Benefits, costs and exploitation of caterpillar-induced odor emissions in maize plants

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Contents

Summary

In response to phytophagous insect-attack, plants produce volatiles that can serve as cues for natural enemies of herbivores to locate their host or prey. A considerable progress has been made in understanding the causes and effects of this induced indirect plant defence. Many studies have demonstrated the attraction of parasitoids and predators to odor cues from different plant-herbivore complexes and have placed the responses in the context of qualitatively and quantitatively variability in odor blends among plant species and varieties attacked by different herbivores. Plant physiological studies on the biochemical pathways, enzymes and genes involved in the induced volatile production have been conducted. Despite of the numerous studies concerning this indirect defence, many questions on its ecological significance and applicability for pest control remain unanswered. We tested the costs and benefits of the induced production of volatiles from the viewpoint of the plant. With the tritrophic system maize (*Zea mays*)-armyworm (*Spodoptera* spp.)-parasitoid (*Cotesia marginiventris*), benefits of attracting parasitoid and costs for the production of volatiles were assessed. If plants are attacked by a parasitized rather than a healthy (unparasitized) caterpillar seed production is higher and is not different from the seeds produced by unharmed plants. In this respect, maize plants attacked by caterpillars can benefit from the action of this parasitoid (Chapter 1). Moreover, inducing young plants to produce the volatiles, for a period that corresponds with caterpillar development, does not affect their eventual seed production indicating a low cost for the production of this defence (Chapter 3). Benefits seem to counterbalance the costs of herbivore-induced volatile production when natural enemies that reduce herbivory are attracted. This is important if we want to exploit this indirect defence to enhance biological control in countries where *Spodoptera* spp. is an important pest on maize. To further assess the feasibility of this approach, a survey of *S. frugiperda* natural enemies

and their potential in reduce feeding by caterpillars was conducted in Mexico, in lowland maize fields (Chapter 2). The wasp *C. marginiventris* is a generalist, solitary endoparasitoid that readily parasitizes *Spodoptera* spp. and is highly responsive to induced maize odors, and could therefore be a biological control agent who's effectiveness can be enhanced by increasing plant attractiveness. Attraction of this wasp to induced volatiles from several specific maize varieties was tested in the present study and it was found that quality and not only quantity of the odor blends is important (Chapter 4). Furthermore, it was determined that green leaf volatiles, which are commonly released by most plants upon fresh damage, are crucial for the initial attraction of *C. marginiventris* inexperienced females (Chapter 5). Induced volatiles could also benefit plants as direct defence against plant pathogens, with antibiotic properties. We tested this hypothesis with downy mildew, *Peronosclerospora sorghi*, a common pathogen on maize, however, we found no evidence that this pathogen is affected by the volatiles (Annexe). The present thesis provides some answers to questions concerning the adaptiveness of herbivore-induced volatiles and their potential to enhance the control of insect pests.

Résumé

Face aux attaques d'insectes phytophages, les plantes produisent des substances volatiles qui servent de signaux aux ennemis naturels de leurs ravageurs. De nombreux progrès ont été fait dans la compréhension des causes et des effets de ce mode indirect de défense induit chez les plantes. De nombreuses études ont démontré l'attraction des parasitoïdes et des prédateurs aux signaux odorants provenant des différents complexes plante / ravageurs. Les réponses se placent dans un contexte qualitatif et quantitatif de la variabilité du mélange des odeurs selon les espèces de plantes et les variétés attaquées par les différents ravageurs. Des études physiologiques sur les modèles biochimiques, les enzymes, et les gènes impliqués dans la production de ces volatiles ont été conduites. Malgré cela beaucoup de questions sur la signification écologique de cette défense et son application dans le contrôle des ravageurs demeurent irrésolus. Nous avons testé et estimé les coûts (production des volatiles) et les bénéfices (attraction des parasitoïdes) de cette défense du point de vue de la plante, avec le système tritrophique maïs (*Zea mays*) / chenille (*Spodoptera* spp.) / parasitoïde *(Cotesia marginiventris*). Si les plantes sont attaquées par une chenille parasitée plutôt qu'une chenille saine (non parasitée), la production de graines est plus forte, et ne diffère pas de la production d'une plante non attaquée. Dans ce contexte, les plants de maïs attaqués par les chenilles peuvent bénéficier de l'action du parasitoïde (chapitre 1). De plus, la production des volatiles induite chez de jeunes plants de maïs lors de la période couvrant le développement des chenilles n'affecte pas leur éventuelle production de graines ; ceci indique un faible coût à la production de cette défense (chapitre 3). Les coûts de la production de ces volatiles induits par les herbivores semblent donc contrebalancés par les bénéfices quand l'ennemi naturel du ravageur est attiré. Ceci est important si nous voulons exploiter cette défense indirecte pour améliorer le contrôle biologique dans les régions oú *Spodoptera* spp. est une importante peste

du maïs. Pour mieux estimer la réalisation de cette approche, une étude des ennemis naturels de *S. frugiperda* et leur potentialité à réduire l'alimentation des chenilles a été menée au Mexique dans des champs de maïs (chapitre 2). *C. marginiventris* est une guêpe endoparasite, solitaire et généraliste. Elle parasite volontiers *Spodoptera frugiperda* et est très sensible aux odeurs induites du maïs. Elle représente donc un agent de contrôle biologique dont l'efficacité peut être accrue en augmentant l'attractivité des plantes. L'attraction de cette guêpe par les volatiles induits à partir de différentes variétés spécifiques de maïs a été testée dans cette étude. Il a été trouvé que non seulement la quantité, mais aussi la qualité du mélange d'odeurs sont importantes (chapitre 4). De plus, il a été déterminé que les green leaf volatiles communément relachés par beaucoup de plantes sous l'effet de dommages récents, sont cruciaux pour l'attraction initiale des femelles inexpérimentées de *C. marginiventris* (chapitre 5). Ces volatiles induits peuvent aussi bénéficier aux plantes comme moyen de défense directe contre les pathogènes grâce à des propriétés antibiotiques. Nous avons testé cette hypothèse avec le mildew *Peronosclerospora sorghi*, un pathogène commun du maïs. Nous n'avons cependant pas pu démontrer que ce pathogène était affecté par les volatiles (anexe). En conclusion, cette thèse apporte quelques réponses aux questions concernant l'adaptation des volatiles induits par les herbivores et leur potentialité à contrôler favorablement les insectes ravageurs.

Traduction Erick Campan

General Introduction

Plants can defend themselves against herbivore attack in different manners. Some plants continuously contain toxic or repellent compounds against herbivores in their leaf tissues, this is considered a *constitutive defence*. Another possible defence for plants is the production of toxic or repellent compounds only when they are attacked by phytophagous organisms: this constitutes an *induced defence*. Furthermore, induced and constitutive defences can be *direct*, acting directly against the herbivore, or *indirect*. Indirect defences are multiple, ranging from the constitutive formation of domatia, which serve as homes for organisms such as ants, mites, and even bacteria to the production of foliar nectaries and nutritive structures which can be used by natural enemies of the herbivores (for reviews see Boethel and Eikenbary, 1986 and Whitman, 1988). Also induced indirect defences exists in plants. During the last decade, it was discovered that when herbivores feed on plants, the injured plants respond by producing and releasing odors that are exploited by natural enemies of the herbivores to locate their preys and hosts (for reviews see Turlings and Benrey, 1998 and Dicke and Vet, 1999). The production of predator and parasitoid attracting volatiles by the plant, beginning from the period of herbivore attack, is considered to be an *induced indirect defence*.

The release of volatiles induced under attack of herbivores has in several cases been shown to constitute an active response of the plant, as is apparent from the *de novo* production of volatile compounds that does not occur in undamaged or mechanical damaged plants (Dicke *et al.*, 1990a; Dicke *et al.*, 1990b; Turlings *et al.*, 1990; Paré and Tumlinson, 1997). Furthermore, the induced volatiles are not only released from the damaged site of the plant, it was shown that the release occurs *systemically* (Turlings and Tumlinson, 1992; Dicke *et al.*, 1993;Röse *et al.*, 1996). Elicitors that trigger this plant response were isolated from the regurgitant of lepidopteran larvae. β-Glucosidase was found to be the active compound in the oral secretion of *Pieris brassicae* (Mattiacci *et al.*, 1994; Hopke *et al.*, 1994) while in the regurgitant of *Spodoptera* spp. the active compound is volicitin (Alborn *et al.*, 1997; Turlings *et al.*, 2000; Gouinguné, 2000). Volicitin was identified as N[17-hydroxy-9,12,15-octadecatrienoyl]-L-glutamine and was found to resemble the structure of precursors of jasmonic acid, a plant defence signal, that induces the lipoxygenase pathway (Alborn *et al.*, 1997). The elicitor volicitin is partially plant (the fatty acid portion) and partially caterpillar (glutamine) derived (Paré *et al.*, 1998, Figure 1).

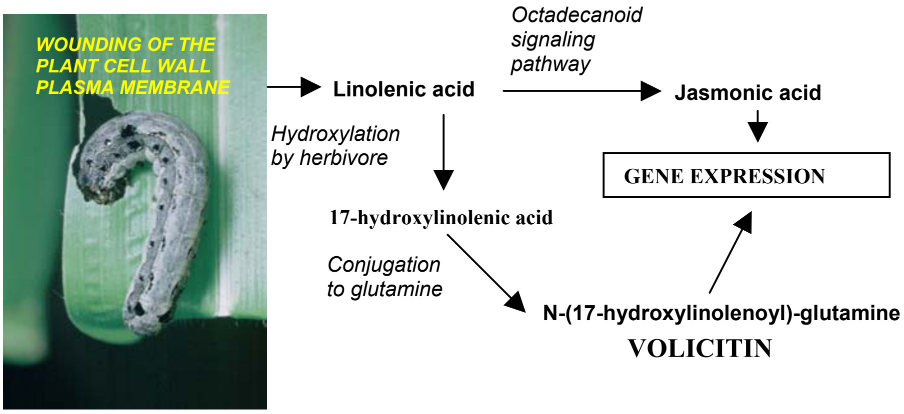

Figure 1: Pathways that leads to volicitin and jasmonic acid synthesis, both volicitin and jasmonic acid are elicitors of the production of induced volatiles.

After treatment with volicitin, maize plants produce the same blend of volatiles as plants attacked by caterpillars, and are highly attractive to parasitoids (Turlings *et al.*, 2000). Spiteller *et al.* (2000) discovered the probable involvement of bacteria, contained in herbivore gut, in the biosynthesis of the elicitor volicitin. This study adds a new trophic level to the complex network of interactions among plant, herbivores and natural enemies of the herbivores. Other elicitors have also been used to induced the plants to produce volatiles: cellulysin and coronatine (Koch *et al.*, 1999), jasmonic acid (Boland *et al.*, 1995; Gols *et al.*, 1999; Thaler, 1999), methyl jasmonate (Halitschke *et al.*, 2000). These elicitors, applied on damaged side of plants, induce them to produce a blend of volatiles that is very similar to the volatiles produced by the plant under herbivore attack. For example, if the elicitor coronatine, which is of pathogenic origin and is a phytotoxin, is applied to the damaged leaves of maize plants, it induces the release of a volatile blend that is virtually the same as that of maize plants treated with *S.littoralis* regurgitant (Figure 2).

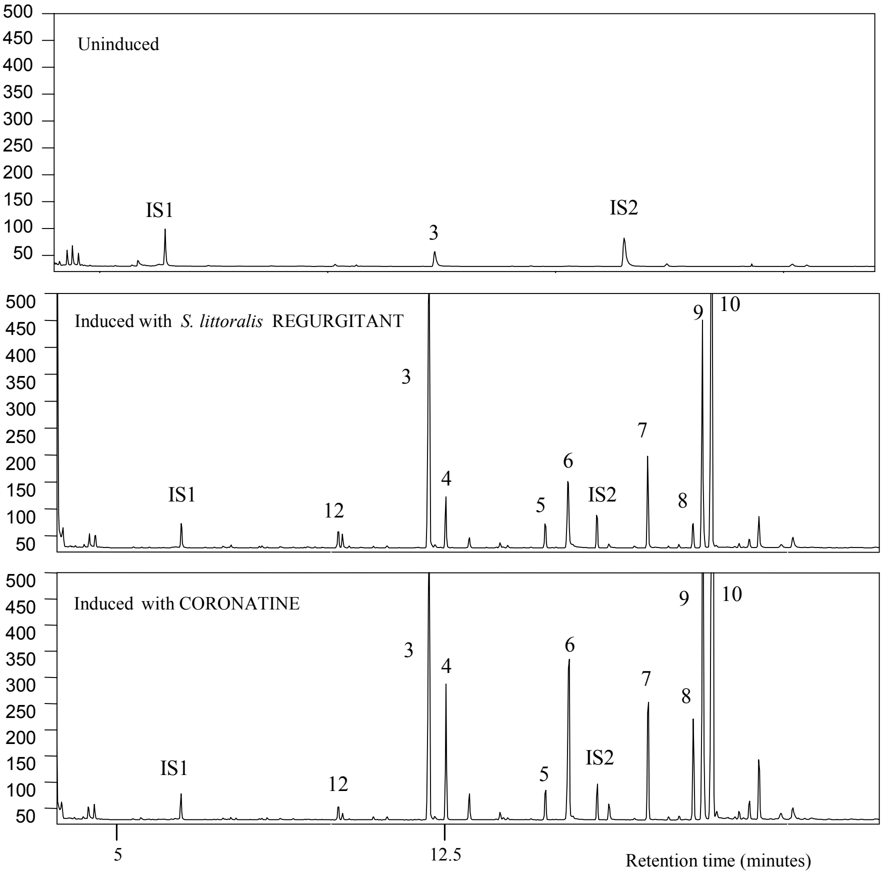

Figure 2: Chromatograms illustrating single compounds (peaks) released by uninduced maize plants (var. Delprim), plants treated with regurgitant of *Spodoptera littoralis* caterpillars and plants treated with coronatine, a pathogen derived elicitor (unpublished data, Fritzsche Hoballah).
The compounds are: **1**, β-myrcene; **2**, (*Z*)-3-hexenyl acetate; **3**, linalool; **4**, (*E*)-4,8-dimethyl-1,3,7-nonatriene; **5**, phenethyl acetate; **6**, 1-H-indole; **7**, geranyl acetate; **8**, (*E*)-β-caryophyllene; **9**, (*E*)-α-bergamotene; **10**, (*E*)-β-farnesene; **17**, **IS1**, n-octane; **IS2**, n-nonyl acetate.

However, in lima bean plants coronatine induced the production of 4,8,12-trimethyltrideca-1,3,7,11-tetraene, but this was not the case for the elicitor jasmonic acid (Koch *et al.*, 1999). This suggests different elicitors may cause different genes to be expressed (Figure 1).

Green leaf volatiles, terpenoids, indole and a few other compounds constitute the blend of induced volatiles in maize. Green leaf volatiles are produced by the *lipoxygenase pathway*, terpenoids by the *mevalonate* and the *alternative isopentenyl pyrophosphate pathway* and indole by *the shikimic acid/tryptophan pathway* (Paré and Tumlinson, 1999). Genes responsible for the expression of these pathways were isolated in some plants (Staswick, 1992; Bell and Mullet, 1993; Arimura *et al.*, 2000). In maize, the gene specifically responsible for production of volatilized indole after treatment of the plant with volicitin was isolated by Frey *et al.* (2000) and a maize gene, responsible for the production of herbivore-induced volatiles, was isolated by Shen *et al.* (2000).

There can be considerable *variability* in quantity and quality of the blends emitted by herbivore damaged plants. This variability is determined by the plant species and genotype, by the herbivore genera and species that feed on the plant and finally by various abiotic factors, like temperature, light, humidity and fertilisation of the soil. There are several induced compounds that are shared among plant species and among plants attacked by different herbivore species (Table 4.2, Chapter 4). Green leaf volatiles are the most common compounds released, while some terpenoids seem to be released only by some species of plants (Table 4.2, Chapter 4). Between varieties of maize, the blends are relatively similar, but certain terpenoids are only released by some varieties and there are extreme quantitative differences in the blends emitted by different varieties (Gouinguené *et al.*, 2001, Figure 3).

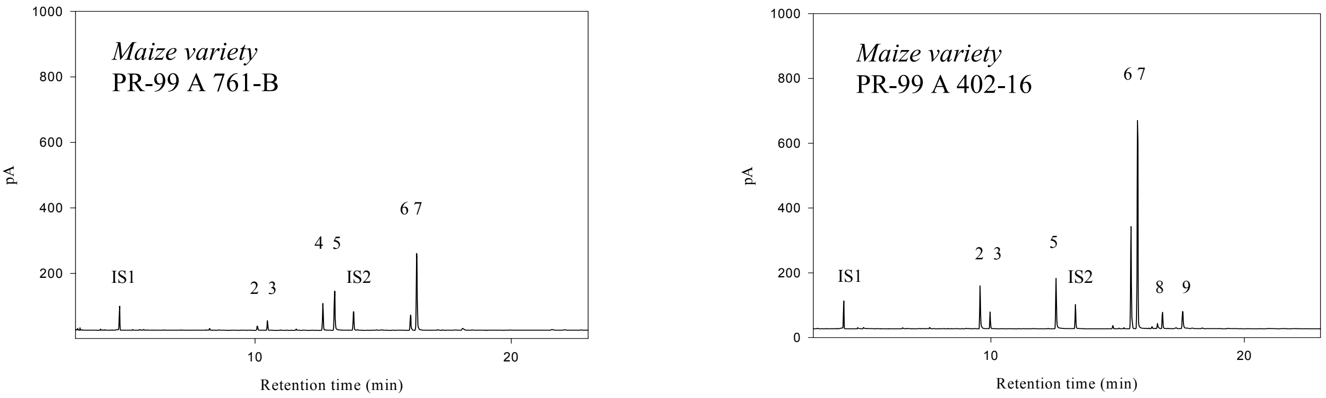

Figure 3: Chromatograms showing the single compounds (peaks) of volatiles collected from two different maize varieties treated with *Spodoptera littoralis* regurgitant. Odors were collected for 3 h 10 h after induction. **2**: linalool; **3**: (*E*)-4,8-dimethyl-1,3,7-nonatriene; **4**: phenethyl acetate; **5**: indole; **6**: (*E*)-α-bergamotene; **7**: (*E*)-β-farnesene; **8**: (*E*)-nerolidol; **9**: (*E,E*)-α-farnesene. **IS1** and **IS2** are the added internal standards n-octane and n-nonyl acetate.

Also among plants of the same species attacked by different herbivores and stages differences in odor blends have been found (Blaakmeer *et al.*, 1994; Takabayashi *et al.*, 1994; Takabayashi and Dicke, 1996; Geervliet *et al.*, 1997; De Moraes *et al.*, 1998; Turlings *et al.*, 1998a). Emissions also differ over time, with same compounds released immediately after the first damage inflicted by herbivores, while other compounds need more time to be synthesised by the plants (Turlings and Tumlinson, 1992; Turlings *et al.*, 1998b; Arimura *et al.*, 2000).

Host habitat location by parasitoids and predators is achieved by the use of very diverse strategies. Foraging strategies of natural enemies of herbivores very often relies on odor signals provided by the habitat of the host, the plant, the host, or emanating from a combination of both plant and its hosts (for reviews see Vinson, 1976; Weseloh, 1981; Stowe *et al.*, 1995). Orientation to odors produced by the interactions of a host and its food, rather than odors released by the host food, would ensure that only habitats containing potential hosts would be searched (Vinson, 1991). This is the case for many natural enemies that have been shown to cueing on odors released by herbivore attacked plants (for review see Turlings and Benrey, 1998; Dicke and Vet, 1999). However, the reliability of cues released by the plants fed on by the host, is limited. In fact, these odors are very similar among different plant-herbivore-complexes and are very *detectable* for natural enemies but not so *reliable* to the host (Vet and Dicke, 1992). The problem of the reliability can be partially solved by the use of *associative learning* that permits parasitoids to associate the odors from the plant-herbivore complex with the suitability of the host (for reviews see Vet and Groenewold, 1990; Turlings *et al.*, 1993). Also learning early during emergence from the cocoon can help parasitoids to orient to the habitat of the host (Hérard *et al.*, 1988).

Much is now know about the mechanisms and elicitors that result in the release of induced plant volatiles, but various questions concerning the function of this response are unanswered. Progresses have been made during the last 12 years in understanding the role of herbivore-induced volatiles in the foraging behaviour of natural enemies of herbivores. However, it remained unexplored if herbivore-induced volatiles truly constitute an indirect defence for plants. Some models that explain the evolutionary stability of such a defence exist (Sabelis and De Jong, 1988; Godfray, 1995), however, experimental evidence of the costs and benefits for the plants of this indirect defence was still lacking. Furthermore, benefits could also comprise antibiotic effects of induced volatiles for plant pathogens, which could even be the primary function of induced volatiles for plants (Turlings and Benrey, 1998). Finally, the feasibility of the often suggested exploitation of the phenomenon of herbivore-induced signalling to enhance biological control (Dicke *et al.*, 1990b; Lewis and Martin, 1990) has not yet been tested. A first step would require testing whether the known differences in odor emissions among crop genotypes is reflected in differential attractiveness of these genotypes for biological control agents.

Thesis outline

The major system used for this study comprises maize *Zea mays*, the herbivore *Spodoptera* spp. and one of its natural enemies, the parasitoid *Cotesia marginiventris* (Figure 4). Maize is an important food crop world wide and *Spodoptera* spp. are important pests of this and other crops, in South America, Africa, the Mediterranean area and Asia. This system was used in studies that attempted to answer some of the remaining questions concerning the costs, benefits and exploitation of herbivore-induced plant volatiles.

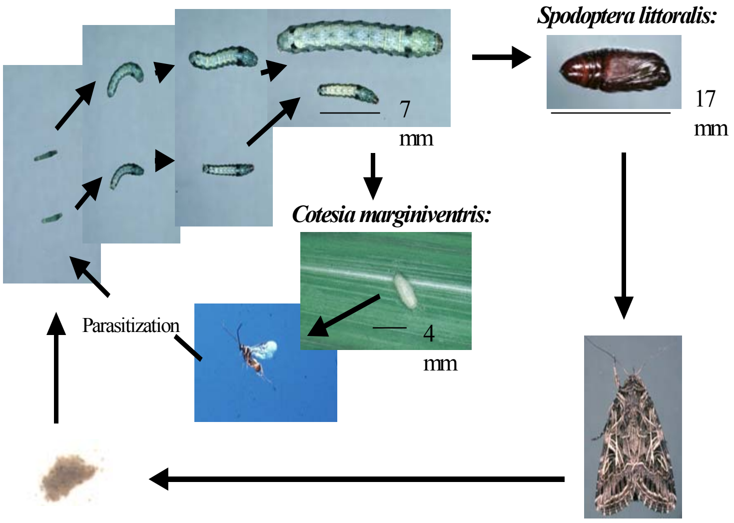

Photo: Y.Borcard & T.Turlings. M.E.Fritzsche

Figure 4: Cycles of the herbivore *Spodoptera littoralis* and the parasitoid *Cotesia marginiventris* that parasitize young caterpillars of the herbivore.

Herbivore-induced volatiles are used by various natural enemies of the herbivores to locate the herbivore host habitat. Thus, the induced volatiles can function as an indirect defence for the plants. In maize, these volatiles are produced within hours after an attack and are known to attract parasitoids such as *C. marginiventris*. To detect the potential of exploiting this indirect defence in biological control programs we first investigated if this parasitoid, once attracted to the maize plant by the induced volatiles and having located and parasitized the herbivore, can benefit the plant in terms of fitness. Secondly, we surveyed the natural enemies in Mexican maize fields that could be used as control agents. Thirdly, we measured the cost of production of volatiles for the plant in terms of fitness. In a subsequent series of experiments, we investigated if it is the quality or the quantity of the odor blend emitted by the plant that is important for the attraction of *C. marginiventris*. Finally, a preliminary study was conducted to test the effects of induced volatiles on infection rate by a pathogen.

The present thesis focuses on the following questions (chapters):

1. *Once a parasitoid is attracted to the plant by herbivore-induced volatiles and a host is parasitized, does the plant directly benefit from the action of this natural enemy?* It has been clearly demonstrated that *C. marginiventris* is attracted to the microhabitat of the host by the use of herbivore induced volatiles. The aim of this study was to assess if plants do indeed benefit from attracting parasitoids. For this purpose, fitness of plants attacked by a parasitized *S. littoralis* caterpillar was compared with the fitness of unharmed plants and plants attacked by a healthy caterpillar. Such comparisons should not only furnish information on the possible role of the natural enemies of the herbivore in the evolution of induced volatiles in plants, but also give a better idea of the potential of using maize varieties that are highly attractive to natural enemies to enhance the efficacy of biological control.

2. *Which insect natural enemies attack* Spodoptera fugiperda *caterpillars on maize and what is there control potential?* In Mexico, different parasitoids attack *Spodoptera frugiperda* caterpillars. Biological control with maize varieties attractive to natural enemies is

envisaged for small scale farming in Latin America. This requires a complete list of the parasitoids and predators of the primary pest, *S. frugiperda*, as well as information on their direct impact of this pest.

3. *What are the costs of the induced production for maize plants?* Costs of induced production of volatiles were analysed in terms of plant performance and fitness. Since maize inbred lines release qualitatively and quantitatively different blends of volatiles after induction by herbivores, a direct comparison could be made between the intensity of the induced response and the production of leaf matter by plants. Plant response was expressed in amount of volatilised and non-volatilised terpenoid production.

4. *Are there certain maize varieties, more attractive than others to* C. marginiventris *and, if so, what determines this differential attractiveness*? Having shown in Chapter 1 that the maize plants attacked by *S. littoralis* caterpillars can directly benefit from the action of the parasitoid *C. marginiventris*, and knowing that different maize varieties release different blends of volatiles if attacked by *S. littoralis*, it was of crucial importance to determine if this parasitoid prefers the odors of specific maize varieties over others.

5. *Which groups of compounds in a specific blend of induced volatiles are the most attractive for* C. marginiventris*?* In Chapter 4 it was found that the odors released by some maize varieties are preferred over others by *C. marginiventris*. From that and other studies was predicted that green leaf volatiles, released by maize plants soon after herbivore attack could be important for the attraction of naive *C. marginiventris*, while experienced wasps are likely to respond to more specifically induced volatile. This

hypothesis was tested and the responses of *C. marginiventris* were compared with those of *Microplitis rufiventris*, a larval parasitoid with more limited host range.

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CHAPTER 1

Experimental evidence that plants under attack may benefit from attracting parasitoids

Based on:

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Some data used for:

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Chattopadhyay, J., Sarkar, R,, Fritzsche Hoballah, M. E., Turlings, T. C. J. and Bersier, L. F.. 2001. Parasitoids may determine plant fitness-a mathematical model based on experimental data. *Journal of Theoretical Biology*, in press.

Abstract

Herbivore-induced plant volatiles have been suggested to function as indirect defence signals that attract natural enemies of herbivores. Several insect parasitoids are known to exploit such plant-provided cues to locate hosts. As yet, it has been unclear if individual plants benefit from the action of parasitoids. We investigated this question in maize plants under attack by *Spodoptera littoralis* larvae and found that parasitization by the endoparasitoids

Cotesia marginiventris and *Campoletis sonorensis* significantly reduced feeding and weight gain in the host larvae. As a result, young maize plants attacked by a single parasitized larva suffered much less feeding damage and at maturity produced about 30% more seed than plants that were attacked by an unparasitized larva. Such fitness benefits may have contributed to selection pressures that shaped the evolution of herbivore-induced indirect defence signals in plants.

Keywords: Cotesia marginiventris, *Campoletis sonorensis, Spodoptera littoralis*, *Zea mays*, parasitoids, plant-insect interactions, plant fitness, indirect defence

Introduction

Herbivore-induced chemical defences in plants can be direct, resulting in antibiosis and antixenosis (as a result of toxic and deterrent chemicals, respectively), or indirect, for example in the form of volatiles that attract natural enemies of the herbivores. Surprisingly few studies demonstrate that herbivore-induced chemical changes in plants enhance plant fitness under field conditions (Karban and Baldwin 1997; Baldwin 1999; Agrawal and Karban 1999). In one such study, Baldwin (1998) showed that tobacco plants benefit from the induced production of toxic compounds (direct defence) under intermediate rates of herbivore attack and produce more seeds if they are induced than when they are not induced. Agrawal (1998, 1999), in a field experiment with wild radish, found that early season caterpillar feeding resulted in a decrease in subsequent herbivory and consequently in enhanced seed production.

Fitness benefits resulting from indirect defences have been well documented for plants that attract ants with domatia and/or food (e.g. Janzen 1966; McKey 1988; Oliveira 1997). But, as pointed out by several authors (Sabelis and de Jong 1988; Faeth 1994; van der Meijden and Klinkhamer 2000; Hare 2001), evidence has been lacking for potential fitness benefits for plants resulting from attracting natural enemies with herbivore-induced volatiles. Although it has been clearly demonstrated that natural enemies such as parasitoids make effective use of induced plant volatiles to locate the microhabitat of their hosts (e.g. Turlings *et al.* 1990; Steinberg *et al.* 1993; McCall *et al.* 1993; Mattiacci *et al.* 1994; Finidori-Logli *et al.* 1996; Ngi-Song *et al.* 1996; Guerrieri *et al.* 1998; De Moraes *et al.* 1998), it is not always evident that plants will benefit from attracting these parasitoids. Many parasitized Lepidoptera larvae continue to feed and in some cases they may even consume more than if they were not parasitized (Rahman 1970; Hunter and Stoner 1975; Slansky 1978; Beach and Todd 1986). On the other hand, numerous solitary parasitoids attack early instar larvae and seem to significantly reduce the development and feeding rate of their host (Rahman 1970; Duodu and Antoh 1984; Hegazi *et al.* 1988; Grossniklaus-Bürgin *et al.* 1994; Schopf and Steinberger 1996; Kruse and Raffa 1999). Evidence that parasitoids benefit plants comes from a study on seed feeding herbivores that show increases in seed production by host plants due to parasitization of the herbivores (Gómez and Zamora, 1994). A notable recent study by van Loon *et al.* (2000) shows that *Arabidopsis thaliana* plants produce considerably less seed after herbivory by healthy larvae of *Pieris rapae* (Lepidoptera: Pieridae) than after herbivory by larvae that were parasitized by the solitary endoparasitoid *Cotesia rubecula*. In such cases where reduction in feeding by the larvae enhances the performance of a plant, it could be a selective advantage to the plant to assist such parasitoids in finding the caterpillars.

The responses to herbivore-induced plant volatiles have been extensively studied for the New World endoparasitoids *Cotesia marginiventris* (Cresson) (Hymenoptera: Braconidae) and *Campoletis sonorensis* (Cameron) (Hymenoptera: Ichneumonidae). These solitary parasitoids attack mostly young lepidopteran larvae (Jalali *et al.* 1987, Rajapakse *et al.* 1992) on various plants, including crops such as maize, soybean and cotton. For *C. marginiventris* it has been shown that females are highly attracted by the odors emitted by caterpillar-damaged maize seedlings (Turlings *et al.* 1990) and that they spend more time on such plants than on artificially damaged or unattacked plants (Loke *et al.* 1983). *C. sonorensis* is also attracted to the odors of plants attacked by its hosts (Elzen *et al.* 1984; McAuslane *et al.* 1991) and was found to remain longer on host-damaged cotton plants than on undamaged plants (Baehrecke *et al.* 1990). The exploitation of plant-provided chemical cues by parasitoids can be expected to enhance parasitism rates, but it is not yet clear if the plants benefit from this interaction. If increased parasitism yields fitness benefits for herbivore-attacked plants, parasitoids may have contributed to selection pressures that shaped the evolution of herbivore-induced indirect defence signals in plants.

In the current study, we tested if parasitism by *C. marginiventris* increased the fitness of maize plants attacked by larvae of *Spodoptera littoralis* (Boisd.) (Lepidoptera: Noctuidae). In a first experiment, we confirmed a dramatic difference in weight gain during development between larvae that were unparasitized or parasitized by either *C. marginiventris* or *C. sonorensis*. In a series of subsequent experiments, we demonstrated that parasitism reduced feeding damage on individual plants and that such a reduction in damage increased seed production.

Material and methods

Origin of insects and plants

Zea mays plants (var. Delprim) were grown from seed in pots (360 ml, 10 cm diam., 8 cm high) with regular potting soil (COOP, Switzerland) and kept in a climate chamber (23°C, 60% r.h., 16D: 8L, 50 000 lm/m^2). Maize seedlings were used for experiments 2 weeks after planting when the plants had 4 fully developed leaves.

Spodoptera littoralis larvae were used as hosts for the parasitoids and reared under ambient laboratory conditions. Host-eggs were supplied by Novartis (Switzerland) weekly and were incubated in a Petri dish (9 cm diam., 1.5 cm high) on humidified filter paper. Eclosed larvae were supplied daily with fresh pieces of maize leaves and kept in plastic-boxes (15x9x5 cm).

The New World endoparasitoids *Cotesia marginiventris* and *Campoletis sonorensis* originated from the USDA-ARS, Biological Control and Mass Rearing Research Unit (Mississippi, USA) and from our own field collections in Poza Rica (Veracruz, Mexico), respectively. For practical reasons parasitoids were reared on the Old World host *S. littoralis*. Both parasitoid species are generalists and their development and survival on this foreign host was the same as on their natural host *S. frugiperda* (Fritzsche-Hoballah, unpublished data). For the rearing of the parasitoid, 25 caterpillars (3-4 days old) were offered to a single mated female parasitoid (3-7 days old) in a plastic-box (9.5 cm diam., 5 cm high). After 2 hr the female was removed and caterpillars were reared on fresh maize leaves until cocoon formation. Cocoons were kept in Petri dishes until adult emergence. Emerging adults were sexed and kept in cages (30x30x30 cm) at a sex ratio of 0.5:1 (male:female), with drops of honey and distilled water on cotton wool. Parasitoids were kept in the laboratory under ambient light and temperature conditions.

Weight gain during development of parasitized and unparasitized S. littoralis larvae

In a first experiment, we compared weight gain during development of parasitized and unparasitized *S.littoralis* larvae. One mated female (3-5 days old) of either *C. marginiventris* or *C. sonorensis* was introduced into a plastic-box (9.5 cm diam., 5 cm high) with 25 *S. littoralis* larvae (3 days old). Larvae were assumed to be parasitized when they were observed to be stung by a parasitoid. From approximately 70% of these larvae a parasitoid emerged. The first 5 larvae stung by a single parasitoid were used for the experiment; the other 20 larvae were discarded. With both parasitoids species 60 larvae were parasitized. An additional 30 control larvae were left unparasitized. Unparasitized larvae originated from the same egg batches as parasitized larvae. The larvae were kept singly in small Petri dishes (5cm diam., 2 cm high) in an incubator (25 ºC, 85% r.h., 11L: 13D). Food (fresh pieces of maize leaves) was replaced daily. Weight of larvae was assessed starting from the 6th day after eclosion until the unparasitized *S. littoralis* larvae pupated or the emergence of parasitoid larvae from parasitized *S. littoralis*. Only data of parasitized larvae that yielded a cocoon and unparasitized larvae that yielded a pupa were used for analysis. Larval weights were ln-transformed to obtain homogeneity of variance. Then, the following statistical model was fitted to each treatment group:

$$\ln(\text{weight}) = a(\text{day})^2 + b(\text{day}) + c$$

and a 95% confidence interval was computed for each day. A t-test was carried out to compare development time between larvae parasitized by *C. marginiventris* and *C. sonorensis*.

Dry weight of young maize plants after an attack by a parasitized or unparasitized S. littoralis *larva*

During a second experiment, dry weight of stems and leaves of maize seedlings attacked by a parasitized or an unparasitized larva was measured. Three-days-old larvae were parasitized by *C. marginiventris* and left for 3 days on fresh maize leaves. Unparasitized larvae of the same egg batch were placed in a separate box on the same diet. After 3 days each larva, parasitized or unparasitized, was placed on an individual 13-day old maize plants. A cellophane bag allowing gas exchange (30x15.5 cm; quality 400P, Celloclair AG, Liestal, Switzerland) was placed over each plant to prevent the larvae from escaping. Plants were kept in a climate chamber (23°C, 60% r.h., 16L:8D, 50 000 lm/m^2) until the last pupae were formed (in the unparasitized treatment). Only data of parasitized larvae that yielded a cocoon and unparasitized larvae that yielded a pupa were used for analysis. At this time, the plants were dried for 4 days at 80 °C and the weight of leaves and stems was determined. Analysis of variance and Fisher's PLSD post-hoc test were used to compare dry weights among treatments.

Seed production by maize plants after an attack by a parasitized or unparasitized S. littoralis *larva*

In a third experiment, we assessed the yield of maize plants after an attack by a single parasitized or unparasitized larva. The initial part of the experiment was the same as in the previous experiment until the larvae stopped feeding on their respective plants. After formation of host pupae (in the unparasitized treatment) the plants were taken from the climate chamber and transplanted in an open plot (5x10 m) in a farmer's maize field (Marin, Switzerland), early during the local maize growing season. The plants were planted randomly, 50 cm apart and left to grow until maturity. One month before maturity the height of each plant was measured (n=13 for control plants, n=16 for plants attacked by *C. marginiventris*-parasitized larvae, n=24 for plants attacked by unparasitized larvae). At maturity, the plants were removed and the number of ears and seeds and the dry-weight of seeds of individual maize plants were determined. The number of replicates was different for each treatment because several plants were destroyed by vandals: n=8 for control plants, n=14 for plants attacked by parasitized larvae, n=17 for plants attacked by unparasitized larvae. Analysis of variance and Fisher's PLSD post-hoc test were used to compare height, dry weight of seeds and number of seeds and ears among treatments.

Results

Weight gain during development of parasitized and unparasitized S. littoralis *larvae*

Weight gain in parasitized larvae was considerably lower than that for unparasitized larvae (Fig. 1A). Fitted functions (program S-Plus) for the 3 treatment groups were:

$\ln(\text{weight}) = = -0.0370(\text{day})^2 + 1.3971(\text{day}) - 12.9651$ (non parasitized larvae, n=29)

$\ln(\text{weight}) = -0.0442(\text{day})^2 + 1.1615(\text{day}) - 11.4221$ (*C. sonorensis* parasitized, n=40)

$\ln(\text{weight}) = = -0.0525(\text{day})^2 + 1.2991(\text{day}) - 11.9859$ (*C. marginiventris* parasitized, n=37)

Estimated values of the non parasitized treatment group were significantly higher then values from the parasitized groups starting from day 7 after parasitization (Fig. 1.1B). There was no difference in weight gain between larvae parasitized by the two parasitoid species *C. marginiventris* and *C. sonorensis* (Fig. 1.1B).

Furthermore, the period of growth was shorter for parasitized larvae (approximately 11 days) than for the unparasitized larvae (approximately 15 days) (Fig. 1.1). The parasitoid larvae emerged from 4th instar hosts, which died soon after, while unparasitized larvae pupated at the 6th instar. The final weight of parasitized larvae was 13.8 % and 14.5 % for *C. marginiventris* and *C. sonorensis* parasitized larvae, respectively, of the weight of unparasitized larvae at the same age and 2.61 % and 2.75 % of the final weight of unparasitized larvae.

Development time until cocoon formation was shorter for the parasitoid *C. marginiventris* than for *C. sonorensis* (13.16±0.1 (mean±SE) vs. 14.15±0.1 days, *P*<0.0001, t =-7.07, d.f.=75). This was also reflected in an earlier emergence of the adults after initial parasitization (18.11±0.1 (mean±SE) vs. 20.57±0.1 days, *P*<0.0001, t=-15.75, d.f.=75).

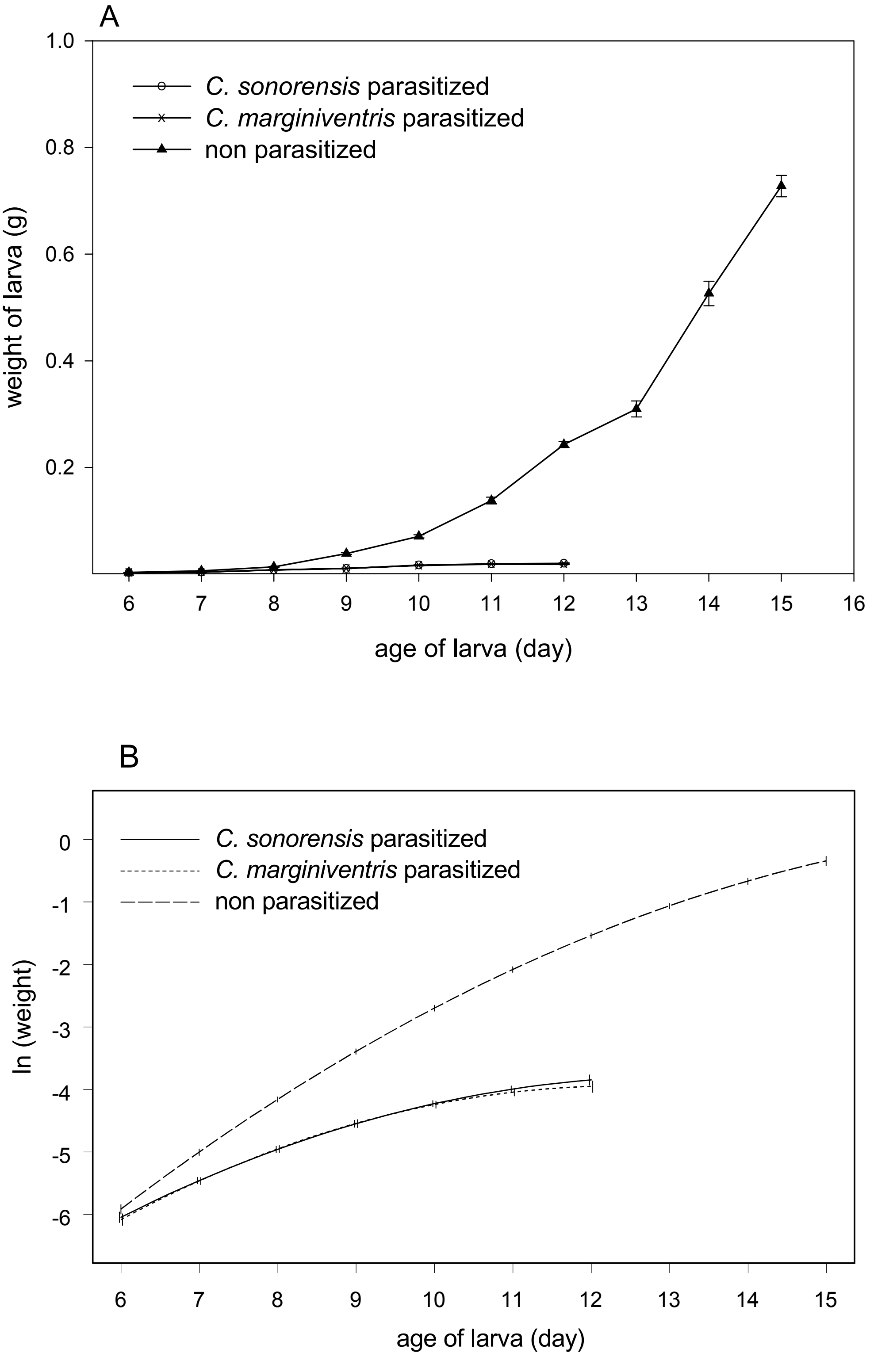

Figure 1.1: A: Mean weight (± SE) during development of *Spodoptera littoralis* larvae: parasitized by *Campoletis sonorensis* (x, n=40) and by *Cotesia marginiventris* (O, n=37) or let unharmed (▲, n=29). B: Fitted functions for the 3 treatment groups with predicted values of the model with 95% confidence intervals of logarithmic transformed data.

Dry weight of young maize plants after an attack by a parasitized or unparasitized S. littoralis *larva*

No difference in dry weight of stems was found between plants attacked by parasitized or unparasitized caterpillars and control plants (Fig. 1.2A, *P*=0.7217, F=0.329, d.f.=2). However, dry weight of leaves of plants attacked by a unparasitized larva was about a third of that of control plants and plants attacked by a parasitized larva (Fig. 1.2B, *P*<0.0001, F=34.921,d.f.=2. Fisher's PLSD post hoc test: parasitized-unparasitized *P*<0.0001, control-parasitized *P*=0.0379, control-unparasitized *P*<0.0001).

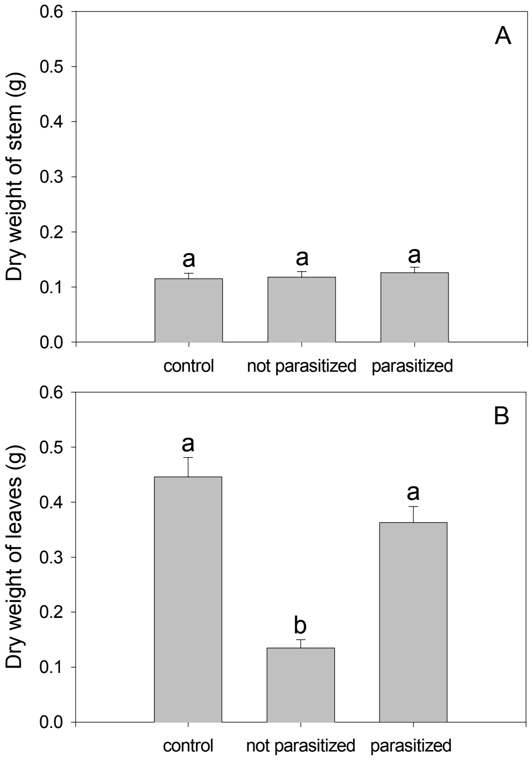

Figure 1.2: Mean dry weight (±SE) of stem (A) and leaves (B) of maize seedlings left unharmed (n=13), infested with one unparasitized larva (n=17), or infested with one *S. littoralis* larva parasitized by *C. marginiventris* (n=19). Different letters above bars indicate significant differences among treatments.

Seed production by maize plants after an attack by a parasitized or unparasitized S. littoralis *larva*

One month after transferring the plants into the field (i.e. 1-month before assessing yield) a significant difference in plant height was measured. Plants that had been attacked by an unparasitized larva were significantly shorter than control plants, while the height of plants that were attacked by a parasitized larva was not significantly different from the height of control plants (Fig. 1.3, *P*=0.0097, F=5.093, d.f.=2. Fisher's PLSD post hoc test: parasitized-unparasitized *P*=0.0578, control-parasitized *P*=0.2520, control-unparasitized *P*=0.0034). At the end of the season, maize seedlings attacked by parasitized *S. littoralis* larvae had the same yield (expressed in number of ears and seeds and dry weight of seeds) as control plants, which was significantly higher than the yield of plants that had been attacked by unparasitized larvae (Fig. 1.4. Fig. 1.4A: *P*=0.0008, F=8.803, d.f.=2. Fisher's PLSD post hoc test: parasitized-unparasitized *P*=0.0038, control-parasitized *P*=0.2558, control-unparasitized *P*=0.0005. Fig. 1.4B: *P*=0.0008, F=8.702, d.f.=2. Fisher's PLSD post hoc test: parasitized-unparasitized *P*=0.0028, control-parasitized *P*=0.3515, control-unparasitized *P*=0.0008. Fig. 1.4C: *P*=0.0010, F=8.432, d.f.=2. Fisher's PLSD post hoc test: parasitized-unparasitized *P*=0.0054, control-parasitized *P*=0.2305, control-unparasitized *P*=0.0006).

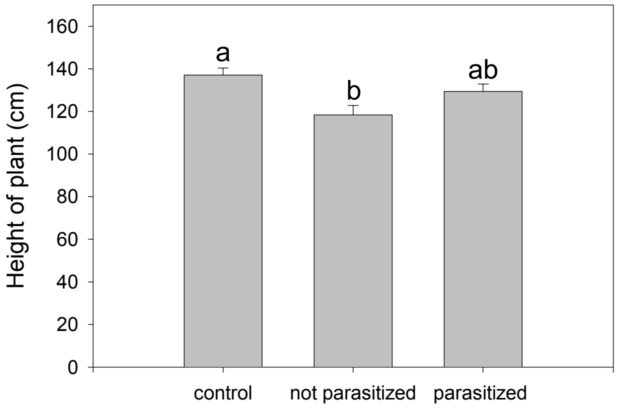

Figure 1.3: Mean height of plants (±SE) measured 1 month before plant maturity: plants were either left unharmed (n=13), infested with one unparasitized larva (n=24), or infested with one *S. littoralis* larva parasitized by *C. marginiventris* (n=16). Different letters above bars indicate significant differences among treatments.

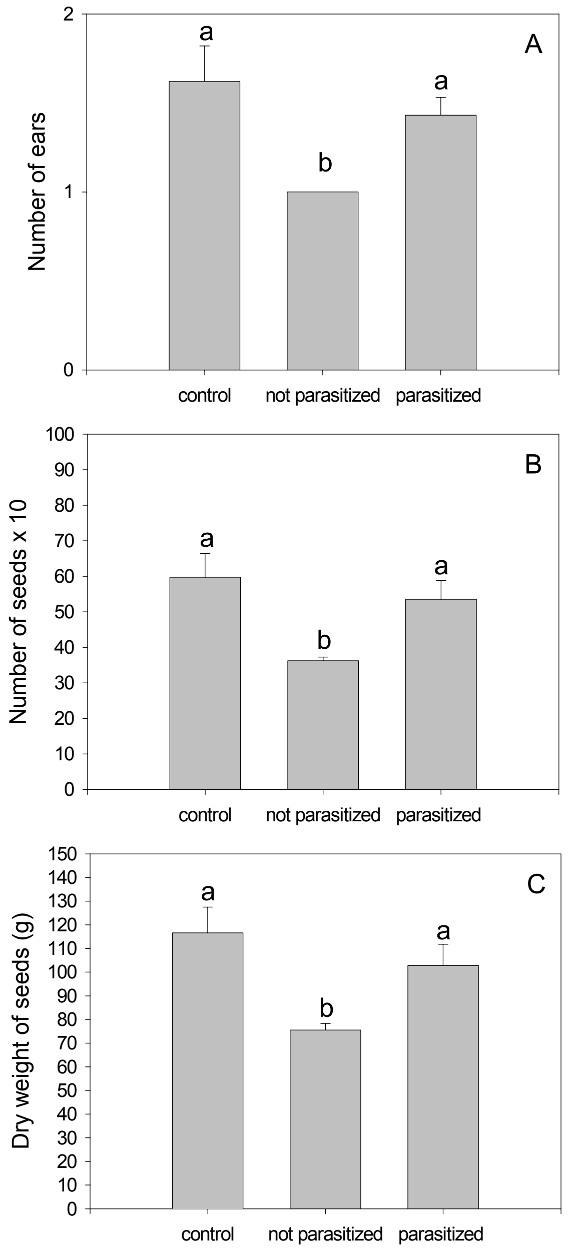

Figure 1.4: Average number of ears (A), number of seeds (B) and mean dry weight of seeds (C) (±SE) of maize seedlings left unharmed (n=8), infested with one unparasitized larva (n=17), or infested with one *S. littoralis* larva parasitized by *C. marginiventris* (n=14). Different letters on bars indicate significant differences among treatments.

Discussion

Our results show that herbivore-infested plants may benefit from the activity of parasitoids even if these do not instantaneously kill their herbivorous hosts. Seed production by maize plants that, at an early stage of development, were subjected to an attack by a single healthy *S. littoralis* larva was about 30% lower than that of plants that had been attacked by a parasitized larva. In fact, the plants that had carried a parasitized larva produced as many seeds as unattacked control plants (Fig. 1.4). This simple experiment shows that the fitness of a plant can increase due to parasitization of herbivores that feed on it. Van Loon *et al.* (2000) obtained comparable results with the *Arabidopsis thaliana*, which produces more seeds when it is eaten by *Pieris rapae* larvae parasitized by *Cotesia rubecula* than when it is eaten by unparasitized larvae. These findings may have important implications for understanding the evolution of plant traits that promote the presence of the third trophic level.

Previous studies had already shown that the solitary endoparasitoid *Cotesia marginiventris* significantly reduces weight gain of its hosts. Ashley (1983) found an average reduction in final weight of 93% in the host *S. frugiperda* and Jalali *et al.* (1988) reported that the host *S. litura* consumes significantly less food starting 72h after being parasitized. Similar results were obtained by McCutcheon *et al.* (1991) for *Pseudoplusia includens* (Lepidoptera: Noctuidae) after parasitization by *C. marginiventris*. No such studies had been conducted with *C. sonorensis*, but a related species *C. flavicincta* caused reduced foliar consumption in the host *S. frugiperda* (Van-Cruz-Maria *et al.* 1997). The current study confirms these results. The average maximum weight of *S. littoralis* larvae parasitized by either *C. marginiventris* or *C. sonorensis* was less than 3% of the final weight of healthy larvae. This considerable effect on host development was shown to significantly reduce the amount of leaf tissue that the larvae consumed (Fig. 2). Despite ample evidence for reduced feeding by parasitized herbivorous hosts, there appear to be no previous studies on the fitness consequences for plants on which these herbivores feed.

The frequently demonstrated emissions of herbivore-induced volatiles have been suggested to have a defence function, in which they serve as signals to attract natural enemies of herbivores (Vet and Dicke 1992; Turlings and Benrey 1998, Dicke 1999). Such a function would require that the attraction of natural enemies enhance plant fitness. It appears obvious that this is the case when predators are attracted, as they can immediately kill a herbivore and instantaneously stop their damaging effects (Sabelis and de Jong, 1988). Experimental evidence for selective pressures favouring such signalling function had been missing. This is especially critical for the attraction of parasitoids (Sabelis and de Jong 1988; Baldwin 1998; Karban *et al.* 1997; Turlings and Benrey 1998; van der Meijden and Klinkhamer 2000), which commonly allow their hosts to further develop and in some cases cause their hosts to eat more than if they are not parasitized (Rahman 1970; Hunter and Stoner 1975; Slansky 1978; Beach and Todd 1986). Van der Meijden and Klinkhamer (2000) and Hare (2001) list several criteria or research objectives that they feel are needed to demonstrate that natural enemies are agents of natural selection on plants. The most important criterion to be satisfied seems to be that "in the presence of both herbivores and natural enemies, plant fitness should be greater for plants expressing the natural enemy-enhancing trait than for those that do not" (Hare 2001). The current study was conducted to investigate whether or not parasitoids can increase plant fitness.

Our results demonstrate that parasitoids have the potential to reduce herbivory to an extent that it can increase a plant's fitness. We used a system for which the role of induced volatiles in parasitoid host location is well studied and the induced odor emissions in cultivated maize are very similar to that of its wild ancestors (Gouinguené *et al.* 2001). The conditions under which the plants were subjected to larval attack were realistic. Although *S. frugiperda* lays clusters of eggs, as a result of rapid dispersal and cannibalism only one larva is commonly found on small plants in maize fields in the sub-tropical lowlands of Mexico (personal observation, Fritzsche-Hoballah). Moreover, of eight species of parasitoids that we reared from *S. frugiperda* larvae collected in maize fields near Poza Rica (Mexico), four always emerged from larvae with a size similar to that of unparasitized 2nd or 3rd instar *S. frugiperda* larvae (unpubl. data). As pointed out by van Loon *et al.* (2000), it appears that all species of solitary parasitoids of Lepidoptera reduce food consumption in their host. How the herbivore-parasitoid interactions affect plant fitness under natural conditions will depend on many factors, but in cases were parasitoids reduce herbivory as drastically as shown here, they are likely to have a significant positive effect. It can therefore be expected that, under certain circumstance, plants that are able to lure in more parasitoids with volatiles will increase their fitness by doing so. However, we have to consider other interactions that will be affected by the volatiles and associated chemical compounds, which can have either positive or negative consequences for the performance of a plant (Turlings and Benrey 1998; Dicke and Vet 1999).

Herbivore-induced volatiles emitted by plants can also play a role in direct defence. In some cases, particularly those involving beetles, induced changes can attract herbivores (Dicke and Vet 1999). However in many cases, the changes render the plants less appealing to herbivores. For example, mites and aphids are repelled by plant volatiles induced by conspecifics (Dicke and Dijkman 1992; Bernasconi *et al.* 1998). Induced plant changes also have been shown to negatively affect consumption by *Spodoptera* species. Edwards *et al.* (1985) found a nine-fold reduction in the area consumed by *S. littoralis* if tomato leaves are artificially damaged. Induced maize plants are less palatable to *S. exigua* larvae (Turlings and Tumlinson 1991). *S. littoralis* larvae take fewer and shorter meals on leaves from previously wounded plants than on control leaves (Barker *et al.* 1995). Both *S. exigua* and *S. littoralis* prefer leaves of undamaged over leaves of damaged cotton plants and *S. littoralis* larvae perform very poorly on previously damaged cotton plants and may eventually die (Alborn *et al.* 1996). These and various other consequences of induced changes in plant defence chemistry suggest that the outcome of their combined effects can be expected to vary tremendously.

Conclusive evidence that, under natural conditions, plant-released volatiles increase the likelihood that natural enemies will attack herbivores on the signalling plants is still lacking, but indirect evidence is accumulating. For instance, field studies by Drukker *et al.* (1995) and Shimoda *et al.* (1997) show that predators (anthocorid bugs and the predatory thrips *Scolothrips takahashii*) are more attracted to attacked plants than to control plants. Most convincing are the direct field observations by De Moraes *et al.* (1998), in which females of the braconid parasitoid *Cardiochiles nigriceps* visited plants that were damaged by its specific host *Heliothis virescens* much more frequently than plants attacked by a non-host or plants that were undamaged. This distinct attractiveness was even observed when the larvae and damaged leaves had been removed. Moreover, Thaler (1999) found that parasitism of *Spodoptera exigua* larvae by the ichneumonid *Hyposoter exiguae* was higher on field-grown-tomato plants sprayed with jasmonic acid to induce volatile emissions compared to controls. These field studies and many preceding laboratory studies strongly suggest that the induced plant odors increase the chances that herbivores are attacked by their natural enemies. The study by van Loon *et al.* (2000) and the study presented here show that increases in attack rates by parasitoids may benefit plants. Therefore, these members of the third trophic level may contribute to the selective pressures shaping plant traits that promote the effectiveness of natural enemies.

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CHAPTER 2

Occurrence and direct control potential of parasitoids and predators of the Fall Armyworm (Lepidoptera: Noctuidae) on maize in the subtropical lowlands of Mexico

Based on:

Fritzsche Hoballah, M. E.; Degen, T.; Bergvinson, D.; Savidan, A.; Tamò, C.; Turlings, T. C. J. Occurrence and direct control potential of parasitoids and predators of the Fall Armyworm (Lepidoptera: Noctuidae) on corn in the subtropical lowlands of Mexico. In preparation for submission to *Biological Control*.

Abstract

Natural enemies have the potential to control fall armyworm *Spodoptera frugiperda* (Smith) in tropical maize grown in Mexico, where this pest insect causes economic losses to farmers. This study focused on the immediate effect of the natural enemies on herbivory by fall armyworm. Caterpillars were collected in maize fields near Poza Rica in the state of Veracruz, Mexico, during January and February 1999, 2000 and 2001. Plants were either naturally infested by *S. frugiperda*, or artificially infested with laboratory-reared larvae. Ten species of parasitoids were reared from the collected larvae and nine species of predators that are known to feed on larvae and eggs were observed on the plants. *Campoletis sonorensis* was the dominant parasitoid species, in 1999 and 2001. Studies on the adaptive role of herbivore-induced plant odors in host location by parasitoids show that solitary wasps that attack early instar larvae may reduce herbivory to such an extent that plant yield is increased. Of the larval parasitoids we collected, five reduce herbivory, while three caused the host to eat more. This has important implications for the evolutionary role of parasitoids in contributing to selection pressures that have shaped indirect defences in plants. Moreover, the results indicate that enhancing the effectiveness of solitary endoparasitoids may benefit subsistence farmers in developing countries immediately through reduced herbivory by parasitized larvae. Improvements on methodology to quantify the impact of native natural enemies of *S. frugiperda* are discussed.

Key Words- Maize, *Spodoptera frugiperda*, fall armyworm, *Diatraea saccharalis*, sugar cane borer, parasitoids, *Aleiodes laphygmae*, *Campoletis sonorensis*, *Chelonus insularis*, *Cotesia marginiventris*, *Euplectrus plathypenae*, *Homolobus truncator*, *Meteorus laphygmae*, *Ophion flavidus*, *Pristomerus spinator*, *Trichogramma atopovirilia*, *Zea mays*, Mexico, biological control, pest management, predators, *Castolus*, *Coleomegilla*, *Chrysopa*, *Doru*, *Orius*, *Podisus sagitta*, *Zelus longipes*, resistance, induced volatiles

Introduction

Spodoptera frugiperda is one of the most destructive insect pests of maize in the Americas (Kranz *et al.*, 1977). First historical yield loss was registered in the United States in 1899 (Cruz, 1995). Since then various efforts have been undertaken to control this pest. Chemical control is still needed during times of outbreaks (Kranz *et al.*, 1977). Maize varieties resistant to fall armyworm have been selected and used in some countries (Mihm *et al.*, 1988). However, to achieve sustainable control of this pest, that delays its adaptation to resistant varieties, an effective biological control would be desirable. The combined use of resistant maize varieties and biological control may be further improved by enhancing the attractiveness of the varieties to natural enemies. It has been shown in field assays that several parasitoids and predators are attracted to odors released by plants that carry the respective host and prey of these natural enemies (Drukker *et al.*, 1995; De Moraes *et al.*, 1998; Thaler, 1999; Kessler and Baldwin, 2001). Volatiles attractive to parasitoids are known to be released also by maize plants under caterpillar attack (Turlings *et al.*, 1991c). The production of these odors is systemic and induced by an elicitor present in caterpillar regurgitant (Alborn *et al.*, 1997). There is high variability among different maize varieties in the quality and quantity of the induced odor blends (Gouinguené *et al.*, 2001), and therefore it may be possible to breed varieties that are highly attractive to natural enemies. It has been shown that plants can directly benefit from the action of parasitoids in terms of fitness (van Loon *et al.*, 2000; Fritzsche Hoballah and Turlings, 2001). Such benefits may contribute to the selection pressures that shape the indirect defence of plant traits (Hare *et al.* 2001, Turlings *et al.* 2001).

A maize plant can produce about 30 % more seeds if the caterpillar attacking the plant is parasitized by *Cotesia marginiventris* (Hymenoptera: Braconidae) than if it is not parasitized (Fritzsche Hoballah and Turlings, 2001). It is unclear how common it is that the various parasitoids attacking *S. frugiperda* reduce feeding by their hosts. For an overall benefit to the plant, parasitizations should lead to reduce herbivory.

The introduction and augmentation of several natural enemies rather than only one has been suggested for biological control of fall armyworm (Miller and Ehler, 1978; Riggin *et al.*, 1993; Takagi and Hirose, 1994; Mills, 1994). However, surveys of potential natural enemies and their efficiency to control armyworm populations are still lacking. Listings of parasitoids of *S. frugiperda* exist, but are still few (Ashley, 1986), as well as studies on predators of fall armyworm (Van Huis, 1981; Andrews, 1988; Cruz, 1995). In the current study, we recorded and identified parasitoids that we obtained from *S. frugiperda* larvae collected from maize plants and predators that were observed feeding on caterpillars, near Poza Rica (20.492N; 97.547 W) (Veracruz, Mexico). Some larvae from another maize pest, the sugarcane borer *Diatraea saccharalis* were also collected and emerging parasitoids recorded. Using our own studies and those of others on the effects of parasitoids on feeding rate by their host, we compared the potential of the recorded parasitoids to directly reduce herbivory by their hosts. The results of this study are discussed in the context of the possibility to improve pest control with native natural enemies in small-scale subsistence farming.

Material and methods

Infestation of maize plants with S. frugiperda *larvae.* This study was part of a project designed to determine the effect of induced volatile emission in maize plants on the herbivores and their natural enemies. We artificially infested maize plants with first instar larvae of *S. frugiperda*. The experiments were carried out at the International Maize and Wheat Improvement Center (CIMMYT) station in Lindero near Poza Rica (state of Veracruz, Mexico, 60 masl). Maize variety Insect Tolerant Synthetic (ITS) G1 (white) was used in 1999 and 2000. Three and 4 field plots (5 m x 10 m), consisting of 400-500 maize plants each (4-5 leaf stage), were used in 1999 and 2000, respectively. Plant spacing was 20 cm within and 50cm between rows with 14 rows per plot. One week prior to infestation, insecticide (Lannate 90, DuPont) was applied in all plots at a rate of 125 g a.i./ha to eliminate *S. frugiperda* larvae and other herbivorous insects on the plants. Groups of 3 neighbouring plants were chosen randomly and infested with fall armyworm larvae, 2 groups per row per plot. *S. frugiperda* larvae were obtained from a colony reared on an artificial diet at CIMMYT (Texcoco, state of Mexico) and were 4 days old (first instar) when they were used for infestation. Each plant was infested with 20 larvae. In 1999, 20 groups of three plants were infested starting January 21 at weekly intervals, for three weeks. In 2000, 20 groups of three plants were infested starting January 31, four times at weekly intervals. Larvae were placed onto plants in the morning. Predators that were observed feeding on *S. frugiperda* larvae during between 9 and 10 am, 12-13 pm and 17-18 pm in january and february 1999 and 2000 in the maize field were recorded. The third day after infestation, infested plants were placed into a paper bag. In the laboratory, all plants were carefully examined for fall armyworm larvae and egg masses of *S. frugiperda* and *Diatraea saccharalis* and *D. saccharalis* larvae. *S. frugiperda* larvae were placed singly in plastic cups (4 cm top dia., 2 cm bottom dia., 4 cm high) because of their cannibalistic behaviour, and reared on artificial diet until formation of the pupa. Parasitoid emergence was recorded.

Collection of naturally occuring S. frugiperda *larvae.* In 2000 and 2001, *S. frugiperda* larvae of different stages were collected from maize fields in two locations, Lindero and Agua Fria, near Poza Rica. Larvae collected from maize plants were brought to the laboratory and placed singly in cups with artificial diet until pupa formation. Parasitoid cocoon formation was checked regularly. Emerging parasitoids were kept and later identified. Identification was done by the use of a manual (Cave, 1995) and confirmation was done by R. Cave (Zamorano Escuela Agrícola Panamericana, Honduras).

Results

Parasitoids of S. frugiperda.

In 1999, more than half of the larvae were recovered 3 days after their release (Table 2.1). In 2000, less then 25% of the caterpillars were recovered after 3 days (Table 2.2). Only the parasitoid *Campoletis sonorensis* was reared from the larvae collected in 1999. The highest parasitization rate among the three replications was 10% (Table 2.1). In contrast, in 2000, several parasitoid species were found, but parasitization rates were low, between 0.7% and 4.1% (Table 2.2).

Table 2.1 Number of insects found on maize plants that were iinfested with 20 *S. frugiperda* larvae 2 days before sampling.

On maize plants	21 January 1999	28 January 1999	5 February 1999
S. frugiperda **larvae**	573/1420*	1024/1420*	833/1260*
aphids	159	298	277
thrips	48	121	70
spiders	20	21	6
***Orius* sp.**	17	4	2
***Chrysopa* sp. eggs**	0	39	26
C. sonorensis	12 (2.1%)	103 (10.05%)	0

*total larvae released on plants, (%) percent parasitized caterpillars

Table 2.2 Number of insects found on maize plants that were infested with 20 *S. frugiperda* larvae 2 days before sampling.

On maize plants	31 January 20007	February 2000	14 February 2000	21 February 2000
N *S. frugiperda* larvae	459/1200*	269/1200*	242/1200*	245/1200*
N aphids	6/60°	3/60°	4/39°	4/35°
N thrips	558	598	497	407
N spiders	15	6	10	33
N *Orius* sp.	55	46	38	52
N *Chrysopa* sp. eggs	36	59	76	70
N parasitoid cocons	8 (1.7%)	2 (0.7%)	10(4.1%)	3 (1.2%)
N parasitoid species	5	2	5	2
N egg batch *S. frugiperda*	5	2	4	0
N egg batch *D. saccharalis*	0	3	1	2

*total larvae released on plants, (%) percent parasitized caterpillars, °number of adults/number of nymphs

The 4 braconid, 4 ichneumonid, 1 eulophid and 1 trichogrammatid parasitoid species found in 2000 are listed in Table 2.4. All are solitary endoparasitoids with exception of *E. plathypenae*. Larvae parasitized by this parasitoid increased feeding by 30-50% compared to non-parasitized larvae (Coudron *et al.*, 1997), whereas all other parasitoid species induce the caterpillars to eat less (Table 2.5). *Ophion flavidus*, *Pristomerus spinator* and *Euplectrus plathypenae* attack third- and fourth-instar larvae, whereas the majority of the other parasitoid species detected parasitize earlier instars of the caterpillars (Table 2.4).

For 2001, a 34.8% parasitism rate was observedest in 2001, with *Campoletis sonorensis* emerging from 23.1% of the recovered caterpillars (Table 2.3). The second most abundant parasitoid was *Cotesia marginiventris* (6.2 %), followed by *Meteorus laphygmae* (1.9%), *P. spinator* and *Chelonus insularis* (1.7% each), *E. plathypenae* (0.2%), and finally *Aleiodes laphygmae* (0.1 %). Two parasitoid species emerged from eggs and larvae of *D. saccharalis* collected during this study: *Trichogramma atopovirilia* and *Apanteles deplanatus* Muesebeck (Hymenopera: Braconidae). The same *Trichogramma* species also parasitized *S. frugiperda* eggs. *A. deplanatus* is a gregarious parasitoid and adults emerge from last instar, *D. saccharalis* caterpillars.

TABLE 2. 3 LOCATION, DATE, NUMBER OF MAIZE PLANTS CHECKED FOR *SPODOPTERA FRUGIPERDA* LARVAE AND NUMBER OF LARVAE COLLECTED, THAT DIED DURING REARING IN THE LABORATORY, THAT FORMED A PUPA (SEX FEMALE, MALE, MALFORMATION), THAT GAVE A PARASITOID COCOON AND NUMBER OF ADULT PARASITOID SPECIES (FOR WHICH SPECIES, SEE TABLE 2.4).

Location	Date	N plants	N larvae	N dead	N pupae (f/m/malformation)	N cocoons	N species
Lindero	31.01.00	396	135	42	88 (41/45/2)	7	4 (2,3,6,10)
Lindero	07.02.00	357	50	19	27 (9/16/2)	1	1 (3)
Lindero	14.02.00	438	69	21	47 (19/28/0)	6	3 (3,4,10)
Lindero	21.02.00	418	70	20	47 (20/27/0)	9	5 (1,3,7,8,10)
Agua fria	09.03.00	random	15	5	3 (1/2/0)	7	1 (4)
Agua fria	19.03.00	random	41	14	19 (8/10/1)	3	1 (4)
Agua fria	03.04.00	random	56	3	-	3	1 (5)
Agua fria	15.02.01	random	1072	159	-	373	9 (1-8)

TABLE 2. 4 PARASITOID SPECIES THAT EMERGED FROM COLLECTED *SPODOPTERA FRUGIPERDA* EGGS AND LARVAE AND PREDATORS OBSERVED EATING *S. FRUGIPERDA* EGGS OR LARVAE IN THE FIELD ON MAIZE PLANTS NEAR POZA RICA (VERACRUZ, MEXICO).

Parasitoids		
1	*Aleiodes laphygmae* (Viereck)	(Hymenoptera: Braconidae)
2	*Campoletis sonorensis*	(Hymenoptera: Ichneumonidae)
3	*Cotesia marginiventris* (Cresson)	(Hymenopetra: Braconidae)
4	*Chelonus insularis* Cresson	(Hymenopetra: Braconidae)
5	*Euplectrus plathypenae* Howard	(Hymenoptera: Eulophidae)
6	*Homolobus truncator* (Say)	(Hymenopetra: Braconidae)
7	*Meterorus laphygmae* Viereck	(Hymenoptera: Ichneumonidae)
8	*Ophion flavidus* Brullé	(Hymenoptera: Ichneumonidae)
9	*Pristomerus spinator* (Fabricius)	(Hymenopetra: Ichneumonidae)
10	*Trichogramma atopovirilia* Oatman & Platner .	(Hymenoptera: Trichogrammatidae)
Predators		
1	*Castolus* sp.	(Heteroptera: Reduviidae)
2	*Coleomegilla* sp.	(Coleoptera: Coccinellidae)
3	unknown Chrysopidae	(Neuroptera: Chrysopidae)
4	*Doru* sp.	(Dermaptera: Forficulidae)
5	*Orius* sp.	(Heteropetra: Anthocoridae)
6	*Podisus sagitta*	(Heteroptera: Pentatomidae)
7	*Spodoptera frugiperda*	(Lepidoptera: Noctuidae) cannibalistic
8	unknown Reduviidae	(Heteroptera: Reduviidae)
9	*Zelus longipes*	(Heteroptera: Reduviidae)

TABLE 2. 5 PARASITOIDS EMERGED FROM *SPODOPTERA FRUGIPERDA* CATERPILLARS, THE INSTAR OF THE HOST THEY ATTACK, THE HOST INSTAR THEY EMERGE FROM AND THE EFFECT THEY HAVE ON THEIR HOST IN TERMS OF HOST FEEDING RATE AND FINAL WEIGHT (FW).

Parasitoid	host instar attacked	instar emergedfrom	% less feeding or % less final weight (fw)
Aleiodes laphygmae (Viereck)	first, second[a]	fourth[a]	similar to *C. sonorensis*[h]
Campoletis sonorensis	2-6 days old[a]	before fourth[a]	85.5% (fw) (*C. flavicincta*, 93% less[e])
Cotesia marginiventris (Cresson)	first[a]	fourth[a]	86.2% (fw)
Chelonus insularis Cresson	egg	fifth[b]	84% less[b]
Euplectrus plathypenae Howard*	third, fourth[d]	-	30-50% more (for host *Heliothis virescens*)[f]
Homolobus truncator (Say)	small[a]	-	-
Meterorus laphygmae Viereck	small[a]	-	similar to *C. sonorensis* [i]
Ophion flavidus Brullé	third, fourth, fifth[a]	-	28-48% less[a]; 17, 20 % less (2 size group)[c]
Pristomerus spinator (Fabricius)	third, fourth[a]	-	-

*is the only gregarious parasitoid, the other listed parasitoids are solitary

[a] (Cave, 1995); [b] (Cruz, 1995), [c] (Rohlfs and Mack, 1983); [d] (Parkman and Shepard, 1982), [e] (Vvan Cruz Figueiredo *et al.*, 1997) [f] (Coudron *et al.*, 1997), [g] (Fritzsche Hoballah and Turlings, 2001), [h] (Isenhour, 1988), [i] personal observation, Thomas Degen and Maria Elena Fritzsche Hoballah

Predators of S. frugiperda.

We observed several predators preying on *S. frugiperda* in the field (Table 2.4). The most common predators were the true bugs *Castolus* sp., *Podisus sagitta*, *Zelus longipes* and an unidentified Reduviidae which all attack larger *S. frugiperda* larvae, and the coccinellid *Coleomegilla* sp., an unidentified Chrysopidae, the forficulid *Doru* sp. and the bug *Orius* sp. that attack newly emerged larvae (Table 2.1, 2.2, 2.3, 2.4).

Discussion

Natural enemies of S. frugiperda *on maize plants.*

Lower parasitism rates in 2000 could be due to higher competition of parasitoids with predators. The higher diversity of parasitoids in 2000 could be explained by the diversity and size of the habitat that was sampled. The location was the same as in 1999, however in 1999, 43 hectares of maize were planted, while in 2000 only a few hectares surrounded by natural vegetation were planted, near the 1999 field plots which had been flooded 4 months earlier. The presence of weedy plants is often associated with higher numbers of natural enemies (Altieri and Whitcomb, 1980; Van Huis, 1981).

Campoletis sonorensis was the dominant parasitoid on the naturally occuring *S. frugiperda* larvae collected in 2001, as was found in the Cascavel region in Brasil with 47% parasitism by *Campoletis* sp. (Valicente and Barreto, 1999). Molina-Ochoa *et al.* (2001) recovered 11 species of parasitoid in their study carried out in four Mexican states. We found the same 6 parasitoid genera in the state of Veracruz. One *Chelonus sp.* and one *Meteorus* sp. were collected, while they listed 3 species of *Chelonus* and 2 species of *Meteorus*. Furthermore, the *Campoletis* found by Molina-Ochoa (2001) was identified as *C. flavicincta*, while the specimens that we collected were identified as *C. sonorensis*. Confusion in the determination of the parasitoids may explain these discrepancies (R. Cave, personal communication). Collections from Honduran maize fields yielded 5 species of parasitoids (Canas and O'Neil, 1998) also found in our study suggesting that these parasitoids are common to a large part of North and Central America. *C. insularis* is outcompeted by *C. marginiventris* and *C. sonorensis* in the case of multiparasitism (Rajapakse *et al.*, 1991; Rajapakse *et al.*, 1992; Escribano *et al.*, 2000), which could explain its low frequency. In contrast, Ashley (1986) found higher parasitism levels of *S. frugiperda* on maize by *C. insularis* than by *C. marginiventris* in the Americas. Parasitism of *S. frugiperda* by *C. marginiventris* on maize was low compared to that by other parasitoids in Florida (Ashley *et al.*, 1980; Ashley *et al.*, 1982) as found in our study.

Higher losses of released caterpillars in 2000 could be explained with the high densities of egg-predators such as *Orius* sp. and the unknown Chrysopidae. These predators also feed on aphids and thrips, which were found in high numbers when predator abundance was also high (Table 2.2). A *Doru* sp. (Dermaptera) was also frequently observed in 2000. This predator attacks eggs and first instar larvae and may be another reason for low recovery of larvae from the plants. *Zelus longipes*, *Castolus* sp., *Podisus sagitta* and the unknown Reduviidae were observed to feed on bigger *S. frugiperda* larvae, of the fourth and fifth instar, whereas *Coleomegilla* sp. fed on younger larvae.

Integrated pest management: 1) *Biological control*

Various efforts have been made to determine which parasitoids can be used in biological control against *S. frugiperda*. Classical biological control was attempted with the parasitoids *Telenomus remus* (Waddill and Whitcomb, 1982) in Florida. Only 4.5% of *S. frugiperda* egg masses collected were parasitized after release of parasitoids, and 50% of larvae reared of eggs collected were parasitized by the native parasitoid *Chelonus insularis* (Waddill and Whitcomb, 1982). The release of *C. insularis* as a biological control agent against *S. littoralis* in cotton fields in Egypt did not reduce the pest population below economic thresholds (Rechav, 1976). *Trichogramma* sp. have also been considered for classical biological control of *S. frugiperda*, but this parasitoid has difficulty reaching the eggs as they are laid in overlapping layers and are protected by scales (Toonders and Sanchez, 1987). *C. marginiventris* was suggested as a biological control agent by Tingle *et al.* (1994) because it attacks a broad range of pests. If different *S. frugiperda* densities are offered to *C. marginiventris*, this parasitoid show a Type II functional response (Riggin *et al.*, 1994), which is desirable in biological control. However, *C. marginiventris* was ineffective in field cage experiments for the control of *Trichoplusia ni* (Boling and Pitre, 1971). *Campoletis grioti* has been suggested for *S. frugiperda* control in Argentina (Valverde *et al.*, 1999). In Mexico, *C. sonorensis* is apparently the most successful species, and could be a candidate for augmented releases.

Some authors have suggested the introduction of more than one natural enemy (Miller and Ehler, 1978; Riggin et al., 1993). Miller and Ehler (1978) considered the concept of r- and K-selection for use in biological control, with a first introduction of most r-selected natural enemies species, like *C. marginiventris* and *C. insularis*, followed by K-selected species. Thus, the species with the highest reproductive potential and inferior competitive ability would be released first. Also Riggin *et al.* (1993), suggested the release of more than one natural enemy to control *S. frugiperda*. Mills (1994) recommended the use of parasitoids that attack young instars of the pest. Half of the larval parasitoids identified in Poza Rica attack first and second instar larvae of *S. frugiperda* (Table 2.5). These parasitoid species are effective in reducing the feeding rate of *S. frugiperda* caterpillars (Table 2.5), which could translate into a higher yield for maize farmers. We previously showed that reduction in feeding rate by *Spodoptera littoralis* caterpillars as a consequence of parasitism by *C. marginiventris* can enhance plant fitness (Fritzsche Hoballah and Turlings, 2001). Predators should also be considered for biological control. In fact, for the control of native insect herbivores on native plants effective biological control is usually due to a suite of generalist predators (Hawkins *et al.*, 1999). For example, *Doru taeniatum* and *Orius albidipennis* can feed on different prey species and can survive on plant pollen or leaves alone when prey is scarce and they can be easily reared in the laboratory (Jones *et al.*, 1988; Fritzsche and Tamó, 2000). *Orius insidiosus* exhibited a typical type II functional response when preying on varying densities of eggs and earlier instar larvae of *S. frugiperda* (Isenhour *et al.*, 1990). The observed *Doru* sp. and *Orius* sp. may be good candidates to control young instars of *S. frugiperda* in tropical agroecosystems of Mexico. Since parasitoids and predators play a primary role in regulating *S. frugiperda* populations (Pair and Gross, 1989) augmentative release of predators and parasitoids in maize fields could prevent outbreaks. In light of increasing objections to introducing non-native species as control agents, it is to be recommended to increase the effectiveness of native agents.

Integrated pest management: 2) The use of resistant varieties

Different methods are used to screen maize for resistance to fall armyworm (Davis and Williams, 1989). Resistance is effective in reducing host larval weight gain during development and the differences among maize genotypes can be high (Wiseman *et al.*, 1980; Wiseman, 1994). Slowing down larval development may significantly prolong their vulnerability to parasitoids (Benrey and Denno, 1997). The augmentative release of natural enemies in addition to the use of resistant maize genotypes could be desirable also because some natural enemies perform even better on resistant than on susceptible maize cultivars. For example, parasitism rates of *C. marginiventris* and *C. sonorensis* are higher on resistant maize varieties than those on susceptible maize varieties (Riggin *et al.*, 1992; Riggin *et al.*, 1993; Pair *et al.*, 1986b; Isenhour and Wiseman, 1987) and predators like *Orius insidiosus* are more efficient in killing *S. frugiperda* larvae if they feed on resistant maize genotypes in comparison to larvae that feed on susceptible maize genotypes (Isenhour *et al.*, 1989).

Integrated pest management: 3) use of varieties attractive to natural enemies

Finally, we suggest a third step to improve *Spodoptera* control: the introduction of maize varieties that release odor blends highly attractive to natural enemies when attacked by the pest. Several studies showed that odor cues released by plants under attack by herbivores are very important for the attraction of natural enemies (for review see, Turlings and Benrey, 1998; Dicke and Vet, 1999). In general, volatiles are attractive to natural enemies and repellent or antifeedant to the herbivores (Turlings and Benrey, 1998). Several predators known to respond to these volatile cues belong to the same families or species of predators that were observed in our study. *Orius* sp. and the Chrysopidae are likely to use induced volatiles to detect *S. frugiperda* larvae, and the same may be the case for the bugs and coccinellids. The attraction of the induced volatiles to these predators should be tested in olfactometers and in field trials. For example, in the study of Drukker *et al.* (1995) *Orius* sp. and *Anthocoris* spp. were attracted more to pear trees infested by the herbivore *Psylla* sp. than to control plants. Several substances released by pear trees under attack by *Psylla* sp. (Scutareanu *et al.*, 1997) are the same as found in the induced maize emissions (Gouinguené *et al.*, 2001). Scutareanu *et al.* (1997), found that *Anthocoris* sp. are attracted to monoterpenes, but not to the green leaf volatiles. Furthermore, this bug can learn to associate herbivore-induced plant odors with its preys (Drukker *et al.*, 2000). In contrast, *Podisus maculiventris* reponds to green leaf volatiles, which are produced by all maize varieties. For some of the parasitoids found in our study, ample information on their attraction to induced volatiles exist. Host habitat location by the use of herbivore induced odors in plants was shown for *C. marginiventris* (Loke and Ashley, 1984a; Loke *et al.*, 1983; Turlings *et al.*, 1990; Turlings *et al.*, 1991c; Turlings *et al.*, 1991a; Baur and Yeargan, 1996; Cortesero *et al.*, 1997; Röse *et al.*, 1998) and for *C. sonorensis* (Elzen *et al.*, 1984a; Elzen *et al.*, 1987; Elzen *et al.*, 1984b; McAuslane *et al.*, 1990; McAuslane *et al.*, 1991b). Some maize varieties are more attractive to the parasitoid *C. marginiventris* than others and it is not only the quantity of the odors released that is important for attraction but also the quality (Fritzsche Hoballah *et al.*, in press). Furthermore, *C. marginiventris* can learn to associate the odor blends with the presence of its hosts (Turlings *et al.*, 1989a; Turlings *et al.*, 1989b; Turlings *et al.*, 1993). *C.*

sonorensis remains longer on host-damaged cotton plants than on undamaged plants (Baehrecke *et al.*, 1990), and responded highly to green leaf volatiles during EAG studies (Baehrecke *et al.*, 1989), but did not show an increase in responsiveness after an oviposition experience (McAuslane *et al.*, 1991a). The egg parasitoid *Trichogramma* sp. is also attracted by plant odors (Romeis *et al.*, 1997), however, it is not known if induced volatiles are more important for this attraction than odors constitutively produced by the plants even in the absence of herbivores.

Two wasp species found in Poza Rica, *O. flavidus* and *M. laphygmae*, would probably not react to induced volatiles because they parasitize hosts during the night (Rohlfs and Mack, 1985; Isenhour, 1988) when induced volatile emissions by maize plants are minimal (Gouinguené, 2000). All other natural enemies of *S. frugiperda* are active during the day and are likely to be attracted by odors released by maize under *S. frugiperda* attack. An important step toward the use of this indirect defence in biological control would be to find out which compounds in the blends of induced maize volatiles are the most important for the attraction of the specific natural enemies, as suggested by (Dicke and van Loon, 2000). Selecting maize varieties very attractive to natural enemies among varieties resistant to *S. frugiperda* in addition to the release of different natural enemies that benefit directly the plants by eating or parasitizing *S. frugiperda* caterpillars could be another strategy to enhance its control. With this study we identified natural enemies of *S. frugiperda* that could be used for this purpose.

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CHAPTER 3

DIRECT COST OF INDUCED VOLATILE PRODUCTION IN MAIZE

Based on:

Fritzsche Hoballah, M. E.; Koellner K. and Turlings, T. J. C.. Direct cost of induced volatile production in maize. In preparation for submission to *Oikos* or *Oecologia*.

Abstract

Herbivore-induced plant volatiles can function as indirect defence signals that attract natural enemies of herbivores. Several parasitoids are known to exploit these plant-provided cues to locate their hosts and some herbivores are repelled by the volatiles. Recently, benefits, in terms of plant fitness, from the action of the parasitoids were shown for a few systems. However, the cost of production of herbivore induced volatiles for the plant remains a topic of discussion. Here, we analysed the costs of the production of herbivore induced volatiles for maize, *Zea mays*. Plants were treated with regurgitant of *Spodoptera littoralis* or with the elicitor volicitin and we measured dry weight of plant parts at specific times after treatments. After a two weeks treatment period, some cost for induced volatile production was detected as the dry-weight of leaves of induced plants was lower than that of uninduced plants. However, maize plants seem to compensate for this loss during subsequent growth, since in a similar experiment seed production at maturity was not different for unharmed plants and plants treated with caterpillar regurgitant. However, for volicitin treated plants some differences in seed production were found between unharmed and treated plants. A comparison of six maize inbred lines with known differences in odor emissions showed a correlation between the intensity of odor emissions and reduction in plant performance as compared to untreated plants. An analysis of the terpenoids inside the leaves of these inbred lines revealed that only those compounds that are also volatilised are induced while non-volatilised compounds are apparently consitutively present in maize. We conclude that benefits of induced volatile production in plants may counterbalance the costs, if natural enemies of the herbivores are present in the environment. Hence, the notion that volatile releases constitute a considerable cost for plants was only partly supported for this system.

Key words-induced defence, induced volatiles, cost, benefit, terpenoids, constitutive defence, *Zea mays*, inbred lines

Introduction

Natural enemies of herbivores can use volatiles that are produced by plants under herbivore attack to locate their prey for reviews see (Whitman, 1988; Vet and Dicke, 1992; Turlings *et al.* 1995, Dicke and Vet 1999). These herbivore-induced volatile emissions are suggested to function as an indirect defence for plants. Indeed, several field studies have shown that parasitism or predation was higher on plants that produced induced volatiles, or on plants that were treated with synthetic volatiles, than on plants that did not produce or carry these volatiles (Titayavan and Altieri, 1990; Drukker *et al.*, 1995; Thaler, 1999; Kessler and Baldwin, 2001). It has also been shown that some induced volatiles can repel herbivores and act as direct defence for the plants. For example, aphids (Bernasconi *et al.*, 1998; Glinwood and Petterson, 2000) and mites (Dicke and Dijkman, 1992) are repelled by induced odors and moths oviposit more on unharmed plants than on plants damaged by caterpillars (Landolt, 1993; De Moraes *et al.*, 2001). Thus, volatiles produced by the plant under herbivore attack can have both direct (by repelling herbivores) and indirect (by attracting natural enemies of herbivores) defence effects. This defence through volatiles will only be maintained over evolutionary time if the plants benefit from induction. Evidence is mounting that insect herbivores impose selection on resistant traits in plants, and that those traits can also influence interactions between herbivores and their natural enemies (for review see Simms and Fritz, 1990). This is in contrast with the scenario proposed by Jermy (1993) that the evolution of insect-plant relationships results primarily from autonomous evolutionary events; namely from heritable functional changes within the insects nervous system that determine plant recognition and ultimately host plant specificity. Similarly, a non-functional accidental release of volatiles as by-products has also been hypothesised (Van der Meijden , 2000). Reasons why accidental release is not probable are discussed by (Dicke and van Loon , 2000).

Possible benefits and costs of induced production of odors are presented in Table 3.1.

Table 3.1: Potential benefits and costs of herbivore induced volatiles are listed.

Herbivore induced volatiles	
Benefits	**Costs**
Attraction of natural enemies of herbivores[a]	Metabolic costs[d]
Repellence of herbivores[c]	Attraction of herbivores[e]
Antibiotic effect on plant pathogens[b]	Antibiotic effects on entomopathogens[f]

a: (Dicke and Sabelis, 1988), (Ponsoby and Copland, 1995), (Shimoda *et al.*, 1997), (Turlings *et al.*, 1990), b: (Shulaev *et al.*, 1997), (Croft *et al.*, 1993), (Harrewijn *et al.*, 1994/1995), c: (Bernasconi *et al.*, 1998), (Dicke and Dijkman, 1992), (Landolt, 1993) d:[Gulmon, 1992 #246], (Gerhenzon, 1994a) (Gerhenzon, 1994b) f: (Landolt *et al.*, 1999), (Loughrin *et al.*, 1996), (Koschier *et al.*, 2000) f: (Brown *et al.*, 1995).

Benefits in terms of seed production were demonstrated for the induced production of the direct defence compounds nicotine (Baldwin, 1998) in tobacco and glucosinolates (Agrawal, 1998) in wild radish. Recent experimental evidence suggests that plants may benefit from attracting parasitoids (van Loon *et al.*, 2000; Fritzsche Hoballah and Turlings, 2001). These studies demonstrate higher plant fitness in terms of seed production if a solitary braconid wasp parasitizes caterpillars on plants as compared to plants attacked by unparasitized caterpillars. Herbivores can be repelled by induced odors (positive effect for the plant), but also attracted (negative effect) (for review see, Dicke and van Loon , 2000).

Besides the positive effects of attracting natural enemies and repelling herbivores, the volatiles may also function as antibiotics against phytopathogens (Croft et al., 1993, Harrewijn *et al.*, 1994/1995; Shulaev *et al.*, 1997). However, the antibiotic function could also act against entomopathogens (Brown *et al.*, 1995), and as such have indirect negative consequence for the plant. Metabolic costs for induced volatile production are high (Gulmon and Mooney, 1986; Gerhenzon, 1994a; Gerhenzon, 1994b). It should be noted that costs are saved by the fact that the defence is only employed in cases of herbivore attack, in contrast to the continuous costs of constitutively produced defences. Quantification of costs of induced production of secondary compounds in plants remains a topic of discussion and disagreement and experimental studies are few. Some authors suggest high fitness costs of induced defence, based on the optimal defence theory (Zangerl and Bazzaz, 1992), while others suggest that the costs are relatively low (Dicke and Sabelis, 1989). Experiments analysing induced direct defence costs are contradictory, depending on which system is used costs are found (Heil *et al.*, 2000; Zangerl *et al.*, 1997) or not (Brown, 1988; Gianoli and Niemeyer, 1997; Ryan, 1992). Models have been proposed for the analysis of the evolutionary stability of induced production of volatiles (Sabelis and De Jong, 1988; Godfray, 1995). The models predict relatively high costs for the production of induced volatiles, but even if costs of volatile production were to be small, this does not imply that they are irrelevant to natural selection (Dicke and Sabelis, 1989). Fitness costs of induced production of volatiles have never been experimentally shown.

The aim of this paper is to assess direct fitness costs of the production of induced volatiles in maize plants. We used maize plants because they are very responsive to herbivore attack (Turlings *et al.*, 1998b), the odors they emit are known to be attractive to various parasitoids (Turlings *et al.*, 1995), and the odor blends of cultivated maize varieties are similar to those of their wild ancestors (Gouinguené *et al.*, 2001). We induced plants to produce volatiles by treating them with regurgitant of *Spodoptera littoralis* (Boisd.) (Lepidoptera: Noctuidae) or with volicitin, an elicitor that has been isolated from *Spodoptera* regurgitant (Alborn *et al.*, 1997; Alborn *et al.*, 2000; Turlings *et al.*, 2000). Induced odor emission in maize is highly polymorphic, with considerable variation of quality and quantity of odors produced after induction by different maize varieties and inbred lines (Gouinguené *et al.*, 2001). The model discussed by Karban *et al.* (1997) emphazises the potential benefits of variability created by induced defences. By using inbred lines that either emit high or low amounts of volatiles after induction, we could correlate this difference with differences in growth parameters between control and induced plants of each specific inbred line. We hypothesised that maize plants incur limited metabolic cost of induced volatile production, which should be reflected in plant performance and in final seed production under conditions where interactions with other organisms are excluded. A problem with the evaluation of the costs could arise if there is a correlation between direct and indirect defence in maize. Perhaps, genotypes that produce small amounts of volatiles produce and store large quantities of other compounds, possibly investing more in direct defence as compared to other genotypes that invest in the production of volatiles (indirect defence). For this reason we included an experiment to correlate the induced production of volatilised and stored terpenoids by different inbred lines. The results obtained are discussed in context of a cost and benefit analysis of induced indirect defence.

Material and methods

Plants and plant treatments. Maize plants (*Zea mays*, var. Delprim, Pactol and several inbred lines) were grown in plastic pots (6 cm high, 8 cm diam.) in fertilised commercial soil (Coop, Switzerland) in a climate chamber (23°C, 60% r.h., and 16L: 8D, 50000 lm/m^2). To induce the plants to produce volatiles we scratched the second and third leaf of each plant with a razor blade (an area of 2-3 cm^2 per leaf) and subsequently 10 µl regurgitant of *S. littoralis* was applied to each damaged site. The regurgitant had been collected from 4th and 5th instar of *S. littoralis* caterpillars fed with maize leaves (variety Delprim), which was kept at –70 °C until 10 minutes before utilisation. Three additional control treatments were used: 1) plants that were left unharmed, 2) plants that were scratched as above, but only 10 µl demineralised water was applied to the damaged sites and 3) plants that were only scratched.

Volatile collections in the laboratory. One week old plants were placed in a climate chamber (Conviron, E95 Mode, Canada). The climate chamber was programmed at 17.5°C during light period and 20°C during dark period, 30 % r.h. and with a light intensity of 14500 lm/m^2 (light provided by 16 Sylvania 150 W VHO Cool White tubes and 12 Sylvania, 100 W Satin bulbs). We induced the plants, starting at the 3-4 leaf stage, 7 times each second day. For the experiment carried out with the variety Delprim, the volatiles of one plant per treatment were collected on the 5th, 6th and 7th treatment day. During the experiment with the variety Pactol, volatiles of one plant per treatment were collected during all 7 periods of induction. For experiments with the varieties Delprim and Pactol, the plants used for the volatile collection were the same for each additional treatment.

For the inbred lines only two treatments were carried out: only scratching of the leaves and scratching with addition of regurgitant. Five different inbred lines were used for this experiment, the volatile collections were carried out 7 times, each time with a different plant.

Volatiles were collected from 5 plants simultaneously in an automated collection system (ASU, ARS, Gainesville, USA). For the first collection, when the plants were still small enough, six glass cylinders (9.5 cm diam., 54 cm high) were placed over the plants and rested on a Teflon disk consisting of two halves with a hole in the middle for the stem of the plant (Turlings *et al.*, 1998b). For the following collections ®Nalophan bags (diam. 150 cm, Kalle Nalo GmbH, Wiesbaden, Germany) were placed over the plants as described by (Turlings *et al.*, 1998a). Air was pushed (1 l/min) through a charcoal filter and introduced from the top of each cylinder or ®Nalophan bag over the plants. Traps containing the adsorbent Super-Q (25 mg, 80/100 MESH, Alltech, Deerfield State) were attached laterally at the base of each cylinder or on the bottom of the ®Nalophan bag. Traps were connected via Tygon tubing to the flowmeters of the automated volatile collection and air was pulled out at 0.8 l/min during 2 hours. The collection period was programmed with the use of the software

TESS (Version 1.0, ARS, Gainesville, USA). The plants were treated in the dark, 7 h before the lights went on in the chamber. Collections started 3 h after lights on. After each collection, traps were rinsed with 150 µl methylene chloride, and 200 ng of *n*-octane and nonyl acetate were added as internal standards. Three µl aliquots of the samples were injected on column, with an automated injection system into a Hewlett Packard model HP 6890 gas chromatograph equipped with a flame ionisation detector. The apolar EC-1 capillary column (30 m, 0.25 mm I.D., 0.25 µm film tickness, Alltech) was held at 50 °C for 3 minutes and then programmed at 8 °C per minute to 230 °C, were it was maintained for 9.5 minutes. The column was preceded by a deactivated retention gap (10 m, 0.25 mm I.D., Connex USA) and a deactivated precolumn (30 cm, 0.530 mm I.D., Connex). Helium (24 cm/s) was used as carrier gas. HP Chemstation software was used to quantify all major components based on the detector response to the internal standards. To compare total amounts of volatiles collected among the three treatments, we used the non-parametric Kruskall Wallis test.

Volatile collections in the field. Some maize plants, variety Delprim, were transferred at the 4-leaf stage from the climate chamber and planted in a field plot near the University of Neuchâtel (Switzerland). Volatiles were collected in a similar way as in the laboratory. Plants were scratched and treated with buffer (phosphate buffer pH8) or volicitin (40ng/µl buffer) at approximately 10 am and several control plants were left unharmed. Volatiles were collected from 5 pm for 3 h in August 1998. In June 1999 plants were treated by scratching the leaves or by scratching and adding regurgitant at 10 am, while volatiles were collected 5 h after treatment for 2 h. Just before each collection, plants were placed in ®Nalophan bags. Purified air was pushed into the bags at the rate of 1.2 l/min at the bottom part of each bag. The filter to trap the volatiles was placed at the upper part of the bag, air was pulled out from the bag trough the filter at a rate of 0.8 l/min by the use of a vacuum pump, air flow was controlled by a flowmeter. For each subsequent collection the same individual plants were used. The Kruskal Wallis test was used to test for differences in volatile emitted among treatments.

Cost experiments under laboratory conditions. Experiments with maize of the varieties Delprim and Pactol were carried out separately. After maize plants of the varieties Delprim or Pactol were treated 7 times over two weeks, leaves, stems and roots were dried in an oven at 80°C for 5 days. Subsequently, dry weight of these plant parts was determined. An ANOVA was used to test for differences in dry weights among treatments of inbred lines. For the inbred lines only the dry weight of leaves was assessed after the 7-fold treatment. A Spearman test was used to correlate difference in dry weight and difference in total amount of volatiles released between the two treatments. In this way, we determined if the production of volatiles through regurgitant induction resulted in a loss of dry weight.

Cost experiments in the field. Maize plants of the variety Delprim were transferred from the climate chamber into a field near Marin (Switzerland) at an age of 2 weeks and treatments started the third week after planting. Three different treatments were carried out which were the same as used for the volatile collections in the field in 1998 and 1999. Short treatments were carried out for two weeks, on Monday, Wednesday and Friday of each week. The longer treatments lasted 6 weeks (with weeks 3 and 4 without treatment) in 1998 and 8 weeks (with weeks 3 and 6 without treamtment) in 1999. For each treatment and treatment period, 10 plants were used (a total of 50 plants). Treatments were carried out between 10 and 13 pm. This experiment was repeated 2 times (2 blocks) in 1998, with a first treatment on June 8 and June 15, and 3 times (3 blocks) in 1999, starting the 7th, the 21st and the 28st of June. During this time of the season Swiss farmers cultivate maize in their fields. Plants parts (leaves, stems, ears) were collected in October. Plants parts were dried at 80°C for 10 days and then their weight were determined. An ANOVA was used to assess differences in dry weights among treatments for each year and each block.

Analysis of volatile and non-volatile terpenoids in maize inbred lines. We collected the volatiles from 2 week old (±3 days, all plants about the same height) unharmed plants and plants scratched and treated with *S. littoralis* regurgitant of 6 different inbred lines (n=4 for each inbred line and treatment). These lines were chosen on the basis of known differences in their inducible emissions (Thomas Degen, unpublished data). Treatment of plants, collection of volatiles, and volatile analyse are described in the section "volatile collections in the laboratory". After volatiles were collected, the plants were frozen at -70°C until analysis of terpenoids inside the leaves of these plants. Frozen leaves (3.3 g) were placed in liquid N_2 and crushed with a mortar. Pentane (10 ml) was added to the leaf powder and mixed for 1 minute. The pentane extract was placed in a glass vial and shaken for 3 h at laboratory temperature, before being centrifuged for 5 min (2000xg). The pentanephase was first separated from the rest and shaken with activated charcoal until discoloration, than centrifuged again for 5 min. The pentanephase was again separated from the rest of the solution and 20 µl iso-octane plus 40 µl nonyl acetate (as internal standard, 20 ng/µl) were added to the sample. Finally, the solution was concentrated under a N_2 flow until 400 µl remained.

For analysis of the extracted terpenoids, 2 µl of the samples was injected splitless into a Hewlett Packard model HP 6890 gas chromatograph coupled to a Hewlett Packard model HP 5973 quadrupole type mass selective detector (transfer line temperature 230°C). The DB-5MS column (30 m, 0.25 mm I.D., 0.25 µm film tickness, J & W Scientific) was held at 40 °C for 3 min and than programmed at 5 °C per min to 195 °C , and from 195 °C to 300 °C at 50 °C per min. Helium (2 ml/min) was used as carrier gas. An ANOVA and the Newman Keuls Student post-hoc test were used to test for differences in amounts of specific terpenoids among inbred lines and between unharmed and treated plants. The Spearman test was used to correlate non-volatile terpenoids inside the plant and volatile terpenoids collected.

Results

Volatile collections. Contrary to our expectetion, volatile emission by Delprim treated with regurgitant was not different from volatile emission from plants that were only scratched (Fig. 3.1). However, there was a clear difference in total odors released between treated and unharmed plants (Kruskal Wallis, *P*=0.066, χ^2=5.422, n=3, Fig. 3.1). Volatile emissions by Pactol were the lowest for unharmed plants and the highest for plants treated with regurgitant (*P*=0.004, χ^2=11.099, n=6), as expected (Fig. 3.1).

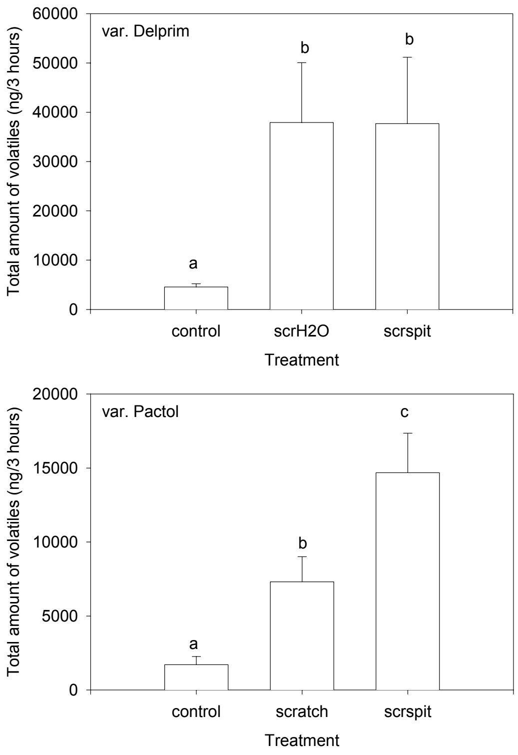


Figure 3.1: Amount of volatiles released by maize plants, variety Delprim and variety Pactol, after different treatments. unharmed plants (control), plants scratched on two leaves (scratch); plants scratched on two leaves and damaged sites treated with water (scrH2O); plants scratched on two leaves and treated with regurgitant (scrspit).

For the total amounts of odors released by Delprim in the field there was also a significant difference between treated and unharmed plants (1998, P=0.038, χ^2=6.515, n=8; 1999, P=0.025, χ^2=7.385, n=4, Fig. 3.2). As for the laboratory experiment, no significant differences in odor emissions were detected in the field between scratched plants and plants treated with regurgitant.

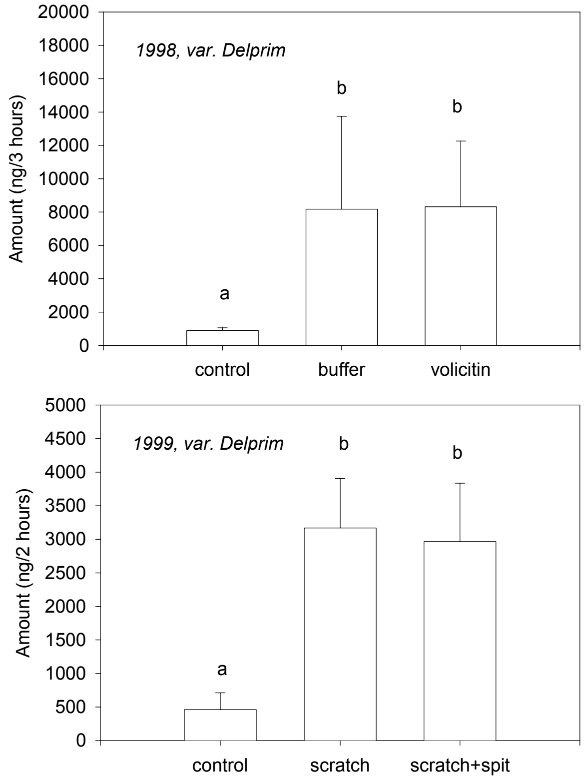
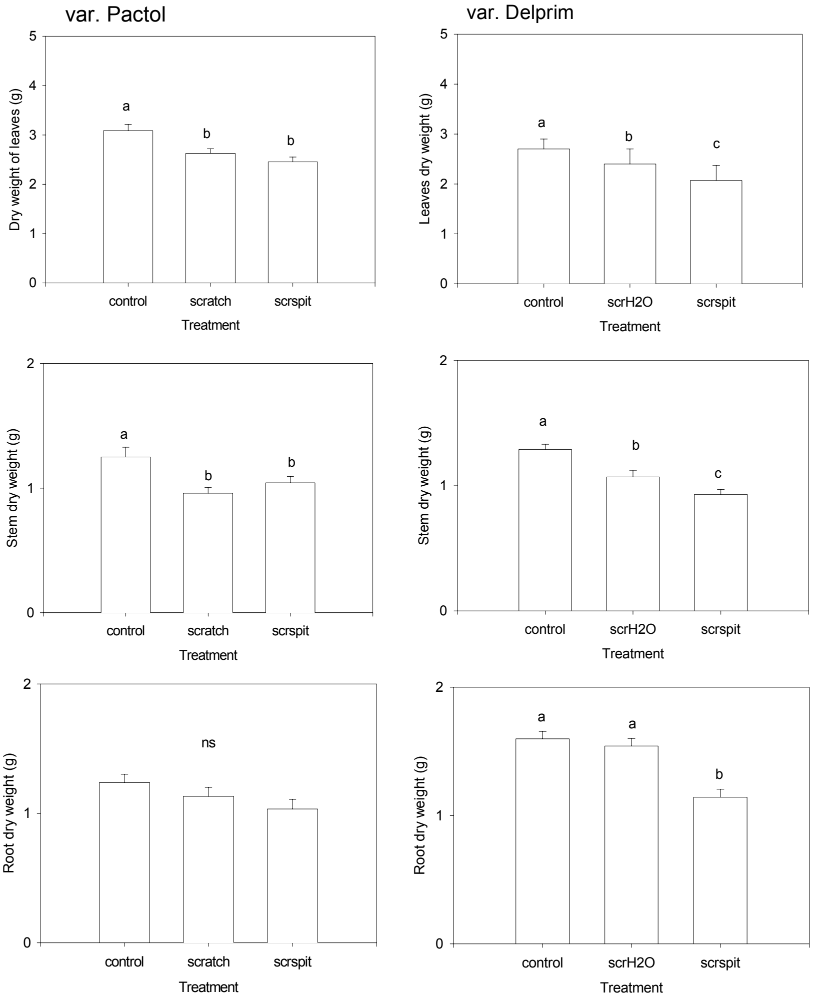


Figure 3.2: Amount of volatiles released by maize plants, variety Delprim on field collections in 1998 and 1999 after different treatments. unharmed plants (control), plants scratched on two leaves (scratch); plants scratched on two leaves and damaged sites treated with buffer (buffer); plants scratched on two leaves and treated with regurgitant (scratch+spit); plants scratched on two leaves and treated with volicitin (volicitin).

Plant performance in the laboratory. For Delprim a significant lower dry weight of leaves, stem and root was found for treated plants compared to unharmed plants (ANOVA, *P*<0.0001, F=18.098, 18.357 and 17.389 respectively, Fig. 3.3). For leaves and stem, a significant difference was also found between plants treated with water and regurgitant, plant treated with regurgitant weighing less (Fig. 3.3). For Pactol, differences among treatments were smaller for the dry-weights of leaves, stem and roots (ANOVA, *P*=0.001, F=9.257, *P*=0.006, F=6.226 and *P*=0.139, F=2.128, respectively, Fig. 3.3). In fact, no significant differences in dry weight of leaves and stems were found between plants scratched and plants treated with regurgitant (Fig. 3.3). The treated Pactol plants differed from the unharmed plants only in terms of leaf and stem dry-weight (Fig. 3.3).

Figure 3.3: Dry weight of leaves, stem and roots for maize plants of varieties Pactol and Delprim after different treatments: unharmed plants (control), plants scratched on two leaves (scratch); plants scratched on two leaves and damaged sites treated with water (scrH2O); plants scratched on two leaves and treated with regurgitant (scrspit).



The difference in dry weight between unharmed and treated inbred lines and the difference in volatiles released between these two treatments were positively correlated (*P*=0.037, Fig. 3.4), which is in accordance with a metabolic cost of volatile emission that results in reduced biomass production. If results for Pactol are added the correlation is even better (*P*=0.019), hbut, the experiment with Pactol was done at a different time period.

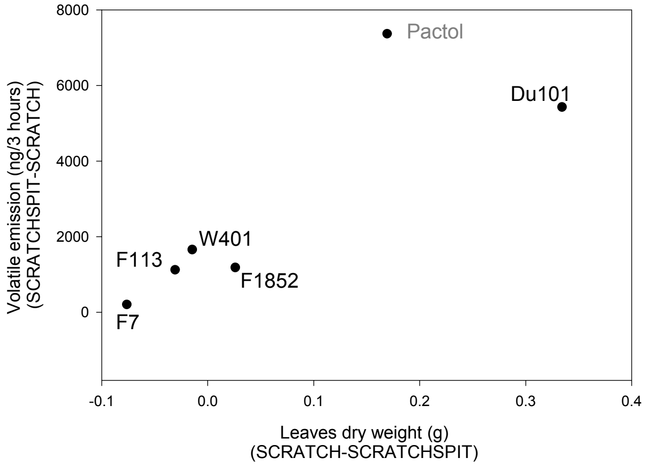


Figure 3.4: Correlation between difference in volatiles released between plants scratched on two leaves with a razor blade and plants scratched on two leaves with addition of regurgitant and difference in dry weight of the leaves of plants treated in these two different ways.

Field experiments. No differences were found in dry weight of leaves and stems among mature plants that were treated differently during growth, this was true for both years (ANOVA, 1998: stem *P*=0.225, leaf *P*=0.0038 not significant after Student Newman Keuls test *P*=0.054, 1999: stem, *P*=0.531, leaf, *P*=0.306). For the dry weight of the seeds, a significative difference was found among treatments in the 1998 experiment (plot 1, *P*=0.035, F=2.838; plot 2, *P*=0.001, F=5.525, Fig. 3.5). For the plot were no fertilizer was added to the soil (plot 1), a significant difference was only found between control plants and plants treated with volicitin during the longer treatment period (Fig. 3.5). For the plot were fertilizer was added (plot 2), the difference in seed production was more significant for the 4 weeks of treatment (Fig. 3.5). However, no significant differences were found between control plants and plants treated for the shorter period in both plots (Fig. 3.5).

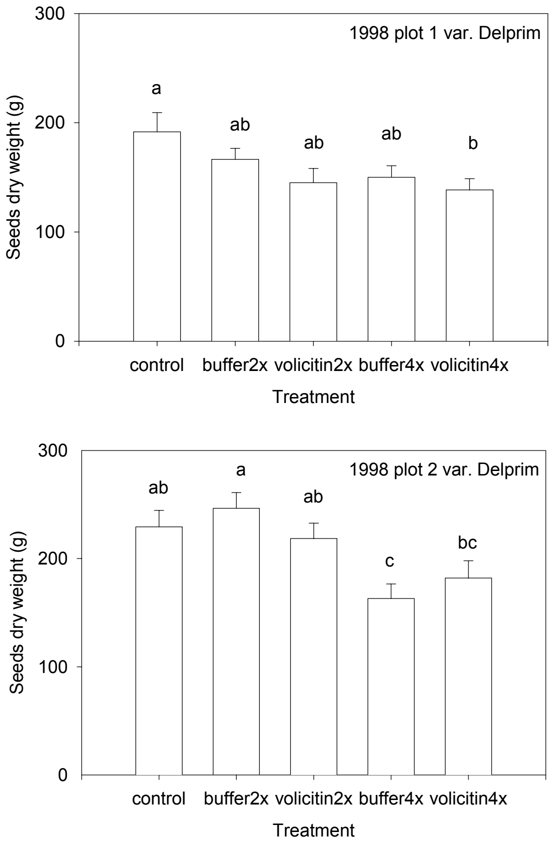


Figure 3.5: Seeds dry weight of plants after treatments: control: unharmed plants, buffer 2x: plants treated with buffer on two damaged leaves for a period of two weeks, volicitin 2x: same as buffer 2x but treated with volicitin, buffer 4x and volicitin 4x: as for 2x but for a treatment period of 4 weeks.

In 1999, even though the longer treatment took 6 weeks instead of 4, no significant differences were found among treatments for seed dry weight (plot 1, *P*=0.723, F=0.518; plot 2, *P*=0.709, F=0.538; plot 3, *P*=0.422, F=0.993, Fig. 3.6), but tendencies were the same as in 1998.

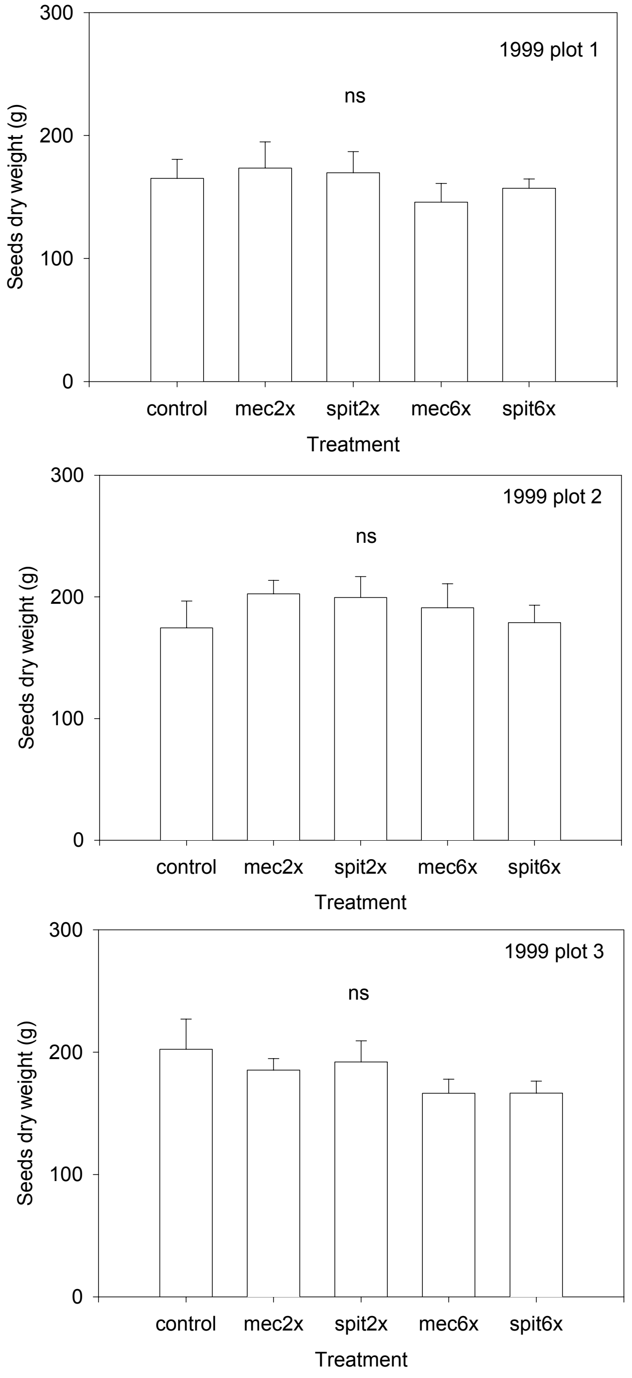
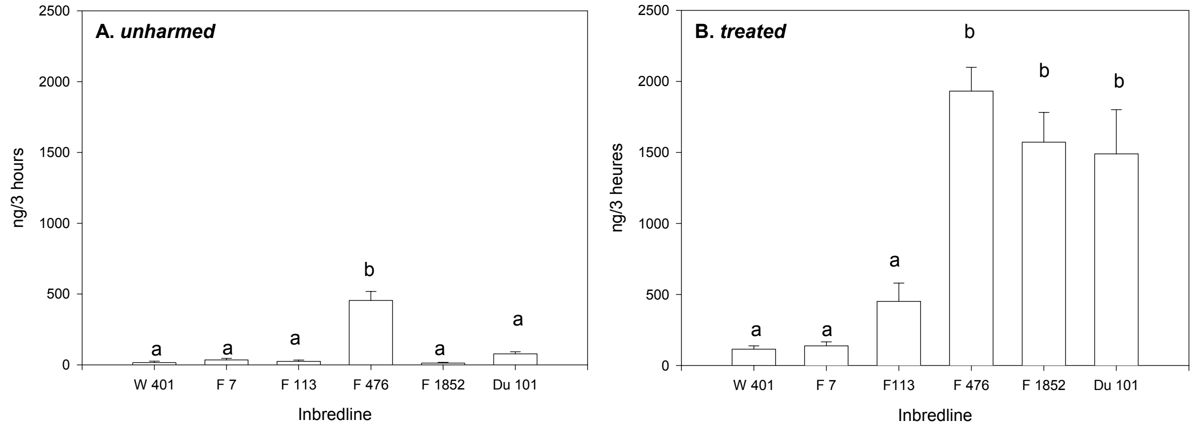


Figure 3.6: Seeds dry weight of plants after treatments: control: unharmed plants, mec 2x: two leaves scratched with a razor blade for a period of two weeks, spit 2x: same as mec 2x but with addition of volicitin on the damaged sites, mec 4x and spit 4x: as for 2x but for a treatment period of 4 weeks.

Analysis of non-volatile terpenoids in maize inbred lines. Total volatiles collected from unharmed plants was significantly higher for the inbred line F 476, no differences were found among the other inbred lines (*P*<0.0001, F=40.276, Fig. 3.7). In contrast, for treated plants, 3 inbred lines released significant smaller amounts of volatiles than the other 3 (*P*<0.0001, F=20.962, Fig. 3.7), which corresponds with previous data (Thomas Degen, unpublished9 that were used to select these lines. For each inbred line, there was a significant difference in total amount volatiles released between unharmed and treated plants (*P*=0.016-0.0001, *F*=11.180-68.210), confirming volatile induction by treatment.

Headspace collections



Extracted from leaves

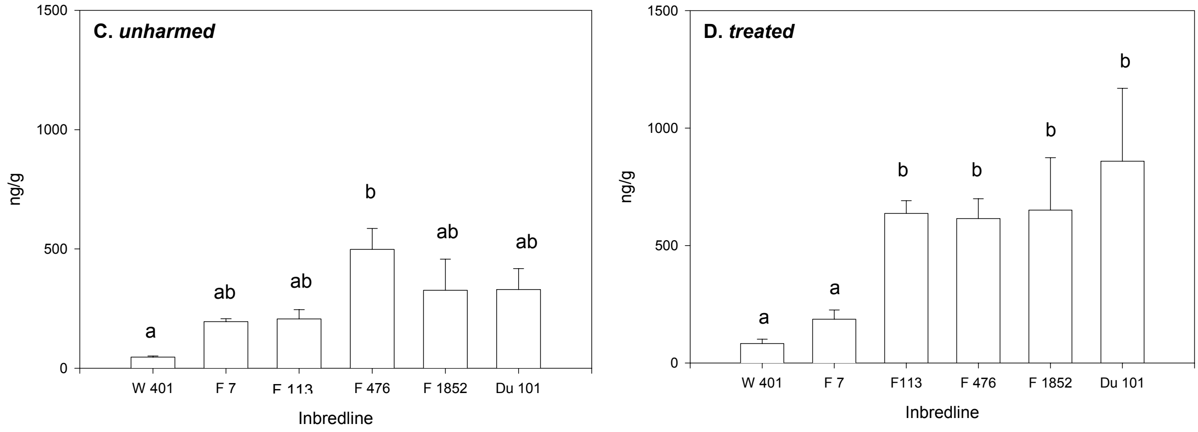


Figure 3.7: Total amount of headspace collected (A and B) and extracted (C-D) terpenoids from unharmed plants (unharmed) and plants treated with regurgitant (treated) of 6 different inbred lines.

Single volatile compounds that were emitted more by treated plants than by unharmed plants were considered to be produced at least in part in response to the treatment and were classified as induced compounds. All volatile compounds (Table 3.1) were induced at least in one of the inbred inbred lines. Myrcene and H-indole were induced only in Du101, while (*E*)-

4,8-dimethyl-1,3,7-nonatriene was induced by all inbred lines with the eception of W401. Linalool and (*E*)-β-farnesene were induced in 4 inbred lines (Du101, F1852, F476, F7, Table 3.1). (*Z*)-3-hexenyl acetate was only induced in the inbred line W 401. A significant difference was detected in the amount of each compound released among inbred lines (separated analysis for the two treatment groups) with the eceptions of H-indole and (*Z*)-3-hexenyl acetate for treated plants and H-indole, (*Z*)-3-hexenyl acetate, phenethyl acetate, (*E*)-α-bergamotene and (*E*)-β-farnesene for the unharmed plants.

Among inbred lines total amount of terpenoids that were not volatilized for unharmed plants was significant different (P=0.029, F=3.287, Fig. 3.7): inbred line W401 contained the lowest and F 476 contained the highest total amount of non-volatile terpenoids. Two inbred lines (W 401 and F7) contained lower amounts of terpenoids inside the leaves than the other four (P<0.0001, F=8.071, Fig. 3.7). Only for two inbred lines, there was a significant difference in total amount of interfoliar terpenoids between unharmed and treated plants (Du 101, P=0.004, F=20.142; F113, P=0.001, F=40.832, Fig. 3.7), indicating that non volatilized terpenoids are probably not produced by every inbred line under induction with regurgitant treatment. Some terpenoids were extracted from only one or a few inbred lines (Table 3.1). (*E*)-β-Farnesene was the dominant induced terpenoid, it was induced in 3 inbred lines (Du101, F113, F7). (*E*)-α-Bergamotene was induced in two inbred lines (Du101, F113). Finally, (*E*,*E*)-4,8,12-trimethyl-1,3,7,11-tridecatetraene and “unknown 17” were induced only in one inbred line, W401 and Du101, respectively. All other compounds were not induced. There were significant differences among inbred lines in quantity of single interfoliar terpenoids extracted from unharmed and treated plants, with the eception of β-caryophyllene, (*E*)-α-bergamotene and (*E*)-β-farnesene for unharmed plants and “unknown 20” for both treatments (Table 3.1).

Table 3.1: Amounts of single compounds collected or extracted from unharmed plants and plants treated with regurgitant of different maize inbred lines.

	Unharmed			*Treated*								
	W401	**F7**	**F113**	**F476**	**F1852**	**Du101**	**W401**	**F7**	**F113**	**F476**	**F1852**	**Du101**
Amounts (ng/g) of single compound amounts inside plant												
1	0	0	0	9±6	27.5±4	0	0	0	0	3.4±3	24.4±6	0
2	0	0	0	19±1	0	14.6±1	0	0	0	13±2	0	14.9±1
3	0	0	0	23.7±9	0	0	0	0	0	33.3±13	0	0
4	0	0	0	48±31	24.9±17	17.4±8	7.4±5	0	13.3±90	33.6±15	11±4	79.8±25
5	0	0	0	6.5±6	14.4±14	0	4.9±3	2.4±2	54.3±12	22.9±8	86.4±39	47.2±11
6	3.1±3	0	2.2±2	10.2±8	36.3±36	5.2±5	29.1±12	8.2±3	271.6±83	44.1±18	186.5±89	214±37
7	0	5.1±5	0	29.7±5	0	41.4±11	0	0	0	30.5±6	0	46.3±8
8	2.2±2	0	0	12.5±1	11.6±1	1.2±4	11±4	0	0	14.7±2	13.2±1	20.9±3
9	0	0	0	75.7±27	58.9±14	0	0	0	0	88±19	40.1±17	0
10	0	75.1±24	0	0	0	0	0	63.4±28	0	0	0	0
11	0	0	0	9±5	16.5±6	0	0	0	0	4.3±4	18.2±8	0
12	0	0	0	20.8±5	0	0	0	0	0	26.8±7	0	0
13	0	0	0	16.8±6	25.1±12	39.6±11	0	0	0	21.2±3	42.1±16	43.3±8
14	0	0	0	0	5.2±5	23.7±8	0	0	0	16.4±12	33±12	37.8±8
15	0	64.8±14	172±38	121±30	37.7±34	112.5±41	0	61.8±15	265.8±70	153.4±17	113.3±36	296±23
16	0	0	0	29.7±5	0	0	0	0	0	31.1±8	0	0
17	15.6±2	32.1±9	3.4	41.2±4	42.7±14.3	10±1	11.1±2	34.9±7	5.3	49.2±10	51±8	13.4±2
18	0	0	0	0	0	25.2±4	0	0	0	0	0	24.1±2
19	0	0	0	0	0	12.9±1	0	0	0	0	0	9.7±1
20	26±4	18.3±3	28.9±4	25.2±5	25.7±9	15.8±3	19.6±3	15.7±2	26.1±4	29.2±9	32.4±11	11.6±3
Single compound amounts (from single plant/3 hours) emitted by plant												
21	4.2±4	4.1±2	6.7±2	19.1±7	1±1	0	1±1	8±3	6.2±1	38.3±13	6.4±1	8.7±2
22	1.3±1	1±1	0	7.3±7	0	3.7±1	7.6±1.6	5.4±2	28.5±7	25.9±9	2.3±2	26±14
23	20.1±9	28.9±11	14.3±5	373.5±51	4±4	70±15	37.8±9	65.6±10	21.9±3	933.6±70	261.7±32	409±88
24	1.4±1	0	1.6±1	24.2±7	0	0	4.7±3	50.1±18	7.2±1	202.6±13	157.7±18	70.3±20
25	0	0	0	0	0	0	1.3±1	0	21.1±10	32.6±3	0	57.4±6
26	1±1	0	0	0	0	1±1	1±1	0	56.1±49	184±98	236.9±10	102±38
27	0	0	0	3.7±2	0	0	0	1.3±1	6.8±3	158±14	0	1±1
28	0	0	0	25.9±8	3.5±1	0	0	1.5±1	10.2±9	84.4±43	26.5±16	75.2±20
29	2.3±2	0	1±1	0	1±1	1±1	11.4±5	0	40.3±18	84.8±8	281.8±43	124±033
30	11.2±11	0	1±1	1.2±01	2±1	2.4±2	50.8±87	5.7±2	238±111	187.5±27	598.2±87	617±167

1 "α-ylangene", **2** α-copaene, **3** unknown, **4** β-caryophyllene, **5** *E*-α-bergamotene, **6** *E*-β-farnesene, **7** germacrene D, **8** α-murolene, **9** unknowm, **10** "α-farnesene", **11** β-bisabolene, **12** unknown, **13** γ-cadinene, **14** nerolidol, **15** (*E,E*)-4,8,12-trimethyl-1,3,7,11-tridecatetraene, **16-20** unknown, **21** myrcene, **22** *Z*-hexenyl acetate, **23** linalool, **24** nonatriene, **25** phenethyl acetate, **26** indole, **27** geranyl acetate, **28** β-caryophyllene, **29** *E*-α-bergamotene, **30** *E*-β-farnesene

No correlation was found between extracted amounts of constitutive and induced terpenoids amount (*P*=0.266, cor. coeff.=0.543), nor between the amounts of extracted constitutive and collected volatile terpenoids (*P*=0.957, corr. coeff.=-0.029). Between constitutive interfoliar terpenoids and induced volatile terpenoids the correlation was almost significant (*P*=0.072, corr. coeff.=0.771). Three terpenoids were extracted from plants, which were also collected as volatiles; β-caryophyllene, (*E*)-α-bergamotene and (*E*)-β-farnesene. For all three compounds a correlation was found between the amounts of extracted and collected terpenoids after treatment (*P*=0.042, corr.coeff.=0.829). The correlation between the amount inside unharmed plants and the amount released by treated plants was positive only for β-caryophyllene (*P*=0.021, corr.coeff.=0.880). Finally, also the correlation between terpenoids inside unharmed plants and the amounts released by treated plants was significant only for β-caryophyllene (*P*=0.015, corr. coeff.=0.898).

Discussion

Cost of production of induced volatiles in maize was only detectable in young plants soon after induction. Unharmed plants had very higher dry weight of leaves than treated plants after a treatment period of 2 weeks (Fig. 3.3) but very low or no fitness costs (expressed in seed dry weight) were detected at maturity (Fig. 3.5 and Fig. 3.6). Hence, during growth, treated plants compensated for the loss of energy spent for production of volatiles in early plant stages. Commonly induced defence compounds are produced in larger amounts in young plants or leaves. For example, terpenoids yields are higher in younger leaves of *Hymenaea*, were the attack of herbivores is more evident (Crankshaw and Langenheim, 1981). We know that also in maize nduced volatile terpenoid production is higher in younger plants (Gouinguené, 2000; Turlings *et al.*, 2001). Production of terpenoids only in stages of the plant or in parts of the plant that are more susceptible to herbivory already contribute to an overall cost saving and is consistent with the optimal defence theory (Crankshaw and Langenheim, 1981). Further costs are saved when defence compounds are only produced when a plant actually suffers on attack by a herbivore or pathogen (Karban *et al.*, 1997). That induced odor emissions bring about some costs can be concluded from the correlation shown in Figure 3.4; it was found that the larger the difference in odor production between fully treated plants and control plants, the larger the difference in biomass (dry weight). Maize plants (var. Delprim), attacked by a parasitized caterpillar produce about 30% more seed than plants attacked by a healthy caterpillar (Fritzsche Hoballah and Turlings, 2001). Cost of production of volatiles in mature plants were a decrease in seed dry weight of 24.3% and 0.04% in 1998 and 0%-5.1% in 1999 for the shorter treatment period and a decrease of seed dry weight of 27.7% and 20.6% in

1998 and 0%-17.7% in 1999 for the longer treatment period (Fig. 3.5 and Fig. 3.6). This large variation between years and plots indicates how the production of volatiles (Fig. 3.2) and its consequences for plant performance are environment dependent for review see (Baldwin and Preston, 1999). However, overall the decrease in seed production as a result of the treatments was minimal, and clearly less than what the plant can potentially gain by attracting natural enemies of herbivores (Fritzsche Hoballah and Turlings, 2001).

In our study, the surface of the leaves was damaged during treatment and as a consequence photosynthetic rate and growth rate of the plant were probably reduced. This would imply that the reduction in leaf-mass production was not only caused by an increased investment in volatiles. The model of Gulmon and Mooney (1986) proposes that the cost, in terms of growth reduction, of the carbon and energy needed for defensive compounds is proportional to photosynthetic rate and leaf allocation fraction. Differences in photosynthetic rate among maize varieties have been shown (Duncan and Hesketh, 1968; Fischer and Palmer, 1984). Production of induced terpenoids in maize plants is strongly light dependent (Turlings *et al.*, 1995; Gouinguené *et al.*, 2001), this was also found for cotton (Loughrin *et al.*, 1994; Paré and Tumlinson, 1997). If different maize varieties have different photosynthetic rates, this could result in differences in induced amounts of defensive compounds. But since growth of the studied inbred lines was comparable, the observed differences are more likely the result of genetic variability in metabolic investment in the synthesis of volatiles such as genetic variation in inducible responses has been found for 14 plant-herbivore systems (for review see Agrawal, 2000).

Reduced growth rate after induction could also be caused by investment in the synthesis of other (defence) compounds. In fact, the common defence hormone jasmonic acid was found to be a growth inhibitor in some plants (Meyer *et al.*, 1984) jasmonic acid is also an elicitor of the release of volatile compounds after wounding of a plant (Paré and Tumlinson, 1999). In tomato and potato, jasmonic acid also induces the accumulation of proteinase inhibitors (Pena-Cortés *et al.*, 1993). Production of non-volatile compounds, toxic to herbivores, could therefore have contributed to the lower dry weight of treated plants.

Different genes seem to be involved in the production of direct and indirect defence compounds in maize. Shen *et al.* (2000) recently identified a sesquiterpene cyclase gene in maize plants that responds to herbivory. Genes responsible for indole release in the atmosphere and indole produced for DIMBOA synthesis are different (Frey *et al.*, 2000), meaning that direct and indirect defences that make use of the same or similar compounds could be triggered by separate genes.

Differences in genes and pathways activation for the production of secondary compounds between constitutive and induced defence or the between direct and indirect defence are poorly understood. There is some evidence that induced and constitutive defence compounds are positively correlated (Björkman and Anderson, 1990; Campbell and Duffey, 1979; Siemens and Mitchell-Olds, 1998; Agrawal, 2000;Scutareanu *et al.*, 1999; Zangerl and Berenbaum, 1993)). Only in one system a negative or lack of correlation between constitutive and induced defence was observed (Brody and Karban, 1992). In our case, no correlation was detected between the production of induced and constitutive terpenoids in maize plants. However a positive correlation was found between terpenoids released under induction and terpenoids extracted from unharmed plants. This means that in maize, a high presence of constitutive constitutive terpenoids that may serve in direct defence corresponds with large amounts of induced volatile terpenoids that may serve in indirect defence. The three dominating terpenoids detected in maize leaf extracts were also the major terpenoids released in the atmosphere under induction (β-caryophyllene, (*E*)-α-bergamotene and (*E*)-β-farnesene, Table 6.2). For all three compounds a positive correlation was found between their presence inside the leafs and the amounts at which they were released after treatment. These terpenoids may act simultaneously as direct and indirect defence for the plant. For example, caryophyllene affects mortality and growth rate of *S. exigua* and quantitative variation in caryophyllene content among *Hymenaea* cultivars result in differential resistance (Crankshaw and Langenheim, 1981). Terpenoid aldehyds in cotton induced by feeding of *S. exigua* are toxic to the caterpillars (McAuslane *et al.*, 1997). It is also known that caryophyllene can attract predators such as the green lacewing (Flint *et al.*, 1979). β-Caryophyllene may be produced by an other pathway than the other two major terpenoids, since correlation between the amount inside unharmed plants and the amount released by treated plants was significant only for this compound. That caryophyllene production is independent of bergamotene and farnesene production is also evident from the fact that there is a difference in timing of production (Turlings *et al.*, 1998b; Gouinguené, 2000). In cotton, *de novo* synthesis of farnesene was demonstrated with ^{13}C labeling, in contrast only low metabolic turnover was found for caryophyllene (Paré and Tumlinson, 1997). Delprim produces caryophyllene under induction, while Pactol does not produce caryophyllene. Difference in dry weight of leaves between scratched and regurgitant-treated plants was higher for Delprim. This may be an indication that caryophyllene is costly to produce.

Conclusions

Domestication is often associated with loss of defences (Loughrin *et al.*, 1995; Rosenthal and Dirzo, 1997), however, the release of volatiles is similar for cultivated maize and its wild ancestors (Gouinguené, 2000). Optimal defence theory predicts that organisms evolve to allocate defences in the way that it maximises individual inclusive fitness and that these defences are costly (Rhoades, 1979). Therefore, poorly defended individuals have higher fitness than well defended individuals when enemies are absent (Rhoades, 1979). The fact that younger maize plants release higher quantities of induced volatiles fits the first prediction of this theory. Our findings that plants that were treated to release volatiles showed a decrease in leaf-mass gain (Fig. 3.4) is also congruent with the second prediction of the theory. However, overall fitness was not reduced as the treated plants compensated for these losses over time and produced just as much seed as control plants. In conclusion, there appears to be some cost of induced volatile production in maize plants but is only in part detectable in the reproductive output of a plant. Benefits of releasing volatiles when under attack by herbivore can be considerable (Fritzsche Hoballah and Turlings, 2001), however, only in environments where natural enemies of the herbivores will be present and responsive to the odors.

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CHAPTER 4

Differential attractiveness of induced odors emitted by eight maize varieties for the parasitoid *Cotesia marginiventris*: is quality or quantity important?

Based on:

Fritzsche Hoballah, M. E.; Tamò, C.; Turlings, T. C. J. Differential attractiveness of induced odors emitted by eight maize varieties for the parasitoid *Cotesia marginiventris*: is quality or quantity important? Submitted to *Journal of Chemical Ecology* (in press).

Abstract

Herbivore-induced plant volatiles can function as indirect defence signals that attract natural enemies of herbivores. Several parasitoids are known to exploit these plant-provided cues to locate their hosts. One such parasitoid is the generalist *Cotesia marginiventris*, which is, among others, attracted to maize volatiles induced by caterpillar damage. Maize plants can be induced to produce the same blend of attractive volatiles by treating them with regurgitant of *Spodoptera* species. We collected and analyzed the regurgitant-induced emissions of two plant species (cowpea and maize) and of 8 Mexican maize varieties and found significant differences among their volatile emissions, both in terms of total quantity and of the quality of the blends. In an Y-tube olfactometer, the odors of the same artificially induced plant species and Mexican varieties were offered in dual choice experiments to naïve mated females of *C. marginiventris*. The wasps showed a significant preference for cowpea over maize odor and in 3 out of 12 combinations with the maize varieties they showed a significant preference for the odors of one of the varieties. A comparison of the odor collection with the results from the behavioral assays indicates that not only the quantity of the volatile emissions, but also the quality (composition) of the volatile blends is important for the attraction of *C. marginiventris*. The results are discussed in the context of the possibility to breed crop varieties that are particularly attractive to parasitoids.

Keywords-induced plant volatiles, parasitoid, olfactometer, synomones, *Cotesia marginiventris*, *Spodoptera littoralis*, *Zea mays*, *Vigna unguiculata*, parasitoid attraction, odor variability

Introduction

Herbivore-induced odors have been suggested to benefit plants in different ways (Vet and Dicke, 1992; Turlings and Benrey, 1998; Sabelis *et al.*, 1999; Dicke and van Loon, 2000). For instance, the odors could signal to herbivores that the plant defence system has been switched on and that the plant is already under attack by herbivore competitors. Over the last 15 years, it has also become evident that these odors are important for the attraction of natural enemies to the microhabitat of their prey (e.g. Dicke *et al.*, 1990a; Turlings *et al.*, 1990a; Steinberg *et al.*, 1993; Du *et al.*, 1998; Röse *et al.*, 1998). The blend of odors released by the plants after herbivore attack is variable, depending on the plant (species and variety) and depending on the herbivore, although some compounds are very commonly emitted (for reviews see Dicke, 1994; Dicke, 1999). For European maize varieties and their wild ancestors it is known that they differ considerably in quality and quantity of odors if induced to produce volatiles by treating scratched leaves with *Spodoptera littoralis* (Lepidoptera: Noctuidae) regurgitant (Gouinguené *et al.*, 2001). By differences in quality of the odor blends we mean differences in the presence of specific compounds in the blend and/or the proportions among compounds . This variation among maize varieties could have important consequences for pest management, as biological control may be more effective for varieties that are highly attractive to natural enemies. This may be particularly true for countries as Mexico and South America were the major pests of maize are leaf-feeding *Spodoptera* caterpillars (for review see Andrews, 1988). Young *Spodoptera* caterpillars can be parasitized by *Cotesia marginiventris* (Hymenoptera: Braconidae), a common solitary endoparasitoid that attacks Lepidopteran larvae during the first and second instar (Jalali *et al.*, 1987). *C. marginiventris* females are strongly attracted by the odors emitted by caterpillar-damaged maize seedlings (Turlings *et al.*, 1990a and 1991a) and spend more time on caterpillar-damaged plants than on artificially damaged or unattacked plants (Loke *et al.*, 1983). Maize plants infested with a *Spodoptera* larva parasitized by *C. marginiventris* produce more seeds than plants infested with a healthy caterpillar (Fritzsche Hoballah and Turlings, 2001), which could translate into instant a higher yields for maize cultivars that are particularly attractive to the parasitoid. Hence, it seems important to know which factors determine the attractiveness of the crop.

Here we report on a study on how differences in induced volatile emissions among different plant genotypes may affect the attractiveness of these genotypes to parasitoids. We first compared the emissions of maize and cowpea and their attractiveness to *C. marginiventris*. The former plant species is know to release large amounts of induced volatiles (e.g. Turlings *et al.*, 1998), while the latter releases relatively little, but can be quite attractive (Whitman and Eller, 1990). Furthermore, we investigated the attractiveness of 8 Mexican maize cultivars. Tests were carried out by offering odors of the two respective plant species and 12 combinations of the Mexican maize varieties to naïve *C. marginiventris* females in an Y-tube olfactometer. Comparison of the results from these behavioral assays with the odor blends that were collected from the various plants, revealed that attractiveness is not simply a matter of quantity of volatiles, but that the quality of the blend is important as well.

Material and methods

Growing and treatment of plants. Plants used in all experiments were grown in plastic pots (6 cm high, 8 cm diameter) in fertilized commercial soil (Coop, Switzerland) in a climate chamber (23°C, 60% r.h., and 16L: 8D, 50000 lm/m^2). Two weeks old *Vigna unguiculata* (var. kpodii-guegue, obtained from IITA, Benin, Africa) and 10-day old *Zea mays* (var.

Delprim and varieties obtained from CIMMYT, Mexico, Table 4.1) plants were used for the experiments.

Table 4.1: Variety number, name, origin and seed color of 8 Mexican corn varieties used for the volatile collection and choice experiments.

Variety	**Name**	**Origin, seeds color**
1	Its 2 Sint. A1# Bulk	PR-99-A 761-A yellow
2	Its 2 Sint. A1# Bulk	PR-99-A 761-B white
3	Its 2 Sint. B3# Bulk	PR-99-A 761-B white
4	Its 2 Sint. A1# Bulk	PR-98-B 5766-A yellow
5	Its 2 Sint. B1# Bulk	PR-98-B 5766-B white
6	P36 C9 HC60-B-1-BBBBBBBBB-2 CL-03618	PR-99 A 402-16 yellow
7	CML287(P24F26*P27F1)-4-1-B-1-1-BB-F-####-BBB	PR-98 A 479-11 yellow
8	CL-00331SINT.AM.TSR-23-3-2-3-2-BB-F-##-B-B CML413	PR-98 A 474-16 yellow

To induce the plants to produce volatiles, we scratched two leaves (the second and third leaf of maize and first and second of cowpea) of each plant with a razor blade (an area of 2-4 cm^2 per leaf) and subsequently applied on each damaged site 10 µl regurgitant. Regurgitant was collected from 4th and 5th instar of *S. littoralis* caterpillars (for method see Turlings *et al.*, 1993a) and kept at –70 °C until just before utilization. After treatment, plants were placed 5 h (plant species) and 6 h (varieties), under 3 fluorescent lamps (Sylvania standard F36W 133-T8 cool white, 5000 lm/m^2 at pot height), before they were used for the volatile collection and olfactometer experiments. It is known that 5-6 h after treatment corn plants emit relatively large quantities of induced volatiles (see Turlings *et al.*, 1998).

Insects. *S. littoralis* caterpillars and eggs were supplied weekly by Syngenta (Basel, Switzerland). Eggs were incubated in Petri dishes (9 cm diam., 1.5 cm high) on moist filter paper. Emerged caterpillars were fed with artificial diet and kept in plastic-boxes (15x9x5 cm) under ambient laboratory conditions. This non-native host causes a similar reaction in plants as the natural host *S. fruguperda* (unpublished data, Fritzsche Hoballah)

The solitary endoparasitoid *C. marginiventris* that was used in the experiments, originated from the USDA-ARS, Biological Control and Mass Rearing Research Unit (Mississippi, USA). For the rearing of parasitoids, 25 young caterpillars (3-4 days old) were offered to a single mated female (4-7 days old) for 3 hours in a plastic-box (9.5 cm diam., 5 cm high). The caterpillars were further reared on artificial diet in an incubator (25°C and 16L: 8D) until cocoon formation. Cocoons were kept in Petri dishes until adult emergence. Emerging adults were sexed and kept in cages (30 x 30 x 30 cm) at a sex ratio of 1:2 (male: female), with honey drops as a food source and distilled water on cotton and plastic flowers which provide hiding places to facilitate mating (personal observation, Betty Benrey). The cages were kept in the laboratory under ambient light and temperature conditions.

Volatile collection. After treatment and light exposure, plants were placed in a climate chamber (CMP4030, Conviron, Winnipeg, Canada) and the volatiles emitted were collected. The climate chamber was programmed at 20 °C and with a light intensity of 14500 lm/m^2 (light provided by 28 lamps, 16x Sylvania 150 W VHO Cool White and 12x Sylvania, 100 W Satin). Volatiles were collected from 6 plants simultaneously in an automated collection system as described by Heath and Manukian (1992), Turlings *et al.* (1998) and Gouinguené *et al.* (2001). Glass cylinders (9.5 cm diameter, 54 cm high) were placed over the plants and rested on a Teflon disk consisting of two halves with a hole in the middle for the stem of the plant. Air was pushed (1 l/min) through a charcoal filter and introduced from the top of each cylinder over the plants. Super-Q traps (25 mg, 80/100 mesh, Alltech, Deerfield State) were attached laterally at the base of each cylinder. Traps were connected to an automated volatile collection system (ASU, ARS, Gainesville, USA) through which the air was pulled out at 0.8 l/min during 2 h. The collection period was programmed with the use of the software TESS (Version 1.0, ARS, Gainesville, USA). After each collection, traps were rinsed with 150 µl methylene chloride and 200 ng of *n*-octane and nonyl acetate were added as internal standards. Three µl aliquots of the samples were injected on column, with an automated injection system into a Hewlett Packard model HP 6890 gas chromatograph equipped with a flame ionisation detector. The apolar EC-1 capillary column (30 m, 0.25 mm I.D., 0.25 µm film thickness, Alltech Associates, Inc, USA) was held at 50 °C for 3 minutes and then programmed at 8 °C per minute to 230 °C, were it was maintained for 9.5 minutes. The column was preceded by a deactivated retention gap (10 m, 0.25 mm I.D., Connex, USA) and a deactivated precolumn (30 cm, 0.530 mm I.D., Connex, USA). Helium (24 cm/s) was used as carrier gas. We collected and analyzed the odor of treated cowpea and maize plants (n=6) as well as of 8 Mexican varieties (n=5 for each variety). HP GC Chemstation software was used to quantify all major components by comparison to the known quantity of internal standards. An ANOVA and the Student-Newman-Keuls post-hoc test were used to compare the total quantity of volatiles emitted among varieties (data ln-transformed). To compare differences in total emission emitted between plant species and among single compounds emitted by the different species and plant varieties the Mann-Whitney Test was used. Tests were carried out with the program SPSS 10.0 for Windows.

Choice experiments. An Y shaped olfactometer was used to test the attractiveness of plant odors to *C. marginiventris* females. The system, based on a design by Sabelis and Van de Baan (1983), consisted of a central tube (13.5 cm long, 4 cm diam.) and two lateral arms (12 cm long, 4 cm diam.) ending with a glass frit (to prevent escape of test insects and homogeneous air flow). The lateral arms tapered off into 0.5 cm tubes to which Teflon tubing was connected. To introduce the odors in the Y tube Teflon tubes were used. Wasps were released singly at the base of the central arm of the Y-tube. In a first experiment they were offered the odor of induced cowpea and maize (var. Delprim). In subsequent experiments they had the choice between the odor of two different induced Mexican maize varieties. Twelve among the 28 possible combinations of the 8 Mexican varieties were randomly chosen to test in the olfactometer. Our goal was not to detect which varieties are the most attractive among the 8 used, but to determine if exclusively the quantity or also the quantities of the blends are important for *C. marginiventris* attraction. To limit visual distractions for the insects, the Y tube olfactometer was placed inside a white paper box, which was open on top (for illumination) and on the front side (for observation). An office lamp (60 W, 750 lm/m^2) illuminated the olfactometer. A black and white striped pattern for visual orientation was placed under the Y-tube. Plants, treated and incubated in the same way as for volatile collections, were placed in plastic bags that were then heat-sealed. Humidified and purified (with activated charcoal) air was pushed into each bag via Teflon tubing and airtight connectors at pot height. The air passed over the plants and into the Teflon tubes connected with the arms of the olfactometer at a rate of 0.8 l/min. Naïve (no experience with hosts or plants) wasps were placed individually in the base of the olfactometer and observed during 10 minutes. If the wasp did not make a choice after this period it was removed and recorded as a "no choice". Wasps that walked to the end of one of the arms and stayed there at least 5 seconds were recorded as having made a choice for the odor offered through that arm. After 4-7 individuals were tested, the olfactometer was turned such that the direction of the arms was reversed and 4-7 other wasps were tested. Each combination of odor sources was tested 6-9 times with different plants and on different days and each time the position of the two different odor sources was switched. A minimum of 67 and maximum of 94 wasps were tested in the twelve combinations of maize odor sources.

A Binomial Test was used to determine significant preferences for an odor. The number of wasps that chose the highest and lowest volatile emitting maize varieties in each experimental day, was compared with a Wilcoxon Signed Rank Test. This test was carried out using data of all experimental days, to determine if the quantity (regardless of quality) of the odor was important for the attraction of the wasps.

Results

Volatile collection. The two plant species, cowpea and maize, differed dramatically in the quantities of volatiles emitted (Fig. 4.1). The total amount of volatiles collected during the experiment was almost 53 times higher in maize (4141.35±460.2 ng/2 h) than in cowpea (77.9±20.9 ng/2 h) (n=6, Mann-Whitney Test: *P*=0.004, Z=-2.882). The two plant species released several identical compounds, but cowpea did not release benzyl acetate, phenethyl acetate, 1-H-indole, (*E*)-β-caryophyllene, "β-sesquiphellandrene" and (*E*, *E*)-4,8,12-trimethyl-1,3,7,11-tridecatetraene and released only traces of (*Z*)-3-hexenyl acetate, (*E*)-β-ocimene, α-(*E*)-bergamotene, (*E*)-β-farnesene, "β-bisabolene" and nerolidol (Fig. 3.1). In contrast, cowpea emitted considerable amounts of (*E*)-2-hexenal and significantly more (*Z*)-3-hexenol than maize plants (Fig. 4.1).

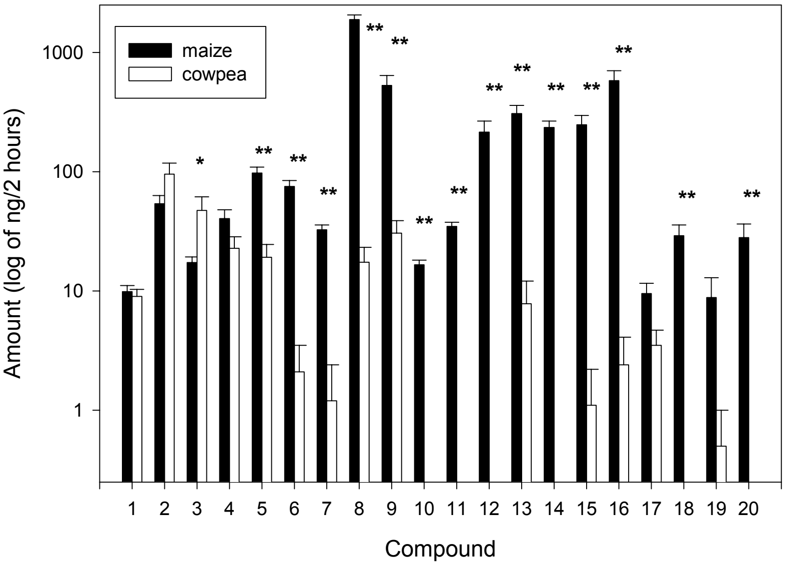

Figure 4.1: Mean amount (±SE) of volatiles collected from cowpea and maize plants that were artificially damaged and treated with regurgitant of *Spodoptera littoralis* caterpillars. Volatiles were collected for 2 h, 5 h after treatment. Asterisks on bars indicate significant differences (Mann-Whitney Test (2-tailed):*$P<0.05$, **$P<0.005$) between single compounds released by two plant species. The compounds are: **1**, "1-(*Z*)-3-hexenal"; **2**, (*E*)-2-hexenal; **3**, (*Z*)-3-hexenol; **4**,"2-hexenol"; **5**, β-myrcene; **6**, (*Z*)-3-hexenyl acetate; **7**, (*E*)-β-ocimene; **8**, linalool; **9**, (*E*)-4,8-dimethyl-1,3,7-nonatriene; **10**, benzyl acetate; **11**, phenethyl acetate; **12**, 1-H-indole; **13**, geranyl acetate; **14**, (*E*)-β-caryophyllene; **15**, (*E*)-α-bergamotene; **16**, (*E*)-β-farnesene; **17**, "β-bisabolene"; **18**, "β-sesquiphellandrene"; **19**, nerolidol; **20**, (*E,E*)-4,8,12-trimethyl-1,3,7,11-tridecatetraene (compounds between "" were identified by the use of a MS-Library; control injections with the pure compound were not made).

Among Mexican maize varieties we also found significant differences in the total amounts of volatiles collected (n=5, ANOVA: d.f.=7, F=10.589, *P*<0.0001, Fig. 4.2). Variety 1 emitted 14 times more volatiles than variety 8 (Fig. 4.2). We compared the emissions of volatiles for the varieties that were shown to be differentially attractive to *C. marginiventris* (see below). Variety 4 released significantly more (*Z*)-3-hexenyl acetate, (*E*)-4,8-dimethyl-1, 3,7-nonatriene, benzyl acetate, phenethyl acetate, (*E*)-α-bergamotene, (*E*)-β-farnesene and "β-sesquiphellandrene" than variety 7 (Fig. 4.3A).

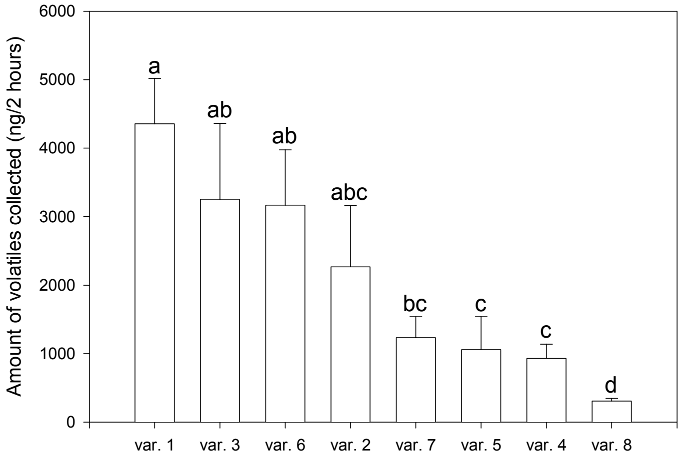

Figure 4.2: Mean amount (±SE) of volatiles collected from different varieties of maize seedlings (10 days old) that were artificially damaged and treated with regurgitant of *Spodoptera littoralis* caterpillars. Volatiles were collected 6 hours after treatment, for 2 hours. Letters above bars indicate significant differences between varieties in the total amount released (Student Newman Keuls Post Hoc Test after ANOVA: d.f.=7, F=10.589, P<0.0001).

Variety 4 did not release geranyl acetate and ocimene and variety 7 did not release cycloisosativene/ylangene and sesquiphellandrene (Fig. 4.3A). Variety 1 released significantly more (*Z*)-3-hexenyl acetate, (*E*)-4,8-dimethyl-1, 3,7-nonatriene, benzyl acetate, phenethyl acetate, geranyl acetate and "cycloisosativene/α-ylangene" than variety 6 (Fig. 4.3B). Variety 6 did not release the compounds: (*Z*)-3-hexenyl acetate, (*E*)-β-ocimene, benzyl acetate and phenethyl acetate (Fig. 4.3B). Variety 1 released significantly more linalool, benzyl acetate, phenethyl acetate, 1-H-indole, geranyl acetate, α-(*E*)-bergamotene, (*E*)-β-farnesene and "β-sesquiphellandrene" than variety 5 (Fig. 4.3C). Variety 5 did not produce "cycloisosativene/α-ylangene" (Fig. 4.3C).

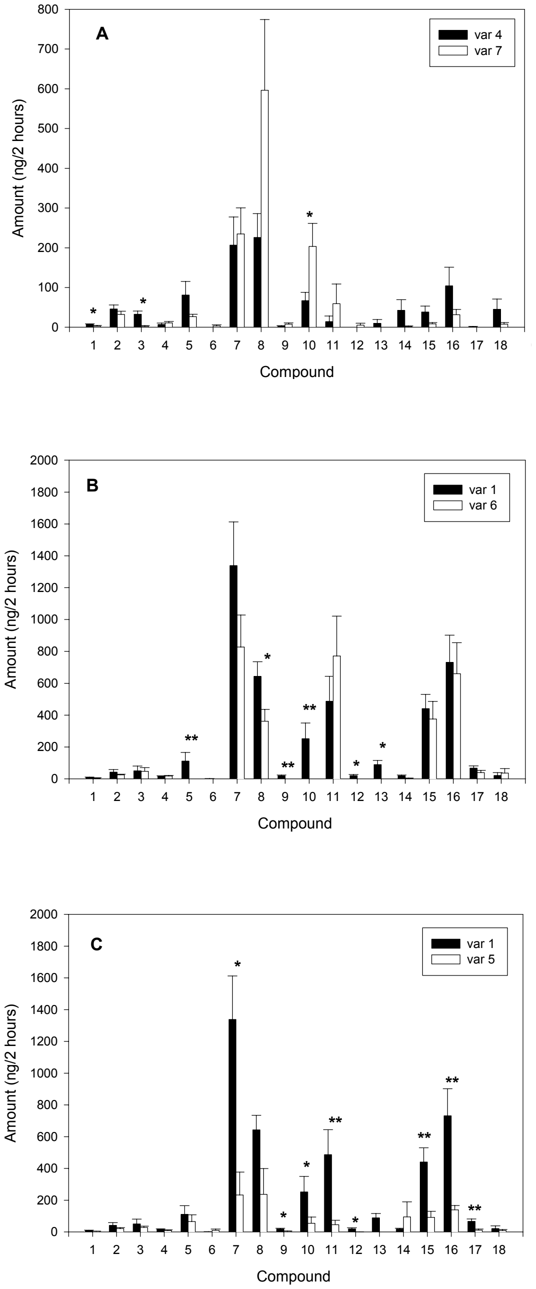

Figure 4.3: Mean amount (±SE) of individual compounds collected from maize varieties that were artificially damaged and treated with regurgitant of *Spodoptera littoralis* caterpillars. Volatiles were collected 6 h after treatment, for 2 h. Asterisks above bars indicate significant differences (Mann-Whitney Test (2-tailed): $*P<0.05$, $**P<0.005$, $***P<0.0005$), between the two plant varieties (**A**: between varieties 4 and 7, **B**: 1 and 6 and **C**: 1 and 5). The compounds are: **1**, "1-(*Z*)-3-hexenal"; **2**, (*E*)-2-hexenal; **3**, (*Z*)-3-hexenol; **4**, β-myrcene; **5**, (*Z*)-3-hexenyl acetate; **6**, (*E*)-β-ocimene; **7**, linalool; **8**, (*E*)-4,8-dimethyl-1,3,7-nonatriene; **9**, benzyl acetate; **10**, phenethyl acetate; **11**, 1-H-indole; **12**, geranyl acetate; **13**, "cycloisosativene/α-ylangene"; **14**, (*E*)-β-caryophyllene; **15**, (*E*)-α-bergamotene; **16**, (*E*)-β-farnesene; **17**, "β-sesquiphellandrene"; **18**, (*E,E*)-4,8,12-trimethyl-1,3,7,11-tridecatetraene (compounds between "" were identified by the use of a MS-Library; control injections with the pure compound were not made).

Choice experiments. Cowpea odors were more attractive to *Cotesia marginiventris* than maize odors (Binomial Test, *P*=0.007, Fig. 4.4). Between different maize varieties the parasitoids showed only a preference in 3 out of the 12 combinations (Fig. 4.4). In one case (var. 1 vs. var. 5) the wasps were attracted to the variety releasing more volatiles (Fig. 4.3C, 4.4). However, in two cases (var. 4 vs. var. 7; var. 1 vs. var. 6), the varieties tested released the same amount of volatiles (Fig. 4.3 A and B, 4.4).

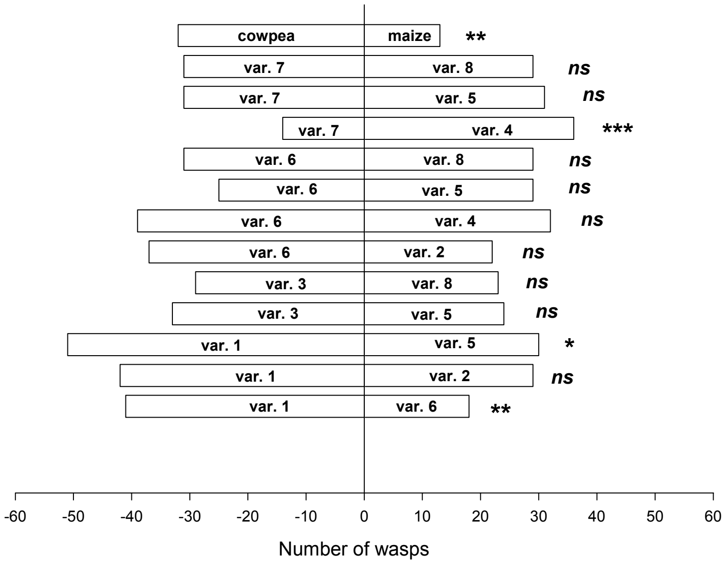

Figure 4.4: Number of choices made by naïve *Cotesia marginiventris* in dual choice tests with various plant combinations offered in a y-tube-olfactometer. Asterisks indicate a significant difference within a choice test (**P*<0.01,***P*<0.001,****P*<0.0001) and ***ns*** indicates no significant difference (Binomial test).

A comparison for all tested combinations between the number of wasps that chose the higher emitting maize variety (4.6±0.25 wasps) and the number of wasps that chose the variety that released a lower amount of odors (3.8±0.24 wasps) was carried out. It revealed a marginal significant preference for the variety that emits larger amounts of volatiles offered in the dual choice test (all single experimental days used, Wilcoxon Signed Rank Test: *P*=0.045, Z=-2.008).

Discussion

Difference in induced volatile emission among plant species and varieties.

Differences in herbivore-induced odor emissions among plant species, genotypes have been demonstrated in several studies (Takabayashi *et al.*, 1991, 1994a; Loughrin *et al.*, 1995; Geervliet *et al.*, 1997; Halitschke *et al.*, 2000; Ozawa *et al.*, 2000). In maize these difference can be considerable (Gouinguené, *et al.*, 2001). The current study confirms that different plant species, but also different varieties release qualitatively and quantitatively different blends of induced odors. The eleven most important compounds that were collected from induced maize plants are partially shared with other plant species for which induced volatiles have been studied (Table 4.2). The only compound released by all plant species is (Z)-3-hexenol and in 12 out of 14 cases, (Z)-3-hexenyl acetate and (E)-4,8-dimethyl-1,3,7-nonatriene are also shared (Table 4.2).

Table 4.2: Volatiles collected from regurgitant-induced maize plants, shared among other plant species

Compound	apple	bean	brussels sprouts	cabbage	cocumber	cotton	gerbera	pear	potato	soybean	tobacco	cowpea
1-(Z)-3-hexenal						+				+		+
(E)-2-hexenal	+(E?)	+	+	+		+		+		+		+
(Z)-3-hexenol	+	+	+	+	+	+	+	+	+	+	+	+
2-hexenol	+		+			+		+				+
β-myrcene	+(β?)		+	+(β?)		+(β?)	+(β?)					+
(Z)-3-hexenyl acetate	+	+	+	+	+	+	+	+		+		+
(E)-β-ocimene	+	+			+	+	+(Z)	+			+	+
linalool	+	+		+			+	+	+		+	+
(E)-4,8-dimethyl-1,3,7-nonatriene	+	+	+	+	+	+	+(Z)	+	+			+
benzyl acetate												
phenethyl acetate												
1-H-indole				+		+	+			+		
geranyl acetate												+
(E)-β-caryophyllene	+(E?)					+	+	+(E?)	+		+	
(E)-α-bergamotene							+		+		+(Z)	+
(E)-β-farnesene	+(E?)		+(α-)		+((E,E)-α)	+(γ-)	+	+(α)	+		+((E,E)-α)	+
β-bisabolene							+		+			+
β-sesquiphellandrene							+		+			
nerolidol							+					+
(E,E)-4,8,12-trimethyl 1,3,7,11-tridecatetraene	+	+	+	+	+	+	+(di-)		+			

References: apple: (Takabayashi *et al.*, 1991), (Takabayashi *et al.*, 1994a); lima bean: (Dicke *et al.*, 1990b); brussels sprouts: (Mattiacci *et al.*, 1994); cabbage: (Agelopoulos and Keller, 1994),(Blaakmeer *et al.*, 1994), (Geervliet *et al.*, 1997); cucumber: (Dicke *et al.*, 1990b), (Takabayashi *et al.*, 1994b); cotton: (McAuslane and Alborn, 1998),(Loughrin *et al.*, 1995),(McCall *et al.*, 1994); gerbera:(Gols *et al.*, 1999); pear: (Scutareanu *et al.*, 1997); potato: (Weissbecker *et al.*, 2000),(Agelopoulus *et al.*, 2000),(Bolter *et al.*, 1997); soybean:(Turlings *et al.*, 1993b); tobacco: (De Moraes *et al.*, 1998), (Halitschke *et al.*, 2000); cowpea: present study.

We found large qualitative and quantitative differences in induced odor blends between the plant species, maize and cowpea, and to a lesser extent among different maize varieties. Cowpea released almost only green leaf volatiles, as was found by Whitman and Eller (1990), and maize released more indole, linalool and a range of terpenoids that differed in presence, proportion and amount among varieties. Among maize varieties the difference in total emission of volatiles was striking, with a 14-fold difference between lowest and highest emitting variety. When comparing the odors released by plants infested by *Pieris* larvae, Geervliet *et al.* (1997) found clear differences in odor blends released by different plant species and cabbage varieties. Takabayashi *et al.* (1991, 1994a) found a qualitative and quantitative difference in volatile compounds emitted between two varieties of apple attacked by the same spider mite. In a comparison of *Spodoptera exigua* infested leaves of different cotton varieties, naturalized cotton released seven times more volatiles than commercial cultivars (Loughrin *et al.*, 1995). One constraint of using insects to induce odor emissions is that observed differences could be simply due to the fact that herbivores eat more or less of a specific plant species or variety. Using elicitors may allow for a more precise comparison. This was done by Halitschke *et al.* (2000), who treated the leaves of three genotypes of *Nicotiana attenuata* with methyl jasmonate and found a difference in the pattern of emitted compounds. The standardized treatment of the Mexican maize varieties rules out any variable treatment effects. The results corroborate the tremendous variability in induced odor emissions in the genus *Zea* (see also Turlings *et al.*, 1998 and Gouinguené *et al.*, 2001).

Responses of natural enemies to plant odors.

It is known that parasitoids show innate preferences to odors of specific undamaged plants (Ngi-Song *et al.*, 1996; Vaughn *et al.*, 1996; Benrey *et al.*, 1997). Furthermore, parasitoids show innate preferences for odors of specific plant varieties. For example, the parasitoid

Campoletis sonorensis was attracted differentially to odors released by different cotton varieties (Elzen *et al.*, 1986). In contrast, the parasitoid *Diaeretiella rapae*, did not distinguish among six grass varieties presented in a flight tunnel assay (Vaughn *et al.*, 1996). If plants are damaged by herbivores, odors subsequently produced and released by the plants are usually more attractive to parasitoids than odors of undamaged plants (Turlings *et al.*, 1991b; Steinberg *et al.*, 1993; Blaakmeer *et al.*, 1994; Agelopoulos *et al.*, 1995; Finidori-Logli *et al.*, 1996; Bertschy *et al.*, 1997; Cortesero *et al.*, 1997; Röse *et al.*, 1998). Choice experiments carried out in flight tunnels and in semi field plots show that parasitoids have preferences for specific herbivore-attacked plant species (Drost *et al.*, 1988; Geervliet *et al.*, 1996; Du *et al.*, 1998, De Moraes and Lewis, 1999).

Our study compared the attraction of *C. marginiventris* females to cowpea and maize and among maize varieties, focusing on odor cues exclusively. Cowpea odors were preferred over maize odors by naïve *C. marginiventris* females. This was somewhat surprising because cowpea released much less odors than maize. We had expected the quantity of odors to be positively correlated with attractiveness, as is indicated by dose-dependent responses of natural enemies in several studies (Eller *et al.*, 1988; Turlings *et al.*, 1990a and 1991b; Ngi-Song *et al.*, 1996; Vaughn *et al.*, 1996; Weissbecker *et al.*, 1999). Although the dual choice experiments with maize varieties showed that in general, *C. marginiventris* is more attracted to high releasing maize varieties, in a few combinations females showed preferences even if the quantities of odors produced by the two varieties did not differ. These results indicate that some compounds are more important than others for attraction of the wasps, and that the proportions among compounds in the blend could play an important role. Cowpea released relatively larger proportions of green leaf volatiles as (*Z*)-3-hexenol, a very common compound among plant induced volatiles (Table 4.2), than maize. Cortesero *et al.* (1997) analyzed the responses of *C. marginiventris* females to cotton plants and suggested that they primarily cue on recently damaged plants, which are known to release green leaf volatiles. Other parasitoids like *Microplitis croceipes* and *Netelia heroica* are also attracted to green leaf volatiles, especially to different hexenols and hexenals, when they are offered individually in a flight tunnel (Whitman and Eller, 1990). The parsitoid *Aphidius rhopalosiphi* was very highly attracted to (*Z*)-3-hexenyl acetate and (*E*)-2-hexenal in an Y tube during no choice experiments, the parasitoid was inexperienced (Wickremasinghe and Van Emden, 1992). These same chemicals are involved in the attraction of *C. glomerata* to infested or regurgitant-treated cabbage plants (Mattiacci *et al.*, 1994). Green leaf volatiles are probably the most common volatiles released by plants damaged by herbivores (Table 4.2), which could explain why natural enemies without previous experience (naïve) are strongly attracted to them. (*Z*)-3-hexenol, which is shared by all plant species listed in Table 4.2, elicits strong responses in the two-spotted stinkbug predator *Perillus bioculatus*, while β-caryophyllene, a terpenoid shared by half of the plant species, elicited only weak responses (Weissbecker *et al.*, 1999). Yet, the importance of specific plant volatiles and mixtures of volatiles has been suggested for the attraction of some natural enemies. The lacewing *Chrysopa carnea*, a predator of small insects, was caught more often in traps containing β-caryophyllene, than in traps that contained limonene, bisabolene or caryophyllene oxide (Flint *et al.*, 1979). In contrast, β-caryophyllene did not elicit antenna palpation of the parasitoid *Campoletis sonorensis*, whereas caryophyllene oxide did (Elzen *et al.*, 1984). The EAG-response of the two spotted stinkbug *Perillus bioculatus* was strong for a α-zingiberene and bicyclogermacrene mixture, but low, when these two compounds were offered singly to the predator (Weissbecker *et al.*, 2000). The volatile compound Borneol, extracted from garry oak, *Quercus garryana*, attracted the tachinid parasitoid *Cyzenis albicans* (Roland *et al.* 1995).

Innate versus learned responses.

Generally, naïve females respond poorly to odor cues, but a contact experience with hosts and/or host feces increases this responsiveness (Dmoch *et al.*, 1985; Wardle *et al.*, 1989; Whitman and Eller, 1990; Steinberg *et al.*, 1992). These experiences can increase the insects motivation to search for a host, but is also the result of associative learning of the encountered odors (Turlings *et al.*; 1993b; Vet and Groenewold, 1990). *C. marginiventris* females too show a dramatic increase in responses to herbivore-damaged maize plants after females contact a host-damaged plant (Turlings *et al.* 1989, 1993b). The wasps prefer plants that have been damaged by caterpillars for more than 10h (old damage) over plants with fresh damage if they experience plants with old damage (Turlings *et al.*, 1993b). However, females that had experienced fresh caterpillar damage, chose equally between plants with fresh and old damage. Naïve females responded very poorly and the few that did respond showed no preference (Turlings *et al.*, 1993b). The olfactometer assays allowed us to better test odor preferences in naïve females. The strong responses to cowpea indicate that the innate preference is for the most common herbivore-attacked plant volatiles, the green leaf volatiles. Once a host is encountered, typical host-induced volatiles, like specific terpenoids, may be associated with host-presence and the wasps will use these more reliable cues in search for more hosts (Vet and Dicke, 1992). The fact that naïve females are less responsive to specific odor cues, could explain why we only found 3 clear preferences among the 12 combinations tested in the Y-tube olfactometer. Nevertheless, the results suggest that *C. marginiventris* has some innate preference for certain odor blends. Additional experiments will have to be conducted to reveal the key compounds in these blends. It would be useful to test the response of the parasitoids to plants that differ only in a single compound as suggested by Dicke and van Loon (2000). Genetic manipulation of odor emissions in plants could make this possible.

Selection of varieties and wasps for their use in biological control.

The selection of varieties that are highly attractive to parasitoids or selection of parasitoids that are particularly responsive to specific odors could be used to enhance biological control programs as suggested by Bottrell and Barbosa (1998) and Loughrin *et al.* (1995). The exploitation of parasitoid associative learning in pest management has also been suggested (Wardle and Borden, 1985; Lewis and Martin, 1990; Papaj and Vet 1990; Vet and Groenewold, 1990).

Some of the Mexican maize varieties tested here appear to be more attractive than others. Field studies will have to reveal if these differences in attractiveness are reflected in parasitism rates. The high genetic variability observed in odor emissions among maize lines (Gouinguené *et al.*, 2001) make it an ideal plant to optimize its attractiveness.

In summary, we confirmed that there are qualitative and quantitative differences in herbivore-induced odor blends among maize varieties. Both quality and quantity of the volatile blends were important for the attraction of inexperienced *C. marginiventris*. We speculate that green leaf volatiles, which are the most common volatiles produced by herbivores-damaged plants, could be important for the attraction of naïve generalist parasitoids as *C. marginiventris*. More specific compounds such as terpenoids, could be learned associatively during host encounters. The odor blends of some maize varieties were preferred over others by naïve wasps. Further studies will need to establish if such differences in attractiveness can be useful to enhance biological control.

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CHAPTER 5

The role of green leaf volatiles in the attraction of parasitic wasps to herbivore-damaged maize plants

Based on:

Fritzsche Hoballah, M. E.; Turlings, T. C. J. The role of green leaf volatiles in the attraction of parasitic wasps to herbivore-damaged maize plants. In preparation for submission to *Journal of Chemical Ecology.*

Abstract

Y-tube and 6-arm olfactometer experiments were carried out to determine the relative importance of green leaf volatiles as compared to induced odors for attraction of parasitic wasps. In this study we tested the hypothesis that the generalist parasitoid *Cotesia marginiventris* should show innate preferences for odor blends containing high quantities of green leaf volatiles and change preference towards more reliable blends containing induced terpenoids by associative learning. We compared the host-location behaviour of *C. marginiventris* with that of *Microplitis rufiventris*. These braconid wasps are both solitary endoparasitoids, but differ in host range and geographical origin. In choice experiments with odor blends containing high amounts of green leaf volatiles versus blends containing high amounts of terpenoids, inexperienced *C. marginiventris* showed a significant preference for green leaf volatiles. In contrast, no such difference was observed with inexperienced *M. rufiventris*. After an oviposition experience in hosts feeding on maize plants, both parasitoid species changed their responses. Experienced *C. marginiventris* were more more attracted by blends of volatiles with intermediate amounts of both green leaf volatiles and terpenoids than by blends containing mostly green leaf volatiles or mostly terpenoids. Surprisingly, the number of *M. rufiventris* females that made no choice increased after an experience. This may indicate that other cues such as volatiles from faeces of the hosts, which was not present in the odor sources offered in the olfactometer, may be used by this wasp after associative learning.

Key words: *Cotesia marginiventris*, *Microplitis rufiventris*, *Spodoptera littoralis*, *Zea mays*, parasitoid, innate preference, associative learning, induced volatiles, green leaf volatiles, terpenoids, host location

Introduction

Parasitoids make use of herbivore induced volatiles to locate their hosts. In fact, several studies showed that odor cues released by plants under attack by herbivores are more important for microhabitat location of the host by parasitoids than the odor cues from the host or host by-products (Turlings *et al.*, 1990; Steinberg *et al.*, 1993; McCall *et al.*, 1993; Agelopoulos and Keller, 1994a; Geervliet *et al.*, 1994; Mattiacci *et al.*, 1994; Vernède and Pak, 1994; Takabayashi *et al.*, 1995). Differential attraction mediated by odors of different plant species attacked by the same herbivore were demonstrated for *Microplitis croceipes* (Drost *et al.*, 1988), *Campoletis sonorensis* (McAuslane *et al.*, 1991), *Cotesia glomerata* and *Cotesia rubecula* (Geervliet *et al.*, 1996), *M. croceipes* and *Cardiochiles nigriceps* (De Moraes and Lewis, 1999), *C. marginiventris* and *Microplitis rufiventris* (Tamò and Turlings, unpublished data). Some parasitoids have shown variable responses to odors emitted by the same plant species attacked by different herbivores: *M. croceipes* (Zanen and Carde, 1991), *Cotesia sesamiae* and *Cotesia flavipes*, (Ngi-Song *et al.*, 1996), *C. glomerata* (Geervliet *et al.*, 1997), *C. nigriceps* (De Moraes *et al.*, 1998; De Moraes and Lewis, 1999). Even more intriguing is the observation that *C. glomerata* and *Cotesia kariyai* can distinguish among the odors produced by the same plant species fed upon by different larval stages of the same host (Mattiacci and Dicke, 1995; Takabayashi *et al.*, 1995). The parasitoid *Apoanagyrus lopezi* are more attracted to the odors of plants infested with unparasitized hosts than to the odors of plants infested with parasitized hosts (Souissi *et al.*, 1998). The odors of the host faeces are also used as cues for host location by some parasitoids. *C. rubecula* can distinguish between the odors of the faeces of its own host and those of other herbivores of the same genera *Pieris* in a flight tunnel; the wasp can also distinguish between second and fourth instar faeces odors of the host (Agelopoulos *et al.*, 1995). However, while *M. croceipes* is attracted to faeces odors (Elzen *et al.*, 1987; Cortesero *et al.*, 1997), there are parasitoids like *Campoletis sonorensis* that are not (Elzen *et al.*, 1987).

These examples show how diverse host location by parasitic wasps can be, with differences being found for example between specialist and generalist ((Baur and Yeargan, 1996; Cortesero *et al.*, 1997; Röse *et al.*, 1998; De Moraes and Lewis, 1999) or between gregarious and solitary parasitoids (Geervliet *et al.*, 1996), respectively.

Some natural enemies solve the detect ability-reliability problem, as described by (Vet and Dicke, 1992), by making use of the volatiles emitted by plants under herbivore attack to locate their hosts because these volatiles are released in larger quantities and therefore easier to detect than odors from herbivores or their by-products. However, odor blends emitted by damaged plants are expected to provide limited information on the identity of the herbivore that has been damaging the plants. In fact, different plant species release similar blends of odor compounds in response to different types of damage. This problem is partially solved by the ability of parasitoids to learn by association (Vet and Groenewold, 1990; Turlings *et al.*, 1993). For various parasitoids it has been shown that they learn to respond to a specific odor when they perceive this odor during contact with the host or host faeces. This ability may allow the wasps to learn subtle differences between odor blends and thus focus on odors that are more reliably associated with the presence of suitable hosts. For example, the generalist parasitoid as *C. marginiventris* shifts its preference for plant odors that it has experienced during an encounter with a host (Turlings *et al.*, 1989b; Turlings *et al.*, 1993). This wasp can learn to distinguish between the odors released by corn fed by two closely related host species of *Spodoptera* (Turlings *et al.*, 1993). *M. croceipes* needs more than one oviposition experience to learn, in fact associative learning take place only if this parasitoid has the possibility to oviposit three times (McCall *et al.*, 1993). Furthermore, there are parasitoids, as *Campoletis sonorensis*, *Cotesia flavipes* and *Diaeretiella rapae*, that seemingly are not capable to learn through experience to associate the host with the odors of the herbivore damaged plant (McAuslane *et al.*, 1991; Potting *et al.*, 1997; Vaughn *et al.*, 1996). Also learning during emergence from the cocoon can help parasitoids to better use specific cues to guide them to host-infested plants. In fact, rearing diet of the host influenced preference of *Microplitis demolitor* (Hérard *et al.*, 1988), *Cotesia plutella* (Bogahawatte and Van Emden, 1996) and *Opius disitus* (Petitt *et al.*, 1992). These wasps preferred the odor of plant species that were the rearing diet of their host than that of other plant species. *Cotesia congregata* females, if exposed to a plant species during adult emergence, showed preferences for that plant species over others (Kester and Barbosa, 1991). Also the attraction of the predator *Phytoseiulus persimilis* can be influenced by the diet of its host during rearing (Krips *et al.*, 1999). In contrast, *C. sonorensis* preferences for specific plant-host complexes did not differ according to the diet of the host on which the parasitoid was reared (McAuslane *et al.*, 1990).

These studies illustrate the great variation of host location cues used and behaviours shown by different parasitoid genera. To determine which odors are important for a specific parasitoid the attraction to single odor compounds or to specific fractions of the blends have to be studied. The attraction and the responsiveness to single compounds released by plants under herbivore attack has been studied with the use of olfactometers, electrophysiological methods or antennal palpation assays, respectively (Elzen *et al.*, 1984; Whitman and Eller, 1990; Vaughn *et al.*, 1996; Du *et al.*, 1998). Studies with fractionated odor blends showed that certain fractions are more attractive to wasps than others (Udayagiri and Jones, 1992; Turlings and Fritzsche, 1999). Such studies with blends of volatiles released from artificial substrates are complicated by lack of control of the rates at which compounds with variable volatility are released.

The aim of the current study was to assess which group of volatile compounds are important for the attraction of the two parasitoid species *C. marginiventris* and *M. rufiventris*

(Hymenoptera: Braconidae). These two parasitoids originate from different continents (the Americas and North-East Africa, respectively), parasitize different host species (*Spodoptera* spp., *Helicoverpa zea*, *Mocis latipes* and other 24 hosts (Maes, 1989) and *Spodoptera littoralis*, *Spodoptera exigua*, *Heliothis armigera* (Hegazi and El-Minshawy, 1979), respectively) that in part are found on the same plant species (cotton, maize, cabbage, sweet potato, tomato and other crops). Two groups of volatile compounds were considered: green leaf volatiles and terpenoids. Green leaf volatiles are released immediately upon mechanical damage of plants (Turlings *et al.*, 1990; Agelopoulos and Keller, 1994b; Mattiacci *et al.*, 1994; Finidori-Logli *et al.*, 1996; Horikoshi *et al.*, 1997; McAuslane and Alborn, 1998; Röse *et al.*, 1998; Agelopoulos *et al.*, 1999; Arimura *et al.*, 2000; Halitschke *et al.*, 2000). Terpenoids, in contrast, are released by several plants primarily in response to herbivory (Dicke and Sabelis, 1988; Turlings *et al.*, 1990) and at the earliest several hours after damage first occurred (Turlings *et al.*, 1998). To circumvent the problem of release rates, we used natural blends produced by maize plants and offered the choice between odor of freshly damaged plants (mainly green leaf volatiles) and the odor of plants with old damage (mainly terpenoids) to the wasps. In all cases, the plants were treated with regurgitant of *S. littoralis* which is known to induce the production of terpenoids (Gouinguené *et al.*, 2001). Studies concerning the attractiveness of odors for *C. marginiventris* are summarised in Table 5.1. From these studies it could be hypothesised that inexperienced *C. marginiventris* females are attracted more to very commonly produced green leaf volatiles (fresh, recent damage) and later to more specific terpenoids when they associate induced odors with the presence of the hosts (Table 4.1). Limited information on the host location behaviour exists for *M. rufiventris*. However, it appears that this parasitoids prefers the odor blends of some plant species over those of others infested with the same herbivore host and does not learn plant odors after association with the hosts (Tamò and Turlings, personal communication).

In this study we compared the attractiveness of green leaf volatiles and terpenoids to *C. marginiventris* and *M. rufiventris* and how experience affects the use of these groups of plant derived cues.

Table 5.1: Studies on the role of odor cues for the attraction of the parasitoid *Cotesia marginiventris* to the microhabitat of its host. Preferred odor sources are underlined. Different combinations of odors were offered to the wasps in olfactometers (O), flight tunnels (FT) or semi field experiments (SFE) or arena studies to detect preferences to specific odors.

Odors offered to *C. marginiventris*	**Reference**
•If experienced with host-damaged leaves, the response to odors was enhanced and associative learning was demonstrated (O)	(Turlings *et al.*, 1989b)
•*S. frugiperda* odors vs. *Trichoplusia ni* odors	
•Corn odors vs. cotton odors	
but preference can shift with associative learning	(Turlings *et al.*, 1989a)
•Herbivore induced plant odors	
vs. other complex-parts odor (faeces, larvae) (FT)	(Turlings *et al.*, 1991c)
•*S:exigua*-maize complex vs. synthetic blend of 11 major compounds (FT)	(Turlings *et al.*, 1991a)
•*S. frugiperda* on corn vs. *S. exigua* on corn, preference with learning (FT)	
•Maize damaged by *S. exigua* for longer period vs. dam. shorter period (FT)	
Can learn to associate old dam. with host but not fresh dam.	(Turlings *et al.*, 1993)
•Upwind flight to green leaf volatiles (FT)	(Baur and Yeargan, 1996)
•Cotton damaged by *S. exigua* vs. undamaged cotton (FT)	
•Artificially damaged cotton plants vs. undamaged plants (FT)	
•Cotton damaged by *S. exigua* vs. artificially damaged cotton plants (FT)	(Röse *et al.*, 1998)
•*S. exigua* damaged cotton until test (48 h)=SEDC vs. undam. plants=UP (FT)	
•SEDC vs. UP + leaf fed by *H.* zea=LF (3 h)	
•SEDC+ LF vs. UP+LF	
•SEDC+LF vs. UP+LF	
•SEDC vs. UP+faeces	(Cortesero *et al.*, 1997)
•Sesquiterpenes and indole particularly attractive (FT)	(Turlings and Fritzsche, 1999)

Material and methods

Plants and insects.

Two weeks old maize plants (var. Delprim) were used for all experiments. Two seeds per pot (200 ml, 7.5 cm top diameter, 5 cm bottom diameter, 6 cm high) were planted in commercial soil (COOP, Switzerland) and grown in a climate chamber (23°C, 60% relative humidity, 16D: 8L, 50 000 lm.m^{-2}). The day before an experiment started, plants were transferred in glass pots that fit the olfactometers (350 ml, 8 cm diameter, 8.5 cm high for Y shaped olfactometer and 250 ml, 4.5 cm diameter, 11 cm high for 6 arm olfactometer). *S. littoralis* eggs were obtained from Syngenta (Basel) weekly. Rearing methods for host and parasitoids are described by (Fritzsche Hoballah and Turlings, 2001). Two parasitoids were used for the study, *C. marginiventris* and *M. rufiventris*. *C. marginiventris* originated from the USDA-ARS, Biological Control and Mass Rearing Research Unit (Mississippi, USA) and *M. rufiventris* from the Faculty of Agriculture, Alexandria University (Egypt). Parasitized larvae and adults *C. marginiventris* were kept until the experimental day in an incubator (25°C and 16L: 8D), whereas parasitized larvae and adults of *M. rufiventris* were kept in the laboratory under ambient light and temperature conditions. The parasitoid females were used for the assays were two to five days old.

Y-tube olfactometer bioassay (inexperienced wasps).

An Y-shaped olfactometer was used to test preferences of inexperienced *C. marginiventris* and *M. rufiventris* females between a blend containing high amounts of green leaf volatiles and a blend containing high amounts of terpenoids. The first odor source consisted of two maize plants treated 30 minutes before and the second odor source consisted of two plants treated 6 hours before the choice Test (the latter plants emit terpenoids in higher amounts and green leaf volatiles in lower amounts in comparison to plants treated and incubated for 30 minutes only, see (Turlings *et al.*, 1998). Treatment consisted of scratching the underside of two leaves over an area of 2-4 cm^2 on both sides of the central vein of the leaf with a razor blade after which 10 µl of *S. littoralis* regurgitant was applied to on each damaged site. After treatment, the plants were placed under 3 fluorescent lamps (Sylvania standard F36W 133-T8 cool white, 5 000 $lm.m^{-2}$ at pot height) until used in bioassays. The olfactometer was based on a design by (Sabelis and Van de Baan, 1983) and consisted of a central tube (13.5 cm long, 4 cm diameter) and two lateral arms (12 cm long, 4 cm diameter) ending with a glass frit (to prevent test insects from walking further and to create an homogeneous air flow). The lateral arms tapered off into 0.5 cm tubes to which Teflon tubing was connected. The odors were introduced via these Teflon tubes. To limit visual distractions for the insects, the Y-tube olfactometer was placed inside a box lined with a white paper, which was open on the top (for illumination) and on the front side (for observation). An office lamp (60 W, 750 lm.m^{-2}) illuminated the olfactometer. A black and white striped pattern for visual orientation was placed under the Y-tube. Plants that served as odor sources were placed into bell-shaped glass chambers. Ground-glass joints of the glass pots in which the plants were growing (male joint: 350 ml, 8 cm diameter, 8.5 cm high) and the glass chamber (female joint: 8.5 cm diameter, 43 cm high) ensured an air-tight connection. At the bottom and top of each chamber a screw-cap connector allowed for the introduction of clean air into the chamber (bottom) and the exhaust of odor-laden air into the olfactometer (top). The air passed over the plants into the Teflon tubes connected with the olfactometer arms at a rate of 1.2 l/min. During experiments, odors of the plants were collected at a rate of 0.6 l/min with the use of Super-Q filter (see Heath and Manukian, 1992) that was attached laterally to a glass piece that connect the two ends of the Y olfactometer with Teflon tubes carrying the odor source chambers (see volatile collection). Wasps were released individually at the base of the olfactometer and observed during 10 minutes. If the wasp did not make a choice after this period it was removed and recorded as a “no choice”. Wasps that walked to the end of one of the arms and stayed there at least 5 seconds were recorded as having made a choice for the odor offered through that arm. After 6 individuals were tested, the olfactometer was turned such that the direction of the arms was reversed and 6 other wasps were tested. The dual choice test was repeated on 7 days for each wasp species, with different plants and each time the position of the two different odor sources was switched. A binomial test was used to test for a preference for one of the two odors offered during the experiment.

Six arm olfactometer bioassay (inexperienced wasps).

The six arm olfactometer is described by Turlings and Tamó (in preparation). This system exposes waspsto 6 air streams that enter a central choice chamber. Each stream entering the chamber via glass tube, can carry a different odor. In our bioassays, only three odor sources were offered to the parasitoids and and each odorous air stream was alternated with a stream of clean air. Two combinations of three odor sources were offered as choice to inexperienced wasps. The combination of odors consisted of:

(1) Two maize plants treated 30 min before (fresh-fresh) *versus* two maize plants treated 6 h before (old-old) *versus* one plant treated 30 min before and one 6 h before (fresh-old) assaying.

(2) Two maize plants treated 30 min before (fresh-fresh) *versus* two maize plants treated 6 h before (old-old) *versus* two maize plants left unharmed, but treated with 20 µl of *S. littoralis* regurgitant on the leaf surface (spit) before assaying.

Accept from this last treatment, all plants were scratched and treated with regurgitant as described for the previous Y-olfactometer experiment.

The wasps were released in groups of 6 into the choice chamber and after 30 min the wasps choices for a particular arm were noted. Wasps that did not enter an arm after 30 min were considered a “no choice”. For the experiments with inexperienced wasps, two groups for each wasp species were tested on one replication day, this was repeated 12 times. For each replication day, the position of the odor sources was shifted. After each replication day, the olfactometer was washed with running tap water and soap, and subsequently rinsed with acetone and pentane before placing it in the oven at 250°C for 3 hours. The Teflon tubes that connect the glass parts of the olfactometer were also rinsed with acetone and pentane.

A G test was used to assess differences in preference among the six choices (three of them being clean air) offered to the wasps. A corrected G test was used to assess differences in choices among the three odor sources offered two the wasps (programmed in S Plus). This test was used after it had been established that the wasps released in groups of 6 do not influence each others choices (Turlings and Tamò, in preparation).

During each bioassay, odors were collected. Charcoal filters purified and a bubbler humidified the air that was pushed into the olfactometer at a rate of 1.2 l/minutes. Odors were collected at a rate of 0.6 ml/min using Super-Q traps that were attached to the glass chambers that contained the treated plants as discribed for the Y-tube olfactometer (see volatile collection).

Six arm olfactometer bioassay (experienced wasps).

To experience wasps they were placed in a plastic box (9 cm top diameter, 8.5 cm bottom diameter, 3.5 cm high) containing pieces of maize leaf (var. Delprim) on which larvae of *S. littoralis* were feeding for 24h. After females parasitized one or two larvae they were considered experienced. This type of experience is known to increase the responsiveness of parasitoids to the perceived odors (Turlings *et al.*, 1993). We gave only one oviposition experience to *M. rufiventris* to obtain approximately the same period of contact with the leaves-host complex for both parasitoid species, since *M. rufiventris* needs more time than *C. marginiventris* to parasitize the same number of hosts (Fritzsche Hoballah, personal observation).

Only one choice bioassay was carried out with experienced wasps:

(1) Two maize plants treated 30 min before (fresh-fresh) *versus* two maize plants treated 6 h before (old-old) *versus* one plant treated 30 min before and one 6 h before (fresh-old) assaying.

For the tests carried out with the experienced wasps, the number of groups released per replication day varied from 2 to 4, only 6 replications were carried out and this was done on different days for the two parasitoid species. Treatment of plants, odor collections and statistical analysis was the same as for the bioassays with inexperienced wasps.

Volatile collection and analysis.

Traps containing Super-Q traps (25 mg, 80/100 mesh, Alltech, Deerfield State) were used to collect odors from the various treatments during the olfactometer experiments. Collections started 20 minutes before olfactometer experiment started and ended after a period of 2 h for the Y-shaped olfactometer and 3 h for the 6 arm olfactometer. After each collection, traps were rinsed with 150 µl methylene chloride and 200 ng of *n*-octane and nonyl acetate were added as internal standards. Three µl aliquots of the samples were injected on column, with an automated injection system into a Hewlett Packard model HP 6890 gas chromatograph equipped with a flame ionisation detector. The HP-1 capillary column (30 m, 0.25 mm I.D., 0.25 µm film thickness, Hewlett-Packard Company, USA) was held at 50 °C for 3 minutes and then programmed at 8 °C per minute to 230 °C, were it was maintained for 9.5 minutes. The column was preceded by a deactivated retention gap (10 m, 0.25 mm I.D., Alltech Associates, USA) and a deactivated precolumn (30 cm, 0.53 mm I.D., Alltech Associates, Inc. USA). Helium (24 cm/s) was used as carrier gas. HP GC Chemstation software was used to quantify all major components by comparison to the known quantity of internal standards. An ANOVA and the Student-Newman-Keuls post-hoc test were used to compare the total quantity of volatiles emitted between and among the sources offered to the wasps in the olfactometer. Tests were carried out with the program SPSS 10.0 for Windows. Statistical analysis for volatile collections carried out in different experiments but carried out with the same treatments were placed together.

Results

Y-tube olfactometer bioassay (inexperienced wasps).

As expected, inexperienced *C. marginiventris* females preferred blends with high amounts of green leaf volatiles over blends with higher amounts of terpenoids (Fig. 5.1). In contrast, inexperienced *M. rufiventris* did not show a preference for one of the two odor sources (Fig. 5.1). The total amount of volatiles released by the plants treated 30 min before was lower from the amount released by plants with 6 h old treatment (mean±SE for 30 min: 518.6±66, 6 h: 1349±300; ANOVA P=0.012, F=7.272).

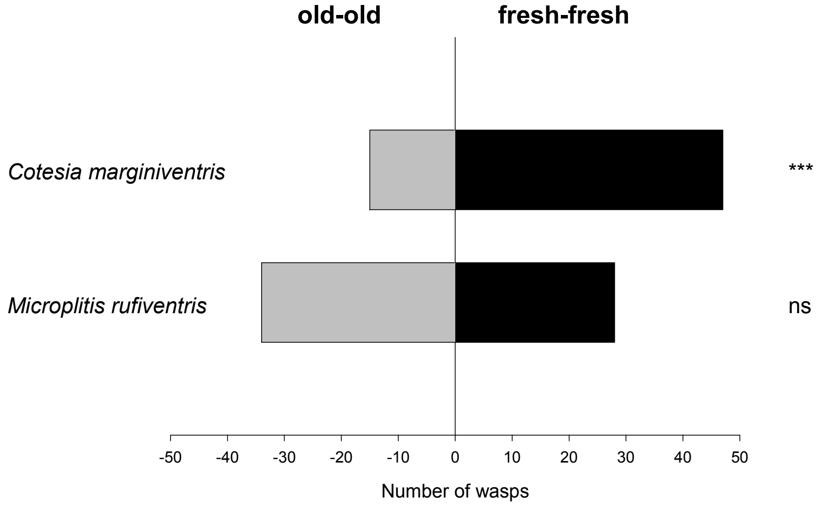

Figure 5.1: Horizontal bars represent number of inexperienced *Cotesia marginiventris* and *Microplitis rufiventris* that made a choice between the two odor sources offered in a Y shaped olfactometer (ns: no significant difference, ***P<0.0001). Offered odor sources were: two maize plants treated 30 minutes (fresh-fresh) or 6 hours (old-old) before experiment started. Treatment consisted in scratching two leaves of a plant and adding *Spodoptera littoralis* regurgitant on the damaged sites.

The amounts of individual compounds collected during the Y-shaped olfactometer experiments clearly confirmed that plants with 30 min old damage release significantly more green leaf volatiles ((*E*)-2-hexenal, (*Z*)-3-hexenol and (*Z*)-3-hexenyl acetate) than plants with 6 h old damage (Fig. 5.2). In contrast, 6 hours incubated plants released significantly more terpenoids (geranyl acetate, (*E*)-β-caryophyllene; (*E*)-α-bergamotene; (*E*)-β-farnesene) (Fig. 5.2).

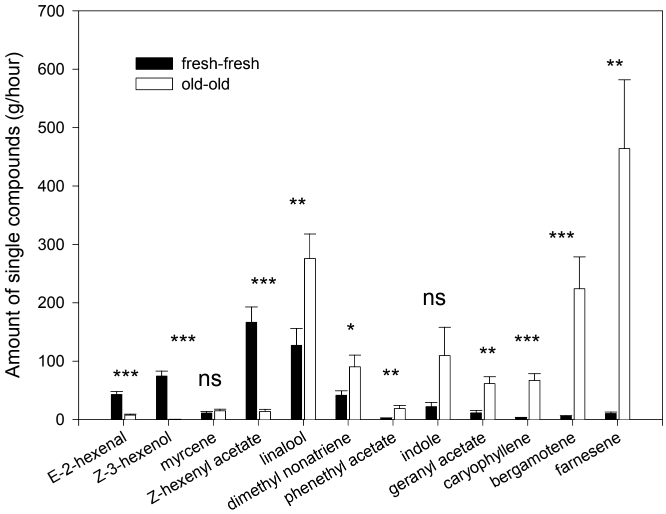

Figure 5.2: Mean (±SE) amount of single volatile compounds collected for 2 hours from plants treated 30 minutes (fresh-fresh) and 6 hours (old-old) before experiments were carried out with inexperienced *Cotesia marginiventris* and *Microplitis rufiventris* wasps in a Y-tube olfactometer (ns= no significant difference among treatments, * P<0.01, **P<0.001, ***P<0.0001).

Six arm olfactometer bioassays (inexperienced wasps).

During the first bioassay it was discovered that inexperienced *C. marginiventris* preferred the blend containing more green leaf volatiles and did not distinguish between “fresh-fresh” and “fresh-old”, indicating that the induced volatiles did not repel the wasp and may contribute to the attractiveness of the blend if they are offered in combination with green leaf volatiles (Fig. 5.3). In contrast, inexperienced *M. rufiventris* was equally attracted to all three treatments (Fig. 5.3). Both wasps rarely entered three arms of the olfactometer carrying pure air but almost one third of the wasps did not choose any arm (Fig. 5.3).

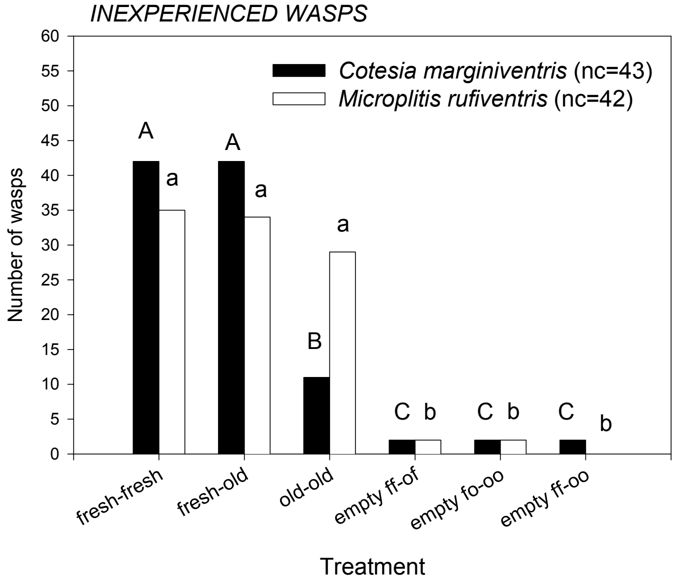

Figure 5.3: Number of inexperienced *Cotesia marginiventris* and *Microplitis rufiventris* females that were attracted to a specific source in a 6 arm olfactometer (nc=number of wasps that did not make a choice). Fresh-fresh: two maize plants treated 30 minutes, fresh-old: one plant treated 30 minutes and one plant treated 6 hours, old-old: two plants treated 6 hours, respectively before experiment started, empty: pure air. Treatment consisted in scratching two leaves of a plant and adding *Spodoptera littoralis* regurgitant on the damaged sites. Different letters above bars indicate significant differences among numbers of wasp that chose a particular odor source.

The total amount of volatiles released by the plants treated in different ways was not significant different (mean±SE fresh-fresh: 613.6±57, fresh-old: 584.5±72, old-old: 610.6±97, ANOVA *P*=0.958, F=0.043). Again, the odors collected during the olfactometer experiences show the clear differences in the amounts of green leaf volatiles and terpenoids emitted by the treatments, with the “fresh-old” treatments showing intermediate release of both groups (Fig. 5.4).

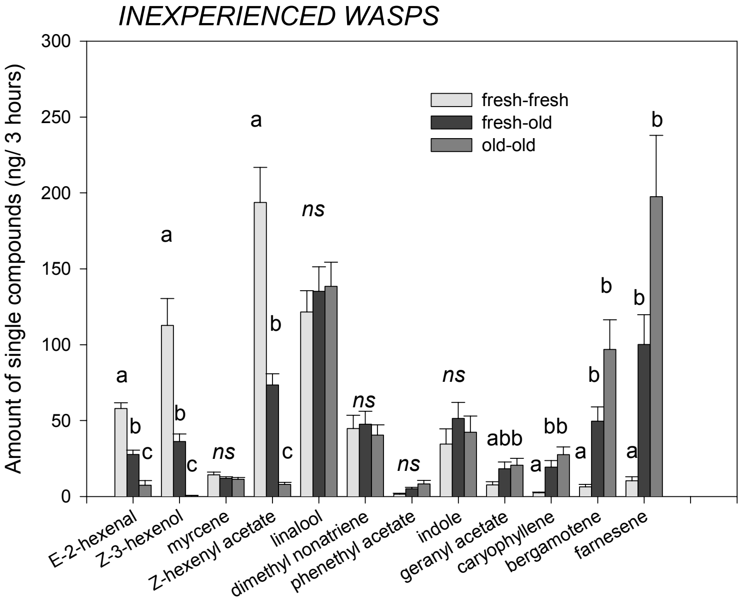

Figure 5.4: Mean (±SE) amounts of single volatile compounds of a blend collected from: two maize plants treated 30 minutes (fresh-fresh), one plant treated 30 minutes and one plant treated 6 hours (fresh-old) and two plants treated 6 hours (old-old) before experiments carried out with *Cotesia marginiventris* and *Microplitis rufiventris* wasps in a 6 arm olfactometer started. Treatment consisted in scratching two leaves of a plant and adding *Spodoptera littoralis* regurgitant on the damaged sites. *ns* indicate no significant difference among treatments and different letters above bars indicate significant differences in amount of single compounds collected among treatments. This graph include the volatile collections of all experiments carried out with those treatments (experiments with unexperienced and experienced wasps).

In the experiment were the wasps were offered the odor of two freshly damaged plants next to the odors of two plants with 6 h old damage and undamaged plants with regurgitant smeared on the leaves, *C. marginiventris* preferred the fresh damage (Fig. 5.5) while inexperienced *M. rufiventris* showed again no significant preference (Fig. 5.5). No preferences exhibited by *M. rufiventris* was unexpected, since the amount of all major compounds released by the plants with just regurgitant on the leaves was significant lower than the other two treatments (Fig. 5.6).

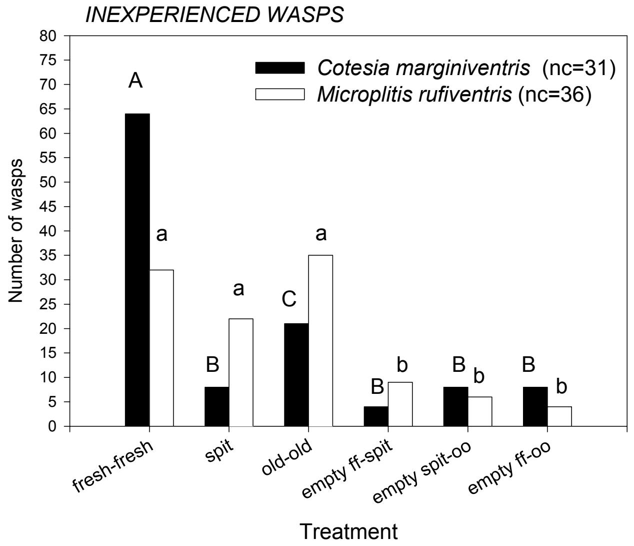

Figure 5.5: Number of inexperienced *Cotesia marginiventris* and *Microplitis rufiventris* females that were attracted to a specific source in a 6 arm olfactometer (nc=number of wasps that did not make a choice). Fresh-fresh: two maize plants treated 30 minutes, spit: two plants on which *Spodoptera littoralis* regurgitant was smeared on the leaves, old-old: two plants treated 6 hours, respectively before experiment started, empty: pure air. Treatment consisted in scratching two leaves of a plant and adding *Spodoptera littoralis* regurgitant on the damaged sites. Different letters above bars indicate significant differences among the numbers of wasp that chose a particular odor source.

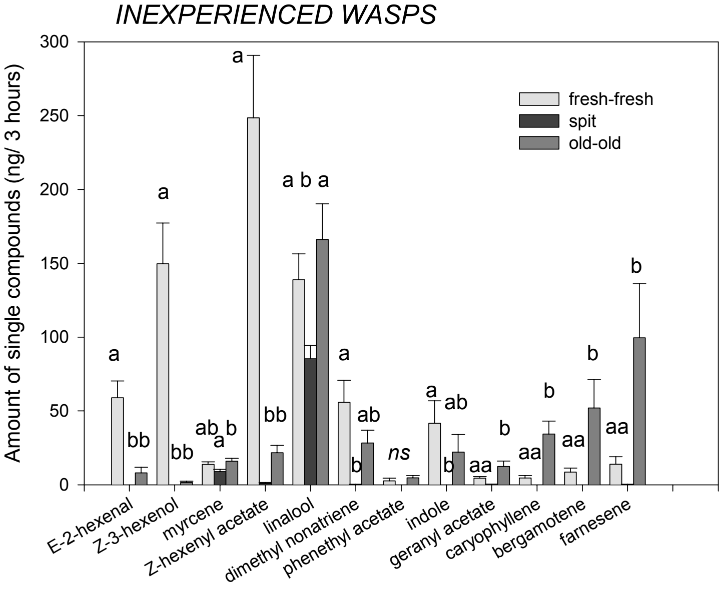

Figure 5.6: Mean (±SE) amounts of single volatile compounds of a blend collected from two maize plants treated 30 minutes (fresh-fresh), two plants smeared with *Spodoptera littoralis* regurgitant (spit) and two plants treated 6 hours (old-old), respectively before experiments carried out with *Cotesia marginiventris* and *Microplitis rufiventris* wasps in a 6 arm olfactometer started. Treatment consisted in scratching two leaves of a plant and adding *S. littoralis* regurgitant on the damaged sites. *ns* indicate no significant difference among treatments and different letters above bars indicate significant differences in amount of single compounds collected among treatments.

6 arm bioassays (experienced wasps).

When *C. marginiventris* was given an oviposition experience on *S. littoralis* caterpillars feeding on maize (var. Delprim) leaves its preference changed. Experienced *C. marginiventris* females significantly preferred the complete blend (fresh-old) over blends containing higher amounts of green leaf volatiles (fresh-fresh) or terpenoids (old-old) (Fig. 5.7). Surprisingly, experienced *M. rufivetris* females preferred the odor source containing more green leaf volatiles (Fig. 5.7). However, the majority of *M. rufiventris* did not make any choice. As for inexperienced wasps, the experienced wasps of both wasp species rarely entered one of the three arms of the olfactometer that carried clean air (Fig. 5.7).

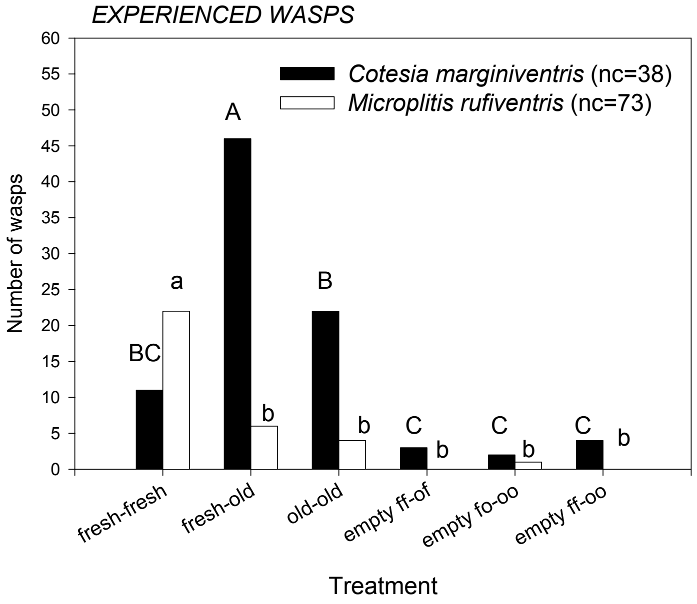

Figure 5.7: Number of experienced *Cotesia marginiventris* and *Microplitis rufiventris* females that were attracted to a specific source in a 6 arm olfactometer (nc=number of wasps that did not make a choice). Experience consisted in oviposition of the host that fed on maize leaves since 24 h. Fresh-fresh: two maize plants treated 30 minutes, fresh-old: one plant treated 30 minutes and one plant treated 6 hours, old-old: two plants treated 6 hours, respectively before experiment started, empty: pure air. Treatment consisted in scratching two leaves of a plant and adding *Spodoptera littoralis* regurgitant on the damaged sites. Different letters above bars indicate significant differences among number of wasp that choose a particular odor source.

Discussion

This study showed that *C. marginiventris* and *M. rufiventris* have different odor preferences, which probably reflects different host location strategies. Both are solitary endoparasitic braconids. Inexperienced *C. marginiventris* preferred blends containing higher amounts of green leaf volatiles, which confirms our hypothesis that green leaf volatiles are important for initial attraction of inexperienced *C. marginiventris.* Other parasitoids like *M. croceipes* and *Netelia heroica* (Whitman and Eller, 1990) and *Aphidius rhopalosiphi* (Wickremasinghe and Van Emden, 1992) have been shown to orient to individual green leaf volatile compounds in a wind tunnel and a y-tube olfactometer, respectively. Furthermore, *M. croceipes* (Li *et al.*, 1992) and *C. sonorensis* (Baehrecke *et al.*, 1989) highest electroantennogram responses are found with green leaf volatiles. These same chemicals are involved in the attraction of *C. glomerata* to infested or regurgitant-treated cabbage plants (Mattiacci *et al.*, 1994). Predators can also react strongly to green leaf volatiles. For example, (*Z*)-3-hexenol elicits strong responses in the two-spotted stinkbug predator *Perillus bioculatus* (Weissbecker *et al.*, 1999) and nymphs of *Podisus maculiventris* are sensitive to (E)-2-hexenal and (E)-2-hexenol (Sant'Ana *et al.*, 1999). In contrast, other predators, like *Anthocoris* spp. are not attacted to green leaf volatiles (Scutareanu *et al.*, 1997).

Not only certain parasitoids and predators, but also a lot of herbivorous insects are very sensitive to green leaf volatiles. 1-hexenol is perceived by several unrelated herbivores: *Spodoptera exigua*, *Leptinotarsa decemlineata* and *Schistocerca gregaria* (Dickens *et al.*, 1993a). Repellency by green leaf volatiles was find for several Coleopteran beetles (Deglow and Borden, 1998; Poland *et al.*, 1998; Poland and Haack, 2000; Zhang *et al.*, 2000). High sensitivity to green leaf volatiles were also detected for the beetle *Leptinotarsa decemlineata* and it was supposed that these volatiles play a role in the host selection behaviour of various phytophagous insects (Visser, 1979). Not only beetles, also moths are highly sensitive to some green leaf volatiles (Lecomte and Pouzat, 1986; Ramachandran *et al.*, 1990; Valterova *et al.*, 1990; Dickens *et al.*, 1993b; Hansson *et al.*, 1994; Anderson *et al.*, 1995). All these studies carried out with Lepidoptera are based on electroantennogram studies and are not indicating if green leaf volatiles are attractive or repellent. However, the study of De Moraes *et al.* (2001) showed that green leaf volatiles may be repellent for the moth *Heliothis virescens*. Also the aphid *Megoura viciae* (Visser and Piron, 1995) and some flies (Diptera) like *Psila rosae* (Guerin and Visser, 1980) and *Dacus dorsalis* (Light and Jang, 1987) are highly sensitive to green leaf volatiles. Further studies are needed to find out if green leaf volatiles are mostly repellent for herbivores and mostly attractive for natural enemies of the herbivores. We may conclude from the previous cited studies that green leaf volatiles, released very commonly by plants are very important odor cues that are used for host location by both herbivores and natural enemies of herbivores.

Through associative learning the preference of natural enemies of herbivores for mixtures of green leaf volatiles can be shifted towards more reliable odor cues containing more terpenoids. *C. marginiventris* that experienced an oviposition on plants that had been damaged by host larvae overnight, preferred the complete blend of green leaf volatiles plus terpenoids (fresh-old). Since the experience of oviposition was associated with the entire complex: host-damaged leaves, host larvae and host by-products, it was to be expected that the wasps were more attracted to the complete blend. Its effect of associative learning was confirmed for *C. marginiventris*. Surprising were the preferences exhibited by *M. rufiventris*. Inexperienced *M. rufiventris* did not distinguish among different odor blends. The attraction to a blend containing almost only linalool (unharmed plants with regurgitant) was not different to the attraction to blends containing various other volatile compounds. It is possible that linalool is important for the attraction of *M. rufiventris* and that some other compounds repel this wasp. The predatory mites *Phytoseiulus persimilis* and *Amblyseius potentillae* are also attracted to linalool (Dicke *et al.*, 1990; Dicke and Sabelis, 1992), whereas for the herbivorous mite *Tetranychus urticae* linalool is a signal for dispersal (Dicke *et al.*, 1990). Another explanation for the non-preference found for inexperienced *M. rufiventris*, could be that it is also attracted to volatiles from host regurgitant (not detected here because of small quantities). Even more surprising was the preference of experienced wasps for the blends containing high amounts of green leaf volatiles. However, the high number of wasps that did not make choice suggests that *M. rufiventris* may have searched for odors that are more specifically associated with the presence with hosts. A related parasitoid, *M. croceipes*, is strongly attracted to host faeces (Elzen *et al.*, 1987; Eller *et al.*, 1988; Eller *et al.*, 1992). This may also be the case for *M. rufiventris*, especially after an experience with a complete plant-host complex, including faeces. It can be concluded that parasitoids with comparable host ranges may employ different strategies in the use of plant-provide cues to locate hosts. Further insight in to the circumstances under which they have to forage may provide explanations for such differences.

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Conclusions and outlook

The following questions were asked in the introduction and are briefly answered here:

1. *Once a parasitoid is attracted to the plant by herbivore-induced volatiles and a host is parasitized, does the plant directly benefit from the action of this natural enemy?*

   Yes, for two larval endoparasitoids it was shown that they can directly enhance plant performance by reducing herbivory of their host. An increase of about 30% of increase in seed production was found for plants if a caterpillar that was feeding on it was parasitized.

2. *Which insect natural enemies attack* Spodoptera fugiperda *caterpillars on maize and what is there control potential?*

   Ten parasitoids and nine predators species of *S. frugiperda* caterpillars were collected over a 3-year period in maize fields in the subtropical region around Poza Rica (Mexico). Five of the parasitoids have the potential to reduce herbivory and directly benefit maize seed production.

3. *What are the costs of the induced production for maize plants?*

   Direct costs of producing induced volatiles were shown to be low, particularly when the potential benefits of attracting parasitoids are considered.

4. *Are there certain maize varieties, more attractive than others to* C. marginiventris *and, if so, what determines this differential attractiveness*?

   Yes, in 3 combinations out of 12, a preference for the odor of one of two varieties was found. Not only odor quantity, but also the quality of the odor blend seems to be important for the attraction of this wasp.

5. *Which groups of compounds in a specific blend of induced volatiles are the most attractive for* C. marginiventris*?*

   For this parasitoid green leaf volatiles are important for initial attraction, if the wasps has not previously experienced plant odors.

   Throughout this dissertation the amounts of the volatiles released were calculated in total weight (ng/h). Hence, the differences in molecular weight among different volatile substances were not taken into account. The molecular weights of the various compounds ranged from 98 for hexenal to 204 for sesquiphellandrene. It is likely that the number of molecules that can potentially hit the insect antenna is more important for the detection of a particular substance than its total weight.

Based on the presented results, various new questions arose.

1. Fitness benefits by the action of a parasitoid have also been shown for the parsitoid *Cotesia rubecula* attacking *Pieris* caterpillars that feed on *Arabidopsis* plants (van Loon *et al.*, 2000) and for parasitoids of the weevil seed predator *Ceutorhyncus* sp. that feed on fruits of the plant *Hormathophylla spinosa* (Gómez and Zamora, 1994). We hypothesised that some parasitoids help shape the evolution of herbivore-induced volatiles if successful attraction of these parasitoids increases plant fitness. Field studies that compare seed production of plants in natural enemies free environments with seed production in environments were natural enemies are present would give a more realistic idea on the effect of natural enemies on plants.

2. Future work should assess which of the natural enemies that attack *S. frugiperda* caterpillars on maize can be best manipulated with changes in herbivore-induced volatiles.

3. Studies carried out with specific parasitoids and predators indicate an enhancement in parasitization rate or predation on plants that produce induced volatiles in comparison to plants of the same species that do not produce these volatiles, but also carry hosts or preys (De Moraes *et al.*, 1998; Thaler, 1999; Kessler and Baldwin, 2001). Field studies would be needed to assess if specific maize genotypes, that differ in quality and quantity of induced volatile production, also differ in the protection they receive from natural enemies like *C. marginiventris*.

4. Green leaf volatiles were found to be important for the initial attraction of *C. marginiventris*. To assess which compounds are the most important among those, electroantennogram studies and olfactometer or flight tunnel studies with specific compounds could be carried out. To test the importance of single compounds for attraction of parasitoids, it would be ideal to determine preferences of the wasp between maize genotypes that differs only in the production of one single compound after herbivore induction. However, the fact that proportions of compounds in the blend are also important for attraction should always be considered.

5. The study of costs of herbivore-induced volatiles in plants is delicate because of the many different reactions that could be expressed in parallel in the plant. For example, it is likely that a plant that is induced to produce volatiles under herbivore attack also produces other substances, such as toxic compounds against herbivores. It may be possible in the future to measure the fitness costs for plants to produce specific compounds. Also for this

purpose, genotypes that differ only in the production of single induced compounds would be helpful.

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ANNEXE

The effect of odor induction with the elicitor volicitin on the susceptibility of maize to the pathogen *Peronosclerospora sorghi*

Based on:

Fritzsche Hoballah, M. E.; Cardwell, K. F.; and Turlings, T. C. J. The effect of odor induction with the elicitor volicitin on the susceptibility of maize to the pathogen *Peronosclerospora sorghi*. In preparation.

Abstract

Various plants, when damaged by insect-herbivores, start to produce and release specific volatiles. The production of volatiles is systemic and is induced by elicitors contained in the regurgitant of the herbivores. Several studies show that these volatiles are used by natural enemies to detect their prey (the herbivores). The attraction of the natural enemies can be an indirect defence for the plant, and may have evolved secondary. The primary function may be a direct defence against herbivores and plant pathogens. However, studies on the effects of induced volatiles on pathogens are still few. With the present study we tested if induced maize volatiles can act against the pathogen *Peronosclerospora sorghi* (downy mildew). We induced young maize plants to produce volatiles by treating them with volicitin, the elicitor contained in the regurgitant of *Spodoptera exigua* caterpillars. The principle experiment, conducted in Nigeria in a screen house, consisted of infesting volicitin treated and control plants with downy mildew spores and subsequently assessing infection symptoms of downy mildew. Furthermore, we analyzed if there was correlation between downy mildew susceptibility of the two maize varieties and feeding preference by larvae of the noctuid *Spodoptera littoralis*. In a first experiment conducted in the laboratory, we found no differences in the total amount of volatiles collected from the two maize varieties after treatment with volicitin. However, there were significant differences between the varieties in the amounts emitted of some sesquiterpenes. Downy mildew symptoms were the same for volicitin-treated and untreated plants. This could indicate that downy mildew spores are not susceptible to maize volatiles produced by the two maize varieties, or that the released quantities were too small to have an effect. It could also be that the treatment period was to short for the volatiles to act against conidia formation. Moreover, induced volatile emissions are minimal in the dark and downy mildew spores germinate only at night. Finally, the treatment enhanced the formation of dew drops which creates an ideal condition for conidia development. The resistant variety was preferred by *S. littoralis* caterpillars, which suggest that a negative correlation exist between pathogen and herbivore resistance.

Key words: *Zea mays*, maize, volicitin, elicitor, *Peronosclerospora sorghi*, pathogen, downy mildew, conidia, volatile collection, induced volatiles, induced resistance, *Spodoptera littoralis*

Introduction

The attraction of insect natural enemies to volatiles produced by plants under attack by herbivores is considered an indirect defence. It has been suggested that this indirect defence evolved secondary and that the volatiles served primarily to defend the plant directly against the herbivores or microorganisms or that the volatiles are by-product of such a direct defence (Turlings and Tumlinson, 1991b; Turlings and Benrey, 1998; Godfray, 1995). In fact, herbivores can be effectively repelled by induced plant odors or they can decrease their feeding rates (Dicke and Dijkman, 1992; Landolt, 1993; Alborn *et al.*, 1996), (Bernasconi *et al.*, 1998; De Moraes *et al.*, 2001; Kessler and Baldwin, 2001). Some evidence suggests that the same volatiles can indeed act against microorganisms. For example, some of the volatiles produced by maize plants under caterpillar attack such as linalool, (*E*)-2-hexenal and (*Z*)-3-hexenol (Fritzsche Hoballah *et al.*, in prep.) are produced also by plants infested with *Pseudomonas* spp. and are bactericidal (Croft *et al.*, 1993). Methyl salicylate is known to be released by various plants under herbivore attack (Dicke *et al.*, 1990; Bernasconi *et al.*, 1998; Dicke *et al.*, 1998; Krips *et al.*, 1999; Kessler and Baldwin, 2001). This same compound is produced also after tobacco mosaic virus inoculation in tobacco plants and activates the expression of defence related genes (Shulaev *et al.*, 1997). Whether induced direct and indirect defences against herbivores and pathogens involve the same biochemical pathways remains unclear. It seems possible that some volatiles produced by plants under herbivory may simultaneously deter herbivores, have antibiotic properties against microorganisms, and at the same time attract natural enemies of the herbivores.

In the early 1990s, it was discovered that the parasitoid *Cotesia marginiventris* (Hymenoptera: Braconidae) was attracted by volatiles emitted from maize seedlings attacked by the larvae of *Spodoptera exigua* (Lepidoptera: Noctuidae) (Turlings *et al.*, 1990; Turlings *et al.*, 1991a). Later, an elicitor that induces the volatile emissions in maize was isolated from *S. exigua* regurgitant and identified as N[17-hydroxy-9, 12,15-octadecatrienoyl]-L-glutamine (Alborn *et al.*, 1997). This compound was named volicitin and found to resemble the structure of substrates of the lipoxygenase pathway, which is one of the processes, stimulated by jasmonic acid, a plant defence signal. The elicitor volicitin is partially plant (fatty acid portion) and partially caterpillar (glutamine) derived (Paré *et al.*, 1998). After treatment with volicitin, maize plants produce the same blend of volatiles as plants attacked by caterpillars, and can therefore attract the parasitoids (Turlings *et al.*, 2000).

Natural enemies can be attracted by odors released by plants under herbivore attack in the field (Drukker *et al.*, 1995; Thaler, 1999; Kessler and Baldwin, 2001). Predators will eat the herbivores and the benefit for the plants is evident. Furthermore, it was shown that plants could directly benefit also from the action of the parasitoid. In fact, if caterpillars were parasitized plants produced more seeds than plants attacked by non parasitized caterpillars (Fritzsche Hoballah and Turlings, 2001; van Loon *et al.*, 2000). One other benefit for the plants that release induced odors is repellency for herbivores as caterpillars and aphids (Turlings and Tumlinson, 1991b; Bernasconi *et al.*, 1998). Escaping, these herbivores will not consume the plant anymore and in this case the volatiles act as direct defence for the plant.

With the current study, we tested if induced plant volatiles also act against a pathogen. By artificially applying volicitin to maize seedlings, we could test how induced maize odors affect the virulence of downy mildew *Peronosclerospora sorghi* ((Weston & Uppal) Shaw), an important pest on maize in different African countries (Bock *et al.*, 1998b; Jeger *et al.*, 1998) and this fact permit us to collect fresh material directly in the maize fields.

We first collected and analyzed volatiles of two maize varieties used for this experiment, one susceptible and one resistant to downy mildew. Secondly, we compared downy mildew symptoms of maize plants that were either treated with volicitin or left unharmed. In addition we determined if the two varieties differed in palatability to freshly emerged *Spodoptera littoralis* caterpillars, an insect pest on maize throughout Africa.

Material and methods

Volatile collection (Switzerland). Maize plants, varieties Pool-16-SR and DMR, were grown in plastic pots (6 cm high, 8 cm diameter) in fertilised commercial soil (Coop, Switzerland) in a climate chamber (23°C, 60% r.h., and 16L: 8D, 50000 lm/m^2). Ten-day old maize seedlings were treated two days in a row by scratching the underside of two leaves of a maize seedling (on an area of 2-3 cm^2 on each site of the leaf vein) with a razor blade. To half of the scratched plants we applied 10 μl of buffer (50 mM phosphate buffer, pH 8) to the damaged sites, while the other received 10 μl of a buffer-volicitin solution (volicitin 40ng/μl). A third set of plants was left unharmed and served as controls. Volatiles were collected during 2 hours in the morning of the third day (after two consecutive treatments in the evenings of the previous days) 2 hours after light was switched on in the collection chamber. The collection chamber (CMP4030, Conviron, Winnipeg, Canada) was programmed at 20 °C, with a light intensity of 14500 lm/m^2 (light provided by 28 lamps, 16x Sylvania 150 W VHO Cool White and 12x Sylvania, 100 W Satin). Volatiles were collected from 6 plants simultaneously in an automated collection system as described by (Turlings *et al.*, 1998) and (Gouinguené *et al.*, 2001). Glass cylinders (9.5 cm diameter, 54 cm high) were placed over the plants and rested on a Teflon disk consisting of two halves with a hole in the middle for the stem of the plant. Air was pushed (1 l/min) through a charcoal filter and introduced from the top of each cylinder over the plants. Super-Q traps (25 mg, 80/100 mesh, Alltech, Deerfield State) constructed as described by (Heath and Manukian, 1992). Super-Q-traps were attached laterally at the base of each cylinder and were connected to an automated volatile collection system (ASU, ARS, Gainesville, USA). Air was pulled through each trap at 0.8 l/min during 2 h. The collection period was programmed with the use of the software TESS (Version 1.0, ARS, Gainesville, USA). After each collection, traps were rinsed with 150 μl methylene chloride and 200 ng of *n*-octane and nonyl acetate were added as internal standards. Three μl aliquots of the samples were injected on column, with an automated injection system into a Hewlett Packard model HP 6890 gas chromatograph equipped with a flame ionization detector. The apolar EC-1 capillary column (30 m, 0.25 mm I.D., 0.25 μm film thickness, Alltech Associates, Inc, USA) was held at 50 °C for 3 minutes and then programmed at 8 °C per minute to 230 °C, were it was maintained for 9.5 minutes. The column was preceded by a deactivated retention gap (10 m, 0.25 mm I.D., Connex, USA) and a deactivated precolumn

(30 cm, 0.530 mm I.D., Connex, USA). Helium (24 cm/s) was used as carrier gas. We collected and analyzed the odor of the two maize varieties treated with the elicitor volicitin. HP Chemstation software was used to quantify all major components by comparison to the known quantity of internal standards. An ANOVA was used to compare the total quantity of volatiles emitted among varieties (data ln-transformed). To compare differences in emission of single compounds between varieties the Mann-Whitney Test was used.

Experimental procedures of downy mildew inoculation experiment (Nigeria). For this experiment, the same two varieties of maize were used as for the volatile collection experiment; a variety of maize that is susceptible to *Peronosclerospora sorghi*, variety Pool-16-SR, and the resistant variety DMR. Seeds were pregerminated before planting them in single pots. Pregermination consisted in washing the seeds with a 10% sodium hypochlorite solution and putting them in flats between moistened paper sheets. Two days later, seeds were planted in pots in a screen house in a complete randomized split plot design. For each treatment 30 plants of each maize varieties were used. The plants were treated two days in a row (see volatile collection experiment) before inoculation with pathogen conidia early in the morning of the third day. Volicitin treatment of plants took place from 16:00 to 19:00 on both days (each day starting from the opposite side of the screen house), ensuring that the plants would start to produce volatiles at sunrise, the moment were the downy mildew spores were invading the plants.

Inoculation procedures. The day before plant inoculation with the pathogen conidia, maize plants carrying *P. sorghi*, were sampled near Akure (southern Nigeria) in a farmer's field. Leaves with the highest incidence of downy mildew were cut and kept in boxes at approximately 5 °C. Back in the laboratory, the leaves were washed under running tap water to remove soil particles, conidiophores, and conidia formed during the previous night. These leaves were subsequently wiped dry with absorbent paper and cut into pieces to fit into PVC containers lined with two layers of moistened paper and aluminum foil. Then they were incubated in the evening at 21:00 by placing them in a dew chamber (21 °C and 90% r.h.). At 2:00 in the night leaves were checked for conidia. At 3.00 conidia were harvested by gently washing the leaves with distilled water. The water suspension containing conidia were adjusted to a concentration of 2.2 x 10^4 spores/ml with the aid of a haemocytometer. Plants were inoculated using freehand spraying, each plant was sprayed with approximately 13 ml of suspension.

Data collection and analysis. The first symptom assessment was carried out on day 17 after planting (1 week after inoculation) and was repeated on day 21 and 24. Symptoms were assessed by use of three reaction classes. low, intermediate and high (for methods see Craig, 1982). The low reaction class showing only minor symptoms and the high reaction class consisting of complete discoloration of the inoculated area of the leaf (Craig, 1982). On day 24, the roots of three plants (chosen randomly) of each treatment and variety were washed and dried at 80°C for 1 day and their dry weight was determined. Dry weight of roots was assessed to determine if the treatments had stressed the plants. The same day, 1 leaf of three plants of each treatment of the variety Pool-16-SR was used to cut out 1 mm^2. Thus, for each treatment we obtained 3 mm^2 of leaf area, which was placed in a glass vial. A total of 9 vials, one for each treatment were then filled with 5 ml distilled water and the number of spores were determined under a microscope. This was repeated 8 times and the sum of spores was used for comparison among treatments. Spore-number was assessed only for the variety Pool-16-SR, since the resistant variety to downy mildew (DMR) did not carry any spores.

Differences among treatments for each symptom assessment day and variety were analyzed by use of ANOVA and the post hoc test Student Newman Keuls test. Root dry weight was compared among treatments with the Kruskall Wallis test. Spore number was compared among treatments for the susceptible variety only, using a G test.

Choice tests with Spodoptera littoralis *larvae*. Eggs of *S. littoralis* were incubated on moist filter paper in Petri dishes (14 cm diam., 2 cm height) and placed in an incubator (25 ºC, 85% r.h., 11L : 13D). Fifty newly emerged larvae were placed in the middle of a glass Petri dish between two pieces of leaves (about 2 x 4 cm), one of each maize variety. The dishes were placed in the dark and after 21 hours the number of larvae on each peace of leaf were counted. The experiment was repeated on 4 different days with a total of 21 replications (dishes). Number of larvae found on the leaf piece of each variety was compared using a Wilcoxon Signed Ranks test.

Results

Volatile collections. The total amount of volatiles collected from volicitin induced plants was not different between the two varieties (*P*=0.499, F=0.496, d.f.=1, Pool-16-SR: 518.34±282.23 ng/2 h, DMR: 247.18±66.83 ng/2 h). There was, however, a qualitative difference in the odors collected from the two varieties (Fig. 6.1). Variety Pool-16-SR plants did not release cycloisosativene and α-ylangene (Fig. 6.1) and variety DMR did not release (*E*)-4,8-dimethyl-1, 3,7-nonatriene, phenethyl acetate and 1-H-indole (Fig. 6.1). Furthermore, the variety Pool-16-SR released significantly more (*E*)-β-farnesene (Fig. 6.1).

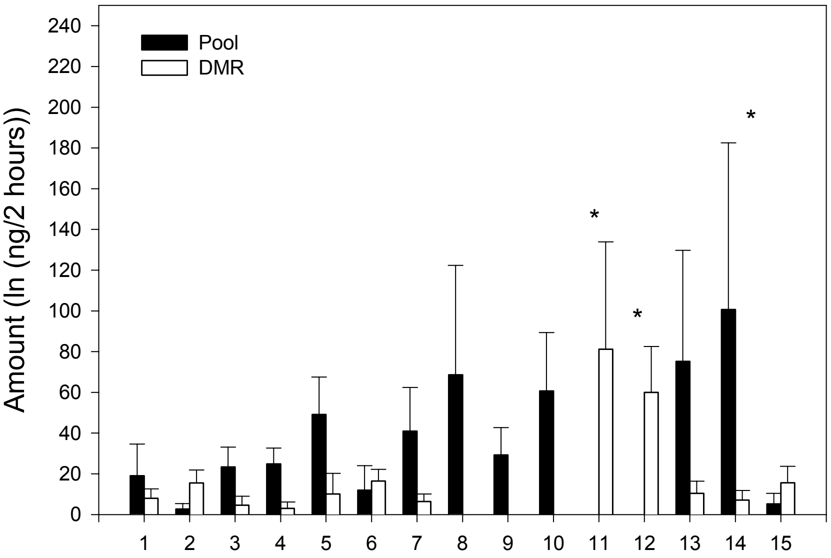

Figure 6.1: Mean amount (±SE) of individual volatile compounds collected from maize varieties (susceptible pool-16-SR, n=7 and resistant DMR, n=4) that were artificially damaged and treated with the elicitor volicitin the two previous days. Volatiles were collected during two hours. Asterisks above bars indicate significant difference ($P<0.05$) between the two varieties. The compounds are: **1**: 2-hexanol; **2**: hexanal; **3**: (*E*)-2-hexenal; **4**: (*Z*)-3-hexenol; **5**: (*Z*)-3-hexenyl acetate; **6**: 2-ethyl hexanol; **7**: linalool; **8**: (*E*)-4,8-dimethyl-1,3,7-nonatriene; **9**: phenethyl acetate; **10**: 1-H-indole; **11**: cycloisosativene; **12**: α-ylangene; **13**: (*E*)-α-bergamotene, **14**: (*E*)-β-farnesene; **15**: β-sesquiphellandrene.

Inoculation with downy mildew. During the first downy mildew symptoms assessment, no differences among treatments were found and almost all plants were classified intermediate (Fig. 6.2 B1). At first assessment no symptoms of the high reaction class were observed on the leaves of either varieties (Fig. 6.2 C1). During the second assessment, plants treated with buffer and volicitin showed more low and intermediate downy mildew symptoms than control plants (Fig. 6.2 A2, B2). In contrast, control plants of the susceptible variety had higher incidence of high infestation (Fig. 6.2 C2). During the third assessment, fewer plants were found with low symptoms for the resistant variety DMR (Fig. 6.2 A3). No significant differences among treatments were found for the resistant variety in the classes intermediate and high and no differences were found for the susceptible variety Pool-16-SR in all three symptom classes (Fig. 6.2 A3, B3 and C3). As expected, the resistant maize variety DMR showed less downy mildew symptoms than the susceptible variety, at least infection was delayed.

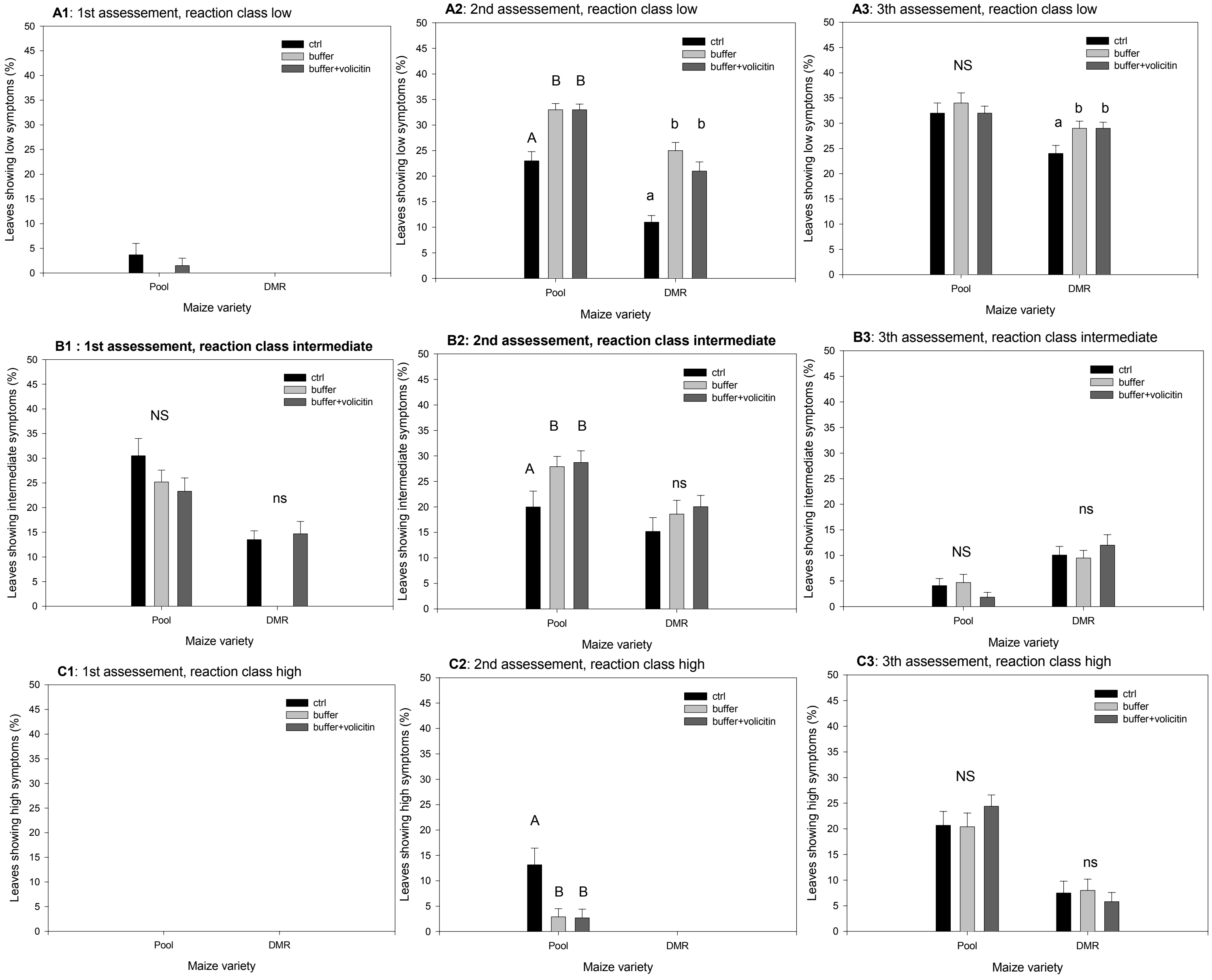

Figure 6.2: Mean percent of leaves (±SE) showing downy mildew symptoms during assessment 1 (7 days after inoculation of plants with downy mildew), 2 (11 days after) and 3 (15 days after) for the susceptible maize variety Pool-16-SR and the resistant maize variety DMR. A: minor symptoms, B: intermediate symptoms, C: high symptoms. Letters above/in bars indicate a significative difference among treatments (a, b: for variety Pool and A,B for variety DMR), ns and NS indicate no differences among treatments for variety Pool and DMR respectively.

Dry weight of roots was not different among treatments for both varieties (ANOVA (n=9), Pool-16-SR, *P*=0.667 and DMR, *P*=0.064). Between the two varieties no difference in root weight was found for control and volicitin-treated plants (ANOVA, *P*=0.281 and *P*=0.729, Fig. 6.3). However a significant difference was found for the plants treated with buffer, dry weight of roots of Pool-16-SR was significantly lower then dry weight of roots of DMR (ANOVA, *P*=0.018, Fig. 6.3). In general, there was a tendency of higher root dry weight for control plants then for treated plants. Numbers of spores was different among treatment for the variety Pool-16-SR, however, the number of spores was to small to realistically detect differences among different treatments (control: 7 spores, buffer: 0 spore, volicitin: 1 spore, G test, *P*=0.004, F=10.64).

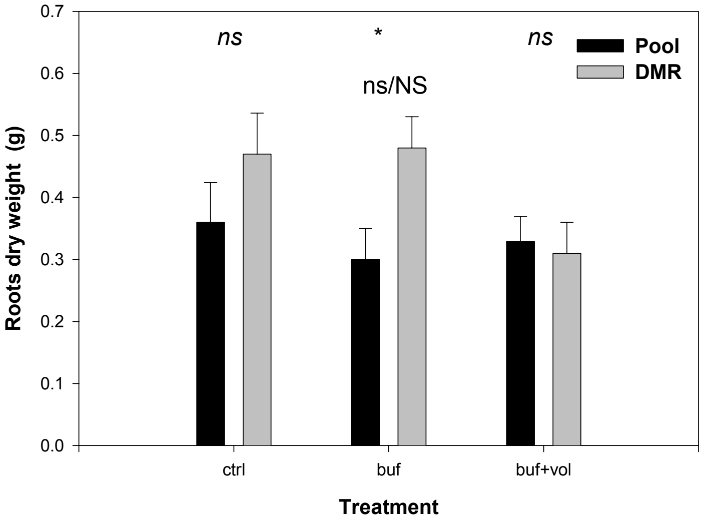

Fig. 6.3: Root dry weight (±SE) of plants of the maize varieties Pool-16-SR and DMR after treatment of the plants and inoculation with downy mildew spores (ns: no difference among treatments for variety Pool; NS: no difference among treatments for variety DMR; *ns*: no difference between varieties, *: P<0.05 difference between varieties).

Choices by Spodoptera littoralis *larvae*. The number of larvae found after 21 h on the DMR leaf was significant higher than on the variety Pool-16-SR (Wilcoxon Signed Ranks test, *P*=0.004, *Z*=-2.904, Fig. 6.4).

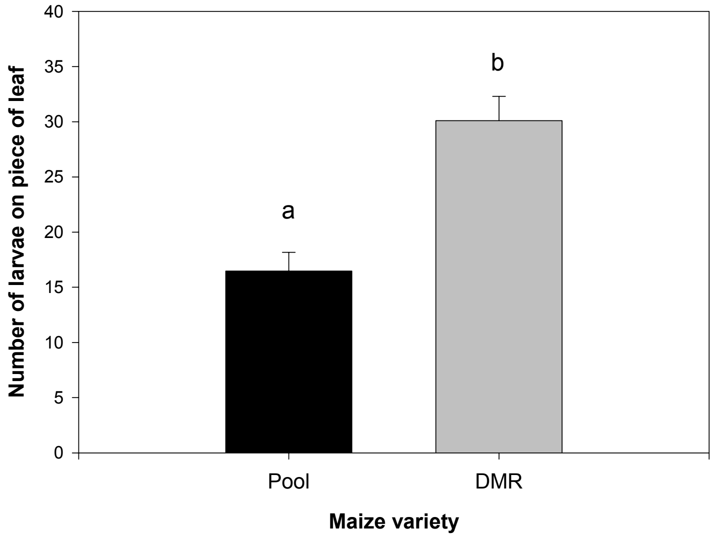

Figure 6.4: Number of caterpillars (±SE) of *Spodoptera littoralis* found back on a piece of leaf of the maize variety Pool (susceptible to downy mildew) and DMR (resistant), respectively in dual choice experiments. Letters above bars indicate a significant difference.

Discussion

A qualitative but not a quantitative difference in volatile emitted after induction of the plants with volicitin was detected between the two maize varieties. The downy mildew resistant variety DMR released the compound cycloisosativene and α-ylangene and the susceptible variety Pool-16-SR released significantly more (*E*)-β-farnesene. Qualitative and quantitative differences in the blends of induced odors among maize genotypes can be considerable (Gouinguené *et al.*, 2001) and Thomas Degen (personal communication).

No differences in downy mildew symptoms were found between treated and control plants at the end of each experiment (the day of the third assessment). This could indicate that downy mildew spores are not susceptible to maize volatiles produced by the two varieties, or that the released quantities were too small to have an effect. It may be better to treat plants for a longer period, to obtain production of volatiles until pathogen spores develop and invade the plants. During the second assessment, plants of the downy mildew susceptible variety Pool-16-SR showed more symptoms on control plants (complete discoloration of the inoculated leaf area extending downward in a wide band along the inoculated portion) on control plants than on treated plants (Fig. 6.2 C2). This could mean that treatment effectively acted against the downy mildew infection, but that over time, this effect waned (Fig. 6.2 C3).

Another reason could be that downy mildew conidia germinate during the night when the plants release only small amounts of volatiles. Plants release induced volatiles mainly during the photophase (Takabayashi *et al.*, 1994; Turlings *et al.*, 1995; Halitschke *et al.*, 2000; Gouinguené, 2000). However, (De Moraes *et al.*, 2001) found that tobacco plants attacked by caterpillars also release several volatiles during the night. For maize plants, volatile emission seem to stop soon after photophase (Gouinguené, 2000). We can not exclude completely volatile production during night.

It is also possible that damage inflicted with a razor blade enhances dew drop formation which could increase downy mildew spores germination. In fact, downy mildew conidia need low temperature and high humidity for germination (Bonde *et al.*, 1978; Williams, 1984; Bock *et al.*, 1998a; Bock *et al.*, 2000). These conditions are actualized during night in Nigeria and could be enhanced by drop formation. This problem may be circumvented with a different treatment method, for example trough injection of the elicitor in the base of the stem (Brown, 1988), Thomas Degen, personal communication) or by growing plants in hydrophobic solutions and adding the elicitors directly in the solution (Baldwin, 1996).

At the end of the experiment a higher number of spores was detected on control plants, however this difference was not sufficient to show that treatment affected downy mildew infection. In retrospect I think that a more simple experiment could provide more information on the effects of volatiles. For example, spores growing and developing on an artificial medium could constantly be aerated with volatiles while the control spores would be aeration with pure air.

In Africa, *S. littoralis* is polyphagous and attacks various crops such as maize, cotton, cowpea and soybean (Kranz *et al.*, 1977; Hill, 1987). However, during the collection of leaves infested with downy mildew near Akure, in Nigeria, no *S. littoralis* caterpillars were found on maize plants. During the choice experiments conducted in the laboratory, more *S. littoralis* caterpillars were found on the downy mildew resistant maize variety. Clearly, the factors that determine pathogen resistance had no negative effect on caterpillar feeding. Additional experiments will need to reveal if there is a general negative correlation between pathogen resistance and herbivore preference. If so, this could be a problem in areas were both downy mildew and *S. littoralis* caterpillars are damaging maize crops. (Thaler, 2000) found a negative correlation between herbivore and pathogen defence in tomato plants. In fact, if plants were treated with BTH (benzothiadiazole-Z-carbothioic acid S-methyl ester, a synthetic mimic of salycilate) to stimulate induced resistance to pathogens, more caterpillars of *Spodoptera exigua* survived compared to survival on unharmed plants. To the contrary, cotton varieties with high levels of constitutive resistance against mites also tended to have high levels of induced resistance against *Verticillum* fungi although this trend was not statistically different (Brody and Karban, 1992). However, if a maize plant is already attacked by a pathogen, the attractiveness of the plant to the herbivore can decrease. For example, antibiosis to *Spodoptera eridania* was associated with a fungal endophyte in grass (Ahmad *et al.*, 1987).

Responses of the plants to pathogens and insects infestation seem to be different. Salicylic acid (SA) is a key compound in a pathway that regulates resistance to fungal, bacterial and viral pathogens while jasmonic acid (JA), produced by the octadecanoid pathway via lipoxygenation of linolenic acid, serves as a signal for expression of chemicals that provide resistance against insect herbivores. SA can inhibit JA biosynthesis, which could compromise the ability of plants to coordinate the defence against pathogens and herbivores simultaneously (Thaler, 2000). (Morris *et al.*, 1998) showed that BTH, which can be used to induce systemic acquired resistance (SAR) in plants, also induced resistance to downy mildew in maize. The effect of JA on downy mildew resistance is not known, but several studies suggest that JA has a direct role in plant defence against both insects and microorganisms (for review see (Karban and Kuc, 2000; Staswick and Lehman, 2000; Stout and Bostock, 2000). Further studies are needed to elucidate the interactions between the JA and SAR pathways and the importance of herbivore-induced volatiles for resistance against pathogens. Such research may lead to a better understanding of the mechanisms behind induced resistance and reveal new opportunities to improve crop protection simultaneous against herbivores and pathogens.

In conclusion, we know that *Spodoptera* induced volatiles in maize can repel herbivores and attract natural enemies, however, further studies are needed to reveal if these volatiles can also act against pathogens.

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Curriculum vitae

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Italian (native language), French and German, all fluent, spoken and written. Good knowledge of English. Some knowledge of Spanish.

Education:

1997-2000: Ph.D. thesis at the University of Neuchâtel (Dr. Ted Turlings and Prof. Martine Rahier): “Exploring Maize Genotypes for Chemical Attributes that Promote the Effectiveness of Biological Control Agents”.

1990-1996: Studies in Biology at the ETH of Zürich, Switzerland. MSc in Biology with specialization in Ecology and Ethology. Thesis at the IITA Cotonou, Benin, Africa (Dr. Manuele Tamò) and at the ETH in Zürich (Prof. Sylvia Dorn): “Beutespektrum von *Orius* sp. (Hemoptera: Anthocoridae) und Wirtspektrum von *Ceranisus menes* (Hymenoptera: Eulophidae), zwei natürlichen Feinden des Blütenthrips *Megalurothrips sjostedti* (Thysanoptera: Thripidae)”.

1986-1990: Liceo cantonale, scientific branch at Locarno, Switzerland.

1982-1986: Secondary school at Minusio, Switzerland.

1977-1982: Primary school at Minusio, Switzerland.

Teaching and practical experiences:

10/2000-12/2000: Training of the laboratory technician student: collection and extraction of odors from cowpea, corn and cotton plants.

1998-2000 (*Summer semesters*): Participation and organisation of practical courses in Entomology. Organisation and supervision of small research projects led by undergraduate students.

11/1996-3/1997: Field assistant of Dr. Laura Cianfanelli during “Atlantic pilot whale project” at the Canary Islands. Data collection and training of participants for analyzing the impact of Whale-watching tourism on the cetaceans, in particularly on the whale *Globicephala macrorynchus*, in Tenerife.

7/1996-9/1996: Assistant in the Applied Entomology Group at the ETH-Zürich. Experiments in maize fields at the Stazioni

Federali di Ricerche Agronomiche, Centro di Cadenazzo, Switzerland.

Attendance of congresses:

25-27.9.00: IIIème Cycle Romand workshop:"Parasitoids: their biology, ecology and application". Oral presentation: "Differential attractiveness of maize varieties to a parasitic wasp". Neuchâtel, Switzerland.

20-26.8.00: XXI International Congress of Entomology. Oral presentation: "Post-emergence host manipulation behavior by larvae of the solitary endoparasitoid *Cotesia marginiventris* (Hymenoptera: Braconidae). Foz do Iguassu, Brazil.

15-18.8.00: 17th Annual Meeting of the International Sociaty of Chemical Ecology. Oral presentation: "Differential attractiveness of maize varieties to a parasitic wasp". Poços de Caldas, Brazil.

5.5.00: ZIL (SDC) Tagung: "Wieder auf dem Boden kommen". Poster presentation: "Exploiting herbivore induced maize odors to promote the effectiveness of biological control agents". ETH, Zürich, Switzerland.

26-30.9.99: XII International Entomophagous Insects Workshop. Oral presentation: "Parasitization of *Spodoptera littoralis* (Lepidoptera: Noctuidae by *Cotesia marginiventris* (Hymenoptera: Braconidae) enhances the performance of infested maize plants". Pacific Grove, California, USA.

Publications:

Fritzsche-Hoballah, M.E., Tamò, C. and Turlings, T.C.J.. Differential attractiveness of induced odors emitted by different maize varieties to the parasiticwasp *Cotesia marginiventris* (Hymenoptera: Braconidae). (accepted *Journal of Chemical Ecology*).

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Book chapters:

Turlings, T.C.J., Gouinguené, S., Degen, T. and Fritzsche-Hoballah M.E. (2001). "The chemical ecology of plant-caterpillar-parasitoid interaction". In: Multitrophic interactions. T. Tscharntke and B. Hawkins Editors. (in press)

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