

# MANIPULATING MAIZE VOLATILES TO IMPROVE THE BIOLOGICAL CONTROL OF INSECT PESTS



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## IMPRIMATUR POUR LA THESE

# Manipulating maize volatiles to improve the biological control on insect pests

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## Summary

Upon herbivore attack, maize plants emit volatile organic compounds (VOCs) that attract parasitic wasps. This natural interaction could be exploited to improve biological control of maize pests, such as the caterpillars of *Spodoptera frugiperda* in the subtropical areas of Central America. To this end, the VOC emission of maize plants was manipulated at three levels: the VOC level, the metabolic level and the genetic level, under field and laboratory conditions.

Results indicate that the dispensing of synthetic so-called green leaf volatiles next to the maize plant increased the plants' emission of inducible VOCs in the field (Chapter I). However, this increased the attack by herbivores, while predation and parasitism of *S. frugiperda* were only slightly influenced by these compounds. Interestingly, the distance at which the maize plants were located from the synthetic VOC dispensers played an important role.

Conversely, when the VOC emission of maize plants was manipulated by the application of two synthetic pathogen and herbivory resistance elicitors in the field (Chapter II), VOC emission was slightly decreased by the pathogen resistance elicitor, and increased by the herbivory resistance elicitor. The application of elicitors did not affect the occurrence of herbivorous insects, nor did it affect parasitism of *S. frugiperda* larvae. The application of resistance elicitors was therefore found compatible with biological control of maize pests.

In Chapter III, the effect of breeding maize for drought tolerance and quality protein was investigated as to their effect on the attractiveness to parasitoids under laboratory conditions. Results indicate that these two breeding goals will have no major impact on the efficacy of these parasitoids as biological control agents. Furthermore, maize inbred lines varied in VOC emission within and between breeding programs. These experiments also indicated that the attractiveness of maize plants could vary depending on the context in which they are tested.

In Chapter IV, we investigated how VOCs affect *Spodoptera littoralis* larval behaviour and feeding rate. Results confirmed that VOCs emitted by maize plants upon conspecific feeding attract caterpillars and that this attraction is higher when the caterpillars have previously fed on maize plants. In addition, fresh damage was more attractive than old damage and this attraction was also increased after maize feeding. In contrast, our results indicate that the VOCs emitted by maize plants decrease the weight gain (probably because of reduced feeding rate) of early instar caterpillars. However, at the later instars, the caterpillars recovered and obtained

a comparable weight to non-exposed caterpillars. We speculate that at close range young caterpillars avoid volatiles from fresh damage in order to escape cannibalism by conspecifics.

The results in this study indicate that slight modifications of plant volatiles in the field affect insect presence. However, the results also indicate that strategies to enhance plant VOCs with the intent of increasing the attraction of beneficial insects, should take in consideration that insect pests may also be attracted, which could lead to an overall negative effect. In conclusion, this work reveals some potential for the improvement of biological control of maize pests through the manipulation of indirect defences, but the intricacies of the various interactions need to be better understood before we can fully deploy this strategy as a way towards a more sustainable agriculture.

## Zusammenfassung

Wenn Maispflanzen von pflanzenfressenden Insekten angegriffen werden, sondern sie flüchtige organische Verbindungen (englisch »*volatile organic compounds*« oder VOCs) ab, die Schlupfwespen anlocken. Dieses natürliche Aufeinanderwirken könnte ausgenutzt werden, um die biologische Schädlingsbekämpfung von Maisschädlingen zu verbessern, wie z.B. die Bekämpfung der Raupen von *Spodoptera frugiperda* in den zentralamerikanischen Subtropen. Mit dieser Absicht wurde die Absonderung von VOCs auf drei Stufen verändert: auf der Stufe der VOCs, auf der Stoffwechselstufe, und auf der genetischen Stufe.

Die Ergebnisse zeigen, dass die Freisetzung von synthetischen VOCs in den Maispflanzen eine erhöhte VOC Absonderung verursachte (Kapitel I). Dies lockte jedoch eine erhöhte Anzahl von Schädlingen an, während die Prädation und die Parasitierung nur leicht beeinflusst wurde. Bemerkenswert ist, dass bei der Freisetzung von VOCs die Distanz der Pflanze vom VOC Spender eine wichtige Rolle spielte.

Bei der Behandlung von Maispflanzen mit einem künstlichen Auslöser der Pathogenabwehr wurde eine verminderte Absonderung von VOCs gemessen (Kapitel II). Eine erhöhte VOC Absonderung wurde nach Anwendung eines Auslösers der Herbivorenabwehr festgestellt. Diese Auslöser hatten weder einen Einfluss auf die Herbivoren, noch auf die Parasitierung von *S. frugiperda* Raupen. Daher steht die Anwendung von Auslösern der Pathogenabwehr im Feld mit der biologischen Schädlingsbekämpfung im Einklang.

Im Kapitel III haben wir die Auswirkungen der Maiszucht für erhöhte Dürretoleranz und verbesserten Proteingehalt auf die Anlockung von Schlupfwespen unter Laborbedingungen untersucht. Die Ergebnisse dieser Studie zeigen, dass diese zwei Zuchtziele keinen massgebenden Einfluss auf die Wirksamkeit von Schlupfwespen für die biologische Schädlingsbekämpfung haben. Die VOC Absonderung der Inzuchtlinien unterscheidet sich zwischen und innerhalb der Zuchtprogramme. Diese Experimente lassen auch vermuten, dass sich die Attraktivität von Maispflanzen für Schlupfwespen kontextabhängig verändern kann.

In Kapitel IV untersuchten wir, wie die VOCs auf das Verhalten und den Frass von Raupen von *Spodoptera littoralis* (Boisduval) (Lepidoptera: Noctuidae) wirken. Die Ergebnisse dieser Studie bestätigen, dass die vom Frass von Artgenossen auf einer Maispflanze ausgelösten VOCs für die Raupen attraktiv sind, und dass diese Attraktivität zunimmt, wenn die Raupen vorher Mais gefressen haben. Zudem werden frisch geschädigte Maispflanzen den alt geschädigten vorgezogen, und diese Attraktivität wurde

durch vorherigen Maisfrass auch erhöht. Erstaunlicherweise zeigen unsere Resultate auch, dass diese VOCs das Wachstum von jungen Raupenstadien hemmen, vermutlich wegen reduziertem Frass. Allerdings passen sich die Raupen in älteren Stadien an die VOCs an und erholen sich vom Wachstumsrückstand. Wir vermuten, dass junge Raupen kleine Abstände zu VOCs von frischem Schaden meiden, um dem Kannibalismus durch Artgenossen zu entgehen.

Die Ergebnisse dieser Studie weisen darauf hin, dass leichte Veränderungen der Pflanzenduftstoffe die Anwesenheit von Insekten beeinflussen. Strategien, die durch verbesserte Pflanzen-VOCs die Anlockung von Nützlingen erhöhen, sollten berücksichtigen, dass damit auch Schädlinge angelockt werden könnten, was zu einer negativen Bilanz führen könnte. Zusammenfassend zeigt diese Arbeit einige Möglichkeiten zur Verbesserung biologischer Schädlingsbekämpfung mittels Manipulation indirekter Abwehrmechanismen. Allerdings sollten die Feinheiten der verschiedenen Interaktionen besser verstanden werden, bevor diese Strategie für eine nachhaltigere Landwirtschaft angewendet werden kann.

## Résumé

Les plantes de maïs attaquées par des herbivores émettent des composés organiques volatiles (de l'anglais « *volatile organic compounds* » ou VOCs) qui attirent les guêpes parasitoïdes. Cette interaction naturelle pourrait être exploitée afin d'améliorer la lutte biologique contre les ravageurs du maïs comme la chenille de *Spodoptera frugiperda* dans les zones subtropicales d'Amérique Centrale. C'est dans ce but que nous avons manipulé l'émission de VOCs à trois différents niveaux : au niveau des VOCs, au niveau métabolique et au niveau génétique, en champ et en laboratoire.

Les résultats indiquent que l'émission de composés volatiles synthétiques (« *green leaf volatiles* ») proche d'une plante de maïs a augmenté l'émission de VOCs inductibles en champ (Chapitre I). Cependant, cela a intensifié l'attaque des herbivores, alors que la prédation et le parasitisme n'ont été influencés que très légèrement par ces composés. Il est à noter que la distance par rapport à l'émetteur de VOCs à laquelle se trouvaient les plantes de maïs a joué un rôle important.

Inversément, lorsque l'émission de VOCs par des plantes de maïs était manipulée en appliquant deux éliciteurs de résistance aux herbivores et aux pathogènes en champ (Chapitre II), l'émission de VOCs était diminuée légèrement par l'éliciteur de résistance aux pathogènes et augmentée par l'éliciteur de résistance aux herbivores. L'application d'éliciteurs n'a affecté ni l'abondance d'insectes herbivores, ni le parasitisme de chenilles de *S. frugiperda*. L'application d'éliciteurs de résistance peut donc être compatible avec la lutte biologique contre les ravageurs du maïs.

Au Chapitre III, l'effet de la sélection de maïs pour une meilleure tolérance aux sécheresses et pour une protéine de meilleure qualité a été étudiée par rapport à l'attractivité pour les parasitoïdes en laboratoire. Les résultats indiquent que ces deux objectifs de sélection n'ont pas d'impact majeur sur l'efficacité de ces parasitoïdes comme agents de lutte biologique. En outre, les VOCs émis par les lignées autofécondées variaient entre et parmi les programmes de sélection. Ces expériences indiquent aussi que l'attractivité des plantes de maïs pourrait varier selon le contexte dans lequel elles sont testées.

Au Chapitre IV, nous avons étudié comment les VOCs affectent le comportement et la vitesse d'alimentation des chenilles de *Spodoptera littoralis*. Les résultats indiquent que les VOCs émis par des plantes de maïs après l'attaque par des congénères attirent les chenilles et que cette attraction est plus élevée lorsque les chenilles ont mangé du maïs par avant. De surcroît, les dégâts récents étaient plus attractifs que les dégâts anciens et cette attraction était également augmentée par le fait de manger du maïs

auparavant. En revanche, nos résultats indiquent que les VOCs émis par des plantes de maïs diminuent le gain de poids des jeunes stades larvaires, probablement à cause de la vitesse d'alimentation réduite. Cependant, les stades larvaires plus avancés se sont remis du retard de croissance et ont atteint un poids comparable aux chenilles non exposées. Nous conjecturons que les jeunes chenilles évitent les composés volatiles émis par des dégâts récents afin d'échapper au cannibalisme de leurs congénères.

Les résultats de cette étude indiquent que de légères modifications de composés volatiles des plantes en champ affectent la présence des insectes. D'autre part, les résultats indiquent aussi que les stratégies qui visent à améliorer les VOCs des plantes dans le but d'attirer plus d'auxiliaires devraient aussi tenir compte de l'éventualité que les nuisibles soient également attirés en plus grand nombre, ce qui pourrait mener à un bilan négatif. En conclusion, ce travail révèle un potentiel pour l'amélioration de la lutte biologique des ravageurs du maïs par la manipulation de défenses indirectes. Cependant, les subtilités des différentes interactions doivent être mieux comprises avant que l'on puisse déployer cette stratégie visant une agriculture plus durable.

## Resumen

Las plantas de maíz al ser atacadas por insectos herbívoros producen compuestos orgánicos volátiles («*volatile organic compounds*» = VOCs) que atraen a las avispas parasitoides. Ésta interacción natural tiene el potencial de ser explotada y utilizada para mejorar el control biológico de plagas del maíz, como las orugas de *Spodoptera frugiperda*, una plaga importante en las zonas subtropicales de América Central. Con este objetivo en mente, se manipuló la emisión de VOCs de plantas de maíz en el campo y en el laboratorio con respecto a tres niveles: el nivel de los VOC, el nivel metabólico y el nivel genético.

Se encontró que al liberar compuestos sintéticos («*green leaf volatiles*») en la proximidad de la planta de maíz, la emisión de VOCs inducibles aumentaba (Capítulo I). Este incremento resultó un aumento en la tasa de herbivoría y solo un ligero incremento de la depredación y parasitismo. La distancia desde la cual se liberaron estos compuestos con respecto a la planta de maíz también tuvo un efecto importante.

Por una parte, la emisión de VOCs disminuyó ligeramente mediante la aplicación de una sustancia activadora que proporciona resistencia a las plantas contra patógenos. Por otro lado, dicha emisión aumentó al aplicar una sustancia que proporciona resistencia contra herbívoros (Capítulo II). Sin embargo, estas sustancias no aumentaron las tasas de herbivoría, ni disminuyeron las tasas de parasitismo. De esta forma se puede considerar que la utilización de estas sustancias activadoras es compatible con el control biológico de plagas de maíz.

En el Capítulo III, se examinó en el laboratorio la atracción de avispas parasitoides hacia plantas de maíz con mayor tolerancia a la sequía y mejor calidad de proteína. Los resultados indican que estas dos características no afectan significativamente a la efectividad de las parasitoides para desempeñar su papel como agentes de control biológico. Además, las líneas autofecundadas variaban en su emisión de VOCs dentro y entre programas de cría. Estos experimentos también indican que la atractividad de plantas de maíz podría variar según el contexto en el cual sea medida.

En el Capítulo IV, investigamos como los VOCs afectan al comportamiento y al crecimiento de orugas de *Spodoptera littoralis*. Los resultados confirman que los VOCs emitidos por las plantas como respuesta al ataque de conoespecíficos atraen a las orugas y que esta atracción aumenta si las orugas han estado comiendo maíz previamente. El daño reciente resultó ser más atractivo que el daño más viejo, así mismo esta atracción fue más intensa tras el un previo consumo de maíz. En contrapartida, nuestros resultados demuestran que los VOCs emitidos por plantas de maíz reducen

el crecimiento de jóvenes estadios de orugas, probablemente debido a un consumo menor. Sin embargo, en estadios mayores, las orugas retomaron un peso comparable con las orugas no expuestas a VOCs. Sugerimos que las orugas jóvenes evitan plantas con daño reciente para escapar del canibalismo por conoespecíficos.

Los resultados de este estudio indican que leves modificaciones de los VOCs emitidos por las plantas en el campo afectan la presencia de insectos. No obstante, los resultados también indican que aún empleando la manipulación de VOCs con el fin de aumentar la acción de insectos benéficos, se debe considerar la posibilidad que existe de atraer otras plagas, lo cual resultaría en un balance negativo. Este estudio se concluye poniendo de manifiesto el potencial que presenta la manipulación VOCs emitidos por las plantas para ser empleada como una herramienta para el manejo de plagas y la mejora de programas de control biológico. Sin embargo, es necesario obtener un mejor conocimiento sobre la complejidad de estas interacciones antes de que se pueda poner en práctica esta estrategia para una agricultura más sostenible.

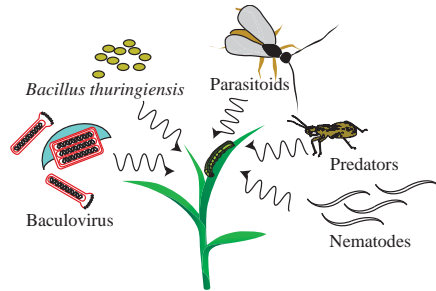
## Introduction and thesis outline

Maize is the world's most important agricultural crop with 822 million tons (MT) produced in 2008 (FAO, 2010), more than rice (685 MT) and wheat (689 MT). In the Americas alone, yearly production reached 438 MT in 2008. However, every year, 16 % of worldwide maize production is lost to animal pests (Oerke, 2006). In many developing countries, pest damage is elevated due to the lack of financial means available for insecticides (Wyckhuys & O'Neil, 2007). Furthermore, the tropical climate favours insect pests (Bale et al., 2002; Porter et al., 1991). In Mexico for instance, *Spodoptera frugiperda* (J. E. Smith) (Lepidoptera: Noctuidae) (Figure 1) causes between 13% and 30% yield losses in maize yield (Andrews, 1988). More recent figures mention damage levels of up to 50% in tropical and sub-tropical areas of Mexico (Bahena Juarez, 2003). The damage is mainly due to the feeding of *S. frugiperda* larvae on growing parts of the maize plant (Hernández-Mendoza et al., 2008). Therefore, farmers in the tropical areas of central and south America are using more and more insecticides (Andrews et al., 1992). With the ensuing concerns for environmental and human safety in agriculture, the use of synthetic pesticides is increasingly restricted, which calls for alternative and sustainable pest control methods. One sustainable method to control pest insects is biological control, defined in 1919 by Harry S. Smith as "the control or regulation of pest populations by natural enemies" (cited in Debach & Rosen, 1991). Biological control is considered especially promising in countries where farmers do not have the means to apply conventional techniques (Kipkoech et al., 2008). In maize, several management techniques have been explored to improve biological control in maize (Figure 2), such as the application of viruses (baculovirus, namely nuclear polyhedrosis viruses), bacteria (such



Figure 1: Distribution of *Spodoptera frugiperda* (J.E. Smith) (Lepidoptera: Noctuidae) in America. After Ashley (1986). The red dot indicates CIMMYT's Ernest W. Sprague station, where the field experiments were conducted (Chapter I and II).

Figure 2: Organisms used in biological control. Examples shown are baculovirus (Moscardi, 1999), *Bacillus thuringiensis* (Gill et al., 1992), hymenopteran parasitoids (Doutt, 1959; Turlings et al., 1995), predatory assassin bugs (Grundy et al., 2000), and entomopathogenic nematodes (Kaya & Gaugler, 1993)



as *Bacillus thuringiensis*, whose bt-toxin is expressed in transgenic crops), predatory bugs and entomopathogenic nematodes. However, in this study, we focus on biological control mediated by insect predators and parasitoids. A worldwide applied biological control strategy is the mass release of parasitoids in European corn borer control programs (Smith, 1996) or for the control of the cassava mealybug in Western Africa (Herren et al., 1987). Another strategy in biological control is the attraction of predator insects to plants surrounding maize plants and the repellence of pests mediating the odour emission of intercropped plants, named the push-pull strategy (Cook et al. 2007). One widely acclaimed example of this strategy is the control of the African stem borer (Khan et al., 2008). However, these strategies do not directly involve the plants' own defences. It is known that plants produce antifeedant compounds (Rostás, 2007; Koul, 2008) that directly defend the plant. Moreover, plants can prevent herbivory

by forming structures that reduce palatability or hamper herbivore mobility, such as trichomes in tomatoes (Boughton et al., 2005). Based on this knowledge, efforts to improve pest resistance by conventional breeding were undertaken in Mexico. Studies under experimental and farmer field conditions showed that maize resistance to *S. frugiperda* was compatible with increased yield (Kumar, 2002). However, the breeding traits focused mainly on constitutive defence mechanisms, such as increased leaf toughness (Bergvinson et al., 1994).

Another promising strategy of biological control of maize pests could involve plants that defend themselves better, by improving their capacity to attract beneficial insects (Turlings & Ton, 2006). Several studies have indeed shown that plants defend themselves against arthropod herbivores by emitting volatile organic compounds (VOCs) that attract parasitoids (Figure 3, Dicke et al., 1990; Heil, 2008; Turlings et al., 1990). Furthermore, parasitoids play an important role in the control of *S. frugiperda* (Andrews et al., 1992; Hoballah et al., 2004; Molina-Ochoa et al., 2004). In this thesis, we investigate how the attractiveness of maize plants for parasitoids can be increased by manipulating their VOC emission, as a strategy to improve biological control. The VOC blend emitted upon herbivory consists of a number of volatiles including green leaf volatiles (GLVs, C6 aldehydes, alcohols and their esters, stemming from the linolenic and linoleic acid-based lipoxygenase pathway, LOX), aromatic compounds, as well as mono, homo and sesquiterpenes (D'Alessandro & Turlings, 2005; Paré & Tumlinson, 1999) (see also Figure 4).

These chemical groups are emitted by plants at different points in time after infestation by herbivores (Turlings et al., 1998). In maize these VOCs have been studied with the goal of finding the key compounds in plant-insect interactions (D'Alessandro & Turlings, 2006). It was found that minor compounds attract parasitoids (D'Alessandro et al., 2009), but also that the VOC emitted upon fresh damage is preferred over old damage (Hoballah & Turlings, 2005). However, the importance in parasitoid attraction of single compounds such as indole has been questioned (D'Alessandro et al., 2006). Interestingly, the VOCs emitted by plants upon herbivory can be perceived not only by parasitic wasps, but also by plants. Upon exposure to herbivore-induced VOCs, plants can prepare for subsequent attack. This so-called priming of plants was observed in several plant species, including maize (Engelberth et al., 2004), poplar (Frost et al., 2008), and wild tobacco (Kessler et al., 2006). What is more, upon exposure to the VOCs emitted by attacked plants, maize plants can also prime their VOC emission (Ton et al., 2007). In addition, VOC-mediated plant-to-plant signalling occurs between plants of the same species (Engelberth

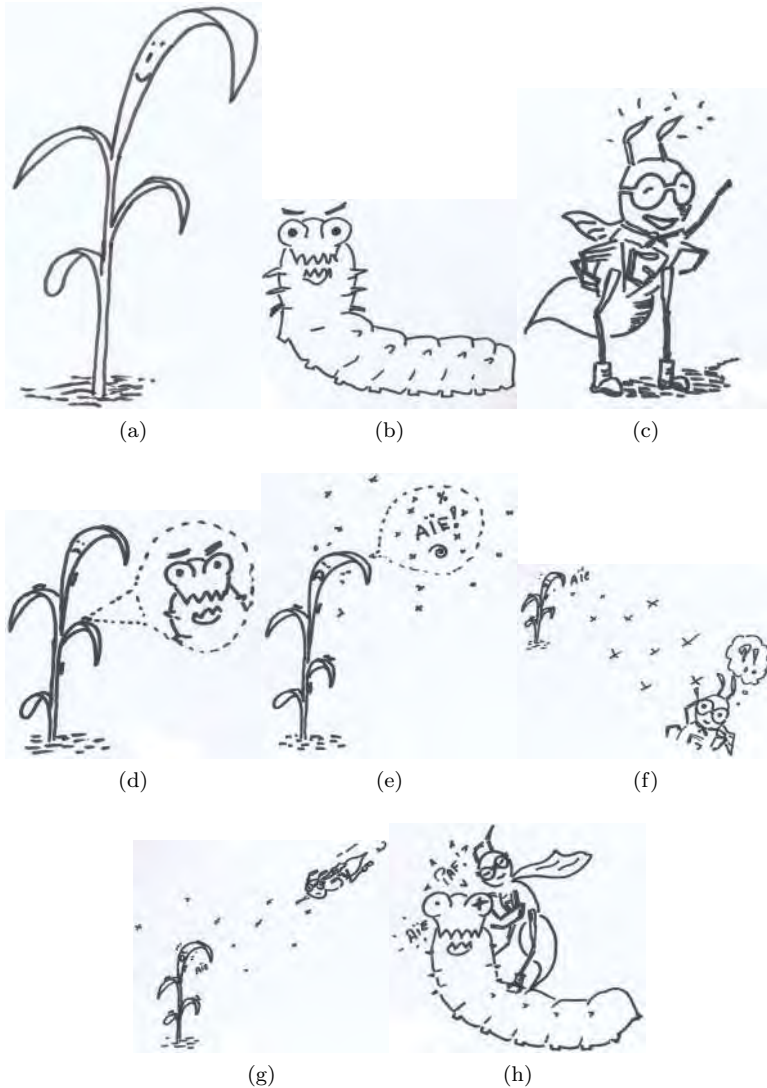


Figure 3: Schematic of tri-trophic interactions: (a) healthy maize plant, (b) *S. frugiperda* caterpillar, (c) parasitoid, (d) herbivore attack on maize plant, (e) herbivore-induced VOC emission by maize plant, (f) parasitoid perception of the VOCs, (g) parasitoid attraction to VOCs, (h) parasitism. Drawn for the tri-trophic workshop of the University of Neuchâtel's children day Graine de Belette, 24<sup>th</sup> June 2007

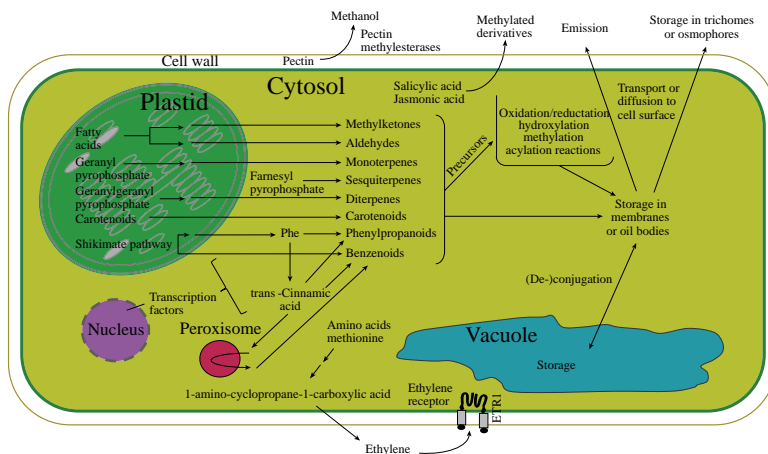


Figure 4: Volatile organic compounds synthesis inside the plant cell as shown by Baldwin (2010).

et al., 2004), between different species (Kessler et al., 2006), and between distant tissues of the same plant (Frost et al., 2007; Rodriguez-Saona et al., 2009). Of all the VOCs, GLVs play an important role in priming (Engelberth et al., 2007; Frost et al., 2008). Moreover, they are also the main volatiles emitted immediately upon fresh damage (Hoballah & Turlings, 2005). GLVs could therefore constitute a promising class of VOCs that may have a role in both priming and parasitoid attraction (D'Alessandro & Turlings, 2005; Turlings & Ton, 2006; Whitman & Eller, 1990).

However, plants are subjected to a complex environment, containing a number of other biotic stresses that influence VOC emission, such as pathogens, parasitisation (Dicke & Baldwin, 2010; Walling, 2009), and abiotic stress factors (Gouinguéné & Turlings, 2002; Heil, 2010; Holopainen & Gershenzon, 2010; Vickers et al., 2009). Some of these stress factors can occur simultaneously, and, in fact, generally do so under field conditions. Plants therefore have to mount a defence strategy that allows them to survive multiple attacks by simultaneous threats. The coordination of these defences is mediated by phytohormones, mainly salicylic acid (SA), jasmonic acid (JA), and abscisic acid (ABA) (Bari & Jones, 2009; Beckers & Spoel, 2006; Flors et al., 2008; Fujita et al., 2006; Kunkel & Brooks, 2002; Walling, 2009). Because of the simultaneous occurrence of different threats, defence mechanisms are interdependent, in order to balance the different metabolic pathways, so called crosstalk (Koornneef & Pieterse, 2008; Kunkel & Brooks, 2002).

When looking at herbivore-induced defences, such as VOC emission, the interdependence of defence pathways plays an important role, because other pathways can operate as regulators for the herbivory-related ones. Crosstalk between metabolic pathways has been demonstrated in several plant species, such as tomato (Doares et al., 1995; Thaler et al., 2002), *Arabidopsis* (van Poecke & Dicke, 2004), and maize (Rostás & Turlings, 2008). However, crosstalk has often been described as an interaction where the activation of one pathway diminishes the activity of another one (Cipollini et al., 2003; Thaler et al., 2002). On the other hand, others have reported that the activation of SA did not affect significantly JA-mediated defence against herbivores (Danielson, 2003; Inbar et al., 2001; Rostás et al., 2006). Rostás & Turlings (2008) have however demonstrated that by activating the SA-pathway with the SA-mimic benzo-(1,2,3)-thiadiazole-7-carbothioic acid S-methyl ester (BTH), maize plants can become more attractive to the parasitoid *Microplitis rufiventris* Kokujev (Hymenoptera: Braconidae). BTH can be applied to induce systemic acquired resistance (SAR) against a number of pathogens (Durrant & Dong, 2004; Shah, 2009). The effect of BTH in maize (Morris et al. 1998), tobacco (Friedrich et al. 1996), and *Arabidopsis* (Conrath et al., 2006) has been investigated extensively. Field studies have shown that BTH could reduce leaf miner densities (Inbar et al., 1998). However, thus far little is known about the effect of BTH application on parasitisation in the field.

Beside the variation of VOC emission due to VOC-mediated priming or metabolic pathway activation by biotic and abiotic stresses, VOC emission also differs between plant species as well as within plant species, between breeding lines and populations (Courtois et al., 2009; Gouinguéné et al., 2005; Khan et al., 2000), which translates into different attraction of parasitoids (Degen et al., 2004; Delphia et al., 2009; Gouinguéné et al., 2002; Hare, 2007; Poelman et al., 2009). This variability in parasitoid attraction constitutes a promising way to improve biological control of maize pests, by breeding for more attractive maize plants. However, biological control traits have thus far not been included in maize breeding schemes. Especially indirect defence traits have not been investigated under field conditions in the context of breeding. Beside seeking to increase yield, present breeding efforts aim to improve the plants tolerance to adverse conditions, such as drought and nitrogen stress, or improved quality of the produce, mainly a protein composition of the grain that is more adapted to human and animal needs. These two breeding goals are currently being pursued by the International Centre for the Improvement of Maize and Wheat (CIMMYT), in Mexico. Due to climatic changes and the increasing scarcity of water, especially in developing countries, maize plants are

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bred for increased drought tolerance (Bänziger et al., 2006). However, abiotic stress, such as heat and drought, influence the VOC emission of maize plants (Gouinguéné & Turlings, 2002). Furthermore, adaptation to drought stress involves abscisic acid, a regulator of stomatal closure in maize plants, which in turn influences the emission of induced VOCs in maize plants (Erb et al., 2009; Fujita et al., 2006). If maize plants become increasingly tolerant to drought, this may affect the emission of VOCs upon herbivory, and therefore the plants' overall attractiveness to parasitoids.

On the other hand, improved protein composition of the maize grain mainly involves increased content of tryptophan and lysine, two essential amino acids in human and animal nutrition (Gupta et al., 2009; Villegas et al., 1992). However, tryptophan is also a component of the shikimic pathway, which leads to the emission of indole (D'Alessandro & Turlings, 2006), an important herbivore-induced VOC. In addition, indole has been shown to mask attractive compounds to parasitoids (D'Alessandro et al., 2006). Maize plants that store increased amounts of tryptophan in their grains could therefore also have a modified emission of indole.

When increasing the attractiveness of maize plants for beneficial insects, one should keep in mind the attractiveness for pests. Indeed, it is not only parasitoids that use volatile cues to locate their host. The same cues can be used by herbivores to locate suitable host plants. For instance, adults of *S. frugiperda* are repelled by plants that are already attacked by conspecifics (De Moraes et al., 2001). On the other hand, larval stages of *S. frugiperda* are attracted towards induced maize plants (Carroll et al., 2006). Other potentially detrimental insects, such as Coleoptera are also often attracted towards VOCs emitted by plants (de Groot et al., 2008; Larsson et al., 2001; Zilkowski et al., 1999; Halitschke et al., 2008). Furthermore, it is not clear how VOCs affect the herbivores in their growth. One could expect VOCs to improve the growth rate of herbivores, as they are attracted towards the source of VOCs.

This thesis aims therefore at improving biological control of insect pests, by manipulating the volatile compounds that are emitted by maize plants. At the same time, the effects of these manipulations on the insect pests is investigated. This effort aims at contributing to the general understanding of induced VOC-mediated beneficial insect attraction and biological control, thereby contributing to a more sustainable agriculture.

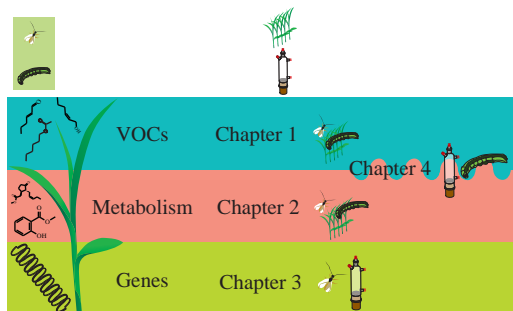


Figure 5: Thesis concept. The VOCs emitted by maize plants are modified at three levels: VOCs, metabolism and genes. In Chapter I, the VOCs surrounding maize plants are modified in the field by dispensing synthetic VOCs. In Chapter II, the VOC emission of maize plants is modified by applying synthetic elicitors in the field. In Chapter III, breeding for specific production-related traits is investigated as to its effect on parasitoid attraction in the laboratory. Finally, in Chapter IV, the attraction of herbivores towards maize plant VOCs and their effect on herbivore growth is observed in the laboratory.

## Thesis outline

There are three levels at which the VOC emission of maize plants can be modified (Figure 5). At the first level, the composition of VOCs surrounding the maize plants can be modified directly through the release of synthetic VOCs (Chapter I). At the second level, the metabolic pathways causing the emission of VOCs can be modified (Chapter II). At the third level, the genetic makeup that determines the emission of VOCs can be altered (Chapter III). Chapter IV looks at how maize VOCs affect the performance and the behaviour of *Spodoptera littoralis* (Boisduval) (Lepidoptera: Noctuidae). larvae.

### Chapter I - The importance of green leaf volatiles for the attractiveness of maize plants to pest and beneficial insects in the field.

The VOC composition surrounding maize plants can be changed to attract more parasitoids. Furthermore, maize plants can be primed for subsequent attack by exposure to VOCs from attacked plants. In this chapter, maize

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plants were exposed to four synthetic green leaf volatiles in a maize field in Mexico. Several parameters were measured. Firstly, the change in VOC emission in plants that had been exposed to GLVs was measured. Secondly, the damage caused by and the number of herbivores on maize seedlings was assessed. Finally, the parasitisation of collected *S. frugiperda* larvae was measured.

## **Chapter II - Effects of two elicitors of insect and pathogen resistance on the volatile emission and the biological control of *Spodoptera frugiperda* in maize fields.**

Synthetic resistance elicitors can induce maize plants' defence mechanisms. These defence mechanisms are controlled by two major metabolic pathways: the salicylic acid (SA) and the jasmonic acid (JA) pathway. The VOC emission of maize plants is influenced by these pathways, either directly through the JA pathway or indirectly through the crosstalk of the SA pathway. In this chapter we induced SA with a synthetic SA mimic benzo-(1,2,3)-thiadiazole-7-carbothioic acid S-methyl ester (BTH), and the JA pathway with methyl jasmonate (MeJA) in plants in a maize field in Mexico. The effects of eliciting these pathways on the VOC emission was measured. Herbivory was then assessed. Finally, caterpillars were collected from the maize plants and the parasitisation measured.

## **Chapter III - Does breeding for quality protein and drought tolerance affect the attractiveness of maize plants to parasitoids?**

Maize plants differ genetically in their VOC emission, both quantitatively and qualitatively. Furthermore, abiotic factors, such as drought, affect the VOC emission of maize plants. Actual breeding schemes aim to increase the tolerance of maize plants to drought. On the other hand, maize breeders seek to improve the protein composition of the maize grain, in order to increase the suitability for human and animal nutrition. The genetic variability of VOC emission could be exploited to increase the attractiveness of maize plants for parasitoids, thereby improving biological control. Here we investigated how breeding for drought resistance and quality protein affects the attractiveness of maize plants to parasitoids.

**Chapter IV - Herbivore-induced maize leaf volatiles affect attraction and early feeding of *Spodoptera littoralis* larvae.**

Upon herbivore attack, maize plants emit VOCs that attract predators. However, not only do predatory insects use these cues to find potential hosts, but so do herbivores. In noctuids, the feeding stages, i.e. the ones that cause most damage to crops, are the larval stages. Despite their reduced mobility, they are capable of changing their host plant, or to find a new host plant once they fall off their previous host plant. Little is known, however, on the effects of VOCs on the feeding of *S. littoralis*.



## **The importance of green leaf volatiles for the attractiveness of maize plants to pest and beneficial insects in the field.**

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**Abstract** Maize plants respond to feeding by arthropod herbivores by producing a number of secondary plant compounds, including volatile organic compounds (VOCs). These herbivore-induced VOCs are not only known to attract natural enemies of the herbivores, but they also prime inducible defences in neighbouring plants, meaning that defence responses are stronger and faster if the plants have previously been exposed to VOCs from other herbivore-infested plants. Among the compounds that cause this priming effect, green leaf volatiles (GLVs) have received particular attention, as they are ubiquitous and rapidly emitted upon damage. In this study, we applied specially devised dispensers to release four synthetic GLVs at physiologically relevant concentrations in several plots of a maize field. We then compared the VOC emission of GLV-exposed maize plants to non-exposed plants and monitored the attraction of herbivores and predators, as well as parasitism of the caterpillar *Spodoptera frugiperda*, the most common herbivore, in GLV-treated plots and control plots. We found that maize plants that were exposed to GLVs in the field emitted increased quantities of sesquiterpenes compared to non-exposed plants. Herbivorous insects, such as adult *Diabrotica* beetles and *S. frugiperda* larvae, were observed more frequently in GLV-treated plots and they caused more damage to GLV-exposed plants than to non-exposed plants. By contrast, parasitism of *S. frugiperda* was only weakly affected by GLVs, with similar parasitism rates of *S. frugiperda* in GLV-exposed and non-exposed plots. These effects depended on the distance from the GLV-dispensers at which the plants were located, indicating that VOC-mediated signalling strongly depends on the distance from the emitter. The results are discussed in the context of strategies to improve biological control by enhancing plant-mediated attraction of natural enemies.

**Key words** Green leaf volatiles, maize, priming, herbivore-induced plant volatiles parasitoids, *Spodoptera frugiperda*.

## I. 1 Introduction

Plants that are attacked by insect herbivores emit volatile organic compounds (VOCs) that attract predators and parasitoids (Arimura et al., 2009; Heil, 2008; Turlings & Wäckers, 2004; Dicke et al., 2003). Besides their role in plant-insect interactions, VOCs have also been implicated in plant-plant interactions (Arimura et al., 2001; Baldwin et al., 2006). For instance, when plants are exposed to herbivory-induced VOCs from other plants their defence mechanisms are triggered faster and with greater magnitude upon subsequent herbivore attack (Engelberth et al., 2004; Heil & Kost, 2006; Kessler et al., 2006). In laboratory experiments with maize seedlings, this so-called "priming" effect has also been shown to initially enhance the emission of VOCs upon feeding damage by larvae of *Spodoptera littoralis*, resulting in a stronger attraction of *Cotesia marginiventris*, an important parasitoid of *Spodoptera* larvae (Ton et al., 2007).

The volatile blend emitted by plants upon herbivore damage consists of a plethora of VOCs of different chemical groups, such as green leaf volatiles (GLVs), alcohols, aromatics, mono-, homo- and sesquiterpenes (D'Alessandro & Turlings, 2006; Kant et al., 2009; Paré & Tumlinson, 1999; Turlings et al., 1990). However, not all VOCs play an equally important role in the attraction of parasitoids (D'Alessandro & Turlings, 2005; D'Alessandro et al., 2009). Particularly the GLVs, C6-alcohols, aldehydes and their esters that derive from the lipoxygenase pathway (LOX) have received increased attention over the last few years. Several studies suggest that GLVs play a key role not only in insect attraction (Halitschke et al., 2008; Shiojiri et al., 2006; Unsicker et al., 2009; Whitman & Eller, 1990), but also in priming of herbivore-induced defences (Engelberth et al., 2004; Frost et al., 2008; Heil & Kost, 2006; Ton et al., 2007), activation of pathogen defence (Yi et al., 2009), or direct induction of defence mechanisms against herbivores (Engelberth et al., 2007; Farag et al., 2005; James & Grasswitz, 2005; Ruther & Kleier, 2005; Walling, 2000). The exact mechanism behind GLV-mediated signalling is yet unknown, however, it is evident that small quantities can already trigger priming in plants (Heil & Ton, 2008; Heil & Walters, 2009). Moreover, several laboratory studies suggest that GLVs are attractive to parasitoids (Degenhardt, 2009; Hoballah & Turlings, 2005; Whitman & Eller, 1990; D'Alessandro & Turlings, 2005). In the field, attraction of insect predators and parasitoids to synthetic GLVs has been confirmed with the use of trapping and monitoring methods (James, 2003a,b). However, certain herbivores, in particular Coleoptera (Halitschke et al., 2008; Hansson et al., 1999; Larsson et al., 2001) are also known to be attracted to GLVs. It is therefore

not clear whether the application of GLVs in the field would result in an overall reduction in herbivore damage.

In this study we investigated the effects of synthetic GLVs on herbivory, as well as on parasitism and predation of *Spodoptera frugiperda* (J.E. Smith) (Lepidoptera: Noctuidae), the main lepidopteran pest on maize in subtropical Mexico. To do so, we developed a device to release physiologically relevant amounts of four GLVs: (*Z*)-3-hexenal, (*Z*)-3-hexenol, (*E*)-2-hexenal, and (*Z*)-3-hexenyl-acetate and we confirmed in a laboratory experiment that maize seedlings that were exposed to these GLVs responded faster and stronger with the emission of certain VOCs upon herbivore-damage. We then investigated the effects of GLVs in a subtropical maize field in Mexico by comparing herbivory on maize plants in plots with and without GLV dispensers at different distances from the dispensers, and we measured predation and parasitism rates by collecting the larvae of *S. frugiperda* and the parasitoids that emerged from the collected larvae.

## I. 2 Material and Methods

### I. 2.1 GLV-dispensers

Amber glass vials (2 ml, Supelco, Sigma Aldrich, Buchs, Switzerland) were filled with 100 mg of glass wool, that were first washed with dichloromethane and heated for 16 hours at 250°C. They were then loaded with 0.2 ml of a mixture of 80% (*Z*)-3-hexenal (>92.5% purity, (NEAT), Bedoukian Research Inc, USA), 10% (*Z*)-3-hexenol (>98% GC, Sigma-Aldrich, Buchs, Switzerland), 8% (*Z*)-3-hexenyl-acetate (>98%, SAFC Supply Solutions, St. Louis, MO, USA), and 2% (*E*)-2-hexenal (>99%, ACROS Organics, New Jersey, USA). The vials were closed with open screw caps that contained a rubber septum. The rubber septa were pierced with a 2 $\mu$ l microcapillary (Drummond) (Figure I.1) and the vials were then wrapped in aluminium foil for heat-protection and to avoid photodegradation of the GLVs. Each such dispenser was then attached to a 50 cm long metal wire, which was stuck into the soil next to an assigned maize plant in the experimental plots, placing the microcapillary close to the whorl of the plant. In control plots, "visual" controls, i.e. aluminium foil wrapped to mimic the aluminium around the GLV-dispenser, were placed similarly as in the plots with GLV-dispensers. In separate experiments, the amounts of GLVs emitted by the dispensers were measured in the laboratory and in the field (Figure I.1). Variations in release rates between summer 2008 and win-

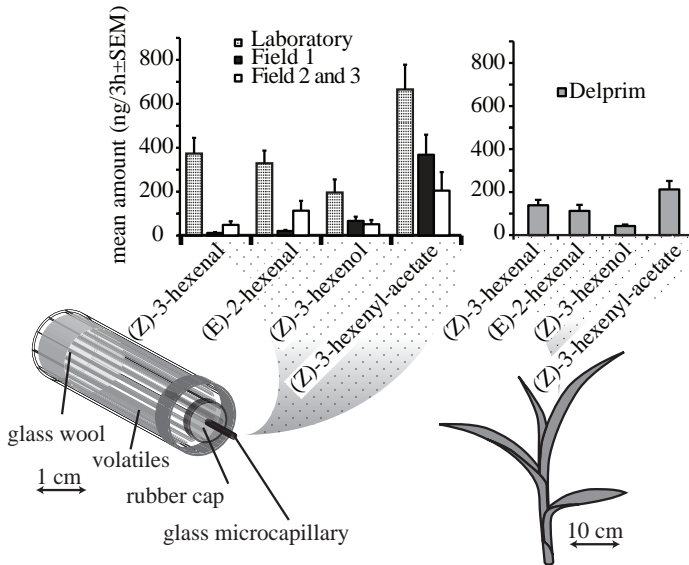


Figure I.1: Schematic of the VOC dispenser and amounts of green leaf volatiles emitted in the laboratory and in the field (summer 2008 and winter 2009). Delprim maize plants emitted comparable amounts.

ter 2009 can be partly explained by differences in temperatures (maxima  $34\pm 1^\circ\text{C}$  in summer 2008 vs.  $28\pm 1^\circ\text{C}$  in winter 2009; minima  $25\pm 1^\circ\text{C}$  in summer 2008 and  $15\pm 1^\circ\text{C}$  in winter 2009 and ).

To confirm that GLVs released from the dispenser prime maize plants, we performed a laboratory experiment with young maize plants under standardised conditions. Nine-day-old maize plants (cv Delprim) were exposed to GLV-dispensers (see above) for 16 h inside a glass bottle with purified, humid air flowing through at a rate of 0.3 l/min passing. After this exposure, the plants were removed from the glass bottles, damaged mechanically and  $10\ \mu\text{l}$  *S. littoralis* regurgitant was applied to the wounds (for details, see Turlings et al., 1998). The plants were then placed in clean glass bottles, with an air flow of 1.2 l/min. VOCs were sampled and analysed as described by Ton et al. (2007) with a sampling time of 2.5 hours at four time-points: 0-2.5h, 2.5-5.0h, 5.0-7.5h, and 24.0-26.5h. Control plants were not exposed to GLVs, but otherwise were treated and sampled similarly.

## I. 2.2 Volatile collection in the field

To collect VOCs from individual plants, maize plants were covered with a 50 cm Nalophan sleeve (Omya AG, Oftringen, Switzerland, 150 mm diameter). At the bottom, the sleeve was closed with a plastic seal below the oldest leaf and at the top it was attached to a metal wire, to prevent mechanical damage due to wind moving the sleeve. A tubular glass device (23 x 17 x 12 mm) with a screw cap was attached to the bottom of the bag (as described by Turlings et al., 1998). Through this device a SuperQ filter (Analytical Research Systems, Inc., Gainesville FL, USA) was inserted into the bag. Air was pulled through the filter tube with the use of an air-sampling pump (SKC 222 series, Blanc Labo S.A., Lonay, Switzerland) for 3 hours at 0.6 l/min. For each of 12 replications, VOCs were collected simultaneously from a GLV-exposed plant and from a control plant. Plants were checked for presence of herbivores before placing them inside the Nalophan sleeve. The VOCs were eluted from the filters and analyzed as described by D'Alessandro & Turlings (2005).

## I. 2.3 Maize fields and plants

Maize plots were planted with the variety "Tuxpeño Sequía" at the Ernest W. Sprague research station in Agua Fría, in the state of Puebla, Mexico (20°26'56.93"N, 97°38'23.98" W, 98 masl). The fields were sown on three dates (16th June 2008, 6th February 2009, and 11th February 2009) in 30 rows of 25 meters length, 70 cm between rows and 20 cm between plants within the rows. The field was divided into 12 plots of 10 rows by 4 meters, leaving 2 meters as a buffer between plots within the same row (Figure I.2). Three to four days after germination (V2-V3 leaf stage, collar of the second-third leaf visible), plants were counted and checked for insect damage, and then exposed to GLV dispensers. The dispensers were placed next to plants in the plot as indicated in Figure I.2.

## I. 2.4 Herbivores and herbivore damage

Herbivores and herbivore damage were measured at different distances from the dispensers. In field 1, two distances were sampled (<0.1m and 0.1-1.0m). In field 2 and 3, three distances were considered (<0.1m, 0.1-0.3m, and 0.3-1.0m). Herbivore damage was measured by rating the feeding damage on the leaves by the chrysomelid adults *Diabrotica* spp. and

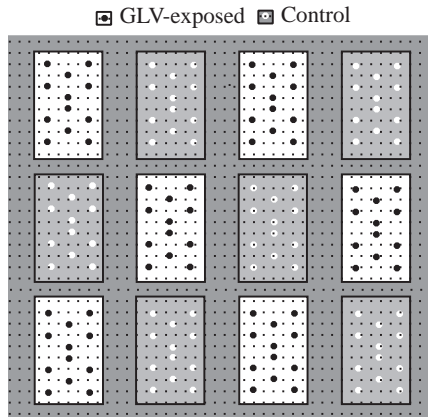


Figure I.2: Design of a field experiment. The field was divided in 4 m x 10 rows-sized plots. White squares were plants exposed to GLVs. Grey squares were control plants. Big black dots represent plants directly exposed to GLV dispensers. Big white dots represent plants exposed to empty control dispensers. Small black dots represent plants that were not exposed directly to dispensers. The distance between rows was 70 cm. Length of rows with in each square (plot) was 4 meters. Plants outside the squares served as a buffer between plots and were not considered in the experiment. Three such field experiments were conducted at different time points.

flea beetles (*Chaetocnema* spp.), and by *S. frugiperda* on maize plants, using a scale from 0 to 5 (0 = absence of feeding, 1 = few small feeding spots, 2 = several feeding spots, 3 = spread feeding spots, 4 = severely damaged plant, 5 = dead plant) (after Wiseman et al., 1966; Montes et al., 1996). At the same time, herbivore numbers of these insects, of aphids (Sternorrhyncha: Aphidoidea) and of planthoppers (Hemiptera: Fulgoromorpha) present on the plants were recorded. The field plants were scored for insect damage and numbers of shoot herbivores in fields 2 and 3. Scorings and counts were performed on two days (day 5 and day 8 after placing dispensers). Finally, the plants were dissected in the field on day 12-13 after placing the volatile dispensers, and all caterpillars were retrieved from the plants. The caterpillars were counted and placed individually in single compartments of 24-well ELISA plates (Jourdie et al., 2008) containing artificial, maize-based diet (Hoballah et al., 2004). In addition, we recorded the number of plants that were infested by *S. frugiperda*, by *Diatraea saccharalis* (Lepidoptera: Pyralidae), or by both of these herbivores. These were the only two Lepidoptera species found on the maize plants of that developmental stage in the field.

## I. 2.5 Parasitoids

Caterpillars of *S. frugiperda* were collected in the field 10 to 13 days after the plants were first exposed to the GLV dispensers. The insects were collected and reared separately, depending on the distance from the GLV dispenser, as described above. The collected caterpillars were placed on a maize-based diet (Hoballah et al. 2004) in 24-well ELISA plates (Jourdie et al., 2008) and reared until adult moths emerged or the parasitoid larvae emerged from their hosts. All parasitoid larvae and pupae were removed, placed in a Petri dish and reared out to adulthood. Parasitoids were identified using an identification manual (Cave, 1995).

## I. 2.6 Predation

Predation was measured in a separate field experiment with potted maize plants. For this purpose, 36 potted maize plants (c.v. Tuxpeño Sequía) were sown in 10 l plastic pots in the greenhouse. At the four-leaf stage, they were placed in a maize field with distances of 2 meters between pots. 18 plants were exposed to GLV dispensers and 18 to an empty control

dispenser, and placed in pairs on the border of a field, each pair consisting of two GLV-exposed plants, or two control plants. All plants were then infested with  $45 \pm 5$  first instar *S. frugiperda* larvae, mixed with grits (Wiseman et al., 1980). One plant of each pair was covered with an insect-proof net of 1 mm nylon sleeve, analogous to predation studies in wheat (Schmidt et al., 2003). On top, the net was tied close and attached to a stake and on the bottom it was fastened to the pot with a rubber band, to prevent soil-born predators from entering. After a period of 14 days, the sleeves were removed; the caterpillars were then retrieved and counted on each plant. Predation was calculated as the ratio (%) of retrieved larvae in uncovered plants to larvae retrieved in covered plants.

## I. 2.7 Statistical analysis

All the statistical analyses in this study were performed using the R programming environment (R Development Core Team, 2010). Laboratory volatile data were analysed using  $F$ -statistics in one-way analysis of variance (ANOVA). Field volatile data were analysed using pairwise  $t$ -test for each treatment and its respective control (paired by sampling day), provided the assumptions of normality and homogeneity of variances were met. The Wilcoxon signed rank test was applied when uniformity of variances was not met.

The herbivory data (*Diabrotica* spp. adults feeding scores and insect number per plant, aphid number per plant, planthopper number per plant, and *S. frugiperda* damage score) were analysed in a linear model with the effect of the field, the effect of the scoring day, the effect of the dispenser and the effect of the distance from the dispenser. For each distance (<0.1m, 0.1-0.3m, and 0.3-1.0m) and time-point (two and three), the data was then compared with one-way analysis of variance (ANOVA).

The number of herbivores were tested using the Kruskal-Wallis rank sum test if assumptions were not met, else they were analysed using ANOVA. The herbivore infestation data were analysed as binomial data in a generalised linear model (GLM), where plants are either infested or not. However, overdispersed data required to adapt the statistical method, as suggested by (Verdon et al., 2007). The difference between fields and between scoring days was tested with a quasi-binomial distribution in a GLM.

Parasitisation data were analysed in a similar way as for the herbivore presence data in a GLM with quasi-binomial distribution, *S. frugiperda* larvae being either parasitized or not. Predation data were analysed in a pair-wise  $t$ -test, comparing the ratio of the number of larvae recovered

from uncovered plants and the number of larvae recovered from covered plants, in GLV-exposed and control plants.

## I. 3 Results

### I. 3.1 VOC emission by GLV-exposed plants in the laboratory

In general, GLV-exposed maize plants emitted larger amounts of VOCs than maize plants not exposed to GLVs (Figure I.3) and the detected VOCs differed between the different sampling periods after induction. At sampling period 0.0-2.5 h the main VOCs found were GLVs. However these were not taken into account, as we could not distinguish between the GLVs emitted by the dispenser from the GLVs emitted by the maize plant. At this time-point, the other VOCs were emitted in slightly higher quantities by GLV-exposed plants, but without statistical difference. At time point 2.5-5.0 h, GLV-exposed maize plants emitted slightly larger amounts of sesquiterpenes. At time-point 5.0-7.5 h, sesquiterpenes were emitted at significantly higher levels ( $F_{1,21} = 4.34$ ,  $P < 0.049$ ), particularly (*E*)- $\beta$ -farnesene ( $F_{1,21} = 4.88$ ,  $P < 0.039$ ). Small amounts of induced volatiles were still emitted at 24.0-26.5 h after mechanical induction and (*E*)- $\alpha$ -bergamotene was emitted in slightly larger amounts by GLV-exposed plants ( $F_{1,21} = 3.04$ ,  $P < 0.096$ ).

### I. 3.2 VOC emission by GLV-exposed plants in the field

In the field, sesquiterpenes were emitted in larger amounts by plants that were exposed to GLVs ( $152.41 \pm 94.08$  ng/3h), compared to non-exposed control plants ( $26.62 \pm 8.36$  ng/3h) (Wilcoxon-test:  $P < 0.047$ ). The emission of aromatic compounds ( $P < 0.194$ ), homoterpenes ( $P < 0.667$ ), and monoterpenes ( $P < 0.313$ ) did not differ significantly between GLV-exposed and control plants (Figure I.4).

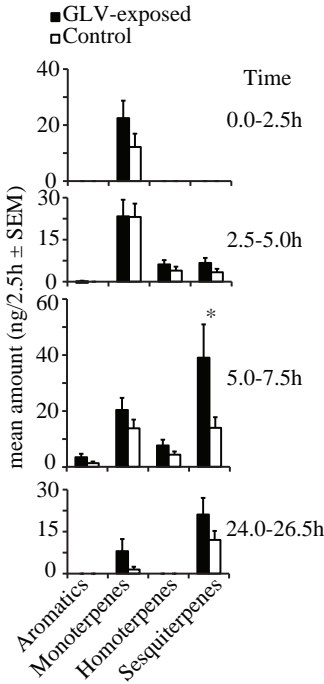


Figure I.3: Mean amount (ng/2.5h±SEM) of volatile compounds emitted by GLV-exposed and control maize plants (cv Delprim), upon mechanical damage. Emission was measured at four different sampling periods after induction (A: 0.0-2.5 h, B:2.5-5.0 h, C: 5.0-7.5 h, and D: 24.0-26.5 h). Data was analysed using one-way analysis of variance (ANOVA). One asterisk indicates statistical difference ( $P < 0.05$ ).

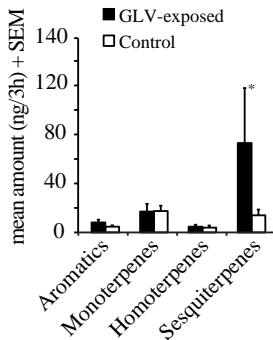


Figure I.4: Mean amount (ng/3h±SEM) of volatile compounds emitted by GLV-exposed and control maize plants (cv Tuxpeño Sequía) in the field. Data was analysed with Wilcoxon pair-wise comparison. One asterisk indicates significant difference  $P < 0.05$ .

### I. 3.3 Herbivore damage

Figure I.5 illustrates the damage caused by *S. frugiperda* and *Diabrotica*, the two most abundant herbivores in the experimental maize fields. In general there was only a tendency of higher damage by *S. frugiperda* in GLV-exposed plants compared to control plants. In particular, in field 2 this tendency towards a higher damage by *S. frugiperda* in GLV-exposed plants on the eighth day was almost significant ( $F_{1,430} = 2.802$ ,  $P < 0.095$ ) (Figure I.5 B). However, there was a significant difference between distances, compared to the plant at  $< 0.1\text{m}$  from the GLV-dispenser ( $0.1\text{-}0.3\text{m}$ :  $t = -2.132$ ,  $P < 0.033$  and at  $0.3\text{-}1.0\text{m}$ :  $t = -1.920$ ,  $P < 0.055$ ) and there were also significant field ( $t = -9.744$ ,  $P < 0.001$ ) and sampling day effects ( $t = 9.188$ ,  $P < 0.001$ ) and their interaction was also significant ( $t = -2.156$ ,  $P < 0.031$ ). But there was no overall difference between GLV-exposed and control plants ( $t = 1.10$ ,  $P < 0.2715$ ).

Conversely, *Diabrotica* spp. damage was significantly increased in GLV-exposed, compared to control plants ( $t = 4.740$ ,  $P < 0.001$ ). When comparing each group of plants following their distance from the GLV-dispenser, *Diabrotica* damage was generally higher on GLV-exposed plants (Figure I.5 E to H). In field 2, five days after placing the dispensers (Figure I.5 E), there was a trend ( $F_{1,424} = 3.32$ ,  $P < 0.07$ ) towards more damage on GLV-exposed plants than on control plants. The main difference was observed at  $0.3\text{-}1.0\text{ m}$  distance from the dispenser ( $F_{1,141} = 7.1485$ ,  $P < 0.008$ ). After eight days (Figure I.5 F), the overall difference in *Diabrotica* spp. damage scores between GLV-exposed and control plants was stronger ( $F_{1,424} = 6.27$ ,  $P < 0.013$ ), mainly due to higher scores for GLV-exposed plants at  $< 0,1\text{m}$  ( $F_{1,70} = 4.26$ ,  $P < 0.043$ ). A similar overall effect was observed in the third field. After five days (Figure I.5 G), significantly more *Diabrotica* spp. damage was observed on plants exposed to dispensers ( $F_{1,142} = 4.25$ ,  $P < 0.041$ ). After eight days (Figure I.5 H), the difference between GLV-exposed and control plants had increased ( $F_{1,430} = 11.70$ ,  $P < 0.001$ ), mainly at  $< 0.1\text{ m}$  ( $F_{1,142} = 6.76$ ,  $P < 0.01$ ) and  $0.3\text{-}1.0\text{m}$  ( $F_{1,142} = 8.08$ ,  $P < 0.005$ ). However, there was no significant distance effect on *Diabrotica* spp. feeding damage, compared to the plants at  $< 0.1\text{m}$  from the GLV-dispenser ( $t = 1.534$ ,  $P < 0.125$  at  $0.1\text{-}0.3\text{m}$  and  $t = 0.467$ ,  $P < 0.641$  at  $0.3\text{-}1.0\text{m}$ ). The *Diabrotica* spp. feeding damage varied significantly between the three fields (multivariate linear model (lm):  $t = -12.43$ ,  $P < 0.001$ ) and sampling day ( $t = -4.145$ ,  $P < 0.001$ ). There was a significant interaction between fields and sampling day ( $t = 10.76$ ,  $P < 0.001$ ).

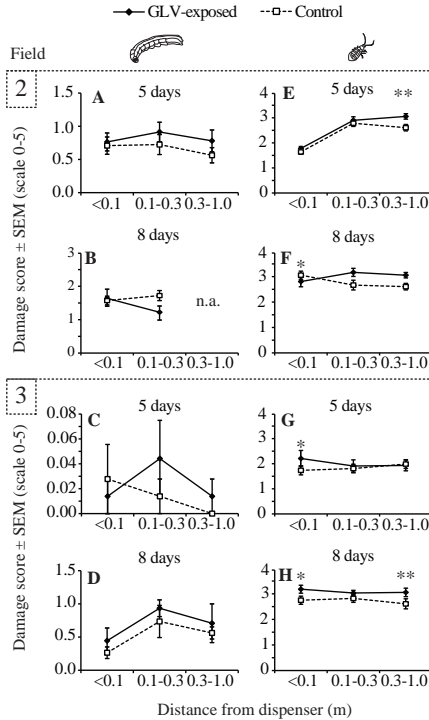


Figure I.5: Herbivore damage (scale 0-5) by *Diabrotica* spp. beetles (A, B, C, D) and *S. frugiperla* larvae (E, F, G, H) on GLV-exposed and Control maize plants. Herbivory was scored in two fields (2 and 3) five and eight days after placing the dispensers. Plants at <0.1m, 0.1-0.3m and 0.3-1.0m were score separately. Data were analysed with one-way analysis of variance (ANOVA). One asterisk indicates significant difference ( $P < 0.05$ ), two asterisks ( $P < 0.01$ )

### I. 3.4 Herbivore abundance

In field 1 (Figure I.6 A), there were significantly more *S. frugiperda*-infested plants in GLV-exposed plots at a distance of 0.1 to 1.0 meters (GLM,  $P < 0.042$ ), than in control plots. This increased infestation was not observed in field 2 (Figure I.6 B) or 3 (Figure I.6 C). The infestation rate (number of insects per plant) of *S. frugiperda* varied significantly between the three fields (Figure I.6 A to C). In field 2, the infestation rate was significantly higher than in the two other fields (GLM,  $P < 0.009$ ), but there was no difference between field 1 and field 3 (GLM,  $P < 0.128$ ).

The tendency towards higher *Diabrotica* spp. damage in GLV-exposed plants was also observed when counting the adults on the plants (Figure I.6 D to G). There were significantly more *Diabrotica* spp. adults on GLV-exposed plants than in control plants ( $t = 3.658$ ,  $P < 0.001$ ). In field 2, five days after placing the dispensers (Figure I.6 D), there was an overall trend towards more *Diabrotica* beetles on GLV-exposed plants than in control plants ( $F_{1,424} = 2.79$ ,  $P < 0.096$ ). This difference was most evident at 0.1-0.3m distance from the dispenser ( $F_{1,137} = 4.05$ ,  $P < 0.046$ ). Eight days after placing the dispensers (Figure I.6 E), the difference in numbers of *Diabrotica* increased. There were significantly more adults on GLV-exposed plants than on control plants ( $F_{1,212} = 7.49$ ,  $P < 0.007$ ), particularly at  $< 0.1$  m ( $F_{1,70} = 6.60$ ,  $P < 0.012$ ). In field 3, the effect of the GLVs on the number of *Diabrotica* was slightly lower. Five days after placing the dispensers (Figure I.6 F), a difference could be observed at  $< 0.1$  m ( $F_{1,142} = 5.49$ ,  $P < 0.020$ ). After eight days (Figure I.6 G), there was a trend towards more *Diabrotica* beetles on GLV-exposed than on control plants ( $F_{1,430} = 3.033$ ,  $P < 0.082$ ), particularly at 0.1-0.3m from the dispenser ( $F_{1,142} = 4.22$ ,  $P < 0.042$ ). Overall, the distance had also a significant effect on the number of *Diabrotica*, compared with the plants at  $< 0.1$  m from the dispenser ( $t = 2.861$ ,  $P < 0.004$  for 0.1-0.3m and  $t = 5.221$ ,  $P < 0.001$  for 0.3-1.0m). The field had also a significant effect ( $t = -6.428$ ,  $P < 0.001$ ).

In fields 2 and 3, we also counted aphids and planthoppers (Figure I.6 H to K). However, they were present only five days after placing the dispenser, therefore, in the linear model, the factors scoring day and the interaction (scoring day x field) were removed. The exposure to GLVs tended to increase the number of aphids ( $t = 1.894$ ,  $P < 0.059$ ). Indeed, in field 2, five days after placing the dispensers (Figure I.6 H), an overall trend towards more aphids on plants with dispensers could be observed ( $F_{1,424} = 3.47$ ,  $P < 0.063$ ). In field 3 (Figure I.6 I), this trend was only observed at a distance of 0.3-1.0m from the dispenser ( $F_{1,142} = 2.78$ ,  $P < 0.097$ ). There

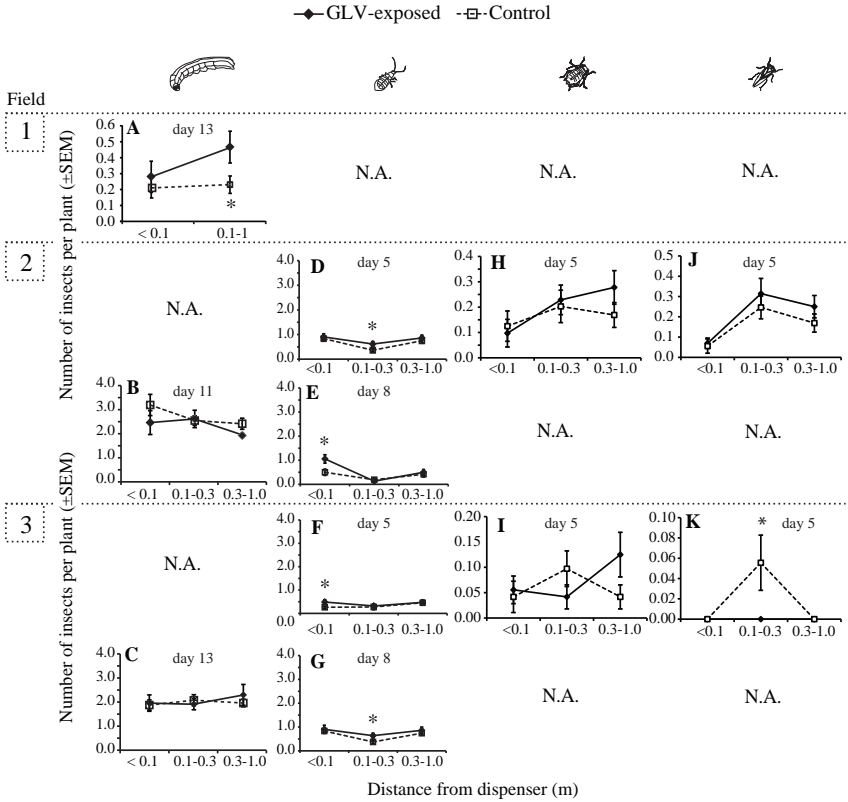


Figure I.6: Number of herbivores on GLV-exposed and control plants. Insects were counted in three fields. *S. frugiperda* (A, B, C) were counted ten to thirteen days after placing the dispensers in all fields. *Diabrotica* beetles (D, E, F, G) were counted in field 2 and 3, five and eight days after placing the dispensers. Aphids (H and J) and planthoppers (J and K) were counted in field 2 and 3 five days after placing the dispensers. Insects were counted separately on plants placed <0.1, 0.1-0.3, 0.3-1.0m from the dispenser, except for field 1, where only two distances were collected separately. Data were analysed with one-way analysis of variance (ANOVA). One asterisk indicates significant difference ( $P < 0.05$ ), two asterisks ( $P < 0.01$ ).

was a significant difference between fields in the number of aphids counted on the plants ( $t = -5.698$ ,  $P < 0.001$ ). Planthoppers were also counted only on day five (Figure I.6 J and K). In field 3, more planthoppers were found on control plants than on GLV-exposed plants at 0.1-0.3m ( $F_{1,142} = 4.17$ ,  $P < 0.042$ ) There were significantly more planthoppers found in field 2 than in field 3 ( $t = -7.857$ ,  $P < 0.001$ )

### I. 3.5 Parasitism

Overall, *S. frugiperda* was parasitized by seven species of parasitoids (Table I.1). Two egg-larval parasitoids, *Chelonus insularis* (Cresson) (Hymenoptera: Braconidae) and *Chelonus cautus* (Cresson) (Hymenoptera: Braconidae), and five larval parasitoids, *Eiphosoma vitticolle* (Cresson) (Hymenoptera: Ichneumonidae), *Cotesia marginiventris* (Cresson) (Hymenoptera: Braconidae), *Campoletis sonorensis* (Cameron) (Hymenoptera: Ichneumonidae), *Pristomerus spinator* (Fabricius) (Hymenoptera: Ichneumonidae). Additionally, the species *Aleiodes laphygmae* (Viereck) (Hymenoptera: Braconidae) and Tachinidae were found only in summer 2008 (Field 1). In field 3 the parasitism by *C. cautus* (GLM,  $P < 0.027$ ) was significantly lower on GLV-exposed than on control plants, whereas *C. insularis* tended to be more attracted by GLV-exposed plants than by control plants at a distance of 0.1-0.3m (GLM,  $P < 0.082$ ). Unfortunately, due to a high mortality of the parasitoid larvae collected in field 1, only few individuals of *Pristomerus spinator*, *Eiphosoma vitticolle*, Tachinidae, and *Aleiodes* spp. could be identified from this field and therefore they are not included in Table I.1.

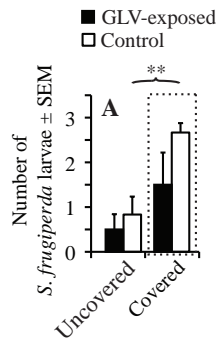
### I. 3.6 Predation

On plants that were covered by a net, the average number of recollected caterpillars after 12 days was  $2.67 \pm 0.21$  for control and  $1.50 \pm 0.72$  for GLV-treated plants ( $V = 13.5$ ,  $P < 0.134$ ) (Figure I.7). For plants that were left uncovered, the average number of recollected caterpillars was  $0.8 \pm 0.40$  for control plants and  $0.50 \pm 0.34$  for GLV-exposed plants (Wilcoxon signed rank test:  $V = 4$ ,  $P < 0.773$ ). There were significantly less *S. frugiperda* larvae recovered on uncovered control plants than on covered control plants ( $P < 0.007$ ). However, there was no difference between uncovered and covered GLV-exposed plants ( $P < 0.276$ ). Thus, the predation rate of *S. frugiperda* caterpillars was estimated at  $69.4 \pm 13.9\%$  for control plants and  $66.7 \pm 33.3\%$  for GLV-exposed plants.

Table I.1: Parasitism of *Spodoptera frugiperda* caterpillars (number of parasitoids retrieved per collected *S. frugiperda*) in %. Parasitoids hatched from caterpillars collected from GLV-exposed and control plants depending on the distance from the dispenser (<0.1m, 0.1-0.3m and 0.3-1.0m). An asterisk indicates a significant difference ( $P<0.05$ ), GLM with quasi-binomial correction, a dot indicates a statistical trend ( $P<0.10$ ).

	Field 2		Field 3	
	GLV-exposed ±SEM	Control ±SEM	GLV-exposed ±SEM	Control ±SEM
Total Parasitism	37.14±4.79	36.98±2.73	38.58±4.66	37.86±5.18
<0.1 m	42.37±10.41	42.04±2.40	45.21±4.53	50.43±9.65
0.1-0.3m	41.25±5.74	37.83±6.01	43.99±9.43	37.01±11.43
0.3-1.0m	35.25±4.86	35.32±2.04	32.46±4.87	34.94±5.47
<i>Chelonus insularis</i>	26.47±3.56	27.23±2.97	24.18±2.86	21.70±3.61
<0.1 m	28.59±7.61	32.36±3.59	29.61±3.85	31.48±6.93
0.1-0.3m	33.53±3.31	27.03±7.27	<b>29.04±5.37</b>	<b>20.57±4.89</b> •
0.3-1.0 m	23.40±3.89	25.71±1.95	19.58±4.03	20.53±4.81
<i>Ephosoma vitticollis</i>	7.05±0.92	6.36±0.65	6.35±1.49	7.17±1.29
<0.1 m	11.03±3.97	5.75±2.39	8.14±2.82	10.03±3.92
0.1-0.3m	3.90±1.56	6.55±2.03	6.08±2.39	7.86±3.48
0.3-1.0 m	8.03±0.96	6.32±0.78	5.43±1.33	5.84±2.72
<i>Pristomerus spinator</i>	1.44±0.69	0.90±0.31	5.38±1.63	4.79±1.89
<0.1 m	2.08±2.08	0.72±0.72	4.42±2.49	0.00±0.00
0.1-0.3m	0.93±0.93	1.19±0.78	6.62±3.71	8.58±4.18
0.3-1.0 m	1.50±0.53	0.93±0.34	5.50±1.57	3.31±1.72
<i>Campoletis sonorensis</i>	1.05±0.54	1.40±0.41	2.36±0.77	2.76±1.42
<0.1 m	0.67±0.67	0.54±0.54	3.05±2.34	4.75±2.23
0.1-0.3m	1.43±1.06	1.87±0.86	2.24±1.65	0.00±0.00
0.3-1.0 m	0.85±0.38	1.52±0.58	1.44±0.77	3.81±1.85
<i>Chelonus cautus</i>	0.28±0.19	0.56±0.46	<b>0.31±0.31</b>	<b>1.45±1.45</b> *
<0.1 m	1.39±1.39	0.60±0.60	0.00±0.00	4.17±4.17
0.1-0.3m	0.00±0.00	1.11±0.71	0.00±0.00	0.00±0.00
0.3-1.0 m	0.17±0.17	0.42±0.42	0.51±0.51	1.45±1.45
<i>Cotesia marginiventris</i>	1.01±0.47	0.83±0.22	0.00±0.00	0.00±0.00
<0.1 m	0.00±0.00	2.68±2.05	0.00±0.00	0.00±0.00
0.1-0.3m	1.11±1.11	0.48±0.48	0.00±0.00	0.00±0.00
0.3-1.0 m	1.48±0.81	0.85±0.33	0.00±0.00	0.00±0.00

Figure I.7:  
 Number of *S. frugiperda* larvae  $\pm$  SEM retrieved from uncovered and netted plants in GLV-exposed and control plants 12 days after artificial infestation with first-instar larvae.



## I. 4 Discussion

In this study we investigated whether applying synthetic GLVs in a maize field would improve the resistance against and improve the biological control of insect pests. In the laboratory we found that exposing maize plants to synthetic GLVs resulted in a stronger and faster emission of sesquiterpenes upon subsequent induction of the VOC-emission in these plants compared to plants that were not exposed the GLVs (Figure I.3). These results confirm findings from earlier studies showing that exposure to GLVs enhances the inducible emission of VOCs in plants (Arimura et al., 2001; Engelberth et al., 2004; Frost et al., 2008). This so-called priming effect (Ton et al., 2007) might also be important in the field, as insect-damaged maize plants emitted higher amounts of sesquiterpenes after exposure to synthetic GLVs (Figure I.4). However, in the field plants were exposed to various extreme biotic and abiotic stresses, including herbivore damage, immediately after germination. Therefore the higher emission of sesquiterpenes in the field was probably not just the result of priming but rather a synergistic effect of GLVs on the emission of these compounds. Indeed, earlier studies showed that GLVs directly affect the emission of sesquiterpenes (Farag et al., 2005; Ruther & Furstenuau, 2005; Ruther & Kleier, 2005). Nevertheless, our study appears to be the first field study to show that physiological realistic concentrations of GLVs (Figure I.1) are sufficient to alter the VOC emission in GLV-exposed plants.

Based on a correlation between sesquiterpene emission and jasmonic acid-related defence mechanisms (Schmelz et al., 2003) we hypothesised that plants with increased sesquiterpenes emission also better resist herbivore attack. This can certainly not be concluded from our field experiments. On

the contrary, plants that were exposed to GLVs generally suffered increased herbivore damage (Figure I.5) and higher numbers of several herbivores were counted on these plants (Figure I.6), as compared to control plants. This is not surprising for Coleoptera, which are known to be commonly attracted to herbivore-induced volatiles (Halitschke et al., 2008; Loughrin et al., 1995; Bolter et al., 1997). Indeed, the increased herbivore presence and herbivory was most significant for *Diabrotica* beetles, which is in agreement with evidence that they are directly attracted to sesquiterpenes (Hammack, 2001). Adults of lepidopteran species, however, are usually repelled by induced VOCs (De Moraes et al., 2001; Landolt, 1993; Huang et al., 2009). This is in contrast with our results, as the infestation by *S. frugiperda* was somewhat higher on GLV-exposed maize plants. This was even more surprising as cage experiments (De Moraes et al., 2001) specifically showed that *S. frugiperda* females are repelled by maize VOCs induced by conspecific larvae. Yet, *S. frugiperda* larvae are attracted to such VOCs (Carroll et al., 2006), which could be one explanation for the field results. For other herbivores, such as aphids and planthoppers, there was no clear effect of GLV exposure. However, in other laboratory studies these piercing-sucking insects were less present on the exposed plants (Bernasconi et al., 1998).

On the other hand, based on evidence from laboratory studies that GLVs are important for parasitoid attraction (Hoballah & Turlings, 2005) we had expected higher parasitisation rates of *S. frugiperda*. Almost 40% of the collected *S. frugiperda* larvae were parasitized by several parasitoids, including *C. insularis*, *E. vitticolle*, *P. spinator*, *C. sonorensis*, *C. cautus*, and *C. marginiventris* (Table I.1), confirming earlier studies that parasitism of this pest is high in the subtropical area of Mexico (Hoballah et al., 2004; Jourdie et al., 2008; Molina-Ochoa et al., 2003, 2004). However, the release of synthetic GLVs did not increase parasitism and the number of some parasitoids, such as the egg-larval parasitoid *C. cautus*, was even reduced in caterpillars collected from GLV-exposed plants. The addition of GLVs also resulted in a higher emission of sesquiterpenes, which might partially explain why there were rather lower numbers of parasitoids in larvae collected in GLV-exposed plants. In fact, in the laboratory we found that the innate attraction of two parasitoids of *Spodoptera* species is stronger to blends with low terpenoid emissions (D'Alessandro & Turlings, 2005; D'Alessandro et al., 2009), and only after the wasps associate terpenoids with host presence do these compounds become important as attractants (Schnee et al., 2006). Moreover, in this study the main parasitoid was the egg-larval parasitoid *C. insularis*. GLVs and other volatiles that are associated with herbivory are less likely to attract parasitoids in search of

insect eggs and may even be repellent as indicated by the lower numbers of another egg-larval parasitoid, *C. cautus* recovered from larvae on exposed plants.

It should be noted that the caterpillars were collected irrespective of their developmental stage, with a large portion of early developmental stages, i.e. first or second instar. Early stages can only be parasitized by a restricted number of species and at a later stage could have been parasitized by other primary parasitoids and/or hyperparasitoids (Marktl et al., 2002; McDonald & Kok, 1991; Tamò et al., 2006). If we had collected more larvae at later stage this would have resulted in a different parasitoid species composition.

Moreover, from the additional experiment with potted maize plants we estimated that as much as two thirds of the caterpillars on a maize plant may be lost through predation (Figure I.7). There was no significant effect of synthetic GLVs on predation rate, despite the fact that predator attraction in the field can be enhanced with synthetic VOCs (James, 2003b).

As can be expected, the effects of the GLV exposure on herbivory and parasitisation depended on the distance of the plants to the dispenser. Parasitoids (Bruinsma et al., 2009; Geervliet et al., 1998; Turlings et al., 2004, 1991; Whitman & Eller, 1992) and herbivorous arthropods (de Bruyne & Baker, 2008; Pophof et al., 2005; Yang et al., 2004) respond to chemical cues in a dose dependent manner. At high concentrations a foraging cue that normally is an attractant can even become a repellent. Since the GLV exposure affected the emission of several VOCs that might be involved in the insects' foraging behaviour, the effects on attraction and repellency might have been blurred.

In conclusion, based on the results of this study the application of synthetic GLVs seems not to be suitable, neither to enhance resistance of maize plants against herbivores nor to improve biological control. This conclusion contrasts with fields studies that demonstrated an increased attraction of both parasitoids and predatory insects in different crop fields treated with GLVs (James, 2003a; James & Price, 2004) or that imply that GLVs are sufficient to improve resistance against herbivores and pathogens (Shiojiri et al., 2006). The results of this study confirm that the application of artificial VOCs also affects the emission of the VOCs of the exposed plants. It is important that such physiological changes in the crop plants be considered in studies on the potential of using VOCs to attract biological control agent.

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# II

## **Effects of two elicitors of insect and pathogen resistance on the volatile emission and the biological control of *Spodoptera frugiperda* in maize fields.**

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**Abstract** Synthetic elicitors can be used to induce resistance in plants against pathogens and arthropod herbivores. Such compounds may also change the emission of herbivore-induced plant volatiles, which are important cues for parasitic wasps to locate their hosts. Therefore, the use of elicitors in the field may affect biological control of insect pests. To test this hypothesis, we treated maize seedlings growing in a subtropical field in Mexico with methyl jasmonate (MeJA), an elicitor of defense responses against many insects, and benzo-(1,2,3)-thiadiazole-7-carbothioic acid S-methyl ester (BTH), an elicitor of resistance against certain pathogens. Volatile emission, herbivore infestation, pathogen infection, and plant performance (growth and grain yield) of treated and untreated maize plants were measured. Application of BTH slightly reduced volatile emission in maize, while MeJA increased the emission compared to control treatments. The elicitors did not consistently affect the number of plants infested by *Spodoptera frugiperda* larvae, the main insect pest found on the maize seedlings, and had only marginal effects on parasitism rates. Moreover, infestation of the maize plants by other herbivores and pathogens, as well as plant performance were similar for all treatments. By contrast, parasitism rates and the composition of parasitoid species differed significantly between summer and winter seasons. Severe biotic and abiotic stresses on young seedlings might explain why the elicitors had only slight effects on pest incidence and biological control in this specific field study. In another field experiment with milder and more standardized conditions, BTH induced significant resistance against *Bipolaris maydis*, a major pathogen in the experimental maize fields.

**Keywords** BTH, methyl jasmonate, *Zea mays*, *Spodoptera frugiperda*, *Bipolaris maydis*, parasitoids, induced plant defenses, systemic acquired resistance, conservation biological control

## II. 1 Introduction

Plants attacked by arthropod herbivores respond by activating a number of defense mechanisms, including the emission of volatile organic compounds (VOCs) that attract predatory and parasitic arthropods (Dicke, 2009; Dicke et al., 1990; Turlings et al., 1990; Turlings & Wäckers, 2004). In maize, these herbivore-induced plant volatiles comprise mainly green leaf volatiles (GLVs), mono-, homo- and sesquiterpenes, as well as aromatic compounds (D'Alessandro et al., 2006; Hoballah & Turlings, 2005; Paré & Tumlinson, 1999). The emission of most herbivore-induced plant volatiles involves a number of well-understood metabolic pathways, such as the jasmonic acid (JA) pathway, the shikimic acid/tryptophan pathway, the mevalonate pathway, as well as the lipoxygenase (LOX) pathway (Bruce & Pickett, 2007; Engelberth et al., 2007; Heil, 2010; Hilker & Meiners, 2010). Similarly, plants infected by fungal and bacterial pathogens respond with the activation of a number of defense mechanisms (van Loon et al., 2006; Walling, 2009), and for many pathogens, the plant hormone salicylic acid (SA) is involved in these defense responses (Bari & Jones, 2009). Resistance against pathogens as well as defenses against insects can be induced with a series of synthetic elicitors (Heil & Walters, 2009; Walling, 2009). Besides increasing the direct defenses in the plants, such treatments may also enhance the volatile defense signals. For instance, spraying plants with methyl jasmonate (MeJA), the volatile derivative of JA, induces the emission of volatiles that are also induced by herbivore feeding (Degenhardt & Lincoln, 2006; Heil & Walters, 2009; Ozawa et al., 2008; Zhang et al., 2009). Caterpillars near tomato plants that were treated with JA were found to have a higher rate of parasitism than caterpillars placed next to control plants, most probably because of the higher amounts of volatiles released by JA-treated plants (Thaler, 1999). Similarly, pathogen resistance can for example be induced by the elicitor benzo-(1,2,3)-thiadiazole-7-carbothioic acid S-methyl ester (BTH), which mimics the effects of salicylic acid and results in a reduction of the impact of several pathogens in different plant species by inducing systemic acquired resistance (SAR) (Friedrich et al., 1996; Görlach et al., 1996) but see (Heidel & Baldwin, 2004). BTH is commercially applied in poaceous and solanaceous crops as well as sunflowers and grapevine as a preventive measure against pathogen growth (Goellner & Conrath, 2008; Perazzolli et al., 2008; Vallad & Goodman, 2004). Interestingly, in many plant species there is crosstalk between the SA and the JA pathways, where the increased activity of one pathway diminishes the activity of the other (Kunkel & Brooks, 2002). Hence, the application of an elicitor to induce one of these two pathways might result in a sup-

pression of defense mechanisms depending on the other pathway (Beckers & Spoel, 2006; Thaler et al., 2002; Walters & Heil, 2007). There are exceptions (Boughton et al., 2006), and in general, herbivorous insects perform better on plants with an activated SA pathway (Rayapuram & Baldwin, 2007; Smith et al., 2009; Taylor et al., 2004; Thaler et al., 2002). However, this may be different if indirect defense signals and tritrophic interactions are also taken into account. In fact, a recent study (Rostás & Turlings, 2008) shows that treatment with BTH not only increases resistance to *Setosphaeria turcica* in maize, but also strongly enhances the attractiveness to the parasitoid *Microplitis rufiventris* (Kokujev) (Hymenoptera: Braconidae) if the treated plants are infested with hosts of the parasitoid, larvae of *Spodoptera littoralis* Boisduval (Lepidoptera: Noctuidae). Ongoing experiments indicate that other parasitoid species also show increased attraction to BTH-treated maize plants (I. Sobhy, personal communication). Hence, both the activation of the emission of volatile compounds and the pathogen-induced defenses could indirectly via the third trophic level enhance resistance against herbivores. The aim of this study was to assess whether treating maize plants with two potent elicitors, BTH and MeJA, affects their direct and indirect defenses against important pest species. Four experiments were conducted under field conditions and repeated at different time-points over the year in maize fields in the subtropical lowlands of Mexico to test effects of elicitors on volatile emission of maize plants, herbivory, parasitism, and plant performance.

## II. 2 Material and Methods

### II. 2.1 Maize fields

Seven field experiments (*Zea mays*, cv Tuxpeño Sequía) planted on six dates were established at the International Wheat and Maize Improvement Center (CIMMYT) experimental station near Agua Fría, Puebla state, México (20°26'56.93"N, 97°38'23.98" W, 98 msl). Two fields were planted in summer 2008 (replicate 1 on 9 June 2008 and replicate 2 on 16 June 2008) and four fields in winter 2009 (replicate 3 on 6 Feb. 2009, replicate 4 on 9 Feb. 2009, replicate 5 on 11 Feb. 2009 and replicate 6 on 13 Feb. 2009). A seventh field was planted on 6 Feb. 2009 to evaluate the treatment effects on disease resistance and kernel production. The fields were planted in 31 rows of 25 meters, with 20 cm distance between plants within the rows and 70 cm distance between rows (Figure II.1). They were

regularly irrigated with sprinklers 2-4 days after sowing, and with flooding from V2 developmental stage (collar of the second leaf visible) onward (Çakir, 2004). Neither seeds nor plants were treated with insecticides until the end of the experimental period. Each field was divided into plots that were used for the different treatments.

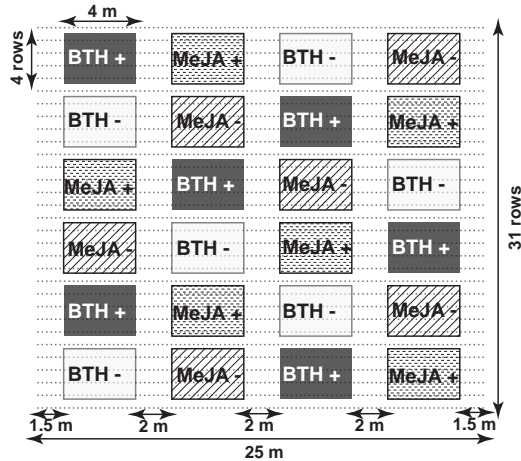


Figure II.1: Design of the field experiments. The field was 25 m by 21.5 m (31 rows 0.7m) wide. Each plot was 4 m by 4 rows, represented by a square. Dots represent the maize plants. On each side of the field, 1.5 m was left untreated. Between plots, 2 m and 1 row was left untreated. The treatments (BTH<sup>+</sup>, BTH<sup>-</sup>, MeJA<sup>+</sup> and MeJA<sup>-</sup>) were applied to young maize seedlings in evenly distributed plots. This treatment was applied on different fields on six occasions throughout the year.

## II. 2.2 Treatment of plants with elicitors

Four types of treatments (two elicitors and corresponding control sprays) were applied to plots of four meters length and four rows wide that were evenly distributed in the field. One meter on each side of the rows and one row between plots were left untreated, as buffer zones (see Figure II.1). For one of the treatments, plants were sprayed with 0.3 mM benzo (1, 2, 3) thiadiazole-7-carbothioic acid S-methyl ester (BTH) (Bion<sup>®</sup>, Syngenta Crop Protection, Pratteln, Switzerland) at V2-V3 developmental stage of the seedlings with 2 to 3 leaves present after the cotyledon (treat-

ment BTH<sup>+</sup>). The control (treatment BTH<sup>-</sup>) consisted of a similar treatment, but contained only wetting powder at 0.3 mM without the active ingredient BTH (Bion 50WG, Syngenta Crop Protection, Basel, Switzerland). For the second elicitor treatment, plants were sprayed with 5 mM methyl jasmonate in 0.5% ethanol solution (treatment MeJA<sup>+</sup>) or with 0.5% ethanol solution (treatment MeJA<sup>-</sup>) as a control treatment. The applied dosages were based on those used in previous studies (Boughton et al., 2005; Boughton et al., 2006) and initial laboratory studies confirmed their effectiveness (data not shown). Before the treatments, herbivore damage and presence (*Diabrotica* spp. (Coleoptera: Chrysomelidae), *Spodoptera frugiperda* (Lepidoptera: Noctuidae), aphids (Homoptera: Aphididae), as well as pathogen presence were checked for all plots.

## II. 2.3 Volatile collection and analysis (*Experiment 1*)

The plants were sampled three to four days after elicitor application. Plants were covered with a Nalophan sleeve (Omya AG, Oftringen, Switzerland) 150 mm caliber, 50 cm length. At the bottom, the sleeve was closed with a plastic seal below the oldest leaf and at the top it was attached to a metal wire, to prevent mechanical damage due to wind moving the sleeve. A tubular glass device (23 x 17 x 12 mm) with an open screw cap was attached to the bottom of the bag in order to insert a SuperQ filter (Analytical Research Systems, Inc., Gainesville FL, USA). Air was pulled through the filter tube with the use of an air-sampling pump (SKC 222 series, Blanc Labo S.A., Lonay, Switzerland) for 3 hours at 0.6 L/min. For each of the 12 replications, volatiles were collected simultaneously from a treated plant (BTH<sup>+</sup> or MeJA<sup>+</sup>) and its respective control (BTH<sup>-</sup> or MeJA<sup>-</sup>). The volatiles were then eluted from the filters and analyzed in GCMS, in accordance with D'Alessandro & Turlings (2005).

## II. 2.4 Herbivory and parasitism (*Experiment 2*)

Seven days after elicitor treatment, six to ten plants in each plot were rated for herbivore damage to the shoot, which could be assigned to different species. The feeding damage by *S. frugiperda* was rated on a scale from 0 to 5: 0 = absence of damage, 1 = very slight damage, 2 = slight damage, 3 = spread damage, 4 = severe damage and 5 = almost complete removal of all foliar tissue, based on other leaf damage studies (Kumar, 2002; Wiseman et al., 1966). A similar scale was used to assess the damage by adult

*Diabrotica* spp. beetles. In addition, while scoring the damage, the number of insect herbivores on each plant was counted. The herbivores that were observed included *Diabrotica* spp. adults, flea beetles (Coleoptera: Chrysomelidae: Alticini), aphids, leafhoppers (Hemiptera: Cicadellidae) and planthoppers (Hemiptera: Fulgoromorpha).

Ten to thirteen days after treatment all plants in the plot were cut off to count and collect the caterpillars feeding on them. The only two species found were *S. frugiperda* and *Diatraea saccharalis* (Fabricius) (Lepidoptera: Pyralidae). The number of plants that were infested by either of these species or by both was recorded. Subsequently all caterpillars were placed individually in single compartments of 24-well ELISA plates containing artificial maize-based diet (Hoballah et al., 2004) and reared at the CIMMYT's entomological facility in Texcoco de Mora, Mexico State, Mexico, under controlled conditions ( $27 \pm 2^\circ\text{C}$  and  $55 \pm 5\%$  R.H.) until adult emergence or, in the case of parasitism, emergence of parasitoids (Hoballah et al., 2004). The adult parasitoids as well as dead parasitoid larvae and cocoons were individually conserved in 90% ethanol in centrifuge tubes. Adult parasitoids were identified under a binocular with the help of a manual (Cave, 1995). Dead larvae and pupae of parasitoids were identified based on the adults that emerged from identical larvae and pupae.

### II. 2.5 Potted plant experiment (*Experiment 3*)

Maize plants (cv Tuxpeño Sequía) were sown on 16 June 2008, individually in black 10l PVC pots in an insect-proof green house, located next to the fields at the experimental field station. Fifteen maize plants per treatment were sprayed with the elicitors (MeJA<sup>+</sup> and BTH<sup>+</sup>) and their respective controls (BTH<sup>-</sup> and MeJA<sup>-</sup>), at the V3-V4 (three to four leaves after cotyledon) developmental stage. After 24 hours, potted maize plants were placed in a maize field with plants at a similar growth stage in groups of four with each treatment. To exclude any position effect, the position of each treatment was rotated between the groups. Groups were evenly distributed over the field with a distance of five meters between groups. Twenty days later, pathogen infestation and herbivore damage were rated. Pathogen infestation was assessed by counting the number of necroses on the most recent, fully developed leaf. In this experiment, herbivory damage was assessed on a scale based on Wiseman et al. (1966). Feeding by *Diabrotica* spp adults was rated using a scale from 0 to 10 (0 = no visible damage, 1 = few pinhole-type injuries, 2 = several small pinholes, 3 =

small amount of shot-hole type injury with few lesions, 4 = several shot-hole type injuries and few lesions, 5 = several lesions, 6 = several lesions, portions of plant eaten away, 7 = several lesions, portions of plant eaten away, few areas dying, 8 = several portions eaten away and areas dying, 9 = most of the leaves eaten away and more areas dying, 10 = plant dead or dying). Flea beetle damage (whitish stripes on leaves) was rated using the same scale. The caterpillars that could be retrieved from the plants were also counted. After pathogen and herbivory assessment, the plants were cut, placed individually in paper bags, heated in an oven at 80°C for 24±1 hours to measure dry weight.

## II. 2.6 Pathogen disease resistance and plant fitness (*Experiment 4*)

One field (25 meters by 30 rows) was planted and treated as described above. No insects were collected from these plants, and the plants were left in the field until maturity to assess kernel production and plant performance parameters. Seven days before harvest, pathogen infestation and disease symptoms were rated. The infection of the plants by the fungal pathogen *Bipolaris maydis* (Y. Nisik. & C. Miyake) Shoemaker (1959), which was the major pathogen found in the fields, was rated visually using a scale from 0 to 5: 0 (no spread), 1 (slight spread), 2 (clearly notable spread), 3 (wide spread), 4 (almost complete spread), 5 (heavy spread) as described by Sharma & Payak (1990). In addition, lodging and stunting of the plants was rated on a similar scale. At harvest, the number of cobs was recorded. Furthermore, we measured the weight of the harvested cobs and estimated the cob humidity by subtracting dry from fresh weight.

## II. 2.7 Statistical analysis

**Volatile emission.** Data of the volatile emissions were tested for homogeneity of variances (Levene test) and analyzed using a pair-wise comparison (*t*-test). Each treatment was compared with its control (i.e. BTH<sup>+</sup> vs BTH<sup>-</sup> and MeJA<sup>+</sup> vs MeJA). When the variances were not homogenous, the data were analyzed using the Wilcoxon pair-wise comparison.

**Herbivory and parasitism.** When comparing the number of herbivores, data were analyzed using analysis of variance (ANOVA) on the same factors. The data were analyzed using the Shapiro-Wilk test of normality.

If the dataset was not normally distributed, it was log-and square root-transformed. In cases where transformation did not improve the data, they were tested using the Kruskal-Wallis rank sum test, with the four treatments BTH<sup>+</sup>, BTH<sup>-</sup>, MeJA<sup>+</sup> and MeJA<sup>-</sup> as explanatory factors. Herbivore damage rating data were analyzed using ANOVA. The data were tested for normality with the Shapiro-Wilk test of normality. If the data were not normally distributed and data transformation did not improve normality, the data were analyzed using the Kruskal-Wallis rank sum test. Data of herbivore-infested plants was analyzed using a generalised linear model (GLM) with binomial distribution, where plants were either infested or not. However, overdispersion of the data required to adapt the statistical method, as suggested by Verdon et al. (2007). The difference between time-points was also tested using the quasi-binomial testing method. The parasitism data were analyzed in a similar way as for the herbivore presence data.

**Disease rating.** Estimates of disease spread were tested using ANOVA with the four treatments BTH<sup>+</sup>, BTH<sup>-</sup>, MeJA<sup>+</sup> and MeJA<sup>-</sup> as explanatory factors. The data were tested for normality using the Shapiro-Wilk test of normality. In cases where the data were not normally distributed, the data were tested using the Kruskal-Wallis rank sum test.

## II. 3 Results

### II. 3.1 Volatile emission (*Experiment 1*)

Maize plants in the field emitted several well-known herbivore-induced VOCs, including GLVs, such as (*Z*)-3-hexenyl acetate, aromatic compounds, such as phenethyl acetate and methyl salicylate, as well as a series of mono-, homo- and sesquiterpenes. Consistent with a previous laboratory study (Rostás & Turlings, 2008), plants treated with BTH (BTH<sup>+</sup>) tended to emit less homoterpenes, sesquiterpenes and aromatic compounds than control-treated plants (BTH<sup>-</sup>), but the differences in the amount of individual compounds were not statistically significant (Table II.1). By contrast, plants treated with MeJA (MeJA<sup>+</sup>) emitted higher amounts of most inducible compounds than the control treated plants (MeJA<sup>-</sup>). This effect was statistically significant for (*E*)- $\alpha$ -bergamotene ( $P < 0.047$ ) and (*E*)- $\beta$ -farnesene ( $P < 0.043$ ) (Table II.1).

Table II.1: Mean amount of VOCs ( $\pm$ SEM) emitted by maize plants treated with BTH<sup>+</sup> (n=12), BTH<sup>-</sup> (n=10), MeJA<sup>+</sup> (n=11) and MeJA<sup>-</sup> (n=12) in the field. Individual compounds are summed into major chemical groups of volatiles and their mean is indicated in bold. Data were analysed using pairwise Student's t-test. Significant differences and trends are in bold and an asterisk indicates significant difference ( $P < 0.05$ ), a large dot indicates a statistical trend ( $0.05 < P > 0.10$ ).

Volatile compounds $\pm$ SEM	BTH <sup>+</sup>	BTH <sup>-</sup>	MeJA <sup>+</sup>	MeJA <sup>-</sup>
1-( <i>Z</i> )-3-hexenal	1.16 $\pm$ 0.47	1.01 $\pm$ 0.52	4.84 $\pm$ 1.85	3.23 $\pm$ 1.45
( <i>E</i> )-2-hexenal	0.00 $\pm$ 0.00	0.00 $\pm$ 0.00	0.00 $\pm$ 0.00	5.41 $\pm$ 5.42
( <i>Z</i> )-3-hexenol	4.51 $\pm$ 3.01	3.04 $\pm$ 1.32	13.51 $\pm$ 6.49	8.89 $\pm$ 4.04
1-butanol, 3- methyl-, acetate <sup>a</sup>	2.49 $\pm$ 0.46	7.95 $\pm$ 2.89	3.45 $\pm$ 0.52	2.58 $\pm$ 0.60
( <i>Z</i> )-3-hexenyl acetate <sup>a</sup>	31.86 $\pm$ 9.48	30.48 $\pm$ 9.83	148.55 $\pm$ 58.88	62.99 $\pm$ 23.15
acetic acid hexyl ester	2.34 $\pm$ 0.49	3.23 $\pm$ 0.79	5.20 $\pm$ 1.13	3.83 $\pm$ 0.50
<b>GLVs</b>	42.35 $\pm$ 12.93	45.70 $\pm$ 14.38	175.57 $\pm$ 66.82	86.96 $\pm$ 29.89
$\alpha$ -pinene	1.18 $\pm$ 0.39	1.96 $\pm$ 0.88	2.03 $\pm$ 0.53	1.73 $\pm$ 0.56
$\beta$ -myrcene	2.88 $\pm$ 1.16	4.88 $\pm$ 2.92	4.49 $\pm$ 1.16	4.14 $\pm$ 1.41
( <i>E</i> )- $\beta$ -ocimene	12.00 $\pm$ 4.85	25.92 $\pm$ 15.62	16.66 $\pm$ 11.90	22.29 $\pm$ 9.69
linalool	22.68 $\pm$ 5.66	19.58 $\pm$ 6.47	34.47 $\pm$ 9.09	27.49 $\pm$ 9.98
<b>monoterpenes</b>	38.74 $\pm$ 9.36	52.35 $\pm$ 25.30	57.67 $\pm$ 19.76	55.67 $\pm$ 21.65
( <i>E</i> )-4,8-dimethyl- 1,3,7-nonatriene (DMNT)	14.44 $\pm$ 3.97	43.23 $\pm$ 26.09	59.24 $\pm$ 37.27	15.31 $\pm$ 7.06
( <i>E,E</i> )-4,8,12- trimethyl 1,3,7,11- tridecatetraene (TMTT)	<b>0.39<math>\pm</math>0.39</b>	<b>4.55<math>\pm</math>2.49</b> •	0.31 $\pm$ 0.25	0.95 $\pm$ 0.86
<b>homoterpenes</b>	14.82 $\pm$ 4.21	47.77 $\pm$ 28.29	59.56 $\pm$ 37.25	16.27 $\pm$ 7.11
cycloisativene	9.55 $\pm$ 3.42	10.09 $\pm$ 4.88	18.42 $\pm$ 7.07	9.38 $\pm$ 5.31
$\alpha$ -copaene	8.53 $\pm$ 4.02	17.64 $\pm$ 12.67	11.20 $\pm$ 3.82	4.52 $\pm$ 2.56
( <i>E</i> )- $\beta$ - caryophyllene	7.07 $\pm$ 2.98	19.29 $\pm$ 10.67	12.90 $\pm$ 3.82	8.58 $\pm$ 3.45
( <i>E</i> )- $\alpha$ -bergamotene	8.85 $\pm$ 4.33	27.28 $\pm$ 14.89	<b>11.74<math>\pm</math>3.58</b>	<b>4.07<math>\pm</math>1.144</b> *
( <i>E</i> )- $\beta$ -farnesene	5.68 $\pm$ 3.64	6.56 $\pm$ 3.91	<b>8.41<math>\pm</math>3.18</b>	<b>1.74<math>\pm</math>0.57</b> *
<b>sesquiterpenes</b>	39.29 $\pm$ 13.17	100.84 $\pm$ 44.58	<b>62.67<math>\pm</math>13.95</b>	<b>27.60<math>\pm</math>11.39</b> •
phenethyl acetate	1.20 $\pm$ 0.95	1.66 $\pm$ 0.71	2.22 $\pm$ 0.67	2.59 $\pm$ 0.97
methyl salicylate	2.30 $\pm$ 0.99	2.75 $\pm$ 1.02	1.34 $\pm$ 0.70	2.07 $\pm$ 0.81
<b>aromatics</b>	3.50 $\pm$ 1.25	4.41 $\pm$ 1.19	3.57 $\pm$ 1.19	4.45 $\pm$ 1.38

<sup>a</sup> Data did not fit with assumptions of equal variance between groups. Hence, a non-parametric test was applied (Wilcoxon pairwise comparison).

### II. 3.2 Herbivory (*Experiment 2*)

Before the treatment with elicitors, 22-25% of the plants were infested in all plots. Ten days after treatment, the number of infested plants did not differ between the treatments in the six replicates, with an average of 42.3% plants (Figure II.2). This similar infestation rate was also reflected in an equal damage by the larvae (0.40-0.58) ( $F_{3,92} = 0.23$ ,  $P < 0.874$ ) and in the number of *S. frugiperda* found per sampled plant (0.75-0.82) ( $F_{3,139} = 0.10$ ,  $P < 0.962$ ) (Table II.2). However, there was a significant difference in the infestation rate for the different replicates sampled at different time-points during the year ( $F_{5,137} = 18.24$ ,  $P < 0.001$ ) (Figure II.2).

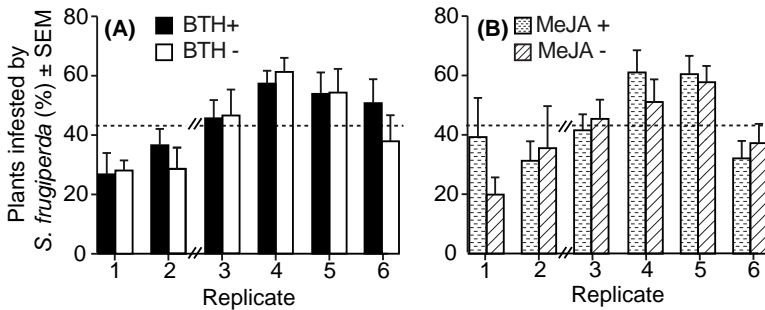


Figure II.2: Infestation of maize plants by caterpillars of *Spodoptera frugiperda* (mean percentage of infested plants  $\pm$  SEM) on **A**) BTH<sup>+</sup> and BTH<sup>-</sup> treated plants and **B**) on MeJA<sup>+</sup> and MeJA<sup>-</sup> treated plants. The dotted line indicates the average infestation rate over the six replicates and four treatments. The experiments were replicated six times over the year. Data were analyzed by GLM.

Other herbivores were also found in similar abundance in all treatments (Table II.2). *Diabrotica* spp. damage ( $F_{3,116} = 0.08$ ,  $P < 0.968$ ) as well as the number of *Diabrotica* beetles ( $F_{3,92} = 0.58$ ,  $P < 0.627$ ) was similar across treatments. The same was true for the presence of aphids ( $F_{3,92} = 0.18$ ,  $P < 0.949$ ). Overall, we collected equal numbers of *S. frugiperda* caterpillars (1420 in BTH<sup>+</sup>, 1441 in BTH<sup>-</sup>, 1379 in MeJA<sup>+</sup>, and 1388 in MeJA<sup>-</sup>), as well as *D. saccharalis* (309 in BTH<sup>+</sup>, 345 in BTH<sup>-</sup>, 327 in MeJA<sup>+</sup>, and 264 in MeJA<sup>-</sup>). *D. saccharalis* was found only in summer 2008.

Table II.2: Mean herbivore damage (scale: 0-5 $\pm$ SEM) and number of herbivores ( $\pm$  SEM) per maize plant treated with BTH<sup>+</sup>, BTH<sup>-</sup>, MeJA<sup>+</sup> and MeJA<sup>-</sup>. Data were analyzed using one-way analysis of variance (ANOVA). n.s.: no significant difference between treatments ( $P>0.05$ ).

	BTH <sup>+</sup>	BTH <sup>-</sup>	MeJA <sup>+</sup>	MeJA <sup>-</sup>	
<b>Herbivore damage</b> $\pm$ SEM <sup>a</sup>					
<i>Diabrotica</i> spp	1.58 $\pm$ 0.11	1.66 $\pm$ 0.13	1.65 $\pm$ 0.11	1.63 $\pm$ 0.12	n.s.
<i>Spodoptera frugiperda</i>	0.54 $\pm$ 0.12	0.58 $\pm$ 0.13	0.40 $\pm$ 0.11	0.46 $\pm$ 0.13	n.s.
<b>Number of herbivores</b> $\pm$ SEM					
<i>Spodoptera frugiperda</i> <sup>a</sup>	0.82 $\pm$ 0.09	0.75 $\pm$ 0.10	0.76 $\pm$ 0.09	0.76 $\pm$ 0.09	n.s.
<i>Diatraea saccharalis</i> <sup>c</sup>	0.54 $\pm$ 0.10	0.61 $\pm$ 0.09	1.02 $\pm$ 0.48	0.45 $\pm$ 0.07	n.s.
<i>Diabrotica</i> spp <sup>b</sup>	0.27 $\pm$ 0.05	0.38 $\pm$ 0.06	0.35 $\pm$ 0.09	0.28 $\pm$ 0.06	n.s.
Aphids <sup>a</sup>	0.08 $\pm$ 0.03	0.13 $\pm$ 0.05	0.05 $\pm$ 0.02	0.08 $\pm$ 0.03	n.s.

### II. 3.3 Parasitism rates and parasitoids (*Experiment 2*)

In replicate 3, parasitism by *C. insularis* was significantly higher in BTH<sup>+</sup> treated plants (14.8 $\pm$ 4.48%) compared to BTH<sup>-</sup> (6.51 $\pm$ 2.42%) treated plants (GLM,  $P<0.014$ ). In this replicate, there was also a trend of increased parasitism by *C. insularis* in MeJA<sup>+</sup> treated plants (7.05 $\pm$ 1.35%), compared with MeJA<sup>-</sup> treated (10.20 $\pm$ 4.73%) plants ( $P<0.051$ ). However, in the other replicates, there were no significant differences between the treatments for any of the wasp species that were retrieved. When cumulating all parasitoid species, there was a trend that parasitism was higher in BTH<sup>+</sup> treated plots than in BTH<sup>-</sup> treated plots ( $P<0.065$ ) (Figure II.3). Particularly, in replicate 2 there was a slightly higher parasitism in BTH<sup>+</sup> plots than in BTH<sup>-</sup> ( $P<0.106$ ), but this trend was reversed in replicate 6, with more parasitism in BTH<sup>-</sup> plots ( $P<0.022$ ). The parasitism rates did not differ between MeJA<sup>+</sup> and MeJA<sup>-</sup> ( $P>0.05$  for all replicates).

The main parasitoid during winter and summer was the egg-larval parasitoid *C. insularis* with almost 20% parasitism rate of *S. frugiperda* (Table II.3). The second most frequent parasitoid was *E. vitticolle*, followed by *P. spinator* and *C. sonorensis*. The next most frequent parasitoid was the braconid *C. marginiventris*, which was also found both in winter and in summer. *A. laphygmae* and *C. cautilus*, as well as *O. flavidus* were found in small numbers with only one occurrence for the latter. For instance, the specialist parasitoid *A. diatraeae* was only reared from *D. saccharalis* larvae during the summer experiments. Tachinidae were also found only

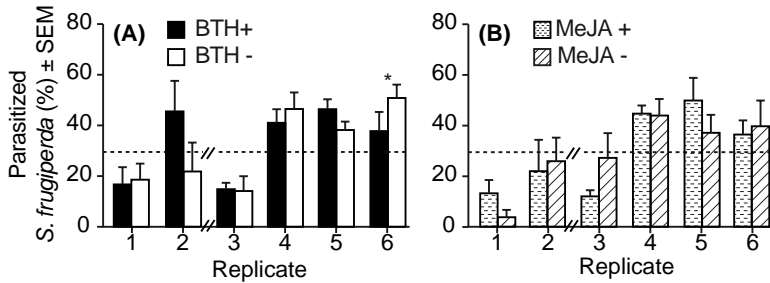


Figure II.3: Parasitism of *Spodoptera frugiperda* (% of collected larvae  $\pm$ SEM) found on plants treated with (A) BTH<sup>+</sup> and BTH<sup>-</sup> and (B) MeJA<sup>+</sup> and MeJA<sup>-</sup>. The dotted line indicates the average parasitism rate over the six replicates and four treatments. The experiments were replicated six times over one year. Data were analyzed by general linear model (GLM).

in summer, as well as *A. laphygmae* and *O. flavidus*. On the other hand, *P. spinator* and *C. sonorensis* were found only in winter. The egg-larval parasitoid *C. cautus* was found only in winter 2009, and *E. vitticollae* was also found mostly in the winter.

Table II.3: Total number of parasitoids emerged from collected *S. frugiperda* larvae. The insects were collected in Summer 2008 and Winter 2009.

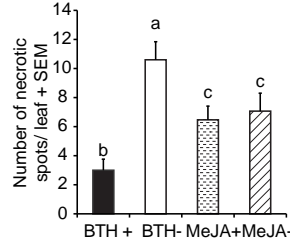
Species	Family	BTH <sup>+</sup>	BTH <sup>-</sup>	MeJA <sup>+</sup>	MeJA <sup>-</sup>	Total
<i>Chelonus insularis</i>	(Hym. Braconidae)	251	290	242	228	1011
<i>Ephosoma vitticollae</i>	(Hym. Ichneumonidae)	58	64	78	73	273
<i>Pristomerus spinator</i> <sup>a</sup>	(Hym. Ichneumonidae)	30	45	41	32	148
<i>Campoletis sonorensis</i> <sup>a</sup>	(Hym. Ichneumonidae)	9	26	14	15	64
<i>Apanteles diatraeae</i> <sup>b</sup>	(Hym. Braconidae)	7	4	4	7	22
<i>Cotesia marginiventris</i>	(Hym. Braconidae)	7	4	2	3	16
Tachinidae <sup>b</sup>	(Diptera)	5	4	2	3	14
<i>Aleiodes laphygmae</i> <sup>b</sup>	(Hym. Braconidae)	2	1	1	1	5
<i>Chelonus cautus</i> <sup>a</sup>	(Hym. Braconidae)	2	0	1	1	4
<i>Ophion flavidus</i> <sup>b</sup>	(Hym. Ichneumonidae)	0	0	1	0	1

<sup>a</sup> insects found only in winter 2009 (Replicate 3, 4, 5, and 6). <sup>b</sup> insects found only in summer 2008 (Replicate 1 and 2); <sup>c</sup> insects emerged from *D. saccharalis*.

### II. 3.4 Potted plant experiment (*Experiment 3*)

The effect of the elicitors was also tested in a more standardized field experiment with potted plants that were divided into groups of equal mean plant

Figure II.4: Number of necrotic spots per last fully developed leaf (n=15) + SEM. Data were analysed with one-way analysis of variance (ANOVA). A different letter indicates a significant difference ( $P < 0.05$ ). Further data of the same experiment are shown in Table II.4.



size and treated in the greenhouse before transfer to the field. Twenty days after treatment, BTH<sup>+</sup> plants weighed significantly more than the plants of the other treatments ( $F_{3,56} = 8.26$ ,  $P < 0.001$ ) (Table II.4). As expected, we observed a significantly lower number of necrosis on BTH<sup>+</sup> plants compared to BTH<sup>-</sup>, MeJA<sup>+</sup> and MeJA<sup>-</sup> plants ( $F_{3,56} = 8.53$ ,  $P < 0.007$ ) (Figure II.4). Interestingly, the BTH<sup>-</sup> treated plants had increased numbers of necrosis, compared to MeJA<sup>+</sup> and MeJA<sup>-</sup>. Flea beetle damage ( $F_{3,56} = 0.72$ ,  $P < 0.542$ ) and *Diabrotica* spp. damage ( $F_{3,56} = 0.45$ ,  $P < 0.721$ ) did not differ between the four treatments. Only few caterpillars were feeding on these plants and therefore no statistical test was applied.

Table II.4: Mean plant dry weight (g±SEM), herbivore damage (scale 0-10 ±SEM) and number of herbivores (±SEM) per maize plant treated with BTH<sup>+</sup>, BTH<sup>-</sup>, MeJA<sup>+</sup> and MeJA<sup>-</sup>. N = 15 for all treatment. Data were analyzed with one-way analysis of variance (ANOVA) and Tukey's post-hoc test. A different letter indicates a significant difference at  $P < 0.05$ .

	BTH <sup>+</sup>	BTH <sup>-</sup>	MeJA <sup>+</sup>	MeJA <sup>-</sup>
Plant dry weight (g ± SEM)	5.70±0.35 a	4.13±0.25 b	4.60±0.22 b	4.13±0.17 b
Herbivore damage ± SEM				
<i>Diabrotica</i> spp	0.80±0.22 a	1.07±0.21 a	0.80±0.26 a	1.07±0.23 a
Flea beetle	3.53±0.59 a	3.53±0.53 a	4.60±0.61 a	4.33±0.81 a
Number of herbivores				
<i>Diatraea saccharalis</i>	4	4	2	5
<i>Spodoptera frugiperda</i>	0	1	1	2

### II. 3.5 Pathogen disease resistance and plant fitness (*Experiment 4*)

In the fourth experiment we grew plants in the fields and treated them with elicitors similarly as in experiment 2. Seven days before harvesting all treatments showed similar *Bipolaris maydis* symptoms, the main fungal pathogen found in the field (Table II.5 : ( $F_{3,92} = 1.66$ ,  $P < 0.181$ ), lodging ( $F_{3,92} = 0.59$ ,  $P < 0.620$ ), or stunting ( $F_{3,92} = 1.90$ ,  $P < 0.135$ ), did not show a significant difference among treatments.) There was also no difference in cob number ( $F_{3,92} = 0.53$ ,  $P < 0.666$ ), cob weight ( $F_{3,92} = 0.44$ ,  $P < 0.730$ ), and kernel humidity ( $F_{3,92} = 0.66$ ,  $P < 0.578$ ) among treatments.

Table II.5: Disease symptoms caused by *Bipolaris maydis* (scale: 0-5) and several fitness measures of maize plants treated with BTH<sup>+</sup>, BTH<sup>-</sup>, MeJA<sup>+</sup> and MeJA<sup>-</sup> at the date of harvesting. Data were analyzed with one-way analysis of variance (ANOVA).

	BTH <sup>+</sup>	BTH <sup>-</sup>	MeJA <sup>+</sup>	MeJA <sup>-</sup>	$P <$
<b>Disease symptoms <math>\pm</math>SEM</b>					
<i>Necrosis</i>	2.08 $\pm$ 0.57	1.58 $\pm$ 0.33	1.21 $\pm$ 0.27	1.42 $\pm$ 0.42	0.181
Lodging	8.29 $\pm$ 0.43	8.54 $\pm$ 0.63	7.63 $\pm$ 1.21	7.67 $\pm$ 0.63	0.621
Stunting	4.96 $\pm$ 0.49	4.25 $\pm$ 0.20	4.42 $\pm$ 0.29	3.67 $\pm$ 0.25	0.135
<b>Fitness measures <math>\pm</math>SEM</b>					
Cobs/plant	0.92 $\pm$ 0.05	0.97 $\pm$ 0.04	0.94 $\pm$ 0.03	0.96 $\pm$ 0.03	0.666
Cob weight (g)	1210 $\pm$ 130	1330 $\pm$ 150	1310 $\pm$ 140	1260 $\pm$ 140	0.728
Grain humidity (%)	38.56 $\pm$ 0.76	38.33 $\pm$ 1.10	37.55 $\pm$ 1.03	36.73 $\pm$ 1.36	0.578

## II. 4 Discussion

In this study we investigated whether the application of two potent elicitors of pathogen resistance (BTH) and insect defense responses (MeJA) affected the herbivores and pathogens in subtropical maize fields in Mexico. In particular, we evaluated the effect of the elicitors on parasitism of caterpillars of *S. frugiperda*, the main pest insect of maize in Mexico. Consistent with laboratory experiments, the treatment with the elicitor BTH (BTH<sup>+</sup>) slightly reduced the volatile emission by herbivore-infested maize seedlings compared to the control treatment BTH<sup>-</sup> (Table II.1; Rostás & Turlings, 2008) and the treatment with MeJA (MeJA<sup>+</sup>) slightly increased the volatile emission of two major sesquiterpenes compared to its control treatment MeJA<sup>-</sup> (Degenhardt & Lincoln, 2006; Rostás & Turlings, 2008).

The differences in volatile emissions that were found among treatments did not affect the infestation by herbivores (Table II.2 and Figure II.2) and only slightly affected the parasitism rate of *S. frugiperda* (Table II.3 and Figure II.3). Our field data do not confirm earlier findings from a laboratory study (Rostás & Turlings, 2008) where BTH treatment strongly increased the attractiveness of caterpillar-damaged maize plants to a parasitoid. Moreover, treatment of tomato plants with jasmonic acid has been shown to increase parasitism in the field (Thaler, 1999), which was also not the case in our study. Application of MeJA with lanolin paste on wild tobacco increases the emission of sesquiterpenes (Halitschke et al., 2000) and the predation of hornworm eggs (Kessler & Baldwin, 2001). In our study on maize, the spraying of MeJA solution also increased the emission of sesquiterpenes while no effect could be measured on herbivory. However, the different application methods (spraying versus lanolin paste) may have caused different effects on the insect herbivory. Indeed, sprayed MeJA may have activated the plant defenses but probably evaporated soon after application, while MeJA in lanolin paste continued to enter the plant, and to evaporate into the airspace surrounding the plant.

Several field-related factors could explain why the treatments had little effect on the responsiveness of the herbivores and parasitoids in our field trials. First of all, the application of elicitors may have occurred after most plants were already induced by the natural infestation with herbivores and pathogens. These early antagonists may have triggered SA and JA-mediated defenses prior to elicitor application. Indeed, flea beetles and leaf bugs were shown to induce the emission of similar amounts of volatiles in wild tobacco (Kessler & Baldwin, 2001). These insects were also observed on the maize plants in our study, already in the first days after the emergence of the cotyledon. Furthermore, a large proportion of the maize plants were damaged prior to application of elicitors, mainly by adult herbivores such as *Diabrotica* spp.

In contrast with the sesquiterpenes, some volatile compounds, including indole, which were shown to be affected by the elicitor treatments in the laboratory (Rostás & Turlings, 2008) were not emitted under the field conditions. This might be due to the different composition of the herbivore population feeding on the plants in the field and/or to additional biotic and abiotic stresses that might have altered the volatile emission in the field. Indeed, biotic and abiotic factors are known to strongly influence quantity and quality of volatile compounds emitted by plants (Gouinguéné & Turlings, 2002; Loreto & Schnitzler, 2010; Takabayashi et al., 1994). Although the fields were irrigated at regular intervals, high temperatures could be measured throughout the experimental period, possibly causing

heat stress. In the study by Gouinguéné & Turlings (2002), maize plants were emitting increased amounts of homoterpenes and sesquiterpenes at 37°C. These temperatures were reached on several days of the experimental period. Furthermore, these temperatures may have dehydrated the top portion of the soil where the plants were growing, which in early stages can have caused some drought stress, which in turn might have affected VOC emission (Peñuelas & Staudt, 2010).

However, the maize plants in our fields were subjected not only to abiotic factors that may have influenced the VOC emission and, ultimately, the parasitism of *S. frugiperda*, but also by a number of biotic factors. First of all, the collected *S. frugiperda* larvae varied in size from first to fourth instar, which might have caused some of the variation in VOC emission and parasitism. However studies with different instars of *S. littoralis* demonstrated that second, third and fifth instars induce similar amounts of VOCs and attract a similar number of parasitoids (Gouinguéné et al., 2003). On the other hand, various other herbivores were observed on the shoots of maize seedlings, such as aphids, flea beetles, planthoppers and leafhoppers, as well as thrips, although in lower and variable numbers between seasons. These herbivores with differing feeding habits might have affected the volatile emission and the attraction of parasitoids (Turlings et al., 1998). Moreover, adult herbivores, such as *Diabrotica* spp., leafhoppers, planthoppers etc., unlike larval herbivores, can rapidly colonize a field without oviposition having to occur first on the plants. It is known that induction of VOCs depends on time (Turlings et al., 1998). Therefore, although feeding damage caused by most of these adult insects was limited, such early damages may still have induced defense responses and affected the outcome of the study. For instance, JA-related defenses and VOC emission may have been activated as soon as the adult herbivores arrived in a field, which in the context of this study was shortly after the emergence of the seedlings. Predictions about the consequences of changes in VOCs emissions for parasitoid attraction are virtually impossible because very little is known about which specific compounds attract parasitoids. In the case of parasitoids that are attracted to host-induced maize VOCs, it is known that their responses are flexible and that their responsiveness to an odor increases if they have encountered hosts while perceiving the odor (Tamò et al., 2006). This is also true for specific compounds within the blend. For instance, Schnee et al. (2006) showed that a typical blend of inducible maize sesquiterpenes that was released from *Arabidopsis* plants transformed with a maize terpene-synthase gene, was only attractive to the parasitoid *C. marginiventris* after female wasps had experienced the sesquiterpenes while contacting hosts. Similarly, another

important compound in the maize blend, the aromatic compound indole, only contributes to attraction of *C. marginiventris* after such a positive experience (D'Alessandro et al., 2006). This effect of associative learning is common among parasitoids (Vet & Dicke, 1992; Tamò et al., 2006) and allows great adaptability to new cues. This might also allow them to adapt to changes caused by elicitor treatments. The compounds that are essential for innate attraction of parasitoids of *S. frugiperda* still need to be determined. These key compounds may be released only in very small amounts (D'Alessandro et al., 2009) and were not detectable in our volatile collection. Alternatively, the fact that a change in VOC emissions did not translate into increased parasitism could be explained by the variable range of species of parasitoids that were found to parasitize *S. frugiperda*. The parasitoid species composition varied strongly with the season and several species occurred only during the winter or only during the summer (Table II.3). The main parasitoid, *C. insularis* was dominant during the winter as well as the summer, representing more than 50% of the wasps collected. This species has also been found in high numbers in previous Mexican field studies (Molina-Ochoa et al., 2003). *C. insularis* is a egg-larval parasitoid, which means that it oviposits in the egg stage of its hosts and then further develops inside the larval stage (Jourdie et al., 2009; Wheeler et al., 1989). *C. insularis* parasitism was increased in one out of six replicates. Yet, other studies have found that certain egg parasitoids are attracted to VOCs emitted by plants upon egg deposition (Moraes et al., 2009). If this is also the case for *C. insularis*, this attraction was not consistently affected by the elicitor treatments (Table II.3). For the other parasitoid species one noteworthy tendency was that fewer *C. sonorensis* wasps were found on BTH treated plants. Although it was not significant, the three-fold lower occurrence on BTH-treated plants suggests a negative effect of the treatment. In the case of *C. sonorensis*, reduced parasitism in BTH<sup>+</sup> plots could be explained by the overall reduction in volatile emissions. In laboratory studies, *C. sonorensis* show strongest attraction to plants that emit the largest amounts of VOCs (unpubl. data).

Previous field studies showed that treatment with BTH can increase the resistance of tomato to herbivory and pathogens (Inbar et al., 2001), and treatment of tomato with jasmonic acid increases the plant's attractiveness to parasitoids (Thaler, 1999). In our experiments with potted plants it was confirmed that treatment with BTH could decrease colonization of plant tissues by pathogens. However, this was not evident in our other field assays and overall the treatment with BTH and MeJA only slightly affected parasitism of caterpillars in our experiments. It is possible that under the severe conditions at the field sites, pathogens may have chal-

lenged the seedlings before treatment, thereby already activating SAR and thus diminishing the effects of subsequent treatments. Maybe such problems could be avoided by treating the seeds with BTH before planting, which also induces the desired resistance to pathogens (Danielson, 2003). Furthermore, the very frequent observation of adults of *Diabrotica* spp. further suggests that root-feeding larvae of these beetles may have been present belowground. Recent studies have shown that root herbivory by *Diabrotica* larvae significantly affects resistance against herbivores and pathogens in the shoot (Erb et al., 2008, 2009).

Previous studies found no effect of BTH treatment on plant performance and fitness (Heil, 2002; Walters & Heil, 2007; Walters & Boyle, 2005). In the potted plant experiments we found that enhanced resistance to pathogens after BTH treatment was reflected in an increased biomass of 21-day old plants. This improved performance was no longer measurable at a later stage of development, possibly again because of the high and diverse disease and herbivore pressure that these plants were subjected to throughout the season. Overall, treatment with BTH had minor positive effects on plant resistance and performance and no measurable negative consequences.

In conclusion, the application of BTH on maize in subtropical field conditions does not increase parasitism of *S. frugiperda* larvae, against expectations based on recent parasitoid attraction studies conducted under laboratory conditions. However, BTH application is compatible with biological control, as it has no negative effect on parasitism of the primary maize pest in Mexico, *S. frugiperda*. Based on previous findings from laboratory assays (Rostás & Turlings, 2008), as well as on the experiment with the potted plants it appears that under more moderate environmental conditions and with early treatment of the plants, BTH treatment could lead to enhanced resistance against pathogens. If under such conditions attraction of key larval parasitoids can be enhanced as well remains to be determined.

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# III

## **Does breeding for quality protein and drought tolerance affect the attractiveness of maize plants to parasitoids?**

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**Abstract** Maize plants respond to herbivory by emitting volatile organic compounds (VOCs) that attract parasitic wasps. The VOCs comprise a blend of several chemical groups, which include green leaf volatiles, nitrogenous and aromatic compounds, as well as mono, homo and sesquiterpenes. The blend of VOCs differs not only between species of plants, but also between genotypes of the same species. Several studies have shown that the difference in VOC emission can lead to different attraction of the parasitoids to the infested plants. In this study, we investigated the attractiveness to *Cotesia marginiventris* and *Campoletis sonorensis* of maize plants from two ongoing breeding programs in tropical maize: drought tolerance and high quality protein. In the drought tolerance program, improved abiotic stress resistance is sought, while in quality protein maize, the content of tryptophan and lysine in the seeds is increased. Top lines from the two programs were compared to understand how breeding for production-related characteristics affects biological control traits. The total VOC emission of the maize plants differed significantly between programs, but not between lines within a program. However, the emission of individual VOCs, particularly sesquiterpenes, varied significantly between breeding lines. Interestingly, these differences in VOC emissions were not correlated with parasitoid attraction. Olfactometer results indicated that the two parasitoid species were attracted similarly by the different maize varieties when the six lines were compared simultaneously. However, when only three lines were compared at the same time, the two wasp species were attracted differently. We hypothesize that the context in which the breeding lines were tested influenced parasitoid attraction. The results are discussed in relation to on-going breeding programs and how biological control parameters can be influenced by breeding goals.

**Key Words** Quality protein maize, drought tolerance, *Campoletis sonorensis*, *Cotesia marginiventris*, parasitoid attraction, olfactometer, volatile organic compounds, biological control.

## III. 1 Introduction

Plants respond to herbivory by emitting volatile organic compounds (VOCs) that are used by parasitoids and predators to locate plants with potential hosts or prey (Dicke, 2009; Dicke et al., 1990; Heil, 2008; Turlings et al., 1990). These VOCs comprise green leaf volatiles (GLVs, C6-alcohols, aldehydes and their esters), aromatic and nitrogenous compounds, as well as mono, homo and sesquiterpenes (D'Alessandro et al., 2006; Dicke & van Loon, 2000; Paré & Tumlinson, 1999). However, the VOC emissions can be highly variable due to biotic and abiotic factors. The herbivore-induced VOC blend emitted by maize plants, for example, is different when different herbivore species feed on the plants (Turlings et al., 1998) and can be affected by the presence of fungal pathogens (Rostás et al., 2006). In maize, the emissions also varies with plant age and size (Degen et al., 2004; Hoballah et al., 2004) and is affected by several abiotic factors, such as drought stress, light conditions, temperature and nutrient supply (Gouinguéné & Turlings, 2002). What's more, the emission of induced VOCs differs between different genotypes of a particular species, as was shown for gerbera (Krips et al., 2001), maize (Degen et al., 2004), horse nettles (Delphia et al., 2009), lima beans (Maeda et al., 1999), wild cabbage (Poelman et al., 2009), and wild tobacco (Schuman et al., 2009). Such differences in VOC emission can lead to different attraction of parasitoids in the laboratory and in the field (Hoballah et al., 2002; Poelman et al., 2009).

Notwithstanding the amount of knowledge about the factors that affect the VOC emission of maize plants and parasitoid attraction, little is known about the specific compounds that mediate this attraction. Several approaches have been explored to identify key parasitoid attractants emitted by maize plants (D'Alessandro & Turlings, 2005; D'Alessandro et al., 2009). These studies showed that not all volatile organic compounds emitted by maize plants contribute equally to the attractiveness of the plants to parasitoids. Certain common maize VOCs like the aromatic compound indole may even mask attractive compounds or directly repel wasps (D'Alessandro et al., 2006). Indole is a product of the shikimic acid pathway, which also involves the amino acid tryptophan. This amino acid is essential for human and animal nutrition (Young & Pellett, 1994), which is why maize breeders have sought to increase its concentration in maize. An ongoing breeding program (Quality protein maize, QPM, aiming at increasing tryptophan and lysine contents in maize) at the International Center for the Improvement of Maize and Wheat (CIMMYT) has contributed significantly to this breeding goal (Bantte & Prasanna, 2004; Villegas et al.,

1992). However, so far little is known how breeding maize plants for increased tryptophan content in the seed may change the emission of VOCs, and consequently the plants attractiveness to parasitoids.

Another important breeding goal pursued by maize breeders is the tolerance of maize plants to drought stress (Byrne et al., 1995). Because abiotic stress factors such as drought also affect the emission of VOCs, maize plants that have been bred for increased drought tolerance may have metabolic features that could affect their attractiveness to parasitoids. For instance, the plant hormone abscisic acid (ABA) plays an important role in drought resistance (Jackson, 1997) and it is also involved in plant defence against herbivores (Erb et al., 2009). However, tropical maize is subjected not only to abiotic stresses, such as drought or shortage of mineral compounds, but also biotic stress factors. In Mexico, up to 50% of the annual yield is destroyed by the fall armyworm *Spodoptera frugiperda* (J.E. Smith) (Lepidoptera: Noctuidae) (Bahena Juarez, 2003). This pest is widely distributed over the tropical and sub-tropical Americas (Clark et al., 2007). Because of its heavy toll on maize crops, several approaches have been explored to mitigate the damage caused by this pest. Besides the conventional use of pesticides, biological control mediated by predators and parasitoids, is receiving increased attention (Lezama-Gutiérrez et al., 2001; Molina-Ochoa et al., 2003, 2004). It would be ideal if breeding for new maize varieties would optimize their attractiveness to biological control agents. *Cotesia marginiventris* (Cresson) (Hymenoptera: Braconidae) and *Camponotus sonorensis* (Cameron) (Hymenoptera: Ichneumonidae) are important larval parasitoids of *S. frugiperda* in subtropical areas of Mexico (Hoballah et al., 2004; Jourdie et al., 2008).

In the current study we used a six-arm olfactometer to investigate the attraction of these two parasitoids to maize plants from the two ongoing tropical maize breeding programs in CIMMYT (QPM and drought tolerance). Three highly drought tolerant lines and three high-tryptophan producing lines out of each of the two programs were compared and parasitoid attraction was correlated with the VOC emission of the lines tested. In a first series of assays, the six lines were compared simultaneously. In the second setup, the three lines from each program were compared separately and, finally, two lines out of each program were compared with one line out of the other program, depending on the volatile profile emitted in the previous experiments.

## III. 2 Material and Method

Six inbred lines from the International Center for the Improvement of Maize and Wheat (CIMMYT) were tested, three highly drought tolerant inbred lines (D1, D2 and D3) and three high-tryptophan content inbred lines (Q1, Q2 and Q3) from the Quality Protein Maize (QPM) breeding program. The three drought tolerant lines were ranked first out of fifty ranks of drought tolerance (Sanchez, C. personal communications). The three QPM lines' tryptophan content in the seeds was increased (Q1: 0.086% of dry weight, Q2: 0.106%, and Q3: 0.109%) (Krivanek, A. personal communications). A detailed reference for each maize line is shown in Table III.1.

Table III.1: Breeding program, breeding line number, and origin of six Mexican breeding lines

Breeding program	Breeding line	Origin
QPM	Q1	CML 150
	Q2	CLQRCWQ116
	Q3	CML 491
Drought tolerance	D1	TL05B6613-1 x CML249
	D2	TL05B6613-48 x CML495
	D3	PR-99A652 2 (Tuxpeño Sequía*)

\* Drought resistant line Tuxpeño Sequía is widely used in drought-resistance breeding schemes

### III. 2.1 Plants

The plants were grown as described in D'Alessandro et al. (2006), but at an average temperature of  $30 \pm 2^\circ\text{C}$ . To induce the emission of VOCs, the lower side of the first and second true leaf of ten-days-old plants was scratched with a razor blade 2-3 cm from the tip  $18 \pm 2$  h before the start of the olfactometer assays (Hoballah & Turlings, 2005). The same leaves were scratched similarly a second time 1.5 h before the assays 2-3 cm from the base of the leaf. Immediately after scratching the leaves,  $10 \mu\text{l}$  of regurgitant from *S. littoralis* caterpillars was applied to the damaged sites. After the second treatment, the plants were transferred into glass bottles that served as the odour source vessels of the olfactometer and clean air was pushed into each bottle at a rate of 1.2 l/min (see Turlings et al. (2004) for details on the olfactometer).

The two wasp species were reared as described in (Tamò et al., 2006). Three groups of six naïve two to four-day old female *C. marginiventris* were released into the release chamber of the olfactometer (Turlings et al., 2004). They were given 30 min to walk into one of the six arms through which the different odours were introduced into this central chamber. After 30 min, all the wasps were counted and removed from the glass trapping bulbs attached to each arm. Wasps that did not enter an arm were considered to have made no choice. Wasps that entered an empty arm without odour, were counted separately, but considered as having made a choice. After three releases, the glass parts that were in contact with the wasps were washed with pasteurized water, rinsed with acetone and with pentane, and finally dried in the oven at 240°C for 120 min. After cooling down the glass parts, the system was set up and kept closed for 30 min. Subsequently the first group of six naïve *C. sonorensis* was released and left to choose for 30 minutes. After three *C. sonorensis* releases the experiment was terminated on a particular day.

Because the plants from different lines may show variation in growth, they were removed from the glass bottles after each experiment, cut at the base of the plant, and their fresh weight was recorded. The plants were then dried at 80°C for 24±2 hours, after which they were removed and placed in a glass jar containing Silica gel to cool down for 24±2 hours. The plants were then weighted again to determine dry weight. Three combinations of plants were tested in the six-arm olfactometer as summarized in Table III.2. In a first setup, one treated plant of all six inbred lines was placed in a glass bottle in each of the arms. The position of the plants was changed between replications (n=6) in a way that none of the possible combinations occurred twice, in order to prevent position effects.

In the second setup, only the three lines within a breeding program were tested, which left three arms empty. The position of the plants was changed between each replication (n=6), but always leaving one empty arm between each plant. In the third setup, one line of the QPM program was compared with two lines of the drought tolerance program, and three empty arms (Q1 versus D1 versus D2). These three lines emitted very similar amounts of volatiles (see Table III.4). One line of drought tolerance program was compared with two lines of the QPM program (Q2 versus Q3 versus D3). These lines emitted very dissimilar amounts of volatiles, Q2 and Q3 emitted very low amounts of volatiles, while D3 emitted high amounts of volatiles. Again, the position of the plants was changed between each replication, always leaving one empty arm between each plant.

Table III.2: Results figure, odour sources and experimental design.

Figure	Odour sources					
	Arm 1	Arm 2	Arm 3	Arm 4	Arm 5	Arm 6
III.2 1 A	Q1	D1	D2	Q2	D3	Q3
III.2 1 B	Q1	D1	D2	Q2	D3	Q3
III.2 2 A	D1	Empty	D2	Empty	D3	Empty
III.2 2 B	D1	Empty	D2	Empty	D3	Empty
III.2 2 C	Q1	Empty	Q2	Empty	Q3	Empty
III.2 2 D	Q1	Empty	Q2	Empty	Q3	Empty
III.2 3 A	Q1	Empty	D1	Empty	D2	Empty
III.2 3 B	Q1	Empty	D1	Empty	D2	Empty
III.2 3 C	Q2	Empty	Q3	Empty	D3	Empty
III.2 3 D	Q2	Empty	Q3	Empty	D3	Empty

## III. 2.2 Statistical analysis

Statistical analysis of wasp preference was performed with the program R (Team, 2010), as described earlier (Tamò et al., 2006; Turlings et al., 2004). Data for fresh and dry weight of the plants, as well as of quantities of emitted volatiles, were analyzed with one-way analysis of variance (ANOVA) on all six breeding lines and Tukey's post-hoc test, performed with the R statistical analysis program (R Development Core Team, 2010).

## III. 3 Results

### III. 3.1 VOC emission of maize plants

Table III.3: Fresh and dry weight ( $g \pm SE$ ) of six breeding lines.

VOC	QPM			Drought tolerance		
	Q1	Q2	Q3	D1	D2	D3
Fresh wt	1.45 $\pm$ 0.17	1.07 $\pm$ 0.07	1.32 $\pm$ 0.14	1.19 $\pm$ 0.11	1.20 $\pm$ 0.08	1.39 $\pm$ 0.10
Dry wt	0.13 $\pm$ 0.01	0.10 $\pm$ 0.01	0.11 $\pm$ 0.01	0.11 $\pm$ 0.01	0.11 $\pm$ 0.01	0.12 $\pm$ 0.01

The six lines did not differ significantly in fresh weight ( $F_{5,85} = 1.62$ ,  $P < 0.164$ ) or dry weight ( $F_{5,85} = 1.89$ ,  $P < 0.105$ ) (Table III.3). In contrast, the two breeding programs differed significantly in the total amount of emitted volatiles, with the lines from the drought tolerance program

emitting more VOCs ( $F_{1,89}=4.95$ ,  $P<0.029$ ). As for the different chemical groups, the lines from the drought tolerant program also emitted more of the sesquiterpenes ( $F_{1,89}=13.58$ ,  $P<0.001$ ) and monoterpenes ( $F_{1,89}=4.26$ ,  $P<0.042$ ) than the lines from the QPM program. The emission of the dominant VOCs is shown in Figure III.1. There was also a significantly higher emission by drought-tolerant than by QPM plants of (*E*)-2-hexenyl-acetate ( $F_{1,89}=4.23$ ,  $P<0.043$ ), phenyl-methyl acetate ( $F_{1,89}=8.14$ ,  $P<0.005$ ) and phenethyl acetate ( $F_{1,89}=5.42$ ,  $P<0.022$ ). The same was true for the individual sesquiterpenes cycloisosativene ( $F_{1,89}=16.05$ ,  $P<0.001$ ),  $\alpha$ -copaene ( $F_{1,89}=6.55$ ,  $P<0.012$ ), (*E*)- $\beta$ -caryophyllene ( $F_{1,89}=12.73$ ,  $P<0.001$ ), (*E*)- $\alpha$ -bergamotene ( $F_{1,89}=14.43$ ,  $P<0.001$ ), and (*E*)- $\beta$ -farnesene ( $F_{1,89}=9.32$ ,  $P<0.003$ ).

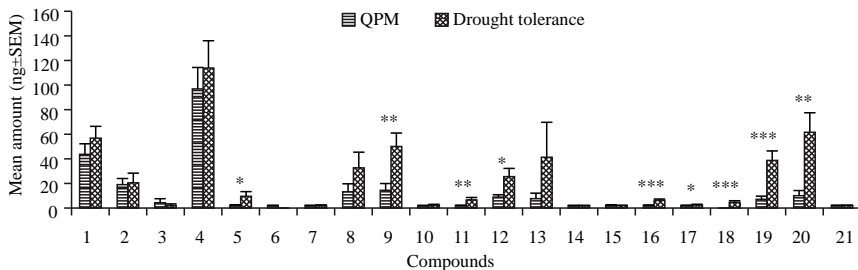


Figure III.1: Mean amount (ng±SEM) of VOCs collected from *Spodoptera*-induced maize plants of two breeding programs (QPM and Drought tolerance). Data was analysed in one-way analysis of variance (ANOVA). The compounds are as follows: 1, (*E*)-2-hexenal; 2, (*Z*)-3-hexenol; 3, (*Z*)-3-hexen-1-ol; 4, (*Z*)-3-hexenyl acetate; 5, (*E*)-2-hexenyl acetate; 6,  $\beta$ -myrcene; 7, (*Z*)- $\beta$ -ocimene; 8, linalool; 9, (3*E*)-4,8-dimethyl-1,3,7-nonatriene (DMNT); 10, (3*E*,7*E*)-4,8,12-trimethyl-1,3,7,11-tridecatetraene (TMTT); 11, phenyl-methyl acetate; 12, phenethyl acetate; 13, indole; 14, methyl-anthranilate; 15, geranyl-acetate; 16, cycloisosativene; 17,  $\alpha$ -copaene; 18, (*E*)- $\beta$ -caryophyllene; 19, (*E*)- $\alpha$ -bergamotene; 20, (*E*)- $\beta$ -farnesene; 21, nerolidol.

The six breeding lines did not differ significantly in the total amount of emitted VOCs ( $P<0.207$ ) (Table III.4), although Q2 and Q3 showed a tendency to emit less than the four other lines. Of the five chemical groups summarized at the bottom of Table III.4, sesquiterpenes were the only group that showed significant differences among breeding lines ( $F_{5,85} = 4.20$ ,  $P<0.002$ ). D1 emitted significantly more sesquiterpenes than Q1, Q2 and Q3. The other groups, GLVs ( $F_{5,85} = 0.35$ ,  $P<0.880$ ), aromatic com-

pounds ( $F_{5,85} = 1.76$ ,  $P < 0.129$ ), monoterpenes ( $F_{5,85} = 1.78$ ,  $P < 0.125$ ), homoterpenes ( $F_{5,85} = 0.96$ ,  $P < 0.450$ ), did not differ between breeding lines. As for individual compounds, (*E*)-2-hexenyl-acetate was emitted in significantly higher amounts by D1 than by Q1, Q2, Q3, and D3 ( $F_{5,85} = 2.48$ ,  $P < 0.038$ ). Quantities of (*E*)-2-hexenyl-acetate emitted by D2 were between D1 and the four other lines. The emission of other detected GLVs, (*E*)-2-hexenal, (*Z*)-3-hexenol, (*Z*)-3-hexenal, and (*Z*)-3-hexenyl-acetate, did not vary significantly among lines. The homoterpene (*E*)-4, 8-Dimethyl-1, 3, 7-nonatriene (DMNT) was emitted in significantly higher amounts by D1 and D2 than by Q2 and Q3, but not Q1 and D3 ( $F_{5,85} = 2.51$ ,  $P < 0.036$ ). The other homoterpene (3*E*, 7*E*)-4,8,12-trimethyl-1, 3, 7, 11-decatetraene (TMTT) was emitted in higher amounts by D3 as compared to the five other lines ( $F_{5,85} = 2.55$ ,  $P < 0.034$ ). The aromatic compound phenyl-methyl-acetate was emitted in higher amounts by D1 than by the five other lines ( $F_{5,85} = 4.10$ ,  $P < 0.002$ ), whereas phenethyl acetate was emitted in significantly higher amounts by Q1 compared to the other five lines ( $F_{5,85} = 4.98$ ,  $P < 0.001$ ). The sesquiterpene cycloisosativene was emitted in higher amounts by D1 and D2, than Q1, Q2 and Q3, but not more than D3 ( $F_{5,85} = 4.18$ ,  $P < 0.002$ ). The sesquiterpene (*E*)- $\beta$ -caryophyllene was emitted in significantly higher amounts by D1 than by the five other lines ( $F_{5,85} = 7.805$ ,  $P < 0.001$ ). (*E*)- $\alpha$ -bergamotene was emitted in significantly higher amounts by D1 than Q1, Q2, Q3 and D2 ( $F_{5,85} = 4.46$ ,  $P < 0.002$ ), but not more than by D3. (*E*)- $\beta$ -farnesene was emitted in higher amounts by Q1, D1, D2 and D3 than by Q2 and Q3 ( $F_{5,85} = 2.75$ ,  $P < 0.024$ ).

Table III.4: Mean amount (ng±SE) of VOCs emitted in 3 hours by regurgitant-induced maize plants of QPM (Q1, Q2, Q3) and drought tolerance (D1, D2, D3) breeding program. Different letters next to the value mean significant difference (ANOVA  $P < 0.05$ )

VOC	Q1	Q2	Q3	D1	D2	D3	ANOVA $P <$
(E)-2-hexenal	44.72±18.92 a	40.41±13.51 a	46.82±12.30 a	44.91±18.87 a	61.3±20.74 a	17.42±6.27 a	0.530
(Z)-3-hexenol	31.72±11.80 a	13.02±6.96 a	13.22±5.84 a	14.83±18.87 a	15.72±5.81 a	30.68±20.36 a	0.723
(Z)-3-hexenal	0.97±0.97 a	0.00±0.00 a	3.58±10.85 a	0.00±0.00 a	3.72±5.74 a	1.52±1.52 a	0.222
(Z)-3-hexenyl acetate	145.27±40.82 a	77.08±23.98 a	70.49±21.10 a	117.00±39.86 a	100.35±46.15 a	123.29±47.22 a	0.673
(E)-2-hexenyl acetate	<b>2.70±1.98 a</b>	<b>0.26±0.26 a</b>	<b>1.26±0.86 a</b>	<b>19.62±39.86 b</b>	<b>7.29±4.67 ab</b>	<b>1.58±1.58 a</b>	<b>0.038</b>
$\beta$ -myrcene	0.51±0.51 a	0.00±0.00 a	0.00±0.00 a	0.00±0.00 a	0.00±0.00 a	0.00±0.00 a	0.363
(E)- $\beta$ -ocimene	0.00±0.00 a	0.49±0.40 a	0.24±0.17 a	0.67±0.32 a	1.43±1.12 a	1.38±0.85 a	0.518
linalool	40.31±18.68 a	1.08±1.08 a	0.00±0.00 a	19.20±4.73 a	53.59±34.30 a	26.46±19.20 a	0.231
(E)-4,8-dimethyl-1,3,7-nonatriene	<b>34.50±16.07 ab</b>	<b>5.98±2.55 b</b>	<b>3.89±1.54 b</b>	<b>50.56±10.23 a</b>	<b>65.67±30.10 a</b>	<b>34.89±12.47 ab</b>	<b>0.036</b>
(DMNT)							
(E,E)-4,8,12-trimethyl-1,3,7,11-tridecatetraene	0.16±0.16 a	0.12±0.12 a	0.00±0.00 a	0.64±0.47 a	0.42±0.29 a	4.72±2.65 b	0.034
(TMTT)							
phenyl-methyl-acetate	1.11±0.72 a	0.06±0.06 a	0.30±0.30 a	13.01±4.60 b	4.91±3.55 a	1.61±0.98 a	0.002
phenethyl acetate	<b>14.82±4.85 a</b>	<b>5.05±1.87 a</b>	<b>7.60±2.09 a</b>	<b>52.55±16.81 b</b>	<b>13.28±4.92 a</b>	<b>10.06±4.98 a</b>	<b>0.001</b>
1-H-indole	23.98±13.30 a	0.00±0.00 a	0.00±0.00 a	22.71±5.88 a	14.84±12.42 a	84.69±83.14 a	0.581
methyl-anthranilate	0.42±0.30 a	0.00±0.00 a	0.00±0.00 a	0.51±0.27 a	0.00±0.00 a	0.00±0.00 a	0.246
geranyl acetate	2.69±1.96 a	0.00±0.00 a	0.14±0.14 a	0.53±0.53 a	0.00±0.00 a	0.20±0.14 a	0.163
cycloisotavene	<b>0.70±0.48 a</b>	<b>1.08±0.74 a</b>	<b>1.08±1.08 a</b>	<b>7.23±2.99 b</b>	<b>7.85±1.78 b</b>	<b>3.47±2.16 ab</b>	<b>0.002</b>
$\alpha$ -copaene	0.00±0.00 a	0.38±0.38 a	0.69±0.69 a	3.64±1.27 a	2.70±1.19 a	1.34±1.34 a	0.130
(E)- $\beta$ -caryophyllene	19.00±0.00 a	0.00±0.00 a	0.00±0.00 a	10.06±2.69 b	0.57±0.57 a	3.16±1.9 a	0.001
(E)- $\alpha$ -bergamotene	<b>18.15±7.12 a</b>	<b>2.04±1.43 a</b>	<b>1.84±0.39 a</b>	<b>54.77±10.80 bc</b>	<b>21.42±6.73 a</b>	<b>38.89±18.48 ac</b>	<b>0.002</b>
(E)- $\beta$ -farnesene	<b>25.28±12.09 a</b>	<b>3.36±2.35 b</b>	<b>2.89±1.07 b</b>	<b>79.63±16.39 a</b>	<b>29.08±10.07 a</b>	<b>74.10±42.56 a</b>	<b>0.023</b>
nerolidol	1.01±0.78 a	0.00±0.00 a	0.00±0.00 a	0.38±0.29 a	0.00±0.00 a	1.15±0.96 a	0.404
Total amounts	<b>45.15±20.11 a</b>	<b>5.40±3.74 a</b>	<b>4.23±2.03 a</b>	<b>145.37±28.70 bc</b>	<b>51.12±16.57 ac</b>	<b>117.80±55.42 abc</b>	<b>0.002</b>
sesquiterpenes	225.39±65.20 a	130.77±43.09 a	145.36±54.87 a	196.42±67.42 a	188.43±55.45 a	174.49±69.22 a	0.880
green leaf volatiles	3.04±2.19 a	0.00±0.00 a	0.14±0.14 a	0.84±0.61 a	0.00±0.00 a	0.20±0.14 a	0.129
aromatics	75.32±54.87 a	7.51±5.52 a	4.13±1.54 a	70.43±13.54 a	120.66±65.46 a	62.70±31.07 a	0.125
monoterpenes	39.91±18.47 a	5.11±1.91 a	7.90±2.15 a	88.27±23.14 a	33.00±20.51 a	96.36±87.69 a	0.460
homoterpenes	391.25±89.34 a	151.52±46.11 a	164.92±57.18 a	513.54±111.99 a	405.50±113.78 a	462.59±229.68 a	0.207

### III. 3.2 Wasp behaviour

In the first setup the six varieties were compared in the same olfactometer. Both *C. marginiventris* (Figure III.2, 1A) and *C. sonorensis* (Figure III.2, 1B) were attracted more by Q1 than by D1, Q2, Q3 and D3 (GLM,  $P < 0.005$ ). D2 was slightly less, but not statistically significantly, attractive than Q1 (GLM,  $P < 0.136$ ). The overall responsiveness was relatively high for both species (*C. marginiventris*: 76% and *C. sonorensis*: 85%). In the second setup, the two breeding programs were compared in separate olfactometers. *C. marginiventris* was attracted similarly by the three lines from the drought tolerance program (Figure III.2, 1A). D2 seemed slightly less attractive to *C. marginiventris* than D1 and D3, but this was not statistically significant. *C. sonorensis* displayed similar preferences (Figure III.2, 1B), except that D2 seemed slightly more attractive than for *C. marginiventris*. *C. sonorensis* showed a higher responsiveness (81%) than *C. marginiventris* (53%). When comparing the lines from the QPM program, *C. marginiventris* preferred Q1 to Q2, but not to Q3 (Figure III.2, 2C). *C. sonorensis* showed similar attraction to all three lines. In the third setup, comparisons were made between the programs. When comparing Q1 from the QPM program with D1 and D2 from the drought tolerance program, *C. marginiventris* showed a clear preference for Q1 (Figure III.2, 3A). *C. sonorensis* did not display this preference (Figure III.2, 3B). The responsiveness of *C. sonorensis* (78%) was higher than *C. marginiventris* (61%) and again more *C. sonorensis* entered empty arms (19%) than *C. marginiventris* (8%). When comparing two lines from the QPM program (Q2 and Q3) with one line from the drought tolerance program (D3), *C. marginiventris* was equally attracted to the three lines (Figure III.2, 3C). Q3 seemed slightly less attractive and was chosen as frequently as the empty arms. *C. sonorensis* was significantly more attracted by D3 than by Q2 and Q3, which were chosen as often as the empty arms (Figure III.2, 3D). In this setup, the responsiveness of *C. sonorensis* (80%) again exceeded the responsiveness of *C. marginiventris* (69%), with only slightly more *C. sonorensis* entering empty arms (15%) than *C. marginiventris* (13%).

## III. 4 Discussion

The total emission of VOCs differed significantly between the two breeding programs, but not between the lines within breeding program. Previous

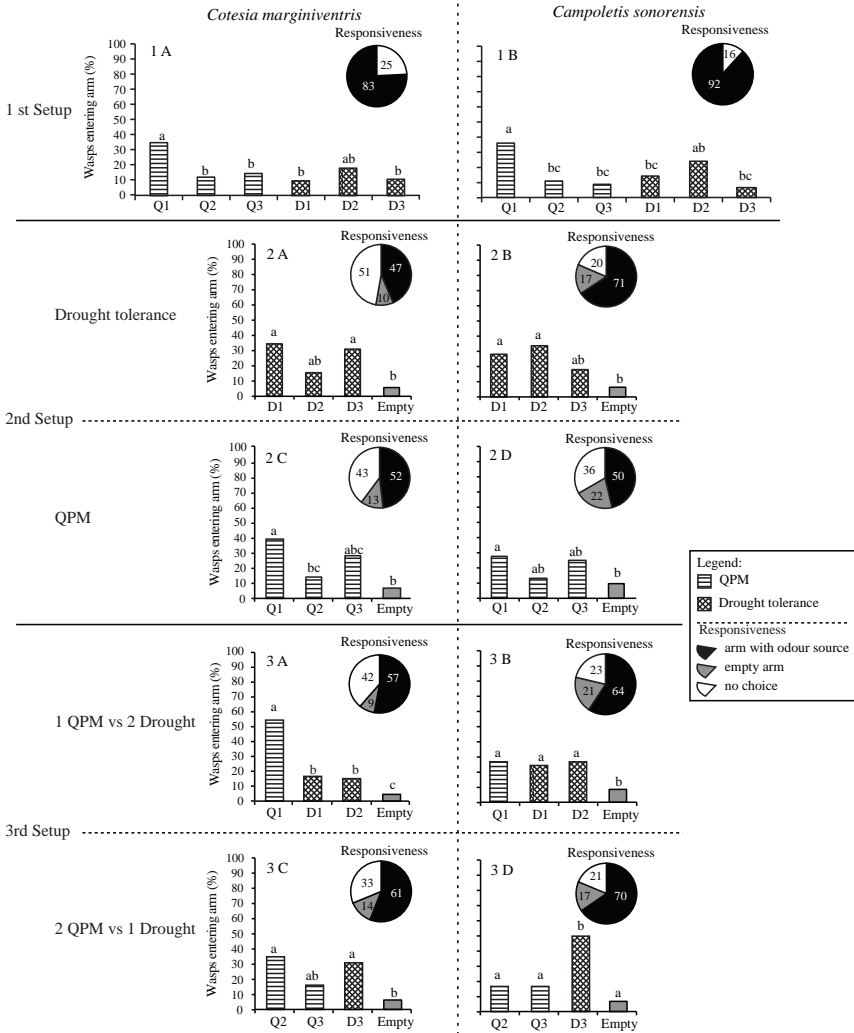


Figure III.2: Olfactometer responses of *Cotesia marginiventris* (left) and *Campoletis sonorensis* (right) to six inbred lines of maize from two breeding programs: QPM (horizontal lines) and drought tolerance (squared). The pie chart in the top right corner of each graph indicates the number of wasps entering an arm with odour source (black), an empty arm (grey), and the wasps that did not make a choice and remained in the center of the olfactometer (white). Three setups were tested: 1st setup with all six lines compared simultaneously (1A and 1B), in the 2nd setup each breeding program separately (Drought tolerance in 2A and 2B, and QPM in 2C and 2D). In the 3rd setup, one line from the QPM program was compared with two lines from the drought tolerance program (3A and 3B), and two lines from the QPM program were compared with one line from the drought tolerance program (3C and 3D). Different letters above the bars indicate significant differences among the inbred lines (GLM:  $P < 0.05$ ).

studies on European and American inbred lines showed more important differences in total quantity of emitted VOCs (Degen et al., 2004; Hoballah et al., 2002). This could suggest that the inbred lines from these two programs share an important common genetic background (Bolaños & Edmeades, 1993; Vasal et al., 1993). Furthermore, the fact that the lines from both breeding programs are grown and selected under similar conditions, i.e. they are subjected to similar selection pressures regarding insect pest resistance, could have had a convergent effect on their indirect defence mechanisms. Field observations have shown that *Spodoptera frugiperda* (J.E. Smith) (Lepidoptera: Noctuidae) infest heavily the maize plants that grow in CIMMYT's Ernest W. Sprague station in the state of Puebla, Mexico (Pers. obs.). Because the two breeding programs QPM and drought tolerance are regularly planted and selected in this station, the six lines may have converged in their VOC emission. Furthermore, the six maize lines emitted equivalent amounts of GLVs. However, D1 emitted increased amounts of (*E*)-2-hexenyl-acetate compared to the five other lines, but not of its isomer (*Z*)-3-hexenyl-acetate, which could be caused by an increased activity of the acetyl CoA: cis-3-hexen-1-ol acetyl transferase (CHAT) enzyme (D'Auria et al., 2007). However, there is no evidence so far of intraspecific variability in activity of this enzyme. Interestingly, the six lines did not differ significantly in their emission of indole. However, Q2 and Q3 did not emit indole, while Q1 emitted comparable amounts with D1. D2 emitted slightly less indole. D3 emitted the highest amount of indole, but only few plants did so. It can therefore be stated that the plants breeding for increased tryptophan content in the seeds did not affect the emission of indole with mechanical damage. D1 also emitted higher amounts of DMNT than Q2 and Q3. Furthermore, D1 also emitted higher amounts of phenyl-methyl acetate and phenethyl acetate, as well as high amounts of sesquiterpenes. Besides the two aromatics phenyl-methyl acetate and phenethyl acetate, the high amounts of TMTT emitted by D3 were the only VOCs that distinguished this line from D1.

The differences in VOC emission were not reflected in the attractiveness to the parasitoids. In the setup with six odour sources, both *C. marginiventris* and *C. sonorensis* were most attracted by Q1, followed by D2 and D1, Q2, Q3 and D3. Even more surprisingly, when comparing the lines only within breeding programs, the pattern of the first setup could not be repeated. Both wasp species did not distinguish between the lines from the drought tolerance program, D1, D2, and D3. Interestingly, while D1 and D3 were slightly less attractive to *C. marginiventris* than D2 in the first setup, this tendency was reversed in the second setup. *C. sonorensis* displayed similar preferences in the second setup than in the first one. When

comparing only lines from the QPM program, Q1 was more attractive to *C. marginiventris* than Q2 but not to *C. sonorensis*. The similarity in the VOC profiles of the three lines from the QPM program could have caused the parasitoids inability to distinguish between them. Indeed, the only VOC that differed significantly between the three QPM lines was (*E*)- $\beta$ -farnesene. Surprisingly, the two lines that did not emit indole, Q2 and Q3, were not more attractive than Q1, which did emit indole. Previous studies on indole suggested a masking effect for naïve parasitoids (D'Alessandro et al. 2006). This could not be observed under these conditions. In the third setup, *C. marginiventris* reproduced a similar attraction than in the first setup, when comparing Q1 with D1 and D2. On the other hand, *C. sonorensis* did not show any preference for any of these three lines. When comparing Q2 and Q3 with D3, *C. marginiventris* was attracted equally to all three lines, while *C. sonorensis* clearly preferred D3. These puzzling results suggest that there could be an effect of the context in which odour sources are tested on the attraction of parasitoid wasps. We speculate that with higher complexity, wasps prefer the odour source that is most similar to the other sources. Conversely, in a simpler context, they are increasingly attracted to odour sources that are more distinctive. However, this only was only observable in two out of four events. So far little is known about wasp attraction in contexts of different complexity. A study by Randlkofer et al. (2007) showed that parasitoids preferred simpler volatile blends to more complex ones, however, the tested odour sources were compared with each other (simple versus complex), the number of odour sources was the same.

Futhermore, this study shows that plants with high VOC emission, i.e. D2, which emitted most sesquiterpenes, do not necessarily attract parasitoids more than plants that emit less VOCs. For instance, in none of the three tested setups did the parasitoids prefer D2 significantly to the other lines. D'Alessandro et al. (2009) showed that minor volatile compounds were actually more attractive to parasitoids than the major compounds. Furthermore, the experiments conducted in two-choice experiments by Hoballah et al. (2002) on Mexican maize varieties have shown that both the quality and the quantity of maize VOCs play an important role in parasitoid attraction. This could be confirmed in this study; however, we also show that two larval parasitoids of the same herbivore species, i.e. *C. marginiventris* and *C. sonorensis* were not equally attracted by the VOCs emitted by the same maize plants. Several studies have shown that parasitoid species differ in the preference for VOCs (Geervliet et al., 1998; Milonas et al., 2009; Tamò et al., 2006). It is therefore important to include more than one parasitoid species when investigating the attractiveness of maize lines. This

should be taken into account when considering breeding for a maize plant that is attractive to parasitoids. Indeed, field studies in the sub-tropical areas of Mexico have shown that certain parasitoid species are present only in one season of the year, while maize is grown in two seasons (Pers. obs.). Therefore, a maize plant should attract beneficial insects from different species, in order to cover the whole growing period.

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**Herbivore-induced maize leaf volatiles affect attraction and early feeding of *Spodoptera littoralis* larvae.**

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**Abstract** Plants under herbivore attack emit volatile organic compounds (VOCs) that can serve as foraging cues for natural enemies. Adult females of Lepidoptera foraging for host plants to deposit eggs are commonly repelled by herbivore-induced VOCs, probably to avoid competition, induced defences, and natural enemies. However, their larval stages have been shown to be attracted to inducible VOCs. We speculate that this contradicting behaviour of lepidopteran larvae is due to a need to quickly find a new suitable host plant if they have fallen to the ground. Once on a plant they might avoid the sites with fresh damage to limit risk of cannibalism by conspecifics and exposure to natural enemies. To test this we studied the effect of herbivore-induced VOCs on the attraction, as well as feeding behaviour of the larvae of the moth *Spodoptera littoralis*. The experiments further considered the effect of previous feeding experience on the responses of the larvae. It was confirmed that herbivore-induced VOCs emitted by maize plants are attractive to the larvae, but they decreased the growth rate of caterpillars at early developmental stages. Larvae that had fed on maize leaves previously were more attracted by VOCs of induced maize plants than larvae that had fed on artificial diet. At relatively high concentrations synthetic green leaf volatiles, indicative of fresh damage, also negatively affected the growth rate of caterpillars, but not at low concentrations. In all cases, feeding by the later stages of the larvae was not affected by the VOCs. The results are discussed in the context of larval foraging behaviour under natural conditions, where there is a possible trade-off between using available host plant signals and avoiding competitors and natural enemies.

**Key words** *Spodoptera littoralis*, green leaf volatiles, maize, larval foraging behaviour, growth rate.

## IV. 1 Introduction

Maize plants attacked by herbivore insects emit volatile organic compounds (VOCs) that attract natural enemies of herbivores (Arimura et al., 2009; Dicke et al., 1990; Turlings et al., 1990; Turlings & Wäckers, 2004). In the case of maize plants, the blend of VOCs emitted by caterpillar-damaged plants is typically composed of green leaf volatiles (GLVs, C-6 aldehydes, alcohols and their esters), nitrogenous and aromatic compounds, as well as mono, homo and sesquiterpenes (D'Alessandro & Turlings, 2006; Paré & Tumlinson, 1999). Among the VOCs that have been identified in this blend, GLVs have received particular attention. They are emitted upon mechanical damage, immediately after feeding on the maize plant begins (Turlings et al., 1998), and have been considered important for the innate attraction of parasitoids, as they are emitted in higher amounts by freshly damaged plants than by plants with only old damage (Hoballah & Turlings, 2005; Whitman & Eller, 1990). Commonly insect herbivores are repelled by inducible plant volatiles (Bernasconi et al., 1998; De Moraes et al., 2001; Rostás & Hilker, 2002). This is particularly evident for Lepidoptera (De Moraes et al., 2001) but several herbivores, in particular coleopterans (Bolter et al., 1997; Landolt et al., 1999), may be attracted to GLVs, as was found for scarabid (Hansson et al., 1999), buprestid beetles (de Groot et al., 2008), and flea beetles (Halitschke et al., 2008).

Interestingly, larval stages of several Lepidoptera are attracted by volatiles emitted by plants that have been damaged by conspecific larvae. This was found for neonates of several Lepidoptera species, including *Ostrinia nubilalis* (Hübner) and *Ostrinia furnacalis* (Guenée) (Lepidoptera: Pyralidae) on maize (Huang et al., 2009; Piesik et al., 2009), *Spodoptera frugiperda* (J.E. Smith) (Lepidoptera: Noctuidae) on maize and cowpea (Carroll et al., 2008, 2006), and *Estigmene acrea* (Drury) (Lepidoptera:Arctiidae) on soybean, tomato, and maize (Castrejon et al., 2009). Furthermore, caterpillars adapt their behaviour depending on plant VOC emission (Shiojiri et al., 2006). This attraction to VOCs emitted by already infested host plants is puzzling, as it will lead to competition and may increase the risk of cannibalism and attack by natural enemies that are attracted to the same volatiles. Cannibalism is common among noctuid larvae, such as *Spodoptera littoralis* (Boisduval) (Abdel Salam, F. and Fokhar, F. cited in Fox, 1975), *S. frugiperda* (Chapman et al., 1999), and *Helicoverpa armigera* (Hübner) (Kakimoto et al., 2009). The attraction of natural enemies to herbivore-induced volatiles has been shown for numerous tritrophic systems (Dicke et al., 1990; Turlings et al., 1990), which makes one wonder why lepidopteran larvae are attracted to the same volatiles. This apparent

maladaptive behavior may be explained by a trade-off between risks: in the field harsh weather conditions and attempts to escape parasitoids and predators cause larvae to frequently fall off plants (personal observ.). In order to find back the same plant or new suitable plants the larvae will have to rely on dependable and readily available VOC signals. Induced VOCs may provide the best cues, as undamaged plants are virtually odourless (Turlings et al., 1990). However, once on a plant, caterpillars may prefer sites with minimal VOC emissions, where it is less likely to encounter competitors and natural enemies.

We therefore hypothesized that caterpillars may initially be attracted to induced VOCs, but once they are on the plant they will feed preferentially in places with low GLV emissions. We tested this for *S. littoralis* larvae. First we confirmed attraction to induced plant volatiles in a four-arm olfactometer and then tested their growth rate as a measure of feeding behavior when they were exposed to GLVs. Previous feeding experiences were also taken into consideration, as larval attraction may be higher for volatiles that are emitted by plant species on which the larvae previously fed (Carlsson et al., 1999).

## IV. 2 Material and Method

### IV. 2.1 Plants and insects.

Maize plants (cv Delprim) were grown individually in plastic pots (10 cm high, 4 cm diameter) with commercial potting soil (Ricoter Aussaaterde, Aarberg, Switzerland) and placed in a climate chamber (23°C, 60% r.h., 16:8 h L:D, 50000 lm/m<sup>2</sup>). Maize plants used for the experiments were ten to twelve days old and had three fully developed leaves. The evening before the experiments, plants were transferred into glass vessels, as described in Turlings et al. (2004) and kept under laboratory conditions (25±2°C, 40±10% r.h., 16:8 h L:D, and 8000 lm/m<sup>2</sup>). *S. littoralis* larvae were reared from eggs provided by Syngenta (Stein, Switzerland). The eggs were kept in an incubator at 30.0±0.5°C until emergence of the larvae, which were then transferred to artificial diet at room temperature (24±4°C).

## IV. 2.2 A four-arm olfactometer to measure larval attraction

Two olfactometer experiments were performed with fourth-instar *S. littoralis* larvae. In the first experiment, the attraction of larvae to an induced maize plant versus healthy maize plant was compared. In the second experiment, the attraction of larvae to a maize plant with fresh damaged was tested against a plant with old damaged. In both experiments, the effect of previous feeding experience (either artificial diet or maize) was compared. All the larvae were initially reared on artificial diet as previously described (Turlings et al., 2004). Twenty-four hours before each experiment, 90 larvae were transferred to fresh maize leaves (maize feeding experience), and 90 on artificial diet (artificial diet feeding experience). A four-arm olfactometer (D'Alessandro & Turlings, 2005) was modified to measure the attraction of *S. littoralis* larvae. The olfactometer consisted of a central glass choice arena (Figure IV.1) (6 cm internal diameter, 5 cm length) with four arms (15mm ID, 5 cm length), each with a glass elbow (5cm length) and an upward connection for a glass bulb (50ml). To avoid visual distraction of the larvae, a white cardboard cylinder was placed around the central choice arena and on top of the choice arena.

## IV. 2.3 Attraction of fourth-instar *S. littoralis* larvae to induced maize plants.

The choice arena was connected to four glass bottles. One bottle contained a maize plant infested with fifteen second-instar *S. littoralis* larvae that had been placed on the plant sixteen hours before the bioassay. The opposite bottle contained a healthy maize plant. The two remaining bottles remained empty. The position of the odour sources was changed between each experimental day, with the two odour sources always opposite to each other.

Thirty fourth-instar larvae were placed in plastic box (2x2x0.8cm) with an open top, which was introduced in the centre of the choice arena. The larvae crawled out of the box and entered one of the four arms. After sixty minutes, the number of larvae in each arm was counted. The larvae that did not leave the choice arena after sixty minutes were considered as having made no choice and all the larvae were removed from the olfactometer. Six such releases were done on a given day.

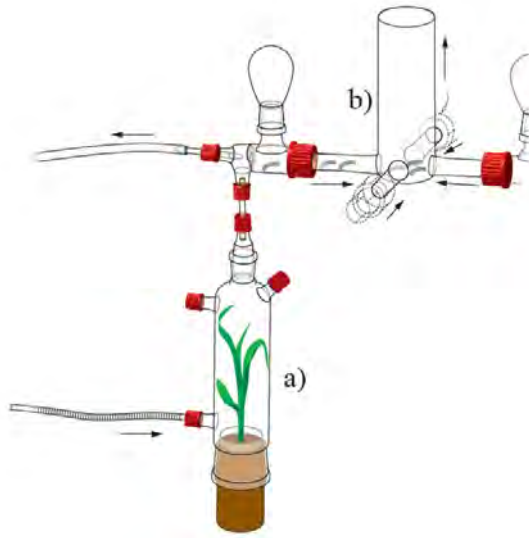


Figure IV.1: Detail of the four-arm olfactometer setup for *S. littoralis* larval behaviour. a) Odour source. b) Choice arena. Arrows indicate airflows. Four odour sources are compared, placed at each of the four arms of the choice arena. Only one of the odour source bottles is shown in this drawing, which was modified after Thomas Degen ([www.thomas-degen.ch](http://www.thomas-degen.ch)).

#### IV. 2.4 Attraction of fourth-instar *S. littoralis* larvae to plants with old versus plants with fresh damage.

The same setup as described above was used, with the same experimental procedure, except for the odour sources. Two maize plants were brought to the laboratory sixteen hours prior to the bioassay. One plant was scratched on the underside of the two oldest leaves, damaging approximately two cm<sup>2</sup>, on both sides of the central vein (Hoballah & Turlings, 2005). Caterpillar regurgitant, collected as described in Turlings et al. (1998), was applied to the two wounds. Both plants were then placed in a glass bottle and exposed to a carbon-filtered, humidified airflow of 300 ml/min for fifteen hours. The second plant was then scratched and regurgitant was applied. The two plants were then placed opposite to each other in the olfactometer, leaving two empty bottles between them. The airflow was then increased to 1200 ml/min. To collect part of the volatiles for subsequent analysis (see below), 700 ml/min of the flow was diverted over a SuperQ filter (D'Alessandro et al., 2009). The position of the treatments was changed for each experimental day. The volatiles trapped on the SuperQ filters were analyzed and quantified using gas chromatography and flame ionisation detector as described by D'Alessandro & Turlings (2005).

#### IV. 2.5 VOC-exposure experiments.

Three experiments were conducted to measure the effect of VOCs on the growth of *S. littoralis* larvae. In the first experiment, the larvae were exposed to the VOCs emitted by *S. littoralis*-infested maize plants. In the second experiment, the larvae were exposed to physiologically relevant amounts of a blend of synthetic GLVs. In the third experiment, they were exposed to high concentrations of synthetic GLVs. In all three experiments we recorded, besides weight gain, mortality and pupation of the larvae.

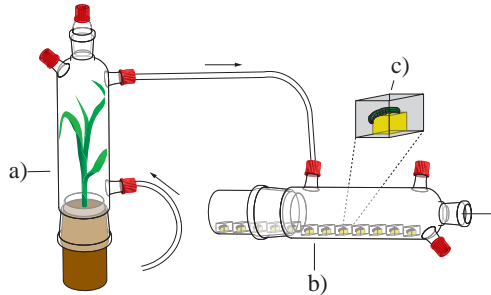


Figure IV.2: Design of growth performance experiment. a) Odour source bottle, which contained either a healthy maize plant or a caterpillar-damaged maize plant. b) Bottles containing 12 larvae inside small plastic boxes. c) Plastic box enlarged showing a *S. littoralis* larva feeding on a cube of artificial diet. Arrows indicate the direction of the airflow.

#### IV. 2.6 Effect of exposure to VOCs emitted by caterpillar-damaged maize plants on feeding rate of *S. littoralis* larvae.

Second-instar *S. littoralis* were placed individually inside small plastic boxes (2x2x1.5cm) that were covered with fine-meshed nylon tissue, fixed with an elastic band. The larvae were provided a 1 cm<sup>3</sup> cube of wheatgerm-based artificial diet (Turlings et al. 2004), which was changed every second day. Twelve such boxes were placed inside a glass bottle lying on its side, connected at its base with a Teflon tube to the top of an odour source bottle (Figure IV.2) (see Turlings et al., 2004, for details on glass bottles and tubing). Odour source bottles contained either a maize plant infested with fifteen second-instar *S. littoralis* larvae (induced plant, VOC<sub>i</sub>, replaced with a new infested plant every third day) or an uninfested maize plant (control plant, VOC<sub>u</sub>, also replaced every third day). The odour source bottle was connected to a four-port air-delivery system (Model VCS-HADS-6AF6C6B; ARS Analytical Research Systems, Gainesville, FL, USA), providing a purified and humidified airflow of 300 ml/minute. Two such four-port air-delivery systems were used simultaneously to introduce odours into eight exposure chambers, resulting in 48 larvae for each treatment.

Before placing the larvae inside the plastic boxes, they were weighted on a microbalance (Model MX5, Mettler Toledo, Greifensee, Switzerland). Weighing was repeated at the following time-points after placing the boxes

inside the glass bottle: five hours, 24h, 48h, 96h, 144h, 192h, 240h, 288h, 312h, 336h, 360h, 408h, and 432h. After this time-point, all larvae had pupated or died and the experiment was terminated.

#### IV. 2.7 Effect of exposure to synthetic GLVs on weight gain of *S. littoralis* larvae.

The same setup as described above was used for this experiment. In this case, the odour source bottles containing a dispenser built up as follows: a 2 ml amber glass vial (11.6 x 32 mm; Sigma-Aldrich, Buchs, Switzerland) containing 100 mg clean fibreglass wool. The vial was sealed with a PTFE/rubber septum (Sigma-Aldrich, Buchs, Switzerland) pierced with a 2l micro-pipette (Drummond, Millan SA, Plan-Les-Ouates, Switzerland). The length of the pipette was calibrated to release a controlled amount of GLVs, similar to the amount emitted by maize plants (cv Delprim). The GLV mixture consisted of 80% (*Z*)-3-hexen-1-al (92.5% purity, (NEAT), Bedoukian Research, Danbury, CT, USA), 10% (*Z*)-3-hexenyl-acetate (98%, SAFC Supply Solutions, St. Louis, MO, USA), 8% (*Z*)-3-hexenyl-Acetate (98%, SAFC Supply Solutions, 3050 Spruce Street, St. Louis, MO 63103, USA) and 2% (*E*)-2-hexenol (99%, ACROS Organics, Geel, Belgium). The same GLV dispenser was kept for the duration of the assay. Control bottles contained no dispenser.

In this experiment, the weighing of the larvae was repeated at 5, 12, 24, 48, 96, 120h, and 144 hours after placement in the bottles. The experiment was terminated at 144 hours because the tests showed that larval weight was not affected by the volatiles at these concentrations.

#### IV. 2.8 Effect of exposure to high concentrations of GLVs on weight gain of *S. littoralis* larvae.

In this experiment, larvae were placed individually in a plastic box (7.5 x 6.5 x 5cm) containing a GLV dispenser (described above), and a piece of diet (2 x 1.5 x 1 cm). The box was closed, in order to increase the concentration of GLVs. As a control, an empty dispenser was placed inside the cage without GLVs inside. There were twelve larvae in each treatment and they were weighed before placing them inside the boxes. They were weighted again after 3h, 6h, 9h, 12h, 15h, 24h, 40h, 48h, 51h, 54h, 58h, 72h, 96h, 120h, and 168h. The larger plastic boxes allowed for more mobility, compared to the cages used in the previous experiments. In order to

observe whether the high concentrations of GLV affected larval mobility, we recorded whether the larvae were on the diet or off the diet during the first eight hours of exposure.

## IV. 2.9 Statistical analysis.

VOC-exposure data were compared using Student's *t*-test, provided they met the assumptions of normality (Shapiro-Wilk test) and equal variance (Levene's test). Else, a Mann-Whitney test was applied. Both treatments (VOC<sub>u</sub> and VOC<sub>i</sub> exposure) were compared at each time-point individually. Data on mortality and pupation of the larvae compared using a one-way analysis of variance (ANOVA). Data was tested with SigmaStat (version 3.5, STATCON, Witzenhausen, Germany). Data on mobility were analysed in a GLM with binomial distribution (the larvae were observed either on the diet or off the diet) family in R (R Development Core Team, 2009). Olfactometer data was analysed using the software package R (R Development Core Team, 2009), in a general linear model (GLM), with the quasi-poisson distribution, allowing to compensate for overdispersed data, as described in D'Alessandro & Turlings (2005).

## IV. 3 Results

### IV. 3.1 Attraction of fourth-instar *S. littoralis* larvae to induced maize plants.

The larvae that had fed on maize and the larvae fed on artificial diet were both more attracted towards caterpillar-damaged maize plants than to intact plants (GLM,  $P < 0.001$  and  $P < 0.002$ , respectively) (Figure IV.3). However, the maize-fed larvae were attracted more strongly by the induced plants than the diet-fed larvae (GLM,  $P < 0.005$ ). In both cases, empty arms were not attractive. Maize-fed larvae also displayed an increased responsiveness (80%) compared to diet-fed larvae (66%).

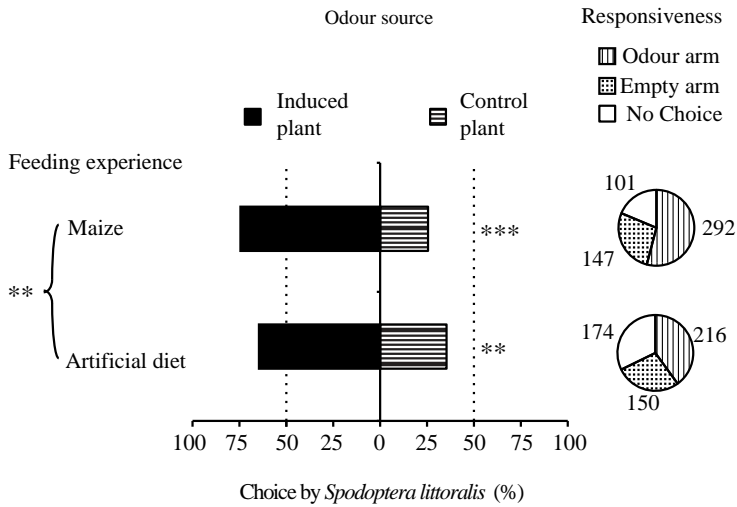


Figure IV.3: Effect of feeding experience on the attraction of *S. littoralis* larvae to induced maize plants. Pie charts indicate overall responsiveness (number of larvae entering the different types of arms). GLMs were performed to test for differences between arms within each group of feeding experience, as well as to compare feeding experiences. Two asterisks indicate a significant difference ( $P < 0.01$ ), three asterisks  $P < 0.001$ .

### IV. 3.2 Attraction of fourth-instar *S. littoralis* larvae to plants with old versus plants with fresh damage.

Freshly damaged plants were more attractive to maize-fed larvae (GLM  $P < 0.003$ ) than plants with older damage (Figure IV.4). Artificial diet-fed larvae did not show a preference between old and fresh damage. This difference in preference between maize-fed and diet-fed larvae was significant (GLM,  $P < 0.001$ ). Also in this case, overall responsiveness of maize-fed larvae (84%) was higher than the responsiveness of artificial diet-fed larvae (62%).

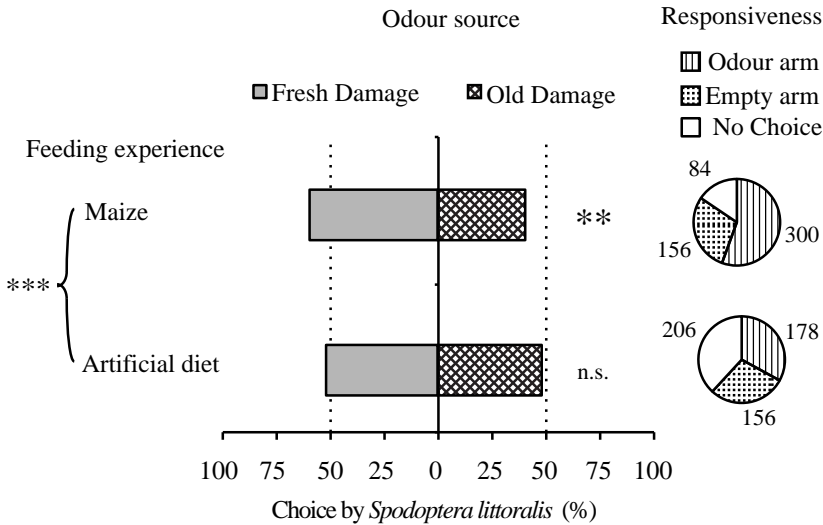


Figure IV.4: Effect of feeding experience on the attraction of *S. littoralis* larvae to old and fresh damaged maize plants. Pie charts indicate overall responsiveness (number of larvae entering the different types of arms). GLMs were performed to test for differences between arms within each group of feeding experience, as well as to compare feeding experiences. n.s.: no significant difference ( $P > 0.05$ ), two asterisks indicate a significant difference ( $P < 0.01$ ), three asterisks  $P < 0.001$ .

Figure IV.5 shows the chromatograms of fresh damage (Figure IV.5 A) and old damage (Figure IV.5 B) maize plants. Quantities emitted corresponded to the ones found by Hoballah & Turlings (2005).

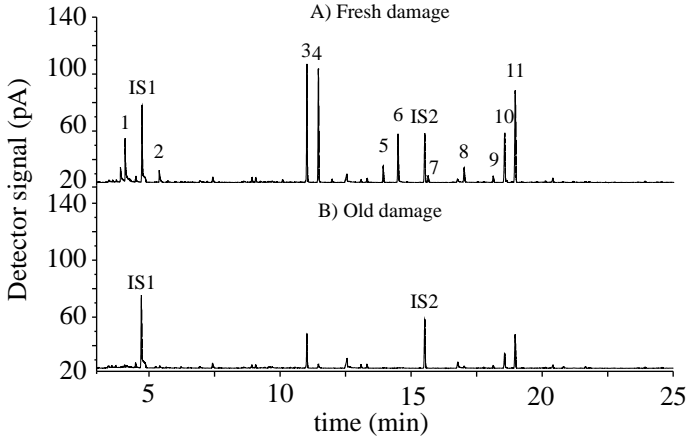
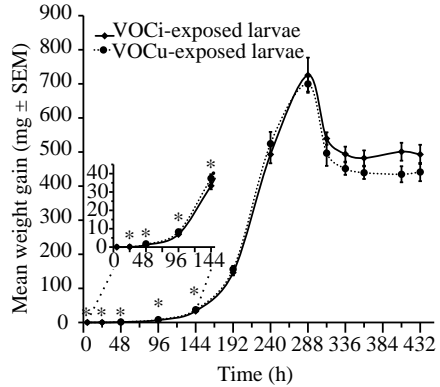


Figure IV.5: Chromatograms of mechanically wounded plants: A) Fresh damage, B) old damage. The VOCs are as follows: 1: (*Z*)-3-hexenal, 2: (*E*)-2-hexenal, 3: linalool, 4: DMNT, 5: phenethyl acetate, 6: indole, 7: methyl anthranilate, 8: geranyl acetate, 9: (*E*)- $\beta$ -caryophyllene, 10: (*E*)- $\alpha$ -bergamotene, 11: (*E*)- $\beta$ -farnesene. IS1 and IS2 correspond to internal standards.

### IV. 3.3 Effect of exposure to VOCs emitted by caterpillar-damaged maize plants on feeding rate of *S. littoralis* larvae.

The larvae that were exposed to the VOCs emitted by caterpillar-damaged maize plant grew more slowly in the early stages of development (Figure IV.6). Initial weight of the larvae was equal across treatments. After five hours, there was still no difference between the two treatment groups ( $P < 0.356$ ). However, after 24 hours, the larvae exposed to VOCs from damaged plants (VOC<sub>i</sub>) had gained significantly less weight than the larvae exposed to VOCs emitted by healthy plants (VOC<sub>u</sub>) ( $P < 0.030$ ). This difference in growth rate persisted throughout the early weighing time points: 48 hours ( $P < 0.030$ ), 96 hours ( $P < 0.012$ ), 144 hours ( $P < 0.033$ ). After this, both treatment groups displayed similar weight gains until pupation. The weight of the pupae did not differ significantly ( $P < 0.916$ ). Furthermore, there was no difference in mortality of the larvae ( $P < 0.839$ ).

Figure IV.6: Mean weight gain ( $\text{mg} \pm \text{SEM}$ ) of *S. littoralis* larvae exposed to VOCs emitted by *S. littoralis*-induced (VOCi) or healthy (VOCu) maize plants. One asterisk indicates a significant difference (Student's t-test,  $P < 0.05$ ).



#### IV. 3.4 Effect of exposure to synthetic GLVs on feeding of *S. littoralis* larvae.

When larvae were exposed to the synthetic volatile blend we measured no difference either in larval weight gain (5h:  $P < 0.759$ , 12h:  $P < 0.286$ , 24h:  $P < 0.267$ , 48h:  $P < 0.502$ , 72h:  $P < 0.506$ , 96h:  $P < 0.833$ , 120h:  $P < 0.833$ , 144h:  $P < 0.646$ ), or mortality (0% in both treatments).

#### IV. 3.5 Effect of exposure to high concentrations of GLVs on feeding of *S. littoralis* larvae.

When larvae were exposed to high concentrations of green leaf volatiles, such as can be expected to be present in the immediacy of the feeding sites on the maize plants, the larvae were found to gain less weight at the early stages of their development (Figure IV.7). After three hours ( $P < 0.514$ ) and six hours ( $P < 0.173$ ), there was still no difference between the treatments. After exposure to green leaf volatiles for nine hours a strong trend of lower weight gain in GLV-exposed larvae was observed ( $P < 0.051$ ) and at twelve hours the difference between the two treatments was significant ( $P < 0.025$ ). This difference persisted throughout the early part of the experimental time (15h:  $P < 0.036$ ; 24h:  $P < 0.027$ ; 40h:  $P < 0.031$ ; 48h:  $P < 0.030$ ; 51h:  $P < 0.033$ ; 54h:  $P < 0.039$ ; 58h:  $P < 0.038$ ; 72h:  $P < 0.047$ ). From 96h, however, there was no longer a difference in weight gain between the treatments. Interestingly, the mobility of GLV-exposed larvae was slightly increased ( $P < 0.060$ ), with a significant difference in number of larvae moving in the box after six hours ( $P < 0.048$ ). However,

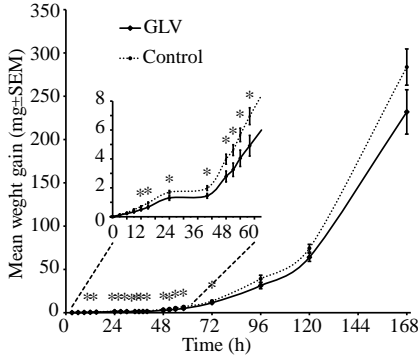


Figure IV.7: Weight gain (mg±SEM) of *S. littoralis* larvae exposed to GLV dispensers or control dispensers. An asterisk above the value point indicates significant difference between treatments ( $P < 0.05$  Students t-test).

at 30 minutes ( $P < 0.410$ ), two hours ( $P < 0.716$ ), four hours ( $P < 0.572$ ), and eight hours ( $P < 0.423$ ), GLV-exposed and control larvae were equally on the diet and off the diet.

## IV. 4 Discussion

This study confirms that *S. littoralis*, are capable of "learning" the odour of their substrate and are increasingly attracted to their host plant, which was already demonstrated in *S. littoralis* larvae on cotton (Carlsson et al., 1999). Maize-fed larvae were increasingly attracted to induced maize plants compared to artificial diet-fed larvae (Figure IV.3). Furthermore, they displayed a preference for fresh-damaged maize plants, which emit elevated amounts GLVs (Figure IV.4). The attraction of *S. littoralis* to fresh damage versus old damage, and its increase with feeding experience, seems contradictory when considering the detrimental effect of exposure to high amounts of GLVs described above.

The question that arises from this finding is whether the risk of facing cannibalism in an already infested host plant or the risk of starvation is most important when identifying a host. Our results suggest that larvae of noctuids prefer to find a suitable host and meet a potentially dangerous conspecific than taking the risk of starvation (Carroll et al., 2006). Furthermore, the preference of freshly damaged maize plants over old damaged maize plants indicates that they prefer host plants that have been colonized recently. This could indicate that the larvae of *S. littoralis* are capable of distinguishing the age of the feeding conspecifics depending on the blend of VOCs emitted by the host plant, supposing that small larvae have fed

shorter time on the maize plants and therefore induce "fresh damage", while large larvae have fed longer time and induce more "old damage". However, it was shown that different larval stages do not cause significant changes in the blend of VOCs emitted by maize plants (Gouinguéné et al., 2003). However, further studies should investigate how the developmental stage of the larvae that have already infested the maize plant influences the choice of the larvae. It could indeed well be that larvae can identify the stage of the infesting host and are repelled by plants infested by hosts that are in a much higher developmental stage, to prevent becoming victims of cannibalism (Chapman et al., 1999, 2000; Richardson et al., 2009). The increased amount of linalool emitted from fresh damaged plants (Figure IV.5) (data not shown, but see Hoballah & Turlings, 2005, who measured equivalent amounts) confirms the results on *S. frugiperda* published by Carroll et al. (2006), which showed a high attraction of *S. frugiperda* to this particular compound. It was shown in wild tobacco that increased linalool decreased oviposition by adult Lepidoptera (De Moraes et al., 2001; Kessler & Baldwin, 2001). Therefore a plant that is not attractive to adult Lepidoptera could represent a safe haven for larvae, as it is less likely to receive oviposition and thus future conspecific competition. Another explanation for the preference for fresh damage could also point towards a preference for plants with lower accumulation of induced direct defences in the leaf tissue, such as de novo synthesized antifeedant compounds. *S. littoralis* larvae would therefore prefer plants that have activated their direct defences against herbivory, besides being plants with lower emission of VOCs, which were here shown to decrease the feeding rate of early developmental stages.

To study the direct defence effect of maize VOCs against their host, we exposed *S. littoralis* larvae to the VOCs induced by their conspecifics on maize plants (Figure IV.6). To our knowledge, so far no study showed the effect of VOCs on the feeding rate of lepidopteran larvae. Our results also suggest that the larvae of *S. littoralis* are capable of adapting to volatile exposure over time, so that the pupal weight attained compares to larvae that were not exposed to these volatiles. This adaptability of lepidopteran larvae to detrimental feeding substrates was exemplified in *Spodoptera exigua* (Hübner) feeding on plants expressing a protease inhibitor (Jongsma et al. 1995; Lara et al. 2000). It was also shown that *S. frugiperda* could adapt to DIMBOA without any decrease in feeding rate or mortality (Rostás, 2007). Furthermore, the sphingid *Manduca sexta* (L.) was shown to adapt to caffeine and nicotine ingestion (Kester et al., 2002). The negative effect of the VOCs on the feeding rate of the larvae could however be due to other effects, such as a comparatively lower concentration of oxygen

or the higher concentration of carbon dioxide in the airstream issued by induced plants (VOC<sub>i</sub>) than by uninduced plants (VOC<sub>u</sub>). These gases could indeed be altered in the airstream of induced plants due to reduced amount of photosynthetic plant tissue and the respiration of the feeding larvae. In the experiment with low concentrations of GLVs the growth rate of *S. littoralis* larvae was not affected, which suggests that the GLVs emitted by the VOC<sub>i</sub> plants were not biologically active on the exposed larvae. However, it can be expected that the concentrations of GLVs that reign locally at the feeding site are much higher than what is measured at plant level (Turlings & Tumlinson, 1992). Our results indicate that high concentrations of GLVs reduce weight gain in early stages (Figure IV.7). However, *S. littoralis* larvae adapted eventually, and had equal final pupal weights. Further studies should investigate which VOCs beside the GLVs reduce the early growth rate of *S. littoralis* larvae, and at which concentrations.

The attraction of the larvae of the agricultural pest *S. littoralis* towards specific VOCs could be exploited in sustainable agriculture practices, such as "push-pull" systems (Cook et al., 2007; Khan et al., 2008, 2000). By finding more attractive host plants, which could be planted close to the crop plants, larvae could be attracted away from their host plant. However, the increased preference of *S. littoralis* for host plants they have previously been feeding on may complicate this approach. Our results suggest however, that in a comprehensive VOC-mediated biological control strategy, besides the attraction of predators, namely parasitoids, the behaviour of the larval and adult stages of the pest should be taken into account.

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## Conclusion and outlook

In the introduction, we evoked three levels at which the volatile organic compound (VOC) emission of maize plants can be manipulated in the field and the laboratory, in order to improve the attraction of beneficial insects: the VOC level, the metabolic level and the genetic level. Our results indicate that the modification of the VOC emission of maize plants indeed affects the insects both in the field and the laboratory. In Chapter I, we showed that the release of synthetic green leaf volatiles in the field increasingly attracted *Diabrotica* beetles, but did not affect the infestation by *Spodoptera frugiperda*. Parasitism of *S. frugiperda* was not affected either, and the parasitism rate by egg-larval parasitoids, on some occasions, was diminished by the synthetic GLVs. The treatment of maize plants with pathogen and herbivore resistance elicitors (Chapter II) in the field did not affect pest insects nor the parasitism of *S. frugiperda*. However, the VOC emission of maize plants was increased by the herbivore resistance elicitor. Furthermore, under more controlled conditions, the pathogen resistance elicitor decreased the infestation by *Bipolaris maydis*. We therefore concluded that the use of pathogen resistance elicitors in the field is compatible with biological control of maize pests. In Chapter III, we showed that maize plants with improved protein content and increased drought tolerance in maize do not affect the efficacy of beneficial insects for biological control. Nonetheless, the breeding lines that were tested varied in their VOC emission, within and between breeding program. Finally, in Chapter IV, we demonstrated that the VOCs emitted by maize plants upon conspecific feeding increasingly attracted *Spodoptera littoralis* larvae

and that this attraction was increased when the larvae had previously fed on maize. Conversely, the same VOCs decreased the feeding rate of the larvae in early instars. We speculate that in a trade-off situation, *S. littoralis* larvae value the finding of a suitable host plant more than the risk of conspecific encounter, and the ensuing cannibalism.

From these findings, we can draw several conclusions:

1. Slight variations in VOCs affect the insect population in the field (Chapter I). However, for improved biological control the attraction of beneficial insects needs to be taken into consideration as much as the attraction of pest insects. These may indeed be attracted to plant VOCs, which could lead to an overall negative effect. This calls for an integrative approach to understand the systems plants are growing in, for example with molecular techniques (Baldwin, 2010). In the field, many herbivores are feeding on one plant, which can influence parasitoid attraction. While recent studies relativize the effect of multiple herbivory on parasitoid attraction, they also indicate that root herbivory and shoot herbivory interact (Erb, 2009; Erb et al., 2008). Furthermore, our studies on *S. littoralis* larvae and on *S. frugiperda* by Carroll et al. (2006) clearly demonstrate that under laboratory conditions these herbivores are attracted towards VOC-emitting plants. Field observations will reveal how these interactions affect biological control of maize pests.
2. The key VOCs that mediate beneficial insect attraction have not been identified yet. Recent studies indicate that some VOCs are repellent (D'Alessandro et al., 2006), while minor VOCs are attractive to parasitoids (D'Alessandro et al., 2009). Further separation techniques of VOC samples will help elucidate the key compounds. In addition to metabolic manipulation of the plants, molecular advances, such as the production of 'mute' plants (Baldwin et al., 2006; Dicke & Baldwin, 2010; Paschold et al., 2006), or the insertion of VOC-emission genes into model plant species (Schnee et al., 2006) can improve our understanding of key VOCs in parasitoid attraction. Promising results with the attraction of nematodes to control a root pest indicate that molecular methods can be successful in the field (Degenhardt et al., 2009).
3. Little is known so far about some of the parasitoids that we found in our fields (Chapter I and II). For instance, the population genetics (Jourdie et al., 2010) and the geographical distribution (Molina-Ochoa et al., 2003, 2004) of *Chelonus insularis* have been investigated, but behavioural data of this important parasitoid is lacking.

Recent studies indicate that some egg-parasitoid are attracted to sexual pheromones emitted by adult females of their host species (Bruce et al., 2009; Huigens et al., 2009). For these species, induced plant VOCs may therefore play a different role than for larval parasitoids. Furthermore, classical biological data, such as life cycle and alternate hosts is lacking for some of the species that were collected. This data could improve the rearing of parasitoids which would allow behavioral and physiological studies in the laboratory.

4. Pathogen resistance elicitors were found to be compatible with biological control (Chapter II). However, in order to increase parasitism under field conditions their application needs to be carried out at the earliest-most time-point, in order to precede pest attack by adult and highly mobile herbivores. This could be achieved by treating seeds with elicitors. The seedlings would then germinate with activated defences and the effect of the elicitor treatment could be measured already in early growth stages (Danielson, 2003). However, it is not clear for how much time after application elicitors affect the plant metabolism. Investigations on alternative pathogen-resistance elicitors may improve our understanding of the increased attraction of parasitoids observed with BTH Rostás & Turlings (2008).

In summary, the intricacies of tri-trophic interactions need to be better understood, before we can apply this strategy as way towards a more sustainable agriculture.



## Bibliography

- Andrews, K. L. (1988). Latin american research on *Spodoptera frugiperda* (Lepidoptera: Noctuidae). *The Florida Entomologist*, 71(4), 630–653.
- Andrews, K. L., Bentley, J. W., & Cave, R. D. (1992). Enhancing biological control's contributions to integrated pest management through appropriate levels of farmer participation. *The Florida Entomologist*, 75(4), 429–439.
- Arimura, G., Matsui, K., & Takabayashi, J. (2009). Chemical and molecular ecology of herbivore-induced plant volatiles: proximate factors and their ultimate functions. *Plant and Cell Physiology*, 50(5), 911–923.
- Arimura, G., Ozawa, R., Horiuchi, J., Nishioka, T., & Takabayashi, J. (2001). Plant-plant interactions mediated by volatiles emitted from plants infested by spider mites. *Biochemical Systematics and Ecology*, 29(10), 1049–1061.
- Ashley, T. R. (1986). Geographical distributions and parasitization levels for parasitoids of the fall armyworm, *Spodoptera frugiperda*. *Florida Entomologist*, 69(3), 516–524.
- Bahena Juarez, F. (2003). Control biológico de la plagas del maíz en México: el caso del "gusano cogollero" *Spodoptera frugiperda* (J.E. Smith) (Lepidoptera: Noctuidae). *RAPAM*, 241–255.
- Baldwin, I. T. (2010). Plant volatiles. *Current Biology*, 20(9), 392 – 397.

- Baldwin, I. T., Halitschke, R., Paschold, A., von Dahl, C. C., & Preston, C. A. (2006). Volatile signaling in plant-plant interactions: "talking trees" in the genomics era. *Science*, *311*(5762), 812–815.
- Bale, J. S., Masters, G. J., Hodkinson, I. D., Awmack, C., Bezemer, T. M., Brown, V. K., Butterfield, J., Buse, A., Coulson, J. C., Farrar, J., Good, J. E. G., Harrington, R., Hartley, S., Jones, T. H., Lindroth, R. L., Press, M. C., Symrnioudis, I., Watt, A. D., & Whittaker, J. B. (2002). Herbivory in global climate change research: direct effects of rising temperature on insect herbivores. *Global Change Biology*, *8*(1), 1–16.
- Bantte, K. & Prasanna, B. M. (2004). Endosperm protein quality and kernel modification in the quality protein maize inbred lines. *Journal of Plant Biochemistry and Biotechnology*, *13*(1), 57–60.
- Bänziger, M., Setimela, P. S., Hodson, D., & Vivek, B. (2006). Breeding for improved abiotic stress tolerance in maize adapted to southern Africa. *Agricultural Water Management*, *80*(1-3), 212–224.
- Bari, R. & Jones, J. (2009). Role of plant hormones in plant defence responses. *Plant Molecular Biology*, *69*(4), 473–488.
- Beckers, G. J. M. & Spoel, S. H. (2006). Fine-tuning plant defence signalling: Salicylate versus jasmonate. *Plant Biology*, *8*(1), 1–10.
- Bergvinson, D. J., Arnason, J. T., Hamilton, R. I., Mihm, J. A., & Jewell, D. C. (1994). Determining leaf toughness and its role in maize resistance to the European corn-borer (Lepidoptera: Pyralidae). *Journal of Economic Entomology*, *87*(6), 1743–1748.
- Bernasconi, M. L., Turlings, T. C. J., Ambrosetti, L., Bassetti, P., & Dorn, S. (1998). Herbivore-induced emissions of maize volatiles repel the corn leaf aphid, shape *Rhopalosiphum maidis*. *Entomologia Experimentalis et Applicata*, *87*(2), 133–142.
- Bolaños, J. & Edmeades, G. O. (1993). Eight cycles of selection for drought tolerance in lowland tropical maize. ii. responses in reproductive behavior. *Field Crops Research*, *31*(3-4), 253–268.
- Bolter, C. J., Dicke, M., Van Loon, J. J. A., Visser, J. H., & Posthumus, M. A. (1997). Attraction of Colorado potato beetle to herbivore-damaged plants during herbivory and after its termination. *Journal of Chemical Ecology*, *23*(4), 1003–1023.
- Boughton, A. J., Hoover, K., & Felton, G. W. (2005). Methyl jasmonate application induces increased densities of glandular trichomes on tomato,

- 
- Lycopersicon esculentum*. *Journal of Chemical Ecology*, 31(9), 2211–2216.
- Boughton, A. J., Hoover, K., & Felton, G. W. (2006). Impact of chemical elicitor applications on greenhouse tomato plants and population growth of the green peach aphid, *Myzus persicae*. *Entomologia Experimentalis Et Applicata*, 120(3), 175–188.
- Bruce, A. Y., Schulthess, F., Mueke, J., & Calatayud, P. A. (2009). Olfactory attraction of egg parasitoids to virgin females of noctuid stemborers. *Biocontrol*, 54(6), 763–772.
- Bruce, T. J. A. & Pickett, J. A. (2007). Plant defence signalling induced by biotic attacks. *Current Opinion in Plant Biology*, 10(4), 387–392.
- Bruinsma, M., Posthumus, M. A., Mumm, R., Mueller, M. J., van Loon, J. J. A., & Dicke, M. (2009). Jasmonic acid-induced volatiles of *Brassica oleracea* attract parasitoids: effects of time and dose, and comparison with induction by herbivores. *J. Exp. Bot.*, 60(9), 2575–2587.
- Byrne, P. F., Bolanos, J., Edmeades, G. O., & Eaton, D. L. (1995). Gains from selection under drought versus multilocation testing in related tropical maize populations. *Crop Science*, 35(1), 63–69.
- Çakir, R. (2004). Effect of water stress at different development stages on vegetative and reproductive growth of corn. *Field Crops Research*, 89(1), 1–16.
- Carlsson, M. A., Anderson, P., Hartlieb, E., & Hansson, B. S. (1999). Experience-dependent modification of orientational response to olfactory cues in larvae of *Spodoptera littoralis*. *Journal of Chemical Ecology*, 25(11), 2445–2454.
- Carroll, M., Schmelz, E., & Teal, P. (2008). The attraction of *Spodoptera frugiperda* neonates to cowpea seedlings is mediated by volatiles induced by conspecific herbivory and the elicitor inceptin. *Journal of Chemical Ecology*, 34(3), 291–300.
- Carroll, M. J., Schmelz, E. A., Meagher, R. L., & Teal, P. E. A. (2006). Attraction of *Spodoptera frugiperda* larvae to volatiles from herbivore-damaged maize seedlings. *Journal of Chemical Ecology*, 32(9), 1911–1924.
- Castrejon, F., Virgen, A., & Rojas, J. C. (2009). Influence of chemical cues from host plants on the behavior of neonate *Estigmene acrea* larvae (Lepidoptera: Arctiidae). *Environmental Entomology*, 35(3), 700–707.

- Cave, R. D. (1995). *Manual para el reconocimiento de parasitoides de plagas agrícolas en América Central*. El Zamorano: Zamorano Academic Press.
- Chapman, J. W., Williams, T., Escribano, A., Caballero, P., Cave, R. D., & Goulson, D. (1999). Fitness consequences of cannibalism in the fall armyworm, *Spodoptera frugiperda*. *Behav. Ecol.*, *10*(3), 298–303.
- Chapman, J. W., Williams, T., Martínez, A. M., Cisneros, J., Caballero, P., Cave, R. D., & Goulson, D. (2000). Does cannibalism in *Spodoptera frugiperda* (Lepidoptera: Noctuidae) reduce the risk of predation? *Behavioral Ecology and Sociobiology*, *48*(4), 321–327.
- Cipollini, D., Purrington, C. B., & Bergelson, J. (2003). Costs of induced responses in plants. *Basic and Applied Ecology*, *4*(1), 79–89.
- Clark, P. L., Molina-Ochoa, J., Martinelli, S., Skoda, S. R., Isenhour, D. J., Lee, D. J., Krumm, J. T., & Foster, J. E. (2007). Population variation of the fall armyworm, *Spodoptera frugiperda*, in the western hemisphere. *Journal of Insect Science*, *7*.
- Conrath, U., Beckers, G. J. M., Flors, V., Garcia-Agustin, P., Jakab, G., Mauch, F., Newman, M. A., Pieterse, C. M. J., Poinssot, B., Pozo, M. J., Pugin, A., Schaffrath, U., Ton, J., Wendehenne, D., Zimmerli, L., Mauch-Mani, B., & Grp, P.-A.-P. (2006). Priming: Getting ready for battle. *Molecular Plant-Microbe Interactions*, *19*(10), 1062–1071.
- Cook, S. M., Kahn, Z. R., & Pickett, J. A. (2007). The use of push-pull strategies in integrated pest management. *Annual Review of Entomology*, *52*, 375–400.
- Courtois, E. A., Paine, C. E. T., Blandinieres, P. A., Stien, D., Bessiere, J. M., Houel, E., Baraloto, C., & Chave, J. (2009). Diversity of the volatile organic compounds emitted by 55 species of tropical trees: a survey in french guiana. *Journal of Chemical Ecology*, *35*(11), 1349–1362.
- D'Alessandro, M., Brunner, V., von Mérey, G. E., & Turlings, T. (2009). Strong attraction of the parasitoid *Cotesia marginiventris* towards minor volatile compounds of maize. *Journal of Chemical Ecology*, *35*(9), 999–1008.
- D'Alessandro, M., Held, M., Triponez, Y., & Turlings, T. C. J. (2006). The role of indole and other shikimic acid derived maize volatiles in the attraction of two parasitic wasps. *Journal of Chemical Ecology*, *32*(12), 2733–2748.

- 
- D'Alessandro, M. & Turlings, T. C. J. (2006). Advances and challenges in the identification of volatiles that mediate interactions among plants and arthropods. *Analyst*, *131*(1), 24–32.
- D'Alessandro, M. & Turlings, T. C. (2005). In-situ modification of herbivore-induced plant odors: a novel approach to study the attractiveness of volatile organic compounds to parasitic wasps. *Chemical Senses*, *30*, 739–753.
- Danielson, S. D. (2003). Induced resistance studied in maize with the fall armyworm, *Spodoptera frugiperda* (J.E. Smith) (Lepidoptera : Noctuidae) and the chemical inducer Actigard. *Journal of the Kansas Entomological Society*, *76*(4), 645–648.
- D'Auria, J. C., Reichelt, M., Luck, K., Svatos, A., & Gershenzon, J. (2007). Identification and characterization of the BAHD acyltransferase malonyl CoA: Anthocyanidin 5-O-glucoside-6"-O-malonyltransferase (At5MAT) in *Arabidopsis thaliana*. *Febs Letters*, *581*(5), 872–878.
- de Bruyne, M. & Baker, T. C. (2008). Odor detection in insects: Volatile codes. *Journal of Chemical Ecology*, *34*(7), 882–897.
- de Groot, P., Grant, G., Poland, T., Scharbach, R., Buchan, L., Nott, R., Macdonald, L., & Pitt, D. (2008). Electrophysiological response and attraction of emerald ash borer to green leaf volatiles (GLVs) emitted by host foliage. *Journal of Chemical Ecology*, *34*(9), 1170–1179.
- De Moraes, C. M., Mescher, M. C., & Tumlinson, J. H. (2001). Caterpillar-induced nocturnal plant volatiles repel conspecific females. *Nature*, *410*(6828), 577–580.
- Debach, P. & Rosen, D. (1991). *Biological control by natural enemies* (2nd ed.). Cambridge University Press.
- Degen, T., Dillmann, C., Marion-Poll, F., & Turlings, T. C. (2004). High genetic variability of herbivore-induced volatile emission within a broad range of maize inbred lines. *Plant Physiology*, *135*, 1928–1938.
- Degenhardt, D. & Lincoln, D. (2006). Volatile emissions from an odorous plant in response to herbivory and methyl jasmonate exposure. *Journal of Chemical Ecology*, *32*(4), 725–743.
- Degenhardt, J. (2009). Indirect defense responses to herbivory in grasses. *Plant Physiol.*, *149*(1), 96–102.
- Degenhardt, J., Hiltbold, I., Kollner, T. G., Frey, M., Gierl, A., Gershenzon, J., Hibbard, B. E., Ellersieck, M. R., & Turlings, T. C. J. (2009).

- Restoring a maize root signal that attracts insect-killing nematodes to control a major pest. *Proceedings of the National Academy of Sciences of the United States of America*, 106(32), 13213–13218.
- Delphia, C. M., Rohr, J. R., Stephenson, A. G., De Moraes, C. M., & Mescher, M. C. (2009). Effects of genetic variation and inbreeding on volatile production in a field population of horsetettle. *International Journal of Plant Sciences*, 170(1), 12–20.
- Dicke, M. (2009). Behavioural and community ecology of plants that cry for help. *Plant Cell and Environment*, 32(6), 654–665.
- Dicke, M. & Baldwin, I. T. (2010). The evolutionary context for herbivore-induced plant volatiles: beyond the 'cry for help'. *Trends in Plant Science*, 15(3), 167–175.
- Dicke, M., Sabelis, M. W., Takabayashi, J., Bruin, J., & Posthumus, M. A. (1990). Plant strategies of manipulating predator-prey interactions through allelochemicals: Prospects for application in pest control. *Journal of Chemical Ecology*, 16(11), 3091–3118.
- Dicke, M. & van Loon, J. J. A. (2000). Multitrophic effects of herbivore-induced plant volatiles in an evolutionary context. *Entomologia Experimentalis Et Applicata*, 97(3), 237–249.
- Dicke, M., van Poecke, R. M., & de Boer, J. G. (2003). Inducible indirect defence of plants: from mechanisms to ecological functions. *Basic and Applied Ecology*, 4(1), 27–42.
- Doares, S. H., Narváez-Vásquez, J., Conconi, A., & Ryan, C. A. (1995). Salicylic-acid inhibits synthesis of proteinase-inhibitors in tomato leaves induced by systemin and jasmonic acid. *Plant Physiology*, 108(4), 1741–1746.
- Doutt, R. L. (1959). The biology of parasitic hymenoptera. *Annual Review of Entomology*, 4(1), 161–182.
- Durrant, W. E. & Dong, X. (2004). Systemic acquired resistance. *Annual Review of Phytopathology*, 42, 185–209.
- Engelberth, J., Alborn, H. T., Schmelz, E. A., & Tumlinson, J. H. (2004). Airborne signals prime plants against insect herbivore attack. *Proceedings of the National Academy of Sciences of the United States of America*, 101(6), 1781–1785.
- Engelberth, J., Seidl-Adams, I., Schultz, J. C., & Tumlinson, J. H. (2007). Insect elicitors and exposure to green leafy volatiles differentially upreg-

- ulate major octadecanoids and transcripts of 12-oxo phytodienoic acid reductases in *Zea mays*. *Molecular Plant-Microbe Interactions*, 20(6), 707–716.
- Erb, M. (2009). *Modification of plant resistance and metabolism by above- and belowground herbivores*. PhD thesis, University of Neuchâtel.
- Erb, M., Flors, V., Karlen, D., de Lange, E., Planchamp, C., D'Alessandro, M., Turlings, T. C. J., & Ton, J. (2009). Signal signature of aboveground-induced resistance upon belowground herbivory in maize. *Plant Journal*, 59(2), 292–302.
- Erb, M., Ton, J., Degenhardt, J., & Turlings, T. C. J. (2008). Interactions between arthropod-induced aboveground and belowground defenses in plants. *Plant Physiol.*, 146(3), 867–874.
- FAO (2010). Faostat database.
- Farag, M., Fokar, M., Abd, H., Zhang, H., Allen, R., & Paré, P. (2005). (Z)-3-hexenol induces defense genes and downstream metabolites in maize. *Planta*, 220(6), 900–909.
- Flors, V., Ton, J., van Doorn, R., Jakab, G., Garcia-Agustin, P., & Mauch-Mani, B. (2008). Interplay between ja, sa and aba signalling during basal and induced resistance against *Pseudomonas syringae* and *Alternaria brassicicola*. *Plant Journal*, 54(1), 81–92.
- Fox, L. R. (1975). Cannibalism in natural populations. *Annual Review of Ecology and Systematics*, 6, 87–106.
- Friedrich, L., Lawton, K., Ruess, W., Masner, P., Specker, N., Rella, M. G., Meier, B., Dincher, S., Staub, T., Uknes, S., Metraux, J. P., Kessmann, H., & Ryals, J. (1996). A benzothiadiazole derivative induces systemic acquired resistance in tobacco. *Plant Journal*, 10(1), 61–70.
- Frost, C. J., Appel, H. M., Carlson, J. E., De Moraes, C. M., Mescher, M. C., & Schultz, J. C. (2007). Within-plant signalling via volatiles overcomes vascular constraints on systemic signalling and primes responses against herbivores. *Ecology Letters*, 10(6), 490–498.
- Frost, C. J., Mescher, M. C., Dervinis, C., Davis, J. M., Carlson, J. E., & De Moraes, C. M. (2008). Priming defense genes and metabolites in hybrid poplar by the green leaf volatile cis-3-hexenyl acetate. *New Phytologist*, 180(3), 722–733.
- Fujita, M., Fujita, Y., Noutoshi, Y., Takahashi, F., Narusaka, Y., Yamaguchi-Shinozaki, K., & Shinozaki, K. (2006). Crosstalk between

- abiotic and biotic stress responses: a current view from the points of convergence in the stress signaling networks. *Current Opinion in Plant Biology*, 9(4), 436–442.
- Geervliet, J. B. F., Ariëns, S., Dicke, M., & Vet, L. E. M. (1998). Long-distance assessment of patch profitability through volatile infochemicals by the parasitoids *Cotesia glomerata* and *C. rubecula* (Hymenoptera: Braconidae). *Biological Control*, 11(2), 113–121.
- Geervliet, J. B. F., Vreugdenhil, A. I., Dicke, M., & Vet, L. E. M. (1998). Learning to discriminate between infochemicals from different plant-host complexes by the parasitoids *Cotesia glomerata* and *C. rubecula*. *Entomologia Experimentalis Et Applicata*, 86(3), 241–252.
- Gill, S. S., Cowles, E. A., & Pietrantonio, P. V. (1992). The mode of action of *Bacillus thuringiensis* endotoxins. *Annual Review of Entomology*, 37(1), 615–634.
- Goellner, K. & Conrath, U. (2008). Priming: it's all the world to induced disease resistance. *European Journal of Plant Pathology*, 121(3), 233–242.
- Görlach, J., Volrath, S., Knauf-Beiter, G., Hengy, G., Beckhove, U., Kogel, K. H., Oostendorp, M., Staub, T., Ward, E., Kessmann, H., & Ryals, J. (1996). Benzothiadiazole, a novel class of inducers of systemic acquired resistance, activates gene expression and disease resistance in wheat. *Plant Cell*, 8(4), 629–643.
- Gouinguené, S., Alborn, H., & Turlings, T. C. J. (2003). Induction of volatile emissions in maize by different larval instars of *Spodoptera litoralis*. *Journal of Chemical Ecology*, 29(1), 145–162.
- Gouinguené, S., Degen, T., & Turlings, T. C. (2002). Variability in herbivore-induced odour emissions among maize cultivars and their wild ancestors (teosinte). *Chemoecology*, 11, 9–16.
- Gouinguené, S., Pickett, J. A., Wadhams, L. J., Birkett, M. A., & Turlings, T. C. J. (2005). Antennal electrophysiological responses of three parasitic wasps to caterpillar-induced volatiles from maize (*Zea mays mays*), cotton (*Gossypium herbaceum*), and cowpea (*Vigna unguiculata*). *Journal of Chemical Ecology*, 31(5), 1023–1038.
- Gouinguené, S. P. & Turlings, T. C. J. (2002). The effects of abiotic factors on induced volatile emissions in corn plants. *Plant Physiol.*, 129(3), 1296–1307.

- 
- Grundy, P. R., Maelzer, D. A., Bruce, A., & Hassan, E. (2000). A mass-rearing method for the assassin bug *Pristhesancus plagipennis* (Hemiptera: Reduviidae). *Biological Control*, 18(3), 243–250.
- Gupta, H. S., Agrawal, P. K., Mahajan, V., Bisht, G. S., Kumar, A., Verma, P., Srivastava, A., Saha, S., Babu, R., Pant, M. C., & Mani, V. P. (2009). Quality protein maize for nutritional security: rapid development of short duration hybrids through molecular marker assisted breeding. *Current Science*, 96(2), 230–237.
- Halitschke, R., Kessler, A., Kahl, J., Lorenz, A., & Baldwin, I. T. (2000). Ecophysiological comparison of direct and indirect defenses in *Nicotiana attenuata*. *Oecologia*, 124(3), 408–417.
- Halitschke, R., Stenberg, J. A., Kessler, D., Kessler, A., & Baldwin, I. T. (2008). Shared signals - 'alarm calls' from plants increase apparency to herbivores and their enemies in nature. *Ecology Letters*, 11(1), 24–34.
- Hammack, L. (2001). Single and blended maize volatiles as attractants for diabroticite corn rootworm beetles. *Journal of Chemical Ecology*, 27(7), 1373–1390.
- Hansson, B. S., Larsson, M. C., & Leal, W. S. (1999). Green leaf volatile-detecting olfactory receptor neurones display very high sensitivity and specificity in a scarab beetle. *Physiological Entomology*, 24(2), 121–126.
- Hare, J. (2007). Variation in herbivore and methyl jasmonate-induced volatiles among genetic lines of *Datura wrightii*. *Journal of Chemical Ecology*, 33(11), 2028–2043.
- Heidel, A. J. & Baldwin, I. T. (2004). Microarray analysis of salicylic acid- and jasmonic acid-signalling in responses of *Nicotiana attenuata* to attack by insects from multiple feeding guilds. *Plant, Cell and Environment*, 27(11), 1362–1373.
- Heil, M. (2002). Ecological costs of induced resistance. *Current Opinion in Plant Biology*, 5(4), 345–350.
- Heil, M. (2008). Indirect defence via tritrophic interactions. *New Phytologist*, 178(1), 41–61.
- Heil, M. (2010). Plastic defence expression in plants. *Evolutionary Ecology*, 24(3), 555–569.
- Heil, M. & Kost, C. (2006). Priming of indirect defences. *Ecology Letters*, 9(7), 813–817.

- Heil, M. & Ton, J. (2008). Long-distance signalling in plant defence. *Trends in Plant Science*, 13(6), 264–272.
- Heil, M. & Walters, D. R. (2009). Ecological consequences of plant defence signalling. *Plant Innate Immunity*, 51, 667–716.
- Hernández-Mendoza, J., López-Barbosa, E., Garza-González, E., & Mayek-Pérez, N. (2008). Spatial distribution of *Spodoptera frugiperda* (Lepidoptera: Noctuidae) in maize landraces grown in Colima, Mexico. *International Journal of Tropical Insect Science*, 28(03), 126–129.
- Herren, H. R., Neuenschwander, P., Hennessey, R. D., & Hammond, W. N. O. (1987). Introduction and dispersal of *Epidinocarsis lopezi* (Hym., Encyrtidae), an exotic parasitoid of the cassava mealybug, *Phenacoccus manihoti* (Hom., Pseudococcidae), in Africa. *Agriculture, Ecosystems and Environment*, 19(2), 131 – 144.
- Hilker, M. & Meiners, T. (2010). How do plants "notice" attack by herbivorous arthropods? *Biological Reviews*, 85(2), 267–280.
- Hoballah, M. E., Degen, T., Bergvinson, D., Savidan, A., Tamò, C., & Turlings, T. C. (2004). Occurrence and direct control potential of parasitoids and predators of the fall armyworm (Lepidoptera: Noctuidae) on maize in the subtropical lowlands of Mexico. *Agricultural and Forest Entomology*, 6, 83–88.
- Hoballah, M. E., Kollner, T. G., Degenhardt, J., & Turlings, T. C. J. (2004). Costs of induced volatile production in maize. *Oikos*, 105(1), 168–180.
- Hoballah, M. E. & Turlings, T. C. J. (2005). The role of fresh versus old leaf damage in the attraction of parasitic wasps to herbivore-induced maize volatiles. *Journal of Chemical Ecology*, 31(9), 2003–2018.
- Hoballah, M. E. F., Tamò, C., & Turlings, T. C. J. (2002). Differential attractiveness of induced odors emitted by eight maize varieties for the parasitoid *Cotesia marginiventris*: Is quality or quantity important? *Journal of Chemical Ecology*, 28(5), 951–968.
- Holopainen, J. K. & Gershenson, J. (2010). Multiple stress factors and the emission of plant vocs. *Trends in Plant Science*, 15(3), 176–184.
- Huang, C.-H., Yan, F.-M., Byers, J. A., Wang, R.-J., & Xu, C.-R. (2009). Volatiles induced by the larvae of the asian corn borer (*Ostrinia furnacalis*) in maize plants affect behavior of conspecific larvae and female adults. *Insect Science*, 16(4), 311–320.

- 
- Huigens, M., Pashalidou, F., Qian, M.-H., Bukovinsky, T., Smid, H. M., van Loon, J. J. A., Dicke, M., & Fatouros, N. E. (2009). Hitch-hiking parasitic wasp learns to exploit butterfly antiaphrodisiac. *Proceedings of the National Academy of Sciences*, *106*(3), 820–825.
- Inbar, M., Doostdar, H., Gerling, D., & Mayer, R. T. (2001). Induction of systemic acquired resistance in cotton by bth has a negligible effect on phytophagous insects. *Entomologia Experimentalis Et Applicata*, *99*(1), 65–70.
- Inbar, M., Doostdar, H., Sonoda, R. M., Leibee, G. L., & Mayer, R. T. (1998). Elicitors of plant defensive systems reduce insect densities and disease incidence. *Journal of Chemical Ecology*, *24*(1), 135–149.
- Jackson, M. (1997). Hormones from roots as signals for the shoots of stressed plants. *Trends in Plant Science*, *2*(1), 22–28.
- James, D. G. (2003a). Field evaluation of herbivore-induced plant volatiles as attractants for beneficial insects: Methyl salicylate and the green lacewing, *Chrysopa nigricornis*. *Journal of Chemical Ecology*, *29*(7), 1601–1609.
- James, D. G. (2003b). Synthetic herbivore-induced plant volatiles as field attractants for beneficial insects. *Environmental Entomology*, *32*(5), 977–982.
- James, D. G. & Grasswitz, T. R. (2005). Synthetic herbivore-induced plant volatiles increase field captures of parasitic wasps. *BioControl*, *50*(6), 871–880.
- James, D. G. & Price, T. S. (2004). Field-testing of methyl salicylate for recruitment and retention of beneficial insects in grapes and hops. *Journal of Chemical Ecology*, *30*(8), 1613–1628.
- Jourdie, V., Alvarez, N., Molina-Ochoa, J., Williams, T., Bergvinson, D., Benrey, B., Turlings, T. C. J., & Franck, P. (2010). Population genetic structure of two primary parasitoids of *Spodoptera frugiperda* (Lepidoptera), *Chelonus insularis* and *Campoletis sonorensis* (Hymenoptera): to what extent is the host plant important? *Molecular Ecology*, *19*(10), 2168–2179.
- Jourdie, V., Alvarez, N., & Turlings, T. C. J. (2008). Identification of seven species of hymenopteran parasitoids of *Spodoptera frugiperda*, using polymerase chain reaction amplification and restriction enzyme digestion. *Agricultural and Forest Entomology*, *10*(2), 129–136.

- Jourdie, V., Alvarez, N., Turlings, T. C. J., & Franck, P. (2009). Isolation and characterization of polymorphic microsatellite loci in two primary parasitoids of the noctuid spodoptera frugiperda: *Chelonus insularis* and *campoletis sonorensis* (hymenoptera). *Molecular Ecology Resources*, *9*(1), 171–173.
- Kakimoto, T., Fujisaki, K., & Miyatake, T. (2009). Egg laying preference, larval dispersion, and cannibalism in *Helicoverpa armigera* (Lepidoptera: Noctuidae). *Annals of the Entomological Society of America*, *96*(6), 793–798.
- Kant, M. M., Bleeker, P. M., Wijk, M. v., Schuurink, R. C., & Haring, M. A. (2009). Plant volatiles in defence. In L. van Loon (Ed.), *Advances in Botanical Research*, volume 51 of *Advances in Botanical Research* (pp. 613–666). Burlington: Elsevier Ltd.
- Kaya, H. K. & Gaugler, R. (1993). Entomopathogenic nematodes. *Annual Review of Entomology*, *38*(1), 181–206.
- Kessler, A. & Baldwin, I. T. (2001). Defensive function of herbivore-induced plant volatile emissions in nature. *Science*, *291*(5511), 2141–2144.
- Kessler, A., Halitschke, R., Diezel, C., & Baldwin, I. T. (2006). Priming of plant defense responses in nature by airborne signaling between *artemisia tridentata* and *nicotiana attenuata*. *Oecologia*, *148*(2), 280–292.
- Kester, K. M., Peterson, S. C., Hanson, F., Jackson, D. M., & Severson, R. F. (2002). The roles of nicotine and natural enemies in determining larval feeding site distributions of *Manduca sexta* L. and *Manduca quinquemaculata* (Haworth) on tobacco. *Chemoecology*, *12*(1), 1–10.
- Khan, Z. R., James, D. G., Midega, C. A. O., & Pickett, J. A. (2008). Chemical ecology and conservation biological control. *Biological Control*, *45*(2), 210–224.
- Khan, Z. R., Pickett, J. A., van den Berg, J., Wadhams, L. J., & Woodcock, C. M. (2000). Exploiting chemical ecology and species diversity: stem borer and striga control for maize and sorghum in Africa. *Pest Management Science*, *56*(11), 957–962.
- Kipkoech, A. K., Mithfer, D., Yabann, W. K., Maritim, H. K., & Schulthess, F. (2008). Assessing yield and efficiency implications of relying on parasitoids for control of cereal stemborers: The case of small-scale maize farmers in Kenya. *Crop Protection*, *27*(10), 1318–1326.

- 
- Koornneef, A. & Pieterse, C. M. (2008). Cross talk in defense signaling. *Plant Physiol.*, *146*(3), 839–844.
- Koul, O. (2008). Phytochemicals and insect control: An antifeedant approach. *Critical Reviews in Plant Sciences*, *27*(1), 1–24.
- Krips, O. E., Willems, P. E. L., Gols, R., Posthumus, M. A., Gort, G., & Dicke, M. (2001). Comparison of cultivars of ornamental crop *Gerbera jamesonii* on production of spider mite-induced volatiles, and their attractiveness to the predator, phytoseiulus persimilis. *Journal of Chemical Ecology*, *27*(7), 1355–1372.
- Kumar, H. (2002). Plant damage and grain yield reduction by fall armyworm and stem borers on certain maize hybrids containing resistance genes from varying sources under experimental and farmers field conditions. *Crop Protection*, *21*(7), 563–573.
- Kunkel, B. N. & Brooks, D. M. (2002). Cross talk between signaling pathways in pathogen defense. *Current Opinion in Plant Biology*, *5*(4), 325–331.
- Landolt, P. J. (1993). Effects of host plant leaf damage on cabbage-looper moth attraction and oviposition. *Entomologia Experimentalis Et Applicata*, *67*(1), 79–85.
- Landolt, P. J., Tumlinson, J. H., & Alborn, D. H. (1999). Attraction of Colorado potato beetle (Coleoptera : Chrysomelidae) to damaged and chemically induced potato plants. *Environmental Entomology*, *28*(6), 973–978.
- Larsson, M. C., Leal, W. S., & Hansson, B. S. (2001). Olfactory receptor neurons detecting plant odours and male volatiles in *Anomala cuprea* beetles (Coleoptera: Scarabaeidae). *Journal of Insect Physiology*, *47*(9), 1065–1076.
- Lezama-Gutiérrez, R., Hamm, J. J., Molina-Ochoa, J., López-Edwards, M., Pescador-Rubio, A., González-Ramírez, M., & Styer, E. L. (2001). Occurrence of entomopathogens of *Spodoptera frugiperda* (Lepidoptera: Noctuidae) in the Mexican states of Michoacán, Colima, Jalisco and Tamaulipas. *Florida Entomologist*, *84*(1), 23.
- Loreto, F. & Schnitzler, J.-P. (2010). Abiotic stresses and induced bvocs. *Trends in Plant Science*, *15*(3), 154–166.
- Loughrin, J. H., Potter, D. A., & Hamilton-Kemp, T. R. (1995). Volative compounds induced by herbivory act as aggregation kairomones for

- the japanese-beetle (*Popillia japonica* Newman). *Journal of Chemical Ecology*, 21(10), 1457–1467.
- Maeda, T., Takabayashi, J., Yano, S., & Takafuji, A. (1999). Response of the predatory mite, *Amblyseius womersleyi* (Acari : Phytoseiidae), toward herbivore-induced plant volatiles: Variation in response between two local populations. *Applied Entomology and Zoology*, 34(4), 449–454.
- Marktl, R. C., Stauffer, C., & Schopf, A. (2002). Interspecific competition between the braconid endoparasitoids *Glyptapanteles porthetriae* and *Glyptapanteles liparidis* in *Lymantria dispar* larvae. *Entomologia Experimentalis Et Applicata*, 105(2), 97–109.
- McDonald, R. C. & Kok, L. T. (1991). Hyperparasites attacking *Cotesia glomerata* (L.) and *Cotesia rubecula* (Marshall) (Hymenoptera: Braconidae) in Southwestern Virginia. *Biological Control*, 1(2), 170–175.
- Milonas, P. G., Martinou, A. F., Kontodimas, D. C., Karamaouna, F., & Konstantopoulou, M. A. (2009). Attraction of different *Trichogramma* species to prays oleae sex pheromone. *Annals of the Entomological Society of America*, 102(6), 1145–1150.
- Molina-Ochoa, J., Carpenter, J. E., Heinrichs, E. A., & Foster, J. E. (2003). Parasitoids and parasites of *Spodoptera frugiperda* (Lepidoptera: Noctuidae) in the Americas and Caribbean basin: an inventory. *Florida Entomologist*, 86(3), 254–289.
- Molina-Ochoa, J., Carpenter, J. E., Lezama-Gutierrez, R., Foster, J. E., Gonzalez-Ramirez, M., Angel-Sahagun, C. A., & Farias-Larios, J. (2004). Natural distribution of hymenopteran parasitoids of *Spodoptera frugiperda* (Lepidoptera : Noctuidae) larvae in Mexico. *Florida Entomologist*, 87(4), 461–472.
- Montes, J. I. L., Pitre, H. N., Meckenstock, D. H., & Gomez, F. (1996). Evaluation of a sorghum population for resistance to fall armyworm (Lepidoptera: Noctuidae). *Tropical Agriculture*, 73(1), 43–48.
- Moraes, M. C. B., Laumann, R. A., Pareja, M., Sereno, F., Michereff, M. F. F., Birkett, M. A., Pickett, J. A., & Borges, M. (2009). Attraction of the stink bug egg parasitoid *Telenomus podisi* to defence signals from soybean activated by treatment with cis-jasmone. *Entomologia Experimentalis Et Applicata*, 131(2), 178–188.
- Moscardi, F. (1999). Assessment of the application of baculoviruses for control of lepidoptera. *Annual Review of Entomology*, 44(1), 257–289.

- Oerke, E.-C. (2006). Crop losses to pests. *The Journal of Agricultural Science*, 144(01), 31–43.
- Ozawa, R., Shiojiri, K., Sabelis, M. W., & Takabayashi, J. (2008). Maize plants sprayed with either jasmonic acid or its precursor, methyl linolenate, attract armyworm parasitoids, but the composition of attractants differs. *Entomologia Experimentalis Et Applicata*, 129(2), 189–199.
- Paré, P. W. & Tumlinson, J. H. (1999). Plant volatiles as a defense against insect herbivores. *Plant Physiol.*, 121(2), 325–332.
- Paschold, A., Halitschke, R., & Baldwin, I. T. (2006). Using 'mute' plants to translate volatile signals. *The Plant Journal*, 45(2), 275–291.
- Peñuelas, J. & Staudt, M. (2010). BVOCs and global change. *Trends in Plant Science*, 15(3), 133–144.
- Perazzolli, M., Dagostin, S., Ferrari, A., Elad, Y., & Pertot, I. (2008). Induction of systemic resistance against *Plasmopara viticola* in grapevine by *Trichoderma harzianum* T39 and benzothiadiazole. *Biological Control*, 47(2), 228–234.
- Piesik, D., Rochat, D., van der Pers, J., & Marion-Poll, F. (2009). Pulsed odors from maize or spinach elicit orientation in European corn borer neonate larvae. *Journal of Chemical Ecology*, 35(9), 1032–1042.
- Poelman, E. H., Oduor, A. M. O., Broekgaarden, C., Hordijk, C. A., Jansen, J. J., Van Loon, J. J. A., Van Dam, N. M., Vet, L. E. M., & Dicke, M. (2009). Field parasitism rates of caterpillars on *Brassica oleracea* plants are reliably predicted by differential attraction of *Cotesia* parasitoids. *Functional Ecology*, 23(5), 951–962.
- Poelman, E. H., van Dam, N. M., van Loon, J. J. A., Vet, L. E. M., & Dicke, M. (2009). Chemical diversity in *Brassica oleracea* affects biodiversity of insect herbivores. *Ecology*, 90(7), 1863–1877.
- Pophof, B., Stange, G., & Abrell, L. (2005). Volatile organic compounds as signals in a plant-herbivore system: electrophysiological responses in olfactory sensilla of the moth *Cactoblastis cactorum*. *Chem. Senses*, 30(1), 51–68.
- Porter, J. H., Parry, M. L., & Carter, T. R. (1991). The potential effects of climatic change on agricultural insect pests. *Agricultural and Forest Meteorology*, 57(1-3), 221 – 240.
- Randlkofer, B., Obermaier, E., & Meiners, T. (2007). Mother's choice of the oviposition site: balancing risk of egg parasitism and need of food

- supply for the progeny with an infochemical shelter? *Chemoecology*, *17*(3), 177–186.
- Rayapuram, C. & Baldwin, I. T. (2007). Increased SA in NPR1-silenced plants antagonizes JA and JA-dependent direct and indirect defenses in herbivore-attacked *Nicotiana attenuata* in nature. *The Plant Journal*, *52*(4), 700–715.
- Richardson, M. L., Mitchell, R. F., Reagel, P. F., & Hanks, L. M. (2009). Causes and consequences of cannibalism in noncarnivorous insects. *Annual Review of Entomology*, *55*(1), 39–53.
- Rodriguez-Saona, C. R., Rodriguez-Saona, L. E., & Frost, C. J. (2009). Herbivore-induced volatiles in the perennial shrub, *Vaccinium corymbosum*, and their role in inter-branch signaling. *Journal of Chemical Ecology*, *35*(2), 163–175.
- Rostás, M. (2007). The effects of 2,4-dihydroxy-7-methoxy-1,4-benzoxazin-3-one on two species of *Spodoptera* and the growth of *Setosphaeria turcica* in vitro. *Journal of Pest Science*, *80*(1), 35–41.
- Rostás, M. & Hilker, M. (2002). Feeding damage by larvae of the mustard leaf beetle deters conspecific females from oviposition and feeding. *Entomologia Experimentalis Et Applicata*, *103*(3), 267–277.
- Rostás, M., Ton, J., Mauch-Mani, B., & Turlings, T. (2006). Fungal infection reduces herbivore-induced plant volatiles of maize but does not affect naïve parasitoids. *Journal of Chemical Ecology*, *32*(9), 1897–1909.
- Rostás, M. & Turlings, T. C. J. (2008). Induction of systemic acquired resistance in *Zea mays* also enhances the plant's attractiveness to parasitoids. *Biological Control*, *46*(2), 178–186.
- Ruther, J. & Furstenu, B. (2005). Emission of herbivore-induced volatiles in absence of a herbivore - response of *Zea mays* to green leaf volatiles and terpenoids. *Zeitschrift Fur Naturforschung C-a Journal of Biosciences*, *60*(9-10), 743–756.
- Ruther, J. & Kleier, S. (2005). Plant–plant signaling: ethylene synergizes volatile emission in *Zea mays* induced by exposure to (*Z*)-3-hexen-1-ol. *Journal of Chemical Ecology*, *31*(9), 2217–2222.
- Schmelz, E., Alborn, H., Banchio, E., & Tumlinson, J. (2003). Quantitative relationships between induced jasmonic acid levels and volatile emission in *Zea mays* during *Spodoptera exigua* herbivory. *Planta*, *216*(4), 665–673.

- 
- Schmidt, M. H., Lauer, A., Purtauf, T., Thies, C., Schaefer, M., & Tschardtke, T. (2003). Relative importance of predators and parasitoids for cereal aphid control. *Proceedings of the Royal Society B: Biological Sciences*, 270(1527), 1905–1909.
- Schnee, C., Kollner, T. G., Held, M., Turlings, T. C. J., Gershenzon, J., & Degenhardt, J. (2006). The products of a single maize sesquiterpene synthase form a volatile defense signal that attracts natural enemies of maize herbivores. *PNAS*, 103(4), 1129–1134.
- Schuman, M. C., Heinzl, N., Gaquerel, E., Svatos, A., & Baldwin, I. T. (2009). Polymorphism in jasmonate signaling partially accounts for the variety of volatiles produced by *Nicotiana attenuata* plants in a native population. *New Phytologist*, 183(4), 1134–1148.
- Shah, J. (2009). Plants under attack: systemic signals in defence. *Current Opinion in Plant Biology*, 12(4), 459–464.
- Sharma, R. C. & Payak, M. M. (1990). Durable resistance to 2 leaf blights in 2 maize inbred lines. *Theoretical and Applied Genetics*, 80(4), 542–544.
- Shiojiri, K., Kishimoto, K., Ozawa, R., Kugimiya, S., Urashimo, S., Arimura, G., Horiuchi, J., Nishioka, T., Matsui, K., & Takabayashi, J. (2006). Changing green leaf volatile biosynthesis in plants: An approach for improving plant resistance against both herbivores and pathogens. *Proceedings of the National Academy of Sciences*, 103(45), 16672–16676.
- Shiojiri, K., Ozawa, R., & Takabayashi, J. (2006). Plant volatiles, rather than light, determine the nocturnal behavior of a caterpillar. *PLoS Biol*, 4(6), e164.
- Smith, J. L., De Moraes, C. M., & Mescher, M. C. (2009). Jasmonate- and salicylate-mediated plant defense responses to insect herbivores, pathogens and parasitic plants. *Pest Management Science*, 65(5), 497–503.
- Smith, S. M. (1996). Biological control with *Trichogramma*: advances, successes, and potential of their use. *Annual Review of Entomology*, 41(1), 375–406.
- Takabayashi, J., Dicke, M., & Posthumus, M. A. (1994). Volatile herbivore-induced terpenoids in plant mite interactions - variation caused by biotic and abiotic factors. *Journal of Chemical Ecology*, 20(6), 1329–1354.
- Tamò, C., Ricard, I., Held, M., Davison, A. C., & Turlings, T. C. J. (2006).

- A comparison of naive and conditioned responses of three generalist endoparasitoids of lepidopteran larvae to host-induced plant odours. *Animal Biology*, 56, 205–220.
- Tamò, C., Roelstra, L.-L., Guillaume, S., & Turlings, T. C. (2006). Odour-mediated long-range avoidance of interspecific competition by a solitary endoparasitoid: a time-saving foraging strategy. *Journal of Animal Ecology*.
- Taylor, J. E., Hatcher, P. E., & Paul, N. D. (2004). Crosstalk between plant responses to pathogens and herbivores: a view from the outside in. *Journal of Experimental Botany*, 55(395), 159–168.
- Team, R. D. C. (2010). R: A language and environment for statistical computing.
- Thaler, J. S. (1999). Jasmonate-inducible plant defences cause increased parasitism of herbivores. *Nature*, 399(6737), 686–688.
- Thaler, J. S., Fidantsef, A. L., & Bostock, R. M. (2002). Antagonism between jasmonate- and salicylate-mediated induced plant resistance: effects of concentration and timing of elicitation on defense-related proteins, herbivore, and pathogen performance in tomato. *Journal of Chemical Ecology*, 28(6), 1131–1159.
- Thaler, J. S., Karban, R., Ullman, D. E., Boege, K., & Bostock, R. M. (2002). Cross-talk between jasmonate and salicylate plant defense pathways: effects on several plant parasites. *Oecologia*, 131(2), 227–235.
- Ton, J., D'Alessandro, M., Jourdie, V., Jakab, G., Karlen, D., Held, M., Mauch-Mani, B., & Turlings, T. C. J. (2007). Priming by airborne signals boosts direct and indirect resistance in maize. *Plant Journal*, 49(1), 16–26.
- Turlings, T. C., Davison, A. C., & Tamò, C. (2004). A six-arm olfactometer permitting simultaneous observation of insect attraction and odour trapping. *Physiological Entomology*, 29, 45–55.
- Turlings, T. C. & Ton, J. (2006). Exploiting scents of distress: the prospect of manipulating herbivore-induced plant odours to enhance the control of agricultural pests. *Current Opinion in Plant Biology*, 9, 421–427.
- Turlings, T. C. & Wäckers, F. (2004). Recruitment of predator and parasitoid by herbivore-injured plants. *Advances in Insect Chemical Ecology*, 2, 21–75.

- 
- Turlings, T. C. J., Bernasconi, M., Bertossa, R., Bigler, F., Caloz, G., & Dorn, S. (1998). The induction of volatile emissions in maize by three herbivore species with different feeding habits: possible consequences for their natural enemies. *Biological Control*, *11*, 122–129.
- Turlings, T. C. J., Lengwiler, U. B., Bernasconi, M. L., & Wechsler, D. (1998). Timing of induced volatile emissions in maize seedlings. *Planta*, *207*(1), 146–152.
- Turlings, T. C. J., Loughrin, J. H., McCall, P. J., Rose, U. S. R., Lewis, W. J., & Tumlinson, J. H. (1995). How caterpillar-damaged plants protect themselves by attracting parasitic wasps. *Proceedings of the National Academy of Sciences*, *92*(10), 4169–4174.
- Turlings, T. C. J. & Tumlinson, J. H. (1992). Systemic release of chemical signals by herbivore-injured corn. *Proceedings of the National Academy of Sciences*, *89*(17), 8399–8402.
- Turlings, T. C. J., Tumlinson, J. H., Eller, F. J., & Lewis, W. J. (1991). Larval-damaged plants: source of volatile synomines that guide the parasitoid *Cotesia marginiventris* to the micro-habitat of its hosts. *Entomologia Experimentalis et Applicata*, *58*(1), 75–82.
- Turlings, T. C. J., Tumlinson, J. H., & Lewis, W. J. (1990). Exploitation of herbivore-induced plant odors by host-seeking parasitic wasps. *Science*, *250*(4985), 1251–1253.
- Unsicker, S. B., Kunert, G., & Gershenson, J. (2009). Protective perfumes: the role of vegetative volatiles in plant defense against herbivores. *Current Opinion in Plant Biology*, *12*(4), 479–485.
- Vallad, G. E. & Goodman, R. M. (2004). Systemic acquired resistance and induced systemic resistance in conventional agriculture. *Crop Science*, *44*(6), 1920–1934.
- van Loon, L., Rep, M., & Pieterse, C. (2006). Significance of inducible defense-related proteins in infected plants. *Annual Review of Phytopathology*, *44*(1), 135–162.
- van Poecke, R. M. P. & Dicke, M. (2004). Indirect defence of plants against herbivores: using *Arabidopsis thaliana* as a model plant. *Plant Biology*, *(4)*, 387–401.
- Vasal, S. K., Srinivasan, G., Pandey, S., Gonzalez C., F., Crossa, J., & Beck, D. L. (1993). Heterosis and combining ability of cimmyt's quality

- protein maize germplasm: I. lowland tropical. *Crop Science*, 33(1), 46–51.
- Verdon, A., Margraf, N., Davison, A. C., Rahier, M., & Naisbit, R. E. (2007). Conserved oviposition preferences in alpine leaf beetle populations despite host shifts and isolation. *Ecological Entomology*, 32(1), 62–69.
- Vet, L. E. M. & Dicke, M. (1992). Ecology of infochemical use by natural enemies in a tritrophic context. *Annual Review of Entomology*, 37, 141–172.
- Vickers, C. E., Gershenzon, J., Lerdau, M. T., & Loreto, F. (2009). A unified mechanism of action for volatile isoprenoids in plant abiotic stress. *Nature Chemical Biology*, 5(5), 283–291.
- Villegas, E., Vasal, S. K., & Bjarnason, M. (1992). *Quality protein maize - what is it and how was it developed*. Quality Protein Maize. St Paul: Amer Assoc Cereal Chemists Inc.
- Walling, L. L. (2000). The myriad plant responses to herbivores. *Journal of Plant Growth Regulation*, 19(2), 195–216.
- Walling, L. L. (2009). Adaptive defense responses to pathogens and insects. In *Plant Innate Immunity*, volume 51 of *Advances in Botanical Research* (pp. 551–612). London: Academic Press Ltd-Elsevier Science Ltd.
- Walters, D. & Heil, M. (2007). Costs and trade-offs associated with induced resistance. *Physiological and Molecular Plant Pathology*, 71(1-3), 3–17.
- Walters, D. R. & Boyle, C. (2005). Induced resistance and allocation costs: What is the impact of pathogen challenge? *Physiological and Molecular Plant Pathology*, 66(1-2), 40–44.
- Wheeler, G., Ashley, T., & Andrews, K. (1989). Larval parasitoids and pathogens of the fall armyworm in honduran maize. *BioControl*, 34(3), 331–340.
- Whitman, D. & Eller, F. (1992). Orientation of *Microplitis croceipes* (Hymenoptera: Braconidae) to green leaf volatiles: Dose-response curves. *Journal of Chemical Ecology*, 18(10), 1743–1753.
- Whitman, D. W. & Eller, F. J. (1990). Parasitic wasps orient to green leaf volatiles. *Chemoecology*, 1(2), 69–76.
- Wiseman, B. R., Davis, F. M., & Campbell, J. E. (1980). Mechanical in-

- festation device used in fall armyworm plant-resistance programs (Lepidoptera, Noctuidae). *Florida Entomologist*, 63(4), 425–432.
- Wiseman, B. R., Painter, R. H., & Wassom, C. E. (1966). Detecting corn seedling differences in greenhouse by visual classification of damage by fall armyworm. *Journal of Economic Entomology*, 59(5), 1211–1214.
- Wyckhuys, K. & O’Neil, R. (2007). Local agro-ecological knowledge and its relationship to farmers’ pest management decision making in rural Honduras. *Agriculture and Human Values*, 24(3), 307–321.
- Yang, Z. H., Bengtsson, M., & Witzgall, P. (2004). Host plant volatiles synergize response to sex pheromone in codling moth, *Cydia pomonella*. *Journal of Chemical Ecology*, 30(3), 619–629.
- Yi, H.-S., Heil, M., Adame-Alvarez, R. M., Ballhorn, D. J., & Ryu, C.-M. (2009). Airborne induction and priming of plant defenses against a bacterial pathogen. *Plant Physiology*, 151(4), 2152–2161.
- Young, V. & Pellett, P. (1994). Plant proteins in relation to human protein and amino acid nutrition. *Am J Clin Nutr*, 59(5), 1203S–1212.
- Zhang, Y., Xie, Y., Xue, J., Peng, G., & Wang, X. (2009). Effect of volatile emissions, especially  $\alpha$ -pinene, from *Persimmon* trees infested by japanese wax scales or treated with methyl jasmonate on recruitment of ladybeetle predators. *Environmental Entomology*, 38(5), 1439–1445.
- Zilkowski, B. W., Bartelt, R. J., Blumberg, D., James, D. G., & Weaver, D. K. (1999). Identification of host-related volatiles attractive to pineapple beetle *Carpophilus humeralis*. *Journal of Chemical Ecology*, 25(1), 229–252.



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# Curriculum Vitae

## Personal details

Date, place of birth: 8 July 1981, Zurich, Switzerland  
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## Work experience

04.2007-present Assistant/PhD student. Teaching and research in chemical ecology at the University of Neuchâtel, Switzerland. Field research at the International Center for Improvement of Maize and Wheat, CIMMYT, Mexico. Thesis title: Manipulating Maize Volatiles to Improve Biological Control of Insect Pests. Thesis successfully defended on 2 July 2010

09.2006-01.2007 Research assistant. Projects on corporate sustainability in China and energy policy in Switzerland. Agri-Food and Agri-Environmental Economics Group. Swiss Federal Institute of Technology Zurich, Switzerland

09.2005-09.2005 Quality control, logistics, packaging. AvM Merisan S.A., Cortébert, Switzerland

07.2005-08.2005 Production internship at Loyal Farms, Warkworth, ON, Canada

- 07.2004-09.2004      Research internship at International Center for Insect Physiology and Ecology (ICIPE), Nairobi, Kenya.
- 03.2003-09.2003      Production internship at organic farm La Colombera, San Antonino, Switzerland.
- 05.2001-08.2001      Research internship at the Instituto Nacional de Pesquisa da Amazônia (INPA), silviculture, Manaus AM, Brazil.
- 01.2001-04.2001      Research and production internship at the Fundação Agraria de Pesquisa Agropecuaria (FAPA), Entre Rios, Guarapuava PR, Brazil.

### Publications and theses

von Mérey, G., Veyrat, N., de Lange, E., Degen, T., Mahuku, G., Lopez Valdez, R., Turlings, T., D'Alessandro, M. Effects of two elicitors of insect and pathogen resistance on the volatile emission and the biological control of *Spodoptera frugiperda* in maize fields. *In prep.*

von Mérey, G., Veyrat, N., Mahuku, G., Lopez Valdez, R., Turlings, T., D'Alessandro, M. The importance of green leaf volatiles for the attractiveness of maize plants to pest and beneficial insects in the field. *In prep.*

D'Alessandro, M., Brunner, V., von Mérey, G. and Turlings, T. C. 2009. Strong attraction of the parasitoid *Cotesia marginiventris* towards minor volatile compounds of maize. *J. Chem. Ecol.* 35(9):999-1008

von Mérey, G. 2007. Forecasting Chinese Pork Demand. *Agrarwirtschaft und Agrarsoziologie* 1(07):159-175.

Mohamed, S. A., Wharton, R. A., von Mérey, G. and Schulthess, F. 2006. Acceptance and suitability of different host stages of *Ceratitidis capitata* (Wiedemann) (Diptera: Tephritidae) and seven other tephritid fruit fly species to *Tetrastichus giffardii* Silvestri (Hymenoptera: Eulophidae). *Biological Control* 39(3):262-271.

Master thesis: The Transformation Process of the Chinese Meat Production Based on Concentrated Feed.

3<sup>rd</sup> Award of the Swiss Society for Agricultural Economics and Rural Sociology.

### Education

- 04.2007-07.2010      PhD in biology, University of Neuchâtel, Switzerland
- 09.2004-03.2005      Erasmus exchange program at Wageningen University, Netherlands

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10.2001-11.2006 Swiss Federal Institute of Technology (ETH) Zurich, Agricultural Engineer (MSc ETHZ in Animal Production), Switzerland

### Languages

German	mother tongue
French	secondary language (bilingual)
Spanish	excellent level
English	excellent level
Italian	high level
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### Computer skills

MS Excel, MS Word, MS PowerPoint, MS Outlook, MS Access  
Graphic design programs: Adobe Illustrator, Photoshop  
Data analysis programs: R, SAS, SyStat, Maple, Matematica, MatLab

### Attended conferences

Swiss Plant Science Web summer school. 23-26.06.2010. Poster title: Importance of green leaf volatiles for pest and beneficial insects in the field. Mürren, Switzerland. Award for the best poster from the Swiss Society of Phytiatry.

25<sup>th</sup> Annual Meeting of the International Society for Chemical Ecology. 23-27.08.2009. Attendee and organizing committee member. Neuchâtel, Switzerland.

1<sup>st</sup> International Conference on Entomophagous Insects. 24-29.07.2009. Presentation title: Does Artificial Induction of SAR also Increase the Parasitoid Attack of Herbivores in a Maize Field? Minneapolis, MN, USA.

5<sup>th</sup> meeting of the International Organisation for Biological Control Working Group. 12-16.05.2009. Presentation title: Green Leafy Volatiles: Priming and Insect Attraction in a Maize Field. Granada, Spain.

Annual PhD Student Meeting. 28.4.2009. Presentation title: Green Leafy Volatiles: Priming and Insect Attraction in a Maize Field. Neuchâtel, Switzerland.

Annual PhD Student Meeting. 07.04.2008. Poster title: Exploiting Scents of Distress. Organizing committee member. Neuchâtel, Switzerland.

Frontiers in Phytohormone Science. 10-11.09.2007. Attendee. Lausanne, Switzerland.