amino acids. These metabolites can negatively impact growth and development or induce feeding deterrents via direct toxicity or mimicry of insect hormones (CHEN, 2008; HUFFAKER et al., 2013).

Herbivory also stimulates emission of a complex blend of volatiles that function as indirect defenses. Indirect defenses include herbivore induced plant volatiles (HIPVs) that can serve as attractants to natural enemies of herbivores i.e., parasitoids or predators. Natural enemies use these chemical cues to search for prey or hosts and can consequently suppress herbivores population (TURLINGS TUMLINSON; LEWIS, 1990; DE MORAES; LEWIS; PARE, 1998; DICKE; VAN LOON, 2000; KESSLER; BALDWIN, 2001; DE MORAES; MESCHER; TUMLINSON, 2001).

Emission of HIPVs is a phenomenon also referred to as 'crying for help' (DICKE et al., 1990) that has been demonstrated for different plant families in response to a wide diversity of herbivorous arthropods in the last two decades (DICKE; SABELIS, 1988; TURLINGS; TUMLINSON; LEWIS, 1990; DRUKKER; SCUTAREANU; SABELIS, 2000; DU et al., 1998; VET; DICKE 1995, VAN TOL et al., 2001; RASMANN et al., 2005, DE LANGE et al., 2016). Most of the studies for tritrophic systems have looked at the volatile attraction to parasitoid wasp species. (TURLINGS; TUMLINSON; LEWIS, 1990; TURLINGS et al., 1995; TAMÒ et al., 2006). Some studies conducted with entomopathogenic nematodes have shown attraction of these natural enemies to roots upon herbivory by root-feeding herbivores (RASMANN et al., 2005; VAN TOL et al., 2001; ALI; ALBORN; STELINSKI, 2010). Nevertheless, there are still few studies about effects of HIPVs in predators (DRUKKER; BRUIN; SABELIS, 2000; SCUTAREANU et al., 1997; VAN LOON; VOS; DICKE, 2000; MAEDA et al., 2015; ARDANUY; ALBAJES; TURLINGS, 2016) and most of them refer to predatory mites (DICKE; SABELIS, 1988; DICKE et al., 1990; DE BOER et al., 2008).

Induction of HIPV is mediated by the phytohormones jasmonic acid (JA), salicylic acid (SA) and ethylene (ET). It is well known that chewing insects, such as caterpillars, elicit plant defense responses regulated by JA, whereas phloem-sucking herbivores frequently activate the salicylic acid (SA) signaling pathway. Even within feeding guilds, different herbivore species elicit different HIPV composition (TURLINGS; TUMLINSON; LEWIS, 1990). Likewise, natural enemies can discriminate the attack by different herbivore species (DU et al., 1998; RASMANN; TURLINGS, 2007). Dicke et al. (1999) showed that the JA application to lima bean plants induces a volatile blend that is similar to the blend induced by spider mite feeding, and predaceous mites can discriminate between induced not induced plants volatiles. Moreover, the emission of HIPV occurs locally at the site of infestation, but also systemically from uninfested organs (TURLINGS; TUMLINSON, 1992). For example, the maize stem borer, *Chilo partellus* (Swinhoe) (Lepidoptera: Pyralidae) induce the emission of volatiles from the foliage of plants whose stem they infest, and consequently attracts parasitoids can localize the stem borer larva (POTTING; VET; DICKE, 1995). Volatile composition could be affected by biotic factors such as plant and herbivore species (DE MORAES; LEWIS; PARE, 1998; DE BOER et al., 2008) herbivory time (TURLINGS et al., 1998; CLAVIJO MCCORMICK et al., 2014) as well

as abiotic conditions such as temperature, humidity and light (GOUINGUENÉ; TURLINGS, 2002; SEIDL-ADAMS et al., 2015).

Induced plant volatiles in general include green leaf volatiles (GLVs: 6-carbon aldehydes, alcohols, and acetates), terpenoids, phenols (phenylpropanoids or benzenoids) and, sulfur and nitrogen containing volatiles. These groups are involved in both toxicity of plant tissues and attraction of natural enemies (DE BOER et al., 2008; DICKE, 2009; MUMM; DICKE, 2010; SCHOONHOVEN et al., 2005)

GLVs are immediately released from early damage leaves and are common in all plant species. Therefore, GLVs provide fast, but nonspecific information to natural enemies about herbivore location. Later blends such as terpenoids are more indicative of actual herbivore damage due to involving specific metabolic routes in plants. Their composition varies among plant genotypes (HOBALLA; TURLINGS, 2005; ALLMANN; BALDWIN, 2010). Both GLVs and terpenes were shown to play a role in host or prey location of parasitic wasps and predators (WEISSBECKER et al., 1999; HOBALLA; TURLINGS, 2005; MAEDA et al., 2015; KESSLER; BALDWIN, 2002). Frequently, specialist species respond to specific plant volatile blends, while generalist species seem to be less selective (DE BOER et al., 2008; PEÑAFLORES et al., 2011).

Thus, plant responses play an important role in population dynamics, because they might affect the performance and the behavior of other species such as herbivores and consequently natural enemies (PRICE et al., 1980; VET; DICKE, 1992; DICKE; BALDWIN, 2010). It has been proposed the study of plant defenses as a new focus for crop pest control (STENBERG et al., 2015). It is supposed that direct and indirect defense strategies act together, however, little is known about their interaction (KANT, 2004)

Direct and indirect plant defenses can be affected by selection pressures through evolution. Domestication of wild plants for agriculture can negatively influence defense strategies by intense selection for traits, such as rapid growth and high yield (ROSENTHAL; DIRZO, 1997; RODRIGUEZ-SAONA et al., 2011). Selective breeding reduces genetic diversity of domesticated crops, which results in lacking or weakly defensive traits (DOEBLEY et al., 2004). While artificial selection of desirable traits increased global crop productivity, there is a tendency to develop modern crop varieties with low levels of diversity. This represents a challenge for improving crop production sustainability as it suggests that modern varieties could

perform poorly in systems with restricted pesticide use (MITCHELL et al., 2016). Several studies have suggested that domesticated plants are less resistant to herbivory than their wild ancestors or land races, including cranberry (RODRIGUEZ-SAONA et al., 2011), cotton (HAGENBUCHE et al., 2016), *Brassica* and *Phaseolus* (BENREY et al., 1998), tomato (WELTER; STEGGALL, 1993), sunflower (CHEN; WELTER, 2005) and maize (ROSENTHAL; DIRZO, 1997; TAMIRU et al., 2011; SZCZEPANIEC et al., 2013; DÁVILA-FLORES et al., 2013; MUTYAMBAL et al., 2015). Hence, plant domestication also affects species interactions in tritrophic systems (BENREY et al., 1998; CHEN et al., 2015). For maize, this effects has not been studied in detail (DE LANGE, 2016)

Native to Mexico, maize (*Zea mays* ssp. *mays*) was domesticated 9 000 years ago, from the wild ancestor, Balsa teosinte (*Zea mays* ssp. *parviglumis* Iltis & Doebley) which resulted in considerable alteration of the plant morphology, growth rate, and yield (MATSUOKA et al., 2002; DOEBLEY, 2004). Maize has spread worldwide to become one of the most important crops in the world at present. The amount of maize produced worldwide is about 1010 million metric tons every year. The highest portion of maize production is used for animal consumption. It is the one most important food source in Africa, Mesoamerica and Asia (FAO, 2014).

The genus *Zea* contains eight taxa classified into two sections and five species. All taxa in *Zea*, besides cultivated maize, are named teosintes (Nahuátl Indian name). The section *Luxuriantes* contains two perennial and two annual species (*Zea diploperennis* Iltis, Doebley & Guzmán, *Zea perennis* (Hitchc.) Reeves & Mangelsdorf, *Zea luxurians* (Durieu & Ascherson) Bird, *Zea nicaraguensis* Iltis & Benz). The section *Zea* contains four subspecies of *Z. mays* (ssp. *mexicana* (Schrader) Iltis, ssp. *parviglumis* Iltis & Doebley, ssp. *huehuetenangensis* (Iltis & Doebley) Doebley, ssp. *mays*) (DOEBLEY; ILTIS, 1980). Populations of teosinte grow in and around maize fields in Mexico and Central America. Indeed, hybridization and introgression between teosintes and maize is a common practice by farmers (TAKAHASHI et al., 2012). These wild ancestors represent potential genetic resources for crop's maize improvement (WANG et al., 2008).

Teosinte and maize have genetic and morphological differences, such as, mutations in a small number of genes, increased seed size and reduced number of tillers in maize (MOYA-RAYGOZA et al., 2016). Aside from this, differences in direct and indirect defenses between wild and modern species have reported in several

studies. Teosinte showed more resistance to the stemborer *Diatraea grandiosella* Dyar (Lepidoptera: Crambidae) (ROSENTHAL; DIRZO, 1997), leafhopper *Dalbulus maidis* (DeLong & Wolcott) (Hemiptera: Cicadellidae) (DÁVILA-FLORES; DEWITT; BERNAL, 2013) and *Spodoptera frugiperda* (J.E. Smith) (Lepidoptera: Noctuidae) (TAKAHASHI et al., 2012) compared to landraces and modern maize varieties. Researches have revealed that variation in production HIPVs which attract the natural enemies of herbivores among maize lines and teosintes (GOUNGUENE; DEGEN; TURLINGS, 2001; DEGEN et al., 2004; DE LANGE 2016). American maize varieties have lost the ability to release (*E*)- β -caryophyllene, key compound to the attraction of entomopathogenic nematodes (KÖLLNER et al., 2008). At field, Takahashi et al. (2012) observed that maize was more frequently infested by *S. frugiperda* larvae than Balsa teosinte.

Maize domestication represents an evolutionary time scale that shows differences in several traits when compared with modern varieties with land races, annual and perennial teosintes. Plant life histories reflect adaptations to different environmental pressures and consequently affect the interaction of plants with their insect herbivores and natural enemies (TAKAHASHI et al., 2012; MUTYAMBA et al., 2015; DE LANGE et al., 2016). For example, perennial species generally allocate more resources in herbivore defenses due to they must survive across growing seasons and are more likely to suffer injury from herbivores over their lifetime, while annual species generally allocate more to growth and reproduction due to their reproductive opportunities are limited to a single growing season (DÁVILA-FLORES; DEWITT; BERNAL, 2013)

Fall armyworm, *S. frugiperda*, is a polyphagous herbivore and is among the most important pests of maize in the Americas (LUGINBILL 1928; MOLINA-OCHOA et al., 2003). The larvae are known for attacking sorghum, rice, sugarcane, pasture and cotton, in which it causes enormous yield losses, although it can also occur in more than 80 host species (WOJCIK; WHITCOMB; HABECH, 1976; GARCÍA-ROA et al., 2002). Outbreaks occur regularly in maize due to their high reproductive rate, multivoltinism, broad host range and ability to migrate long distances (CAPINERA, 2000). Similar *Diatraea saccharalis* (Fabricius) (Lepidoptera: Crambidae), the sugarcane borer, is a pest that occurs from the southern United States to Argentina (PEAIRS; SAUNDERS, 1980) and attacks crops of economic importance such as sugarcane, sorghum, rice, wheat and maize (PINTO; GARCIA; BOTELHO, 2006).

According to Cruz (2007), it has been pointed out as a serious threat to maize culture in tropical regions. The direct damages are caused by penetration of caterpillars in the stalks and opening galleries, leading to an increase in sterility, reduction of spike and grain size (PEAIRS; SAUNDERS, 1980). *S. frugiperda* and *Diatraea* spp. occurred on teosinte plants at field, indicating that these herbivores also use wild ancestors of maize as a food source (PAINTER, 1955). Chemical control and genetically modified crops are the most used strategies to caterpillar's management. Further, reports of arthropod resistance to pesticides and *Bt* technologies (DIEZ-RODRIGUEZ; OMOTO, 2001; KIKUCHI et al., 2012; BINNING et al., 2014) raise concerns about the use of these strategies in the future.

The earwig, *Doru luteipes* Scudder (Dermaptera: Forficulidae) is an important generalist predator that naturally occurs in maize crops. It has shown voraciously consuming eggs and larvae of Lepidoptera. This includes, *S. frugiperda* (REIS et al., 1988), *D. saccharalis* (ROMERO-SUELDO; VIRLA, 2008), corn earworm *Helicoverpa zea* (Boddie) (Lepidoptera: Noctuidae) (CRUZ et al., 1995), and also other herbivores such as aphids (ALVAREGA; VENDRAMIM; CRUZ, 1996). *Doru* spp. individuals have been already observed in teosinte fields (PAINTER, 1955) showing a long evolutionary history with *Zea* plants. In general, earwigs (Dermaptera) are distributed worldwide and most have nocturnal habits (LAMB; WELLINGTON, 1975). Many species are recognized as potential biological control agent in agricultural systems including *Doru taeniatum* Dohrn and *Doru lineare* Eschs. (Dermaptera: Forficulidae), *Labidura riparia* (Pallas) (Dermaptera: Labiduridae), *Euborellia annulipes* (Lucas) (Dermaptera: Anisolabididae), and the European earwig *Forficula auricularia* Lin. (Dermaptera: Forficulidae) (LAMB; WELLINGTON, 1975; EVANS; LONGÉPÉ 1996; HAILE; HOF SVANG, 2001; ALVAREGA; VENDRAMIM; CRUZ, 1996; HE; WANG; XU, 2008; ROMERO-SUELDO; VIRLA, 2008). Despite of this, the use of earwigs in integrated pest management (IPM) is not common. That is due to lack of knowledge about behavior and predation activity of these species in special to *D. luteipes*.

Earwigs are considered subsocial insects due to maternal care for their offspring and temporary formation of family groups (LAMB; WELLINGTON, 1975). The maintenance of these groups is mediated through pheromone communication. Previous studies in European earwig *F. aurieularia*, confirmed the existence of aggregation pheromones facilitated by cuticular hydrocarbons, tibial glands and feces

(SAUPHANOR; SUREAU, 1993; WALKER et al., 1993; EVANS; LONGÉPÉ, 1996; HEHAR et al., 2008; LORDAN et al., 2014). These studies suggest that the knowledge of aggregation pheromones could promote an efficient use of earwigs as a tool in biological control programs. Besides that, pheromones that induce aggregation can act as a mean of conveying information through trail formation in non-social insects, such as cockroaches (MILLER; KOEHLER, 2000; JEANSON; DENEUBOURG, 2006) and caterpillars (FITZGERALD; UNDERWOOD, 1998; FITZGERALD; WEBSTER, 1993; RUF et al., 2001). According to Lordan et al. (2014), pheromonal communication in earwigs should be more complex than a simple aggregation pheromone. Pheromones scent trails that elicited arrestment behavior, therefore, could contribute to exploit earwigs at field as important biocontrol agents. Even though some biology studies have been conducted with earwigs, the knowledge about behavior and communication systems of *D. luteipes* remains poorly understood.

Predator contribution could be maximized in agrosystems by understanding the mechanisms that affect foraging behavior and olfactory communication. In addition, almost nothing is known about the third trophic level natural enemies that feed on herbivore insects of maize and teosintes (MOYA-RAYGOZA et al., 2016), over all on predators. Thus, knowledge about plant defenses and chemical communication between insects allows elucidating different alternatives to optimize crop breeding strategies and development of methods that promote the conservation and increase of natural enemies in crops.

1.1 Thesis outline

The aim of this thesis was to study chemical interactions of the trophic system composed of maize and teosinte plants, the herbivores *S. frugiperda*, *D. saccharalis*, and the predator *D. luteipes*.

Due to lack of information about the olfactory behavior of earwigs, we initially performed some experiments aiming to establish a methodology that allowed study the behavior of *D. luteipes*. From these observations, in **Chapter 2**, we asked whether *D. luteipes* can deposit scent-trails by walking on a surface and whether these scents can influence orientation of conspecific. In **Chapter 3**, we questioned whether earwigs can use HIPVs of maize plants when attacked by different herbivore

species to find their prey and if so, whether they prefer general (GLVs) or specific volatiles. For this, we studied the tritrophic interaction among maize plants, the herbivorous caterpillars *S. frugiperda* (leaf chewing) and *D. saccharalis* (borer) and the predatory earwig *D. luteipes*. The predation activity of *D. luteipes* has not been studied previously, so we conducted tests during the photo and scotophase to confirm the most appropriate time to study the olfactive attraction. Finally, in **Chapter 4**, we explore how the life-history of *Zea* influences tritrophic interactions through plants defense. We measured food intake and utilization by larvae, releases of HIPVs and predator attraction. We chose two wild teosintes and a modern maize (*Z. mays* ssp. *mays*, variety Delprim). The wild teosintes include *Z. diploperennis* Ittis, Doebley & Guzmán, a perennial weed and *Z. mays* ssp. *mexicana* (Schrader) Ittis, a wild annual subspecies of *Z. mays*. This species represents a gradient in life-history evolution of *Zea* genus (from perennial to annual life cycle, and finally the evolution to modern cultivar) that could contribute to knowledge about how the effect on defense strategies through domestication influences the tritrophic interactions.

This study is the first report about predation strategies and HIPVs in Dermaptera, as well to explore the ecological relevance of life history in tritrophic interactions involving generalist predators.

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2 TRAIL-FOLLOWING BEHAVIOR IN THE EARWIG *Doru luteipes* SCUDDER

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Abstract

Trails pheromones are used by social and non-social insects to orient between their shelter and forage. The ability to produce and use scent-trails represents early stage of evolution to cooperative foraging. Gregarious insects such as earwigs are considered subsocial that play an important role as generalist predators in different crop systems. We study the trail-following behavior of the earwig *Doru luteipes* Scudder (Dermaptera: Forficulidae). We hypothesize that when earwigs are looking for food would deposit scent-trails by the successive walking than influence orientation of starving conspecific as a foraging strategy. First, we collected the scent-trails deposited by an earwig that walks in a glass tube. Subsequently, the behavior of starving and non-starving earwigs towards extract collected were observed in a multiple-choice arena. Finally, we analyses the chemical composition of the extract. The results showed that *D. luteipes* produced scent-trails that modified the behavior of the conspecific individuals. Earwigs showed a clear preference toward scent-trails extracts when they were starved. We highlighted six main compound 2,4-Dimethyl-1-heptene, 5-Hydroxy-4-octanone, 1-Decanol, 2-hexyl- and the cuticular hydrocarbons Tetradecane, Heneicosade and Tetrapentacontane,

however confirmation of the compounds is still necessary. The studies about earwigs pheromones allow exploit strategies at field to maintain predator populations in crops. The behavior observed in this study could be common in other species of earwigs.

Keywords: Foraging strategy. Generalist predators. Gregarious. Pheromones. Subsocial. Starving.

2.1 Introduction

It has long been known that social insects rely on chemical cues as trail pheromones for recruitment and directional orientation between food sources and the nest. Among the most studied groups stand ants (Kohl et al. 2003, Morgan 2009, Califano and Chaves-Campos 2011, Czaczkes et al. 2016), termites (Sillam-Dussès et al. 2007, Sillam-Dussès et al. 2010, Wen et al. 2014) and bees (Reichle et al. 2011, Schorkopf et al. 2011, John et al. 2012, Reichle et al. 2012). In these groups, foragers can use trail pheromone and direct siblings to find the most profitable food as a foraging strategy (Ruf et al. 2001). Although most of the studies on scent-trails have been performed in social species using sophisticated means of communication, few studies have evaluated whether non-social insects can produce and use trails to orient in the path between their shelter and resources range (Jeanson and Deneubourg 2006). It has been shown that non-social insects (e.g. gregarious species) can use trail following behavior to forage and return to their harborages, such as some caterpillars (Fitzgerald and Underwood 1998; Fitzgerald and Webster 1993, Ruf et al. 2001) and Germanic cockroach (Miller and Koehler 2000, Jeanson and Deneubourg 2006).

Within the gregarious insects are included species of the order Dermaptera, commonly called earwigs. Earwigs are omnivores, nocturnally active that shelter in dark crevices during the day. They can be considered subsocial insects due to the temporary formation and maintenance of aggregated family groups and maternal care to their offspring (Lamb and Wellington 1975). Earwig species have been shown to play an important role as generalist predators, being able to limit herbivore populations in orchards (Evans and Longépé, 1996; He et al. 2008), vineyards (Buccholz and Schruft 1994) maize (Reis et al. 1988, Romero-Sueldo and Virla 2009) sorghum (Alvarenga et al. 1996), cotton (Soares and Busoli 2000) and sugar-cane

crops (Soussa-Silva et al. 1992, Romero-Sueldo and Virla 2009) around the world. Previous studies confirmed the existence of aggregation pheromones in European earwig *Forficula auricularia* L. (Dermaptera: Forficulidae) mediated by cuticular hydrocarbons and feces (Sauphanor 1992, Walker et al. 1993, Evans and Longepe 1996, Hehar et al. 2008). These studies suggest that the knowledge of aggregation pheromones could promote the use of earwigs as a tool in biological control programs.

As well as in other earwig species, *Doru luteipes* Scudder (Dermaptera: Forficulidae) is known as an important predator of herbivorous in different crops for its voracious consumption of eggs and larvae of *Spodoptera frugiperda* (J. E. Smith) (Lepidoptera: Noctuidae) in maize (Reis et al. 1988, Romero-Sueldo and Virla 2009), *Diatraea saccharalis* (Fabricius) (Lepidoptera: Crambidae) in sugar-cane (Soussa-Silva et al. 1992) and aphids in general (Alvarenga et al. 1996). Even though some biology studies have been conducted, the knowledge about behavior and communication systems of *D. luteipes* remains poorly understood. In addition, studies about trail pheromones have not been applied for any earwig species and according to Lordan et al. (2014), earwigs are considered subsocial insects, therefore, pheromonal communication should be more complex than a simple aggregation pheromone. *Doru luteipes* could be considered as an interesting biological model to study whether organisms with a simple social organization have the ability to rely on chemical trails to foraging strategy.

Thereby, the purpose of this study was to investigate whether *D. luteipes* can produce and follow scent-trails. Additionally, we aimed to determine if this trail-following behavior is motivated by the physiological condition (starvation). We hypothesize that when earwigs are looking for food (foraging earwigs) would deposit scent-trails by the successive walking that influence orientation of starving conspecific to follow it.

2.2 Methodology

Insects

Adults of *D. luteipes* were collected in field (from maize crops in Piracicaba-SP, Brazil) and reared under laboratory conditions ($25 \pm 1^{\circ}\text{C}$, $70 \pm 10\%$ RH, photoperiod 12 L:12 D). In nature, *D. luteipes* lives between the leaf axils of the

grass, ensuring a safe, dark and humid environment with frequent provisioning of food. The leaf axils also providing conditions for their thigmotropic and cryptic habits, such as response to the stimulus of direct contact and remain hidden (Butnariu et al. 2013). Therefore, we simulated these conditions keeping the insects in closed transparent plastic cages (23 cm length x 7 cm wide x 14 cm height), where it was placed transparent straws containing wetted cotton as oviposition substrate and refuge. Rearing methodologies were adapted from Pasini et al. (2007) and Butnariu et al. (2013). These adaptations were: (i) the plastic cages were sealed with aluminum foil to reduce light incidence; (ii) due to high occurrence of cannibalism, paper strips were folded in W-shape and placed inside of the cages to provide shelter for the earwigs; and (iii) the insects were fed with a diet based on cat food (Cruz 2009). Cotton was re-wetted or changed and food renewed twice a week. Straws contained mothers with her egg postures were transferred to new cages.

Experimental trial included two successive phases: (1) Scent-trails pheromones collection, and (2) bioassay. Both phases were made during the scotophase (19:00-20:00, starting 1h after dark) and under laboratory conditions ($25 \pm 1^\circ\text{C}$, $70 \pm 10\%$ RH).

Scent-trails pheromones collection

Scent-trail pheromones were collected through “walking arenas” made by a glass tube (0.5 cm internal diameter, 15 cm length) and Petri dishes (6 cm diameter) connected to each tip (Fig. 1A). Ten arenas were built, totalizing 10 repetitions. One female earwig per arena (starved for 48h) was released in one Petri dish, thus individuals could walk freely by the glass tube during 1 h. We offered a food source (artificial diet) to motivate the forging behavior and consequently earwigs explore the arena. A piece of voile was placed on the tip of the glass tube in order to prevent direct contact of the individuals with the food source, hence, avoiding possible contamination in the glass tube. Subsequently, the glass tubes were washed with hexane (2 ml) and the content was collected in vials (Fig. 1B), which were stored at -80°C to further bioassays and chemical analyses. The extracts obtained were concentrated and mixed with 10 μL of Nonanone (internal standard solution at 100 ng/ μL).

Trail-following behavior bioassays

To answer our hypothesis about whether starving earwigs follow scent-trails previously deposited by foraging conspecifics, we observed the behavior of starving and non-starving earwigs towards extract collected. In a multiple-choice arena, the extracts vs. control (hexane) were evaluated. Each arena had five acrylic plates (6 cm diameter), one of them in the center and another four arranged at each end of the arena (cross shaped), connected to the center by acrylic runners (10 cm x 0.5 cm). One female (starved or not) was released in the central plate, while the other four contained just shelters (filter paper folded in a W-shape). Pieces of filter paper (10 X 0.3 cm) were arranged inside the runners, and were all impregnated with 20 μ l of extract or hexane (control) and the treatments were placed in opposite sites (Fig. 1C).

Chemical analyses

A 2 μ l aliquot of extracts was injected with a splitless injector into a gas chromatograph coupled to mass spectrometer (GC–MS, Shimadzu GC-2010) equipped with Rtx1-MS capillary column (CA; 30 m 9 0.25 mm 9 0.25 μ m), using helium as the carrier gas. The column temperature was held at 40°C for 5 min, increased to 250°C (7°C /min) for 50 min. Compound quantification was estimated by the peak area relative to internal standard amount. Compound identifications were based by comparing obtained mass spectra retention times with mass spectra retention and by calculating Kovats index (KI) using nalkane (C7–C30) standards.

Statistical analysis

Logistic regression and Wald's Chi-square test was adopted to analyze earwig choices assessed in the arenas. Statistical analysis was performed using software package R 3.0.2 (www.r-project.org).

2.3 Results

Earwigs showed a clear preference toward scent-trails left by foraging conspecifics (extract) when they were starved ($F=69.59$; $P<0.001$). Nevertheless, when they were no starved, there were no differences with the control ($F=91.44$; $P=0.81$) (Fig. 2). More earwigs responses when they were starved (95%) than when

they were not starved (82%). It is evident that the starvation condition affected the path selection and the individual decision was dependent on the presence of a trail. These results suggest that *D. luteipes* produces scent-trails that could modify the conspecifics' behavior, they can follow these trails probably motivated by physiological conditions such as starvation and, consequently, by searching for food resources.

We highlighted six main compounds, among which are 2,4-Dimethyl-1-heptene, 5-Hydroxy-4-octanone, 1-Decanol, 2-hexyl- and the cuticular hydrocarbons Tetradecane, Heneicosane and Tetrapentacontane (Table 1). Confirmation of the compounds is still necessary with authentic standards.

2.4 Discussion

The results obtained in this study support our hypothesis that *D. luteipes* deposits chemical scents that influence the directional orientation of their conspecifics. *D. luteipes* employs a scent-trail communication system that recruits other earwigs when they are in starvation conditions and looking for food. The starvation act as a negative reinforcement, increased the earwig's movement during the collection step. The pheromone concentration could help to guide individuals in the direction of their conspecific groups and probably point profitable foraging sites.

In accordance with the approach proposed by Miller and Koehler (2000), the ability to produce and use scent-trails brings multiple advantages for the population, such as that the individuals would not have to locate resources individually, but would follow the chemical cues of their conspecifics. Consequently, trail-following behavior would enhance survival and fitness of explorer earwigs by reducing the amount of energy expended to locate resources in their environment. This represents an early stage in the evolution of cooperative foraging that could be motivated by the necessity to feed (Fitzgerald and Underwood 1998), where members of the population would be able to use and contribute to these trails during their foraging excursions (Miller and Koehler 2000). Aside from this, trail-following represents an advantageous behavior for nocturnal species due to directional response to scent and chemical stimuli allowing free movement in the dark when physical landmarks and visual cues are not important for navigating between their foraging areas and the shelter (Miller and Koehler 2000, Jeanson and Deneubourg 2006).

Many gregarious species use pheromones not just to ensure group cohesion, but also as a mean of conveying information through trail formation. These scent trails, therefore, could contribute to optimal foraging strategies and efficient exploitation of environmental resources (Jeanson and Deneubourg 2006). The potential role of cuticular hydrocarbons in scent-trail has been shown to induce aggregation and to act as a trail pheromone in non-social insects groups, such as caterpillars (Fitzgerald and Webster 1993, Fitzgerald and Underwood 1998, Ruf et al 2001) and cockroaches (Miller and Koehler 2000, Jeanson and Deneubourg 2006, Imen et al 2015). For earwigs, the existence of aggregation pheromones mediated by cuticular hydrocarbons, tibial glands and feces has been confirmed (Sauphanor and Sureau 1993, Walker et al. 1993, Evans and Longép  1996, Hehar et al. 2008, Lordan et al 2014). Nevertheless, only Walker et al. (1993) performed a chemical analysis. They identified cuticular extracts among which are Tricosane, Pentacosane, Heptacosane, Nonacosane. This study represents the first report about chemical cues acting as trail-following in Dermaptera.

Based on previous studies about chemical defense in other earwig species (Eisner et al. 2000, Gash et al. 2013, Gasch and Vilcinskas 2014), we could question whether the scent-trails used by *D. luteipes* actually derived from deposition of hydrocarbons to substrate through the multiple passages of individuals or maybe from the defensive exocrine glands. There are many discrepancies in the literature concerning the origin of Dermaptera aggregation pheromone (Sauphanor and Sureau 1993, Walker et al. 1993, Evans and Longepe 1996, Hehar et al. 2008, Lordan et al 2014). Many species of Dermaptera are provided for dischargeable glands that offer chemical protection (Eisner et al. 2000). Most thoroughly studied has been the European earwig, *F. auricularia* which discharges a secretion consisting of a mixture of 2-methyl-1,4-benzoquinone and 2-ethyl-1,4-benzoquinone (Gasch and Vilcinskas 2014). Eisner et al (2000) showed that *Doru taeniatum* Dohrn has a pair of defensive exocrine glands as well, opening on the 4th abdominal tergite, from which it discharges a spray of methyl-1,4-benzoquinone and 2,3-dimethyl-1,4-benzoquinone when disturbed. Recently, we had the opportunity to study the morphology of *D. luteipes* and we observed these glands on the same location showed by Eisner et al (2000) in *D. taeniatum*. Social insects also have some glands located in different parts of the body that release trail pheromones on the substrate. Venom glands, present from the middle part to the tip of the abdomen of the leaf-

cutting ants, for instance, produce molecules as 3-ethyl-2,5-dimethylpyrazine which is the main compound in some species trail pheromone (Tumlinson et al. 1971, Tumlinson et al. 1972, Cross et al. 1979, Evershed et al. 1982). Trail-following pheromone composition and the behavior triggered by these chemicals are well-studied in social insects (Jackson and Morgan 1993, Morgan 2009, Jaffe et al. 2012, Reichle et al. 2012, Czaczkes et al. 2016), but underestimated in other insects groups, as Dermaptera. Therefore, further work should examine whether cuticular hydrocarbons or glands extracts applied on the substrate can also elicit trail-following behaviors.

Pheromones that elicited arrestment behavior may allow us to exploit earwigs at field as important biocontrol agents. Aggregation pheromone emitted by earwigs is not commercially available, nonetheless from field studies made by Evans and Longép   (1996), Sauphanor and Sureau (1993), Hehar et al. (2008) and Lordan et al. (2014) we can assume that shelters impregnated with different pheromone extracts can be useful to maintain earwig populations in crops. This trail-following and consequently aggregation behavior is beneficial in terms of earwigs' role as generalist predators. *D. luteipes* has been shown to be efficient predator of aphids (Alvarega et al. 1996), insect eggs (Reis et al. 1988, Romero-Sueldo and Virla 2009) and young caterpillars (Reis et al. 1988, Soussa-Silva et al. 1992, Romero-Sueldo and Virla 2009) which are known to remain aggregate in high densities. In conclusion, our results indicate that *D. luteipes* can produce and use chemical cues by conspecifics to orient in their foraging range. This behavior could be common in other species of earwigs. Thus, this work brings a contribution regarding the development methods that can promote maintenance and enhancement of earwigs that occur naturally in crops.

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Tables

Table 1. Mean (\pm SE) amounts of scent-trails pheromones of *Doru luteipes* females collected in hexane.

No.	Compound*	Ret. Time (min)	Amount (ng)
1	2,4-Dimethyl-1-heptene	7.549	0.05 \pm 0.03
2	5-Hydroxy-4-octanone	8.334	0.18 \pm 0.06
3	Tetradecane	18.823	0.02 \pm 0.01
4	Heneicosane	26.569	0.03 \pm 0.01
5	1-Decanol, 2-hexyl-	27.390	0.05 \pm 0.02
6	Tetrapentacontane	29.632	0.05 \pm 0.01

*Confirmation of the compounds is still necessary with authentic standards

Figures

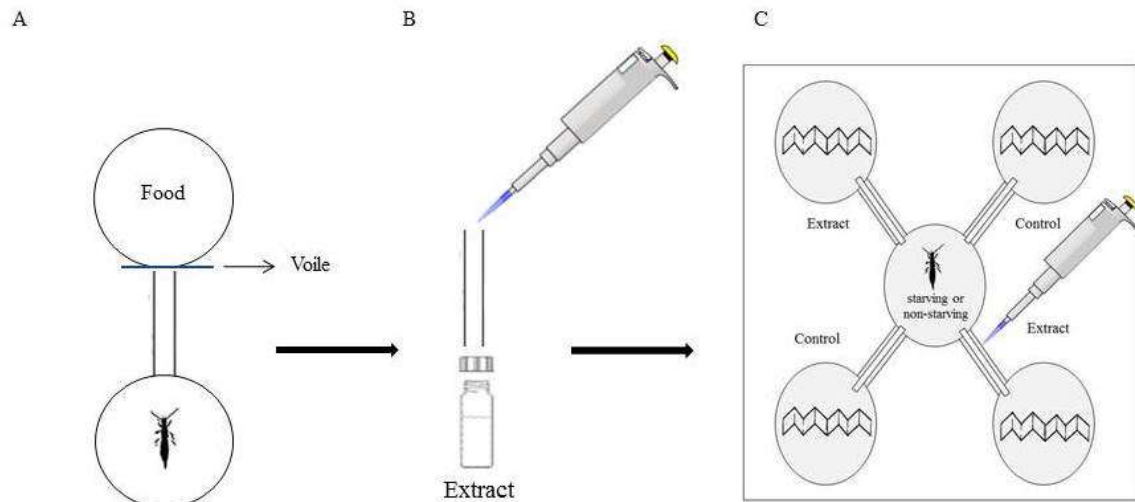


Figure 1. Methodology to study trail-following behavior in *Doru luteipes*. A. Walking arena made by a glass tube and petri dishes. B. Collection of scent-trails extracts by washing glass tube with hexane (2ml). C. Multiple choice arena to do the bioassays. Voile: used as a barrier to prevent contact of earwigs with food resource.

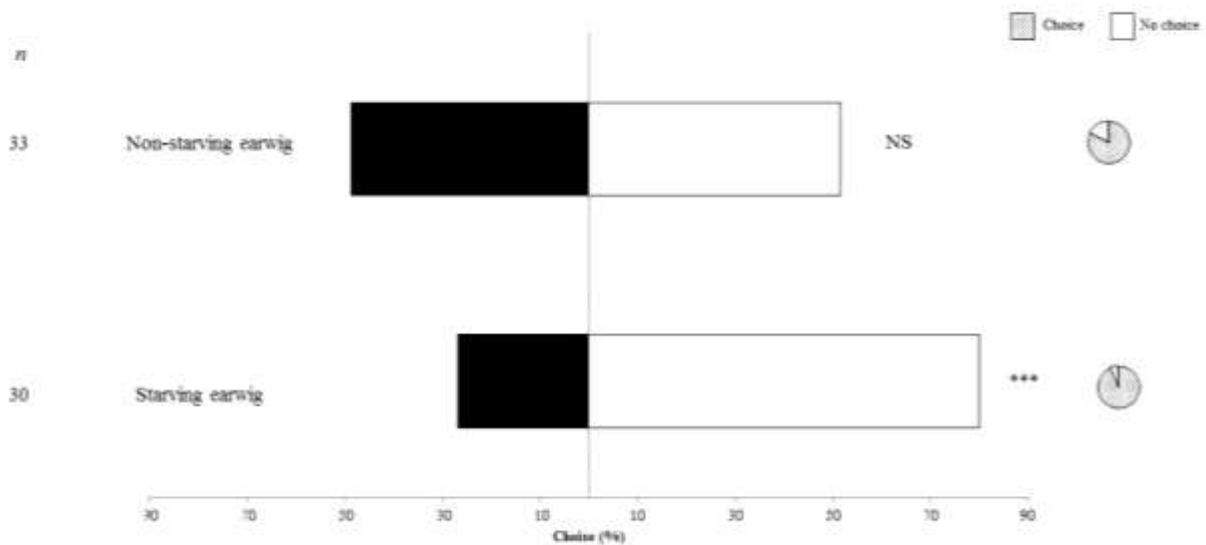


Figure 2. Responses of non-starving and starving earwigs *Doru luteipes* females to scent-trails pheromones or hexane (control). Pie charts in right side show proportions of responsive (choice) and non-responsive (no choice) earwigs in the assay. Asterisks indicate significant difference between treatments according to chi-square test ($*P \leq 0.05$; $**P \leq 0.01$; $***P \leq 0.001$) and NS no significant differences.

3 GENERALIST PREDATORY EARWIGS (*Doru luteipes* SCUDDER) USE NOCTURNAL HERBIVORE-INDUCED MAIZE VOLATILES TO FORAGING

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Abstract

Over the last few decades, numerous studies have demonstrated that entomophagous arthropods use herbivore-induced plant volatiles (HIPVs) in search for their prey/host. However, so far, no study has focused on the response of nocturnal predators to volatile blends emitted by prey-damaged plants. Here, we investigated the olfactory behavior of the night-active and generalist predatory earwig *Doru luteipes* Scudder (Dermaptera: Forficulidae) to diurnal and nocturnal volatile blends emitted by maize plants under attack of either a stemborer (*Diatraea saccharalis*) or leaf chewing caterpillar (*Spodoptera frugiperda*), both suitable lepidopteran preys. Additionally, we examined whether the earwig preferred odors emitted from early- or old-damaged maize. We first determined the earwig diel foraging rhythm and confirmed that *D. luteipes* is a nocturnal predator. Olfactometer assays showed that, although earwigs were actively walking, they did not

discriminate volatiles of undamaged maize from herbivore-damaged maize plants during the day. In contrast, at night, earwigs exhibited preference to volatiles emitted by maize plants under attack of *D. saccharalis* or *S. frugiperda* over undamaged plants, and early- over old-damaged maize. Our GC-MS analysis revealed that early-damaged nocturnal plant volatile blends mainly comprised of fatty acid derivatives, while old-damaged plant volatile blend mostly contained terpenoids. This pattern was similar comparing the blends emitted by maize damage by either of the caterpillars. Our results showed that *D. luteipes* innately uses nocturnal herbivore-induced plant volatiles in search for preys. Moreover, attraction of the earwig to early-damaged plant is likely mediated by fatty acid derivatives

Keywords: *Diatraea saccharalis*. Maize. Night-active predator. Plant induced defenses. Tritrophic interaction. *Spodoptera frugiperda*.

Introduction

It is widely recognized that damage by arthropod herbivores induces different plant metabolic processes, resulting on the emission of herbivore-induced plant volatiles (HIPVs) (Turlings and Tumlinson 1992; Karban and Baldwin 1997; Pare and Tumlinson 1997a; Turlings et al. 2000; Kessler and Baldwin 2002; Dicke and Hilker 2003; Rodriguez-Saona et al. 2011). They play important ecological roles by mediating interactions with several trophic levels (Dicke et al. 1990; Dicke and Van Loon 2000; De Boer et al. 2008; Bukovinszky et al. 2012). For example, herbivore natural enemies, parasitoids and predators, use HIPVs as cues to locate their host or prey (Price et al. 1980; Dicke et al. 1990; Turlings et al. 1990; Vet and Dicke 1992; De Moraes et al. 1998; Kessler and Baldwin 2001; Rasmann et al. 2005; Dicke and Baldwin 2010; De Lange et al. 2016). As HIPV blend varies depending on the herbivore identity and developmental stage, natural enemies have evolved to exploit information about the host/prey suitability based on HIPVs (Takayashi et al. 2006; De Moraes et al. 1998; De Boer et al. 2008; Tamiru et al. 2011; McCormick et al. 2012).

HIPV blends usually comprise terpenoids, phenylpropanoids/benzenoids, and fatty acid derivatives (commonly known as green leaf volatiles – GLVs) (Dudareva et al. 2006). In general, herbivore-damaged plants show a temporal dynamic of volatile emission (Turlings et al. 1998b), being GLVs (6-carbon aldehydes, alcohols, and

acetates) the first volatiles to be released and terpenoids thereafter (Turlings et al. 1998a; D'auria et al. 2007). Both GLVs and terpenoids have been shown to be key-compounds on the recruitment of parasitic wasps or predators to host/prey- damaged plants (Weissbecker et al. 1999; Hoballa and Turlings 2005; Maeda et al. 2015). GLVs are common among plant species and provide fast, but nonspecific information about host/prey location for natural enemies, while terpenoids are present in later blends and are more specific of the herbivore identity (Hoballah and Turlings 2005; Allmann and Baldwin 2010). Therefore, it is expected that generalist natural enemies respond to earlier HIPV blends, in which GLVs are predominant, and specialists later blends, mainly comprised of terpenoids (De Boer et al. 2008; Peñaflor et al. 2011).

Plants respond differently to herbivory depending on the time of the day the damage was inflicted (reviewed by Greenham and McClung 2015). For example, nocturnal herbivory triggers higher levels of jasmonic acid in lima bean plants than diurnal herbivory, reflecting in a different composition of the HIPV blend (Arimura et al. 2008). Moreover, diurnal and nocturnal plant volatile emissions are also different because the synthesis of some HIPVs are photosynthesis-dependent (Paré and Tumlinson 1997b). These differences on the blend can affect plant-insect, such as host plant selection by herbivores (De Moraes et al. 2001; Chamberlain et al. 2006) and parasitoids attraction (Turlings et al. 1995; Shiojiri et al. 2006; Signoretti et al. 2012).

So far, the few studies that have examined prey-searching behavior of predators guided by nocturnal HIPVs tested day-active species (Maeda et al. 2000; Signoretti et al. 2012). We expect that night-active are responsive to HIPVs released during the night either because they forage at night or they are only attracted to nocturnal HIPV blends.

The order Dermaptera includes several generalist predators with nocturnal habits (Lamb and Wellington 1975). For example, *Doru taeniatum* Dohrn and *Doru lineare* Eschs. (Dermaptera: Forficulidae), *Labidura riparia* (Pallas) (Dermaptera: Labiduridae), *Euborellia annulipes* (Lucas) (Dermaptera: Anisolabididae), and the European earwig *Forficula auricularia* Lin. (Dermaptera: Forficulidae), have been reported as important predators of stem borers (Haile and Hofsvang 2001), leaf-chewing caterpillars (Jones et al. 1988; Romero-Sueldo and Virla 2008) as well as garden and orchard pests, such as aphids, scales and mites (Evans and Longépé 1996; He and Xu 2008). It is common to find the predatory earwig *Doru luteipes*

Scudder (Dermaptera: Forficulidae) in maize plantations in the Americas (Reis et al. 1988; Romero-Sueldo and Virla 2008). This species has shown to be an important biological control agent of lepidopterans (eggs and caterpillars), such as the fall armyworm, *Spodoptera frugiperda* (J.E. Smith) (Lepidoptera: Noctuidae) (Reis et al. 1988), sugarcane borer *Diatraea saccharalis* (Fabricius) (Lepidoptera: Crambidae), maize earworm *Helicoverpa zea* Boddie (Lepidoptera: Noctuidae) (Cruz et al. 1995), *Ascia monustes* orseis (Godart) (Lepidoptera: Pieridae) (Picanço et al. 2003), and aphids (Alvarega et al. 1996). Despite the importance of dermapterans in agroecosystems, to the best of our knowledge, there is no study reporting their searching behavior guided by plant volatiles. Within this context, we studied the innate behavior of the earwig *D. luteipes*, a generalist and night-active predator, to maize plant volatiles under attack of two lepidopterans that usually consume different plant organs, *S. frugiperda* (leaf chewing) and *D. saccharalis* (stem borer), both considered important pests. *Spodoptera frugiperda* is the major pest in maize of the Americas (Luginbill 1928; Molina-Ochoa et al. 2003), and *D. saccharalis* occurs also in maize of the mid-southern United States, the Caribbean, Central America and the warmer areas of South America to Argentina (Capinera 2001).

We first confirmed that the earwig *D. luteipes* is a night-active predator by observing its diel foraging rhythm. Then, we addressed the following questions: (i) does the predatory earwig use HIPVs to find its preys?; (ii) does the predatory earwig prefer HIPVs emitted at early or late time interval (early- or old- damaged plants)?; (iii) is the predatory earwig attracted to HIPVs released at day and/or night? As a generalist predator, we expected that *D. luteipes*: (i) is able to recognize HIPV blend released by maize damaged; (ii) earwigs would be guided by the early HIPV by either of the prey species as this blend mainly comprises GLVs and; (iii) as a night-active predator, we also expected that the earwig would be attracted to HIPVs mainly during the night. To answer these questions, we conducted a series of olfactometer assays during the photo- and scotophase. As earwigs responded to plant volatiles only at night, we chemically characterized the volatile profiles of the night treatments. Our results help to understand the role of HIPVs on the foraging behavior of night-active and generalist predators, such as the predatory earwig *D. luteipes*.

Materials and methods

Plants and Insects

Commercial hybrid maize seeds (*Zea mays* L., var. Delprim; Delley Semences et Plants SA, Delley, Switzerland) were sown in Basiplant® potting soil (250 ml) with no additional fertilization, and maintained in an insect-free greenhouse under natural light and temperature (March-June 2015, Piracicaba, SP, Brazil). Plants used in experiments were eight-to-ten day old and had three fully expanded leaves.

All insects used in the experiments were obtained from laboratory rearings kept under controlled conditions ($25 \pm 3^{\circ}\text{C}$, $65 \pm 5\%$ RH, 12 L:12 D). Rearings of the lepidopterans *S. frugiperda* and *D. saccharalis* were maintained using artificial diets as described in Parra (2001).

Founding population (≈ 50 individuals) of *D. luteipes* laboratory rearing was collected in maize plantations in Piracicaba-SP, Brazil. We tried to simulate *D. luteipes* natural habitat (they live between leaf axils of grasses, a dark and humid environment that meets their thigmotropic habits) in the laboratory. Our method was adapted from the ones described in Pasini et al. (2007), Cruz (2009) and Butnariu et al. (2013). Briefly, we kept insects in closed plastic boxes (23 cm x 7 cm x 14 cm) covered with aluminum foil to reduce light incidence. Inside these boxes, we distributed pieces of straws partially fulfilled with wet cotton, which served as oviposition substrate and refuge, and pieces of paper folded in W shape, to avoid high incidence of cannibalism. Earwigs were fed on cat food. Cotton was watered and food renewed twice a week. Eggs laid on the wet cotton inside straws, together with the female, were individually placed in Petri dishes (14 cm diameter). Two-day-old nymphs were transferred to new boxes.

Earwig diel rhythm

To determine the appropriate time interval to study *D. luteipes* foraging activity, we observed its prey consumption along the day and night. Females starved for 48 h were individually enclosed in Petri dishes (14 cm in diameter), where five third-instar *S. frugiperda* caterpillars were offered hourly. The number of consumed caterpillars was recorded every hour during 12h for day or night (day: 07:00 AM - 06:00 PM, or night: 07:00 PM - 06:00 AM). A total of 30 females per period were

observed. The experiment were conducted in acclimatized room ($25 \pm 1^{\circ}\text{C}$, $70 \pm 10\%$ RH).

Treatments

One day prior to experiments, plants were transferred from greenhouse to laboratory, where they were exposed to supplementary lighting (60-80 μmol , 12 L: 12 D). A single third-instar caterpillar of *S. frugiperda* or *D. saccharalis* starved for 24 h was used to inflict herbivory in maize plants. The sugarcane borer was enclosed in a cage attached to the basis of the maize stem, while the fall armyworm was placed on the leaves. Then, both caterpillar-infested and uninfested maize plants were covered with voile bags (22 x 30 cm) for 1-3h (named early damaged plants), or 6-8h (old damaged plants). These treatments were performed in the photophase (diurnal herbivory) or scotophase (nocturnal herbivory). Therefore, we obtained the following treatments for diurnal and nocturnal herbivory: (i) early damaged plant with *S. frugiperda* (ED-SF); (ii) old damaged plant with *S. frugiperda* (OD-SF); (iii) early damaged plant with *D. saccharalis* (ED-DS); (iv) old damaged plant with *D. saccharalis* (OD-DS); and (v) undamaged plants (UP).

Earwig olfactory behavior

The olfactory response adult females, (up-to-two day old after adult emergence) starved for 48h, was evaluated in a glass Y-tube olfactometer. The olfactometer was designed to have long arms and narrow diameter (main arm: 25 cm long, side arms: 20 cm long, 0.9 cm internal diameter) as earwigs are motivated to move forward by the contact (thigmotropism). The olfactometer system was connected to an ARS Volatile Collection System (Analytical Research Systems, Gainesville, FL, USA), which contained a charcoal filter, humidifier and flow meters. Clean air was pushed through tubes into two glass chambers (10 cm diameter x 5 cm height), each containing a single plant, and then into the olfactometer side arms. Air flow was adjusted to 0.8 L/min/arm. Assays were conducted in the laboratory under controlled conditions ($25 \pm 1^{\circ}\text{C}$, $70 \pm 10\%$ RH) at day (08:00 _{AM} - 02:00 _{PM}) or night (06:00 _{PM} - 12:00 _{AM}). Insects were individually introduced into the olfactometer central arm and observed for 5 min. Earwigs were considered to have made a choice when they crossed the threshold line (located in the middle of each arm). Only insects that made a choice for one arm within 5 min were considered for statistical

analyses. Insects were tested only once. After each trial, olfactometer was rotated and the sides were inverted to avoid side bias. According to our observations (unpublished data), *D. luteipes* females deposit trail pheromone when they are foraging. Therefore, after every replicate, the olfactometer was washed with acetone and hexane (v/v 90%), and dried at 160 °C for 2 min. Every five insects, a new pair of plants was tested. We tested at least 46 earwigs for each assay.

Plant volatile collection and chemical analyses

As earwigs only responded to maize volatiles at night, we only collected and analyzed the volatiles of the nocturnal treatments. Six plants per treatment were individually placed into glass chambers (10 cm diameter x 5 cm height) connected to the ARS Volatile Collection System. Clean humidified air was injected into the chambers through PTFE tubes at 1 L/min/chamber. A column with 30 mg of the adsorbent polymer HayeSep™ (Supelco, Bellefonte, PA, USA) was connected to an air outlet of each chamber. Vacuum pump pulled air through the polymer columns for 2 h (from 10:00 PM to 12:00 AM) at around 1.0 L/min. Thereafter, filters were eluted with 150 µL µl dichloromethane (Merck, Kenilworth, NJ, USA) mixed with 10 µl of nonyl acetate (internal standard solution at 100 ng/µL). Extracts were stored at -80°C until analyses. A 2-µl aliquot of each extract was injected in splitless into a gas chromatograph coupled to mass spectrometer (GC-MS, Varian 4000) equipped with HP5-MS capillary column (JeW Scientific, Folsom, CA; 30 m; 0.25 mm; 0.25 µm), using helium as the carrier gas. The column temperature was held at 40°C for 5 min, increased to 150°C (5°C /min) and maintained for 1 min, and then raised (5 °C /min) until reaching 250 °C. Relative quantification was estimated based on the peak area relative to the internal standard, and corrected by plant dry weight. Compounds were identified by comparing their mass spectra with those of the NIST08 library and by calculating Kovats index (KI).

Statistical analyses

Normality and homogeneity of the relative amounts of volatiles were tested by Shapiro-Wilk and Levene tests ($P < 0.05$). We used the logistic regression and Wald's *Chi-square* test for analyzing the earwig choice in olfactometer assays. Plant volatile composition were transformed [$\log (x + 0.5)$] to analyzed by multivariate analysis of variance (MANOVA). In addition, we also performed a principal

component analysis (PCA). The quantifications of individual volatiles were analyzed by analysis of variance (One-Way ANOVA) followed by Tukey's HSD ($P < 0.05$). Statistical analyses were performed using software package R 3.0.2 (www.r-project.org).

Results

Earwig diel rhythm

Earwigs only fed on fall armyworm caterpillars during the scotophase (Fig. 1). The peak of prey consumption occurred during the first three hours of the scotophase (07:00 PM - 9:00 PM). Even though earwigs were starved for 48 h prior to diurnal assays, they did not consume any fall armyworm caterpillar (data not shown).

Olfactory behavior

Doru luteipes adult females were responsive in olfactometer tests conducted during the photophase, however, they did not show any preference to the treatments (Fig. 2; $P > 0.05$). By contrast, earwigs showed preference to the treatments in the assays performed in the scotophase. First, they oriented preferentially to odors of undamaged plant (UP) over clear air (CA) ($\chi^2 = 13.25$, $P < 0.001$). Nonetheless, earwigs preferred early- or old-damaged plant with either *S. frugiperda* or *D. saccharalis* over UP (ED-SF: $\chi^2 = 17.11$, $P < 0.001$; OD-SF: $\chi^2 = 30.47$, $P < 0.001$; ED-DS: $\chi^2 = 5.82$, $P = 0.0160$; OD-DS: $\chi^2 = 13.25$, $P < 0.001$). Earwigs exposed to early- and old-damaged plants (*S. frugiperda* or *D. saccharalis*) preferred early-damaged plants (ED-SF vs. OD-SF: $\chi^2 = 5.82$, $P = 0.016$; ED-DS vs. OD-DS: $\chi^2 = 11.36$, $P < 0.001$).

Plant volatiles and chemical analyses

A total of 10 compounds was detected in the blend emitted by maize plants (Table 1). Multivariate analysis of volatile emissions showed that the composition significantly differed among the treatments (MANOVA, Wilk's $\lambda = < 0.001$; $F_{(4,25)} = 28.684$; $P < 0.001$). Analysis of individual compounds revealed that the concentrations of all volatiles differed among treatments (One-Way ANOVA, $P < 0.05$).

Composition analysis of plant volatile emissions using PCA showed separation of the early-damaged plant treatments from the old-damaged plant treatments (damage either by *S. frugiperda* or *D. saccharalis*) along the PC 1 (57% of the variation) (Fig. 3). Aggregation of the early-damaged plant treatments was influenced by the presence of GLVs in the blend, while old-damaged plant treatments by indole and sesquiterpenes. PC 2, which explained 19.2% of the variation, separated plant volatile emissions released by maize plants damaged by *S. frugiperda* from those damaged by *D. saccharalis* (both early- and old-damaged maize blends). Thus, separation was mainly influenced by the induction time (early vs. old) and, to a lesser extent, by herbivore species.

GLVs and linalool were predominant in early-damaged plant volatile blends, but they were at much lower concentrations in old damaged plant volatile blends, which mostly comprised of sesquiterpenes (Online Resource 1).

Discussion

To the best of our knowledge, the role of HIPVs on the foraging behavior of predators has only been studied on day-active predatory arthropods, such as predatory mites (Dicke et al. 1990; De Boer et al. 2008) mirids (Drukker et al. 2000; Moayeyri et al. 2007), pentatomids (Dickens 1999; Weissbecker et al. 1999; Van Loon et al. 2000), anthocorids (Drukker 1995; Scutareanu et al. 1997; Ardanuy 2016) and coccinellids (Le Rü and Makaya Makosso 2001; Ninkovic et al. 2001; Maeda et al. 2015; Wang et al. 2015). Our study has focused on a predatory dermapteran, the earwig *D. luteipes* that, different from those, exhibits a night-active foraging behavior, as we confirmed in our experiments.

The literature was unclear about the diel foraging rhythm of *D. luteipes*, especially because this earwig is seen in the field during the day. Although a previous study has reported that *D. luteipes* exhibit a cryptic behavior, remaining hidden during the day and is active at night (Lamb and Wellington 1975), a more recent work suggested that this earwig is active during the day (Romero-Sueldo and Virla 2009). Our results allowed us to confirm that *D. luteipes* foraging behavior occurred exclusively in the night. Interestingly, Y-tube olfactometer assays did not show that *D. luteipes* is inactive during the day, but it walks randomly.

As predicted, our olfactometry assays confirmed that (i) the earwig *D. luteipes* was attracted to HIPV blends triggered by herbivory of either preys, *S. frugiperda* or *D. saccharalis*; (ii) the earwig preferred early-damaged over old-damaged maize volatile blends; (iii) the earwig responded to plant volatiles only at night. As a generalist and night-active predator, these innate behavioral strategies of *D. luteipes* to HIPVs are likely adaptive to find suitable preys, as we explain below.

According to our PCA, we detected different volatile compositions of early- and old-damaged maize plants, but a similar composition comparing damage by either of the herbivores. In general, we observed that early-damaged plant volatile blends in the night mainly comprised of fatty acid derivatives (or green-leaf volatiles, GLVs), while old-damaged plant volatile blend mostly contained terpenoids, in a similar fashion as reported for the day-light emissions of the same maize hybrid (Turlings et al. 1998a; Peñaflores et al. 2011). Both plant volatile blends were attractive to the earwig, but females preferred early- over old-damaged plant volatiles. The strong attraction of the earwig to early-damaged plant is likely mediated by the mixture of GLVs or individual compound(s). Similarly, preferential orientation of the earwig to old-damaged over undamaged plant volatile blend may be guided by GLVs, which were present in the blend, but at lower concentrations than early-damaged plant volatile blend. Alternatively, another biologically active terpenoid in the old-damaged plant volatile blend was responsible for the earwig attraction.

Orientation of generalist natural enemies to GLVs seems to be a common strategy for host/prey searching, as it has been previously reported for day-active predators (Maeda et al. 2015), larval (Hoballah and Turlings 2005; Shiojiri et al. 2006) and egg parasitoids (Peñaflores et al. 2011). For example, James (2003) observed that the predatory mirid *Deraeocoris brevis* (Uhler), the anthocorid *Orius tristicolor* (White), and the lady beetle *Stethorus punctum picipes* (Casey) were attracted to cards baited with (*Z*)-3-hexenyl acetate in hop yards.

The attraction of *D. luteipes* to GLVs possibly increases the chances of the earwig finding suitable preys as damage by caterpillars and aphids in crop plants elicit emission of GLVs (De Moraes et al. 1998; Ninkovic et al. 2001). At the same time, we expect that this strategy may also lead earwigs to orient to plants under attack of non-prey insects (e.g., adult beetles), unless they also use other more reliable chemical cues derived from the prey. In the case of caterpillars and aphids, these chemicals could be derived from frass and honeydew, which are known as

attractants to natural enemies (Reddy et al. 2002; Rogers and Potter 2002). Notwithstanding, prey-derived odors are not usually detected by natural enemies at long distances as plant volatiles (Shu and Jones 1989; Vet et al. 1990; Vet and Dicke 1992), and combination of both odors would be necessary for the earwig to find preys.

The orientation to nocturnal maize volatile blends by *D. luteipes* may have evolved from the feeding diel rhythm of its preferred prey, *S. frugiperda* (Reis et al. 1988), which feeds more actively during the night, under mild temperatures (Sparks 1979). However, we cannot discard the fact that most natural enemies forage during the day (Hassell and Southwood 1978) creates a competition-free space for nocturnal predators, and may also have shaped the earwig foraging strategies.

Although we did not chemically characterized diurnal plant volatile emissions in our study, plants, in general, release higher total amounts of volatiles during the day than night (Loughrin et al. 1995; Maeda et al. 2000; Gouinguené and Turlings 2002; Arimura et al. 2008; Seidl-Adams et al. 2015), as part of the synthesis is light-dependent (Paré and Tumlinson 1997a). Moreover, darkness seems to attenuate herbivore-induced plant responses (Morker and Roberts 2011). Despite of that, earwigs did not show an odor-oriented behavior in the photophase, in contrast to the scotophase, when earwigs preferred undamaged plant volatiles over clean air, and herbivore-damaged over undamaged plant volatiles. Therefore, our data suggest that the lack of olfactory preference of the earwig in diurnal assays is not because of the composition of diurnal plant volatile blends, but the earwig diel foraging rhythm. Moreover, we speculate that, as a night-active predator, *D. luteipes* is able to detect low amounts of plant volatiles, such as nocturnal emissions, allowing them to efficiently locate their in the dark, when visual cues are absent.

Our work demonstrated that the predatory dermapteran and night-active predator *D. luteipes* innately uses nocturnal herbivore-induced plant volatiles in search for preys. The fact that the earwig does not respond to diurnal plant volatiles needs to be further investigated at the physiological and molecular levels. As the earwig - *S. frugiperda* or *D. saccharalis* represents an important system in South American maize crops, future studies on the key-compounds responsible for the attraction of the earwig can help developing strategies to enhance prey searching of this biological control agent.

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Tables

Table 1 Relative amounts of nocturnal volatile emissions (mean \pm SE ng.g⁻¹ of dry weight) released by undamaged maize and maize damaged with *Spodoptera frugiperda* or *Diatraea saccharalis* at 1-3h (early-damaged) or 6-8h (old-damaged)

Compounds	<i>S. frugiperda</i>			<i>D. saccharalis</i>		Statistical Significance ^a
	Undamaged Plants	Early	Old	Early	Old	
		Damage (1-3h)	Damage (6-8h)	Damage (1-3h)	Damage (6-8h)	
<i>Fatty acid derivates</i>						
(<i>E</i>)-3-hexenal	-	0.045±0.005	0.009±0.002	0.030±0.001	0.001±0.0008	<0.0001
(<i>E</i>)-2-hexenal	-	0.050±0.004	0.011±0.006	0.056±0.004	0.011±0.008	<0.0001
(<i>Z</i>)-3-hexen-1-ol	-	0.051±0.040	0.003±0.001	0.063±0.006	0.015±0.0015	<0.0001
(<i>Z</i>)-3-hexenyl acetate	-	0.349±0.030	0.072±0.013	0.147±0.008	0.006±0.004	<0.0001
Decanal	0.625±0.331	0.030±0.024	0.156±0.012	0.048±0.006	0.153±0.098	<0.0001
Total	0.625±0.331	0.525±0.021	0.255±0.007	0.344±0.005	0.186±0.022	
<i>Benzenoids</i>						
Indole	-	-	0.247±0.088	-	0.031±0.013	<0.0001
Total	-	-	0.247±0.088	-	0.031±0.013	
<i>Terpenes</i>						
Linalool	0.042±0.008	0.366±0.047	0.273±0.040	0.039±0.003	0.062±0.014	<0.0001
(<i>E</i>)-β-caryophyllene	-	-	0.391±0.046	-	0.123±0.040	<0.0001
(<i>E</i>)-α-bergamotene	-	-	0.089±0.028	-	0.100±0.019	<0.0001
(<i>E</i>)-β-farnesene	-	-	0.009±0.002	-	0.015±0.007	0.0090
Total	0.042±0.008	0.366±0.047	0.761±0.030	0.039±0.003	0.3±0.0200	

^aSignificant difference between treatments according to One-Way ANOVA followed by Tukey's HSD ($P < 0.05$).

Figures

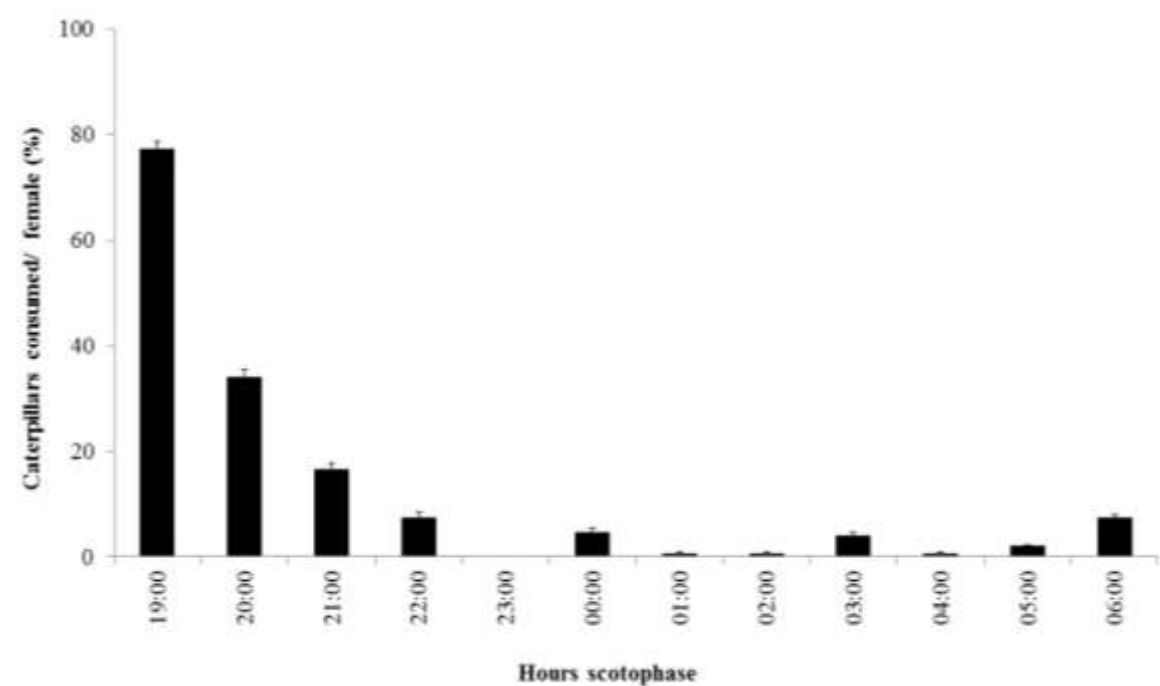


Fig. 1 Foraging activity of the earwig *Doru luteipes* preying *Spodoptera frugiperda* caterpillars in the scotophase.

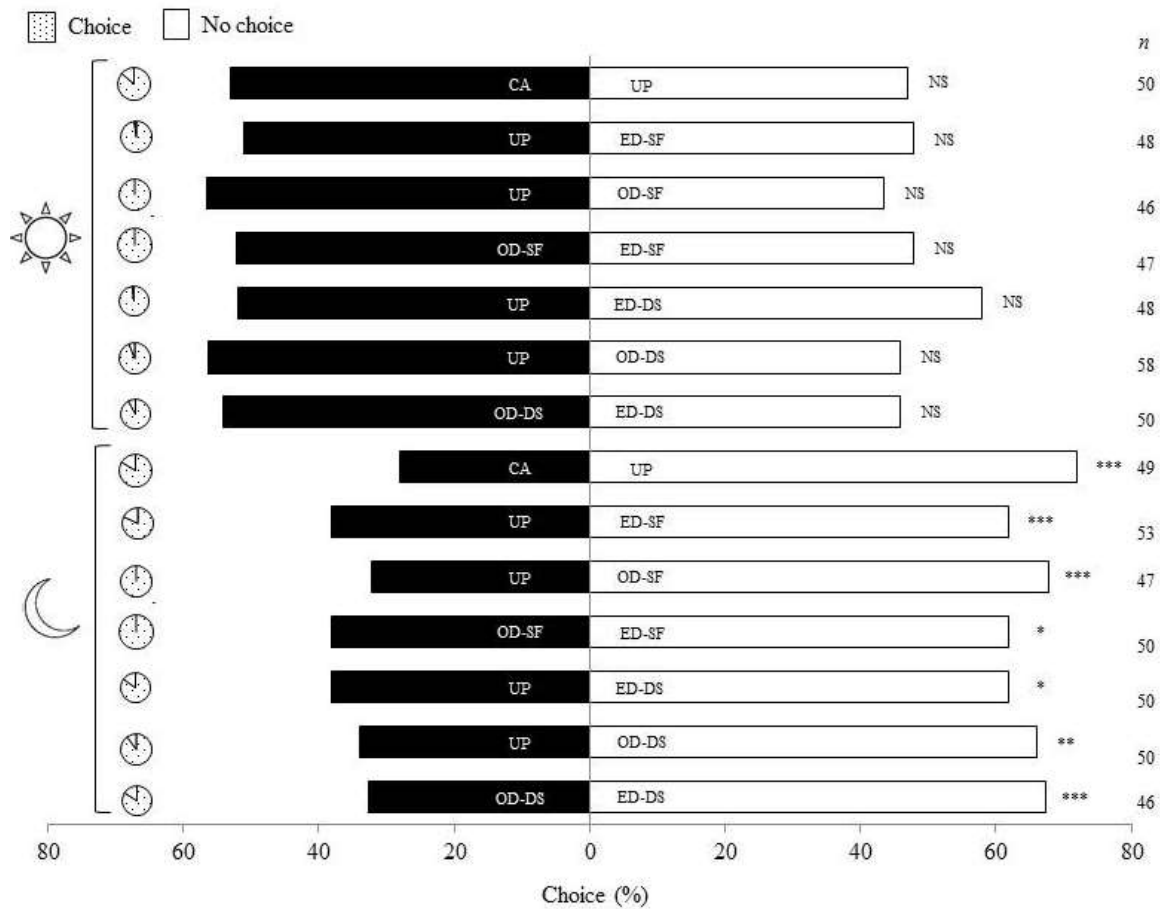


Fig. 2 Olfactory preference of *Doru luteipes* females to plants volatiles induced by herbivory of leaf-chewing and steam-borer caterpillar during photo- and scotophase. Early damaged plant with *S. frugiperda* (ED-SF); old damaged plant with *S. frugiperda* (OD-SF); early damaged plant with *D. saccharalis* (ED-DS); old damaged plant with *D. saccharalis* (OD-DS); undamaged plants (UP); and clean air (CA). Pie charts on right represent non-responsive (no choice) and responsive (choice) earwigs. Asterisks indicate a significant difference between treatments according to the chi-square test (* $P \leq 0.05$, ** $P \leq 0.01$, *** $P \leq 0.001$).

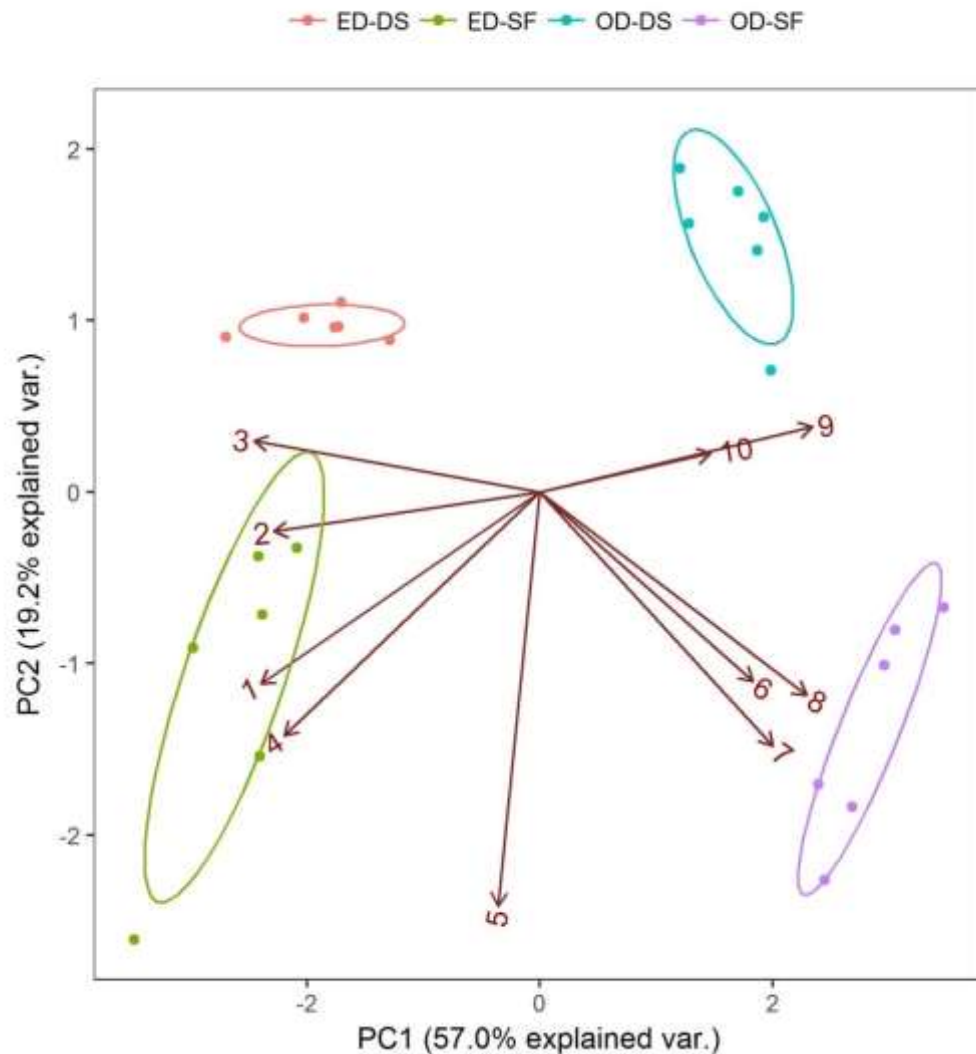


Fig. 3 Score plot for Principal Component Analysis (PCA) for volatile composition emitted by undamaged maize (UP), maize infested with chewing-leaf *Spodoptera frugiperda* or steam borer (*Diatraea saccharalis*) caterpillars at 1-3h (ED-SF and ED-DS) and 6-8h (OD-SF and OD-DS). Volatiles (x1) (*E*-3-hexenal, (x2) (*E*-2-hexenal, (x3) (*Z*-3-hexen-1-ol, (x4) (*Z*-3-hexenyl acetate, (x5) linalool, (x6) Decanal, (x7) indole, (x8) (*E*- β -caryophyllene, (x9) (*E*- α -bergamotene, and (x10) (*E*- β -farnesene).

Online Resource 1 – Naranjo et al.

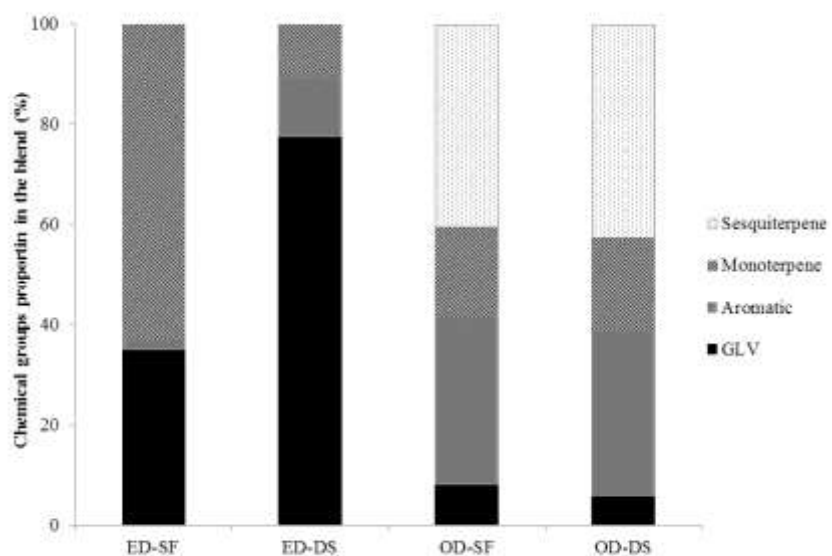


Fig. S1 Proportions of the volatile chemical groups in the blends nocturnally released by maize induced with *Spodoptera frugiperda* (SF) or *Diatraea saccharalis* (DS) at 1-3 h (early damage, ED) or 6-8 h (old damage, OD).

4 LIFE-HISTORY EVOLUTION OF *Zea* spp. DETERMINE THE ECOLOGICAL CONTEXT OF PLANTS DEFENSE STRATEGIES IN TROPHIC INTERACTIONS

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Abstract

Plants have developed strategies to protect themselves against herbivores by direct defences (production of toxins and digestibility reducers) and by indirect defenses (herbivore induced plant volatiles-HIPVs- that attract natural enemies). However, domestication of plants for desired traits such as increased yield has result in weakening or losses of these plant defenses. We study how life-history of *Zea* plants affects trophic interactions through plants defense. We chose three species: perennial teosinte, annual teosinte and modern maize cultivar that represent a gradient evolution in the *Zea* genus. An herbivorous *Spodoptera frugiperda* (J.E. Smith) (Lepidoptera: Noctuidae) and a predator, *Doru luteipes* Scudder (Dermaptera: Forficulidae) were included. We assessed: (i) consumption and food utilization by *S. frugiperda* caterpillars feed with leaf sections of plants, (ii) nocturnal production of HIPVs, and (iii) olfactive behavior of earwigs *D. luteipes* towards plants induced by herbivory. Caterpillars perform better on modern maize than in teosinte species. Maize fed caterpillars survived more time and only these ones reached a pupal stage. Maize leaves were more suitable food and requires low metabolic costs than teosintes leaves, especially compared with perennial teosinte. Teosinte plants

showed a predominance of GLV and the sesquiterpenes (*E*)- β -caryophyllene and (*E*)- β -farnesene were higher in maize. Earwigs showed a significant preference for the odours of teosintes over the odours of maize plants. Life plant histories carry different defense strategies that affect how the plants interact with their environments, including insect herbivores and predators. Understanding plant evolution could optimize crop breeding and biological control strategies.

Keywords: Earwigs. Food utilization. HIPV. Maize. Olfactory behavior. Teosinte

4.1 Introduction

In natural environments, plants are exposed to many attacks, including those by herbivorous arthropods. To defend themselves, plants have developed complex mechanisms such as constitutive and induced defenses. Constitutively, plants can affect herbivores from morphological alterations like lignified cell walls, trichomes and callose deposits (Karban and Baldwin 1997) or by the synthesis of secondary metabolites (Wittstock and Gershenzon 2002). Plants can induce both direct and indirect defenses in response to herbivore attacks. Directly, plants produce toxins or digestion inhibitors. Indirectly, plants use herbivore induced plant volatiles (HIPVs) to attract natural enemies of herbivores i.e., predators or parasitoids, which used these chemical cues to search for prey or hosts (Turlings et al. 1990; De Moraes et al. 1998, 2001; Dicke and van Loon 2000; Kessler and Baldwin 2001).

Domestication of wild plants for agriculture, however, alters these defense strategies by intensely selecting for traits, such as rapid growth and high yield. Selective breeding causes a genetic bottleneck and reduction on genetic diversity, which result in weakening or losses of important traits, such as defenses against herbivores (Doebley 2004; Rosenthal and Dirzo 1997). On the other hand, toxins that protect against herbivory may have actively been selected against to increase palatability (Heaney et al. 1987; Paris 1989). The assumption of weaker defenses in crops are based on the resource allocation hypothesis, which predicts a trade-off between resource allocation to competing functions, such as productivity and defense (Bernal et al. 2015). An increasing number of studies have shown that wild plants are better defended against herbivores compared with corresponding crops including, cranberry (Rodriguez-Saona et al. 2011), cotton (Hagenbuche et al. 2016),

Brassica and *Phaseolus* (Benrey et al. 1998), tomato (Welter and Steggall 1993), sunflower (Chen and Welter 2005) and maize (Rosenthal and Dirzo 1997; Szczepaniec et al. 2012; Dávila-Flores et al. 2013; Mutyambai et al. 2015). Other studies have suggested that plant domestication can influence tritrophic interactions in various ways (Benrey et al. 1998; Chen et al. 2015).

Maize (*Zea mays* ssp. *mays*) is native to Mexico and was domesticated 9 000 years ago, from Balsas teosinte (*Zea mays* ssp. *parviglumis* Iltis & Doebley) and subsequently spread throughout the Americas and worldwide (Doebley 2004). Although morphologically very distinct from maize, introgression between teosinte plants and maize is a common practice among Mexican farmers in order to improve the crop's germplasm (Takahashi et al. 2012). The term teosinte is of Nahuátl Indian name that commonly referred to all taxa within the genus *Zea*, except for maize (Doebley 2004).

Zea genus includes eight taxa classified into two sections and five species (Doebley and Iltis 1980). To develop this study, we chose three species based on of ecological and evolutionary comparability. The group comprises two wild teosintes and a modern maize (*Z. mays* ssp. *mays*, variety Delprim). The wild teosintes include *Z. diploperennis* Iltis, Doebley & Guzmán, a perennial weed, probably the most primitive species in the genus (Doebley and Iltis 1980) and *Z. mays* ssp. *mexicana* (Schrader) Iltis, a wild annual subspecies of *Z. mays*. This species represents a gradient in life-history evolution of *Zea* genus (from perennial to annual life cycle, and finally the evolution to modern cultivar) that could contribute to knowledge about how the effect on defense strategies through domestication influences the tritrophic interactions. This supposition is supported by prior studies. Rosenthal and Dirzo (1997) observed that wild perennial teosinte grew the slowest, had the lowest grain production and had highest defense to herbivore attacks, while a modern cultivar grew the fastest, had the highest grain yield and showed the highest attack levels. A wild annual (Balsa teosinte) and a land-race cultivar were intermediate. Dávila-Flores et al. (2013) observed that the performance of maize leafhopper (*Dalbulus maidis* DeLong & Wolcott) was poorest on perennial teosinte, intermediate on Balsa teosinte and landrace maize, and best on hybrid maize.

In the current study, we included an herbivorous *Spodoptera frugiperda* (J.E. Smith) (Lepidoptera: Noctuidae), and a predator, *Doru luteipes* Scudder (Dermaptera: Forficulidae). These insects have a long evolutionary history with *Zea*

genus and are frequently observed on both, maize and teosinte (Painter 1955; Takahashi et al. 2012). *S. frugiperda*, known as fall armyworm (FAW) stands out to be a highly polyphagous caterpillar with a strong preference for grasses. It is one of the most economically important pests in the Americas (Molina-Ochoa et al. 2003). The species is known to induce direct defences and indirect defenses in maize (Rosenthal and Welter 1995; Rosenthal and Dirzo 1997; Szczepaniec et al. 2012; Dávila-Flores et al. 2013). Regardless of the large economic importance of this herbivore in maize crops, chemical control and Bt-transgenic maize are the most common practice for FAW management (Diez-Rodriguez and Omoto 2001; Binning et al. 2014). The earwig *D. luteipes* is one of the most important generalist predators of maize pests by voraciously consuming eggs and larvae of wide range of herbivores including FAW (Reis et al. 1988; Romero-Sueldo and Virla 2009). Previously we observed that *D. luteipes* are attracted to maize plants attacked by *S. frugiperda* and *Diatraea saccharalis* (Fabricius) (Lepidoptera: Crambidae). Earwigs are nocturnal predators recognized as potential biological control agent in agricultural systems around the worldwide (Lamb and Wellington 1975; Evans and Longepe 1996; Haile and Hofsvang 2001; Alvarega et al. 1996; He et al. 2008; Romero-Sueldo et al. 2008). Despite of this, they are not used in integrated pest management (IPM) programs due to few studies conducted with these species.

Aiming to understand how life-history of *Zea* genus influence tritrophic interactions through plants defense, we asked the following questions: (i) is the consumption and food utilization by FAW caterpillars lower in maize than in teosinte plants? (ii) are there differences in the nocturnal production of HIPVs between *Zea* plants? And if so, (iii) are there differences in the olfactory behavior of earwigs towards *Zea* plants? For that, in the tritrophic system proposed (*Zea* plants-FAW-earwigs) we measured food intake and utilization by larvae, releases of HIPVs and predator attraction. Based on studies cited above we hypothesized perennial teosinte plants, would be more defended than the annual and maize plants. Determining links between plant defense strategies contributes to understanding plant evolution and allow optimize crop breeding and biological control strategies (Erb et al. 2011). The current study is the first to explore whether differences in expression of plant defenses in teosinte and maize have an ecological relevance on generalist predators.

4.2 Methodology

Plants and insects

Perennial teosinte *Z. diploperennis*, Annual teosinte *Z. mays* ssp. *mexicana* (Embrapa milho e sorgo, germplasm bank, Sete Lagoas-MG, Brazil) and commercial hybrid maize seeds (*Z. mays* L., var. Delprim; Delley Semences et Plants SA, Delley, Switzerland) were sown in Basiplant® potting soil (5L). Plants were maintained in an insect-free greenhouse (12/12h day/night cycle) and were watered daily.

All the insects were maintained under controlled laboratory conditions ($25 \pm 3^{\circ}\text{C}$, $65 \pm 5\%$ RH, photoperiod 12 L:12 D). *S. frugiperda* caterpillars were obtained from insect rearing already established in the Laboratory of Chemical Ecology and Insect Behavior (ESALQ / USP). Adults were fed a 10% honey solution and kept in PVC-cages (15 cm high) lined with bond paper to serve as a surface for oviposition. Eggs were collected daily and incubated in glass tubes (2.5 cm diameter and 8 cm high). An artificial diet was formulated using beans, wheat germ and brewer's yeast as described by Parra (2001).

Adults of *D. luteipes* were collected at field (maize crops in Piracicaba-SP, Brazil). In nature, *D. luteipes* lives between the leaf axils of the grass, because this habitat provides a safe, dark and humid environment with frequent provisioning of food. The leaf axils also providing conditions for their thigmotropic and cryptic habits, such as response to the stimulus of direct contact and remain hidden (Butnariu et al. 2013). Therefore, we simulated these conditions keeping the insects in closed transparent plastic cages (23 cm length x 7 cm wide x 14 cm height). Transparent straws containing wetted cotton, which functioned as oviposition substrate and refuge were placed in these cages. Methodology adaptations were carried out from proposed by Pasini et al. (2007) and Butnariu et al. (2013). These adaptations were: (i) the plastic cages were sealed with aluminum foil to reduce light incidence; (ii) due to high incidence of cannibalism, paper folded in a W-shape to provide shelter for the earwigs, were placed inside of the cages; and (iii) the insects were fed with a diet based on cat food (Cruz 2009). Cotton was re-wetted or changed and food renewed twice a week. The egg postures made in the straws together with the female were individually placed in petri dishes (14 cm diameter), until to two days after hatching nymphs, which were transferred to new cages.

Food intake and utilization

To evaluate the resistance of teosintes and maize plants to *S. frugiperda* herbivory we measured consume and food utilization. For that, 15 larvae were fed with the three plant species and measured weight gain, food ingested, total excretion, and larval survival. Leaf sections (third-youngest leaf, 4 x 3 cm) of V9-10 plants stage, were cut weighed on a monopan balance (± 0.01 mg) and put in petri dishes. Newly exuviated second instar caterpillar ($n = 15$) reared on artificial diet were weighing and transferred separately at the petri dishes. Every 48 hours the caterpillars were again weighed and leaf sections were replaced until their death. The weight gain during the period of study was estimated by determining the difference in fresh weight of caterpillar (by subtracting initial and final weight during the period of study). Death caterpillars, remaining food and feces were weighed, oven dried ($60^{\circ}\text{C}/24\text{h}$) and reweighed. Aliquots of ten sample leaves from each species were weighed, oven dried and reweighed to estimate percent dry weight conversion to allow estimation of the dry weight of the food supplied to caterpillars. The quantity of the food ingested was estimated by determining the difference between the dry weight of remaining food every 48h and total dry weight of initially provided food. Additionally, caterpillars that reached pupal state were also weighed during the first 24h. Food utilization indices (based on dry weight) were calculated in order to assess consumption and feeding efficiencies follows Waldbauer (1968) modified by Parra et al (2012):

Growth rate (GR) = P/T

Consumption rate (CR) = I/T

Relative consumption rate (RCR) = I/AT

Relative growth rate (RGR) = P/AT

Approximate digestibility (AD) (%) = $100(I-F)/I$

Efficiency of conversion of digested food (ECD) (%) = $P/(I-F) 100$

Efficiency of conversion of ingested food (ECI) (%) = $P/I100$

Where, A: mean dry weight of insect during T; I: dry weight of food consumed during T; F: dry weight of excretory products; P: dry weight gain of insect; T: duration of feeding period.

Plant induction

At the start of inductions, the plants were 8-10-day old and had 3 fully expanded true leaves. One day prior to the experiments, they were transferred to laboratory conditions with supplementary lighting (12 L:12 D). To promote volatile emissions, plants were induced with *S. frugiperda* caterpillars. Third instar caterpillars were kept without food for 24 h. Afterwards, one caterpillar was placed in the plant stem. Plants were covered with voile bags (22 x 30 cm) to prevent insects escape or migration to other plants. Undamaged plants were also covered to reduce any possible effect of the bag on the emission of HIPVs. Inductions were allowed for 12h. The following treatments were established: (i) induced perennial teosinte; (ii) undamaged perennial teosinte; (iii) induced annual teosinte; (iv) undamaged annual teosinte; (v) induced maize; and (vi) undamaged maize. After induction the plants were used in HIPVs collection and olfactory behavior experiments and.

Plant volatiles and chemical analyses

HIPVs were collected aiming relate the results of plant releases with earwig olfactory behavior. Previously, we showed that *D. luteipes* are attracted to plants attack by *S. frugiperda* and *D. saccharalis* in Y-tube olfactometer experiments and have nocturnal foraging behavior (Naranjo-Guevara et al. Unpublished data). Therefore, volatile collection was performed during the night. For that, plants were individually placed into 15-L glass chambers and airflow of 1 L/min was introduced into each chamber. Six plants per treatment were sampled for 12h using a column filter with 30 mg of HayeSep® (Supelco, Bellefonte, PA, USA). Filters were eluted with 150 μ L dichloromethane (Merck, Kenilworth, NJ, USA) mixed with 10 μ L of nonyl acetate (internal standard solution at 100 ng/ μ L). Extracts were stored at -80°C until analyses. After collections, plants were harvested and fresh weight was determined.

The extracts (2 μ L), were injected in splitless in an Agilent 6850 (Agilent J&W Scientific, USA) gas chromatograph with a flame ionization detector (GC-FID). GC-FID was equipped with an HP5 apolar capillary column (JeW Scientific, Folsom, CA; 30 m x 0.25 mm x 0.25 μ m) and using helium as the carrier gas. After injections, the column temperature was held at 40°C for 5 min, increased to 150°C ($5^{\circ}\text{C}/\text{min}$) and maintained for 1 min, and then raised ($5^{\circ}\text{C}/\text{min}$) until the final temperature of 250°C

for 5 min. The detected volatiles were quantified based on a comparison of their peak areas with the internal standard (D'Alessandro and Turlings 2005).

Compound identification was analyzed using a gas chromatograph (Agilent 6890 Series GC System G1530A) coupled to a mass spectrometer (GC–MS; Agilent 5973 Network Mass Selective Detector; transfer line 230 °C, source 230 °C, ionization potential 70 eV) equipped with an HP1 MS apolar capillary column (JeW Scientific, Folsom, CA; 30 m x 0.25 mm x 0.25 µm). An aliquot of 2 µl was injected in the pulsed splitless mode with the same conditions in the column as described above. Compound identities were based by comparing obtained mass spectra retention times with mass spectra retention reporter in NIST98 library and by calculating Kovats index (KI) using nalkane (C7–C30) standards.

Olfactory behavior

Olfactory behavior bioassays were conducted to test the olfactory attraction of *D. luteipes* to HIPVs from perennial teosinte, annual teosinte and maize. Earwigs were naïve females (until 30 days old), starving (48h) and the experiments were performed during the first three hours after light-off (Naranjo-Guevara et al. Unpublished data). A glass Y-tube olfactometer adapted to meet earwig habits such as legs adapted for running and positive thigmotropism was used. The olfactometers consisted of a central tube (25 cm long and 0.9 cm diameter) and two arms (20 cm long and 0.9 cm diameter, offset by 120°). After induction, single plants were placed inside 15-L glass bottles, which were connected to the extremities of the olfactometer and to an ARS Volatile Collection System (Analytical Research Systems, Gainesville, FL, USA). The air flow was adjusted to 0.8 L/min for each arm using calibrated flowmeters. Insects then were positioned individually at the beginning of the central arm of the Y-tube and observed for 5 min. Earwigs were considered to have made a choice when they crossed the threshold line (located in the middle of each arm). Only insects that made a choice for one arm within 5 min were considered for statistical analysis. Each female was tested only once to prevent associative learning. After each trial, the odor source position was alternating to avoid any position effect. According with our observations (unpublished data) *D. luteipes* deposits pheromone trails that affect behavior of conspecifics when they are looking for food, thus every repetition the olfactometer was washed with hexane and acetone (v/v 90%) and dried

in at 160 °C for 2 minutes. After every five repetitions plants were changed and at least 40 repetitions were executed.

Statistical analysis

Shapiro-Wilk and Levene tests ($P < 0.05$) were carried out to determine normality and homogeneity of the data respectively. Effects of plant genotype on *Spodoptera frugiperda* fed were analysed by Kruskal-Wallis test, followed by Tukey and Kramer post-hoc test, $P < 0.05$. Food utilization indices were subjected to ANOVA in a randomized complete block design with unequal replication and Tukey's HSD ($P < 0.05$) was used to separate means. Relative amounts of volatiles were corrected by the plant fresh weight and analyzed through One-Way ANOVA followed by Tukey's HSD ($P < 0.05$). The composition of the volatile blend was analyzed by Principal Component Analysis - PCA ($P \leq 0.05$). We used the logistic regression and Wald's Chi-square test for analyzing the earwig choice in olfactometer assays. Statistical analysis was performed using software package R 3.0.2 (www.r-project.org).

4.3 Results

Food intake and utilization

A significant effect of plant genotype on *S. frugiperda* caterpillars was observed (Fig. 1). Caterpillars fed with perennial teosinte showed the lowest weight gain followed by annual teosinte and maize ($\chi^2 = 39.1304$, $P < 0.0001$). The same pattern was observed in the amount of food ingested ($\chi^2 = 31.806$, $P < 0.0001$) and total excretion ($\chi^2 = 26.4139$, $P < 0.0001$). Maize fed caterpillars lived on average more time than those fed with teosinte species ($\chi^2 = 38.608$, $P < 0.0001$) and only these ones reached a pupal stage (0.18 ± 0.03 mg).

Food intake and conversions of ingested and digested food by *S. frugiperda* varied considerably among the three plant species (Table 1). Recorded values of Growth rate (GR), Consumption rate (CR), Relative consumption rate (RCR) Approximate digestibility (AD) and Efficiency of conversion of digested and ingested food (ECD, ECI) were higher for caterpillars fed on maize ($\chi^2 = 38.9599$, $P = < 0.001$; $\chi^2 = 12.2118$ $P = 0.002$; $\chi^2 = 31.319$ $P < 0.001$; $\chi^2 = 34.417$ $P < 0.001$; $\chi^2 = 39.1304$, $P < 0.001$; $\chi^2 = 38.9573$, $P < 0.001$, respectively). Both, Relative consumption rate

(RCR) and Approximate digestibility (AD) values were greater in perennial teosinte ($\chi^2 = 31.319$, $P < 0.001$; $\chi^2 = 34.417$, $P < 0.001$) and just Relative growth rate (RGR) was higher in annual teosinte ($\chi^2 = 33.2947$, $P < 0.001$).

Plant volatiles and chemical analyses

It was observed that teosinte and maize plants released a complex mixture of volatiles during the scotophase, due to continuous attack of *S. frugiperda* caterpillars. Different chemical classes were observed as GLVs [(*E*)-3-hexenal, (*E*)-2-hexenal, (*E*)-3-hexen-1-ol and (*Z*)-3-hexenyl acetate]; aromatics (phenylmethyl acetate, decanal and indole); monoterpenes (β -myrcene and linalool); and sesquiterpenes [(*E*)- β -caryophyllene, (*E*)- α -bergamotene, (*E*)- β -farnesene and β -sesquiphellandrene]. Annual teosinte releases the highest amount of volatiles, followed by perennial teosinte and maize (Table 2). GLVs were predominant in perennial and annual teosinte mixtures (63 and 50%, respectively) followed by aromatic compounds (13 and 19%, respectively). The monoterpenes and sesquiterpenes constituted less than 20% in these two species. In mixtures emitted by maize, sesquiterpenes (44%) were predominant followed by VFVs (24%), aromatics (16%) and monoterpenes (16%) (Fig. 2). Statistical differences were observed between the three species. Perennial teosinte release significantly more concentration of (*Z*)-3-hexen-1-ol and β -Sesquiphellandrene. Annual teosinte release higher amount of (*E*)-3-hexenal, Phenylmethyl acetate and Indole. (*E*)- β -caryophyllene and (*E*)- β -farnesene compounds were observed in larger quantities in maize plants. The geranyl acetate compound was just founded in maize plants (Fig. 3).

The PCA analysis showed two principal components that together resented major clusters of samples. The separation was influenced by the plant genotip. It was possible to observe that tree species were clearly separated (Fig. 4).

Olfactory behavior

Olfactometer Bioassays showed that *D. luteipes* females distinguished the odor of undamaged plant and clear air ($P < 0.001$; $df=98$), since undamaged plants were used as a control (Fig. 5). They were significantly attracted to induced perennial teosinte ($P < 0.001$; $df=78$) and induced annual teosinte ($P < 0.001$; $df=96$) over induced maize. In the same way, earwigs preferred induced perennial teosinte ($P=0.01$; $df=100$) and induced annual teosinte ($P=0.03$; $df=86$) over undamaged

teosintes. No differences were observed when induced perennial teosinte and induced annual teosinte were contrasted ($P=0.683$; $df=96$)

4.4 Discussion

Life-history features can define plant defenses against herbivores through diverse plant traits such as direct and indirect defenses. We found that life-history of *Zea* plants had a strong influence on plant defense and consequently in tritrophic interactions which involve plant, herbivore and predators. Our data are consistent with results of prior studies suggesting that direct (Rosenthal and Welter 1995; Rosenthal and Dirzo 1997; Szczepaniec et al. 2012; Dávila-Flores et al. 2013) and indirect (Gouinguene et al. 2001; Mutyambai et al. 2015; De Lange 2016) herbivore defenses are weaker in modern maize compared with their wild ancestors.

The results are consistent with the prediction that FAW caterpillars would perform better on modern maize than in wild species. We observed an evident gradient which larvae feed on perennial teosinte gained less weight, ingested less food and defecated less than annual teosinte, followed by maize. Likewise, larvae lived less time on perennial teosinte, follow by annual teosinte. In contrast, just in maize, caterpillars reached the pupa stage. Differences in larval growth and development of FAW feeding on teosinte plants were reported before (Takahashi et al. 2012; Szczepaniec et al. 2012). Nevertheless, in some cases, such as caterpillars feed with Balsa teosinte, survivorship was not affected (Takahashi et al. 2012). Rosenthal and Welter (1995), and Rosenthal and Dirzo (1997) observed differences in tolerance of *Zea* plants damaged from diverse herbivorous arthropods, associated to evolution from perennial to annual habits. They showed a gradient of attack that suggests wild perennial (*Z. diploperennis*) is most tolerant, followed in descending order by the wild annual (*Z. m. ssp. parviglumis* Ittis & Doebley), the land-race cultivar (*Z. m. ssp. mays* L.) and the modern high-yielding variety (*Z. m. mays*).

Clear differences were also observed in terms of food intake and utilization. Surprisingly, when feed on maize, caterpillars showed lower RGR and CI values compared with those than when fed on teosinte leaves. However, larvae were more efficiently converting maize tissues into biomass than teosintes tissues, as observed by ECD and ECI values that showed highest efficiency of conversion of digested and ingested food to maize. Xue et al. (2009) obtained similar results feeding *Spodoptera*

litura (Fabricius) (Lepidoptera: Noctuidae) with tobacco. They, affirm that these results can be explained due to homeostatic adjustment of consumption rates of an insect to achieve ideal growth rate even with foods of different quality. The higher caterpillar growth rate (GR) observed in caterpillars feed on maize resulted also from a high ECD and ECI values. Nutritional requirements for insect growth depend on the ability of the insect to ingest, assimilate and convert food into body tissue (Roy and Barik 2013). In this way, maize leaves were more suitable food and requires low metabolic costs than teosintes leaves, especially compared with perennial teosinte that showed the lowest values to GR, ECD and ECI. This indicated that wild species are lower suitability to herbivores than modern maize.

Variation of *Zea* plants related to resistance against herbivores also was detected in field. Rosenthal and Welter (1995) reported that *Z. diploperennis* was more tolerant to damage by a stem borer, *Diatraea grandiosella* Dyar (Lepidoptera: Crambidae), by producing more tillers and leaves compared with maize. Studies have shown that maize plants suffered higher FAW infestation rates and those larvae performed better on maize compared with Balsas teosinte (Takahashi et al. 2012). The same effect has been observed in maize land races, which were less consumed by lepidoptera caterpillars than the commercial hybrid variety and larval survival was significantly lower in the landraces compared to hybrid maize (Rasmann et al. 2005; Tamiru et al. 2011; Mutyambai et al. 2014).

Reduced feeding on teosinte tissues by FAW larvae suggests that teosinte plants could have poor nutritional value or have an antibiotic effect on larvae that interfere with the optimal utilization of the ingested food. Different factors can influence larval feeding, including induced secondary metabolites such as the hydroxamic acid DIMBOA (2,4-dihydroxy-7-methoxy-1,4-benzoxazin-3-one) (Mutyambai et al. 2014) and/or physical characters, such as hairiness and leaf toughness (Bernal et al. 2015). Another explanation of a general lack of defense in maize plants is the lost ability to express genes related with herbivore resistance. Szczepaniec et al. (2012) found that the greater susceptibility of maize to *S. frugiperda* is related with the reduced expression of wip1, PR1, and chitinase, genes associated with protease inhibitors, when compared with wild *Zea* plants. Analysis of these factors in teosinte and maize can provide a comprehension about resistance mechanism employed by *Zea* plant and could generate tools to the management of *S. frugiperda*. Our work demonstrates that the effect on FAW performance observed

in prior studies is due to differences in the food utilization capacity of caterpillars when they feed on teosintes or maize tissues.

Wild plants from diverse families are also more resistant to herbivores than their cultivated relatives. Hagenbucher et al. (2016) showed that domesticated cotton plants supported a better performance for the moth *S. littoralis* than wild plants. Likewise, insect herbivores also performed better on cultivated crucifers (*Brassica oleracea* L.) and beans (*Phaseolus*) when compared with wild plants (Benrey et al. 1998). In tomato *Solanum lycopersicum* L., wild plants showed more tolerance to defoliation (Welter and Steggall 1993). Rodríguez-Saona et al. (2011) found that the defensive chemistry was reduced in the high-yielding American cranberry cultivar compared to ancestral cultivars, and the performance of gypsy moth, *Lymantria dispar* L. (Lepidoptera: Lymantriidae) was better on a this high-yielding.

Our results also showed effects of domestication on production of HIPV. Maize and teosinte plants emits a qualitatively blend similar, but the quantities were different. Fourteen dominating compounds were present in the blends that are in accordance with earlier reports about headspace composition of herbivore-induced maize and teosinte varieties (Gouinguene et al. 2001; De Lange et al. 2016). According to Dicke (1999), within plant species, the variation in volatile composition among genotypes is mostly restricted to quantitative differences in the contribution of each component to mixture composition. The low amount of compounds collected also agree with the volatiles blend that we observed previously in induced maize during the night (Naranjo-Guevara et al., unpublished data). A positive correlation between light intensity and HIPVs emission has been shown before (Loughrin et al. 1994; Seidl-Adams et al. 2014).

Different from prior studies by Gouinguene et al. (2001), De Lange et al. (2016) (carried out in light conditions), the volatile mixtures observed here in teosinte plants showed a predominance of GLV and the sesquiterpenes (*E*)- β -caryophyllene and (*E*)- β -farnesene were higher in maize. These results could indicate that domestication may affect the GLV production, at least in dark conditions. Although annual teosinte released in total more quantity of volatiles, the highest GLV production was observed in perennial teosinte and the lowest in maize. This could support our hypothesis about the gradient in HIPVs production from 'most ancestral' to 'most domesticated' *Zea* plants.

Doru luteipes females showed strong responses to volatiles from undamaged plants when compared with clean air. They preferred volatiles from induced plants over undamaged ones in all cases, indicating that earwigs are able to associate *Zea* volatiles with the presence of potential prey. Besides, earwigs showed high attraction to volatiles from induced perennial and annual teosinte over induced maize. However, females did not distinguish between two teosinte species. Considering the high GLVs emissions in teosinte plants, we suggest that (*E*)-3-hexenal, (*E*)-2-hexenal, (*E*)-3-hexen-1-ol are important compounds for innate attraction of *D. luteipes*, or even the mixture of them. This fact corresponds with our earlier observations of olfactory behavior in earwigs.

The attraction towards GLVs compounds could represent an adaptive value to generalist predators such earwigs that allows them discriminate between infested and uninfested plants (Naranjo-Guevara et al., unpublished data). It has been demonstrated that some generalist parasitoids (Hoballah and Turlings 2005; Peñaflor et al. 2011) and predators (Maeda et al. 2015) are attracted to GLVs. The data suggest that teosinte plants could recruit more efficiently generalist predators than maize during the night. This assumption adds an important value of the ancestral features to biological control, given earwigs are considered voracious predators by feeding on a wide range of herbivores that attack different crops (Evans and Longépé 1996; Haile and Hofsvang 2001; Alvarega et al. 1996; He et al. 2008; Romero-Sueldo et al. 2008). Even if the FAW populations are diminished by the strong direct defenses of teosinte plants, earwigs could still be attracted by other potential prey that triggers GLV releases.

Several studies on *Zea* have shown significant variation among maize cultivars and teosintes in HIPV that attract the natural enemies of herbivores. (Gouinguene et al. 2001; Degen et al. 2004; Köllner et al. 2008; Mutyamba et al. 2015). Modern North American maize varieties, for example, lose the ability to produce (*E*)- β -caryophyllene that attracts entomopathogenic nematodes (Rasman et al. 2005; Köllner et al. 2008). Tamiru et al. (2011 and 2012) showed that some maize landraces respond to *Chilo partellus* (Swinhoe) (Lepidoptera: Pyralidae) oviposition by producing HIPVs both which attract parasitoids. On the other hand, Erb et al. (2011) reported on a positive correlation between HIPVs and insect resistance in 20 maize inbred lines. However, the attractiveness of teosinte compared with maize volatiles to parasitoids is being studied just recently (De Lange et al. 2016),

and so far no work has been performed with predators. Except to our studies, the olfactory response to HIPVs on earwigs had never been studied before.

Life plant histories, perennial and annual, carry different metabolic resource allocation strategies that affect how the plants interact with their environments, including insect herbivores (Takahashi et al. 2012,), and natural enemies (Mutiyamba et al. 2015; De Lange et al. 2016). Annual species, for example, may be selected to maximize reproduction focused on increased productivity. In contrast, perennial species grow slower and produced fewer seeds than annual species (Rosenthal and Dirzo 1997). Domestication process have resulted in weaker herbivore defenses per the resource allocation hypothesis (Davila-Flores et al. 2013).

Overall, this study was consistent with prior observations that direct and indirect defenses of maize against herbivores are weakened by domestication (Rosenthal and Dirzo 1997; Gouinguene et al. 2001; Szczepaniec et al. 2012; Dávila-Flores et al. 2013; Mutiyambai et al. 2015; de Lange 2016).

We presented evidence that the life-history of *Zea* can determine the ecological tritrophic interactions through shifts in plants defense strategies. This change may result in fitness cost to plants by reducing resistance and volatile production. The knowledge of evolutionary and ecological history in systems with a high degree of domestication, such as maize, may allow understanding the defense plants mechanisms aiming sustainable alternatives to pest control. The challenges to be addressed in future studies are the identification of specific defense mechanism such as secondary metabolites (Mutiyambai et al. 2014) that act directly o herbivores or active volatile compounds that attract natural enemies (Dicke and van Loon 2000) as well as creation of strategies for the use of earwigs in IPM programs.

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Tables

Table 1. Nutritional indices for *Spodoptera frugiperda* fed on perennial teosinte (*Zea diploperennis*) annual teosinte (*Z. mays* L. ssp. *mexicana*) and modern maize (*Z. mays* L. spp. *mays*) leaves.

	Perennial teosinte	Annual teosinte	Modern maize	Statistical Significance
Growth rate (GR)	0.4±0.2b	1.9±0.7b	17.4±4.0a	<0.001
Consumption rate (CR)	83.1±19.0b	79.4±18.4b	114.3±37.1a	0.002
Relative growth rate (RGR)	0.3±0.2b	1.1±0.7a	0.1±0.02b	<0.001
Relative consumption rate (RCR)	80.2±31.3a	54.6±24.3b	0.5±0.2c	<0.001
Approximate digestibility (AD) (%)	99.2±0.4a	71.1±10.2c	87.4±71.1b	<0.001
Efficiency of conversion of digested food (ECD) (%)	0.5±0.3b	3.5±1.5b	21.7±14.6a	<0.001
Efficiency of conversion of ingested food (ECI) (%)	0.5±0.3b	2.4±0.9b	19.4±14.9a	<0.001
larval survival (T) in days	4.3±0.9c	10.8±2.9b	18.7±2.4a	<0.001

Table 2. Amount of volatiles (ng \pm SD) released by perennial teosinte (*Zea diploperennis*) annual teosinte (*Z. mays* L. ssp. *mexicana*) and modern maize (*Z. mays* L. spp. *mays*) seedlings induced during the scotophase by *Spodoptera frugiperda* caterpillars in contrast with undamaged plants.

Compounds	Undamage Plants	Perennial Teosinte	Annual Teosinte	Modern Maize	Statistical Significance ^a
<i>Green leaf volatiles</i>					
(E)-3-hexenal	-	0.098 \pm 0.015	1.234 \pm 0.085	0.141 \pm 0.050	0.001
(E)-2-hexenal	-	0.905 \pm 0.086	0.789 \pm 0.074	0.610 \pm 0.061	0.134
(Z)-3-hexen-1-ol	-	0.995 \pm 0.073	0.619 \pm 0.085	0.090 \pm 0.02	<0.001
(Z)-3-hexenyl acetate	-	0.550 \pm 0.106	0.428 \pm 0.085	0.341 \pm 0.885	0.110
<i>Aromatic</i>					
Phenylmethyl acetate	-	0.078 \pm 0.012	0.332 \pm 0.096	0,065 \pm 0.016	0.011
Decanal	0.149 \pm 0.017	0.306 \pm 0.051	0.262 \pm 0.090	0.198 \pm 0.051	0.456
Indole	-	0.159 \pm 0.050	0.606 \pm 0.142	0.357 \pm 0.093	0.008
Geranyl acetate	-	-	-	0,0819 \pm 0,023	-
<i>Monoterpenes</i>					
β -myrcene	-	0.089 \pm 0.011	0.133 \pm 0,060	0.053 \pm 0,026	0.423
Linalool	0.149 \pm 0.027	0.424 \pm 0.061	0.448 \pm 0.134	0.660 \pm 0.010	0.063
<i>Sesquiterpenes</i>					
(E)- β -caryophyllene	-	0.054 \pm 0.015	0.201 \pm 0.071	0.494 \pm 0.125	0.014
(E)- α -bergamotene	-	0.026 \pm 0.004	0.507 \pm 0.111	0.426 \pm 0.100	0.013
(E)- β -farnesene	-	0.016 \pm 0.004	0.480 \pm 0.131	1.005 \pm 0.128	<0.001
β -Sesquiphellandrene	-	0.329 \pm 0.085	0.115 \pm 0.034	0.081 \pm 0.028	0.018
Total	-	4.030 \pm 1.389	6.155 \pm 1.312	4.603 \pm 1.062	

^aSignificant difference between treatments according to One-Way ANOVA test ANOVA followed by Tukey's HSD ($P<0.05$).

Figures

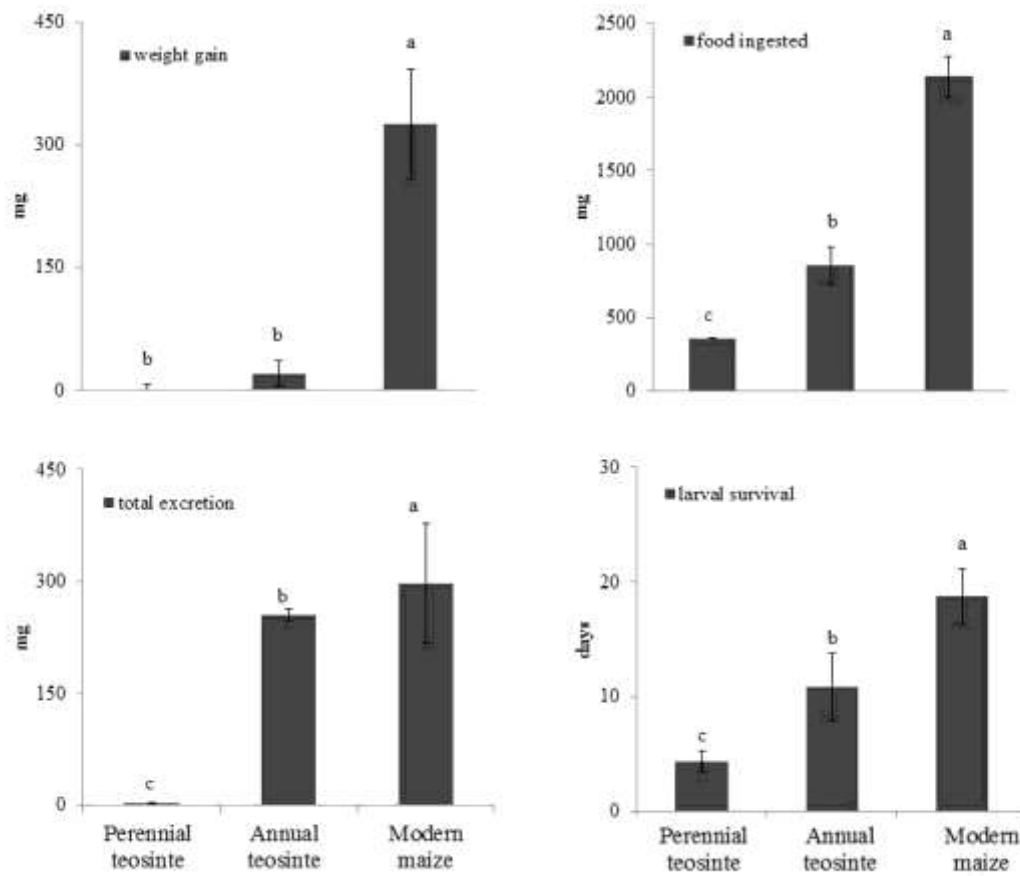


Figure 1. Effects of plant genotype on *Spodoptera frugiperda* fed on perennial teosinte (*Zea diploperennis*), annual teosinte (*Z. mays* L. ssp. *mexicana*), and modern maize (*Z. mays* L. spp. *mays*) leaves. Different letters indicate significant differences between treatments (Kruskal-Wallis test, followed by Tukey and Kramer post-hoc test, $P < 0.05$).

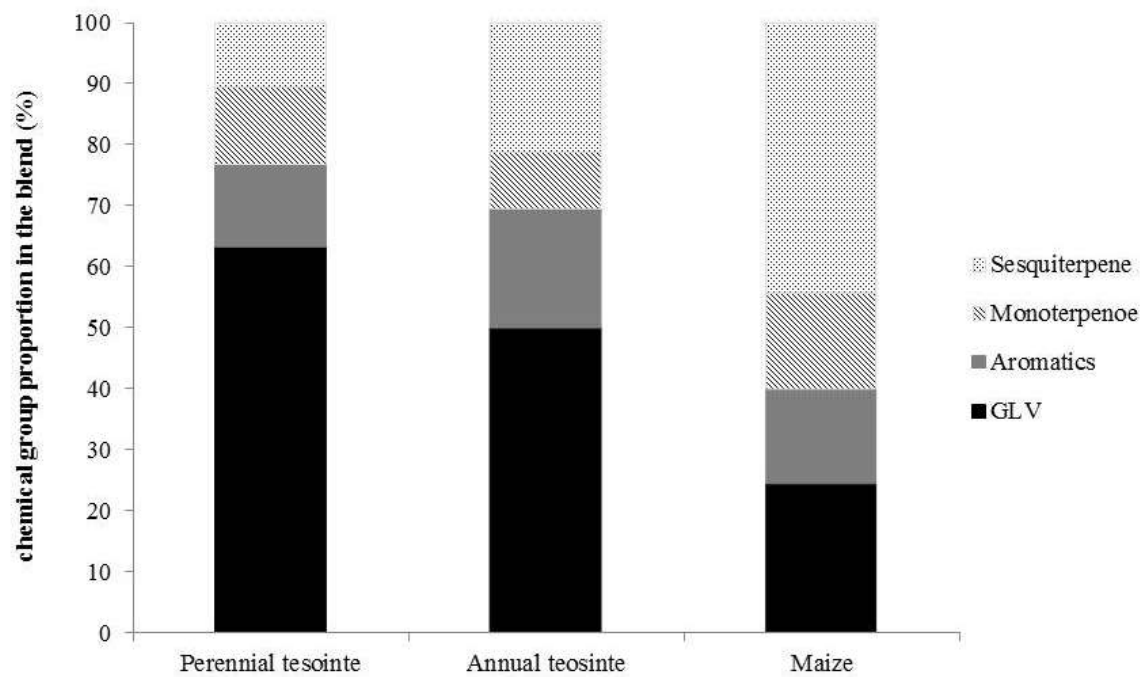


Figure 2. Chemical groups proportions on mixtures released by perennial teosinte (*Zea diploperennis*) annual teosinte (*Z. mays* L. ssp. *mexicana*) and modern maize (*Z. mays* L. spp. *mays*) induced with *Spodoptera frugiperda*.

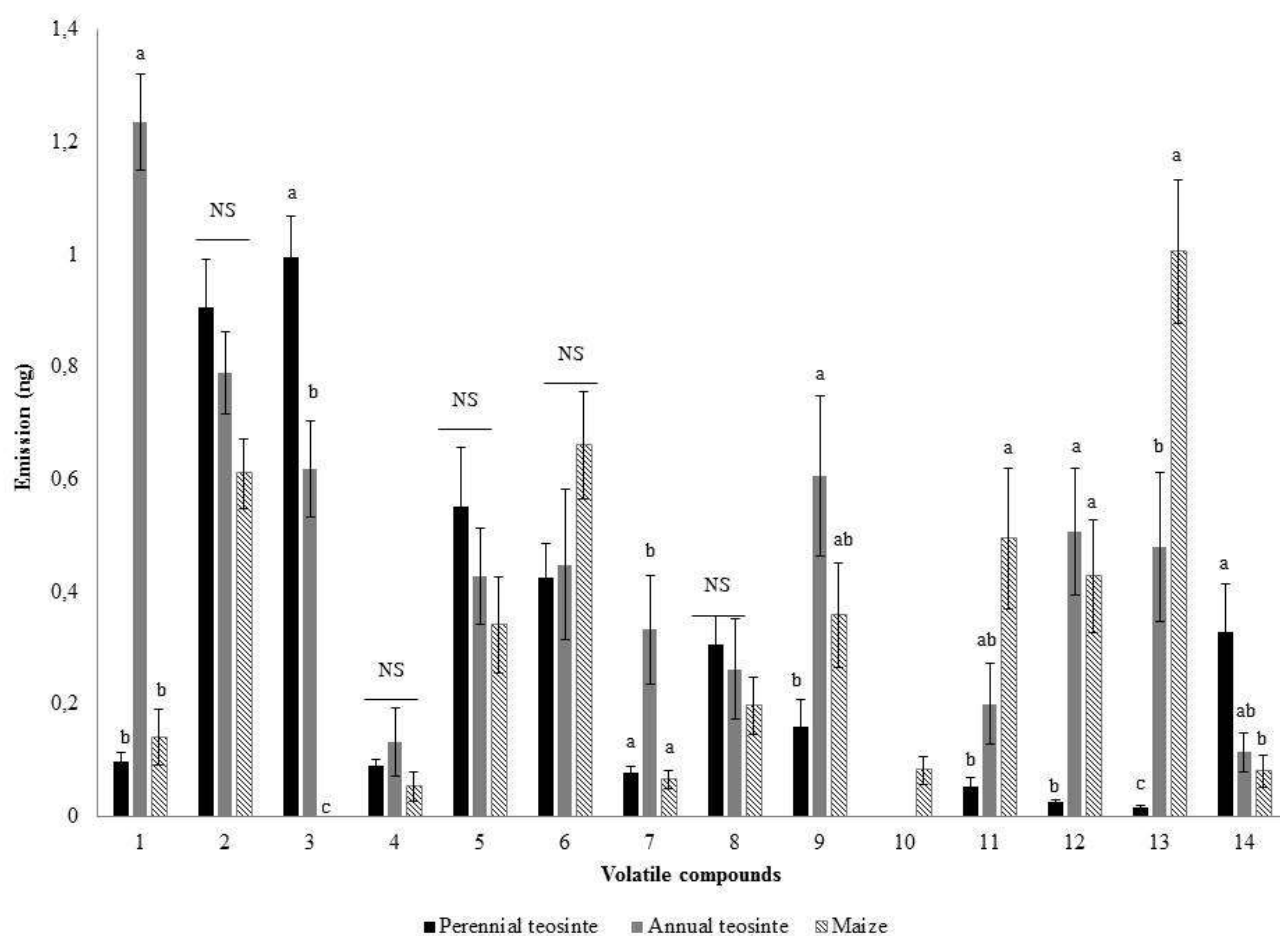


Figure 3. Mean (\pm SE) amounts of nocturnal volatiles emitted by perennial teosinte (*Zea diploperennis*) annual teosinte (*Z. mays* L. ssp. *mexicana*) and modern maize (*Z. mays* L. ssp. *mays*) induced with *Spodoptera frugiperda*. Volatile compounds: (1) (E)-3-hexenal; (2) (E)-2-hexenal; (3) (Z)-3-hexen-1-ol; (4) β -myrcene; (5) (Z)-3-hexenyl acetate; (6) linalool; (7) phenylmethyl acetate; (8) decanal, (9) indol; (10) geranyl acetate; (11) (E)- β -caryophyllene; (12) (E)- α -bergamotene; (13) (E)- β -farnesene; (14) β -Sesquiphellandrene. Different letters indicate significant differences (One-Way ANOVA followed by Tukey's HSD ($P < 0.05$)).

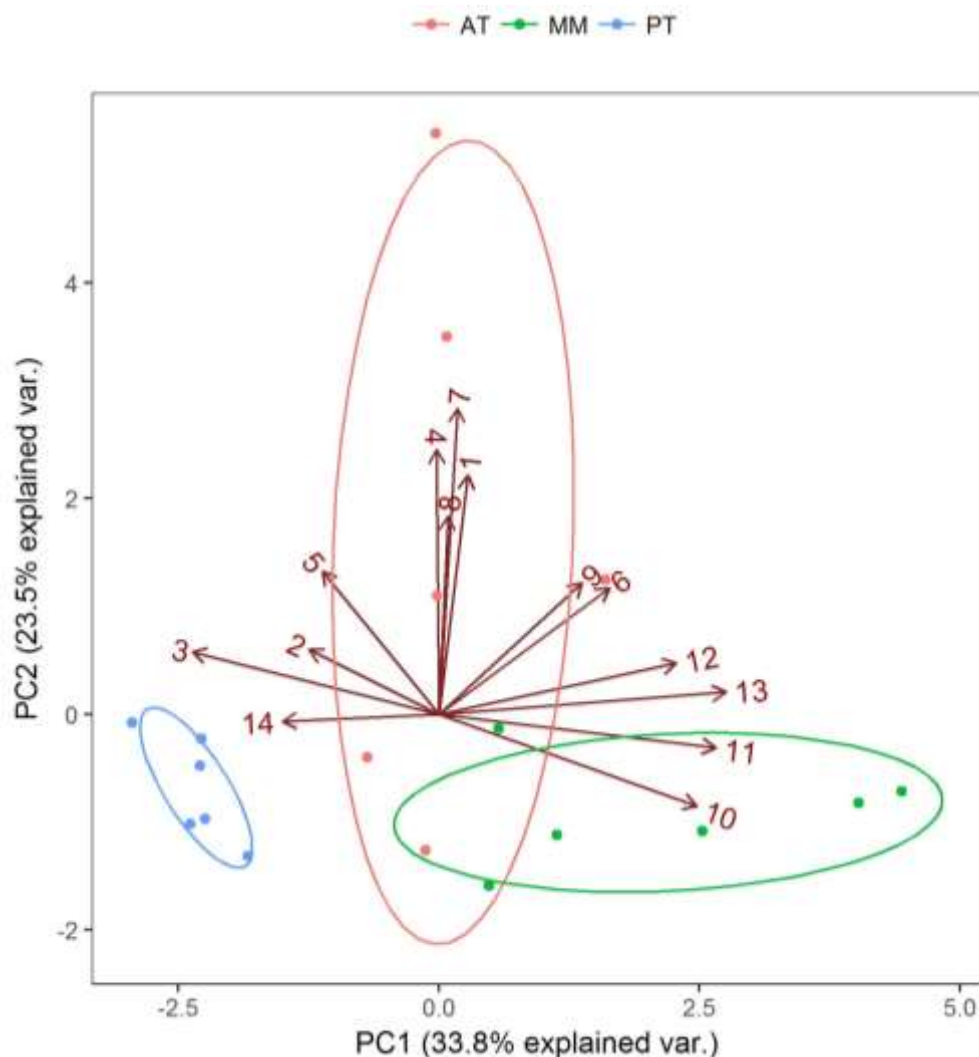


Figure 4. Separation of headspace composition of plants using Principal Component Analysis - PCA ($P \leq 0.05$) after seedlings infested with *Spodoptera frugiperda*. a (*Z. mays* L. spp. *Mays*); b (*Z. mays* L. spp. *mexicana*); c (*Z. diploperennis*). Score plot visualizing the grouping pattern of the samples according to the first two principal components (PCs). (x1) (*E*)-3-hexenal; (x2) (*E*)-2-hexenal; (x3) (*Z*)-3-hexen-1-ol; (x4) β -myrcene; (x5) (*Z*)-3-hexenyl acetate; (x6) linalool; (x7) phenylmethyl acetate; (x8) decanal; (x9) indol; (x10) geranyl acetate; (x11) (*E*)- β -caryophyllene; (x12) (*E*)- α -bergamotene; (x13) (*E*)- β -farnesene; (x14) β -Sesquiphellandrene. AT: annual teosinte, MM: moden maize, PT: perennial teosinte.

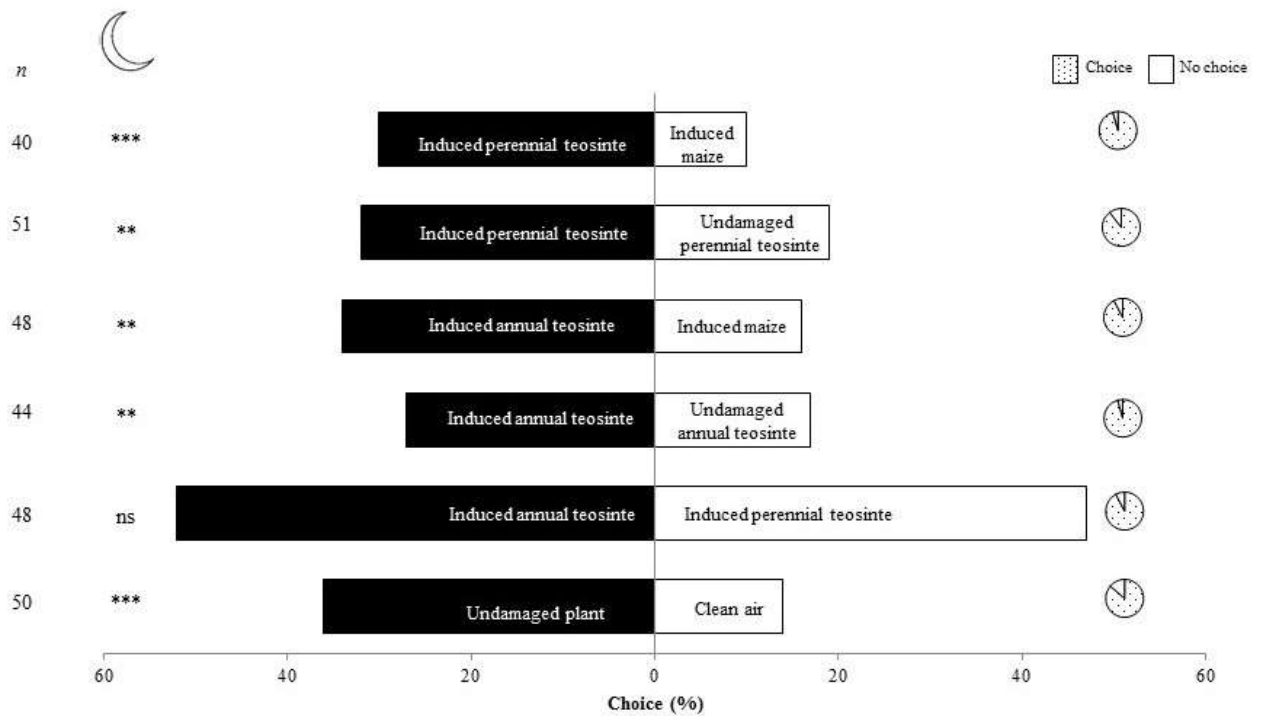


Figure 5. Olfactory preference of *Doru luteipes* females towards perennial teosinte (*Zea diploperennis*) annual teosinte (*Z. mays* L. ssp. *mexicana*) and modern maize (*Z. mays* L. spp. *mays*) induced or not with *Spodoptera frugiperda*. Pie charts on right represent non-responsive (no choice) and responsive (choice) earwigs, *D. luteipes*. Asterisks indicate a significant difference between treatments according to the Chi-square test (* $P \leq 0.05$; ** $P \leq 0.01$; *** $P \leq 0.001$) and ns = no significance