

# Tritrophic interactions on cultivated maize and its wild ancestor, teosinte



PhD thesis presented by

**Elvira S. de Lange**

University of Neuchâtel, Switzerland

September 13<sup>th</sup> 2013

Thesis director

**Prof. Ted C.J. Turlings**



# **Tritrophic interactions on cultivated maize and its wild ancestor, teosinte**

Dissertation submitted to the University of Neuchâtel  
for the Degree of Doctor in Natural Sciences by

**Elvira S. de Lange**

Faculty of Science, Institute of Biology  
Laboratory of Fundamental and Applied Research in Chemical Ecology  
University of Neuchâtel

Accepted by recommendation of the thesis committee:

Prof. Ted C.J. Turlings, University of Neuchâtel, Switzerland (thesis director)  
Dr. Christophe J. Praz, University of Neuchâtel, Switzerland  
Prof. Florian P. Schiestl, University of Zürich, Switzerland  
Dr. Alisa Huffaker, USDA-ARS, United States of America

Defended on September 13<sup>th</sup>, 2013

University of Neuchâtel  
2014



## IMPRIMATUR POUR THESE DE DOCTORAT

---

**La Faculté des sciences de l'Université de Neuchâtel  
autorise l'impression de la présente thèse soutenue par**

**Madame Elvira DE LANGE**

Titre:

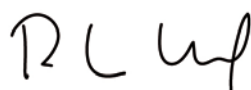
**Tritrophic interactions on cultivated maize and  
its wild ancestor « teosinte »**

**sur le rapport des membres du jury composé comme suit:**

- Prof. Ted Turlings, Université de Neuchâtel, directeur de thèse
- Dr Christophe Praz, Université de Neuchâtel
- Prof. Florian Schiestl, Université de Zürich
- Dr Alisa Huffaker, USDA-ARS, Center for medical, Agricultural and veterinary Entomology, Gainesville, FL, USA

Neuchâtel, le 13 janvier 2014

Le Doyen, Prof. P. Kropf





## Key words

*Campoletis sonorensis*, Chemical ecology, *Cotesia marginiventris*, Corn, Domestication, Fall armyworm, Green leaf volatiles, Herbivore-induced plant volatiles, Host plant, Insect herbivore, Maize pest, Mexico, Natural enemy, Parasitoid wasp, Plant defense, *Spodoptera frugiperda*, Teosinte, Tritrophic interactions, Volatile organic compounds, *Zea mays*

## Mots clés

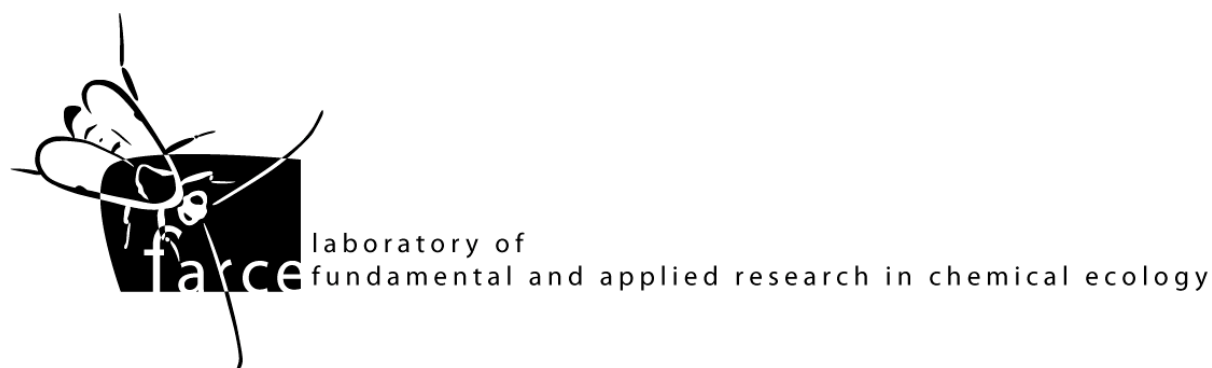
*Campoletis sonorensis*, Écologie Chimique, *Cotesia marginiventris*, Maïs, Domestication, Légionnaire d'automne, Green leaf volatiles, Odeurs induites, Plante hôte, Insecte herbivore, Ravageur du maïs, Mexique, Ennemi naturel, Guêpe parasitoïde, Défense des plantes, *Spodoptera frugiperda*, Téosinte, Interactions tritrophiques, Composés organiques volatils, *Zea mays*





## Contents

Abstract .....	7
Résumé .....	9
Resumen .....	13
Zusammenfassung .....	15
Samenvatting .....	19
General introduction .....	23
Chapter 1     Insect and pathogen resistance in cultivated maize and its wild ancestor, teosinte .....	39
Chapter 2     Natural presence of insect pests on teosinte, the ancestor of maize, in Mexico .....	59
Chapter 3     Has maize lost some of its ability to attract parasitoids during domestication? .....	75
Chapter 4     Behavior and performance of fall armyworm and its parasitoids on cultivated maize and its wild ancestor, teosinte .....	93
Chapter 5     Parasitic wasps can reduce mortality among caterpillar-infested teosinte plants .....	115
Chapter 6     Are green leaf volatiles important for parasitoid wasp attraction in the field? .....	137
General discussion .....	157
References .....	163
Acknowledgements .....	183
Annex 1 .....	I
Annex 2 .....	V
Curriculum vitae .....	XIX



Front cover pictures: A female parasitoid wasp, *Campoletis sonorensis*, localizes and parasitizes her lepidopteran host, *Spodoptera frugiperda*, while the latter is feeding on a maize plant. Pictures: Matthias Held



## Abstract

Modern maize plants (*Zea mays* ssp. *mays*, Poaceae) are characterized by large cobs that contain juicy grains, although they have not always had these characteristics. Approximately 9000 years ago, maize was domesticated from teosinte (*Z. mays* ssp. *parviglumis*), its wild ancestor, which produces much less and much smaller seeds. Teosinte still grows in the wild in southern Mexico, while cultivated maize is produced all over the world. Continuous selection for improved yield and quality has had a cost for the plant in terms of the loss or alteration of other potentially useful traits, such as resistance to pathogens and herbivorous insects (reviewed in Chapter 1). This thesis focuses on the resistance of maize and teosinte against insect pests, in particular with respect to the emission of herbivore-induced volatiles. This feature is considered an indirect defense trait, as the volatiles can betray the presence of prey or hosts to predators and parasitoids, natural enemies of herbivorous insects. Among these natural enemies are parasitoid wasps, of which females have an ovipositor with which they can lay single or multiple eggs in individual hosts. When the wasp larvae develop inside the host they will eventually kill it, potentially benefitting the plant.

At first, we assessed in nature which insect pests occur on teosinte (Chapter 2). Fall armyworm, *Spodoptera frugiperda* (Lepidoptera: Noctuidae), is reported as one of the main maize pests in the Americas, and is frequently attacked by many species of parasitoids. However, little is known about the presence of this herbivore and its associated parasitoids on teosinte.

In a laboratory setting, we then assessed whether maize and teosinte emit a similar blend of volatiles when induced by lepidopteran herbivores. We also evaluated the attractiveness of the odor blends to females of two species of solitary koinobiont endoparasitoid wasps, *Campoletis sonorensis* (Hymenoptera: Ichneumonidae) and *Cotesia marginiventris* (Hymenoptera: Braconidae). Although the odor blends appeared similar upon gas chromatography analysis, the foraging parasitoids responded differentially to them. *C. sonorensis* did not distinguish between the odors, whereas *C. marginiventris* showed a preference for the odors of teosinte over those of maize (Chapters 3, 4). We obtained similar results when testing real plants and extracts of collected odors (Chapter 3) indicating that these extracts can be used to identify the key compounds that are responsible for parasitoid wasp attraction.

Interestingly, we observed that the host plant, i.e. cultivated maize or wild teosinte, can have an effect on the development of parasitoid larvae within their host. While *C. marginiventris* developed equally well when their host, *S. frugiperda*, had been feeding on maize or teosinte, *C. sonorensis* cocoons were larger when their host had fed on maize plants. Since *C. marginiventris* wasps preferred the odors of teosinte, and *C. sonorensis* wasps did not display a preference for the odors of teosinte or maize, these results indicate that the parasitoids were not necessarily attracted to the plants that were best suited for the development of their offspring (Chapter 4).

There is surprisingly little field evidence for fitness benefits for plants due to the action of natural enemies. This is still an important point in the discussion on the possible indirect defense role of inducible plant volatiles, especially in the case of parasitoid wasps that, unlike predators, do not directly kill their hosts. Therefore, we evaluated how parasitoid wasps can affect plant performance in a semi-natural setting in Morelia, Mexico. Our tritrophic system comprised teosinte, devoid of the pleiotropic effects of artificial selection, the herbivore *S. frugiperda* and the wasp *C. sonorensis*, all organisms that co-occur in nature. Teosinte was planted in large screen tents, which allowed us to assess how the herbivore, in the absence or in the presence of parasitoid wasps, affected plant growth and survival. Also, we collected volatiles and confirmed that teosinte plants emit odors in response to herbivore attack under semi-field conditions. The presence of parasitoid wasps in the tents significantly reduced herbivore damage, which, for the smallest plants, resulted in a reduction in plant mortality. These findings support the notion that plants may benefit from the presence of parasitoids and may help to resolve the current debate on the defensive function of herbivore-induced volatiles (Chapter 5).

To further explore the importance of volatiles for the attraction of parasitoids under field conditions, we studied maize *lox10* mutants, impaired in the biosynthesis of green leaf volatiles (GLVs). These volatiles, responsible for the smell of cut grass, are commonly emitted by plants when wounded or attacked by herbivores. Previously, laboratory studies have shown that GLVs can be used as foraging cues by predators and parasitoids. We planted maize *lox10* mutants alongside wildtype plants that do emit GLVs in an experimental field in Texas, USA. The plants were artificially infested with *S. frugiperda* larvae and were harvested after a week. The collected larvae were monitored until the emergence of an adult moth or, in the case of parasitism, a parasitoid wasp. *C. marginiventris* and *Chelonus* spp. (Hymenoptera: Braconidae), an egg parasitoid, were identified as the major parasitoids. Overall, parasitism levels were low but, contrary to expectations, tended to be highest on non-GLV-emitting *lox10* plants. This result implies that GLVs are not of key importance for parasitoid attraction in the field (Chapter 6).

Collectively, these studies provide novel insights into the importance of herbivore-induced volatiles for the attraction of parasitoid wasps, and their indirect importance for plant growth and survival. We identified differences between modern, cultivated maize and its wild ancestor, teosinte, with respect to the attraction of parasitoid wasps that could possibly be exploited for ecologically sound methods to better protect cultivated maize against insect pests.

## Résumé

Le maïs moderne (*Zea mays* ssp. *mays*, Poaceae) est caractérisé par des larges épis renfermant des graines juteuses. Pourtant, ces plantes n'ont pas toujours présenté ces caractéristiques. Il y a environ 9000 ans, le maïs a été domestiqué à partir de la téosinte (*Z. mays* ssp. *parviglumis*), une espèce sauvage ancestrale qui produite de faibles quantités de graines de petite taille. Alors que la téosinte ne pousse qu'au sud du Mexique, le maïs cultivé est répandu à travers le monde entier. Une sélection continue pour améliorer le rendement et la qualité des graines a influencé d'autres traits, comme la résistance contre les pathogènes et les insectes herbivores (discuté dans le chapitre 1). Cette thèse s'intéresse à la résistance du maïs et de la téosinte face aux insectes ravageurs et plus particulièrement aux composés organiques volatiles émis en réponse à leurs attaques. Ces odeurs, considérées comme des défenses indirectes, peuvent être utilisées par les ennemis naturels des ravageurs, des prédateurs et des parasitoïdes, comme signaux pour trouver leurs proies et leurs hôtes. Les guêpes parasitoïdes sont un exemple d'ennemi naturel. Les femelles peuvent pondre un ou plusieurs œufs à l'intérieur de leur hôte à l'aide de leur ovipositeur. Au cours de son développement, la larve du parasitoïde se nourrira de son hôte et finira par le tuer, pouvant ainsi avantager la plante.

D'abord nous avons étudié quels insectes ravageurs sont associés avec la téosinte dans la nature (Chapitre 2). La chenille *Spodoptera frugiperda* (Lepidoptera: Noctuidae) est un des plus importants ravageurs du maïs sur le continent américain et est associée avec plusieurs espèces de guêpes parasitoïdes. Cependant, il n'y a pas beaucoup d'informations concernant la présence de cet herbivore et de ses parasitoïdes associés sur la téosinte.

En conditions de laboratoire, nous avons comparé les odeurs émises par le maïs et la téosinte lorsqu'ils sont attaqués par des chenilles. Nous avons ensuite évalué l'attractivité de ces odeurs pour deux espèces de guêpes parasitoïdes solitaires, à savoir *Cotesia marginiventris* (Hymenoptera: Braconidae) et *Campoletis sonorensis* (Hymenoptera: Ichneumonidae). Bien que les analyses par chromatographie gazeuse révèlent une similarité dans les mélanges odorants émis, les guêpes parasitoïdes les perçoivent différemment. Alors que *C. sonorensis* ne semble pas faire de distinction entre les odeurs émises par le maïs et celles émises par la téosinte, *C. marginiventris* semble quant à elle préférer les odeurs émises par la téosinte (Chapitres 3, 4). Nous avons obtenu des résultats similaires en testant de vraies plantes ainsi que des extraits d'odeurs, ce qui indique que ces extraits pourraient permettre l'identification des composés clés responsables de l'attraction des guêpes parasitoïdes (Chapitre 3).

De façon intéressante, nous avons observé que la plante hôte, c'est à dire le maïs cultivé ou la téosinte sauvage, peut influencer le développement des larves de parasitoïdes dans leurs hôtes. Tandis que *C. marginiventris* se développait de façon similaire, que son hôte se soit nourri sur du maïs ou sur de la téosinte, les cocons de *C. sonorensis* étaient plus gros lorsque l'hôte s'est nourri de maïs. Ces résultats montrent que les guêpes parasitoïdes ne

sont pas nécessairement attirées par les meilleures plantes pour le développement de leur progéniture (Chapitre 4).

Il y a étonnement peu de preuves sur le terrain indiquant que les ennemis naturels confèrent un avantage aux plantes en termes de fitness. Cet aspect reste un élément important au cœur de la discussion du rôle des composés volatiles dans le cadre des défenses indirectes, et plus particulièrement en ce qui concerne les parasitoïdes qui, à la différence des prédateurs, ne tuent pas directement leurs hôtes. Pour éclaircir ce point, nous avons évalué l'impact des guêpes parasitoïdes sur la performance des plantes dans un environnement semi-naturel à Morelia, au Mexique. Notre système tritrophique comprenait la téosinte, dénuée d'effets pléiotropiques liés à la sélection artificielle, l'insecte ravageur *S. frugiperda* et la guêpe parasitoïde *C. sonorensis*, un des principaux ennemis naturels de *S. frugiperda* avec lequel il partage une longue association évolutive. La téosinte a été plantée dans de vastes tentes grâce auxquelles nous avons pu évaluer l'influence de l'herbivore sur la croissance et la survie des plantes et ce, en présence ou en absence de guêpes parasitoïdes. Parallèlement, des collectes d'odeurs nous ont permis de confirmer que la téosinte émet des substances volatiles en réponse à l'attaque d'insectes herbivores dans la nature. La présence des guêpes parasitoïdes dans les tentes a réduit les dégâts causés par les herbivores sur les jeunes plantes, réduisant ainsi la mortalité de la téosinte. Ces résultats étayaient l'hypothèse selon laquelle les plantes bénéficient de la présence de guêpes parasitoïdes et peuvent de ce fait aider à résoudre le débat actuel sur le rôle défensif des volatils induits par les herbivores (Chapitre 5).

Pour explorer en plus amples détails l'importance des substances volatiles pour l'attraction des parasitoïdes sur le terrain, nous avons étudié des mutants de maïs *lox10*, produisant peu ou pas de « green leaf volatiles » (GLVs). Ces composés volatiles, à l'origine de l'odeur d'herbe coupée, sont généralement émis par des plantes blessées ou attaquées par des herbivores. Des études en laboratoires ont montré que ces GLVs peuvent être utilisés par les prédateurs et parasitoïdes pour localiser leurs proies et leurs hôtes. Nous avons planté des mutants de maïs *lox10* ainsi que du maïs dépourvu de cette mutation, pouvant donc produire des GLVs, dans un champ expérimental au Texas, aux Etats Unis. Les plantes ont été artificiellement infestées avec des larves de *S. frugiperda* et ont été récoltées après une semaine. Les larves collectées ont été observées jusqu'à l'émergence d'un papillon ou, en cas de parasitisme, d'une guêpe parasitoïde. *C. marginiventris* et *Chelonus* spp. (Hymenoptera: Braconidae), un parasitoïde oophage, ont été identifiés comme les principaux parasitoïdes. Globalement, les taux de parasitisme étaient faibles, mais, contrairement à nos attentes, ils tendaient à être plus élevés sur les plantes mutantes *lox10*. Ces résultats impliquent que les GLVs ne sont pas d'une importance capitale pour l'attraction des guêpes parasitoïdes sur le terrain (Chapitre 6).

Dans l'ensemble, ces études ouvrent de nouvelles perspectives au sujet de l'importance des odeurs des plantes pour l'attraction des guêpes parasitoïdes et leur importance indirecte pour la croissance des plantes et leur survie. Nous avons mis en évidence l'existence de différences entre le maïs cultivé et son ancêtre sauvage, la téosinte, concernant l'attraction

de guêpes parasitoïdes. Ces nouvelles connaissances peuvent être utilisées dans le cadre du développement de nouvelles méthodes écologiques visant à mieux protéger les cultures de maïs contre les insectes ravageurs.





## Resumen

El maíz moderno (*Zea mays* ssp. *mays*, Poaceae) se caracteriza por grandes mazorcas con granos jugosos de alto contenido nutricional comparado con los presentes en la especie de maíz nativo, el teosinte. Estos caracteres, han ido modificándose desde hace aproximadamente 9000 años por medio de la domesticación hecha por el hombre. Cabe mencionar, que en la actualidad, aunque el maíz está distribuido por casi todo el mundo, podemos encontrar algunas poblaciones de teosinte en el sur de México. La selección artificial impuesta por los humanos en esta especie ha llevado un costo para las plantas, debido a que se han alterado caracteres tales como la resistencia en contra de patógenos y a insectos herbívoros (revisión capítulo 1). En esta tesis, se evaluó la resistencia del maíz y del teosinte contra sus plagas (artrópodos), enfocándose en la emisión de volátiles inducidos después de la herbivoría. Este carácter es considerado como una defensa indirecta, porque los enemigos naturales, como depredadores y parasitoides, de los herbívoros pueden utilizar estas señales para buscar sus presas y hospederos. Un ejemplo son las avispas parasitoides que ponen huevos en su hospedero (herbívoro) y cuando están es la última etapa de desarrollo matan al hospedero beneficiando a la planta.

En primer lugar se evaluó en la naturaleza cuyos insectos plagas ocurren en teosinte (Capítulo 2). El gusano cogollero, *Spodoptera frugiperda* (Lepidoptera: Noctuidae), es una plaga importante del maíz en las Americas. Es frecuentemente atacado por varias especies de avispas. Pero no hay mucha información de la presencia de este herbívoro y sus avispas asociadas sobre teosinte.

Con experimentos de laboratorio se evaluó si las plantas de maíz y de teosinte emiten olores similares después de haber sido dañadas por larvas de lepidópteros. Después, se evaluó la acción de estos olores sobre la atracción de dos avispas parasitoides solitarias *Cotesia marginiventris* (Hymenoptera: Braconidae) y *Campoletis sonorensis* (Hymenoptera: Ichneumonidae). Aunque los resultados de estos análisis de olores entre plantas no muestran diferencias significativas; cuando fueron analizados bajo cromatografía de gases, las especies de parasitoides respondieron de forma diferente. La especie *C. sonorensis* no distinguió entre olores, mientras que *C. marginiventris* mostró preferencias en los olores emitidos por las plantas de teosinte comparado con las plantas de maíz (capítulo 3, 4). Resultados similares fueron obtenidos cuando se probaron con plantas *versus* los extractos colectados en el campo (capítulo 3), lo cual sugiere que estos extractos podrían ser usados para identificar algunos compuestos implicados en la atracción de los parasitoides.

También, se encontró que existe un efecto de la planta sobre el desarrollo de las larvas del parasitoide dentro de su hospedero. Mientras que en *C. marginiventris* no se encontraron diferencias entre el desarrollo de los parasitoides provenientes de herbívoros que se alimentaron de teosinte y de maíz, la especie *C. sonorensis* mostró diferencias significativas en el tamaño de los capullos, los cuales fueron más grandes cuando los herbívoros se alimentaron de plantas de maíz. Estos resultados indican que los parasitoides

no fueron necesariamente atraídos a las plantas que representan un recurso de mejor calidad para el desarrollo de su progenie (capítulo 4).

Existe escasa evidencia en la literatura sobre los beneficios en términos de adecuación para las plantas por la acción de los enemigos naturales de los herbívoros. Aunque el rol de la posible defensa indirecta mediada por los volátiles que son inducidos por la planta después del daño esta aun en discusión, y aún más particularmente en el caso particular de los parasitoides que durante su ciclo de vida no matan directamente a los herbívoros. Se evaluó el cómo los parasitoides pueden afectar el rendimiento de las plantas en condiciones seminaturales en Morelia, México. Nuestro sistema tritrofico incluyó el teosinte, el gusano cogollero *S. frugiperda* y la avispa parasitoide *C. sonorensis*, las cuales en su conjunto comparten una historia evolutiva definida. El diseño consistió en plantas de teosinte acomodadas en tiendas las cuales permitieron evaluar como la presencia del herbívoro con o si parasitoidismo afecta el crecimiento y la supervivencia de las plantas. También se colectaron volátiles y se confirmó que las plantas de teosinte emiten olores en respuesta al ataque de los herbívoros bajo condiciones de campo. La presencia de las avispas parasitoides en las tiendas redujo significativamente el daño por herbívoros, lo cual para plantas muy pequeñas resultó en una reducción de la mortalidad de las plantas. Estos resultados sugieren que la presencia de los parasitoides podría ayudar a resolver el debate sobre el rol defensivo de los volátiles inducidos por los herbívoros (capítulo 5).

Para explorar con más detalles la importancia de olores para atraer las avispas bajo condiciones de campo, se estudiaron plantas del maíz mutantes *lox10*, que no pueden sintetizar “volátiles verdes de las hojas” (GLVs). Estos volátiles, responsables por el olor de hierba cortada, son rápidamente emitidos por las plantas cuando ellas son dañadas o atacadas por insectos herbívoros. Estudios en el laboratorio han mostrado que los depredadores y los parasitoides pueden utilizar GLVs para localizar sus presas u hospederos. Para este experimento se plantó maíz *lox10* mutantes al lado de plantas variedad “silvestre” que pueden emitir GLVs en un campo experimental en Texas, EEUU. Infestamos las plantas con *S. frugiperda* y se colectaron una semana después, las larvas colectadas fueron almacenadas y puestas bajo condiciones controladas hasta la emergencia de herbívoro o depredador. Los niveles de infestación por parasitoides fueron muy bajos pero a diferencia de nuestra hipótesis, se encontraron más parasitoides en las plantas mutantes *lox10*. Estos resultados sugieren que los GLVs no son compuestos cruciales en la atracción de avispas parasitoides en el campo (capítulo 6).

En resumen, estos estudios en su conjunto proveen evidencia de la importancia de los volátiles inducidos por herbivoría para la atracción de parasitoides, y el rol que ejercen en la defensa indirecta de las plantas. Las diferencias encontradas entre la variedad silvestre del maíz (teosinte) y el maíz actual con respecto a la atracción de parasitoides que podría ser explotado en un futuro para crear estrategias de protección de cultivos de maíz en contra de sus insectos herbívoros.

## Zusammenfassung

Moderne Maispflanzen (*Zea mays* ssp. *mays*, Poaceae) zeichnen sich durch grosse Maiskolben mit saftigen Körnern aus, obwohl sie diese Eigenschaften nicht immer hatten. Vor etwa 9000 Jahren wurde Mais von Teosinte (*Z. mays* ssp. *parviglumis*), dem wilden Vorfahren, domestiziert, welche viel weniger und viel kleinere Samen produziert. Teosinte wächst noch immer wild im Süden Mexikos, während kultivierter Mais auf der ganzen Welt angebaut wird. Kontinuierliche Selektion für erhöhten Ertrag und bessere Qualität war mit Kosten für die Pflanze verbunden, bezüglich dem Verlust oder der Modifikation anderer potentiell nützlicher Eigenschaften, wie beispielsweise der Resistenz gegenüber Pathogenen und pflanzenfressenden Insekten (Überblick im Kapitel 1). Diese Dissertation bezieht sich auf die Resistenz von Mais und Teosinte gegen Pflanzenschädlinge, insbesondere bezüglich der Emission von durch Herbivoren induzierten Duftstoffen. Diese Eigenschaft ist als indirekter Verteidigungsmechanismus angesehen, da die Duftstoffe den Prädatoren und Parasitoiden, welche die natürlichen Feinde der pflanzenfressenden Insekten sind, die Anwesenheit von Beute- oder Wirtstieren signalisieren. Parasitische Wespen gehören zu den natürlichen Feinden. Die Weibchen legen mit ihrem Legebohrer einzelne oder mehrere Eier in einzelne Wirtstiere. Wenn die Wespenlarven sich im Wirt entwickeln, werden sie ihn letztendlich töten, was von Vorteil für die Pflanze sein kann.

Zuerst haben wir im Freiland ermittelt, welche Pflanzenschädlinge auf Teosinte vorkommen (Kapitel 2). Der Eulenfalter *Spodoptera frugiperda* (Lepidoptera: Noctuidae) ist eine der schwerwiegendsten Maisplagen in Amerika, und er wird regelmässig von vielen Schlupf- und Brackwespenarten parasitiert. Allerdings ist wenig bekannt bezüglich der Präsenz dieses Pflanzenfressers und seiner Parasitoide auf Teosinte.

Im Labor haben wir daraufhin untersucht, ob Mais und Teosinte ähnliche Mischungen von Duftstoffen ausstossen, wenn sie von Pflanzenfressern (Lepidoptera) induziert werden. Wir haben zusätzlich die Attraktivität des Duftgemisches gegenüber Weibchen zweier Arten von solitär lebenden endoparasitischen Wespen, *Campoletis sonorensis* (Hymenoptera: Ichneumonidae) und *Cotesia marginiventris* (Hymenoptera: Braconidae), untersucht. Obwohl die Duftgemische in der Gaschromatographie ähnlich erschienen, reagierten die Parasitoide unterschiedlich auf sie. *C. sonorensis* konnte nicht zwischen den Düften unterscheiden, während *C. marginiventris* den Duft von Teosinte über den der Maispflanzen bevorzugte (Kapitel 3, 4). Wir erhielten ähnliche Ergebnisse in einem Test mit lebenden Pflanzen und Extrakten des gesammelten Duftes (Kapitel 3), was darauf deuten lässt, dass diese Extrakte zur Identifizierung der Schlüsselkomponenten, welche für die Anziehung der parasitischen Wespen wichtig sind, genutzt werden können.

Interessanterweise haben wir beobachtet, dass die Wirtspflanze, also kultivierter Mais oder wilde Teosinte, eine Auswirkung auf die Entwicklung der Parasitoidlarven in ihrem Wirt haben kann. Während *C. marginiventris* sich gleich gut entwickelt, wenn ihr Wirt, *S. frugiperda*, an Mais oder Teosinte gefressen hat, waren die Kokons von *C. sonorensis*

grösser, wenn ihr Wirt an Mais gefressen hat. Da *C. marginiventris* Wespen den Duft der Teosinte bevorzugt haben, und *C. sonorensis* Wespen keine Präferenz für Teosinte- oder Mais-Düfte gezeigt haben, deuten diese Ergebnisse darauf hin, dass Parasitoide nicht unbedingt von den Pflanzen angezogen waren, die am besten für die Entwicklung ihres Nachwuchses geeignet waren (Kapitel 4).

Es existieren überraschend wenige Nachweise von Fitnessvorteilen für Pflanzen durch die Aktivität natürlicher Feinde im Freiland. Dies ist noch immer ein wichtiges Argument in der Diskussion um die mögliche Rolle von induzierbaren pflanzlichen Duftstoffen in indirekter Verteidigung, insbesondere im Fall parasitischer Wespen, die, im Gegensatz zu Prädatoren, ihren Wirt nicht direkt töten. Deshalb haben wir evaluiert, wie parasitische Wespen die Leistung der Pflanzen in einer halbnatürlichen Umgebung in Morelia, Mexiko, beeinflussen. Unser tritrophes System umfasste Teosinte, frei von pleiotropen Effekten von künstlicher Selektion, den Herbivoren *S. frugiperda* und die Wespe *C. sonorensis*; jeweils Organismen, die in der Natur zusammen vorkommen. Teosinte wurde in grossen Fliegengitterzelten gepflanzt, was uns ermöglichte zu untersuchen, wie der Pflanzenfresser, in Abwesenheit oder Anwesenheit parasitischer Wespen, das Wachstum und Überleben der Pflanzen beeinflusst. Zusätzlich haben wir Duftstoffe gesammelt und bestätigt, dass Teosintepflanzen unter halbnatürlichen Konditionen Düfte als Reaktion auf den Angriff von Pflanzenfressern abgeben. Die Anwesenheit parasitischer Wespen in den Zelten reduzierte den Schaden durch die Pflanzenfresser, welches für die kleinsten Pflanzen eine Reduktion der Mortalitätsrate bedeutete. Diese Ergebnisse unterstützen die Ansicht, dass Pflanzen von der Gegenwart der Parasitoide profitieren können, und sie könnten helfen, die jetzige Debatte um die Verteidigungsfunktion von durch Herbivoren induzierte Duftstoffe beizulegen (Kapitel 5).

Zur weiteren Erforschung der Bedeutung von flüchtigen Stoffen für die Anziehung von Parasitoiden im Feld, haben wir Mais *lox10* Mutanten untersucht, welche in der Biosynthese von Blattduftstoffen („green leaf volatiles“, GLVs) beeinträchtigt sind. Diese Duftstoffe, verantwortlich für den charakteristischen Duft von geschnittenem Gras, werden häufig von Pflanzen abgegeben, die verletzt oder von Schädlingen befallen wurden. Es wurde zuvor gezeigt, dass GLVs von Prädatoren und Parasitoiden als Hinweise während der Nahrungssuche genutzt werden. In einem Freilandversuch in Texas (USA) wurden Mais *lox10* Mutanten neben Wildtyppflanzen, welche GLVs ausstossen, ausgebracht. Die Pflanzen wurden künstlich mit *S. frugiperda* Larven infiziert und nach einer Woche geerntet. Die eingesammelten Larven wurden beobachtet bis zum Schlüpfen der adulten Motten oder, im Fall von Parasitismus, der parasitischen Wespen. *C. marginiventris* und *Chelonus* spp. (Hymenoptera: Braconidae), ein Eiparasitoid, wurden als hauptsächliche Parasitoiden identifiziert. Insgesamt waren die Parasitismusraten niedrig, aber, im Gegensatz zu unserer Erwartung, tendierten sie dazu, am höchsten in nicht-GLV-emittierenden *lox10* Pflanzen zu sein. Dieses Ergebnis lässt darauf schliessen, dass GLVs nicht die Haupt-Ursache für die Anziehung von Parasitoiden im Feld sind (Kapitel 6).

Gemeinsam bringen diese Studien neue Einsicht in die Bedeutung von durch Schädlinge induzierten Duftstoffen für die Anziehung von parasitischen Wespen, und ihre indirekte Bedeutung für das Wachstum und Überleben der Pflanzen. Wir identifizierten Unterschiede zwischen modernem, kultiviertem Mais und seinem wilden Vorfahren, Teosinte, bezüglich der Anziehung parasitischer Wespen, welche möglicherweise für ökologische Methoden genutzt werden können, um kultivierten Mais besser gegen Pflanzenschädlinge zu schützen.



## Samenvatting

Moderne, gecultiveerde maisplanten (*Zea mays* ssp. *mays*, Poaceae) hebben grote kolven met vele sappige maiskorrels. Deze planten hebben er echter niet altijd zo uitgezien. Ongeveer 9000 jaar geleden is mais gedomesticeerd vanuit zijn wilde voorouder, teosinte (*Z. mays* ssp. *parviglumis*). Teosinte produceert veel minder en veel kleinere zaden dan mais. Terwijl mais overal ter wereld gecultiveerd wordt, groeit teosinte in het zuiden van Mexico nog steeds in het wild. Voortdurende kunstmatige selectie heeft geleid tot een betere gewasopbrengst en een hogere kwaliteit van de maiskorrels. Deze vooruitgang is echter onbedoeld ten koste gegaan van nuttige natuurlijke eigenschappen, zoals resistentie tegen pathogenen en planteneterende insecten (samengevat in hoofdstuk 1). Dit proefschrift gaat over de resistentie van gecultiveerde mais en wilde teosinte tegen plaaginsecten, voornamelijk met betrekking tot de uitstoot van geurstoffen in reactie op een aanval van deze insecten. Deze eigenschap wordt over het algemeen geïnterpreteerd als een mechanisme van indirecte afweer. Geurstoffen kunnen de aanwezigheid van een prooi of gastheer verraden aan hun natuurlijke vijanden, predatoren en parasitoiden. Een sluipwesp is een voorbeeld van een parasitoïde die zich in of op een gastheer ontwikkelt. Vrouwtjes hebben een legboor waarmee ze één of meerdere eitjes kunnen leggen per gastheer en de ontwikkeling van de wespen, die zich voeden met de gastheer, leidt uiteindelijk tot zijn dood.

Eerst hebben we onderzocht welke herbivore insecten voorkomen op teosinte in de natuur (Hoofdstuk 2). De larven van de nachtvlinder *Spodoptera frugiperda* (Lepidoptera: Noctuidae), zijn verantwoordelijk voor één van de belangrijkste plagen van mais op het Amerikaanse continent. Vele verschillende soorten parasitoïde wespen zijn geassocieerd met deze rups. Er is echter nog weinig bekend over het voorkomen van de rups en zijn parasitoiden op teosinte.

Daarna hebben we in het laboratorium gekeken of mais en teosinte vergelijkbare geurstoffen uitstoten in reactie op rupsenvraat. We hebben ook de aantrekkelijkheid van deze geurstoffen geëvalueerd voor vrouwtjes van twee soorten solitaire sluipwespen, *Cotesia marginiventris* (Hymenoptera: Braconidae) en *Campoletis sonorensis* (Hymenoptera: Ichneumonidae). Hoewel het er na analyse met behulp van gaschromatografie op leek dat mais en teosinte een vergelijkbaar geurprofiel produceren in reactie op insectenvraat, reageerden de sluipwespen verschillend op deze geuren. *C. sonorensis* had geen voorkeur voor een bepaalde plantengeur, maar *C. marginiventris* verkoos de geur van teosinte boven die van mais (Hoofdstuk 3, 4). We verkregen vergelijkbare resultaten wanneer we het experiment uitvoerden met echte planten en met geurextracten van door herbivoren aangevreten mais en teosinte (Hoofdstuk 3). Dit geeft aan dat geurextracten mogelijk gebruikt kunnen worden voor het identificeren van de sleutelcomponenten die verantwoordelijk zijn voor het aantrekken van de sluipwespen.

Interessant genoeg hebben we geobserveerd dat de waardplant waarop de gastheer zich bevindt, dat wil zeggen mais of teosinte, een effect kan hebben op de ontwikkeling van sluipwespen. Terwijl *C. marginiventris* zich vergelijkbaar ontwikkelde in rupsen die zich voedden met mais of teosinte, waren de poppen van *C. sonorensis* groter wanneer hun gastheer mais te eten kreeg. Deze resultaten geven aan dat de sluipwespen niet noodzakelijkerwijs aangetrokken werden tot de waardplanten die het beste zijn voor de ontwikkeling van hun nageslacht (Hoofdstuk 4).

Er is verrassend weinig bewijs gebaseerd op veldexperimenten voor de bewering dat het aantrekken van natuurlijke vijanden een positieve invloed heeft op de fitness van planten. Wetenschappers bediscussiëren volop of induceerbare plantengeuren wel of niet beschouwd moeten worden als een indirect afweermecanisme, voornamelijk omdat de aantrekking van sluipwespen, in tegenstelling tot predatoren, niet direct leidt tot het doden van herbivoren. Daarom hebben we in een semi-natuurlijke omgeving in Morelia, Mexico, bestudeerd hoe sluipwespen de ontwikkeling van planten beïnvloeden. Ons tritrofe systeem bestond uit teosinte, een plant die niet is beïnvloed door veredeling, de rups *S. frugiperda* en de sluipwesp *C. sonorensis*, een belangrijke natuurlijke vijand van *S. frugiperda* in de regio. Door teosinte te planten in grote tenten werd het mogelijk om te bestuderen hoe de herbivoren in de aan- of afwezigheid van sluipwespen de groei en overleving van de planten beïnvloedden. Ook verzamelden we geurstoffen, wat bevestigde dat teosinte in het veld geurstoffen uitstoot in reactie op rupsenvraat. Wanneer er sluipwespen in de tenten aanwezig waren, veroorzaakten de rupsen minder schade aan de planten, wat voor de kleinste planten resulteerde in een grotere overlevingskans. Deze resultaten bewijzen dat planten baat hebben bij het aantrekken van sluipwespen en kunnen daarom een bijdrage leveren aan het voortdurende debat over de mogelijke functie van plantengeuren, geïnduceerd door herbivoren, als indirect afweermecanisme (Hoofdstuk 5).

Om het belang van specifieke plantengeuren voor het aantrekken van sluipwespen te bestuderen onder natuurlijke omstandigheden gebruikten we mais *lox10* mutanten, planten die niet langer in staat zijn om „green leaf volatiles“ (GLVs) te produceren. Deze componenten zijn verantwoordelijk voor de geur van versgemaaid gras en worden razendsnel uitgestoten door planten in reactie op verwonding en insectenvraat. Studies in laboratoria hebben aangetoond dat sluipwespen en predatoren GLVs kunnen gebruiken als signaalstoffen voor het vinden van gastheren en prooien. In een experimenteel veld in Texas, Verenigde Staten van Amerika, hebben we mais *lox10* mutanten geplant naast wildtype maisplanten die wel GLVs produceren. Deze planten werden geïnfesteerd met *S. frugiperda* rupsen en werden na een week geoogst. Van de verzamelde rupsen werd genoteerd of ze zich ontwikkelden tot een mot of dat er, in het geval van parasitisme, een sluipwesp larve tevoorschijn kwam. De meest voorkomende parasitoiden werden geïdentificeerd als *C. marginiventris* en *Chelonus* spp. (Hymenoptera: Braconidae), een parasitoïde van eieren. Het aantal geparasiteerde rupsen was laag, maar neigde, in tegenstelling tot onze verwachtingen, het hoogst te zijn op mutante *lox10* planten. Dit resultaat impliceert dat



GLVs niet de belangrijkste signaalstoffen zijn voor het aantrekken van sluipwespen in het veld (Hoofdstuk 6).

Gezamenlijk geven deze studies nieuwe inzichten in het belang van door rupsen geïnduceerde plantengeuren voor de aantrekking van sluipwespen en hun indirecte belang voor de groei en overleving van planten. We hebben verschillen ontdekt tussen moderne, gecultiveerde mais en zijn wilde voorouder, teosinte, met betrekking tot het aantrekken van natuurlijke vijanden van herbivore insecten. Deze kennis kan mogelijk gebruikt worden voor het ontwikkelen van nieuwe, duurzame methodes om gecultiveerde mais beter te beschermen tegen insectenplagen.



## General introduction

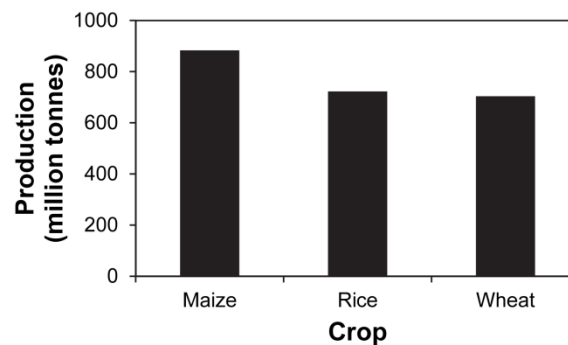
Despite the large variety of crop protection methods available, insects still account for at least 15% of the world's crop losses (Oerke, 2006). This is an alarming fact in the view of a human population that is projected to rise from today's 7.1 billion to 8.9 billion by 2050 (United Nations, 2004) and will therefore require an equally dramatic increase in the amount of food that is produced. Current pest control measures rely mostly on chemicals and although they are quite effective, they may have severe non-target effects. One of the first of such effects was shown for dichlorodiphenyltrichloroethane (DDT), which even adversely affects humans after exposure and was found to display enormous bioaccumulation and persistence in the ecosystem (Carson, 1962). Its use has therefore been severely restricted (United Nations, 2001). Most recently, it has been suggested that neonicotinoids may have adverse effects on bees, killing pests and beneficial insects alike (Gill et al., 2012; Henry et al., 2012; Whitehorn et al., 2012), reasons to temporarily ban general use in the European Union (European Union, 2013). Furthermore, pests are increasingly developing resistance to insecticides (Denholm et al., 2002; Whalon et al., 2008). It is generally recognized that novel methods are needed for more sustainable ways to handle pests. Recent focus has been on strengthening the strategies that plants themselves use to combat their enemies (e.g. Turlings and Ton, 2006; Bruce, 2010; Gurr et al., 2012).

Plants are far from helpless when it comes to defending themselves against their herbivorous enemies. They have access to a broad spectrum of defense mechanisms to fend off their attackers, which can be constitutively present or specifically induced in response to herbivore attack. Plant defenses can be physical in nature, such as thorns and trichomes, but also chemical, like toxic compounds and proteinase inhibitors, which are molecules that inhibit the digestion of proteins in the insect gut. Because the presence of these defenses has a direct negative impact on the herbivore, they are termed direct defenses (Schoonhoven et al., 2005)

Plants also show traits that have been interpreted as indirect defenses, because they act through the recruitment of natural enemies of herbivores. For instance, *Acacia* (Fabaceae) and *Cecropia* (Urticaceae) trees have hollow thorns that can house ants, and they may produce extrafloral nectar and food bodies to provide food for the ants, who in turn protect the trees against herbivory (Heil and McKey, 2003; Bronstein et al., 2006). Another well-studied possible indirect defense trait is the emission of volatile organic compounds (VOCs) in response to herbivory, which can be exploited by predators and parasitoids in their search for prey or hosts (Turlings et al., 1990; Turlings and Wäckers, 2004). This thesis will focus on the importance of these herbivore-induced VOCs in cultivated maize (*Zea mays* L. ssp. *mays*, Poaceae) and its wild ancestor, teosinte (*Z. mays* ssp. *parviglumis* Iltis & Doebley).

### Differences and similarities in cultivated maize and wild teosinte

Maize is the most abundantly grown cereal, with an annual production of about 880 million tonnes, above wheat and rice (Fig. 1; FAO, 2013). It is the most important staple food in Mexico and Central America and is also an important food source in other countries worldwide. Furthermore, it is used as feed for livestock and as raw material for industry (FAO, 1992). Despite this importance, its wild ancestor has only recently been revealed. There is no plant with a fruiting structure that looks similar to a maize cob, so the origin of maize has been the topic of considerable controversy (Mangelsdorf and Reeves, 1939; Bennetzen et al., 2001; Doebley, 2001). An extensive genetic study based on microsatellite genotyping eventually put an end to the controversy, as Balsas teosinte (*Z. mays* ssp. *parviglumis*) was confirmed to be the wild ancestor of cultivated maize (Matsuoka et al., 2002; Doebley, 2004). This plant is still growing nowadays in the area where maize was thought to be domesticated some 9000 years ago, in southern Mexico (Sánchez González and Ruiz Corral, 1995; Matsuoka et al., 2002).



**Fig. 1** Worldwide production of maize, rice and wheat, the world's most important cereals, in 2011. Adapted from FAO (2013)

All taxa in the genus *Zea*, besides cultivated maize, are collectively referred to as teosintes. Occasionally, teosinte is still referred to as *Euchlaena*, the genus in which it was placed initially. The genus *Zea* contains a total of eight taxa classified into two sections and five species (Table 1), although new classifications are continuously being proposed (Fukunaga et al., 2005; Sánchez G. et al., 2011). The section *Zea* contains four subspecies of *Z. mays* and in *Z. mays* ssp. *mexicana* (Schrader) Iltis, different races are distinguished based on ethnobotanical information, geography, and morphology. The section *Luxuriantes* contains four more distantly related species.

Maize and teosinte are monocots with C4 carbon fixation. They are monoecious, that is, they contain separate male and female flowers on the same plant. Maize, as well as most teosintes, is diploid ( $2n=20$ ), with the exception of *Z. perennis* (Hitchc.) Reeves & Mangelsdorf, which is tetraploid ( $2n=40$ ). All maizes and teosintes are annual with the exception of two perennial species, *Z. diploperennis* Iltis, Doebley & Guzmán and *Z. perennis*. Maize and teosinte differ greatly, both morphologically and genetically, although they frequently hybridize (Wilkes, 1977; Fig. 2,3). During domestication, the process of genetic

modification of a wild species to create a new form of a plant suited to human needs (Doebley et al., 2006), maize stopped producing as many branches as teosinte. Instead, maize developed a large cob with more individual kernels. Those are, contrary to teosinte, firmly attached to the cob so that they do not disperse, facilitating harvest by humans (Fig. 2,3). A common set of traits, including but not limited to apical dominance, seed dispersal, and a decrease in bitter substances in edible structures, distinguishes most seed and fruit crops from their wild ancestors and is known as the “domestication syndrome” (Doebley et al., 2006). Although defining this “domestication syndrome” tends to be biased towards cereals, when comparing a great number of different crops, changes in secondary metabolites affecting flavor, pigments and toxicity appear to be the most common traits associated with breeding and cultivation (Meyer et al., 2012). Charles Darwin already addressed domestication as an adaptation (Darwin, 1859, 1868). After initial domestication, there has been a large improvement of maize. Traditional landraces, locally adapted varieties managed by local farmers, used to be major sources of crop variability. However, nowadays, a small number of hybrids and other high-yielding varieties are mostly planted. These varieties were mainly developed during the “Green Revolution” in the sixties, and in combination with improved cultivation practices, such as applying nitrogen fertilizers and enhanced methods of irrigation, worldwide crop yield increased tremendously (Khush, 2001).

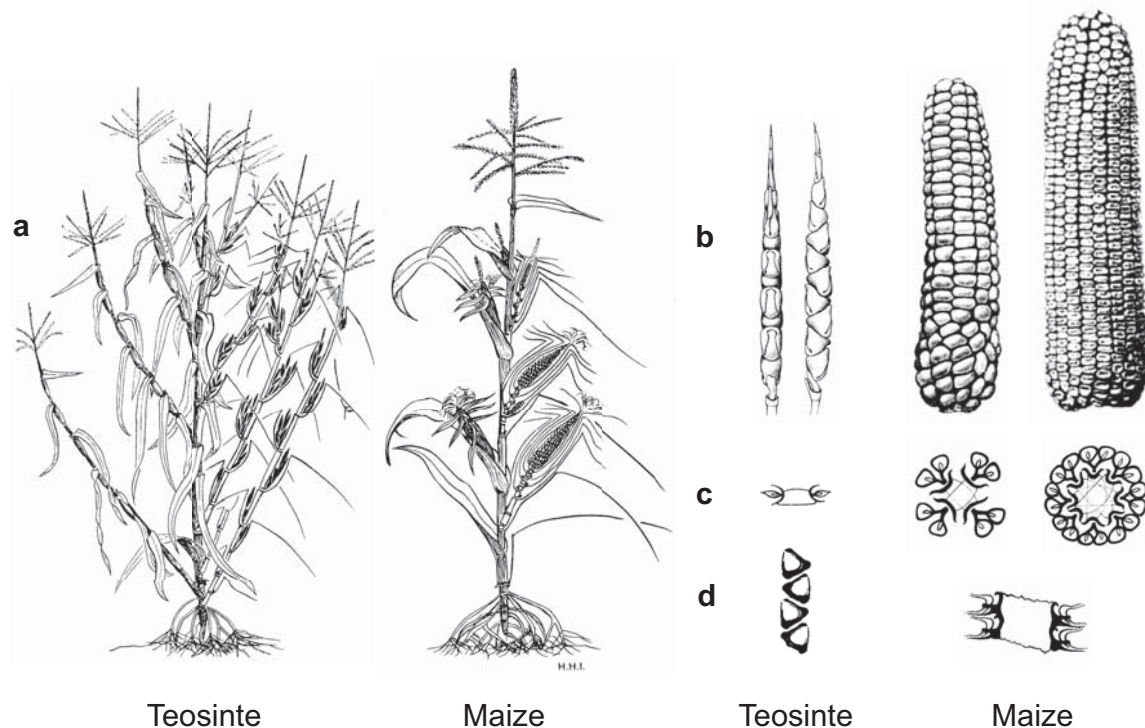
**Table 1** The taxonomy of *Zea*

<b>Section Luxuriantes Doebley &amp; Iltis</b>
<i>Zea diploperennis</i> Iltis, Doebley & Guzmán
<i>Zea perennis</i> (Hitchc.) Reeves & Mangelsdorf
<i>Zea luxurians</i> (Durieu & Ascherson) Bird
<i>Zea nicaraguensis</i> Iltis & Benz
<b>Section Zea</b>
<i>Zea mays</i> L.
ssp. <i>mexicana</i> (Schrader) Iltis
Race Chalco
Race Central Plateau
Race Durango
Race Nobogame
ssp. <i>parviglumis</i> Iltis & Doebley
ssp. <i>huehuetenangensis</i> (Iltis & Doebley) Doebley
ssp. <i>mays</i>

After Iltis and Doebley (1980), Doebley (1990), Iltis (2000) and Iltis and Benz (2000)

While maize is grown all over the world nowadays, teosinte occurs only in the tropical and subtropical areas of Mexico, Guatemala, Honduras and Nicaragua (Fig. 4). Teosinte populations are not uniformly distributed, but populations are isolated and rather vary in size. Teosintes commonly grow at roadsides, in abandoned fields and next to maize fields, but the plants can also invade maize fields and in some areas are notorious as weeds (Vibrans and Estrada Flores, 1998; Mondragón-Pichardo and Vibrans, 2005). Overall, similar

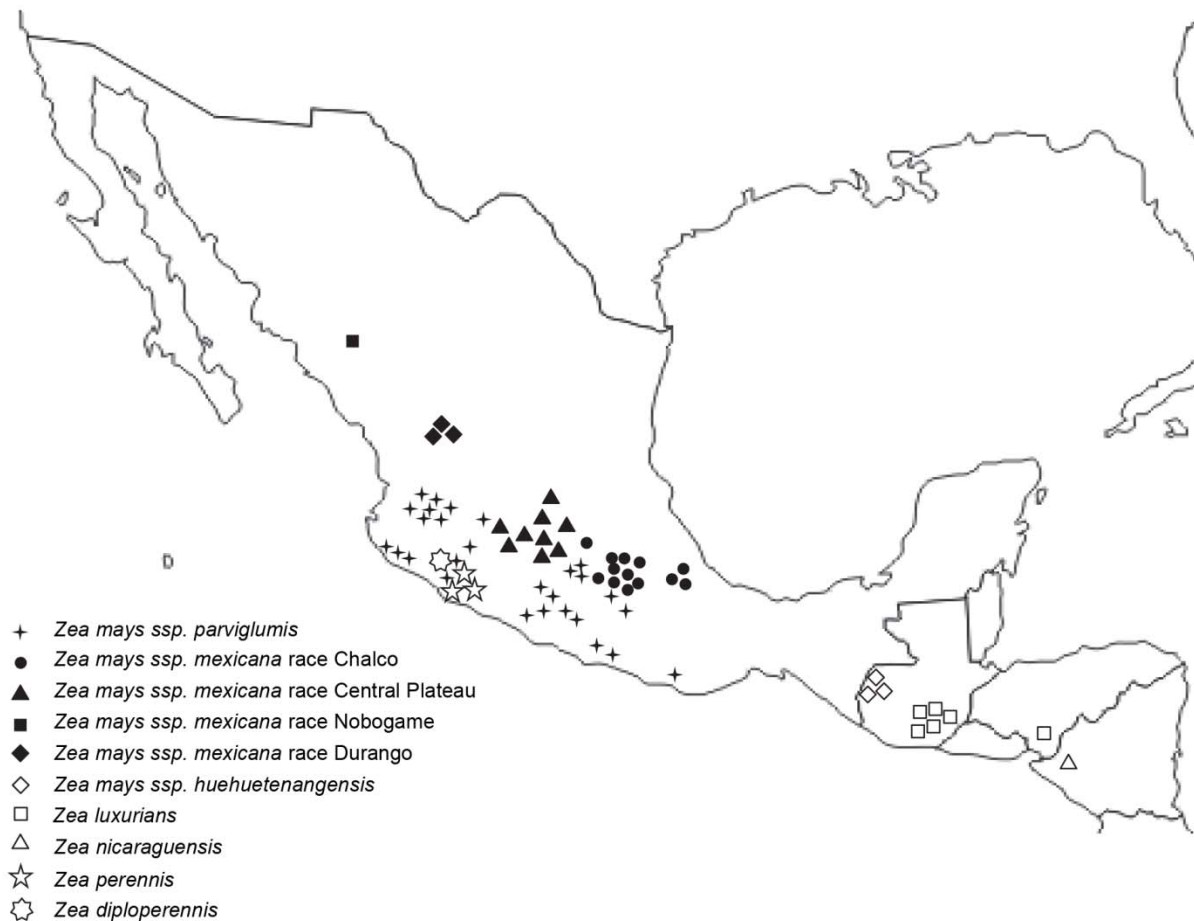
insects and pathogens occur on maize and teosinte in the area of distribution of teosinte, although there is ample local variability (Sánchez González and Ruiz Corral, 1995).



**Fig. 2** Morphological differences between wild teosinte and cultivated maize. While teosinte plants have long lateral branches, each terminated by a tassel, and multiple ears growing from secondary branches in the nodes of the leaves on these lateral branches, maize plants have a single stalk bearing one or two short lateral branches tipped by an ear (a). While teosinte ears contain 5-12 kernels, one on top of the other, maize ears can bear 500 or more kernels arranged in 4-20 ranks. The teosinte ear contains abscission layers that allow the ear to shatter when mature, while maize kernels are firmly attached to the central axis of the ear and do not disperse (b). While teosinte bears one kernel on each rank or cupule (kernel-bearing structure), maize has two kernels on each rank (c). Teosinte kernels contain a protective casing, the cupulate fruitcase, while maize kernels are naked on the ear (d). Adapted from several sources (Iltis, 1983; Doebley et al., 1990; Doebley, 1992; Doebley et al., 1995) by Thomas Degen



**Fig. 3** Morphological differences between maize (left) and teosinte (right) seeds. Picture: Matthias Held



**Fig. 4** Teosinte distribution in Mexico. Adapted from Sánchez González and Ruiz Corral (1995)

### Herbivore and pathogen resistance in maize and other plants

Maize plants contain a variety of constitutive and induced defenses against microbes and herbivores. It is known that the plant hormones jasmonic acid (JA), salicylic acid (SA) and ethylene (ET) play an important role in the regulation of these defenses (Balmer et al., 2013b; Lyons et al., 2013). The best-investigated classes of direct defense compounds in maize are benzoxazinoids (Macias et al., 2009; Niemeyer, 2009). Benzoxazinoid-glucosides, weakly active compounds, are stored in the vacuole, and only upon contact with the enzyme  $\beta$ -glucosidase, which is located in the plastid, more toxic aglucons are produced. The compound and the enzyme come in contact when a pathogen or pest attacks the plant (Frey et al., 2009). Benzoxazinoids are also known to be present in teosinte (Shahid and Esen, 1998).

When it comes to VOC-mediated attraction of parasitoids, maize is one of the best studied plants. Non-attacked maize plants hardly emit any odors, but caterpillars feeding on maize plants induce the emission of copious amounts of VOCs that attract various species of parasitoid wasps (Turlings et al., 1990, 1995; Turlings and Fritzsche, 1999; Tamò et al., 2006a). Lima bean (*Phaseolus lunatus* L., Fabaceae) and tobacco (*Nicotiana attenuata* Torr. Ex Wats, Solanaceae) serve as model systems to study the attractiveness of herbivore-induced VOCs to predators. Spider mites feeding on lima bean induce the emission of VOCs

that are attractive to predatory mites (Dicke and Sabelis, 1988) and caterpillars feeding on tobacco induce the emission of VOCs that are attractive to predatory bugs (Kessler and Baldwin, 2001). But the capacity to emit VOCs and to attract natural enemies is not limited to these plant species; the trait appears to be rather common and has been demonstrated in at least 49 plant species in 25 families (Mumm and Dicke, 2010). For instance, caterpillar-induced VOCs of cabbage (*Brassica oleracea* L., Brassicaceae) attract parasitoid wasps (Geervliet et al., 1996), as well as VOCs emitted by the model plant *Arabidopsis thaliana* (L.) Heynh. (Brassicaceae) (Van Poecke et al., 2001). Interestingly, maize roots also emit odors upon herbivory by root-feeding herbivores. When larvae of the beetle *Diabrotica virgifera virgifera* LeConte (Coleoptera: Chrysomelidae) attack the roots, those emit an odor that attracts insect-killing nematodes (Rasmann et al., 2005). Similarly, roots of *Thuja occidentalis* L. (Pinales) emit odors attractive to parasitic nematodes in response to root feeding by weevil larvae (Van Tol et al., 2001), as do roots of citrus plants (Ali et al., 2010).

Odor emission by maize leaves in response to herbivory has been studied for various species of folivorous caterpillars, like beet armyworm *Spodoptera exigua* Hübner (Lepidoptera: Noctuidae) (Turlings et al., 1990), Egyptian cotton leafworm *S. littoralis* Boisduval (Turlings et al., 1998a; Degen et al., 2004), fall armyworm *S. frugiperda* Smith (Carroll et al., 2006), and corn earworm *Heliothis armigera* Hübner (Lepidoptera: Noctuidae) (de Lange, 2008). Two stemborers, the European corn borer *Ostrinia nubilalis* Hübner (Lepidoptera: Crambidae) (Turlings et al., 1998a) and the Asian corn borer *O. furnacalis* Guenée (Huang et al., 2009) also induce odor emission, as does an attack of piercing-sucking insects, such as the corn leaf aphid *Rhopalosiphum maidis* Fitch (Hemiptera: Aphididae) (Turlings et al., 1998a) and the leafhopper *Euscelidius variegatus* Kirschbaum (Hemiptera: Cicadellidae) (Erb et al., 2010). However, maize does not tend to emit odors in response to attack of a pathogen, such as *Setosphaeria turcica* (Rostás et al., 2006) or *Colletotrichum graminicola* (Gregory Röder, Elvira S. de Lange, Dirk Balmer and Ted C.J. Turlings, unpublished data).

The interactions between plants, herbivores and natural enemies are generally termed tritrophic interactions, as they take place at three levels of the food chain (Price et al., 1980). Although herbivore-induced VOCs are extensively studied, for many study systems it remains unclear which of the compounds in the blend are of key importance for parasitoid and predator attraction (D'Alessandro and Turlings, 2006).

### **Herbivore-induced VOCs and their biosynthesis**

Various plant organs emit odors, but these odors may have different functions: while floral volatiles serve, among others, to attract pollinators, volatiles emitted from vegetative parts, both from shoots and from roots, tend to play a role in protection against herbivory (Pichersky and Gershenzon, 2002). The blends emitted from floral and vegetative parts are distinct, although they can have several compounds in common (Lucas-Barbosa et al., 2011; Tholl et al., 2011). Maize plants are wind pollinated and these types of flowers tend to emit



less odors than insect-pollinated flowers (Faegri and Van der Pijl, 1979). But, as stated above, maize vegetative parts emit copious amounts of odors in response to herbivory.

There are four major VOC biosynthetic pathways (Maffei, 2010; Dudareva et al., 2013). The first, the lipoxygenase (LOX) pathway, leads to the emission of green leaf volatiles (GLVs; Paré and Tumlinson, 1997; Turlings et al., 1998b), six-carbon aldehydes, alcohols and their acetates, which are named after their distinctive “green” scent, like the smell of cut grass (Hatanaka, 1993; Matsui, 2006). This pathway also leads to the biosynthesis of the plant hormone JA, which plays an important role in the regulation of defenses against herbivory. GLVs, such as (Z)-3-hexenal, (E)-2-hexenal, (Z)-3-hexen-1-ol and (Z)-3-hexenyl acetate, are rapidly formed after leaves are damaged, whether that is by insects or mechanically, for instance by wind or a lawnmower. Within half an hour after wounding, GLVs are released from the ruptured plant cells (Loughrin et al., 1994; Turlings et al., 1995; Turlings et al., 1998b) while *de novo* production of other compounds might take at least 4-5 hours (Turlings et al., 1998b). GLVs are formed from linolenic and linoleic fatty acids and the first step of their synthesis is catalyzed by the enzyme lipoxygenase (LOX). Tissue disruption is suggested to trigger the provision of free fatty acids for the formation of GLVs, but the details of this process have yet to be revealed (Matsui, 2006). Plants impaired in producing GLVs in response to wounding, such as the recently characterized maize *lox10* mutant (Christensen et al., 2013) and the *A. thaliana* mutant *all84* (Shiojiri et al., 2006b), attracted fewer wasps of some species in a laboratory setting, indicating that GLVs are important cues for parasitoid wasps in their search for hosts.

The second VOC biosynthetic pathway is the shikimate/phenylalanine pathway, which results in the emission of aromatic compounds like indole and methyl anthranilate, the emission of benzenoids such as methyl salicylate, and phenylpropanoids. The mevalonic acid pathway, the third VOC biosynthetic pathway, produces 15-carbon sesquiterpenes, the most abundant compounds that are emitted by herbivore-induced maize plants (D'Alessandro and Turlings, 2005). Examples of sesquiterpenes are (E)- $\beta$ -caryophyllene, (E)- $\alpha$ -bergamotene, (E)- $\beta$ -farnesene,  $\beta$ -sesquiphellandrene and (E)-nerolidol. The fourth pathway, the methylerythrol phosphate pathway, produces 10-carbon monoterpenes such as linalool and (Z)- $\beta$ -ocimene, as well as 20-carbon diterpenes. Then, there are also terpenoids with irregular acyclic C<sub>16</sub> and C<sub>11</sub> carbon skeletons, so called homoterpenes: 4,8-dimethyl-1,3(E),7-nonatriene and 4,8,12-trimethyl-1,3(E),7(E),11-tridecatetraene. They are believed to be derived from geranyl-linalool and (E)-nerolidol, respectively (Maffei, 2010; Dudareva et al., 2013).

### The relevance of VOC emission in an ecological context

Emitting floral scent is important for attracting pollinators and thereby ensuring fertilization. Therefore, floral scent is evidently important for plant fitness (Kessler et al., 2008; Majetic et al., 2009). Despite general agreement that VOCs emitted by plants in response to herbivory are used by natural enemies to locate herbivorous insects, it remains to be demonstrated that recruitment of predators and parasitoids indeed confers adaptive advantages to the

plants (Allison and Hare, 2009; Agrawal, 2011; Hare, 2011; Kessler and Heil, 2011). VOCs are also emitted in response to mechanical damage and might therefore not be all that reliable, although there are distinct differences compared to herbivore-induced VOCs (Turlings et al., 1990). This might be due to the presence of elicitors in the oral secretions of insects, as has been detected in caterpillars and grasshoppers, which trigger the emission of specific VOCs (Alborn et al., 1997, 2007; Schmelz et al., 2006). However, the saliva could also contain repressors that can manipulate the emission of VOCs (Musser et al., 2002).

Most studies on tritrophic interactions have been performed in the laboratory (Reviewed by Hunter (2002)). Only few studies have shown that herbivore-induced VOCs act to increase parasitism and predation of herbivores in the field. In Africa, intercropping of cereal crops with odor-emitting, native molasses grass significantly decreased crop infestation by stemborers and increased larval parasitism (Khan et al., 1997a). In the USA, *Cardiochiles nigriceps* Viereck (Hymenoptera: Braconidae) wasps were preferentially attracted to tobacco plants infested by their hosts over plants infested by non-host lepidopteran larvae (De Moraes et al., 1998). Inducing odor emission in tomato plants with JA increased parasitism of caterpillar pests in an agricultural field (Thaler, 1999). And application of ecologically relevant concentrations of synthetic VOCs to plants, resulting in the augmented emission of these compounds, increased predation of lepidopteran eggs on wild tobacco (Kessler and Baldwin, 2001).

Even fewer studies provide evidence that parasitoids help to increase plant fitness in terms of seed production. A parasitoid exclusion experiment with seed-weevils showed that the presence of parasitoids benefits the plant *Hormathophylla spinosa* (L.) Küpfer (Brassicaceae) in terms of seed dispersal, but the relevance of volatiles in attracting the parasitoids, chalcid wasps, is unknown (Gomez and Zamora, 1994). Other studies, where it was shown that *A. thaliana* (Van Loon et al., 2000), *Brassica nigra* L. (Brassicaceae) (Smallegange et al., 2008) and maize plants (Fritzsche Hoballah and Turlings, 2001) under attack by parasitized caterpillars produced more seeds than plants attacked by healthy larvae, included plants and insects that do not co-occur together or were conducted under relatively artificial conditions.

A recent study with transformed tobacco plants has convincingly shown that plants produce fewer flowers when they are unable to recruit predatory bugs, which probably affects seed production (Schuman et al., 2012). However, parasitoid wasps do not immediately kill their hosts, rendering the proposed defense function of herbivore-induced VOCs quite controversial. Therefore, whether plants benefit from the emission of VOCs and the attraction of parasitoid wasps remains the topic of heavy debate (Allison and Hare, 2009; Agrawal, 2011; Hare, 2011; Kessler and Heil, 2011).

### **The influence of domestication on plant-insect interactions**

Obviously, the emergence of agriculture and the planting of monocultures have shaped insect communities. At the individual plant level, there are indications that artificial selection of crop plants for increased yield and quality also influenced resistance to insects and

diseases (Wink, 1988). Indeed, teosinte, the wild ancestor of maize, displays superior resistance to the stemborer *Diatraea grandiosella* Dyar (Lepidoptera: Crambidae) (Rosenthal and Dirzo, 1997) and to the leafhopper *Dalbulus maidis* DeLong & Wolcott (Hemiptera: Cicadellidae) (Dávila-Flores et al., 2013) compared to traditional landraces and modern maize varieties. Similarly, defenses against pathogens tend to be impaired in cultivated compared to wild plants in young seedlings (before the appearance of true leaves) of the common bean, *Phaseolus vulgaris* L., the scarlet runner bean, *P. coccineus* L., and lima bean (Lindig-Cisneros et al., 1997; Lindig-Cisneros et al., 2002). However, there seems to be no distinct difference in direct defenses against insects between wild and cultivated lima bean (Ballhorn et al., 2008).

Domestication has undeniably influenced plant VOC emission. Although teosinte releases a very similar bouquet of volatiles as has been reported for cultivated maize (Gouinguéné et al., 2001), substantial differences have been reported for other crops. The volatiles associated with tomato flavor tend to be reduced in cultivated tomato varieties compared to wild tomatoes, with one interesting exception, 6-methyl-5-hepten-2-one. This compound, derived from lycopene, is linked to the bright red color of tomato and reflects the breeders' preferences for selecting brightly colored fruits (Goff and Klee, 2006). Also, for instance, wild and cultivated strawberries vary tremendously in their VOC profile (Aharoni et al., 2004). Interestingly, a feral cotton variety emitted higher amount of volatiles than commercial cultivars (Loughrin et al., 1995), indicating that the domestication effect on plant VOC emission can be reversible.

An increasing number of studies show that domestication has had an impact on complex tritrophic interactions, and most examples are of negative interactions (Macfadyen and Bohan, 2010). The parasitoid wasp *Cotesia rubecula* Marshall (Hymenoptera: Braconidae) seems to be more attracted to wild than to cultivated cabbage plants in an experimental field (Gols et al., 2011). Furthermore, wild and cultivated cabbage plants differ in their suitability as a hostplant for the herbivores on which different parasitoids rely for their development (Gols et al., 2008). The parasitoid *Dolichogenidea homoeosomae* Muesebeck (Hymenoptera: Braconidae) was more efficient in finding and parasitizing its host on wild compared to cultivated sunflowers (*Helianthus annuus* L., Asteraceae) (Chen and Welter, 2003, 2007). Also, the parasitoid *Psytalia lounsburyi* Silvestri (Hymenoptera: Braconidae) more effectively parasitized its host in the small fruits of wild olives (*Olea europaea* L., Oleaceae) compared to the larger fruits of cultivated olives (Wang et al., 2009). However, also positive interactions have been described. For instance, the parasitoid *Cotesia glomerata* L. was more attracted to cultivated than to wild cabbage, and similarly, the parasitoid *Stenocorse bruchivora* Crawford (Hymenoptera: Braconidae) preferred cultivated over wild bean (Benrey et al., 1998). These observations indicate that parasitoids are able to change their behavior in an agricultural environment.

A recent example of how crop breeding may have effected and disrupted tritrophic interactions comes from maize. As mentioned before, when attacked by the root-feeding larvae of the beetle *D. v. virgifera*, the roots of European maize varieties, as well as maize's

wild ancestor, teosinte, emit VOCs that attract insect-killing nematodes (Rasmann et al., 2005). Interestingly, most American maize varieties have lost the ability to release the key nematode attractant (*E*)- $\beta$ -caryophyllene, making their roots much more vulnerable to *Diabrotica* damage (Köllner et al., 2008). By transforming a deficient maize line with a gene from oregano, this signal could be restored, enhancing the protection that nematodes provide to the roots (Degenhardt et al., 2009). Similarly, commercial maize varieties have lost the ability to emit egg-parasitoid-attracting VOCs in response to egg deposition to the stemborer moth *Chilo partellus* Swinhoe (Lepidoptera: Crambidae), while maize landraces did have this ability (Tamiru et al., 2011). The attractiveness of teosinte VOCs to natural enemies has thus far not been assessed.

### Study system

My thesis concerns herbivore-induced VOC emission and parasitoid wasp attraction of cultivated maize and its wild ancestor, teosinte. The herbivore of choice was fall armyworm, *S. frugiperda*, one of the main herbivores of maize in the Americas (Fig. 5; Annex 1; Luginbill, 1928; Ortega C. 1987; Andrews, 1988; Steffey et al., 1999). Adult moths lay hundreds of eggs on young plants, from which larvae emerge that may rapidly consume large amounts of plant tissue. Early instars inflict typical window-pane damage, ingesting the epidermis and mesophyll tissue of only one side of the leaf, while leaving the epidermis of the other side intact (Fig. 6; Annex 1). Since they frequently hide in the whorl of the plant, they also inflict important damage to the growing meristem. Older instars can completely defoliate an entire plant (Luginbill, 1928). *S. frugiperda* larvae can be recognized by a distinctive y-shape on their forehead and four distinct dots on their abdomen (Fig. 7a; Luginbill, 1928). The adults are typical night-active moths (Fig. 7b). *S. frugiperda* larvae are also frequently observed on teosinte (Luginbill, 1928; Mondragón-Pichardo and Vibrans, 2005; De la Paz-Gutiérrez, 2008; Takahashi et al., 2012). *S. frugiperda* is known to induce VOC emission in maize (Carroll et al., 2006; E. de Lange, 2008).

Before a rearing colony of *S. frugiperda* was available, we instead studied *S. littoralis*, the Egyptian cotton leafworm. This caterpillar has been reported to consume maize in Northern Africa and the Near East, where it is mainly a pest of cotton (Hill, 1987). Although differences exist in the way *S. frugiperda* and *S. littoralis* cope with plant defenses (Glauser et al., 2011) and the quantities of odors that they induce, they induce an odor blend with a similar composition of compounds (de Lange, 2008).

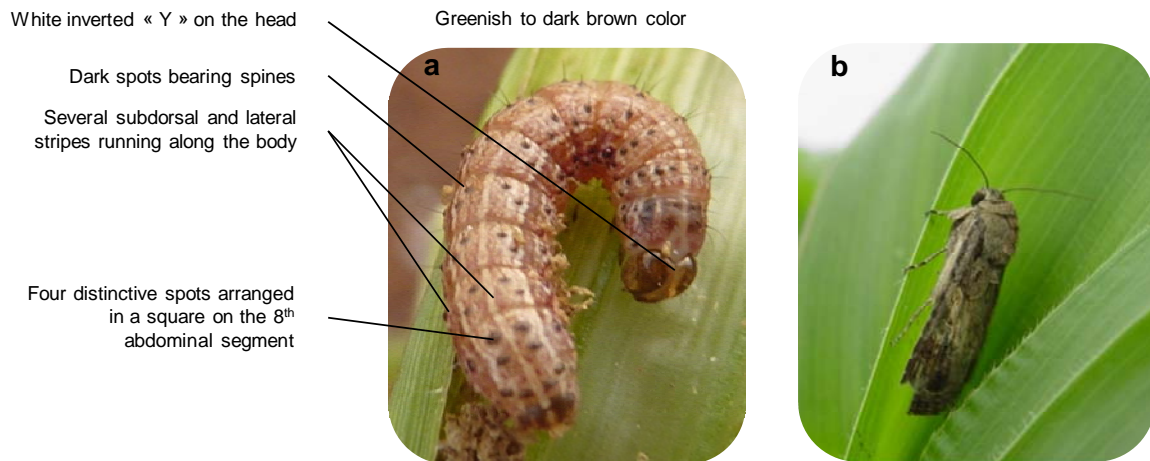
*S. frugiperda* has many natural enemies, among which parasitoid wasps, parasitic Tachinid flies, and various predators of insect but also vertebrate nature (Luginbill, 1928). Furthermore, there are parasitic nematodes, viruses, fungi, protozoa and bacteria (Gardner and Fuxa, 1980). Parasitoid wasps attack the larvae in various life stages. The egg-larval parasitoid *Chelonus* spp. (Hymenoptera: Braconidae), for instance, parasitizes the eggs, and the solitary endoparasitoids *Cotesia marginiventris* Cresson (Fig. 8d) and *Campoletis sonorensis* Cameron (Hymenoptera: Ichneumonidae) (Fig. 8e), parasitize first-, second- or third-instar larvae. Larvae parasitized by the latter two wasp species are known to consume



**Fig. 5** Distribution of *Spodoptera frugiperda* in the Americas. Adapted from Commonwealth Institute of Entomology (1985) and Von Mérey (2010)



**Fig. 6** Typical “windowpane” damage caused by early *Spodoptera frugiperda* instars. The larvae ingest the epidermis and mesophyll tissue of only one side of the leaf, while the epidermis of the other side remains intact. Picture: Elvira de Lange



**Fig. 7** Characteristics of *Spodoptera frugiperda* larvae (a) and adults (b). Pictures: Elvira de Lange (a) and Fernando Bahena-Juárez (b)

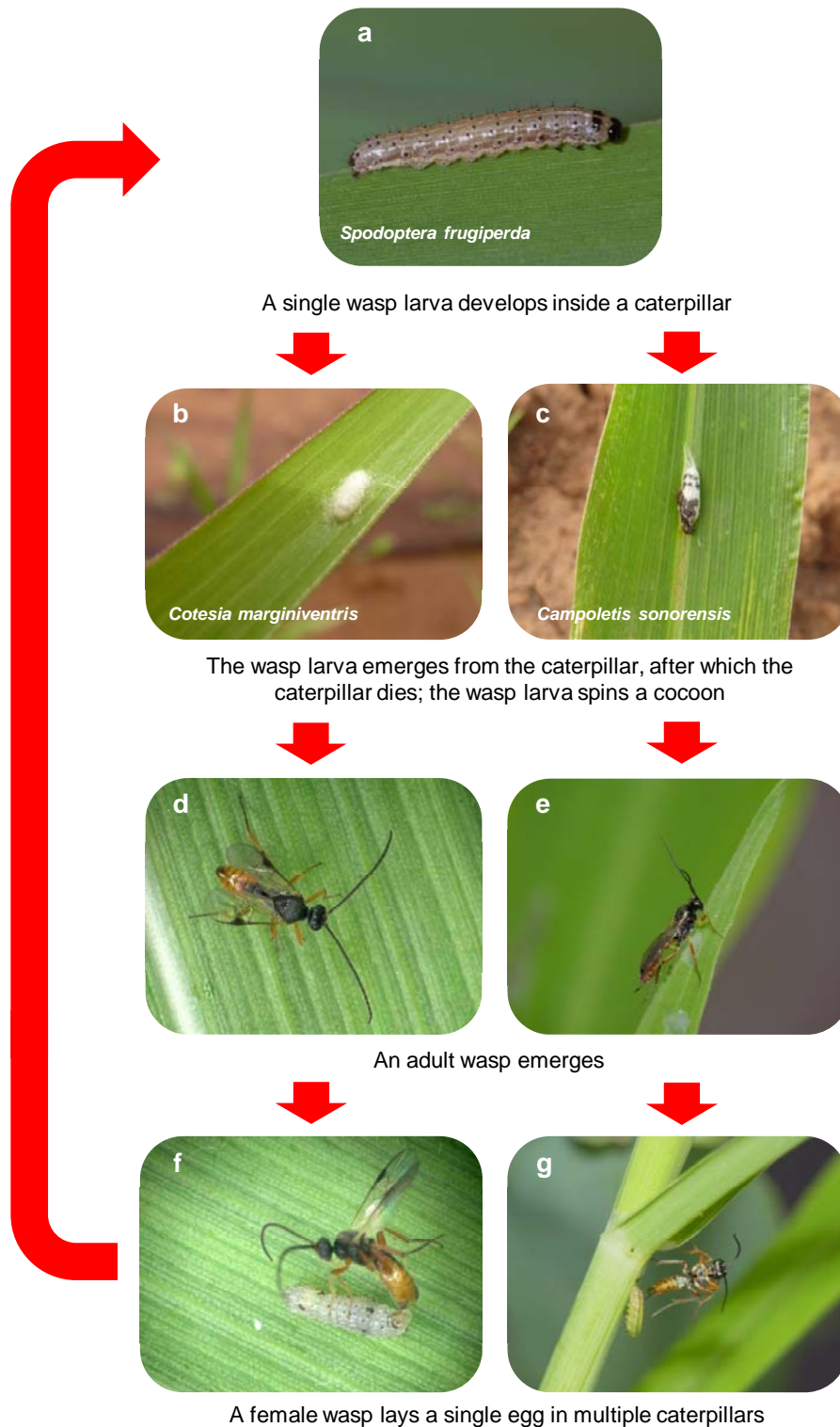
considerably less leaf tissue than healthy larvae and die before pupating (Fritzsche Hoballah and Turlings, 2001). In a survey in a tropical area in Mexico, up to 23% of *S. frugiperda* larvae collected was infested by *C. sonorensis*, and up to 6.2% by *C. marginiventris* (Hoballah et al., 2004), indicating that they co-occur with *S. frugiperda* and are important biological control agents of this pest. More recent surveys also mention the occurrence of the two wasps as important parasitoids of *S. frugiperda* (Jourdie et al., 2008; Bahena-Juárez et al., 2010). Because of their relative importance and the possibility to readily rear *C. marginiventris* and *C. sonorensis* in the laboratory (Turlings et al., 2004), they have been our parasitoids of choice.

The lifecycle of both parasitoids is quite similar. Female wasps lay eggs in early instar caterpillars, usually one egg per caterpillar. The wasp larva emerges when the caterpillar reaches its third or fourth instar, and soon afterwards, the caterpillar dies. Upon emerging, the wasp larva directly starts to spin a silk cocoon inside which it will complete its metamorphosis. By then, an adult wasp will emerge. When male and female wasps have mated, the females will try to find a host for oviposition (Fig 8; Cave, 1995). Indeed, only female wasps are attracted by herbivore-induced plant odors, whereas males are in fact solely attracted to females (Turlings et al., 2004).

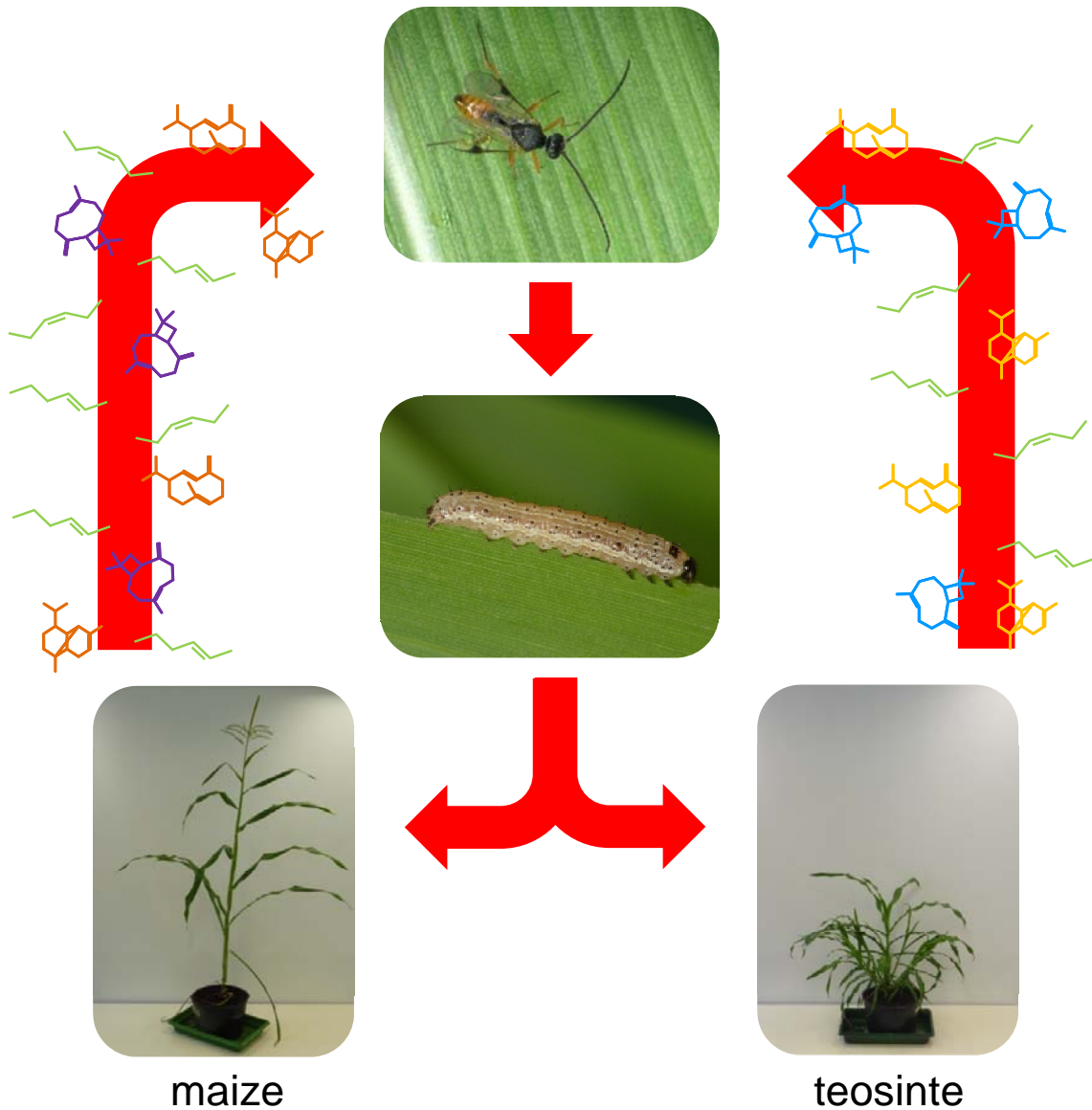
## Thesis outline

The aim of this PhD thesis was to study the importance of herbivore-induced plant volatiles from an evolutionary perspective, using maize and its wild ancestor as study models (Fig. 9).

**Chapter one** contains a review that addresses our current knowledge of the defense mechanisms of maize and teosinte against pathogens and insects. Also, the pathogens, herbivores and beneficial insects that occur on teosinte and maize in their native habitats are discussed. Teosinte appears to be more resistant to a number of pests and diseases than maize. Therefore, studying the defenses of teosinte could possibly lead to improved resistance in cultivated maize.



**Fig. 8** Life cycle of the solitary endoparasitoids *Cotesia marginiventris* (b,d,f) and *Campoletis sonorensis* (c,e,g). (a) A single wasp larva develops inside a lepidopteran larva and eventually bores through the skin to exit, after which the caterpillar will die. (b,c) The wasp larvae will immediately start spinning a silk cocoon. (d,e) An adult wasp emerges from the cocoon. Males and females mate and female wasps will search for novel hosts. (f,g) When a new host is found, the female wasp lays a single egg. Pictures: Elvira de Lange (b,c) and Matthias Held (a,d,e,f,g)



**Fig. 9** The study system. The plants, cultivated maize and its ancestor teosinte, emit odors upon attack by important pest species in the genus *Spodoptera*. Parasitoid wasps use these odors to locate insect herbivores as hosts for their offspring. As cultivated maize has been selected for increased yield and quality, its odor profile is expected to have changed in response. We evaluated the attractiveness of maize and teosinte odors to parasitoid wasps. Furthermore, we studied the importance of parasitoid wasp attraction for the growth and survival of herbivore-attacked plants. Pictures: Mickaël Gaillard, Gaétan Glauser and Elvira de Lange (plants) and Matthias Held (herbivore, parasitoid wasp)



**Chapter two** explores the pest insects that occur on teosinte plants in their natural environment. At eight different locations in Mexico, we sampled arthropods on teosinte leaves and attempted to confirm whether fall armyworm, *S. frugiperda*, and its parasitoids co-occur with teosinte.

**Chapter three** focuses on the attraction of parasitoid wasps to VOCs emitted by maize and teosinte plants. Two species of parasitoid wasps, *C. marginiventris* and *C. sonorensis*, were allowed to make a choice between the odors of maize and those of teosinte in a six-arm olfactometer. By this means, we could identify possible differences in the attraction of parasitoids by these plants. Since cultivated maize is reportedly compromised in direct defenses against a stemborer compared to its wild ancestor and certain modern varieties have lost a root signal that is implied in indirect defenses, it would not be unthinkable that maize plants have also become compromised in aboveground indirect defenses during domestication.

In **chapter four**, we studied whether parasitoid wasps developed better in host larvae feeding on the plants the wasps were preferentially attracted to. Also, we assessed whether non-parasitized *S. frugiperda* larvae developed better on maize or on teosinte plants. Since cultivated maize has reduced defenses compared to teosinte to several insect species, *S. frugiperda* might perform better on cultivated maize than on wild teosinte. Because parasitoids are directly influenced by the quality of their host's food source, we expected that teosinte as the host's food source would negatively impact parasitoid wasp development compared to maize as the host's food source.

**Chapter five** explores whether the presence of parasitoid wasps is beneficial for teosinte plants in a semi-natural setting in Mexico. Although it sounds logical that attracting natural enemies and thereby eliminating herbivores would be beneficial to plants, there is surprisingly little field evidence for this in naturally evolved systems. We evaluated how parasitoid wasps can affect plant performance in a tritrophic system comprising teosinte, the herbivore *S. frugiperda* and the wasp *C. sonorensis*. Plants were planted in large screen tents, where it was assessed how the presence of the herbivore with and without the wasp affected plant growth and survival.

**Chapter six** is devoted to the importance of GLVs in the attraction of parasitoid wasps under field conditions. In an experimental field in Texas, USA, we planted maize *lox10* mutants, impaired in GLV biosynthesis, alongside wildtype plants, infested the plants with *S. frugiperda* herbivores, and assessed the degree of parasitism. Since the *lox10* mutants were previously found to be less attractive to parasitoid wasps in olfactometer assays, we hypothesized that also in the field, mutants compromised in GLV emission would display a lower degree of parasitism.

Finally, in the **general discussion**, the data presented in this thesis are discussed in a broader context.



## Chapter 1



## **Insect and pathogen resistance in cultivated maize and its wild ancestor, teosinte**

Elvira S. de Lange<sup>1</sup>, Dirk Balmer<sup>2</sup>, Brigitte Mauch-Mani<sup>2</sup> and Ted C.J. Turlings<sup>1,\*</sup>

<sup>1</sup> *Laboratory of Fundamental and Applied Research in Chemical Ecology, Institute of Biology,  
University of Neuchâtel, Rue Emile-Argand 11, 2000 Neuchâtel, Switzerland*

<sup>2</sup> *Laboratory of Molecular and Cell Biology, Institute of Biology, University of Neuchâtel,  
Rue Emile-Argand 11, 2000 Neuchâtel, Switzerland*

\*e-mail: [ted.turlings@unine.ch](mailto:ted.turlings@unine.ch)

Tel: (+41) 32 718 3158, Fax: (+41) 32 718 3001



## Abstract

Maize (*Zea mays* ssp. *mays*) is one of the most important crops, and abundant literature can be found on its pathogen and insect defenses. However, research on its wild ancestor, teosinte, with respect to resistance is limited. Teosinte is a collective term for all taxa in the genus *Zea* besides cultivated maize. The subspecies *Zea mays* ssp. *parviglumis*, or Balsas teosinte, has only recently been identified as the wild ancestor of maize. Its origin is in Mexico and teosintes occur in Mexico, Nicaragua and Guatemala, where these plants are often attacked by similar pathogens and insects as cultivated maize. Here, we discuss the different arthropods and microbes that are associated with cultivated maize and wild teosinte in the area where the plants naturally occur, and evaluate the mechanisms of resistance of the plants. Teosinte appears to have superior resistance against a number of pathogens and pests compared to its cultivated counterpart. We therefore highlight the need to study wild teosinte in order to identify and potentially transfer resistance traits to improve protection of cultivated maize. In this regard, since teosinte is at risk of becoming extinct, there is a need to protect the germplasm for future generations.

## Key words

Arthropods, bacteria, fungi, oomycetes, plant defense, viruses, *Zea mays*

## Introduction

Maize (*Zea mays* L. ssp. *mays*) is one of the most remarkable crops in the world. It is grown worldwide and is the most important staple in many countries. With an annual production of over 880 million tons, it is the world's most abundantly grown cereal, surpassing wheat and rice (FAO 2013). A considerable amount of research is devoted to the improvement of the crop under varying climatic environments, biotic and abiotic stresses (Bennetzen & Hake 2009). Interestingly, the wild ancestor of maize, teosinte, was not clearly agreed on until only recently. There is no plant with a fruiting structure that resembles a maize cob very closely, and therefore, initially, its ancestor was assumed to be extinct (Mangelsdorf & Reeves 1939). The origin of maize has been the topic of considerable controversy, and various theories have been suggested (initially reviewed in Mangelsdorf & Reeves 1939). These authors have then proposed their own theory: *Tripsacum*, a closely related grass, has been discussed as possible crossing partner with a maize-like plant giving rise to modern maize (Mangelsdorf & Reeves 1939). Another proposition was that a cross between *Tripsacum* and a teosinte yielded the crop plant known as maize nowadays (Eubanks 1995).

Although not the first to have the idea (Vinson 1877, Schuman 1904, Blaringhem 1906), Beadle (1936) was the first to propose teosinte as the direct ancestor of maize based on genetic evidence. However, the plants are morphologically so distinct that this hypothesis has always been seriously questioned (reviewed in Doebley, 2001). The availability of modern molecular tools allowed disregarding *Tripsacum* as an ancestor of maize, while strengthening the evidence that maize derived from teosinte (Bennetzen *et al.* 2001). An extensive genetic study based on microsatellite genotyping eventually put an end to the

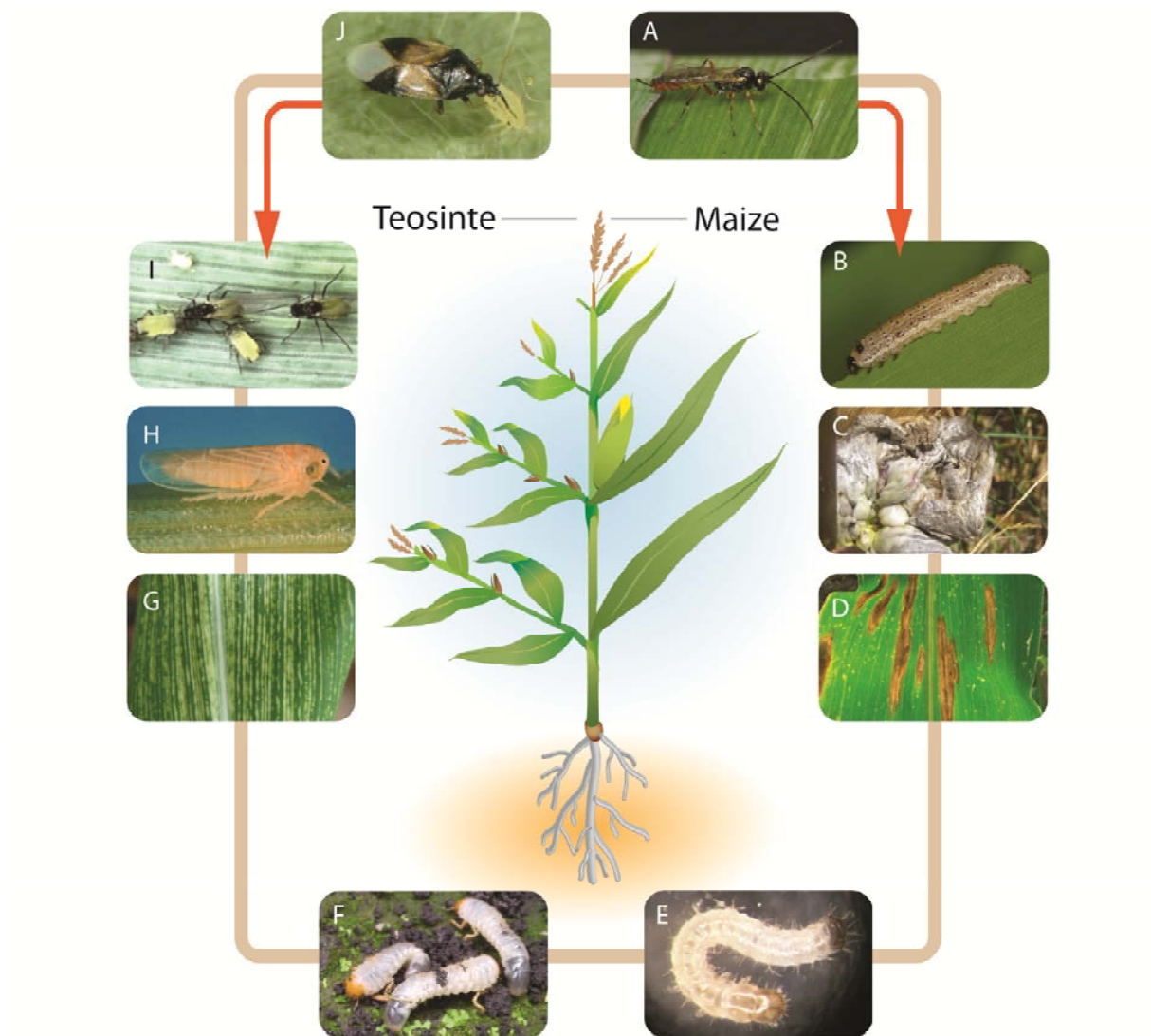
controversy and Balsas teosinte (*Z. mays* ssp. *parviglumis* Iltis & Doebley) was confirmed to be the wild ancestor of cultivated maize (Matsuoka *et al.* 2002). Domestication has been estimated to have started about 9000 years ago as a single event in southern Mexico, where teosinte is still growing nowadays. Teosinte grows in isolated populations, but also as a weed in maize fields (Sánchez González & Ruiz Corral 1995).

Teosinte is a collective term for all taxa that comprise the genus *Zea* besides cultivated maize. All are annual except two perennial species that occur in western Mexico, *Z. diploperennis* Iltis, Doebley & Guzmán and *Z. perennis* (Hitchc.) Reeves & Mangelsdorf. The latter is tetraploid, while all other teosintes are diploid. Besides Mexico, teosintes occur in Nicaragua (*Z. nicaraguensis* Iltis & Benz), northwestern Guatemala (*Z. mays* ssp. *huehuetenangensis* (Iltis & Doebley) Doebley), southeastern Guatemala (*Z. luxurians* (Durieu & Ascherson) Bird) and Honduras (*Z. luxurians*), although the latter population got extinct (Wilkes 1995; Iltis 2000; Iltis & Benz 2000). The teosintes that are most closely related to cultivated maize are considered subspecies of *Z. mays*. These are: the above-mentioned *Z. mays* ssp. *huehuetenangensis*, *Z. mays* ssp. *mexicana* (Schrader) Iltis which grows in northern and central Mexico, and Balsas teosinte, growing in southwest Mexico in the mountains of the Rio Balsas (Sánchez González & Ruiz Corral 1995).

Teosinte contains a number of traits that are not suitable for cultivation and consumption of the grains. During domestication - the process of genetic modification of a wild species to create a new form of a plant suited to human needs (Doebley, Gaut & Smith 2006) - several of these traits have been modified. The “domestication gene” *teosinte branched1* is responsible for a remarkable difference in plant architecture: while teosinte plants have many branches with numerous tassels (male inflorescences) and small ears (female inflorescences), maize plants typically have a single stalk with a terminal tassel and few short lateral branches on which large ears grow (Fig. 1; Doebley, Stec & Hubbard 1997; Doebley 2004). The tiny teosinte ears have 5 to 12 kernels, which are easily dispersed at maturity. In contrast, maize ears can bear 500 or more kernels, each firmly attached to the central axis of the ear so that they do not disperse, which allows for easy harvest. Teosinte kernels are enclosed in a hard casing that provides excellent protection against pathogens and herbivores, but also prevents the easy use of the grain by humans. However, due to a modification in the “domestication gene” *teosinte glume architecture1*, the protective casing was lost, leading to maize grains that are “naked” on the ear and that can readily be consumed (Dorweiler *et al.* 1993; Wang *et al.* 2005). The obvious trade-off is that this trait exposes the inner seeds and makes them highly vulnerable to antagonists.

Five major genes have been proposed to be responsible for the transformation from teosinte to maize (Beadle 1972; Beadle 1980; Doebley & Stec 1991), of which only two, the above-mentioned “domestication genes”, have been identified. Nevertheless, an estimated 1200 genes were targeted during maize domestication, representing 2-4% of the maize genome (Wright *et al.* 2005). The transcriptomes of maize and teosinte also differ considerably (Swanson-Wagner *et al.* 2010; Hufford *et al.* 2012; Swanson-Wagner *et al.* 2012). Despite these genotypic and phenotypic differences – two maize varieties might





**Fig. 1** Insects and pathogens associated with cultivated maize and wild teosinte in Mexico and Latin-America. Teosinte is depicted on the left, and maize is depicted on the right. There are distinct phenotypic differences between the plants, but the insect fauna and pathogens associated with the plants tend to be similar. Examples of pathogens and insects that occur on teosinte and maize: (a) *Campoletis sonorensis*, a parasitoid of *Spodoptera frugiperda* (picture: M. Held); (b) fall armyworm, *Spodoptera frugiperda*, a folivorous herbivore (picture: M. Held); (c) the fungus *Ustilago maydis* or corn smut (picture: D. Begerow); (d) the fungus *Setosphaeria turcica*, causing Northern leaf blight (picture: R.J. Nelson); (e) the root-herbivore *Diabrotica* spp. (picture: I. Hiltbold); (f) the root-feeder *Phyllophaga* spp. (Picture: M. Najera); (g) the Maize streak virus (picture: F. J. Kloppers); (h) the corn leafhopper, *Dalbulus maidis* (picture: L.R. Nault); (i) the aphid *Rhopalosiphum maidis* (picture: T.C.J. Turlings); (J) the predator *Orius* spp. (picture: F. Bahena-Juárez)

genetically differ as much as a human from a chimpanzee (Buckler, Gaut & McMullen 2006) – maize and teosinte still frequently hybridize (Wilkes 1977).

Cultivated maize is usually referring to modern cultivars as the result of hybridizations of two parent inbred lines (Duvick, 2001). But there are many more kinds of cultivars, for example landraces. These are dynamic populations of a cultivated plant that have an historical origin, a distinct identity and that lack formal crop improvement. Often, they are genetically diverse, locally adapted and associated with traditional farming systems (Camacho Villa *et al.* 2005). These different landraces are adapted to specific needs, displaying increased yield under specific environmental conditions such as drought, lodging, or abundance of insect pests.

Artificial selection of crop plants for increased yield and quality has been shown to influence resistance to pathogens and insects, among others for crops as diverse as carrot (Leiss *et al.* 2013), bean (Córdova-Campos *et al.* 2012) and maize (Rosenthal & Dirzo 1997). This could have important consequences for crop protection, as microbes and animal pests, including insects, account for 18% and 16% of the world's crop losses, respectively (Oerke 2006). Most crops are bred to contain fewer toxins and thereby increase palatability (e.g. with respect to alkaloids in potato (Johns and Alonso, 1990) and lupins (Enneking and Wink, 2000) and cucurbitacins in cucumber and squash (Schoonhoven *et al.* 2005)). Indeed, it appears as if maize has lost some of its direct defenses against a stemborer and a leafhopper during selection for increased yield and quality, modern varieties more so than landraces (Rosenthal & Dirzo 1997; Dávila-Flores *et al.* 2013), although different defense traits seem to be variably affected by domestication (Bellota *et al.* 2013). Plants have probably not intentionally been selected for increased emission of volatile organic compounds (VOCs) and attraction of natural enemies, an indirect defense mechanism (Rodríguez-Saona *et al.* 2012). The trade-off between yield and resistance could lead to the disruption of species interaction, possibly causing reduced biological control in the shape of predators or parasitoids of pest insects (Macfadyen & Bohan 2010). Maize may also have lost indirect defenses during cultivation, with possible consequences for resistance (Köllner *et al.* 2008).

In this review, we discuss the various insects and pathogens that occur on maize and teosinte in the area of natural distribution of teosinte (Fig. 1), and evaluate insect and pathogen resistance mechanisms in the wild and cultivated plants. We highlight the need of studying and protecting wild maize as a precious resource for improved crop protection.

### **Herbivores of maize and teosinte**

Maize is attacked by a myriad of animals, from seeding until harvest and storage. About 9.6% of maize production is lost due to herbivores as various as insects, slugs, and rodents (Oerke 2006). We will focus on the insect pests that occur on roots and shoots, in the areas where cultivated maize and its wild ancestor, teosinte, co-occur, allowing a comparative analysis of cultivar-specific defense.

Major pests of maize in Mexico include a number of leaf-feeding insects (Table 1), such as fall armyworm, *Spodoptera frugiperda* Smith (Lepidoptera: Noctuidae), a polyphagous

species with a strong preference for grasses (Luginbill 1928) that is specifically adapted to cope with maize defenses (Glauser *et al.* 2011). The highly polyphagous corn earworm, *Helicoverpa zea* Boddie (Lepidoptera: Noctuidae) is also an important pest of maize (Fitt 1989). The corn leafhopper, *Dalbulus maidis* DeLong & Wolcott (Hemiptera: Cicadellidae), is considered an important maize pest in Mexico, and although the insect can cause damage by itself (Bushing & Burton 1974), its pest status is mainly due to its activity as a vector for various plant viruses (Ortega C. 1987; Steffey *et al.* 1999). The corn leaf aphid, *Rhopalosiphum maidis* Fitch (Homoptera: Aphididae), is also an important pest that can transmit plant viruses. Weevils, thrips and grasshoppers may also become serious pests, as well as a number of leaf-feeding beetles and their root-feeding larvae (Table 2). For instance corn rootworm, *Diabrotica* spp. (Coleoptera: Chrysomelidae), the larvae of cucumber beetles, can consume maize roots to such extent that the plants dry and fall (Ortega C. 1987; Steffey *et al.* 1999).

It is generally assumed that a similar insect fauna occurs on maize and teosinte (Sánchez González & Ruiz Corral 1995). A study performed in Guatemala revealed that most, but not all, of the insects collected on teosinte were also found on maize (Painter 1955). Another study, comparing root insects on maize and teosinte, reported the occurrence of identical insect species on the roots of teosinte and maize. The number of taxa retrieved from teosinte roots was higher, possibly because the perennial teosinte that was studied provided roots all year long, while the roots of maize as an annual plant died in winter. Additionally, the estimated total insect biomass around teosinte roots was higher than around maize roots, but since the root biomass itself increased as well, teosinte appeared to be more tolerant to herbivory than maize (Moya-Raygoza 1987; Moya-Raygoza, Bedoy V. & Santana C. 1990). In a study comparing the occurrence of leafhoppers on maize and teosinte, more taxa were collected from teosinte than from maize. Interestingly, on maize, one species represented 79% of all taxa, while on teosinte, the most abundant species represented 33% of all taxa, indicating that teosinte populations can sustain a higher insect diversity. The author did not draw conclusions about the insect resistance of the teosinte plants, but rather suggested that the perennial teosinte studied provided food all year long, and occurred in a more diverse environment, causing the increased variability of insects (Moya-Raygoza 1994).

Two recent studies have extensively compared insect populations on maize and teosinte roots and shoots, corroborating the fact that teosinte and maize harbor a similar insect fauna (De la Paz-Gutiérrez 2008; Jorge E. Ibarra, unpublished data, but preliminary results available in Jofre y Garfias *et al.* (2010)). De la Paz *et al.* found an overall higher incidence of organisms on maize than on teosinte, both on roots and on shoots. While aphids were dominant on maize, teosinte was mostly colonized by aphids and thrips on the leaves, and white grub - root-feeding larvae of scarab beetles, *Phyllophaga* spp. (Coleoptera: Scarabaeidae) - on the roots. The stemborer *Diatraea* spp. (Lepidoptera: Crambidae) and the weevil *Sphenophorus* spp. (Coleoptera: Curculionidae), as well as white grub, were most

**Table 1** Herbivores associated with teosinte and maize shoots

Order	Family	Genus / species	Common name	Feeding guild	Reference
<b>Coleoptera</b>	Chrysomelidae	<i>Diabrotica</i> spp.	Cucumber beetle	Chewing	De Lange & Degen, unpublished; Painter 1955; De la Paz-Gutiérrez 2008; Jofre y Garfias <i>et al.</i> 2010
<b>Coleoptera</b>	Curculionidae	<i>Geraeus senilis</i>	Weevil	Chewing	De la Paz-Gutiérrez 2008
<b>Coleoptera</b>	Curculionidae	<i>Nicentrites</i> spp.	Weevil	Chewing	De Lange & Degen, unpublished; De la Paz-Gutiérrez 2008; Jofre y Garfias <i>et al.</i> 2010
<b>Coleoptera</b>	Curculionidae	<i>Sphenophorus</i> spp.	Weevil	Chewing	De la Paz-Gutiérrez 2008; Jofre y Garfias <i>et al.</i> 2010
<b>Coleoptera</b>	Scarabaeidae	<i>Macrodactylus</i> spp.		Chewing	De Lange & Degen, unpublished; Jofre y Garfias <i>et al.</i> 2010
<b>Diptera</b>	Ulidiidae	<i>Euxesta major</i> (larvae)	Picture-winged fly	Chewing	Painter 1955
<b>Hemiptera</b>	Cicadellidae	<i>Dalbulus maidis</i>	Corn leafhopper	Piercing-sucking	Moya-Raygoza & Trujillo-Arriaga 1993; Moya-Raygoza 1994
<b>Hemiptera</b>	Cicadellidae	<i>Dalbulus</i> spp.		Piercing-sucking	Moya-Raygoza & Trujillo-Arriaga 1993; Moya-Raygoza 1994; De la Paz-Gutiérrez 2008
<b>Hemiptera</b>	Delphacidae	<i>Peregrinus maidis</i>	Delphacid, planthopper	Piercing-sucking	Brewbaker 1979
<b>Hemiptera</b>	Lygaeidae	<i>Blissus</i> spp.	Chinch bug	Chewing	De Lange & Degen, unpublished
<b>Homoptera</b>	Aphididae	<i>Rhopalosiphum maidis</i>	Corn leaf aphid	Piercing-sucking	De la Paz-Gutiérrez 2008
<b>Lepidoptera</b>	Crambidae	<i>Diatraea</i> spp. (larvae)	Stemborer	Chewing	Painter 1955; Mondragón-Pichardo & Vibrans 2005; De la Paz-Gutiérrez 2008; Jofre y Garfias <i>et al.</i> 2010
<b>Lepidoptera</b>	Noctuidae	<i>Agrotis ipsilon</i> (larvae)	Dark sword-grass	Chewing	Jofre y Garfias <i>et al.</i> 2010
<b>Lepidoptera</b>	Noctuidae	<i>Helicoverpa zea</i> (larvae)	Corn earworm	Chewing	De Lange & Degen, unpublished; Jofre y Garfias <i>et al.</i> 2010
<b>Lepidoptera</b>	Noctuidae	<i>Spodoptera frugiperda</i> (larvae)	Fall armyworm	Chewing	De Lange & Degen, unpublished; Mondragón-Pichardo & Vibrans 2005; De la Paz-Gutiérrez 2008; Jofre y Garfias <i>et al.</i> 2010
<b>Orthoptera</b>	Acrididae	<i>Melanoplus differentialis</i>	Grasshopper	Chewing	De la Paz-Gutiérrez 2008
<b>Orthoptera</b>	Acrididae	<i>Sphenarium purpurascens</i>	Grasshopper	Chewing	De la Paz-Gutiérrez 2008
<b>Thysanoptera</b>	Thripidae	<i>Frankliniella</i> spp.	Thrips	Piercing-sucking	Painter 1955; De la Paz-Gutiérrez 2008

**Table 2** Herbivore larvae associated with teosinte and maize roots

Order	Family	Genus	Common name	Feeding guild	Reference
<b>Coleoptera</b>	Chrysomelidae	<i>Colaspis</i> spp.		Chewing	Moya-Raygoza 1987; Moya-Raygoza, Bedoy V. & Santana C. 1990; De la Paz-Gutiérrez 2008
<b>Coleoptera</b>	Chrysomelidae	<i>Diabrotica</i> spp.	Corn rootworm	Chewing	Moya-Raygoza 1987; Moya-Raygoza, Bedoy V. & Santana C. 1990; De la Paz-Gutiérrez 2008
<b>Coleoptera</b>	Chrysomelidae			Chewing	Moya-Raygoza 1987; Moya-Raygoza, Bedoy V. & Santana C. 1990
<b>Coleoptera</b>	Curculionidae			Chewing	Moya-Raygoza 1987; Moya-Raygoza, Bedoy V. & Santana C. 1990
<b>Coleoptera</b>	Elateridae	<i>Conoderus</i> spp.	Wireworms	Chewing	Moya-Raygoza 1987; Moya-Raygoza, Bedoy V. & Santana C. 1990
<b>Coleoptera</b>	Elateridae	<i>Melanatus</i> spp.		Chewing	Moya-Raygoza 1987; Moya-Raygoza, Bedoy V. & Santana C. 1990
<b>Coleoptera</b>	Elateridae			Chewing	De la Paz-Gutiérrez 2008
<b>Coleoptera</b>	Scarabaeidae	<i>Anomala</i> spp.		Chewing	Moya-Raygoza 1987; Moya-Raygoza, Bedoy V. & Santana C. 1990; De la Paz-Gutiérrez 2008
<b>Coleoptera</b>	Scarabaeidae	<i>Cyclocephala</i> spp.		Chewing	De la Paz-Gutiérrez 2008
<b>Coleoptera</b>	Scarabaeidae	<i>Diploaxis</i> spp.		Chewing	Moya-Raygoza 1987; Moya-Raygoza, Bedoy V. & Santana C. 1990
<b>Coleoptera</b>	Scarabaeidae	<i>Macroductylus</i> spp.		Chewing	De la Paz-Gutiérrez 2008
<b>Coleoptera</b>	Scarabaeidae	<i>Phyllophaga</i> spp.	White grub	Chewing	Moya-Raygoza 1987; Moya-Raygoza, Bedoy V. & Santana C. 1990; De la Paz-Gutiérrez 2008; Jofre y Garfias <i>et al.</i> 2010
<b>Coleoptera</b>	Tenebrionidae			Chewing	De la Paz-Gutiérrez 2008

**Table 3** Predators and parasitoids associated with teosinte and maize

Order	Family	Genus / species	Common name	Feeding guild	References
<b>Coleoptera</b>	Carabidae	<i>Calosoma</i> spp.	Ground beetle	Predator	De Lange & Degen, unpublished
<b>Coleoptera</b>	Coccinellidae	<i>Hippodamia convergens</i>	Convergent lady beetle	Predator	De Lange & Degen, unpublished
<b>Dermaptera</b>	Forficulidae	<i>Doru</i> spp.	Earwig	Predator	Hoballah <i>et al.</i> 2004; Mondragón-Pichardo & Vibrans 2005
<b>Hemiptera</b>	Anthocoridae	<i>Orius</i> spp.	Minute pirate bug	Predator	De Lange & Degen, unpublished; Hoballah <i>et al.</i> 2004
<b>Hymenoptera</b>	Braconidae	<i>Cotesia marginiventris</i>		Parasitoid of <i>S. frugiperda</i>	De Lange & Degen, unpublished; Hoballah <i>et al.</i> 2004
<b>Hymenoptera</b>	Braconidae	<i>Meteorus laphygmae</i>		Parasitoid of <i>S. frugiperda</i>	De Lange & Degen, unpublished; Hoballah <i>et al.</i> 2004
<b>Hymenoptera</b>	Drynidae	<i>Gonatopus bartletti</i>		Parasitoid of <i>D. maidis</i>	Moya-Raygoza & Trujillo-Arriaga 1993
<b>Hymenoptera</b>	Drynidae	<i>Gonatopus flavipes</i>		Parasitoid of <i>D. elimatus</i>	Moya-Raygoza & Trujillo-Arriaga 1993
<b>Hymenoptera</b>	Ichneumonidae	<i>Campoletis sonorensis</i>		Parasitoid of <i>S. frugiperda</i>	De Lange & Degen, unpublished; Hoballah <i>et al.</i> 2004

frequent in teosinte, while aphids, thrips, corn leafhopper, corn rootworm and fall armyworm were most abundant in maize (De la Paz-Gutiérrez 2008). Likewise, in another study where the incidence of *S. frugiperda* on maize and on teosinte was compared, maize plants were found to be more frequently infested by these herbivorous larvae than teosinte (Takahashi, Kalns & Bernal 2012). Jofre y Garfias et al. (2010) reported a high incidence of the stem borer *Diatraea grandiosella* Dyar, the caterpillar *Agrotis ipsilon* Hufnagel (Lepidoptera: Noctuidae), larvae of the scarab beetle *Phyllophaga* spp. and larvae of the weevil *Sphenophorus* spp. feeding on teosinte, which are similar insects as those found on maize plants in the area (Jofre y Garfias et al. 2010).

A number of other studies only reported the incidence of insects on teosinte. A questionnaire among 63 farmers revealed that some did not notice any insect damage on teosinte plants, but others reported *S. frugiperda* and *Diatraea* spp. on the plants (Mondragón-Pichardo & Vibrans 2005). Another study reported that *Diabrotica* spp. adults were found on teosinte plants, and laboratory studies confirmed that the larvae readily fed on teosinte roots (Branson & Reyes R. 1983). The larvae did not distinguish between the odors of roots of maize and teosinte, both host plants, when given a choice in a surface olfactometer. However, when given a choice between moist roots or a wad of moist cotton (blank), they preferred maize roots, and also teosinte roots, over blank (Branson 1982). A recent preliminary study of the insects that occur on teosinte reported that *S. frugiperda*, weevils, aphids and thrips are among the most abundant insects found on teosinte (de Lange & Degen, unpublished or Chapter 2), similar insects as are reported on maize (Ortega C. 1987; Steffey et al. 1999).

### Insects as vectors of diseases

As mentioned above, the corn leafhopper, *D. maidis*, is mainly important as it plays a role as vector for diseases. The insect transmits the bacteria *Corn stunt spiroplasma* and *Maize bushy stunt mycoplasma*, as well as the *Maize rayado fino virus* (Nault 1980; Gámez & León 1988). Teosinte is reportedly susceptible to these diseases (Nault 1980; Nault et al. 1980), although data on the infection rates in nature are lacking. Interestingly, while the majority of maize pests is thought to have evolved on other plants and later adapted to maize (e.g. the native host of European corn borer, *Ostrinia nubilalis* Hübner (Lepidoptera: Crambidae), an important pest of maize in the USA since its introduction from Europe, was probably wild hop or millet (Caffrey and Worthley 1927; Steffey et al. 1999)), it appears that the corn leafhopper evolved on teosinte and expanded its host range to include maize following domestication, suggesting that agriculture facilitated the expansion of this herbivore's host range (Nault & DeLong 1980; Nault 1990; Medina, Reyna & Bernal 2012). It seems that the leafhoppers and their associated viruses shaped teosinte evolution and distribution (Nault & DeLong 1980).

Examples of other virus vectors are a planthopper, the corn delphacid, *Peregrinus maidis* Ashmead (Hemiptera: Delphacidae), which is responsible for vectoring *Maize mosaic virus* and *Maize stripe virus* (Falk & Tsai 1985; Nault & Gordon 1988). Both viruses appear to infest

maize and teosinte alike (Brewbaker 1979; Nault *et al.* 1982). The corn leaf aphid, *R. maidis*, transmits *Maize dwarf mosaic virus* (Steffey *et al.* 1999), to which teosinte is also susceptible (Nault *et al.* 1982).

### **Beneficial insects associated with maize and teosinte**

Maize and teosinte are not only associated with pest insects. Many beneficial insects, such as parasitoids and predators, are attracted to herbivore-infested plants. Upon an attack by herbivores, plants emit odors that can be used by natural enemies to find their hosts or prey (Turlings, Tumlinson & Lewis 1990; Turlings & Wäckers 2004; Degenhardt 2009). Among the predators that are associated with maize are a number that prey on *S. frugiperda* larvae: bugs, coccinellids and earwigs (Hoballah *et al.* 2004). A preliminary study observing insects on teosinte showed that *Orius* spp. (Hemiptera: Anthocoridae), and *Calosoma* spp. (Coleoptera: Carabidae) are predators of *S. frugiperda* that frequently occur on teosinte. Also, *Hippodamia convergens* (Coleoptera: Coccinellidae), an aphid predator, is associated with teosinte (Table 3; de Lange & Degen, unpublished or Chapter 2).

A large number of parasitoids attacking various insects is associated with maize. Among them are numerous parasitoids that attack *S. frugiperda*, like *Camponotus sonorensis* Cameron (Hymenoptera: Ichneumonidae), *Cotesia marginiventris* Cresson (Hymenoptera: Braconidae) and *Meteorus laphygmae* Viereck (Hymenoptera: Braconidae) (Hoballah *et al.* 2004; Molina-Ochoa *et al.* 2004). *S. frugiperda* larvae obtained from teosinte plants are also frequently parasitized, and wasps of the three above-mentioned species have been observed (de Lange & Degen, unpublished or Chapter 2), indicating that indirect defenses are active in teosinte as well as maize. On teosinte, the leafhopper *D. maidis* was parasitized by *Gonatopus bartletti* Olmi (Hymenoptera: Drynidae), and *Dalbulus elimatus* Ball was found to be parasitized by *Gonatopus flavipes* Olmi (Moya-Raygoza & Trujillo-Arriaga 1993). Also, a number of unidentified hymenopteran parasitoids were collected from *Dalbulus* spp. on maize and teosinte (Moya-Raygoza & Trujillo-Arriaga 1993; Moya-Raygoza 1994). This suggests that teosinte can attract the natural enemies of herbivores when attacked. More parasitoids were found in the teosinte than in the maize habitat, indicating that the teosinte habitats provided better conditions for these natural enemies (Moya-Raygoza 1994).

There are many species of natural enemies of insect herbivores, and among the enemies of *S. frugiperda* are various organisms such as vertebrates, parasitic Tachinid flies, parasitic nematodes, viruses, fungi, protozoa and bacteria (Luginbill 1928; Gardner & Fuxa 1980). There is no information about the occurrence of these organisms on teosinte.

### **Pathogens associated with maize and teosinte**

To decipher evolutionary dynamics of plant-pathogen interactions, a comparison of defense responses between modern maize and teosinte would be a valuable approach to elucidate the influence of crop breeding on defense traits. However, teosinte has been widely neglected as phytopathological model organism. Only a few studies dating from decades ago provide a scarce idea of pathogens that are able to infect both teosinte and maize. No



comparative studies are available truly supporting the fact that either teosinte or maize would be more or less susceptible to pathogens. Nevertheless, it is assumed that most of the pathogens of maize are able to infect teosinte (Table 4; Wilkes 1967). For instance, the most widespread fungus infecting teosinte in the Guatemalan highlands is *Setosphaeria turcica* (Melhus 1953), one of the dominant fungal diseases of modern maize. Other fungal pathogens detected in both teosinte and maize are *Puccinia polyspora*, *P. sorghi*, *Physopella zae* (Robert 1962); *Physoderma zae-maydis* (Eddins 1933); *Sorosproium* spp., *Ustilago kellermanii* (Borlaug 1946); *Claviceps gigantea* (Fuentes et al. 1964); *Gibberella fujikuroi* (Steenkamp et al. 2001); and *Cochliobolus heterostrophus* (Hesseltine and Ellis, 1971). Another well-described fungal pathogen, which is also known to attack teosinte, is *Ustilago maydis* (Banuett 1995). Intriguingly, this pathogen might have played an important role in the domestication process of maize (Martínez-Soriano & Avina-Padilla 2009). Crosses of maize and teosinte were found to be highly susceptible to *U. maydis*. As *U. maydis* serves as food delicacy in Mexican diet, highly susceptible maize/teosinte hybrids could have been favored for domestication subsequently leading to modern maize varieties (Martínez-Soriano & Avina-Padilla 2009).

Apart from fungal diseases, a few bacterial, oomycete (downy mildews) and viral pathogens were described for both teosinte and maize (Table 4). Both *Pseudomonas andropogonis* and *P. avenae* were found on teosinte (Vidaver & Carlson 1987). As stated above, teosinte as well as maize is susceptible to *Corn stunt spiroplasma* and the *Maize bushy stunt mycoplasma*, bacterial pathogens vectored by *D. maidis* (Nault 1980). Teosinte is susceptible to various downy mildews, like *Sclerospora graminicola* (Hiura 1930; Kenneth 1981) *Peronosclerospora sacchari* (Suma and Magarey 2000), *P. sorghi* (Basandrai and Singh, 2002) and *Sclerophthora macrospora* (Smith and White 1988). A comparative study suggests that some teosinte species could be more resistant against certain viruses than maize. After testing maize and teosinte varieties against *Maize dwarf mosaic virus*, *Maize chlorotic dwarf virus*, *Maize chlorotic mottle virus*, *Maize stripe virus* and *Maize streak virus*, *Z. perennis* was only found susceptible to *Maize stripe virus*. However, the annual *Z. mays* ssp. *mexicana*, *Z. mays* ssp. *parviglumis* and *Z. luxurians* were susceptible to all tested maize viruses (Nault et al. 1982). The enhanced virus resistance of *Z. perennis* could be based on the tetraploid genetic background, which could encode potential resistance genes likely functioning also in modern maize. Intriguingly, comparative genetic studies of defense genes in maize and teosinte are scarce. For instance, both defense genes *hm1* and *hm2*, which confer resistance to the fungus *Cochliobolus carbonum* race 1, were studied simultaneously (Zhang et al. 2002). Thereby, it was found that both defense genes probably were not subjected to artificial selection, as they retain > 80% of the level of genetic variation in modern maize relative to teosinte.

Overall, there is an eminent lack of phytopathological studies on teosinte, which makes it elusive whether teosinte is more resistant against pathogens than modern maize. Nevertheless, it is clear that teosinte bears a yet not well-explored potential for enhancing pathogen resistance in modern maize, as some teosinte species are resistant against various

pathogens. For example, teosinte was also found to be resistant to *Colletotrichum graminicola* (Ces.) Wils. (M1.001) (Elvira S. de Lange and Dirk Balmer, unpublished data). Future phytopathological studies are required to exploit the defensive potential of teosinte in favor of modern maize varieties.

**Table 4** Pathogens infecting teosinte and maize

Pathogen type	Pathogen name	Common name	Reference
<b>Bacteria</b>	<i>Pseudomonas andropogonis</i>	Bacterial leaf spot	Vidaver and Carlson 1978
	<i>Pseudomonas avenae</i>	Bacterial brown stripe	Vidaver and Carlson 1978
<b>Mollicutes</b>	<i>Spiroplasma kunkelii</i>	Corn stunt spiroplasma	Nault 1980
	Maize bushy stunt phytoplasma		Nault 1980
<b>Fungi</b>	<i>Claviceps gigantea</i>	Ergot of maize	Fuentes <i>et al.</i> 1964
	<i>Cochliobolus heterostrophus</i>	Southern leaf blight	Hesseltine and Ellis 1971
	<i>Gibberella fujikuroi</i>	Bakanae, foot rot	Steenkamp <i>et al.</i> 2001
	<i>Physoderma zeae-maydis</i>	Brown spot of corn	Eddins 1933
	<i>Physopella zeae</i>	Rust	Robert 1962
	<i>Puccinia polyspora</i>	Southern corn rust	Robert 1962
	<i>Puccinia sorghi</i>	Common rust	Robert 1962
	<i>Setosphaeria turcica</i>	Northern leaf blight	Melhus 1953
	<i>Sorosporium</i> spp.	Long smut	Borlaug 1946
	<i>Ustilago kellermanii</i>	Corn smut	Borlaug 1946
	<i>Ustilago maydis</i>	Corn smut	Banuett 1995
<b>Oomycetes</b>	<i>Sclerospora graminicola</i>	Downy mildew	Hiura 1930; Kenneth 1981
	<i>Peronosclerospora sacchari</i>	Downy mildew	Suma and Magarey 2000
	<i>Peronosclerospora sorghi</i>	Downy mildew	Basandrai and Singh 2002
	<i>Sclerophthora macrospora</i>	Downy mildew	Smith and White 1988
<b>Viruses</b>	Maize chlorotic dwarf virus		Nault <i>et al.</i> 1982
	Maize chlorotic mottle virus		Nault <i>et al.</i> 1982
	Maize dwarf mosaic virus		Nault <i>et al.</i> 1982
	Maize mosaic virus		Brewbaker 1979
	Maize rayado fino virus		Nault <i>et al.</i> 1980
	Maize streak virus		Nault <i>et al.</i> 1982
	Maize stripe virus		Nault <i>et al.</i> 1982

### Mechanisms of insect and pathogen resistance of maize and teosinte

As stated above, maize and teosinte differ considerably, phenotypically and genetically. However, besides the genetics of domestication, the genetics of other traits, such as insect and pathogen resistance, has received little attention until recently. Maize plants contain a variety of constitutive and induced defenses (Balmer, Planchamp & Mauch-Mani 2013b). The plant hormones salicylic acid (SA), jasmonic acid (JA) and ethylene (ET) play an important role in the regulation of these defenses (Harfouche *et al.* 2006; Erb *et al.* 2009a; Shivaji *et al.* 2010; Sobhy *et al.* 2012; Christensen *et al.* 2013; Lyons, Manners & Kazan 2013). Whereas SA appears to be important in resistance against piercing-sucking insects and biotrophic pathogens, JA and ET tend to be most important in resistance against chewing herbivores and necrotrophic pathogens (Glazebrook 2005). All three hormones are known to influence plant volatile emission (Schmelz, Alborn & Tumlinson 2003; Sobhy *et al.* 2012). Furthermore, recently, members of the maize plant elicitor peptide (Pep) family were discovered, and while ZmPep3 is important in regulating defense against herbivores (Huffaker *et al.* 2013),

ZmPep1 is important for resistance against fungal pathogens (Huffaker, Dafoe & Schmelz 2011a). Whether these peptides are also present in teosinte is not known.

Maize direct defenses against insects include protease inhibitors, molecules that inhibit the digestion of proteins in the insect gut (Erb *et al.* 2009a) and phytoalexins, secondary metabolites with a low molecular weight that are active against a wide variety of biotic stressors (Schmelz *et al.* 2011). Also, a defensive cysteine protease, mir1-CP, increases plant resistance against feeding of insects as diverse as *S. frugiperda*, aphids, and *Diabrotica* spp. (Lopez *et al.* 2007). No information about the presence of these compounds in teosinte is available, although the presence of mir1-CP in teosinte is currently being tested (Dawn S. Luthe, personal communication). Maysin, a flavonoid that appears to be related to resistance to *H. zea* and *S. frugiperda* has been detected in both maize and teosinte (Gueldner *et al.* 1991). But chlorogenic acid, a related secondary metabolite associated with stress resistance, has thus far only been found in maize (Erb *et al.*, 2009b). The best-investigated classes of defense compounds in maize are benzoxazinoids (Balmer *et al.*, 2013a). Indeed, caterpillar feeding induces a variety of benzoxazinoids in maize (Erb *et al.* 2009b; Glauser *et al.* 2011) and these compounds are highly toxic to the insects. They also play a role in resistance against aphid attack (Ahmad *et al.* 2011). These secondary compounds have also been detected in teosinte (Shahid & Esen 1998).

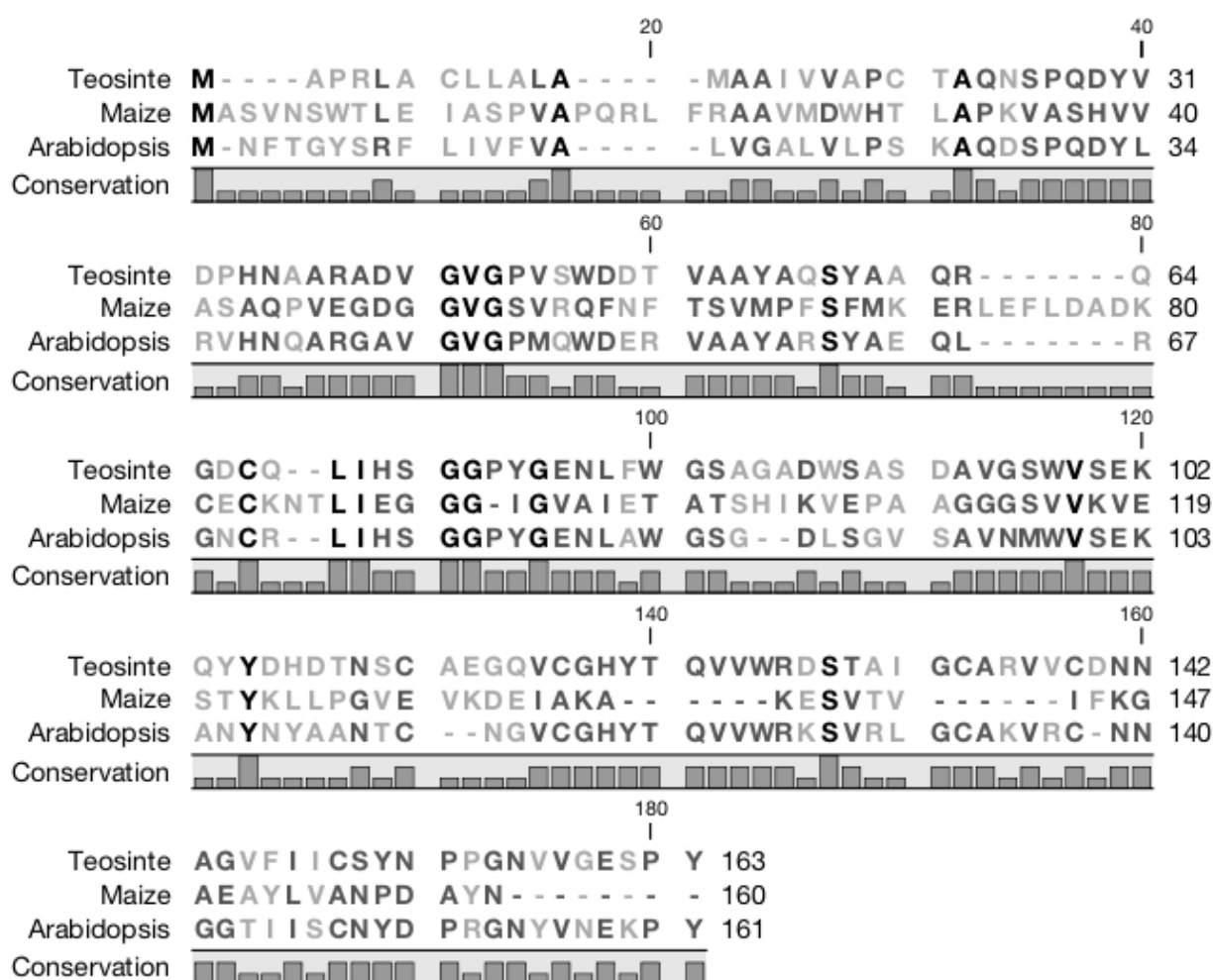
Surprisingly, the constitutive level of benzoxazinoids seems to be lower in teosinte roots than in maize roots (Robert *et al.* 2012), raising the question whether cultivated maize has been selected for increased resistance against herbivores. However, when observing the shoots, levels of benzoxazinoids appear to be higher in teosinte than in maize (Daniel Maag, personal communication), indicating the exact opposite, that cultivated maize has lost some of its direct defenses during domestication. The latter seems to be the case with respect to the specialist stemborer *D. grandiosella*, which caused more damage on cultivated plants than on wild teosinte (Rosenthal & Dirzo 1997). These results are further supported by studies showing that certain teosinte varieties expressed higher levels of four defense-related genes in response to herbivory by *S. frugiperda* than cultivated maize (Szczepaniec *et al.*, 2013), and that *S. frugiperda* larvae can perform better and develop faster on maize than on teosinte plants (Szczepaniec *et al.*, 2013; Takahashi *et al.*, 2012). However, maize and teosinte responded in a similar way to combined root herbivory by *D. virgifera* and shoot herbivory by *S. frugiperda*, although teosinte seemed slightly more resistant to both herbivores (Erb *et al.*, 2011b). Interestingly, extracts of both maize and teosinte can have a toxic effect on *S. frugiperda* larvae (Farias-Rivera *et al.*, 2003) and the exact compounds responsible for the observed differences between maize and teosinte remain to be elucidated. Similar to *S. frugiperda*, maize appears to be more suitable than teosinte for the development of *D. maidis*, since the insects developed faster on maize (Nault & Madden 1985). This effect could also hold true for aphids, for when comparing maize, teosinte and maize x teosinte hybrids in a greenhouse study, only maize became infested by the aphids (Gernert 1917).

In addition to direct defenses, maize plants emit VOCs in response to herbivory. These VOCs can be used by the natural enemies of herbivores, predators and parasitoids, in their search for prey or hosts (Turlings, Tumlinson & Lewis 1990; Turlings & Wäckers 2004; Degenhardt 2009). Different maize varieties emit a highly variable bouquet of odors in response to herbivory (Degen *et al.* 2004; Erb *et al.* 2011a), but overall, teosinte leaves release a very similar bouquet of volatiles as has been reported for cultivated maize (Gouinguéné, Degen & Turlings 2001). However, since it remains unclear which compounds are biologically active and function in the attractiveness of predators and parasitoids, it has been difficult to pinpoint differences and similarities (D'Alessandro & Turlings 2006). Preliminary studies show that the odors of maize and teosinte differ in their attractiveness to various species of parasitoid wasps (Chapter 3, 4), and teosinte odors might be more attractive. Interestingly, it has been shown that commercial maize varieties have lost the ability to emit egg-parasitoid-attracting VOCs in response to egg deposition to the stemborer moth *Chilo partellus* compared to African maize landraces (Tamiru *et al.* 2011). Also, the roots of several European maize varieties emit a volatile signal, (*E*)- $\beta$ -caryophyllene, that attracts entomopathogenic nematodes when attacked by the root-feeding larvae of the beetle *D. virgifera* (Rasmann *et al.* 2005). Roots of teosinte also emit this signal in response to root herbivory (Rasmann *et al.* 2005), but it has been lost in most American maize varieties (Köllner *et al.* 2008). Therefore, it can be reasonably assumed that maize has partially lost indirect defense mechanisms during domestication compared to its wild ancestor. Considering that maize and teosinte, at least in Mexico and around, have to defend themselves against similar insects, this could potentially be detrimental for maize.

Many of the above-mentioned insect resistance mechanisms are also involved in pathogen resistance. Kauralexins, specific phytoalexins, are active against pathogens (Schmelz *et al.* 2011) as well as zealexins, another family of phytoalexins (Huffaker *et al.* 2011b). Benzoxazinoids play a role in resistance against fungal attack as well (Ahmad *et al.* 2011). Furthermore, maize plants strengthen the cell wall around a fungal penetration site by depositing callose (Morris *et al.* 1998), and respond to an attack by a biotrophic pathogen with a hypersensitive response (Morris *et al.* 1998). It has thus far not been confirmed that these resistance mechanisms also function in teosinte. Also, maize plants produce pathogenesis-related proteins (PR-proteins) in response to pathogen attack. Population genetic studies showed that *PR* genes are present in the teosinte genome (Moeller & Tiffin 2005). Besides one recent study (Szczepaniec *et al.* 2013), it remains unknown how different stresses affect the expression of *PR* genes. Comparing the amino acid sequences of the pathogenesis-related protein 1 (PR-1) of teosinte (accession: ABA34070.1), maize (accession: ACG39585.1) and *Arabidopsis* (accession: NP\_179068), it is clear that PR-1 is conserved in all species (Fig. 2). All three sequences share 14% pairwise similarity and 23 identical sites (14%). Interestingly, teosinte and maize share 26% pairwise similarity and 6% identical sites, whereas teosinte and *Arabidopsis* share 54% pairwise similarity and 54% identical sites. It remains to be elucidated whether the sequence differences are due to domestication processes or due to independent evolutionary events.

### Pest resistance in maize x teosinte crosses

Farmers occasionally use teosinte x maize crosses for improvement of their crop (Wilkes 1977). Experimental crosses have been evaluated for pest resistance, and could display increased resistance with respect to hybrid varieties to ear-infesting insects (Barry *et al.* 1992) and various pathogens (Wang *et al.* 2008b). Teosinte has also been evaluated for resistance to other factors, such as the parasitic weed *Striga*, for which no resistance in cultivated maize is known (Rich & Etja, 2008), and flooding (Mano & Omori 2007). In accordance, experimental crosses and analysis of the offspring by means of QTL analysis, as has been done for identification the genes responsible for morphological differences, the “domestication genes” (e.g. Doebley & Stec 1993) could shed a new light on genes that are important in maize resistance.



**Fig. 2** Sequence alignment of the teosinte, maize and *Arabidopsis* pathogenesis-related protein 1 (PR-1). The alignment was performed using the CLC sequence viewer (CLC Bio, 2013). Conservation of sequences is illustrated with bars (high bars for a high conservation)

### Conclusion and outlook

Despite the large amount of crop protection methods available, insects and pathogens still account for at least 15% of the world's crop losses (Oerke 2006). This is an alarming fact in view of an increasing human population that will require an equally increasing amount of food (Bruce, 2010). In addition, recent climate changes have had a measurable impact on crop yield trends. Each degree increase in growing season temperature has thus far caused a roughly 17% relative decrease in US corn yield (Lobell & Asner 2003). Therefore, protecting and improving the maize crop, the most important staple at the moment, is an imminent task.

Cultivated maize might have lost both direct and indirect defenses against insect feeding during domestication (Rosenthal & Dirzo 1997; Köllner et al., 2008; Dávila-Flores et al., 2013). Although similar insects occur on maize and teosinte, the latter appears to be more tolerant (Rosenthal & Welter 1995) and resistant to certain pests and pathogens. Much information about teosinte is written in Spanish and published in local Mexican journals or books (e.g. Jofre y Garfias et al. 2010), so these studies are hard to obtain for a broad public. Studying the defense mechanisms of teosinte offers interesting possibilities for the protection of our modern, numerous maize crops. Overall, as a genetic resource, teosinte is highly valuable for possible improvement of the crop.

Indeed, the use of variation from wild and landrace germplasms for the improvement of maize has been advocated (e.g. Flint-Garcia 2013). Unfortunately, teosinte is threatened with extinction by deforestation, urbanization and cattle (Sánchez González & Ruiz Corral 1995), but most of all by abandoning the cultivation of landrace maize to feed the farmers in favor of cash crops grown to make profit, and where the presence of teosinte as a weed becomes more obvious. The existing populations should be actively conserved in order to maintain the teosinte germplasm for future generations (Wilkes 1995, 2007).

*Acknowledgements* - The authors were supported by the National Centre of Competence in Research (NCCR) "Plant Survival" and by SNF Grant 31003A-122132, both research programs of the Swiss National Science Foundation.

## Chapter 2





## **Natural presence of insect pests on teosinte, the ancestor of maize, in Mexico**

Elvira S. de Lange<sup>1</sup>, Thomas Degen<sup>1</sup>, Jorge E. Ibarra<sup>2</sup>, George Mahuku<sup>3</sup> and Ted C.J. Turlings<sup>1,\*</sup>

<sup>1</sup> *Laboratory of Fundamental and Applied Research in Chemical Ecology, Institute of Biology,  
University of Neuchâtel, Rue Emile-Argand 11, 2000 Neuchâtel, Switzerland*

<sup>2</sup> *Departamento de Biotecnología y Bioquímica, CINVESTAV, Apdo Postal 629, 36500 Irapuato,  
Guanajuato, Mexico*

<sup>3</sup> *Centro Internacional para el Mejoramiento de Maíz y Trigo (CIMMYT), El Batán, Texcoco,  
Estado de México, CP 56130, Mexico*

\*e-mail: [ted.turlings@unine.ch](mailto:ted.turlings@unine.ch)



## Abstract

Fall armyworm, *Spodoptera frugiperda*, is one of the most devastating pests of maize in the Americas. Maize, like other plants, is not completely helpless against herbivore attack. The plant may respond to herbivory with the production of toxic compounds, but also with the emission of odors that can be used by parasitoid wasps as a means to find caterpillars that serve as hosts for their offspring. However, artificial selection of crops has reportedly led to a diminished resistance to herbivory. To determine if fall armyworm and its associated parasitoids share an evolutionary history with teosinte, the wild ancestor of maize, we conducted a field survey of arthropods associated with teosinte at eight different sites across Mexico, the country of origin of maize and teosinte. The main organisms that were collected from the leaves of teosinte were insects. We obtained specimens belonging to at least seven orders and 29 families. Fall armyworm was among the most abundant herbivores, along with weevils, *Diabrotica* spp., aphids and thrips. About 15% of the obtained fall armyworm larvae was infested by parasitoid wasps, indicating that teosinte, fall armyworm and its natural enemies co-occur.

## Key words

Fall armyworm, herbivore-induced plant volatiles, herbivore resistance, parasitoid wasps, tritrophic interactions

## Introduction

The fall armyworm, *Spodoptera frugiperda* Smith (Lepidoptera: Noctuidae), is a major pest of maize in the Americas (Luginbill, 1928; Ortega C., 1987). Adult moths oviposit egg-batches that can contain hundreds of eggs on young plants (Sparks, 1979; Pitre et al., 1983). The emerging larvae first cause characteristic ‘windowpane-feeding’, where leaf tissue is consumed from one side only, leaving the opposite epidermal layer intact. Further instars make holes in the leaves and cause considerable defoliation. The larvae also occasionally cause damage to corn cobs (Ortega C., 1987; Steffey et al., 1999).

To fight off herbivores like the fall armyworm, plants can employ direct defenses, such as the production of toxic compounds, to repel or even kill their attackers (Schoonhoven et al., 2005). Benzoxazinoids are among the best-studied toxins produced by maize (Macias et al., 2009; Niemeyer, 2009). In addition, herbivory is known to induce the emission of volatile organic compounds (VOCs) and the resulting odors can be used by natural enemies of herbivores, predators and parasitoids, in their search for prey or hosts (Turlings et al., 1990; Turlings and Wäckers, 2004). Maize plants are particularly responsive to herbivory and emit copious amounts of odors when attacked by various lepidopteran larvae, among which fall armyworm (Carroll et al., 2006; de Lange, 2008). Fall armyworm serves as food for a wide variety of predators (Andrews, 1988; Hoballah et al., 2004) and is frequently parasitized by numerous species of parasitoid wasps (Hoballah et al., 2004; Molina-Ochoa et al., 2004; Bahena-Juárez et al., 2010). Parasitoids are highly valued in reducing larval populations in agricultural fields (Luginbill, 1928; Vickery, 1929; Barbosa, 1998).

Importantly, artificial selection of crop plants for increased yield and quality has had a negative influence on resistance to herbivores (Wink, 1988). In maize, both toxin levels (Rosenthal and Dirzo, 1997) and herbivore-induced VOC emission (Köllner et al., 2008) have reportedly been affected, which could partially explain the devastating pest outbreaks in agricultural environments (Barbosa et al., 2012). For example, most American maize varieties have lost the ability to release a volatile from their roots that attracts insect-killing nematodes in response to an attack by the root-feeding larvae of the beetle *Diabrotica virgifera virgifera* LeConte (Coleoptera: Chrysomelidae) (Rasmann et al., 2005; Köllner et al., 2008). Their roots are therefore significantly more vulnerable to *Diabrotica* damage than the roots of plants that still emit this key nematode attractant, such as teosinte, the wild ancestor of maize (Rasmann et al., 2005). When the volatile signal was “restored” by transforming a non-producing maize line with a gene derived from oregano, the protection that nematodes provide to the roots was greatly enhanced (Degenhardt et al., 2009). Therefore, restoring or enhancing natural defense traits might be an effective way to combat pests (Turlings and Ton, 2006).

In the context of studying natural defenses in the wild ancestors of crop plants, the aim of the current study was to obtain information about the occurrence of fall armyworm, as well as other herbivorous, carnivorous and parasitoid arthropods on teosinte, the wild ancestor of maize (Matsuoka et al., 2002). Native to Mexico, teosinte grows alongside maize in Central America (Sánchez González and Ruiz Corral, 1995). Its natural habitat ranges from deciduous thorn shrub to oak woodland (Wilkes, 1995), but mostly open habitats (Sánchez González and Ruiz Corral, 1995). Teosinte could potentially serve as a source of plant defense traits that can be transferred for the improvement of resistance in cultivated maize (e.g. Barry et al., 1992; Wang et al., 2008b).

## Methods and Materials

**Survey Sites** We surveyed teosinte plants at eight different locations in three Mexican states in June and July 2009 (Table 1; Fig. 1). This is during the rainy period, when teosinte plants are a few weeks old. The plants’ growing season is broadly from June to October and they have reached midflowering stage by August-September. Flowering time mostly corresponds to that of the local maize landraces (Wilkes, 1977). The sites we visited differed substantially in parameters such as soil composition, average minimum and maximum temperature and rainfall (Víctor M. Hernández, personal communication). Typically, teosinte grows as a weed in maize fields or alongside roads nearby maize fields (Sánchez González and Ruiz Corral, 1995; Vibrans and Estrada Flores, 1998). Teosintes belong to the genus *Zea* and comprise annual and perennial grasses that are native to Mexico and Central America. Balsas teosinte (*Z. mays* ssp. *parviglumis*) is considered the wild ancestor of cultivated maize (Matsuoka et al., 2002). This annual species occurs in the Balsas River valley in southern and western Mexico, where the mean annual temperature is between 20 and 25°C and annual rainfall ranges from 125 to 200 cm. It grows at an altitude between 400 and 1700 meters (Sánchez González and Ruiz Corral, 1995). Closely related Chalco and Central Plateau teosinte

(*Z. mays* ssp. *mexicana*) are annuals that grow on the plains and valleys of central and northern Mexico at altitudes of 1800 to 2500 meters. Here, mean annual temperature varies from 15 to 20°C and rainfall ranges from about 50 to 100 cm per year (Sánchez González and Ruiz Corral, 1995). We did not survey the other known teosintes, which include the perennial *Z. diploperennis* and *Z. perennis* from Mexico, the annual *Z. luxurians* from Guatemala, *Z. mays* ssp. *huehuetenangensis* from Guatemala or *Z. nicaraguensis* from Nicaragua (Iltis and Doebley, 1980; Doebley, 1990; Iltis, 2000; Iltis and Benz, 2000).

**Field Survey** We sampled 69-247 whorl-stage corn plants per site, on one, two or three individual days, not more than ten days apart (Table 1). To estimate plant developmental stage, we measured plant height from stem base to the tip of the longest leaf, as well as stem diameter for 10-20 plants per site. In addition, we counted the total number of developing leaves, including small emerging leaves (Annex 2). Herbivore damage was visually estimated as a percentage of the total plant. To determine the teosinte-related aboveground arthropod fauna, we scored arthropod presence on each plant. Representative insects were collected and stored in 70% ethanol in Eppendorf tubes and brought to the laboratory for identification as described below. We specifically collected larvae of the noctuid moth *Spodoptera frugiperda* (fall armyworm), which is one of the most devastating pests of maize in the New World (Ortega C., 1987). To estimate parasitism of these and other lepidopteran larvae, we kept the collected larvae in the laboratory on an artificial maize-based diet. Parasitoids were reared out as described below. In addition, plants exhibiting infestation symptoms were further surveyed for caterpillar presence to increase the sample size. Young maize and teosinte plants are very much alike. Teosinte plants could be recognized by their finer leaf shape and their random distribution in the field, which contrasts with the cultivated maize plants that grew in neat rows. An additional feature of teosinte was the formation of tillers (Mondragón-Pichardo and Vibrans, 2005). Each surveyed plant was pulled out of the soil to verify the presence of a teosinte seed, which can be easily distinguishable from a maize seed.

**Parasitoid Recovery** Recovered fall armyworm larvae were placed in individual compartments of a 24-well ELISA plate supplied with artificial diet. They were reared at room temperature until adult emergence or, in the case of parasitism, the emergence of parasitoids (Hoballah et al., 2004). The adult parasitoids, as well as dead parasitoid larvae and cocoons, were individually conserved in 70% ethanol in Eppendorf tubes for later identification. To estimate percent parasitism, the number of collected larvae was corrected by subtracting the number that died from unknown causes. Occasionally, larvae tentatively identified as *Heliothis* spp. (site 8) and *Pseudaletia* spp. (site 1,2,6,7) were retrieved and reared out in a similar way. The latter was sometimes found to be parasitized by *Campoletis sonorensis* wasps. These samples were excluded from the analysis of fall-armyworm associated parasitoids.

*Arthropod Identification* Teosinte-related arthropods were visually examined under a binocular and identified to the species level where possible, or otherwise to genera or family levels. All arthropods were grouped according to their trophic function: herbivores, parasitoids, predators, saprophages and decomposers. Hymenopteran parasitoids were identified according to Cave (1995).



**Fig. 1** Approximate location of the survey sites of eight teosinte populations in Mexico

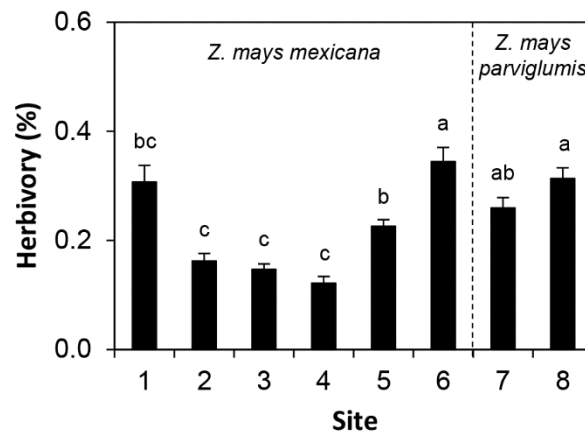
**Table 1** Survey of eight teosinte populations in Mexico

Site no.	Site name	State	Altitude (m)	gps coordinates	Teosinte species	Teosinte race	Site type	Sampling dates	Sample size
1	Ejidos de la Magdalena (Texcoco)	Estado de México	2246	N 19°32.075' W 098°55.422'	<i>Zea mays ssp. mexicana</i>	Chalco	Maize field, fallow land	June 26,27	236
2	La Purificación (Texcoco)	Estado de México	2295	N 19°31.614' W 098°50.144'	<i>Zea mays ssp. mexicana</i>	Chalco	Fallow land	June 30, July 1,4	246
3	Ejido de San Sebastián (Metepec, Toluca)	Estado de México	2581	N 19°13.664' W 099°32.790'	<i>Zea mays ssp. mexicana</i>	Chalco	Maize field	July 2	124
4	Santa Ana Maya	Michoacán	1837	N 20°02.776' W 101°04.573'	<i>Zea mays ssp. mexicana</i>	Central Plateau	Road site	July 6	70
5	Jaripeo	Michoacán	2012	N 19°41.117' W 101°04.841'	<i>Zea mays ssp. mexicana</i>	Central Plateau	Maize field	July 7,8	273
6	Cuitzeo	Michoacán	1848	N 19°57.851' W 101°11.485'	<i>Zea mays ssp. mexicana</i>	Central Plateau	Road site	July 10,20	221
7	Tzitzio	Michoacán	1316	N 19°32.994' W 100°55.161'	<i>Zea mays ssp. parviglumis</i>	Balsas	Fallow land	July 9	138
8	Guachinango	Jalisco	1311	N 20°37.973' W 104°26.192'	<i>Zea mays ssp. parviglumis</i>	Balsas	Maize field	July 15,16	231

Part of this table is reproduced in Annex 2

## Results

**Herbivory** The plants in each site suffered herbivore damage, but there were significant differences between sites (Kruskal-Wallis test,  $P < 0.001$ ), indicating differential herbivore pressures (Fig. 2).

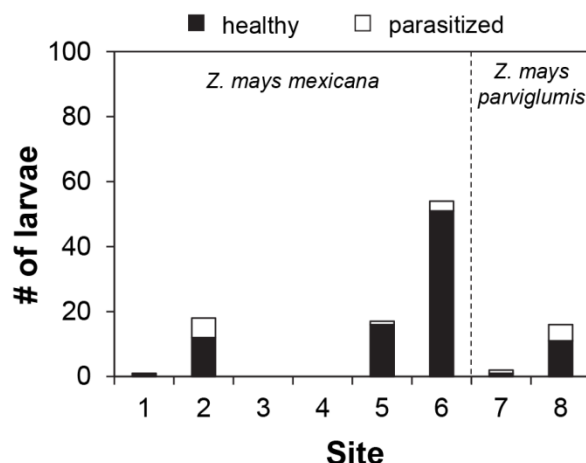


**Fig. 2** Herbivore damage of teosinte plants at eight different locations in Mexico. Different letters indicate significant differences (Kruskal-Wallis test,  $P < 0.001$ ). For more information about the sites, see Table 1 and Fig. 1

**Fall Armyworm Presence** Overall, about 8% of the surveyed plants was infested with *S. frugiperda* larvae. The highest fall armyworm pressure was observed in site 6 (0.3 larvae/plant), while the lowest pressure was observed in site 4, the only site where not a single fall armyworm larva was recovered (Annex 2). On plants where larvae were present, we found on average one larva per plant.

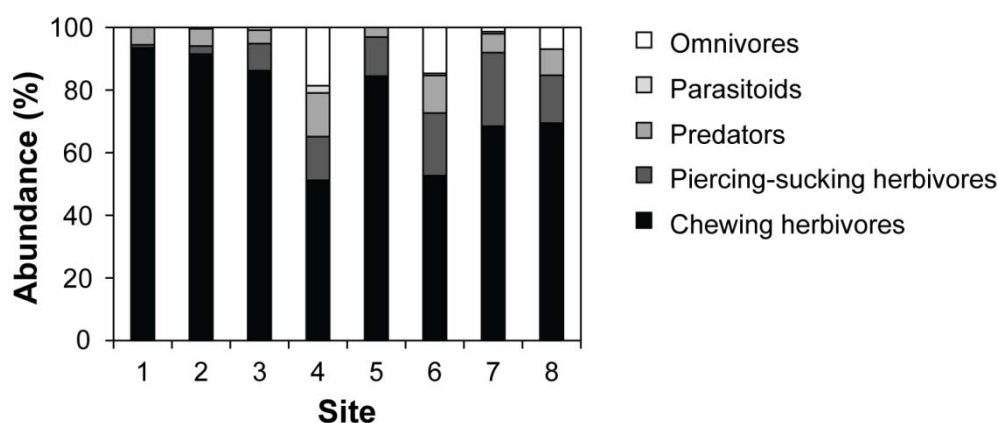
**Fall Armyworm-associated Parasitoids** There was an overall parasitism rate of 15%. No parasitoids were found in site 3 (Fig. 3). The parasitoids that emerged from the fall armyworm larvae were identified as *Campoletis sonorensis* Cameron (Hymenoptera: Ichneumonidae) (populations 2,5,6,8), *Cotesia marginiventris* Cresson (Hymenoptera: Braconidae) (population 6) and *Meteorus laphygmae* Viereck (Hymenoptera: Braconidae) (populations 6 and 8). All parasitoids are common natural enemies of fall armyworm in Mexico (Hoballah et al., 2004; Bahena-Juárez et al., 2010). One cocoon from an unknown parasitoid was obtained from population 7.





**Fig. 3** Parasitism of *S. frugiperda* larvae at eight different locations in Mexico. For more information about the sites, see Table 1 and Fig. 1

**Teosinte-associated Arthropods** We observed a total of 2700 arthropods on the leaves of teosinte plants. Most arthropods belonged to the class Insecta, where we tentatively identified at least ten orders and at least 32 different families (Table 2). The relative abundance of herbivores varied among survey sites (Fig. 4). Overall, the most abundant chewing herbivores were weevils (0.44/plant), fall armyworm larvae (0.09/plant) and adults of *Diabrotica* spp. (0.02/plant). The most abundant piercing-sucking herbivores were aphids and thrips. On average, a plant carried about two insect herbivores.



**Fig. 4** Herbivore, predator and parasitoid abundance associated with teosinte plants at eight different locations in Mexico. For more information about the sites, see Table 1 and Fig. 1

Table 2 Teosinte-associated arthropods

Class	Order	Family	Tribe	Species	Trophic function	Tentative feeding mode
Arachnida	Acari	Tetranychidae				
Arachnida	Acari					
Arachnida	Araneae	Argiopidae			Predator	
Arachnida	Araneae	Lycosidae			Predator	
Collembola						
Insecta	Blattaria	Blattidae			Omnivore	
Insecta	Coleoptera	Anthicidae			Herbivore	Chewing
Insecta	Coleoptera	Cantharidae		<i>Discodon</i> spp.	Herbivore	Chewing
Insecta	Coleoptera	Carabidae		<i>Calosoma</i> spp.	Predator	
Insecta	Coleoptera	Chrysomelidae	Alticini			
Insecta	Coleoptera	Chrysomelidae	Cassidini			
Insecta	Coleoptera	Chrysomelidae		<i>Chaetocnema</i> spp.		
Insecta	Coleoptera	Chrysomelidae		<i>Diabrotica</i> spp.	Herbivore	Chewing
Insecta	Coleoptera	Chrysomelidae		<i>Diabrotica undecimpunctata</i>		
Insecta	Coleoptera	Chrysomelidae		<i>Diabrotica multipunctata</i>		
Insecta	Coleoptera	Chrysomelidae		<i>Zigospila</i> spp.	Herbivore	Chewing
Insecta	Coleoptera	Chrysomelidae		<i>Zygogramma</i> spp.	Herbivore	Chewing
Insecta	Coleoptera	Chrysomelidae				
Insecta	Coleoptera	Cleridae				
Insecta	Coleoptera	Cleridae (1)			Herbivore	Chewing
Insecta	Coleoptera	Cleridae (2)			Predator	
Insecta	Coleoptera	Coccinellidae (brown)			Herbivore	Chewing
Insecta	Coleoptera	Coccinellidae (red)		<i>Hippodamia convergens</i>	Predator	
Insecta	Coleoptera	Curculionidae		<i>Nicentrites</i> spp.	Herbivore	Chewing
Insecta	Coleoptera	Curculionidae				
Insecta	Coleoptera	Curculionidae	Rhynchophorini			
Insecta	Coleoptera	Elateridae			Herbivore	Chewing
Insecta	Coleoptera	Melyridae		<i>Collops</i> spp.	Herbivore	Chewing
Insecta	Coleoptera	Pentatomidae		<i>Euschistus</i> spp.	Herbivore	Chewing
Insecta	Coleoptera	Phalacridae			Herbivore	Chewing

Table 2 (continued)

Class	Order	Family	Tribe	Species	Trophic function	Tentative feeding mode
Insecta	Coleoptera	Scarabaeidae		<i>Macrodactylus</i> spp.	Herbivore	Chewing
Insecta	Coleoptera	Staphylinidae			Predator	
Insecta	Coleoptera	Tenebrionidae		<i>Lobometopon</i> spp.	Herbivore	Chewing
Insecta	Dermaptera	Forficulidae			Omnivore	
Insecta	Diptera					
Insecta	Hemiptera	Aleyrodidae			Herbivore	Piercing-sucking
Insecta	Hemiptera	Anthocoridae		<i>Orius</i> spp.	Predator	
Insecta	Hemiptera	Aphididae			Herbivore	Piercing-sucking
Insecta	Hemiptera	Cicadellidae			Herbivore	Piercing-sucking
Insecta	Hemiptera	Coreidae				
Insecta	Hemiptera	Lygaeidae		<i>Blissus</i> spp.	Herbivore	Piercing-sucking
Insecta	Hemiptera	Nabidae			Predator	
Insecta	Hemiptera	Pentatomidae		<i>Euschistus</i> spp.		
Insecta	Hemiptera	Pentatomidae				
Insecta	Hemiptera	Reduviidae (nymph)			Predator	
Insecta	Hymenoptera	Chalcididae				
Insecta	Hymenoptera	Formicidae		<i>Pseudomyrmex</i> spp.		
Insecta	Hymenoptera	Sphecidae				
Insecta	Lepidoptera	Arctiidae			Herbivore	Chewing
Insecta	Lepidoptera	Noctuidae		<i>Spodoptera frugiperda</i> (larvae)	Herbivore	Chewing
Insecta	Lepidoptera	Noctuidae		<i>Pseudaletia</i> ssp. (larvae)	Herbivore	Chewing
Insecta	Lepidoptera	Noctuidae		<i>Heliothis</i> ssp. (larvae)	Herbivore	Chewing
Insecta	Orthoptera	Acrididae			Herbivore	Chewing
Insecta	Orthoptera	Tettigoniidae			Predator	
Insecta	Phasmatodea	Phasmatidae			Herbivore	Chewing
Insecta	Thysanoptera				Herbivore	Piercing-sucking

## Discussion

Our survey demonstrates that teosinte, the wild ancestor of maize, serves as a niche for many different species of arthropods simultaneously. These results are in accordance with earlier studies of arthropod abundance on different members of the *Zea* family (Painter, 1955; Moya-Raygoza et al., 1990; Moya-Raygoza, 1994; De la Paz-Gutiérrez, 2008; Jorge E. Ibarra, unpublished data). Fall armyworm abundantly occurred on teosinte plants, indicating that it can readily use the wild ancestor of maize as a food source. In all cases where we observed that surrounding maize plants suffered fall armyworm damage (Elvira S. de Lange and Thomas Degen, personal observations), teosinte was also attacked by the herbivore. As the fall armyworm is able to detoxify the main maize defensive compounds (Glauser et al., 2011), potential differences in defenses between maize and teosinte might not affect its performance. However, fall armyworm reportedly occurs more abundantly on maize plants than on surrounding teosinte plants (De la Paz-Gutiérrez, 2008; Takahashi et al., 2012). There are indications that teosinte is more resistant to pests than cultivated maize (reviewed in Chapter 1), which would confirm that modern maize has lost part of its defensive ability during cultivation. This would offer extensive opportunities to exploit wild teosinte to develop new maize varieties with increased resistance against insect pests (e.g. Barry et al., 1992; Wang et al., 2008b).

About 15% of retrieved fall armyworm larvae was infested by parasitoid wasps, implying that teosinte is well able to attract parasitoids, as shown in the olfactometer experiments presented in Chapters 3 and 4. Three species of parasitoids could be identified, which are commonly reported as natural enemies of fall armyworm on maize (Hoballah et al., 2004). A more elaborate survey, comparing parasitoids from fall armyworm retrieved from maize and from teosinte, might bring to light differences in parasitoid attractiveness of maize and teosinte under field conditions. However, an elaborate study comparing the parasitoids associated with fall armyworm on maize and sorghum in Mexico did not demonstrate a difference in species composition (Jourdie, 2008).

Besides fall armyworm, *Diabrotica* spp. adults were abundantly present on teosinte plants. Although the adults can cause some damage to the leaves and silk, the root-feeding larvae of *Diabrotica* spp. are considered an important maize pest. They consume and cut off root tissue to such extent that the plants dry and fall, causing substantial economical damage (Metcalf, 1986; Ortega C., 1987; Steffey et al., 1999). *Diabrotica* spp. reportedly occurs more abundantly on maize plants than on teosinte (De la Paz-Gutiérrez, 2008). Another group of insects that was frequently observed on teosinte was weevils or billbugs. Both the root-feeding larvae and the leaf-chewing adults can cause considerable damage to maize plants (Ortega C., 1987; Steffey et al., 1999). Furthermore, weevils can become important pests of stored maize grains (Ortega C., 1987).

A limited number of studies have compared the insect fauna on teosinte and cultivated maize and reported mostly similarities (Moya-Raygoza, 1987; Moya-Raygoza, 1994; Sanchez Gonzalez and Corral, 1995; De la Paz-Gutiérrez, 2008; Jorge E. Ibarra, unpublished data). Also for other crops, only a limited number of studies compared herbivore and natural enemy

occurrence on wild and cultivated plants. Examples are hop in Idaho (Gardiner et al., 2003), cereals in Kenya (Mailafiya et al., 2009) and lesser burdock in Argentina (Salvo et al., 2005). None of these studies show evident effects of domestication on herbivore and natural enemy presence on plants. However, a recent investigation on rice showed that cultivated rice fields supported less insect taxa than wild rice fields (Chen et al., 2013). When comparing the insect fauna on maize and teosinte, a lower species diversity may be obtained on cultivated maize compared to its wild ancestor (Moya-Raygoza, 1994; De la Paz-Gutiérrez, 2008). Studying wild plants may increase the knowledge on complicated multitrophic interactions

Arthropod abundance on teosinte shows major seasonal variability (Moya-Raygoza et al., 1990; De la Paz-Gutiérrez, 2008). Therefore, our results are not fully representative for the arthropods that may occur on teosinte. Furthermore, we only surveyed young whorl-stage plants, which is the most relevant in terms of vulnerability of the plants to the detrimental effects of pest insects. But a more thorough survey of all arthropods associated with teosinte would require sampling of teosinte plants from the early stages until seed production. Furthermore, by only evaluating arthropods associated with teosinte leaves, we missed out on soil-dwelling organisms (Moya-Raygoza, 1987; Moya-Raygoza et al., 1990; De la Paz-Gutiérrez, 2008) that could potentially also influence the complex multitrophic interactions aboveground (Soler et al., 2005). It is recommended to include the sampling of soil around teosinte plants, in order to disentangle and fully appreciate multitrophic interactions in nature.

In summary, we found that the wild ancestor of maize provides a niche for many different species of insect herbivores, predators and parasitoids. We confirm that teosinte, *S. frugiperda* and its associated parasitoid wasps co-occur. Further research on defensive toxins and volatile signals that attract parasitoids to teosinte is currently being performed. The aim is to identify novel defensive mechanisms that might have gone lost during artificial selection of maize. We argue that wild plants should be included in studies on herbivore resistance of agricultural crops in order to obtain ecologically relevant data that could be used for the development of novel crop protection methods based on biological control.

*Acknowledgements* – We thank Carlos Muñoz, Emiliano Gutiérrez and the Entomología team at the International Maize and Wheat Improvement Center (CIMMYT) for help rearing the caterpillars, Dr. Victor Chávez, Marcial Rivas and Dr. Suketoshi Taba for help localizing teosinte populations, Dr. Victor Hernández for climatic data and Ricardo Romero for field assistance. We are grateful to Rafael Aguilar-Romero at Universidad Nacional Autónoma de México (UNAM) for field assistance. We thank Prof. Gustavo Moya-Raygoza at the University of Guadalajara for help localizing teosinte populations and Prof. Jesus Sánchez and Dr. Salvador de la Paz for helpful discussions on the resistance of teosinte against insects. We also thank Dr. Martin Heil at Centro de Investigación y de Estudios Avanzados del Instituto Politécnico Nacional (CINVESTAV) for helpful discussions on the design of field experiments.



## Chapter 3





## **Has maize lost some of its ability to attract parasitoids during domestication?**

Elvira S. de Lange<sup>1</sup>, Kevin Farnier<sup>1,2</sup>, Benjamin Gaudillat<sup>1,3</sup> and Ted C.J. Turlings<sup>1,\*</sup>

<sup>1</sup> *Laboratory of Fundamental and Applied Research in Chemical Ecology, Institute of Biology,  
University of Neuchâtel, Rue Emile-Argand 11, 2000 Neuchâtel, Switzerland*

<sup>2</sup> *Department of Zoology, La Trobe University, Melbourne, VIC 3086, Australia*

<sup>3</sup> *UMR 'Peuplements Végétaux et Bio-agresseurs en Milieu Tropical', CIRAD/Université de La  
Réunion, Pôle de Protection des Plantes, 7 chemin de l'IRAT, F-97410 Saint-Pierre, France*

\*e-mail: [ted.turlings@unine.ch](mailto:ted.turlings@unine.ch)

Tel: (+41) 32 718 3158, Fax: (+41) 32 718 3001



## Abstract

Artificial selection of crop plants for increased yield and quality has had a cost for other potentially useful traits, including resistance to herbivores. Besides direct defenses, such as the production of toxins, plants may also indirectly protect themselves by emitting volatile organic compounds (VOCs) that attract the natural enemies of herbivores. Parasitoid wasps are known to use these VOCs to localize hosts for their offspring. However, domestication and selective breeding of crop plants have reportedly led to the loss of such signals. The aim of this study was to identify possible differences in the attraction of parasitoid wasps by modern, cultivated maize and its wild ancestor, teosinte. In a six-arm olfactometer, we compared the capacity of teosinte and maize to attract the parasitoid wasps *Cotesia marginiventris* and *Campoletis sonorensis*. We tested plants in which VOC emission was induced by the application of artificial damage and caterpillar regurgitant, as well as extracts of collected volatiles of plants exposed to caterpillar herbivory. *C. sonorensis* did not distinguish between the odors of maize and teosinte, whereas *C. marginiventris* showed a significant preference for the odors of teosinte over the odors of maize, indicating that the modern maize variety has reduced capacity to attract this parasitoid. The fact that we obtained very similar results with extracts of volatiles implies that we can use these extracts to identify the key compounds that are responsible for wasp attraction. Restoring and/or enhancing such key parasitoid attractants in cultivated plants could be an effective way to increase natural pest control.

## Key words

*Campoletis sonorensis*, *Cotesia marginiventris*, Plant defenses, Tritrophic interactions, Volatile organic compounds (VOCs)

## Introduction

Artificial selection of crop plants for increased yield and quality can negatively influence other agronomically important traits, such as resistance to herbivores (Wink, 1988; Welter and Steggall, 1993; Rosenthal and Dirzo, 1997; Rodriguez-Saona et al., 2011). In response to herbivory, plants can mobilize a variety of defense mechanisms. Direct defenses include the production of toxic compounds that have a direct negative effect on insect feeding (Howe and Jander, 2008). In addition, indirect defenses comprise the emission of volatile organic compounds (VOCs) that can be perceived by natural enemies of herbivores in their search for prey or hosts (Dicke and Sabelis, 1988; Turlings et al., 1990; Turlings and Wäckers, 2004). An increasing number of studies show that domestication has influenced complex tritrophic interactions, both in negative (Chen and Welter, 2002, 2003, 2005; Wang et al., 2009; Gols et al., 2011) and in positive ways (Benrey et al., 1998).

An example of how selective breeding can disrupt tritrophic interactions comes from maize (*Zea mays* L. ssp. *mays*). When attacked by the root-feeding larvae of the beetle *Diabrotica virgifera virgifera* LeConte (Coleoptera: Chrysomelidae), maize roots emit VOCs that attract entomopathogenic nematodes (Rasmann et al., 2005). Interestingly, most

American maize varieties have lost the ability to release a key nematode attractant, making their roots much more vulnerable to *Diabrotica* damage (Rasmann et al., 2005; Köllner et al., 2008). By transforming a deficient maize line with a gene from oregano, this signal could be restored, thereby enhancing the protection that nematodes provide to the roots (Degenhardt et al., 2009). Similarly, commercial maize varieties have lost the ability to emit parasitoid-attracting VOCs in response to egg deposition by the stemborer moth *Chilo partellus*. By contrast, maize landraces, locally adapted varieties not produced by breeders, but kept by farmers, retained this ability (Tamiru et al., 2011). Furthermore, a feral cotton variety emitted higher amounts of VOCs in response to herbivore feeding than varieties that remained cultivated (Loughrin et al., 1995). Restoring and/or optimizing the release of the attractants in cultivated plants could therefore be an effective way to control pests (Bottrell et al., 1998; Cortesero et al., 2000; Turlings and Ton, 2006).

The wild ancestor of modern maize is Balsas teosinte, *Z. mays* ssp. *parviglumis* Iltis & Doebley (Matsuoka et al., 2002). Maize and teosinte greatly differ, morphologically as well as genetically (Doebley, 2004; Doebley et al., 2006). The “domestication gene” *teosinte branched1* is responsible for the most striking morphological difference: while maize plants typically have a single stalk, contain one tassel (male inflorescence) and two or three short lateral branches on which the ears (female inflorescences) grow, teosinte plants are much more branched and contain many tassels and slender ears (Doebley et al., 1997). The differences between maize and teosinte are however not big enough to prevent hybridization (Doebley, 2004).

It has been suggested that cultivated maize is less defended against insect feeding than wild teosinte as a result of domestication (Rosenthal and Dirzo, 1997; Takahashi et al., 2012; Dávila-Flores et al., 2013). Although it is known that there is some variability in the capacity of maize and teosinte to emit VOCs in response to herbivory (Gouinguéné et al., 2001), no study has yet looked at how these wild and cultivated plants may differ in their attractiveness to parasitoids. As stated above, modern maize seems to have lost the ability to respond to egg deposition (Tamiru et al., 2011) and many maize varieties have lost a belowground signal, known to be present in teosinte (Rasmann et al., 2005; Köllner et al., 2008). We therefore hypothesized that an analogous phenomenon could be observed with aboveground attractants. We tested this hypothesis by comparing the capacity to attract parasitoids between cultivated maize, its wild ancestor Balsas teosinte, and the closely related Central Plateau teosinte.

## Methods and Materials

**Plants** Maize (*Z. mays* ssp. *mays*, variety Delprim, European commercial hybrid; Delley semences et plantes SA, Switzerland), Balsas teosinte (*Z. mays* ssp. *parviglumis*, Michoacán, Mexico; ARS-USDA, USA) and Central Plateau teosinte (*Z. mays* ssp. *mexicana* (Schrader) Iltis, Michoacán, Mexico; ARS-USDA, USA) seeds were sown in plastic pots (4 cm diameter, 10 cm high) with fertilized commercial soil (Ricoter Aussaaterde, Aarberg, Switzerland). All plants were kept in a climate chamber (maize:  $25 \pm 2^\circ\text{C}$ ; teosinte:  $30 \pm 2^\circ\text{C}$ ; 60% relative humidity; 16 h light / 8 h dark;  $50.000 \text{ lm/m}^2$ ). At the start of the experiments, the plants were 10-20 days old.

**Insects** The caterpillar *Spodoptera littoralis* Boisduval (Lepidoptera: Noctuidae) and the solitary endoparasitoids *Cotesia marginiventris* Cresson (Hymenoptera: Braconidae) and *Campoletis sonorensis* Cameron (Hymenoptera: Ichneumonidae) were reared as described by Turlings et al. (2004) and Tamò et al. (2006a,b). *S. littoralis* regurgitant was collected as described by Turlings et al. (1993).

**Induction of Plants** Odor emission in plants used for six-arm olfactometer bioassays was elicited by scratching the undersides of two leaves with a razor blade over an area of approximately  $1 \text{ cm}^2$  on both sides of the central vein. Immediately after wounding,  $10 \mu\text{l}$  of *S. littoralis* regurgitant was applied to the wounds. This treatment was performed 15 h as well as 1 h before the start of the experiments. Artificial damage was applied in order to prevent bias in VOC emission due to differential feeding of the herbivores on maize and teosinte plants (Sandrine P. Gouinguéné, unpublished data). For the collection of plant volatile extracts, plants were infested with 20 second-instar *S. littoralis* larvae the night prior to odor collections. We corrected for differential VOC induction as described below.

**Odor Collection and Analysis** Odor collections from all three plant genotypes were conducted simultaneously, during the six-arm olfactometer bioassays with plants described below. Plants were placed in glass vessels into which purified air entered through a Teflon tube at a rate of 1.1 l/min. Air was pulled out of the vessels at a rate of 0.7 l/min through a trapping filter containing 25 mg of 80-100 mesh SuperQ adsorbent (Alltech Associates, Inc., Deerfield, Illinois, USA). Before each collection these filters were rinsed with 3 ml dichloromethane (Suprasolv, GC-grade, Merck, Dietikon, Switzerland). Odor collections lasted for 3 h, and on each plant either one collection was performed in the morning (1-4 h after the last induction) or two collections were performed, one in the morning and one in the afternoon (4-6 h after the last induction,  $n = 16$  odor collections on 11 individual plants). After each collection, the filters were removed and eluted with  $150 \mu\text{l}$  of dichloromethane. Two internal standards (n-octane and nonyl acetate, each 200 ng in  $10 \mu\text{l}$  dichloromethane) were added to each sample. The samples were stored at  $-80^\circ\text{C}$  before analysis (Turlings et al., 2004).

VOCs were analyzed using an Agilent 6850 gas chromatograph with a flame ionization detector. A  $3\text{-}\mu\text{l}$  aliquot of each sample was injected in pulsed splitless mode onto an apolar

capillary column (HP-1ms, 30 m, 0.25 mm ID, 0.25  $\mu$ m film thickness; Agilent J&W Scientific, USA). Helium at constant pressure (18.71 psi) was used as carrier gas. After injection, the column temperature was maintained at 40°C for 3 min, and then increased to 100°C at 8°C/min and subsequently to 200°C at 5°C/min followed by a post-run of 3 min at 250°C. The detected VOCs were quantified based on a comparison of their peak areas with those of the internal standards and identified by comparison of retention times with those from previous analyses (D'Alessandro and Turlings, 2005). After VOC collection, plant shoots were harvested and fresh weight was determined.

To confirm the identities of the different peaks, at least one odor sample per plant genotype was analyzed using a gas chromatograph (Agilent 6890 Series GC System G1530A) coupled to a mass spectrometer (Agilent 5973 Network Mass Selective Detector; transfer line 230°C, source 230°C, ionization potential 70 eV). An aliquot of 2  $\mu$ l was injected in the pulsed splitless mode onto the same type of column as described above. Helium at constant flow (0.9 ml/min) was used as carrier gas. After injection, the column temperature was maintained at 40°C for 3 min, and then increased to 100°C at 8°C/min and subsequently to 220°C at 5°C/min followed by a post-run of 3 min at 250°C. The detected VOCs were identified by comparison of their mass spectra with those of the NIST05 library and by comparison of their spectra and retention times with those of authentic standards. Eleven principal compounds were identified.

*Preparation of Volatiles in Solution* Maize and Balsas teosinte plants were placed in a multiple air-delivery VOC collection setup as described by Turlings et al. (2004) and Ton et al. (2007). VOCs were collected in a similar way as in the six-arm olfactometer as described above, with the exception that 200  $\mu$ l of dichloromethane was used to elute the filters. Three collections of 3 h were performed on each day. The eluted samples of all individual collections, here referred to as volatile extracts, were combined for maize and for teosinte separately and were stored at -80°C before analysis. Two samples of the maize and teosinte volatile extracts were analysed by means of GC-FID as described above, but without the addition of internal standard. The relative peak area of the 30 principal peaks was calculated. According to these numbers the maize extract was diluted with dichloromethane to have a similar total VOC concentration as the teosinte extract.

*Six-arm Olfactometer Bioassays with Plants* Bioassays were performed in a six-arm olfactometer as described by Turlings et al. (2004). Maize, Balsas teosinte and Central Plateau teosinte plants were placed on randomized positions in the olfactometer on each experimental day. Three arms were left empty; arms with a plant were always alongside an arm without a plant. While half of the incoming air that passed over the plants was pulled through trapping filters for odor collection, the other half entered the chamber where the wasps were released. Mated two- to six-day-old female *C. marginiventris* (n = 234) and *C. sonorensis* (n = 180) wasps were given 30 min to make a choice for an arm, thereby assessing their preferences for the odor sources. The females were naïve, which means that

as adults they had never been in contact with a plant or a host before. Three to eight releases of groups of six wasps were performed on each experimental day, on a total of five to eight experimental days. Only the experimental days where > 50% of the wasps made a choice were included in the analysis ( $n = 210$  for *C. marginiventris* and  $n = 180$  for *C. sonorensis*). Because the response of *C. marginiventris* is known to be affected by the odor of *C. sonorensis* (Tamò et al., 2006b), the two species were tested in separate olfactometers.

*Six-arm Olfactometer Bioassays with Volatile Extracts* An aliquot of 50  $\mu$ l of volatile extract was applied on a filter paper (Whatman grade No. 1), which was then rolled up and introduced into a clean glass tube. Control tubes contained filter paper treated with 50  $\mu$ l of solvent (dichloromethane) only. The tubes containing volatile extracts were placed on opposite sides of a six-arm olfactometer that was adapted for application of the extracts (Fig. 1; see also D'Alessandro and Turlings, 2005 and D'Alessandro et al., 2009). About 15 minutes after assembling the olfactometers, experiments began, testing mated naïve two- to six-day-old female *C. marginiventris* ( $n = 336$ ) and *C. sonorensis* ( $n = 252$ ) wasps. Only the experimental days where > 50% of the wasps responded were included in the analysis ( $n = 252$  for both wasp species). We collected odors during the bioassays, but the quantities were too low for analysis by means of gas chromatography (data not shown).

*Statistics* VOC emission data were analyzed using the non-parametric Kruskal-Wallis test, post-hoc Tukey test, as the assumptions of normality of the data were not met (Shapiro-Wilk test). The analyses were performed using SigmaPlot version 12 (Systat Software, San Jose, CA, USA). Wasp choice data were analyzed using a generalized linear model (GLM) fitted by maximum quasi-likelihood estimation according to Turlings et al. (2004). These analyses were performed in the software package R version 3.0.2 (R Core Team, 2013).

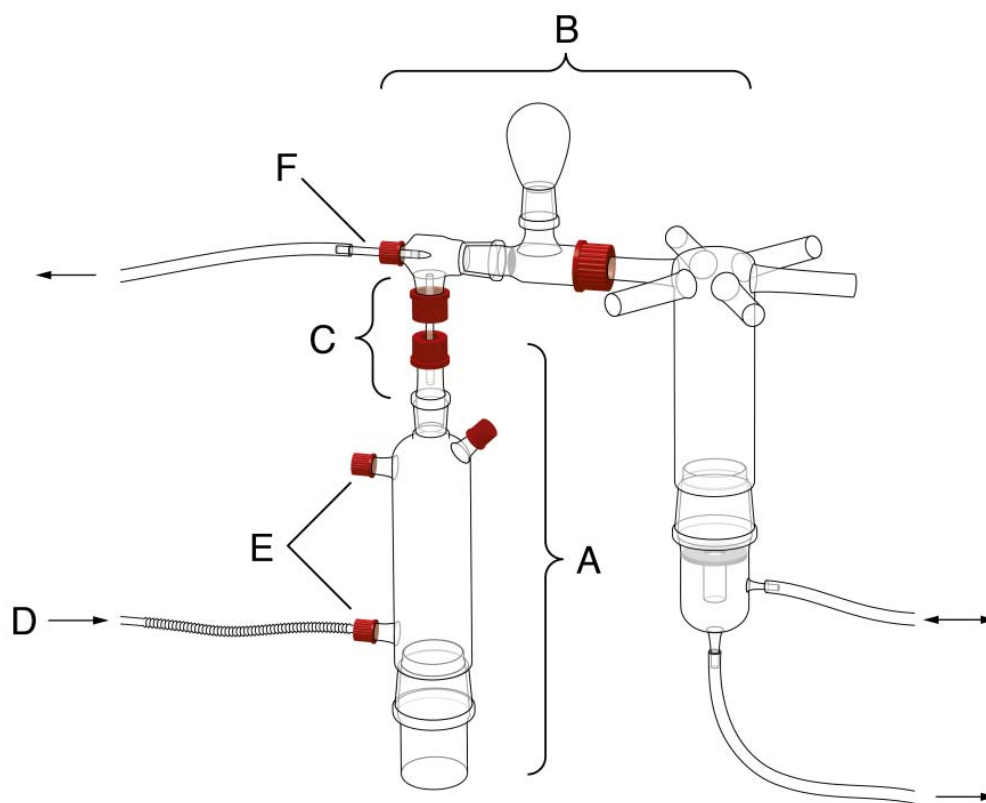
## Results

*Odor Emission in Maize and Teosinte* Maize, Balsas teosinte and Central Plateau teosinte look rather alike (Fig. 2a, b, c). Maize and Balsas teosinte emitted similar amounts of odors, significantly more than Central Plateau teosinte (*Kruskal-Wallis test*,  $P = 0.003$ ; Fig. 2d). On average, maize had a 50% higher shoot biomass than its wild relatives. If corrected for biomass, Balsas teosinte released significantly more odors per amount of fresh weight than Central Plateau teosinte and maize (*Kruskal-Wallis test*,  $P < 0.001$ ; data not shown). Differences among the three plants were also significant at the level of individual compounds in 10 out of 11 cases (for these 10 compounds: *Kruskal-Wallis test*,  $P < 0.05$ ; for (Z)-3-hexenyl acetate: *Kruskal-Wallis test*,  $P = 0.071$ ; Fig. 2e), but the chemical profiles, i.e. relative proportions of the individual compounds in the odor blend, were quite similar.

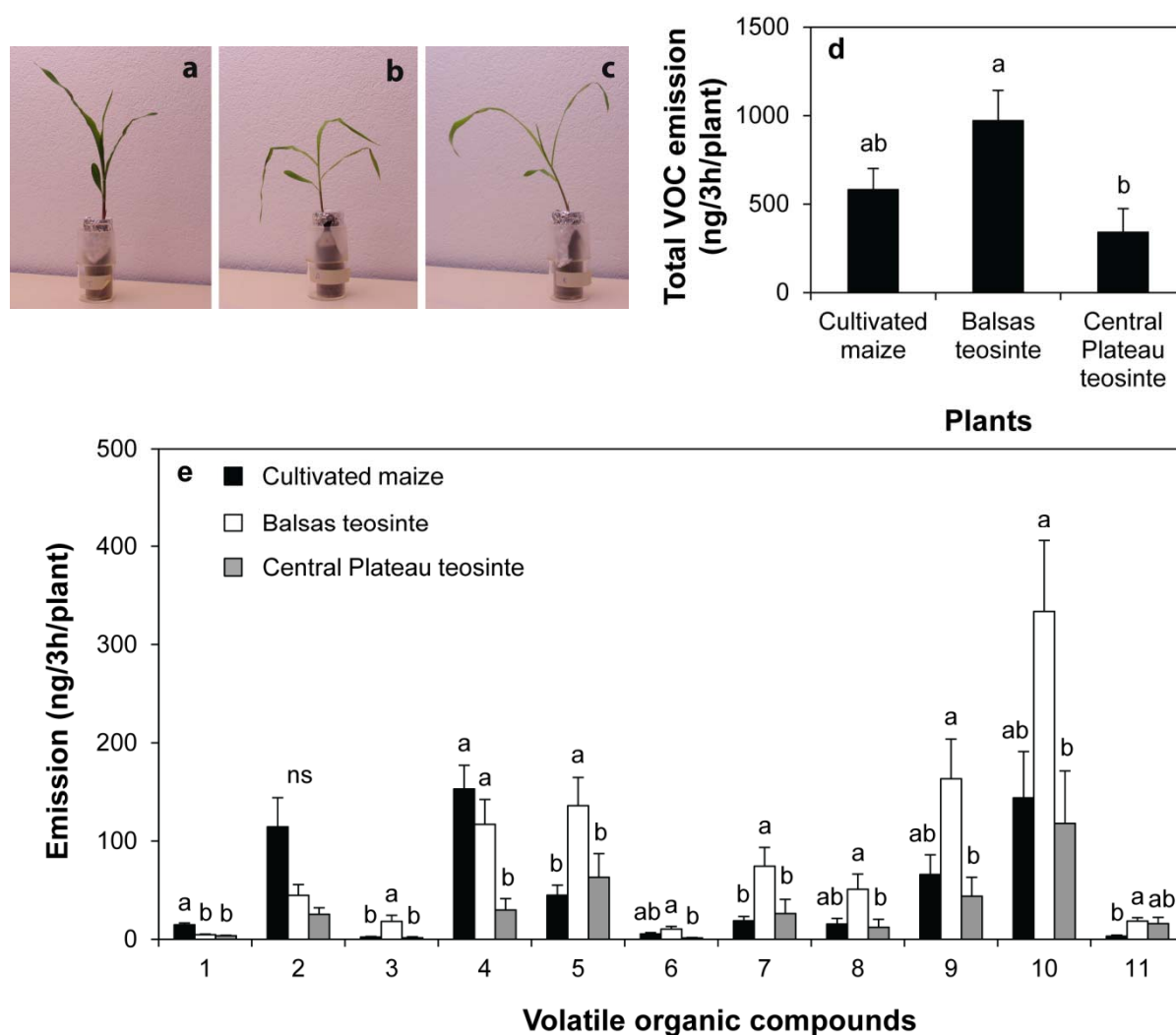
*Six-arm Olfactometer Bioassays with Plants* Both wasp species preferred the arms with the plants over the empty arms (*GLM*,  $P < 0.001$ ). *C. marginiventris* preferred Balsas and Central Plateau teosinte over maize (*GLM*,  $P = 0.009$  and  $P = 0.011$ , respectively) and did not distinguish between the two teosintes (*GLM*,  $P = 0.93$ ; Fig. 3a). *C. sonorensis* was not selectively attracted to any of the offered plants (*GLM*,  $P > 0.05$ ; Fig. 3b).

*Six-arm Olfactometer Bioassays with Volatile Extracts* The volatile extracts of maize and teosinte appeared very similar (Fig. 4). Both wasp species preferred the arms with the volatile extracts over the arms that contained solvent only (*GLM*,  $P < 0.001$ ). As with actual plants, *C. marginiventris* preferred the volatile extract of Balsas teosinte over the odor extract of maize (*GLM*,  $P = 0.01$ ; Fig. 5a), while *C. sonorensis* did not distinguish between the odor blends (*GLM*,  $P = 0.85$ ; Fig. 5b).

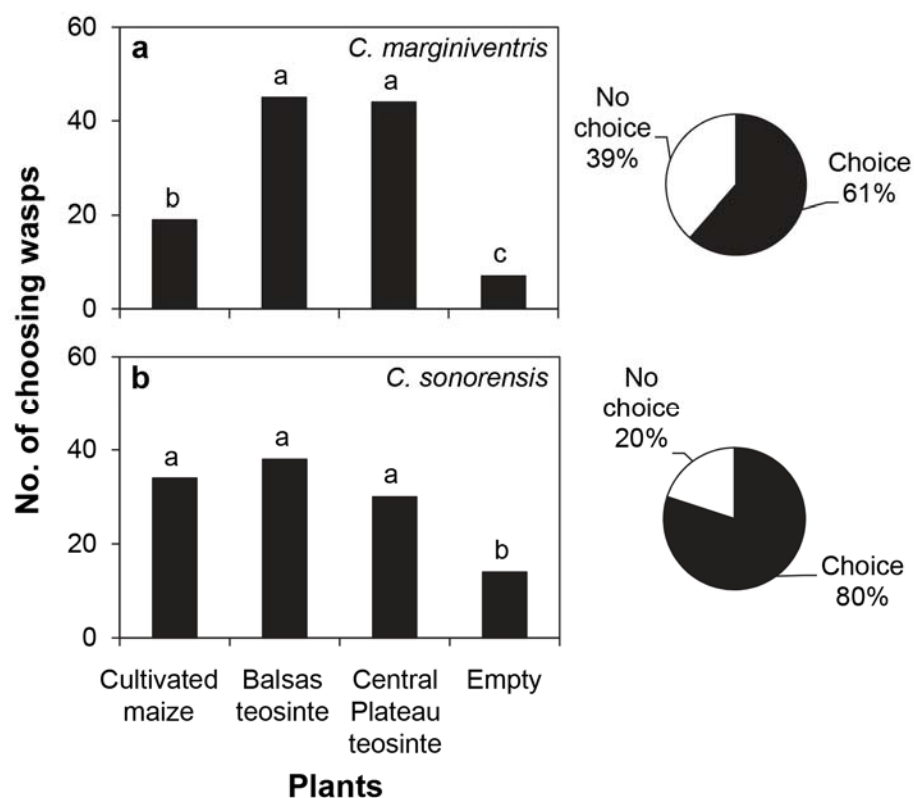




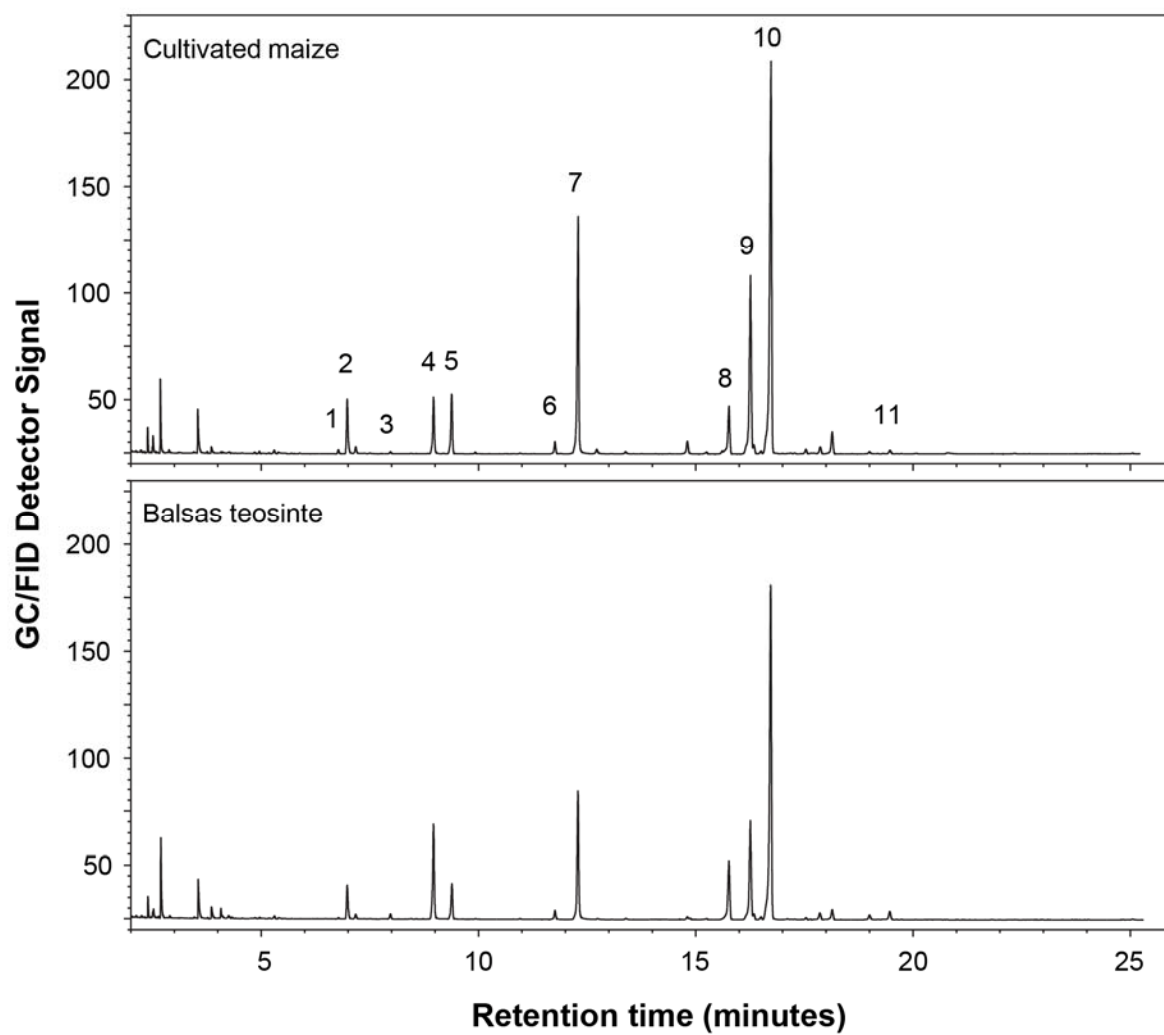
**Fig. 1** An overview of the six-arm olfactometer, suitable to test the attractiveness of extracts of collected plant volatiles for parasitoid wasps. A: Glass bottle. B: Wasp central choice chamber and insect trapping bulb. C: Odor source, glass tube with filter paper containing maize or teosinte volatile extract. D: Air inlet. E: Teflon-coated GL-screwcap fittings. F: Volatile collection filter. Adapted from Turlings et al. (2004) and D'Alessandro and Turlings (2005)



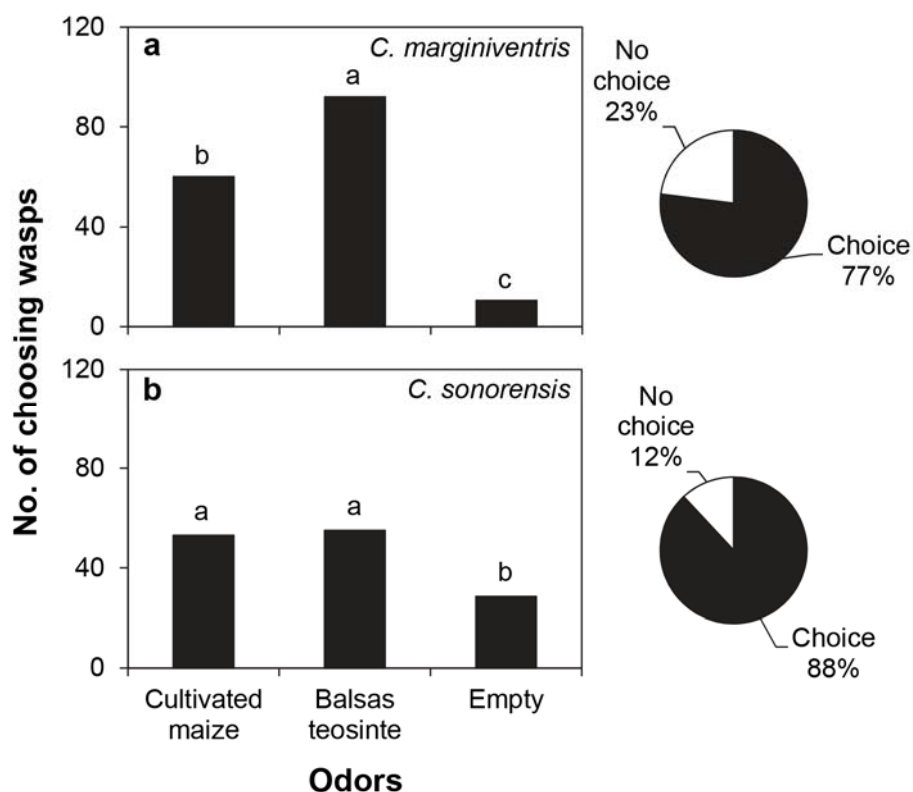
**Fig. 2** VOC emission by cultivated maize and wild teosintes and pictures of the tested plants. **a** Cultivated maize. **b** Balsas teosinte. **c** Central Plateau teosinte. **d** Cumulative amount of the eleven principal VOCs (+SE). Different letters indicate significant differences (*Kruskal-Wallis test*,  $P = 0.003$ ). **e** Individual emission of the eleven principal VOCs (+SE) by maize (black bars), Balsas teosinte (white bars) and Central Plateau teosinte (grey bars). The numbered compounds are: 1= $\beta$ -myrcene; 2=(Z)-3-hexenyl acetate; 3=(Z)- $\beta$ -ocimene; 4=linalool; 5=(3E)-4,8-dimethyl-1,3,7-nonatriene; 6=phenethyl acetate; 7=indole; 8=(E)- $\beta$ -caryophyllene; 9=(E)- $\alpha$ -bergamotene; 10=(E)- $\beta$ -farnesene; 11=4,6,12-trimethyl-1,3,7,11-tridecatetraene (TMTT). Different letters indicate significant differences (*Kruskal-Wallis test*,  $P < 0.05$ )



**Fig. 3** Responsiveness of parasitoid wasps to herbivore-induced odor emissions of cultivated maize and wild teosintes. **a** Choices made by *C. marginiventris* wasps. **b** Choices made by *C. sonorensis* wasps. Empty = control, empty vessels (average value of three vessels). Pie charts indicate the proportion of wasps choosing an arm. Different letters indicate significant differences (GLM,  $P < 0.05$ ). Composition of the plant odors is displayed in Fig. 2



**Fig. 4** Chromatograms of extracts of collected volatiles from herbivore-damaged maize and teosinte plants. For an explanation of the numbered compounds, see Fig. 2



**Fig. 5** Responsiveness of parasitoid wasps to extracts of volatiles of cultivated maize and its ancestor teosinte. **a** Choices made by *C. marginiventris* wasps. **b** Choices made by *C. sonorensis* wasps. Empty = control, empty vessels (average value of four vessels). Pie charts indicate the proportion of wasps choosing an arm. Different letters indicate significant differences (*GLM*,  $P < 0.05$ ). Composition of the plant volatile extracts is displayed in Fig. 4

## Discussion

The cultivated maize variety we chose emits upon herbivory an odor blend qualitatively similar to that of its wild ancestor Balsas teosinte and closely related Central Plateau teosinte, but the total quantities of released volatiles were rather different (Fig. 2). These results are in accordance with earlier findings stating that different maizes and teosintes vary mainly quantitatively in their VOC emission profiles upon herbivory (Gouinguéné et al., 2001).

In order to prevent bias in VOC induction due to differential feeding of leaf-chewing herbivores on maize and teosinte plants (Sandrine P. Gouinguéné, unpublished data), we applied artificial damage to induce plant VOC emission. As this type of damage is not continuous as with feeding caterpillars, we observed differences in odor samples collected in the morning, right after the second wounding treatment, compared to those collected in the afternoon, a few hours later. In morning samples, a higher quantity of green leaf volatiles (GLVs) was observed, whereas more sesquiterpenes were emitted later during the day (Turlings et al., 1998b). This effect of timing could potentially have an influence on plant attractiveness to parasitoid wasps in the olfactometer bioassays. However, since most of the

tests were performed in the morning, no objective comparison could be made between the attractiveness of the odor blend emitted by the plants in the morning or the afternoon.

We hypothesized that the different VOC profiles of maize, Balsas teosinte and Central Plateau teosinte would result in differential attractiveness to parasitoid natural enemies of herbivores. Interestingly, the two solitary endoparasitoids that were tested responded differently to the proposed odors. While *C. sonorensis* did not distinguish between the odor blends (Fig. 3b), *C. marginiventris* preferred the odors of both teosintes over those of maize (Fig. 3a). It is likely that the two species orient towards different compounds in the volatile blend (Gouinguéné et al., 2005; Tamò et al., 2006a). As the parasitoids co-occur throughout North America and compete for lepidopteran hosts (Tamò et al., 2006b), this might be an adaptive strategy aiming to avoid sharing the same niche. The observed results provide evidence for the fact that cultivated maize and wild teosinte emit an odor blend that is biologically distinguishable for at least one species of parasitoid, where the blend of teosinte is the most attractive. Therefore, during domestication, maize might have lost signals important in indirect defense.

Similar results were obtained when using odor extracts with equal VOC concentrations: *C. marginiventris* preferred the odor extracts of Balsas teosinte over those of maize (Fig. 5a), while *C. sonorensis* did not distinguish between the two offered odor extracts (Fig. 5b). These results confirm that the ecologically relevant differences between the odors of modern maize and its ancestor teosinte are qualitative rather than quantitative (D'Alessandro et al., 2009).

Extracts of collected volatiles are frequently used to test the attractiveness of odor blends to various insects in wind tunnels (Du et al., 1998) and in diverse multiple choice bioassays such as static air olfactometers (Steiner et al., 2007), y-tube olfactometers (Du et al., 1998; Colazza et al., 2004) and four-arm olfactometers (D'Alessandro and Turlings, 2005; D'Alessandro et al., 2009). In the process of identifying individual attractive compounds, the use of volatile extracts has an advantage over the use of intact plants or plant parts. The need for availability of plant pairs that differ only in a single trait has been recognised already in 2000 by Dicke and Van Loon. Treatment with different insects (Turlings et al., 1998a), elicitors (Dicke et al., 1999; Sobhy et al., 2012) and inhibitors (Bruinsma et al., 2010a,b) as well as the use of mutant plants (Van Poecke and Dicke, 2003; Kessler et al., 2004; Christensen et al., 2013) are commonly used to manipulate plant VOC emission, but these approaches generally affect multiple traits. Both the use of plants and the use of volatile extracts provide the opportunity to manipulate the odor blend, either by subtracting (D'Alessandro and Turlings, 2005) or adding (De Boer and Dicke, 2004) specific compounds in different concentrations. However, only the use of volatile extracts makes it possible to fractionate the odor blend, so that independent fractions could be tested for their insect attractiveness (Colazza et al., 2004; Steiner et al., 2007; D'Alessandro et al., 2009).

Although it has proven to be very difficult to identify the bioactive compounds in a complex odor blend (D'Alessandro and Turlings, 2006), in several model systems, “key compounds” have been identified that play an important role in natural enemy attraction

(Du et al., 1998; Powell et al., 1998; De Boer and Dicke, 2004; De Boer et al., 2004). An example concerning maize is (*E*)- $\beta$ -caryophyllene, a sesquiterpene that is emitted by herbivore-attacked maize roots and that attracts insect-killing nematodes (Rasman et al., 2005). Indeed, *C. marginiventris* is not as much attracted to the major components of the maize odor blend (e.g. sesquiterpenes; Fritzsche Hoballah et al., 2002; D'Alessandro and Turlings, 2005; Degen et al., 2012), although it can perceive most of them (Gouinguéné et al., 2005; Ngumbi et al., 2009), but rather to key compounds that are released in very small amounts (D'Alessandro et al., 2009). Bioassay-guided fractionation, using the adapted six-arm olfactometer (Fig. 1), would be a useful tool in the putative identification of compounds that are attractive to *C. marginiventris*, a natural enemy of important lepidopteran pests (Jalali et al., 1987).

As effective as bioassay-guided fractionation of volatile extracts might be, in order to identify compounds attractive to parasitoids, appropriate starting material should be selected. Different maize and teosinte varieties vary enormously in their VOC-emission response towards herbivory (Gouinguéné et al., 2001; Degen et al., 2004; Erb et al., 2011a; Degen et al., 2012). At this point, we cannot generalize our results, as more different plant varieties should be tested in order to obtain a robust dataset on the attractiveness of parasitoid wasps to cultivated maize and wild teosinte. Nevertheless, our results provide a first indication that during domestication, maize may have lost some of its ability to attract parasitoids. Therefore, we suggest the use of teosinte, the ancestor of maize, to identify key parasitoid attractants.

In conclusion, we found evidence that there are ecologically relevant differences, mainly quantitative, between the odors of cultivated maize and its wild ancestor teosinte, possibly indicating the loss of aboveground indirect defense signals. We suggest the use of the ancestor of maize to identify key signals that are involved in parasitoid attractiveness. Such information will not only provide new insights into plant-mediated tritrophic interactions, but may help in producing new maize varieties that are optimally compatible with biological control.

**Acknowledgements** - We thank Matthias Held for advice on statistical analysis and Thomas Degen and Yury Andrea Alvear Mahmod Smith for technical assistance. We thank ARS-USDA (Agricultural Research Service - United States Department of Agriculture) for providing us with teosinte seeds. We are grateful to Yves Borcard and the students of the University of Neuchâtel for parasitoid rearing and to Syngenta (Stein, Switzerland) for the weekly shipments of *S. littoralis* eggs. Fig. 1 was created by Thomas Degen ([www.thomas-degen.ch](http://www.thomas-degen.ch)).





## Chapter 4



## **Behavior and performance of fall armyworm and its parasitoids on cultivated maize and its wild ancestor, teosinte**

Elvira S. de Lange<sup>1</sup>, Benjamin Gaudillat<sup>1,2</sup>, Kevin Farnier<sup>1,3</sup> and Ted C.J. Turlings<sup>1,\*</sup>

<sup>1</sup> *Laboratory of Fundamental and Applied Research in Chemical Ecology (FARCE), Institute of Biology, University of Neuchâtel, Rue Emile-Argand 11, 2000 Neuchâtel, Switzerland*

<sup>2</sup> *UMR 'Peuplements Végétaux et Bio-agresseurs en Milieu Tropical', CIRAD / Université de La Réunion, Pôle de Protection des Plantes, 7 chemin de l'IRAT, F-97410 Saint-Pierre, France*

<sup>3</sup> *Department of Zoology, La Trobe University, Melbourne, VIC 3086, Australia*

\*e-mail: [ted.turlings@unine.ch](mailto:ted.turlings@unine.ch)



## Abstract

Selective breeding of crop plants for increased yield and quality is expected to have negatively affected other traits, such as resistance to herbivores. Besides the production of toxic compounds, herbivory induces the emission of volatiles in plants, which are used by parasitoid wasps as a means to find their hosts. However, as parasitoid wasps develop inside their hosts, they are indirectly affected by the presence of toxins in their hosts' diet. In a six-arm olfactometer, we confirmed the results of a previous study, showing that the parasitoid wasp *Cotesia marginiventris* was more attracted to the herbivore-induced odors of teosinte, the wild ancestor of maize, than to the odors emitted by modern maize. The aim of this study was to investigate whether this preference is adaptive and if *C. marginiventris* develops better in host larvae feeding on the preferred plants. For this we studied fall armyworm (*Spodoptera frugiperda*), a major maize pest, and its parasitoids *C. marginiventris* and *Campoletis sonorensis*. The latter does not appear to distinguish between maize and teosinte odors. Healthy and parasitized fall armyworm larvae were forced to feed on either maize or teosinte and we measured several performance traits of the developing insects. Healthy fall armyworm larvae developed equally well when feeding on maize and teosinte. Also, *C. marginiventris* developed equally well when their host was feeding on wild or cultivated plants. In contrast, *C. sonorensis* developed best in hosts feeding on maize, indicating variable effects of plant chemistry on the development of organisms in higher trophic levels. These results suggest that parasitoid wasps are not necessarily attracted towards the plants that are best suited for the development of their offspring.

## Key words

*Campoletis sonorensis*, *Cotesia marginiventris*, herbivore-induced plant volatiles, herbivore resistance, *Spodoptera frugiperda*, tritrophic interactions, *Zea mays*

## Introduction

About 9.000 years ago, maize (*Zea mays* L. ssp. *mays*) was domesticated from its wild ancestor, teosinte (*Z. mays* ssp. *parviglumis* Iltis & Doebley), in southern Mexico (Matsuoka et al., 2002). Since then, it has become one of the main food sources worldwide (Oerke, 2006). Although artificial selection of crop plants has had enormous positive effects on yield and quality (Duvick and Donald, 2005), it also has had a cost for other potentially useful traits, including resistance to herbivores (Wink, 1988; Evans, 1996; Rosenthal and Dirzo, 1997; Massei and Hartley, 2000; Córdova-Campos et al., 2012; Dávila-Flores et al., 2013). Plants employ a wide variety of structural and chemical mechanisms to defend themselves against herbivory. Direct defenses include the production of toxins that can repel, intoxicate and even kill many herbivores (Schoonhoven et al., 2005). Levels of toxic compounds are frequently lower in cultivated plants than in their wild conspecifics. For example, levels of glucosinolates in cabbage (Josefsson, 1967; Gols et al., 2008), cyanogenic glycosides in lima bean (Kaplan, 1965), and alkaloids in potatoes (Johns and Alonso, 1990) have been reduced, most likely when selecting crops for palatability and nutritional value.

Unintentionally, domestication has also altered the complex interactions between plants, herbivores and organisms of higher trophic levels (Macfadyen and Bohan, 2010). Besides direct defenses, plants respond to herbivore attack with the emission of volatile organic compounds (VOCs) that can guide natural enemies of herbivores to suitable prey or hosts (Turlings and Wäckers, 2004). Emission of VOCs is therefore considered an indirect defense mechanism. Positive (Benrey et al., 1998) as well as negative (Chen and Welter, 2005; Köllner et al., 2008; Gols et al., 2011) effects of domestication on plant indirect defenses have been reported.

Parasitoid wasps constitute an important group of natural enemies of mainly insect herbivores. Once adult females have located their host, they oviposit inside or near that host and the larvae that hatch from the eggs feed from this single host. As a consequence, they are indirectly influenced by the quality of their host's food source (Bottrell et al., 1998; Turlings and Benrey, 1998; Gols and Harvey, 2009). Therefore, not only is the performance of most herbivores negatively correlated with the presence of toxins in their host plant (Bennett and Wallsgrave, 1994; Awmack and Leather, 2002) but, also, the performance of parasitoids tends to be negatively affected by the presence of noxious substances in their hosts' diet (Campbell and Duffey, 1979; Sznajder and Harvey, 2003; Harvey et al., 2005, 2007). Both reduced host quality and direct negative effects of the toxins on their development are mentioned as causes (Harvey, 2005). The opposite may be true if plant defense compounds suppress the herbivore immune system, and thereby their host's ability to encapsulate parasitoid eggs (Bukovinszky et al., 2009). As yet, little is known about the correlation between direct and indirect defenses. In the case of lima bean (*Phaseolus lunatus* L.) there appears to be a trade-off between direct and indirect defensive mechanisms (Ballhorn et al., 2008), whereas for maize there is a positive correlation between direct resistance to caterpillars and the amount of volatiles that the plants emit in response to the caterpillar attack (Erb et al., 2011a).

In the current study, we compared the behavior and performance of an herbivore and two of its parasitoids on cultivated maize and its wild ancestor, teosinte. Cultivated maize has been intensively studied in the context of tritrophic interactions (Turlings et al., 1990, 1995), but, as yet, its ancestor has received only little attention in this respect (Gouinguéné et al., 2001). Maize and teosinte co-occur in Mexico, their country of origin (Sanchez Gonzalez and Corral, 1995; Matsuoka et al., 2002). The plants differ in a great number of morphological and biochemical traits (Doebley et al., 2006). Recently, it has been discovered that selected maize varieties have lost the ability to release an attractant to soil-dwelling natural enemies in response to root-feeding by a beetle larva (Rasman et al., 2005; Köllner et al., 2008). It is known that maize and teosinte display great variability in the release of herbivore-induced VOCs from the leaves (Gouinguéné et al., 2001), but their attractiveness to natural enemies has only recently been investigated (Chapter 3).

Fall armyworm, *Spodoptera frugiperda* Smith (Lepidoptera: Noctuidae), is a major pest of maize in the Americas (Luginbill, 1928; Kranz et al., 1977). Although it can consume various plant species, the larvae have an innate preference for grasses (Walton and Luginbill, 1916;

Luginbill, 1928). Young larvae chew characteristic “windowpane-like” holes in maize leaves, while older larvae can bite entire holes in the leaves and are even reported to consume parts of the cobs. In nature, fall armyworm larvae are frequently reported to occur on teosinte as well (De la Paz-Gutiérrez, 2008). *Cotesia marginiventris* Cresson (Hymenoptera: Braconidae) and *Campoletis sonorensis* Cameron (Hymenoptera: Ichneumonidae) are generalist solitary koinobiont endoparasitoids that attack a wide variety of early instar lepidopteran larvae (Cave, 1995; Bahena-Juárez, 2008). They are important natural enemies of *S. frugiperda* in nature (e.g. Hoballah et al., 2004; Molina-Ochoa et al., 2004; Jourdie et al., 2008). *C. marginiventris* primarily feeds on the hemolymph and fat body of its host (Harvey et al., 2005), while *C. sonorensis* feeds on all tissues (Wilson and Ridgway, 1975). Final instars of both species chew their way through the larval skin to spin a cocoon outside the host, which eventually dies.

In a recent study, we found that the parasitoid wasp *C. marginiventris* was significantly more attracted to the herbivore-induced odors of teosinte than to the odors of maize, indicating that the modern maize variety has reduced capacity to attract this parasitoid. *C. sonorensis* did not distinguish between the odors of maize and teosinte (Chapter 3). Here, we aimed to determine whether this differential attractiveness was linked to a difference in plant suitability for the development of the parasitoid wasps and their host fall armyworm. As previous studies were conducted with another herbivore, *Spodoptera littoralis* Boisduval (Chapter 3), we first established that the two parasitoid wasps were also differentially attracted to fall armyworm-induced odors of maize and teosinte. Then, we measured various performance characteristics of healthy and parasitized fall armyworm larvae feeding on maize and teosinte. We discuss the results in the context of crop-insect interactions.

## Methods and Materials

**Plants** Seeds of maize (*Z. mays* ssp. *mays*, variety Delprim, Delley semences et plantes SA, Switzerland) and teosinte (*Z. mays* ssp. *parviglumis*, ARS-USDA, USA) were sown in plastic pots (4 cm diameter, 10 cm high) with fertilized commercial soil (Ricoter Aussaaterde, Aarberg, Switzerland). All plants were kept in a climate chamber (maize:  $25 \pm 2^\circ\text{C}$ ; teosinte:  $30 \pm 2^\circ\text{C}$ ; 60% relative humidity; 16 h light / 8 h dark;  $50.000 \text{ lm/m}^2$ ). At the start of the experiments the plants were 10-20 days old.

**Insects** Larvae of *S. frugiperda* were obtained from an in house rearing on artificial diet, similar to the rearing described by Turlings et al. (2004). *C. marginiventris* and *C. sonorensis* wasps were reared as described by Hoballah et al. (2004). In order to obtain parasitized *S. frugiperda* larvae for experimental purposes, five second-instar larvae were put in a small plastic box with artificial diet and one gravid female wasp for 24 h. Unparasitized larvae received a similar treatment, but in the absence of a wasp.

**Odor Extract Preparation** Maize and teosinte plants (n = 15) were placed in glass vessels, in a VOC collection setup under experimental conditions as described by Turlings et al. (2004)

and Ton et al. (2007). Each plant was infested with five second-instar *S. frugiperda* larvae the night prior to VOC collections. At the start of odor collections, a flow of 1.1 l/min purified and humidified air was pushed into the vessels. Air was pulled out of the vessel at a rate of 0.7 l/min through a trapping filter containing 25 mg of 80-100 mesh SuperQ adsorbent (Alltech Associates, Inc., Deerfield, Illinois, USA). On each day, three collections of 3 h were performed with the same plants and this procedure was repeated with different plants on several days. Before use, the filters were rinsed with 3 mL dichloromethane (Suprasolv, GC-grade, Merck, Dietikon, Switzerland). After each collection, the filters were removed and eluted with 150 µL of dichloromethane. The extracts of all individual collections were combined for maize and for teosinte to be used in the six-arm olfactometer bioassays. The samples were stored at -80°C before analysis.

In order to estimate total VOC quantity, two 150 µL samples of the maize extract and two 150 µL samples of the teosinte extract were taken for analysis using an Agilent 6850 gas chromatograph with a flame ionization detector. Two internal standards (n-octane and nonyl acetate, each 200 ng in 10 µl dichloromethane) were added to each sample before analysis. A 3-µl aliquot of each sample was injected in pulsed splitless mode onto an apolar capillary column (HP-1ms, 30 m, 0.25 mm ID, 0.25 µm film thickness; Agilent J&W Scientific, USA). Helium at constant pressure (18.71 psi) was used as carrier gas. After injection, the column temperature was maintained at 40°C for 3 min, and then increased to 100°C at 8°C/min and subsequently to 200°C at 5°C/min followed by a post-run of 3 min at 250°C. The 30 principal detected VOCs were quantified based on a comparison of their peak areas with those of the internal standards and were identified by comparison of retention times with those from previous analyses (D'Alessandro and Turlings, 2005). Based on the absolute total quantities that were measured, the teosinte extract was diluted with dichloromethane to have the same total VOC concentration as the maize extract (Fig. 1).

*Six-arm Olfactometer Bioassays* Volatile extracts were used as odor sources and not herbivore-attacked plants, in order to correct for differential feeding of larvae on maize and teosinte plants (Sandrine P. Gouinguéné, unpublished data), which could influence VOC emission (Gouinguéné et al., 2003; Turlings et al., 2004; Heil, 2009). An aliquot of 50 µl of odor extract was applied on a filter paper (Whatman grade No. 1), which was then rolled up and introduced into a clean glass tube. Control tubes contained filter paper treated with 50 µl of solvent (dichloromethane) only. The tubes containing odor extracts were placed on opposite sides of an adapted six-arm olfactometer that was compatible with the mode of application of the extracts (Chapter 3; Turlings et al., 2004; D'Alessandro and Turlings, 2005; D'Alessandro et al., 2009). About 15 min. after assembling the olfactometers, the assays began. Mated two- to seven-day-old female *C. marginiventris* (n = 108) and *C. sonorensis* (n = 96) wasps were released in groups of six and given 30 min. to make a choice for an arm, thereby allowing them to reveal the odor source of their preference. Wasps that did not enter an arm were considered having made “no choice”. The females were naïve, which means that as adults they had never been in contact with a plant or a host before. The



experiment was repeated on several consecutive days and to avoid any bias, positions were changed in such a way that each treatment was once on each position. All experiments were run between 11:00 AM and 4:00 PM. Because the response of *C. marginiventris* is known to be affected by the odor of *C. sonorensis* (Tamò et al., 2006b), the two species were tested in separate olfactometers. We collected odors during the bioassays, but the quantities were too low for analysis by means of gas chromatography (data not shown).

*Herbivore and Parasitoid Performance Bioassays on Whole Plants* Experiments were carried out under light benches in a climatized laboratory ( $25 \pm 2^\circ\text{C}$ ,  $40 \pm 10\%$  r.h., 16:8 h l:d, and  $148 \mu\text{mol m}^{-2}\text{s}^{-1}$ ). Individual maize and teosinte plants were infested with a single second-instar *S. frugiperda* larva, which was unparasitized, parasitized by *C. marginiventris* or parasitized by *C. sonorensis* ( $n = 20$ ). All larvae had a similar starting weight ( $10 \pm 1.8$  mg). To prevent the larvae from escaping, poly-ethylene (PET) bottles with the bottom cut out (30 cm height, cone-shaped, maximum diameter 8 cm) were placed upside down over the plants and were attached to the pots with Parafilm (Erb et al., 2011a). The tubes were left open at the top to guarantee air circulation. Three times a week, the larvae were recovered from the plants, weighted, and put back on the same plants. Plants that were close to being completely eaten were replaced. Weighing was repeated for each larva until they died or developed into a pupa in the case of unparasitized larvae, or until the emergence of a wasp larva in the case of parasitized larvae. Moth pupae were weighed as a measure of herbivore fitness. Wasp cocoons were measured, adult wasps were sexed and the length of the left hind tibia was taken as a measure of parasitoid fitness (Honek, 1993; Godfray, 1994; Turlings and Benrey, 1998; Awmack and Leather, 2002). Larvae that underwent parasitism treatment, but did not yield a parasitoid were excluded from analysis (1-4 larvae per treatment).

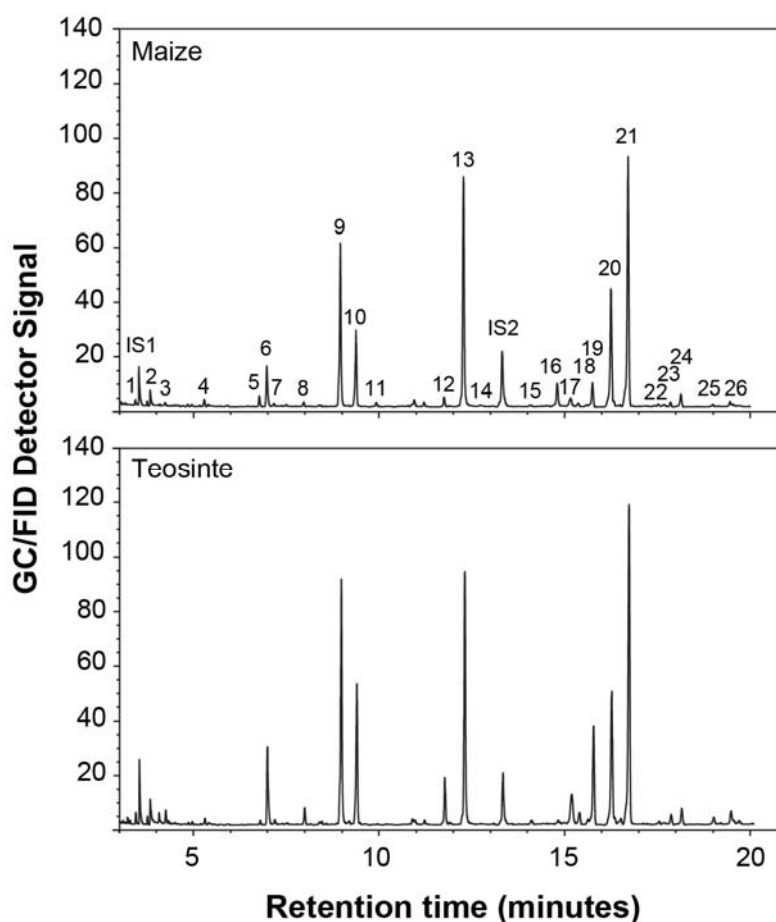
*Herbivore Performance Bioassays on Leaf Disks* To account for differences in plant size, we also conducted the herbivore performance assays with only leaf disks. Leaf disks (surface:  $100 \text{ mm}^2$ ) of maize ( $n = 30$ ) and teosinte ( $n = 26$ ) plants were put in a small plastic box on humid filter paper. To each box, one second-instar *S. frugiperda* larva was added. The boxes were kept in the dark for 12 h, after which the leaf disks were scanned and damage was calculated using NIH ImageJ software (Rasband, 1997-2012). Larvae were weighed before and after the experiment to determine their absolute weight gain.

*Statistics* The results of the olfactometer experiments were analysed using a generalized linear model (GLM) fitted by maximum quasi-likelihood estimation according to Turlings et al. (2004). To compare differences between larval weight gain when feeding on whole maize and teosinte plants, we used Student's t-test when the data were normally distributed and Mann-Whitney test when the assumptions of normality of the data were not met (Shapiro-Wilk test). Student's t-test was also used to compare *S. frugiperda* pupal weights, and a two-way ANOVA was used to compare wasp cocoon and tibia lengths, taking sex into account. As

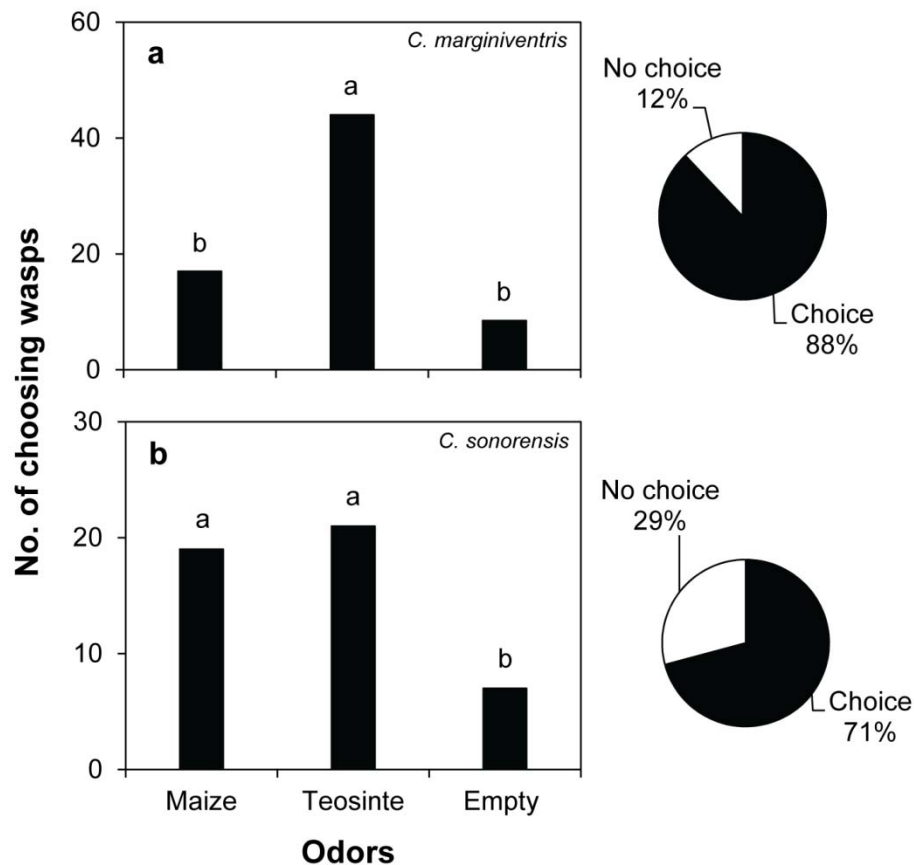
the assumptions of normality of the data were not met (Shapiro-Wilk test), Mann-Whitney test was used to compare differences in larval weight gain and leaf area eaten on leaf disks of maize and teosinte plants. To compare correlations between these parameters, statistical testing was performed after application of Fisher's *r*-to-*z* transformation. ANCOVA was used to test whether insect growth rates differed between the treatments, i.e. maize and teosinte. Statistical analyses were performed in R version 3.0.2 (R Core Team, 2013) and in SigmaPlot version 12.0 (Systat Software, San Jose, CA, USA).

## **Results**

*Six-arm Olfactometer Bioassays* Maize and teosinte extracts were strikingly similar (Fig. 1). Both wasp species preferred the two arms with the volatile collection extracts as compared to the four arms that contained solvent only (*GLM*,  $P < 0.001$ ), even though there was only a trend for *C. marginiventris* to prefer maize extracts over arms containing solvent only (*GLM*,  $P = 0.06$ ). *C. marginiventris* females were significantly more attracted to the odor extract of herbivore-induced teosinte than to the odor extract of herbivore-induced maize (*GLM*,  $P = 0.008$ ; Fig. 2a). *C. sonorensis* females did not display a preference between the odors of either maize or teosinte (*GLM*,  $P = 0.736$ ; Fig. 2b).

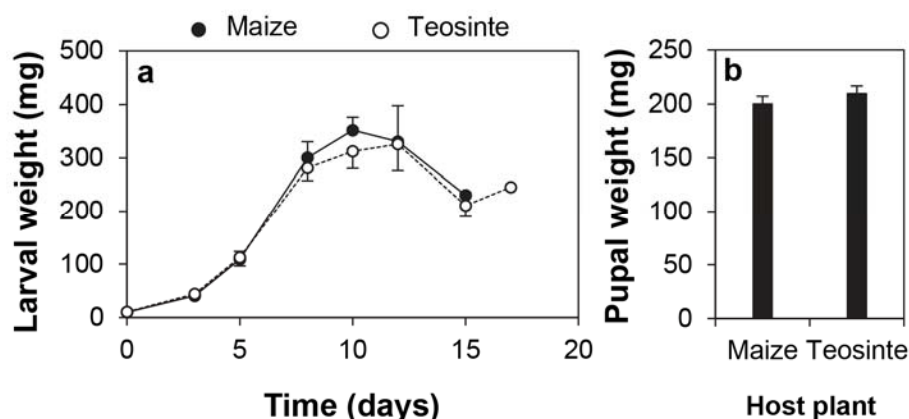


**Fig. 1** Chromatograms of odor extracts of herbivore-damaged maize and teosinte plants. The labeled compounds are: 1) (*Z*)-3-hexenal, 2) (*E*)-2-hexenal, 3) (*Z*)-3-hexenol, 4) (*Z*)-2-penten-1-ol acetate<sup>N</sup>, 5)  $\beta$ -myrcene, 6) (*Z*)-3-hexenyl acetate, 7) (*E*)-2-hexenyl acetate, 8) (*Z*)- $\beta$ -ocimene, 9) linalool, 10) (3*E*)-4,8-dimethyl-1,3,7-nonatriene, 11) benzyl acetate, 12) phenethyl acetate, 13) indole, 14) unknown, 15) methyl anthranilate, 16) geranyl acetate, 17) unknown, 18) unknown, 19) (*E*)- $\beta$ -caryophyllene, 20) (*E*)- $\alpha$ -bergamotene, 21) (*E*)- $\beta$ -farnesene, 22) unknown sesquiterpenoid, 23) unknown sesquiterpenoid, 24)  $\beta$ -sesquiphellandrene<sup>N</sup>, 25) (*E*)-nerolidol, 26) (3*E*, 7*E*)-4,8,12-trimethyl-1,3,7,11-tridecatetraene. IS1 and IS2, internal standards (*n*-octane and nonyl-acetate). <sup>N</sup>tentative identification



**Fig. 2** Parasitoid wasp responsiveness to odor extracts of cultivated maize and its ancestor teosinte. **a** Choices made by *C. marginiventris* wasps. **b** Choices made by *C. sonorensis* wasps. Empty = solvent only (average value of four vessels). Pie charts indicate the proportion of wasps choosing an arm. Different letters indicate significant differences ( $P < 0.05$ ). Composition of the plant odor extracts is displayed in Fig. 1

*Herbivore Performance Bioassays on Whole Plants* There was no significant difference in weight gain for *S. frugiperda* larvae when feeding on maize or teosinte (*Student's t-test* or *Mann-Whitney test*,  $P > 0.05$ ; Fig. 3a; Table 1). Fall armyworm goes through six instars and stops feeding in prepupal stage, when its weight decreases. Then, a pupa is formed (Luginbill, 1928). There was no significant difference in average pupal weight of larvae fed on either food source (*Student's t-test*,  $P = 0.359$ ; Fig. 3b).



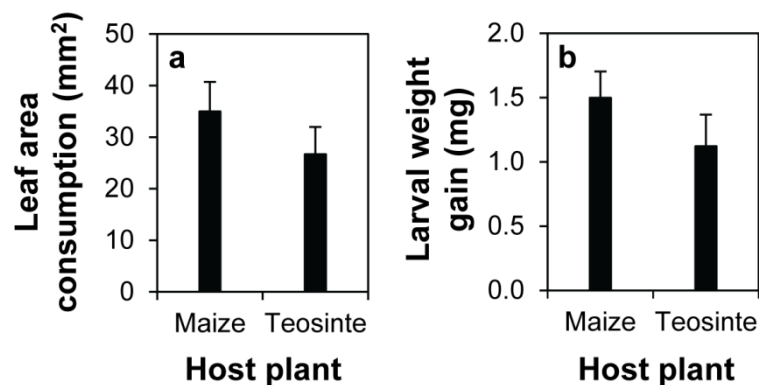
**Fig. 3** Performance of *S. frugiperda* larvae on maize and teosinte plants. **a** Comparison between average weights (+ or -SE) of healthy larvae feeding on maize or teosinte during their development; **b** Comparison between pupal average weights (+SE) for larvae fed with maize (n = 14) or teosinte (n = 13). There were no significant differences ( $P > 0.05$ )

**Table 1** Larval numbers and statistical values corresponding to the performance of *S. frugiperda* larvae on maize and teosinte plants

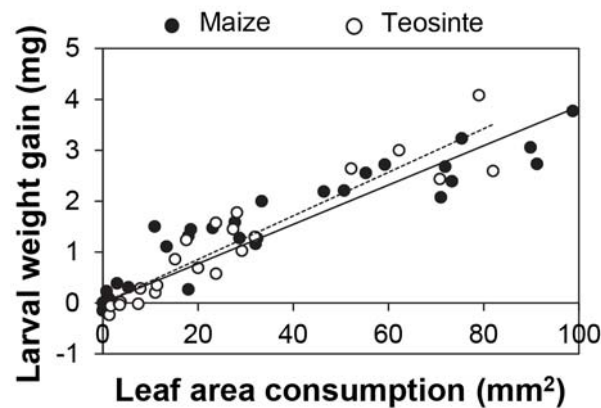
Day	Maize				Teosinte				Statistics	
	n	Nd	Pupa	Dead	n	Nd	Pupa	Dead	Test	P-value
0	19	0	0	0	17	0	0	0	Student's t-test	0.457
3	19	0	0	0	16	1	0	0	Student's t-test	0.580
5	17	2	0	0	17	0	0	0	Mann-Whitney test	0.863
8	16	2	0	1	15	0	0	2	Student's t-test	0.628
10	17	0	0	2	14	1	0	2	Student's t-test	0.326
12	4	11	2	2	4	5	6	2	Student's t-test	0.958
15	2	1	14	2	2	1	12	2	-	-
17	0	0	14	5	1	0	12	5	-	-

n = number of *S. frugiperda* larvae that was weighted. Nd = number of larvae that was not weighted because the larvae could not be retrieved. Pupa = number of larvae that was not weighted because the larvae had entered the pupal stage. Dead = number of larvae that was not weighted because the larvae were dead. No statistical tests were performed when the number of larvae that was weighted for maize or teosinte was below four. There were no significant differences ( $P > 0.05$ )

*Herbivore Performance Bioassays on Leaf Disks* Fall armyworm larvae performed equally well on maize and teosinte leaf disks. There was neither a significant difference in leaf area consumption (*Mann-Whitney test*,  $P = 0.536$ ; Fig. 4a) nor in larval weight gain (*Mann-Whitney test*,  $P = 0.536$ ; Fig. 4b). For both maize and teosinte, there was a linear correlation between food consumption and weight increase of the fall armyworm larvae. When larvae consumed a similar amount of food, their relative weight gain was not statistically different for those that had fed on maize or teosinte (*ANCOVA*,  $P = 0.350$ ; Fig. 5). These results suggest that there was no difference in host plant chemistry influencing growth and development of *S. frugiperda* larvae.

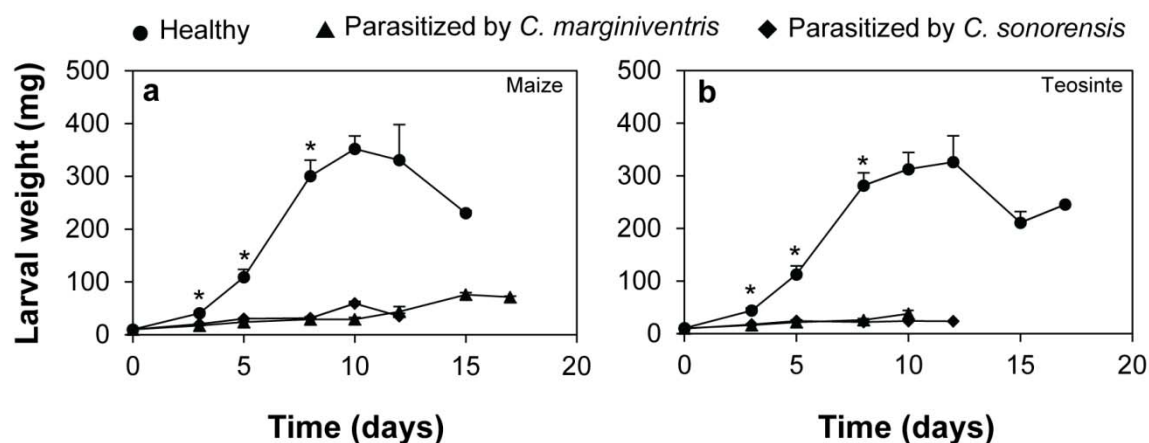


**Fig. 4** *S. frugiperda* performance on maize and teosinte leaf disks after 12 h of feeding. **a** Leaf area consumption. **b** Larval weight gain. There were no significant differences ( $P > 0.05$ )



**Fig. 5** Relationship between leaf area consumption and weight gain of *S. frugiperda* larvae after 12 h of feeding on maize and teosinte leaf disks. The solid line represents maize ( $R^2 = 0.87$ ) and the dashed line represents teosinte ( $R^2 = 0.88$ )

**Parasitoid Performance Bioassays** Parasitized larvae grew significantly less than healthy larvae (Fig. 6; Table 2, 3). There was no effect of host plant on growth of *S. frugiperda* larvae that had been parasitized by *C. marginiventris* (*Student's t-test* or *Mann-Whitney test*,  $P > 0.05$ ; Fig. 7a; Table 4). Two wasp larvae in hosts feeding on maize took a relatively long time to emerge, indicating that wasp developmental time may be prolonged on maize (Fig. 7a; Table 5). Taking the sex of the wasps into account, the host plant did not influence parasitoid cocoon length (*two-way ANOVA*,  $P_{\text{plant}} = 0.667$ ,  $P_{\text{sex}} = 0.886$ ,  $P_{\text{interaction}} = 0.323$ ; Fig. 7), nor hind tibia length (*two-way ANOVA*,  $P_{\text{plant}} = 0.266$ ,  $P_{\text{sex}} = 0.085$ ,  $P_{\text{interaction}} = 0.924$ ; Fig. 7c). In contrast, there was an effect of host plant on growth of *S. frugiperda* larvae that were parasitized by *C. sonorensis*. For two measuring points during the developmental period of the parasitoid larvae we found that *S. frugiperda* weight was higher when feeding on maize than on teosinte (*Student's t-test* or *Mann-Whitney test*,  $P < 0.05$ ; Fig. 7d; Table 3). Also, *C. sonorensis* produced significantly larger cocoons when their host had fed on maize (*two-way ANOVA*,  $P_{\text{plant}} = 0.003$ ,  $P_{\text{sex}} = 0.413$ ,  $P_{\text{interaction}} = 0.171$ ; Fig. 7e). Nonetheless, no differences were observed in adult hind tibia length as a result of host diet (*two-way ANOVA*,  $P_{\text{plant}} = 0.752$ ,  $P_{\text{sex}} = 0.636$ ,  $P_{\text{interaction}} = 0.752$ ; Fig. 7f).



**Fig. 6** Performance of healthy and parasitized *S. frugiperda* larvae on maize (a) and teosinte (b) plants. Shown is the average weight (+SE) of the larvae during their development. An asterisk indicates a significant difference ( $P < 0.05$ )

**Table 2** Larval numbers and statistical values corresponding to the performance of *S. frugiperda* larvae on maize plants

	Healthy	Parasitized by <i>C. marginiventris</i>	Parasitized by <i>C. sonorensis</i>	Statistics	
Day	N	N	n	Test	P-value
0	19	19	18	Kruskal-Wallis test	0.855
3	19	18	18	Kruskal-Wallis test	<b>&lt;0.001</b>
5	17	15	15	Kruskal-Wallis test	<b>&lt;0.001</b>
8	16	17	14	Kruskal-Wallis test	<b>&lt;0.001</b>
10	17	7	2	-	-
12	4	4	5	-	-
15	2	2	0	-	-
17	0	2	0	-	-

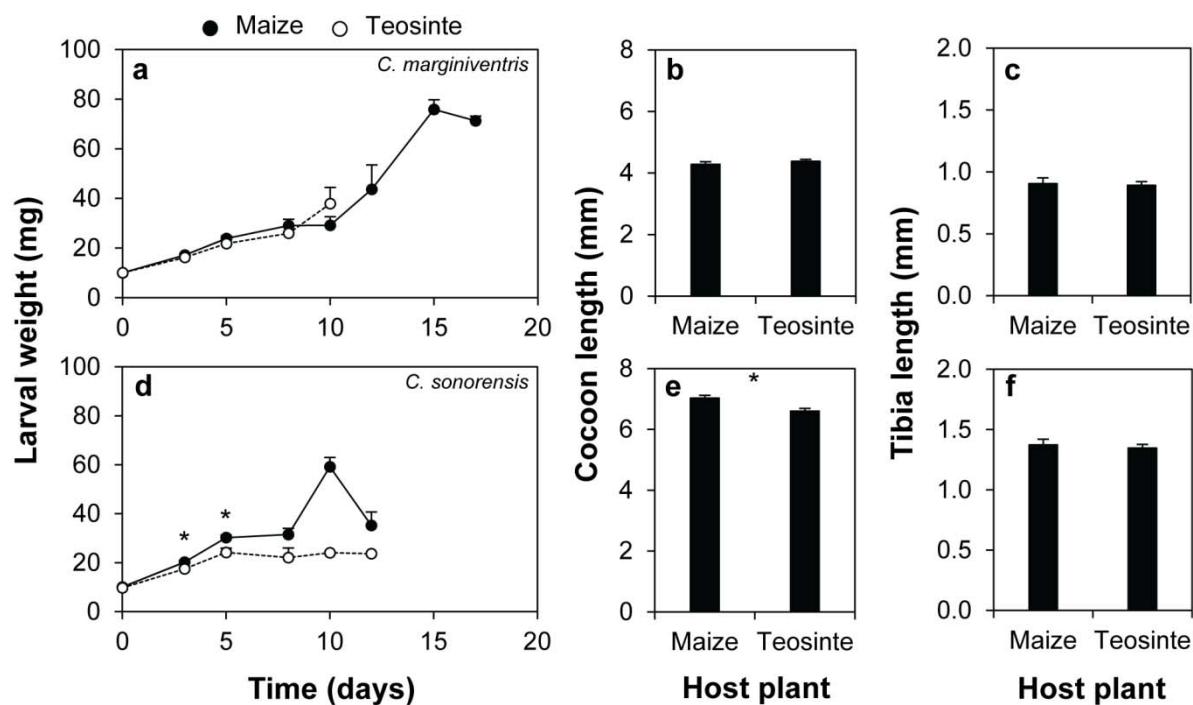
n = number of *S. frugiperda* larvae that was weighted. No statistical tests were performed when the number of larvae in one of the treatments was below four. P-values in bold represent a significant difference ( $P < 0.05$ )

**Table 3** Larval numbers and statistical values corresponding to the performance of *S. frugiperda* larvae on teosinte plants

	Healthy	Parasitized by <i>C. marginiventris</i>	Parasitized by <i>C. sonorensis</i>	Statistics	
Day	N	N	n	Test	P-value
0	17	16	16	Kruskal-Wallis test	0.611
3	16	15	16	Kruskal-Wallis test	<b>&lt;0.001</b>
5	17	11	13	Kruskal-Wallis test	<b>&lt;0.001</b>
8	15	12	4	Kruskal-Wallis test	<b>&lt;0.001</b>
10	14	2	2	-	-
12	4	0	1	-	-
15	2	0	0	-	-
17	1	0	0	-	-

n = number of *S. frugiperda* larvae that was weighted. No statistical tests were performed when the number of larvae in one of the treatments was below four. P-values in bold represent a significant difference ( $P < 0.05$ )





**Fig. 7** Performance of parasitized *S. frugiperda* larvae on maize and teosinte plants. Comparison between average weights (+SE) of larvae feeding on maize or teosinte when parasitized by *C. marginiventris* (a) or *C. sonorensis* (d). Average cocoon length (+SE) of *C. marginiventris* (maize: n = 11; teosinte: n = 10) (b) and *C. sonorensis* (maize: n = 10; teosinte: n = 10) (e) and average length of the left hind tibia (+SE) of adult *C. marginiventris* (maize: n = 9; teosinte: n = 10) (c) or *C. sonorensis* (maize: n = 9; teosinte: n = 9) (f) when the host had been consuming maize or teosinte. An asterisk indicates a significant difference ( $P < 0.05$ )

**Table 4** Larval numbers and statistical values corresponding to the performance of *S. frugiperda* larvae parasitized by *C. marginiventris* wasps on maize and teosinte plants

Day	Maize				Teosinte				Statistics	
	N	Nd	Cocoon	Dead	n	Nd	Cocoon	Dead	Test	P-value
0	19	0	0	0	16	0	0	0	Mann-Whitney test	0.987
3	18	1	0	0	15	1	0	0	Student's t-test	0.407
5	15	2	0	0	11	1	0	0	Mann-Whitney test	0.533
8	17	0	1	1	12	1	0	3	Student's t-test	0.428
10	7	0	6	2	2	1	7	3	-	-
12	4	0	11	4	0	0	12	4	-	-
15	2	0	12	5	0	0	12	4	-	-
17	2	0	12	5	0	0	12	4	-	-

n = number of *S. frugiperda* larvae that was weighted. Nd = number of larvae that was not weighted because the larvae could not be retrieved. Cocoon = number of larvae that died because a wasp cocoon had developed. Dead = number of larvae that died for unknown reasons. No statistical tests were performed when the number of larvae that was weighted for maize or teosinte was below four. There were no significant differences ( $P > 0.05$ )

**Table 5** Larval numbers and statistical values corresponding to the performance of *S. frugiperda* larvae parasitized by *C. sonorensis* wasps on maize and teosinte plants

Day	Maize				Teosinte				Statistics	
	N	Nd	Pupa	Dead	n	Nd	Pupa	Dead	Test	P-value
0	18	0	0	0	16	0	0	0	Mann-Whitney test	0.546
3	18	0	0	0	16	0	0	0	Student's t-test	<b>0.024</b>
5	15	0	0	2	13	0	0	0	Student's t-test	<b>0.005</b>
8	14	0	2	2	4	1	8	3	Student's t-test	0.088
10	2	6	6	4	2	2	9	3	-	-
12	5	0	8	5	1	0	10	5	-	-
15	0	0	12	6	0	0	10	6	-	-
17	0	0	12	6	0	0	10	6	-	-

n = number of *S. frugiperda* larvae that was weighted. Nd = number of larvae that was not weighted because the larvae could not be retrieved. Cocoon = number of larvae that died because a wasp cocoon had developed. Dead = number of larvae that died for unknown reasons. No statistical tests were performed when the number of larvae that was weighted for maize or teosinte was below four. P-values in bold represent a significant difference ( $P < 0.05$ )

## Discussion

Upon herbivory by leaf-chewing herbivores, cultivated maize and its wild ancestor, teosinte, overall emit similar VOCs, although the total quantities and the proportions of individual compounds rather vary (Gouinguéné et al., 2001; Chapter 3). In a previous study, performed with *S. littoralis* as the herbivore, we showed that parasitoid wasps are differentially attracted to maize and teosinte odors (Chapter 3). However, it remains unclear which individual compounds are responsible for wasp attractiveness (D'Alessandro and Turlings, 2006). Here, we found comparable results using *S. frugiperda* as the herbivore: naïve female *C. marginiventris* wasps preferred the odors of herbivore-induced teosinte over those of maize (Fig. 2a), while naïve *C. sonorensis* wasps did not distinguish between the two odor blends (Fig. 2b). These results are interesting, as *S. littoralis* and *S. frugiperda* induce a somewhat different odor signature (de Lange, 2008). Earlier studies showed that naïve *C. marginiventris* females are not attracted to the main maize VOCs, but might depend on “key compounds” that are released in very small amounts (D'Alessandro et al., 2009). Indeed, since the odor extracts that were used to assess wasp attractiveness were standardized to contain equal total amounts of the major VOCs, minute amounts of “key compounds” might explain the observed difference in wasp response. Conversely, attractiveness of *C. sonorensis* tends to be rather correlated to total VOC quantity (Elvira S. de Lange, unpublished data). These results suggest that the two tested wasp species presumably perceive or interpret the odors emitted by maize and teosinte differently.

We further show that *S. frugiperda* develops equally well on a maize and a teosinte variety (Fig. 3). As artificial selection of crop plants for increased yield and quality has reportedly resulted in a decrease in plant defenses (Wink, 1988; Rosenthal and Dirzo, 1997; Dávila-Flores et al., 2013), it is generally assumed that domesticated plants are better suited for herbivore development than their wild relatives. Indeed, in a field setting, fall armyworm seems to occur more frequently on maize than on teosinte (De la Paz-Gutiérrez, 2008; Takahashi et al., 2012), although earlier studies noted a preference for native grasses over the maize crop (Walton and Luginbill, 1916). Even though fall armyworm is able to feed on a multitude of plant species, it is typically regarded as a specialist of maize. Recently it was found that fall armyworm can detoxify benzoxazinoids, the main direct defense compounds of maize and several other grasses (Glauser et al., 2011). Preliminary experiments showed that *S. littoralis*, a generalist leaf-chewing herbivore, performed better on maize than on teosinte (Mickaël D.P. Gaillard, personal communication). These data suggest that *S. frugiperda* is indeed well-adapted to the defenses of plants in the genus *Zea*, even those of wild plants. Our observation that fall armyworm larvae obtained an equal amount of biomass when consuming a given amount of leaf tissue of maize and teosinte (Fig. 5) suggests that the nutritive value, at least of the leaves, is similar for both plants. This can be expected to be considerably different for the fruiting structures, since maize has been selected for centuries for increased yield and quality of the cobs (Palaisa et al., 2003; Flint-Garcia et al., 2009).

Fall armyworm larvae frequently get parasitized by a broad number of parasitoid wasp species (e.g. Molina-Ochoa et al., 2004). We observed that parasitized larvae grow considerably less than healthy larvae (Fig. 6), confirming the findings of earlier studies (Fritzsche Hoballah and Turlings, 2001). Plant direct defense compounds have been reported to have a negative effect on the development of members of the third (Sznajder and Harvey, 2003) and even fourth (Harvey et al., 2007) trophic level. Indeed, fall armyworm larvae that were parasitized by *C. sonorensis* obtained a higher weight when feeding on maize compared to when feeding on teosinte at two timepoints during their developmental period (Fig. 7d). The increased cocoon length of *C. sonorensis* from hosts that fed on maize further suggests that the wasps develop better in caterpillars feeding on cultivated maize (Fig. 7e), although no differences were observed in adult wasp tibia length (Fig. 7f), an important measure of fitness (Honek, 1993; Godfray, 1994). It has been reported that larvae of the small cabbage white, *Pieris rapae* L. (Lepidoptera: Pieridae) grew larger when feeding on cultivated Brussels sprouts (*Brassica oleracea* L.) than on wild cabbage. Also, the larvae displayed increased rates of encapsulation of the eggs of the parasitoid *Cotesia glomerata* L. when feeding on cultivated plants (Bukovinszky et al., 2009), suggesting that plant domestication and the resulting changes in plant chemistry indeed influence parasitoid performance. However, a better host plant for the herbivore is not necessarily a better host plant for the parasitoid. In a field study on aphids, it was shown that feral *Brassica* plants were better hosts for the aphids and also, indirectly, for their parasitoids than the domesticated plants (Bukovinszky et al., 2008). Indeed, parasitoids can be larger when their aphid hosts feed on wild Brassicaceae plants compared to cultivars (Le Guigo et al., 2011b), although performance of the host and its parasitoid are not always correlated (Le Guigo et al., 2011a).

For *C. marginiventris* wasps we found no differences in performance depending on the plant their host was developing on (Fig. 7a,b,c). However, wasp development time may be increased on maize (Fig. 7a), which may be detrimental to the wasps, as this would potentially increase the time they are vulnerable to their own natural enemies, like hyperparasitoids and predators (Turlings and Benrey, 1998). This would mean that, opposed to *C. sonorensis*, *C. marginiventris* would develop best on wild plants. Since the wasps were monitored only every other day, it would be worthwhile to repeat the experiment and to monitor wasp developmental time more closely, in order to verify these results.

Overall, we could not establish any adaptive link between the attractiveness to parasitoids and the suitability for parasitoid development of maize and teosinte. We found a slight but significant effect of host plant food source on *C. sonorensis* development, which may be due to the fact this wasp is larger than *C. marginiventris* and consumes the entire content of its host before spinning a cocoon. Its development and performance may therefore be more tightly linked to the size and quality of its host.

In summary, we have demonstrated that host plant, cultivated maize or its wild ancestor teosinte, did not influence the herbivore *S. frugiperda*, nor its parasitoid *C. marginiventris*. However, the parasitoid *C. sonorensis* developed better when its host had

been feeding on maize. These data potentially support the idea that wild plants have higher levels of defenses than cultivated varieties. *C. marginiventris* parasitoids preferred the odors of wild teosinte to those of cultivated maize, indicating a possible loss of parasitoid-attracting compounds during domestication. However, *C. sonorensis* did not distinguish between the two odor blends, suggesting that this parasitoid is not necessarily attracted towards the plants that are best suited for its development. As different maize varieties vary significantly in their herbivore-induced VOC emission capacities and their suitability as food plant for the herbivores (Degen et al., 2004; Erb et al., 2011a), an evaluation of multiple maize and teosinte varieties is needed to draw more robust conclusions about possible differences in parasitoid attractiveness and performance between cultivated maize and wild teosinte.

*Acknowledgements* - We thank Matthias Held for his help with the statistical analysis and Marianne Duployer for technical assistance. We are grateful to Yves Borcard, Christelle Robert and the students of the University of Neuchâtel for rearing of herbivores and parasitoid wasps.



## Chapter 5





## Parasitic wasps can reduce mortality among caterpillar-infested teosinte plants

Elvira S. de Lange<sup>1</sup>, Kevin Farnier<sup>1,2</sup>, Thomas Degen<sup>1</sup>, Benjamin Gaudillat<sup>1,3</sup>, Rafael Aguilar-Romero<sup>4</sup>, Fernando Bahena-Juárez<sup>5</sup>, Ken Oyama<sup>6</sup> and Ted C.J. Turlings<sup>1,\*</sup>

<sup>1</sup> *Laboratory of Fundamental and Applied Research in Chemical Ecology, University of Neuchâtel, Rue Emile-Argand 11, 2000 Neuchâtel, Switzerland*

<sup>2</sup> *Department of Zoology, La Trobe University, Melbourne, VIC 3086, Australia*

<sup>3</sup> *UMR 'Peuplements Végétaux et Bio-agresseurs en Milieu Tropical', CIRAD/Université de La Réunion, Pôle de Protection des Plantes, 7 chemin de l'IRAT, F-97410, Saint-Pierre, France*

<sup>4</sup> *Laboratorio de Ecología Genética y Molecular, Centro de Investigaciones en Ecosistemas, Universidad Nacional Autónoma de México, Antigua Carretera a Pátzcuaro No. 8701, Col. Ex-Hacienda de San José de la Huerta, C.P. 58190, Morelia, Michoacán, Mexico*

<sup>5</sup> *Campo Experimental Uruapan, Instituto Nacional de Investigaciones Forestales, Agrícolas y Pecuarias, Av. Latinoamericana No. 1101, Col. Revolución, C.P. 60150, Uruapan, Michoacán, Mexico*

<sup>6</sup> *Escuela Nacional de Estudios Superiores Unidad Morelia, Universidad Nacional Autónoma de México, Antigua Carretera a Pátzcuaro No. 8701, Col. Ex-Hacienda de San José de la Huerta, C.P. 58190, Morelia, Michoacán, Mexico*

\*e-mail: [ted.turlings@unine.ch](mailto:ted.turlings@unine.ch)

Tel: (+41) 32 718 3158, Fax: (+41) 32 718 3001



## Abstract

Many parasitic wasps use volatiles emitted by plants under herbivore attack to find their hosts. It has therefore been proposed that inducible plant volatiles serve an indirect defense function by recruiting parasitoids and other natural enemies. This suggested function remains controversial because there is little evidence that plants benefit from the action of natural enemies, particularly parasitoids, which do not immediately kill their hosts. With the use of large screen tents we conducted a semi-natural field experiment in Mexico, to evaluate how parasitoids can affect plant performance. The tritrophic study system comprised teosinte, the ancestor of maize, larvae of the herbivore *Spodoptera frugiperda* and the parasitic wasp *Campoletis sonorensis*, which have a long evolutionary history together. We assessed how the presence of the herbivore at realistic densities with and without the wasp affected plant growth and survival during early and later plant growth stages. In all trials, *S. frugiperda* larvae inflicted severe damage to the plants in the absence of parasitic wasps, which caused significant mortality in the trial with young plants, but not in trials where the larvae were placed on somewhat older plants. The presence of parasitic wasps decreased the damage inflicted by the larvae, resulting in a significant reduction of mortality in the trial with the young plants. Our findings support the notion that, under certain circumstances, plants may benefit from recruiting parasitic wasps.

## Key words

Indirect plant defenses, inducible volatiles, parasitoid attraction, *Spodoptera frugiperda*, tritrophic interactions, *Zea mays*

## Introduction

Plant defense traits are commonly recognized as important determinants of the tremendous success and diversity of plants in natural ecosystems. Insects are important evolutionary drivers of these defenses, selecting for numerous chemical and physical traits that plants use to defend themselves against herbivory (Howe and Jander, 2008). Several defense responses are specifically induced by herbivore attack (Karban and Balwin, 1997). In addition to attributes such as leaf toughness, toxic compounds and digestibility reducers, which have direct negative effects on the herbivores, plants possess traits that have been interpreted as indirect defenses, because they act through the recruitment of natural enemies of herbivores (Dicke and Sabelis, 1988; Heil, 2008; Turlings *et al.*, 1995).

The current study addresses a well-studied phenomenon that has been interpreted as an indirect plant defense: the attraction of natural enemies by insect-damaged plants through the emission of volatile attractants (Heil, 2008; Turlings and Fritzsche, 1999). It remains unclear, however, whether these attractants confer fitness benefits to the plants, and the function of inducible volatiles is still hotly debated in the literature (Agrawal, 2011; Allison and Hare, 2009; Dicke and Baldwin, 2010; Hare, 2011; Kaplan, 2012b; Kessler and Heil, 2011; Steiger *et al.*, 2011). Yet, it is evident that the volatiles are attractive to parasitoids and predators and are specifically produced in response to feeding by arthropod herbivores, with

spider mites (De Boer and Dicke, 2004; Dicke and Sabelis, 1988; Takabayashi *et al.*, 1994) and caterpillars (De Moraes *et al.*, 1998; Geervliet *et al.*, 1996; Turlings and Fritzsche, 1999; Turlings *et al.*, 1990) providing the best-studied examples. In the case of caterpillars, several elicitors contained in their oral secretions have been found to trigger or greatly enhance the emission of specific volatiles (Alborn *et al.*, 1997; Schmelz *et al.*, 2006). These volatiles provide natural enemies with potentially reliable cues, which for instance allow *Cardiochiles nigriceps* Viereck (Hymenoptera: Braconidae), a highly specialized parasitoid, to distinguish plants attacked by hosts from plants attacked by non-hosts (De Moraes *et al.*, 1998). Despite general agreement that volatiles produced by plants in response to herbivory are used by natural enemies to locate herbivores, it remains to be demonstrated that, from the plant's perspective, this attraction is adaptive.

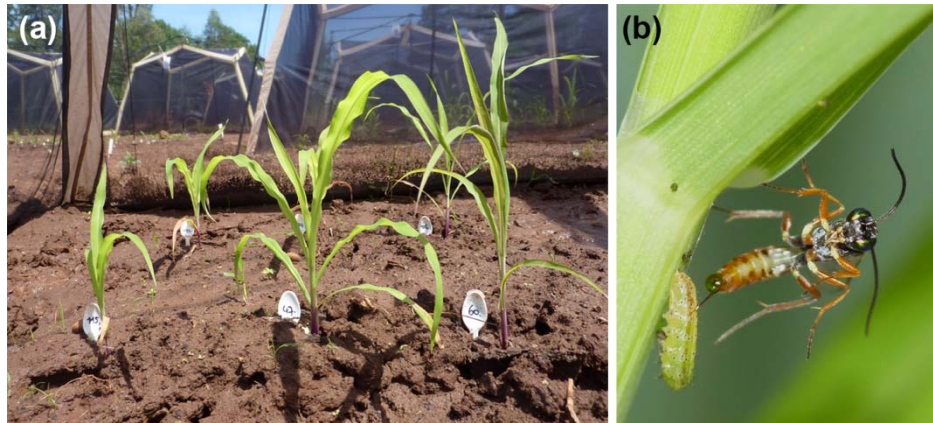
Predators kill herbivorous prey immediately and this is likely to significantly reduce plant damage. Indeed, for wild tobacco (*Nicotiana attenuata* Torr. Ex S. Wats.) it was recently shown that the recruitment of a predator has a positive effect on flower production, which is likely to increase seed production (Schuman *et al.*, 2012). For parasitic wasps this is not as straightforward. They kill their hosts only after some time, and it has even been suggested that in some cases parasitized herbivores feed more than unparasitized herbivores (Coleman *et al.*, 1999, but see Van Loon *et al.*, (2000) for evidence against this notion). It has also been shown that herbivore-induced volatiles are effective in attracting parasitoids and predators in the field (Bernasconi Ockroy *et al.*, 2001) and this may increase parasitism or predation of insect herbivores (De Moraes *et al.*, 1998; Kessler and Baldwin, 2001; Khan *et al.*, 1997a; Thaler, 1999). Current evidence for plant fitness benefits mediated by parasitoid wasps is limited to a parasitoid exclusion experiment with seed-weevils, where the relevance of volatiles in attracting the parasitoid, a chalcid wasp, is unknown (Gomez and Zamora, 1994), as well as studies of plants and insects that did not coevolve together and/or were conducted under relatively artificial conditions (Fritzsche Hoballah and Turlings, 2001; Smallegange *et al.*, 2008; Van Loon *et al.*, 2000). As is evident from a number of recent critical reviews (Agrawal, 2011; Allison and Hare, 2009; Hare, 2011; Kessler and Heil, 2011), none of these studies has been accepted as providing conclusive evidence for fitness benefits conferred to plants through parasitoid recruitment. Here we present a study that was conducted to seek such evidence for a tropical tritrophic system involving teosinte, the wild ancestor of maize (Fig. 1A) and insects that have interacted with this plant over evolutionary time (Fig. 1B).

Maize is one of the most studied plants when it comes to herbivore-induced volatile emissions, in particular in the context of parasitoid attraction (Turlings and Fritzsche, 1999; Turlings *et al.*, 1990). Maize responds strongly to herbivory, and caterpillar-damaged plants produce large quantities of a blend of predominantly green leaf volatiles, terpenoids and some aromatic compounds (Degen *et al.*, 2004; Turlings *et al.*, 1995). The blend is highly attractive to various parasitoids (Tamò *et al.*, 2006a), although it remains unclear which of the compounds in the blend are of key importance for the attraction (D'Alessandro and Turlings, 2006). The wild ancestors of cultivated maize are teosintes, a small species complex

of *Zea* grasses found in Mexico, Guatemala and Nicaragua (Matsuoka *et al.*, 2002). Different species and sub-species of teosinte release a very similar bouquet of volatiles as has been reported for cultivated maize (Gouinguéné *et al.*, 2001). Teosinte species and the insects associated with them are therefore ideal for a study that aims to test if a plant derives fitness benefits from attracting parasitoids.

Here, we studied the herbivore *Spodoptera frugiperda* Smith (fall armyworm) and its natural enemy *Campoletis sonorensis* Cameron (Fig. 1B). Larvae of the moth *S. frugiperda* are polyphagous but particularly well-adapted to feed on grasses, and constitute one of the most devastating maize pests in North-America. *S. frugiperda* larvae are also reported to feed on teosinte (Luginbill, 1928). *C. sonorensis*, an ichneumonid parasitoid, is one of the main natural enemies of *S. frugiperda* (Hoballah *et al.*, 2004; Von Mérey *et al.*, 2012). This parasitoid wasp attacks the early caterpillar instars, which then feed and grow much less than unparasitized caterpillars. The parasitoid larvae emerge long before their host has reached full size potential, which further reduces feeding damage, as the host dies at this point (Turlings and Fritzsche, 1999).

Teosinte, *S. frugiperda* and *C. sonorensis* share a long evolutionary history, and it is well established that *C. sonorensis* and other parasitoid species exploit *Spodoptera*-induced plant volatiles in their search for hosts (McAuslane *et al.*, 1991; Tamò *et al.*, 2006a). For the current study, we first conducted a survey of different teosinte populations in Mexico to confirm this association and found a high frequency of both herbivore and parasitoid on teosinte plants (described in detail in the Supplementary data, see Supplementary Fig. S1 and Supplementary Table S1). Seeds from three teosinte populations, “Tzitzio” (*Zea mays* L. ssp. *parviglumis* Iltis & Doebley), “Cuitzeo” (*Z. mays* ssp. *mexicana* (Schrader) Iltis) and “Hidalgo” (*Z. mays* ssp. *mexicana*), were used to grow plants in large screen tents. The young teosinte plants in each tent were exposed to realistic levels of *S. frugiperda* infestation, and different parasitoid densities in the tents were used as a proxy for differential parasitoid attraction. Tents without herbivores and tents with herbivores but without parasitoids served as controls. Our results indicate that the presence of parasitoid wasps can be a matter of life or death for young teosinte plants, as parasitoids strongly reduced plant mortality. This parasitoid-mediated fitness benefit was much less evident for larger plants.



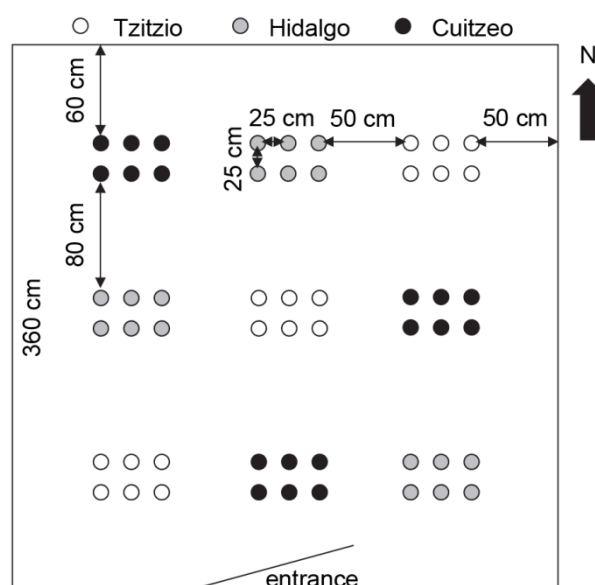
**Fig. 1** Tritrophic study system. (A) Teosinte plants in the experimental field. (B) A *Campoletis sonorensis* wasp attacking a *Spodoptera frugiperda* larva. Photograph credits: (A) Elvira S. de Lange, (B) Matthias Held

### Materials and methods

**Plants** Seeds of annual teosintes were obtained from three different locations in the state of Michoacán, Mexico: near Tzitzio (*Z. mays* ssp. *parviglumis*; N 19°34.310', W 100°55.358'; altitude 1354 m), near Cuitzeo (*Z. mays* ssp. *mexicana*; N 19°59.742', W 100°53.397'; altitude 2096 m) and near Ciudad Hidalgo (*Z. mays* ssp. *mexicana*; N 19°41.696', W 100°37.544'; altitude 2086 m). Seeds were sown in biodegradable pots filled with a mixture of soil and vermiculite in three batches on July 12, July 28 and August 19 2010. All seedlings were kept in a greenhouse for 10-20 days before being planted in a field on the UNAM campus in Morelia, Michoacán, Mexico (N 19°38.960', W 101°13.636'; altitude 1966 m). As the field experiment was replicated three times, the field was divided into three parcels. Each parcel was divided into seven plots with at least 60 cm between plots. At five randomly chosen positions, a tent was placed (12'x12' Dual Identity Screenhouse and Canopy, Gigatent, Wayne NJ, USA) to enclose the plants with specifically chosen insect treatments and to exclude other insects. The sides of the tents were burrowed 15 cm into the soil to stabilize them against strong winds and rain. In each plot, plants were arranged in nine mini-plots of six teosinte plants each, with the different populations randomly distributed in a three-by-three Latin square design (i.e., 54 plants per tent). Plants were spaced at least 25 cm apart within a mini-plot with 50 cm between mini-plots (Fig. 2). Plants were not irrigated until the end of the rainy season in September. Weeds were regularly removed by hand. Prior to insect treatments (see below), the numbers of plants present and absent in each plot were scored. The very few plants that failed to establish after transplantation were excluded from the analyses.

**Insect Rearing** Rearing colonies of *S. frugiperda* (Lepidoptera: Noctuidae) originated from insect collections in various maize fields in the state of Michoacán, Mexico, in the summer of 2010 (Bahena-Juárez *et al.*, 2010). The larvae were kept at room temperature and were

reared on fresh castor bean (*Ricinus communis* L.) leaves. Pupae were sexed and adults were kept in mating boxes at a 50:50 sex ratio, with cotton wool soaked in honey-water solution as a food source. Females were allowed to oviposit on paper lining the walls of the boxes. The rearing colony of the solitary endoparasitoid *C. sonorensis* (Hymenoptera: Ichneumonidae) originated from wasps that emerged from *S. frugiperda* larvae that were collected in the summer of 2010. To maintain the colony, 20 second-instar *S. frugiperda* caterpillars were offered to two mated female wasps for five hours in a small plastic box. The parasitized caterpillars were kept on castor bean leaves until the parasitoid larvae emerged and formed cocoons, which were kept in petri dishes until adult emergence. The adult wasps were sexed and housed in cages (30 x 30 x 30 cm) with a sex ratio of 25:75 (male:female), with cotton wool soaked in honey-water solution as a food source. For experiments, second-instar *S. frugiperda* larvae and two- to seven-day-old mated female wasps were used.



**Fig. 2** Field design. Plants from the three different teosinte varieties, Tzitzio, Hidalgo and Cuitzeo, were placed in a tent in nine mini-plots in a randomized three-by-three Latin square, with six plants per mini-plot. Plants were placed in an identical arrangement in the open plots that were not covered by a tent

**Insect Treatments** Five different treatments were applied to the plants in the tents: C - control, no insects; SF - infestation with five second-instar *S. frugiperda* larvae per plant, but no release of parasitoids; and three treatments (+W, ++W and +++W) that also involved infestation with five second-instar *S. frugiperda* larvae per plant, but in these cases we added female *C. sonorensis* wasps at different densities, of 3, 9, or 54 wasps per tent, respectively. The lowest wasp density treatment (+W) was absent from the first experimental trial and was added to the next two trials because the medium wasp density treatment (++W) resulted in an unexpectedly high parasitism rate. Two additional treatments were applied to the plants in open plots, similar plots without tents: OC - control, no insects; O+SF - infestation with five second-instar *S. frugiperda* larvae per plant.

Infestation with *S. frugiperda* larvae took place on August 23, September 6 and October 6 2010, for trials one, two and three, respectively. The five larvae were placed in the whorl of each plant with a brush. *S. frugiperda* moths readily lay batches that may contain hundreds of eggs. Of the larvae that hatch from these eggs only few survive the first stages (Luginbill, 1928). Five second-instar larvae per plant are within the range that is naturally observed on teosinte (Supplementary data) and maize plants (Von Mérey *et al.*, 2011).

Parasitoid wasps were released in three equally sized batches on days one, two and three following larval infestation (i.e., one, three and 18 wasps were released on each of three consecutive days for the low, medium and high wasp density treatments, respectively). Inside each tent, cups with cotton wool soaked in honey-water solution were provided as a food source for the wasps.

*First Harvest* Seven days after the larval infestations, the shoot of every second plant in each plot was harvested, placed in a paper bag, and transported to the laboratory nearby to evaluate insect presence and herbivory. Nine to ten days later, the tents were removed to allow the remaining plants to continue to develop under natural conditions.

*Parasitism* In the laboratory, the harvested plants were carefully removed from the paper bags and the number of caterpillars present on each plant was counted. Individual larvae were weighed and subsequently placed in individual compartments of a 24-well ELISA plate. Larvae were supplied with artificial diet and reared at room temperature until adult emergence or, in the case of parasitism, until parasitoid emergence occurred (Hoballah *et al.*, 2004). The adult parasitoids, dead parasitoid larvae and cocoons were individually preserved in 70% ethanol in Eppendorf tubes. The wasps were visually examined to identify the species (Cave, 1995). We calculated the parasitism rate by dividing the number of parasitized larvae by the total number of collected larvae minus the number of larvae that escaped or died for unknown reasons.

*Herbivory and Shoot Dry Biomass* After removing the larvae, the leaves of the harvested plants were scanned for herbivore damage using NIH ImageJ 1.44I software (Rasband 1997-2012). After scanning, plant shoots were dried in an oven at 80°C for two days and their dry weight was recorded.

*Volatile Emission* Plant volatiles were collected as described previously by Von Mérey *et al.*, (2012). Plants were enveloped in Nalophan sleeves (Omya AG, Oftringen, Switzerland) of 150 mm diameter and 50 cm length and the top and bottom of the sleeves were closed with a plastic seal. The sleeves were attached to a metal wire to prevent them from falling over. Tubular glass parts (23 x 17 x 12 mm) with an open screw cap were attached to the bag to insert a trapping filter containing 25 mg of 80-100 mesh SuperQ adsorbent (Alltech Associates, Inc., Deerfield, Illinois, USA). Air was pulled through the trapping filters using an air-sampling pump (SKC 222 series, Blanc Labo S.A., Lonay, Switzerland) for six hours at



0.6 L/min. The volatiles were eluted from the filters with 150  $\mu$ l dichloromethane and the extracts were stored at -20°C before analysis. For each trial, we simultaneously collected volatiles from all three teosinte varieties, in tents with plants infested with *S. frugiperda* larvae without parasitoids (treatment “SF”). Collections started one day after larval infestations and were repeated on five or six consecutive days, using different plants.

Volatiles were analyzed using a gas chromatograph (Agilent 6890 Series GC System G1530A) coupled to a mass spectrometer (Agilent 5973 Network Mass Selective Detector; transfer line 280°C, source 230°C, ionization potential 70 eV). An aliquot of 2  $\mu$ l was injected in split mode (65.8:1) onto an apolar capillary column (HP-Ultra 2ms, 30 m, 0.25 mm ID, 0.25  $\mu$ m film thickness; Agilent J&W Scientific, USA). Helium at constant flow (1.1 ml/min) was used as the carrier gas. The column temperature was maintained at 40°C and increased after injection at 8°C/min to 150°C followed by a post-run of 2 min at 300°C. The detected volatiles were identified by comparing their I) mass spectra with those of the NIST05 library, II) mass spectra and retention times with those of authentic standards, and III) retention times with those from previous analyses (D'Alessandro and Turlings, 2005). Volatiles that met only one of those criteria are labelled as tentatively identified. Comparisons of odor quantities between the three teosinte populations were based on peak areas. These data were corrected for the volume of air that was pulled through the trapping filters. We subtracted compounds that were also present in three blank collections (odor collections that were performed in a similar way as described above, but without a plant). To calculate VOC emission per g shoot dry weight, plant total odor emission was divided by the average shoot dry weight in the “SF” tent for the corresponding teosinte population.

*Final Shoot Dry Biomass and Plant Survival* On November 8 2010, all plants died during an unusually early freezing event (See Supplementary Fig. S2). One week later, the aboveground plant tissue was harvested in paper bags and left to dry in a drying chamber for one week. Final shoot dry biomass was then determined, which is strongly correlated with seed production (Fritzsch Hoballah and Turlings, 2001). The numbers of plants present and absent in each plot were scored as a measure of plant survival.

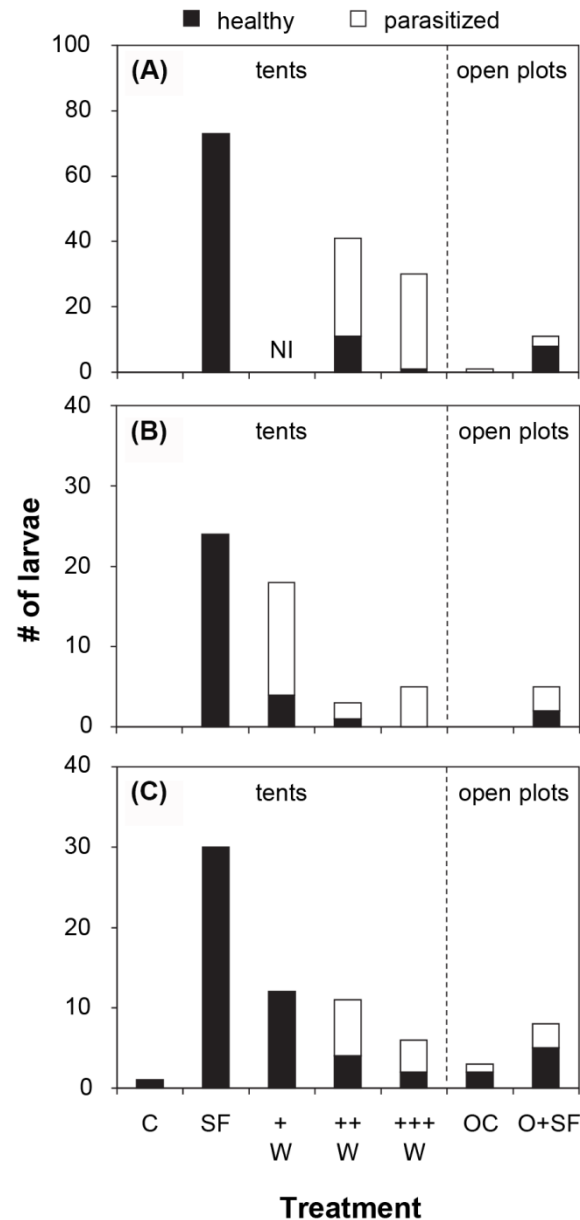
## Results

*Survival and Parasitism of Caterpillars* To assess parasitism by *C. sonorensis* of the released *S. frugiperda* caterpillars, we harvested every second plant in each plot one week after larval infestations and determined the presence of parasitized larvae. Overall, we recovered 36 $\pm$ 4%, 15 $\pm$ 2% and 17 $\pm$ 2% of all released larvae in trials one, two and three, respectively. More larvae were retrieved from tent-covered plots than from open ones (Supplementary Fig. S3). In the first trial, larvae had a lower weight in the presence of wasps than in the absence of wasps (Supplementary Fig. S4), indicating that they were parasitized (Fritzsch Hoballah and Turlings, 2001). There was a negative correlation between the number of larvae retrieved from each tent and the number of parasitoid wasps that had been introduced (Supplementary Fig. S5). When the recovered larvae were reared on artificial

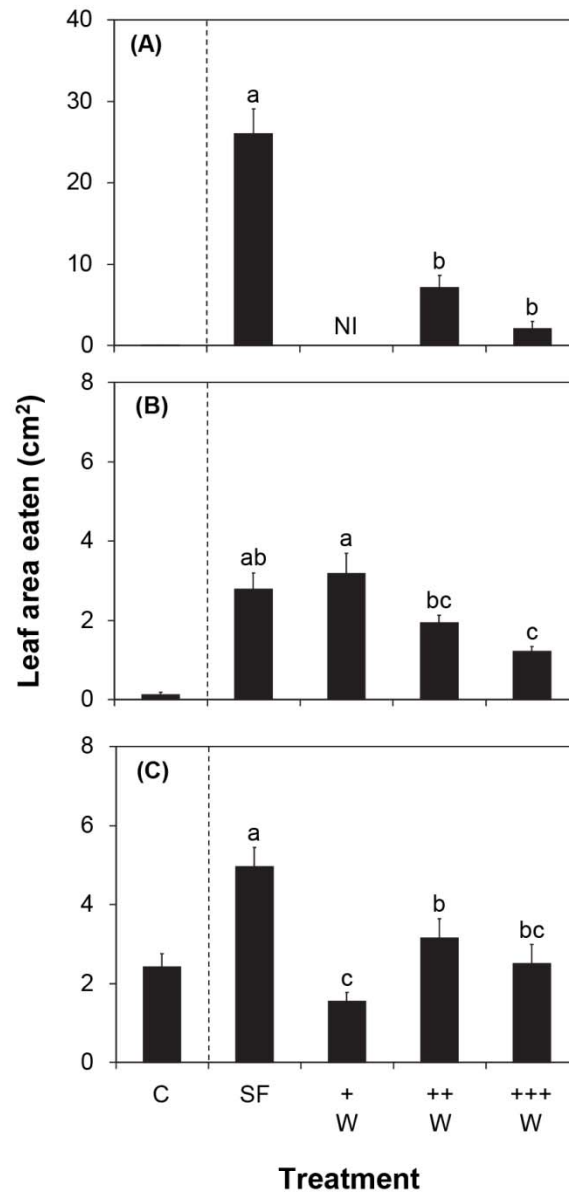
diet, larvae from tents without wasps developed into healthy adult moths. Most of the larvae recovered from tents with wasps were found to be parasitized (trial one: 73% for medium and 97% for high wasp density, trial two: 78% for low, 67% for medium and 100% for high wasp density, trial three: 0% for low, 64% for medium and 67% for high wasp density; Fig. 3A,B,C). Hence, the only exception was the lowest wasp density (i.e., three) in the third trial (Fig. 3C). When combining all trials, there was a significant difference between the proportion of parasitism in “Tzitzio” (0.84) and “Hidalgo” (0.59), while “Cuitzeo” (0.75) was in between (chi-square test for all varieties,  $P = 0.039$ ; Supplementary Fig. S6; all statistical analyses were performed using SigmaPlot version 12.0 (Systat Software, San Jose, CA)).

*Herbivory* The leaves of the harvested plants were scanned in order to calculate herbivore damage. In tents without wasps, larvae consumed an average leaf area of 28, 3 and 5 cm<sup>2</sup> per plant in trials one, two and three, respectively. In the presence of wasps, larvae inflicted significantly less damage, implying that parasitoid wasps can effectively protect the plants against herbivory (Fig. 4A,B,C). Damage data in the third trial might be confounded as a result of grasshoppers invading the tents. There were no significant differences in herbivory between teosinte varieties, except for the second trial, where “Hidalgo” was more damaged than “Tzitzio” (GLM, trial 1:  $P_{\text{treatment}} < 0.001$ ,  $P_{\text{population}} = 0.719$ ,  $P_{\text{interaction}} = 0.650$ , trial 2:  $P_{\text{treatment}} < 0.001$ ,  $P_{\text{population}} = 0.014$ ,  $P_{\text{interaction}} = 0.240$ , trial 3:  $P_{\text{treatment}} < 0.001$ ,  $P_{\text{population}} = 0.161$ ,  $P_{\text{interaction}} = 0.503$ ).

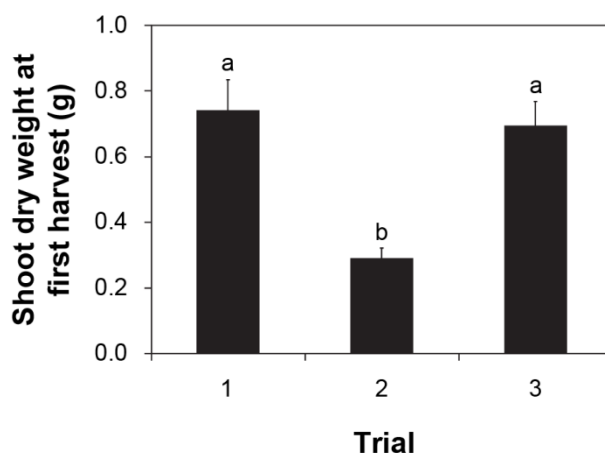
*Shoot Dry Biomass at First Harvest* After scanning the leaves, plant shoots were dried in order to record dry weight. In the first trial, herbivore-attacked plants had more aboveground biomass than control plants at harvest. Conversely, in the second trial, herbivory significantly decreased plant shoot biomass production relative to control plants, both in tents and in open plots, whereas the presence of wasps in the tents could nullify this effect. Compared to the presence of herbivores only, addition of wasps to the tents did not affect shoot biomass in the third trial (Supplementary Fig. S7). Plants from the population “Hidalgo” generally had a higher biomass than plants of the other populations. It is important to note that in the second trial, average plant biomass was significantly lower than in the other two trials at first harvest (GLM,  $P_{\text{trial}} < 0.001$ ,  $P_{\text{population}} < 0.001$ ,  $P_{\text{interaction}} = 0.288$ ; Fig. 5).



**Fig. 3** Retrieval and parasitism of *Spodoptera frugiperda* larvae in tents in the absence (SF) and in the presence of increasing densities of parasitoid wasps (+W, ++W, +++W), and natural parasitism of larvae in plots not covered by a tent (OC, O+SF). (A) First trial. (B) Second trial. (C) Third trial. Overall, *Campoletis sonorensis* was responsible for 25, 66 and 75% of the parasitized larvae retrieved from the open plots in trial one, two and three, respectively; for the remaining parasitized larvae, the responsible species was not identified. There were significant differences between the numbers of healthy and parasitized larvae retrieved from the different tents (chi-square test,  $P_{\text{trial1}} < 0.001$ ,  $P_{\text{trial2}} < 0.001$  and  $P_{\text{trial3}} < 0.001$ ). When comparing the tent with only herbivores to all tents with wasps combined, there was an overall significant difference (chi-square test,  $P_{\text{trial1}} < 0.001$ ,  $P_{\text{trial2}} < 0.001$  and  $P_{\text{trial3}} < 0.001$ ). Furthermore, there were significant differences between the numbers of parasitized larvae in tents with different wasp densities (chi-square test,  $P_{\text{trial1}} = 0.022$ ,  $P_{\text{trial2}} = 0.432$ ,  $P_{\text{trial3}} = 0.002$ ). Only tents where larvae were released were included in the statistical analyses. NI = not included. For a more detailed explanation of the insect treatments, see Materials and methods

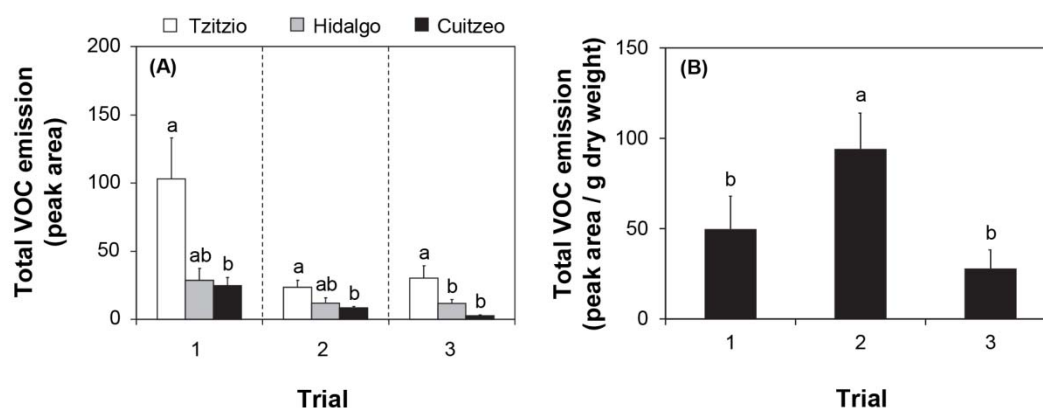


**Fig. 4** Average amount of damage inflicted (+SE) by *Spodoptera frugiperda* larvae in tents in the absence (SF) and in the presence of increasing densities of parasitoid wasps (+W, ++W, +++W). (A) First trial. (B) Second trial. (C) Third trial. Different letters indicate significant differences (GLM, trial 1:  $P_{\text{treatment}} < 0.001$ ,  $P_{\text{population}} = 0.719$ ,  $P_{\text{interaction}} = 0.650$ , trial 2:  $P_{\text{treatment}} < 0.001$ ,  $P_{\text{population}} = 0.014$ ,  $P_{\text{interaction}} = 0.240$ , trial 3:  $P_{\text{treatment}} < 0.001$ ,  $P_{\text{population}} = 0.161$ ,  $P_{\text{interaction}} = 0.503$ ). Only plots where larvae were released were included in the statistical analyses. Damage data of the third trial might be confounded due to grasshoppers invading the tents. NI = not included. For a more detailed explanation of the insect treatments, see Materials and methods



**Fig. 5** Average shoot dry weight (+SE) of control, non-herbivore infested plants in tents (treatment “C”) for each trial of the experiment at first harvest, seven days after infestation with herbivores. Different letters indicate significant differences (GLM,  $P_{\text{trial}} < 0.001$ ,  $P_{\text{population}} < 0.001$ ,  $P_{\text{interaction}} = 0.288$ )

**Volatile Emission** We collected volatiles from individual herbivore-infested plants of each teosinte variety. All three teosinte varieties emitted similar volatile blends (Supplementary Fig. S8), which are typical for herbivore-induced maize (Gouinguéné *et al.*, 2001). However, the varieties differed remarkably in the total emission of volatiles upon herbivory, with plants from “Tzitzio” emitting the largest quantities and those from “Cuitzeo” the lowest. This was consistent across all three trials (one-way ANOVA,  $P_{\text{trial1}} = 0.024$ ,  $P_{\text{trial2}} = 0.031$  and  $P_{\text{trial3}} = 0.003$ ; Fig. 6A; see also Supplementary Fig. S8 and Supplementary Table S2). Plants in the second trial emitted more volatiles per g shoot dry weight than those in the other two trials (GLM,  $P_{\text{trial}} < 0.001$ ,  $P_{\text{population}} < 0.001$ ,  $P_{\text{interaction}} = 0.682$ ; Fig. 6B), suggesting that younger plants show a more intense odor production in response to herbivory than older plants.

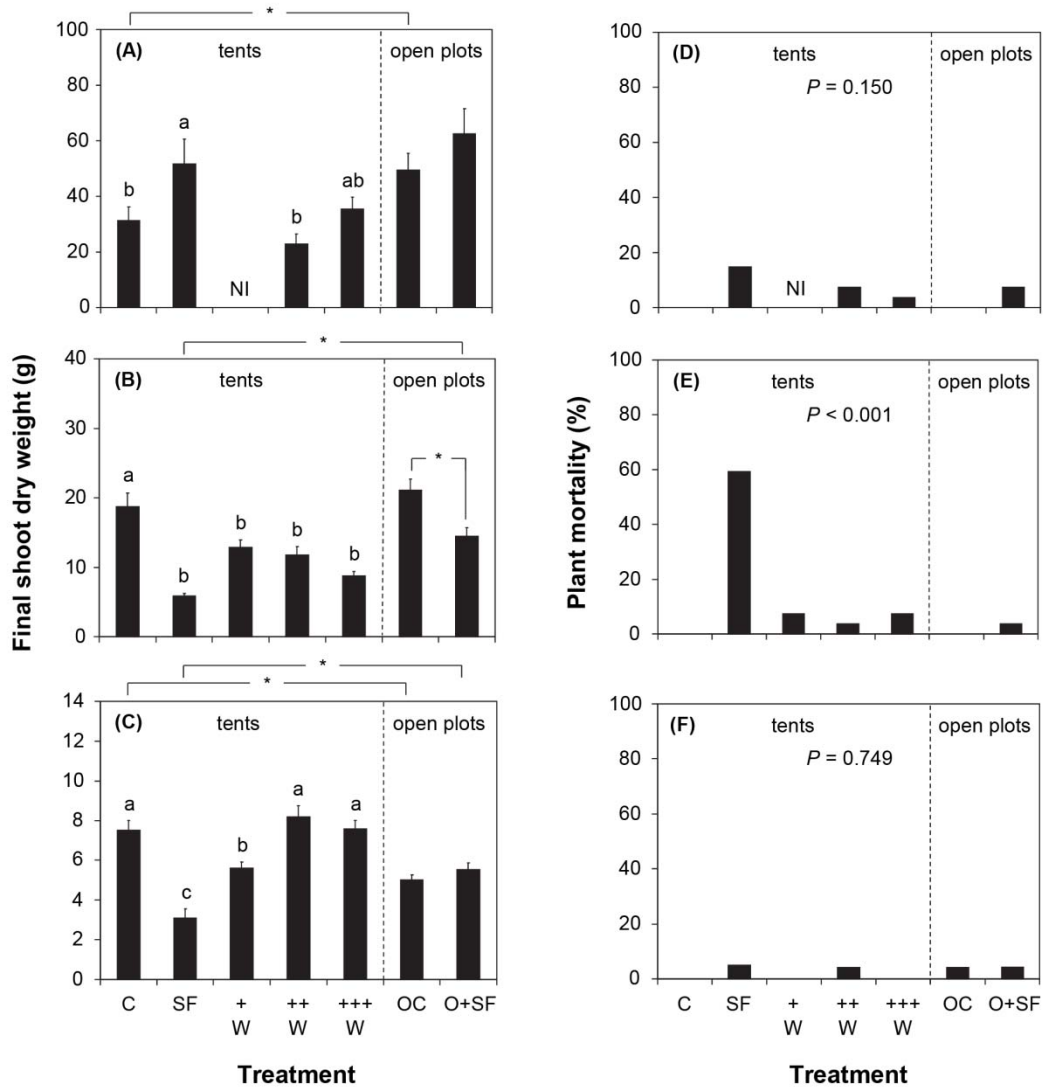


**Fig. 6** Plant volatile emission. (A) Total volatile emission per plant (+SE) for each trial and for each teosinte variety. Different letters indicate significant differences (one-way ANOVA,  $P_{\text{trial1}} = 0.024$ ,  $P_{\text{trial2}} = 0.031$  and  $P_{\text{trial3}} = 0.003$ ). (B) Total volatile emission per plant corrected for the amount of shoot dry biomass for all three teosinte varieties combined. Different letters indicate significant differences (GLM,  $P_{\text{trial}} < 0.001$ ,  $P_{\text{population}} < 0.001$ ,  $P_{\text{interaction}} = 0.682$ )

*Final Shoot Dry Biomass and Plant Survival* After the first harvest, the tents were removed and the remaining plants were allowed to continue development under natural conditions. Due to an unexpected frost event just before plant maturity, we could not fully assess the true consequences of the treatments for plant fitness. We harvested plant shoots shortly after the freezing event and determined final shoot dry biomass, a parameter closely correlated with fitness in terms of seed production (Fritzsche Hoballah and Turlings, 2001).

In the first trial, herbivore-attacked plants had a higher shoot biomass than control plants in tents (GLM,  $P_{\text{treatment}} = 0.003$ ,  $P_{\text{population}} < 0.001$ ,  $P_{\text{interaction}} = 0.060$ ). There was no significant difference between control and herbivore-infested plants in the open plots (GLM,  $P_{\text{treatment}} = 0.269$ ,  $P_{\text{population}} < 0.001$ ,  $P_{\text{interaction}} = 0.733$ ; Fig. 7A). In sharp contrast, herbivory had a detrimental effect on shoot biomass in the second trial, both in open plots (GLM,  $P_{\text{treatment}} < 0.001$ ,  $P_{\text{population}} < 0.001$ ,  $P_{\text{interaction}} = 0.280$ ) and in tents (GLM,  $P_{\text{treatment}} < 0.001$ ,  $P_{\text{population}} = 0.295$ ,  $P_{\text{interaction}} = 0.659$ ). There was a trend towards the presence of parasitic wasps to increase plant biomass (Fig. 7B). In the third trial, herbivory also significantly reduced shoot biomass, while wasp presence alleviated this negative effect (GLM,  $P_{\text{treatment}} < 0.001$ ,  $P_{\text{population}} < 0.001$ ,  $P_{\text{interaction}} = 0.810$ ). We observed no significant differences between control and herbivore-infested plants in the open plots (GLM,  $P_{\text{treatment}} = 0.122$ ,  $P_{\text{population}} = 0.006$ ,  $P_{\text{interaction}} = 0.474$ ; Fig. 7C). “Tzitzio” plants were overall smaller than plants of the other populations.

As an ultimate measure of plant fitness, we monitored the survival of plants in each plot until the freezing event. There were no differences in plant mortality between the three teosinte varieties, neither in individual replicates (chi-square test,  $P_{\text{trial1}} = 0.085$ ,  $P_{\text{trial2}} = 0.698$ ,  $P_{\text{trial3}} = 0.777$ ) nor overall (chi-square test,  $P = 0.578$ ). No difference in plant mortality between plots was observed in either the first (chi-square test,  $P_{\text{trial1}} = 0.150$ ; Fig. 7D) or third (chi-square test,  $P_{\text{trial3}} = 0.749$ ; Fig. 7F) trials. However, in the second trial, plants in the herbivore-attacked, previously tented plots suffered considerable mortality (chi-square test,  $P_{\text{trial2}} < 0.001$ ). Plant mortality was reduced from almost 60% to less than 10% in the presence of wasps (Fig. 7E). Hence, a positive effect of parasitoid presence on plant survival was observed in the trial with the smallest plants, at the time of herbivore infestations, only.



**Fig. 7** Plant fitness parameters. (A-C) Average final shoot dry weight (+SE) of plants previously covered with tents that were not infested with herbivores (treatment “C”) or that were infested with *Spodoptera frugiperda* larvae in the absence (SF) and in the presence of increasing densities of parasitoid wasps (+W, ++W, +++W), and plants in plots that had never been covered by a tent (OC, O+SF). (A) First trial. (B) Second trial. (C) Third trial. (D-F) Plant mortality. (D) First trial. (E) Second trial. (F) Third trial. About two weeks after larval infestations, tents were removed and plants were allowed to continue development under natural conditions. Since all plants died during an unusually early freezing event, plants from all three trials were harvested at the same time, and as they were planted several weeks apart, they did not have the same age at the time of harvest. For (A-C): Different letters indicate significant differences between treatments in tents (GLM, trial 1:  $P_{\text{treatment}} = 0.003$ ,  $P_{\text{population}} < 0.001$ ,  $P_{\text{interaction}} = 0.060$ , trial 2:  $P_{\text{treatment}} < 0.001$ ,  $P_{\text{population}} = 0.295$ ,  $P_{\text{interaction}} = 0.659$ , trial 3:  $P_{\text{treatment}} < 0.001$ ,  $P_{\text{population}} < 0.001$ ,  $P_{\text{interaction}} = 0.810$ ). An asterisk indicates significant differences between treatments in the open plots (GLM, trial 1:  $P_{\text{treatment}} = 0.269$ ,  $P_{\text{population}} < 0.001$ ,  $P_{\text{interaction}} = 0.733$ , trial 2:  $P_{\text{treatment}} < 0.001$ ,  $P_{\text{population}} < 0.001$ ,  $P_{\text{interaction}} = 0.280$ , trial 3:  $P_{\text{treatment}} = 0.122$ ,  $P_{\text{population}} = 0.006$ ,  $P_{\text{interaction}} = 0.474$ ), between corresponding control treatments in tents and open plots (GLM, trial 1:  $P_{\text{treatment}} = 0.006$ ,  $P_{\text{population}} < 0.001$ ,  $P_{\text{interaction}} = 0.144$ , trial 2:  $P_{\text{treatment}} = 0.297$ ,  $P_{\text{population}} < 0.001$ ,  $P_{\text{interaction}} = 0.272$ , trial 3:  $P_{\text{treatment}} < 0.001$ ,  $P_{\text{population}} = 0.004$ ,  $P_{\text{interaction}} = 0.144$ ) or between corresponding herbivore-infestation treatments in tents and open plots (GLM, trial 1:  $P_{\text{treatment}} = 0.460$ ,  $P_{\text{population}} = 0.005$ ,  $P_{\text{interaction}} = 0.740$ , trial 2:  $P_{\text{treatment}} < 0.001$ ,  $P_{\text{population}} = 0.140$ ,  $P_{\text{interaction}} = 0.236$ , trial 3:  $P_{\text{treatment}} < 0.001$ ,  $P_{\text{population}} = 0.012$ ,  $P_{\text{interaction}} = 0.985$ ). For (D-F): Statistical tests included all treatments (chi-square test). NI = not included. For a more detailed explanation of the insect treatments, see Materials and methods

## Discussion

Whether or not plants benefit from attracting natural enemies has recently been heavily debated and is the topic of several reviews (Agrawal, 2011; Allison and Hare, 2009; Dicke and Baldwin, 2010; Hare, 2011; Kessler and Heil, 2011). These reviews emphasize that, as yet, there is at most marginal evidence that attracting natural enemies has any consequences for plant fitness. A recent study with transformed tobacco plants has convincingly shown that plants produce fewer flowers when they are unable to recruit predatory bugs (Schuman *et al.*, 2012). The present study addresses the benefits to plants of attracting parasitic wasps, which do not immediately kill their hosts. These wasps rely heavily on herbivore-induced plant volatiles for host location (De Moraes *et al.*, 1998; Fritzsche Hoballah and Turlings, 2001; Geervliet *et al.*, 1996; Turlings *et al.*, 1990). Using a tritrophic system with organisms that have a longstanding evolutionary history together, we mimicked plants with differential attractiveness to parasitoids under realistic conditions and found a strong effect of parasitism on plant survival in the trial with the smallest plants at the time of herbivore infestations (Fig. 7E). Although plants were the same age across all trials when infested with herbivores, they varied in size throughout the season, due to different weather conditions (Supplementary Fig. S2). The physiological age of the plants at time of treatment was therefore considerably different for each trial.

In all trials, we observed that the presence of parasitic wasps reduced the amount of damage inflicted by *S. frugiperda* larvae (Fig. 4), confirming laboratory experiments showing that parasitized larvae consume and grow considerably less than healthy larvae (Fritzsche Hoballah and Turlings, 2001). Moreover, overall parasitism rates on the three teosinte genotypes were significantly different, with higher parasitism rates on plants from the population “Tzitzio” (Fig. S5). This nicely matches the total amounts of emitted volatiles, which was also highest for this genotype (Fig. 5A) and is therefore in line with the hypothesis that the volatiles help the plants recruit parasitoids. Yet, most relevant to the current study is the positive impact that parasitoid presence had on plant survival. In the trial with the youngest plants, the presence of parasitoids significantly reduced plant mortality (Fig. 7E), which implies that the recruitment of parasitoids can provide fitness benefits to these plants.

In the second trial, even at the lowest wasp density (i.e., three) most larvae were parasitized (Fig. 3), implying that the wasps very efficiently locate and parasitize their hosts. *C. sonorensis* prefers to lay eggs in second- and third-instar *S. frugiperda* larvae (Cave, 1995), which are the stages that most of the larvae remaining on the plants were in. Additional wasps increased *S. frugiperda* mortality, resulting in a lower retrieval rate (Fig. 3 and see Supplementary Fig. S3 and Supplementary Fig. S5), possibly due to mortality after excessive stinging and superparasitism (Van Lenteren *et al.*, 2006) or because larvae dropped off the plants in order to evade the parasitoids (Luginbill, 1928).

It is evident from the above data that the action of parasitoids can decrease herbivore-inflicted damage teosinte plants. The most dramatic and relevant effect of the presence of parasitic wasps was observed on plant mortality in the second trial (Fig. 7E), in which plants



had the lowest biomass upon caterpillar infestation (Fig. 5). Evidently, small plants are much more vulnerable to the effects of caterpillar damage than larger plants and benefit more from attracting parasitoids. The fact that natural enemies of *S. frugiperda* can significantly reduce plant mortality supports the hypothesis that their recruitment may be an evolved function of inducible volatile signals (Kessler and Heil, 2011). This notion is also supported by the fact that young maize plants are particularly responsive to caterpillar attack and emit higher amounts of volatiles, corrected for shoot biomass, than older plants (Gouinguéné, 2000; Köllner *et al.*, 2004), as also observed in the present study (Fig. 6B) and observed for other wild plant species, such as *Datura wrightii* Regel (Hare, 2010) and wild tobacco (Diezel *et al.*, 2011).

A strong effect of herbivory and parasitism on plant mortality was apparent in the second trial, but not in the other trials. In fact, in the first trial, plants grew taller after herbivory by *S. frugiperda* than in the absence of the herbivore (Fig. 7A, Supplementary Fig. S7A). This is likely due to compensatory growth (Agrawal, 2000) and may have been facilitated in the first trial by favorable climatic conditions (i.e., high rainfall). It is therefore important to address compensatory growth in field studies. Compensatory growth may mask the detrimental effects of herbivory and positive effects of parasitoid presence. However, in the second and third trial, plants accumulated more biomass in the absence than in the presence of the herbivore, and suggesting severe tissue loss due to herbivory (Fig. 7B,C). Because of logistical constraints, planting of teosinte in the field tents occurred relatively late in the season, and all of the plants were hit and killed by early frost before seed set. This rare and very unfortunate event limits the conclusive power of this study, as we cannot say anything about the ultimate fitness effects of the treatments, which could be best measured in terms of seed number and quality. However, as plant growth and fitness tend to be strongly correlated in maize plants (Fritzsche Hoballah and Turlings, 2001), it seems safe to propose that reduced plant biomass implies a negative influence of insect herbivores on the fitness of teosinte, which can in part be alleviated by the presence of parasitoid wasps.

Although tent experiments have limitations, they also offer several advantages. The experimental design enabled precise control of herbivory and parasitoid densities and avoided other potentially confounding factors, biotic or abiotic, among treatments. Moreover, upon assessment of parasitism a week after wasp release, the tents were removed and the plants were exposed to the same natural conditions as the plants in the open plots. The use of tents did not obviously affect plant survival (Fig. 7).

Alternative approaches to test for potential advantages of attracting parasitoids could include the use of genotypes with known differences in volatile emissions. Such an approach was used by Degen and co-authors (2012), who evaluated larval parasitism on maize lines with distinct differences in total odor emissions under field conditions in Mexico. However, the differences between such lines are logically not just limited to the emission of volatiles, and herbivore as well as plant performance will be affected by other phenotypic differences among plant genotypes. For example, caterpillar growth on a plant is negatively correlated with the extent of volatile emission (Degen *et al.*, 2012; Erb *et al.*, 2011a). Moreover, the fact

that it remains largely unknown which volatiles are of key importance for parasitoid attraction (D'Alessandro and Turlings, 2006) complicates the choice of appropriate genotypes for such studies. A transgenic approach (Schuman *et al.*, 2012) and the use of elicitors (Alborn *et al.*, 1997; Thaler, 1999; Von Mérey *et al.*, 2012), inhibitors (Bruinsma *et al.*, 2010a; D'Alessandro *et al.*, 2006) or pure compounds (De Boer and Dicke, 2004; Von Mérey *et al.*, 2011) to manipulate volatile emissions are also very useful to demonstrate the role of volatiles in predator or parasitoid attraction, but they present similar problems: the treatments may have other unknown effects on the performance of the plants, herbivores or parasitoids. We therefore assert, depending on the research objectives, that such approaches do not offer advantages over those used in the present study.

In conclusion, we found that attack by *S. frugiperda* can be a significant mortality factor for young teosinte plants. In tents containing *S. frugiperda* larvae in the absence of wasps, up to 60% of the plants died as a result of herbivory. In the case of young plants, the presence of parasitoids in the tents reduced damage and mortality dramatically, representing a fitness gain that leaves no room for alternative interpretation. Although our findings will not fully resolve the long-standing debate on the many possible functions of herbivore-induced plant volatiles, the study presents solid support for the hypothesis that plants can benefit from recruiting parasitoids.

*Supplementary data* - Supplementary data can be found in Annex 2.

**Supplementary Table S1** Survey of eight teosinte populations in Mexico in 2009.

**Supplementary Table S2** Absolute amounts of volatiles collected from herbivore-damaged teosinte plants.

**Supplementary Fig. S1** Plant height, herbivore and parasitoid presence in eight teosinte populations in Mexico.

**Supplementary Fig. S2** Minimum temperatures measured at a weather station nearby the experimental field.

**Supplementary Fig. S3** Recovery of *Spodoptera frugiperda* larvae.

**Supplementary Fig. S4** *Spodoptera frugiperda* larval weight.

**Supplementary Fig. S5** Correlation between wasp release and larval recovery.

**Supplementary Fig. S6** Parasitism of *Spodoptera frugiperda* larvae, retrieved from the three different teosinte populations, in the presence of *Campoletis sonorensis* wasps.

**Supplementary Fig. S7** Shoot dry weight of plants at first harvest, one week after larval infestations.

**Supplementary Fig. S8** Chromatograms of volatiles collected from herbivore-damaged teosinte plants.

*Funding* - This work was supported by a grant from the Swiss National Science Foundation [grant number 31003A-122132].

*Acknowledgements* - We thank Matthias Held for advice on statistical analysis and Marianne Duployer for help with the analysis of herbivore damage. We thank Víctor Rocha-Ramírez for his valuable support in the field and Yolanda García-Rodríguez for assistance with the chromatographic analysis. We are grateful to George Mahuku and Carlos Muñoz at the International Maize and Wheat Improvement Center (CIMMYT), Mexico, for technical assistance and to Suketoshi Taba for contributing teosinte seeds. Thanks to Gaylord Desurmont and Gaétan Glauser for many useful comments on the manuscript.



## Chapter 6



## Are green leaf volatiles important for parasitoid wasp attraction in the field?

Elvira S. de Lange<sup>1</sup>, Mickaël D. P. Gaillard<sup>1</sup>, Yuanxin Yan<sup>2</sup>, Ted C. J. Turlings<sup>1</sup>, Michael V. Kolomiets<sup>2</sup> and Shawn A. Christensen<sup>2,3,\*</sup>

<sup>1</sup> *Laboratory of Fundamental and Applied Research in Chemical Ecology, Institute of Biology, University of Neuchâtel, Rue Emile-Argand 11, 2000 Neuchâtel, Switzerland*

<sup>2</sup> *Department of Plant Pathology and Microbiology, Texas A&M University, 2132 TAMU, College Station, TX 77843, USA*

<sup>3</sup> *Chemistry Research Unit, US Department of Agriculture, Center for Medical, Agricultural & Veterinary Entomology (CMAVE), 1700 Southwest 23<sup>rd</sup> Drive, Gainesville, FL 32608, USA*

\*e-mail: [shawn.christensen@ars.usda.gov](mailto:shawn.christensen@ars.usda.gov)

Tel: (+1) 352 374 5877, Fax: (+1) 352 374 5707

Based on a publication:

**De Lange, E. S.**, Gaillard, M. D. P., Yan, Y., Turlings, T. C. J., Kolomiets, M. V. and Christensen, S. A. 2013. Are green leaf volatiles important for parasitoid wasp attraction in the field?  
*IOBC-WPRS Bulletin* Vol. 89, p. 291-294





## Abstract

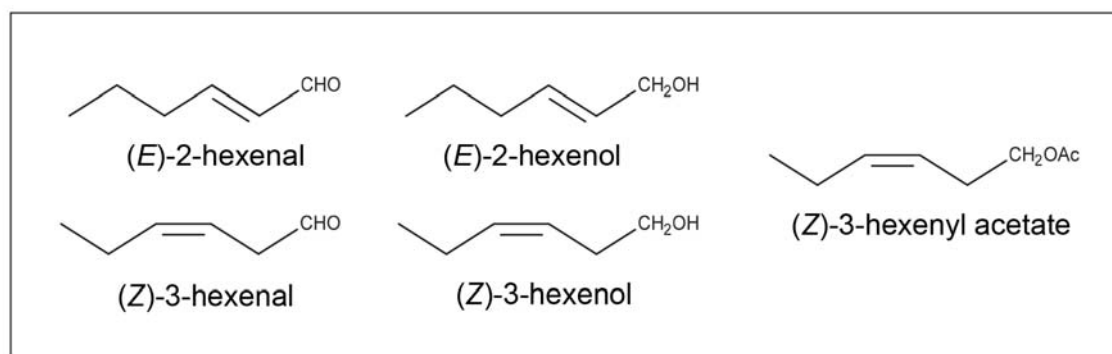
Green leaf volatiles (GLVs) are commonly emitted by plants under herbivore attack. A broad range of laboratory studies have shown that GLVs can be used by predators and parasitoids of these herbivores to find their prey or hosts. The aim of this study was to evaluate the function of GLVs in the attraction of parasitoid wasps to maize plants under field conditions. In an experimental field, we planted maize *lox10* mutants impaired in GLV biosynthesis alongside wildtype plants and *lox8* mutants, which are, similar to *lox10* mutants, impaired in the jasmonic acid pathway, but produce normal levels of GLVs. The plants were infested with herbivorous *Spodoptera frugiperda* larvae, an important pest of maize that abundantly occurs in the area. A week later, plants were harvested and larvae were recollected to determine the degree of parasitism. Overall parasitism levels were low, but contrary to expectations, tended to be highest on non-emitting *lox10* plants, implying that GLVs were not of key importance for parasitoid attraction in the field.

## Key words

*Cotesia marginiventris*, herbivore-induced plant volatiles, herbivore resistance, jasmonic acid, lipoxygenase, *Spodoptera frugiperda*, tritrophic interactions, *Zea mays*,

## Introduction

When attacked by herbivores or otherwise wounded, most green plants immediately emit green leaf volatiles (GLVs; Paré and Tumlinson, 1997; Turlings et al., 1998b). GLVs are six-carbon aldehydes, alcohols and their acetates, which are named after their distinctive “green” scent (Fig. 1; Hatanaka, 1993; Matsui, 2006). GLVs have many ecological functions, but were first studied in the context of host plant location by herbivores (Visser, 1986). GLVs could potentially repel (De Moraes et al., 2001; Piesik et al., 2013), as well as attract (Schütz et al., 1997; Reddy and Guerrero, 2000; Von Mérey, 2010; Allmann et al., 2013; Piesik et al., 2013) insect herbivores. Since then, they have also been implicated in the attraction of natural enemies of herbivores to herbivore-damaged plants (Turlings and Wäckers, 2004), such as parasitoids (Whitman and Eller, 1990; Birkett et al., 2003; Hoballah and Turlings, 2005; Wei et al., 2007) and predators (Kessler and Baldwin, 2001; Allmann and Baldwin, 2010). GLVs also play a role in direct resistance of plants against herbivorous insects (Hildebrand et al., 1993; Vancanneyt et al., 2001) and pathogens (Croft et al., 1993; Shiojiri et al., 2006a; Christensen and Kolomiets, 2011). Furthermore, GLVs are involved in plant-plant communication, where they function in inducing and priming defenses (Arimura et al., 2001; Engelberth et al., 2004; Ruther and Kleier, 2005; Kost and Heil, 2006; Frost et al., 2008b; Shiojiri et al., 2012). For a recent review on the functions of GLVs in plants, see Scala et al. (2013).



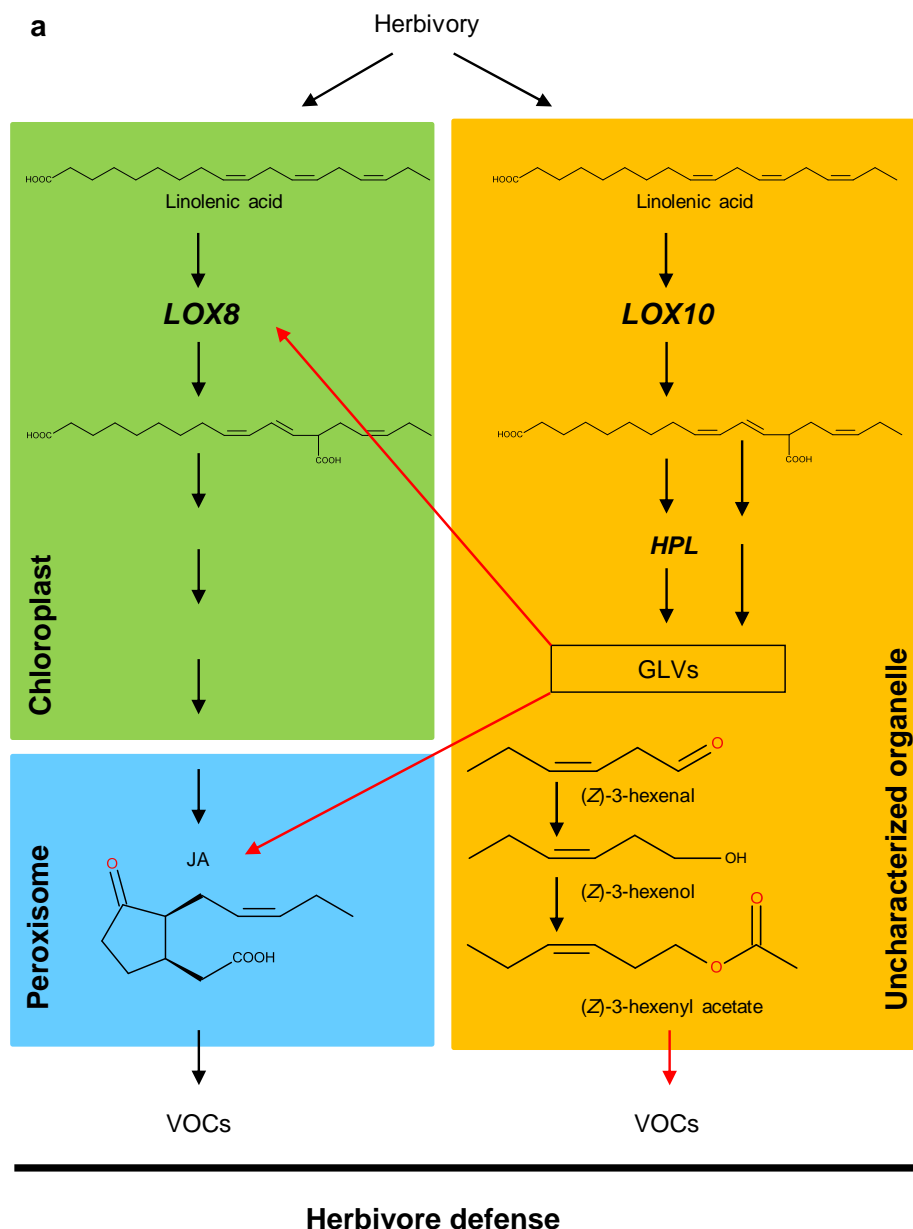
**Fig. 1** Various structures of GLVs that are commonly found in plants. Adapted from Matsui (2006)

The biosynthesis of GLVs is tightly linked to the production of jasmonic acid (JA), a hormone that plays a key role in plant protection against chewing herbivores and is also important for plant growth and development (Fonseca et al., 2009; Wasternack and Kombrink, 2010; Lyons et al., 2013). GLVs and JA are produced from linolenic and linoleic acid with the involvement of lipoxygenases (LOXs), nonheme iron-containing enzymes that catalyze the addition of molecular oxygen to polyunsaturated fatty acids (Porta and Rocha-Sosa, 2002). Some plant species possess different LOX isoforms that are specifically involved in the JA or the GLV branch of the signaling pathway (for an example in maize, see Fig. 2a and Christensen et al. (2013)). Mutations in this pathway that render plants impaired in the herbivore-induced emission of GLVs have been found to diminish attractiveness to parasitoid wasps, as has been shown for *Arabidopsis thaliana* (L.) Heynh. (Shiojiri et al., 2006a; Shiojiri et al., 2006b), Brussels sprouts, *Brassica oleracea* L. (Bruinsma et al., 2010a; Bruinsma et al., 2010b), and recently also for maize, *Zea mays* L. (Christensen et al., 2013).

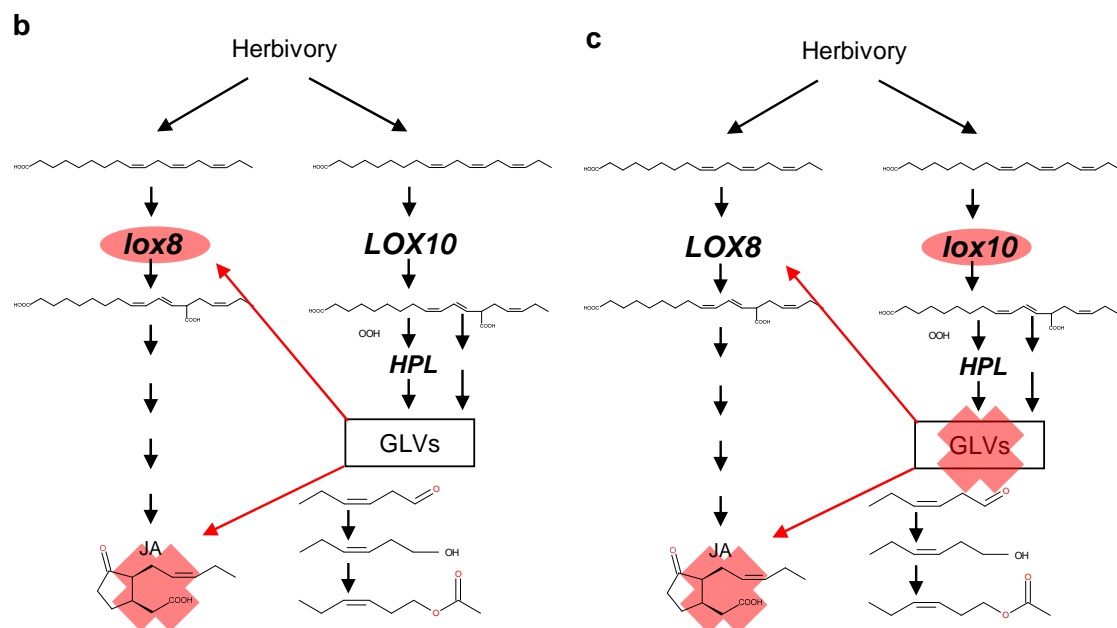
Maize is one of the most studied plants with respect to herbivore-induced volatile emission and parasitoid attraction (Turlings et al., 1990; Turlings and Fritzsche, 1999). Undamaged, healthy maize plants are practically odorless, whereas plants that are under caterpillar attack release large quantities of a blend of predominantly GLVs, terpenoids and some aromatic compounds (Turlings et al., 1991; Turlings et al., 1998b). This blend is highly attractive to various species of parasitoids (Tamò et al., 2006a). However, the key compounds that are responsible for the genetically fixed attraction of parasitoids have not yet been identified (D'Alessandro and Turlings, 2006). Various laboratory studies have indicated an important role of GLVs in wasp attraction to herbivore-attacked maize plants (Gouinguené et al., 2005; Hoballah and Turlings, 2005). A recent study characterizing maize *lox10* mutants, impaired in GLV biosynthesis, provides further indications of the importance of GLVs in attracting parasitoids, as these mutants were less attractive to *Cotesia marginiventris* Cresson (Hymenoptera: Braconidae) in olfactometer assays (Christensen et al., 2013). Nevertheless, the ecological relevance of GLVs in a field setting with respect to the attraction of natural enemies is still unclear. As they are emitted by a wide range of plant species and not only in response to herbivory, but also as a result of mechanical damage (Matsui, 2006), they might not represent the most reliable signal to find a suitable prey or host. There are a few examples of GLV attractiveness to predators in the

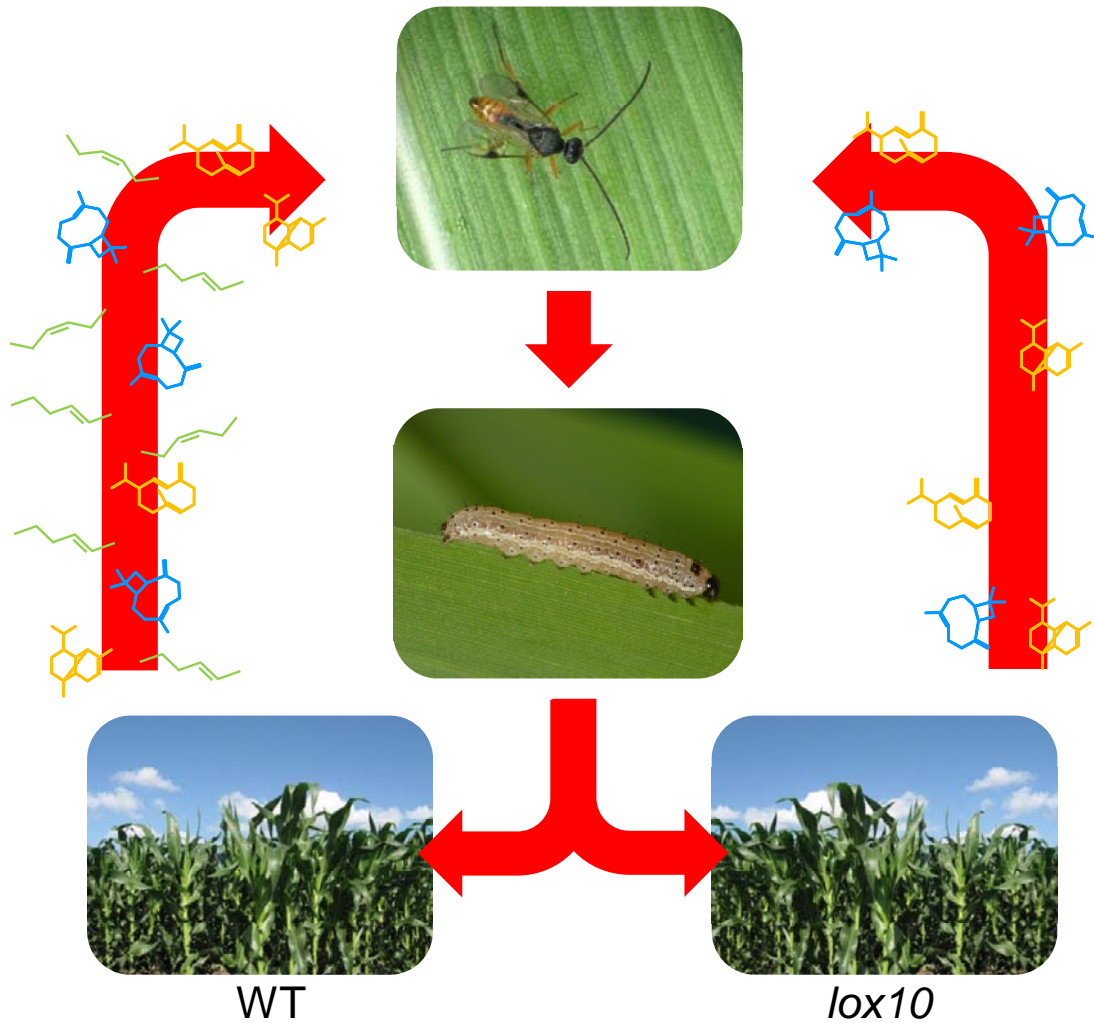
field, in a hop yard (James, 2003) and a wild tobacco population (Kessler and Baldwin, 2001). But attraction of predators and parasitoids seems to be dependent on the species studied and specific environmental conditions, as neutral and repellent effects of synthetic GLVs have also been observed (James and Grasswitz, 2005; Yu et al., 2008; Simpson et al., 2011). Interestingly, a recent study with transformed tobacco plants convincingly showed that plants silenced in the production of GLVs had less predation of herbivores and produced fewer flowers as a result (Schuman et al., 2012). However, a field study where maize plants were supplemented with synthetic GLVs that were released from dispensers did not show an increase in attraction of parasitoids and there was no significant effect on plant fitness (Von Mérey et al., 2011).

The aim of the current study was to evaluate the importance of GLVs released by maize plants for the attraction of parasitoid wasps under field conditions (Fig. 3). We hypothesized that *lox10* mutants, that because of their reduced GLV emission showed diminished parasitoid attraction in the laboratory (Christensen et al., 2013), would be less attractive to parasitoid wasps in a field setting. To test this hypothesis, we planted maize *lox10* mutants alongside wildtype plants and *lox8* mutants, which are, similar to *lox10* mutants, impaired in the jasmonic acid pathway, but emit normal quantities of GLVs, in an experimental field. The lepidopteran pest *Spodoptera frugiperda* Smith (Lepidoptera: Noctuidae) and several of its natural enemies, among which the parasitoid *C. marginiventris*, are frequently recorded in this area (Pair et al., 1986a,b). Plants were infested with *S. frugiperda* larvae and harvested a week later in order to assess several characteristics of plant performance. The larvae were retrieved from the harvested plants and parasitism was assessed. Our results indicate no difference in parasitoid attraction between GLV-emitting and non-emitting *lox10* plants, although the total number of parasitized larvae was too low to draw solid conclusions.



**Fig. 2** The JA and GLV biosynthesis pathways in maize. (a) The transcripts of both *LOX8* and *LOX10* are involved in metabolizing linolenic and linoleic acid, and although there is crosstalk between the pathways, *LOX8* is mainly involved in JA biosynthesis while *LOX10* plays a major role in GLV production. GLV and JA biosynthesis occur in separate and distinct organelles. (b) *lox8* Mutants are impaired in JA biosynthesis, but GLV production is not affected. (c) *lox10* Mutants are impaired in both GLV and JA biosynthesis. Genes are characterized in bold. *LOX* = lipoxygenase; *HPL* = hydroperoxide lyase; GLV = green leaf volatile. Black arrows indicate known mechanisms and red arrows designate hypothetical pathways. Adapted from Christensen et al. (2013)

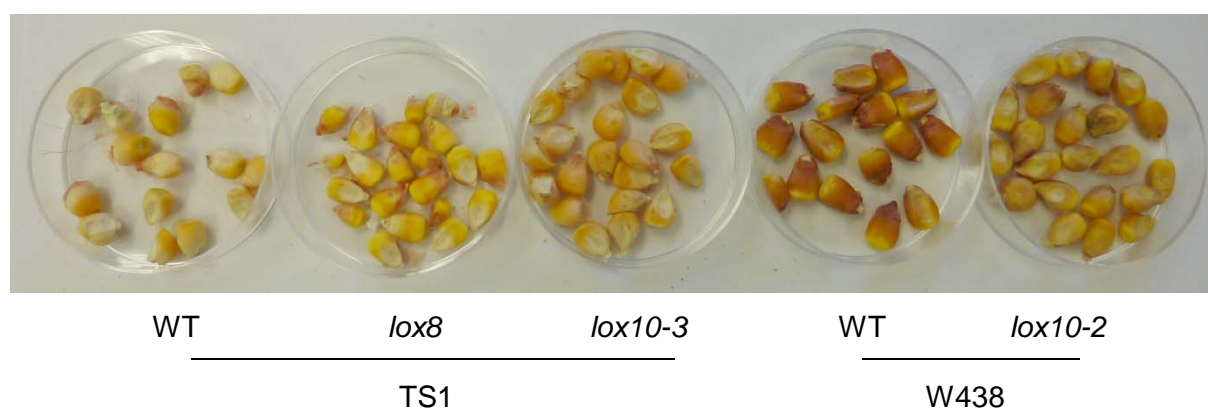




**Fig. 3** Schematic representation of the study system. In response to herbivory by leaf-chewing herbivores, wildtype (WT) maize plants emit an odor blend that is attractive to the natural enemies of the herbivores, parasitoid wasps. A maize mutant (*lox10*) deficient in the production of green leaf volatiles (GLVs) emits similar odors as WT plants, except it does not emit GLVs. We tested the attractiveness of WT and *lox10* plants for parasitoid wasps in a field setting. Pictures of herbivore and parasitoid wasp: Matthias Held

## Methods and Materials

**Plants** Field experiments were performed with wildtype and mutant maize plants of two genetic backgrounds (*Z. mays* ssp. *mays* variety TS1 and variety W438; Fig. 4). Plants with mutations in the *ZmLOX10* gene (*lox10-3* in TS1 genetic background and *lox10-2* in the W438 genetic background) were derived from the *Mutator*-transposon insertional genetics resource at Pioneer Hi-Bred (Gao et al., 2007; Christensen et al., 2013). Plants with mutations in the *LOX8* gene (*lox8* in TS1 genetic background) were derived from a population with a spontaneous mutation (Acosta et al., 2009). These plants have deformed sexual structures (Fig. 5). The *lox10* mutants in both genetic backgrounds are impaired in GLV biosynthesis as well as JA biosynthesis (Fig. 2c). The *lox8* mutant, which is only impaired in JA biosynthesis, was included to account for effects not due to the lack of GLVs, but rather the decrease in other herbivore-induced plant volatiles regulated by JA (Fig. 2b).



**Fig. 4** Seeds of the different plant genotypes

Seeds were sown in plastic pots (3.8 cm diameter, 14 cm high) with commercial soil (Metro-Mix 366; Scotts-Sierra Horticultural Products, Marysville, OH, USA). Three batches of seeds were sown on August 11, 18 and 25, 2011, respectively. All plants were kept at 25-28°C under a 12 hour photoperiod ( $120 \mu\text{mol m}^{-2} \text{s}^{-1}$ ; Quantum Meter; Apogee Instruments, Logan, UT, USA). As the *lox8* mutant was obtained as a segregating population, individual plants were visually inspected as well as genotyped with gene specific primers to confirm the presence of the mutation (P1767: CTAGTCCACCGGCGATTGTG; P1768: ACGATGATTCGGTAAGATCGATG).

When about 10 days old, the plants were transplanted to a field located near Navasota, Texas, USA (N 30°16'59.44", W 96°06'15.68"; Fig. 6). The field was divided into 4 rows and 10 plots. In every plot, 16 plants were planted of every genotype, with a total of 32 plants per genotype per trial (Fig. 7). There was 30 cm distance between plants in a row and 60 cm between rows. Sweetcorn was planted around the experimental field. Plants were regularly irrigated and fertilized and weeds were removed manually.



**Fig. 5** The *lox8* mutant with deformed sexual structures (a,b)

*Insects* Eggs of *S. frugiperda* (Lepidoptera: Noctuidae) were incubated at room temperature and larvae were reared on artificial diet until they had reached the second instar.

*Insect Treatments* One night prior to infestation, 25 second-instar larvae were placed in an Eppendorf tube containing a piece of a wildtype maize leaf. Each plant was infested with the content of one Eppendorf tube. The larvae were applied into the whorl with a brush. Plants in the first trial were used as an uninfested control. Plants in the second and the third trial were infested on September 20 and September 28, 2011, respectively. By then, the plants were 34-35 days old, with 5-8 developing leaves.



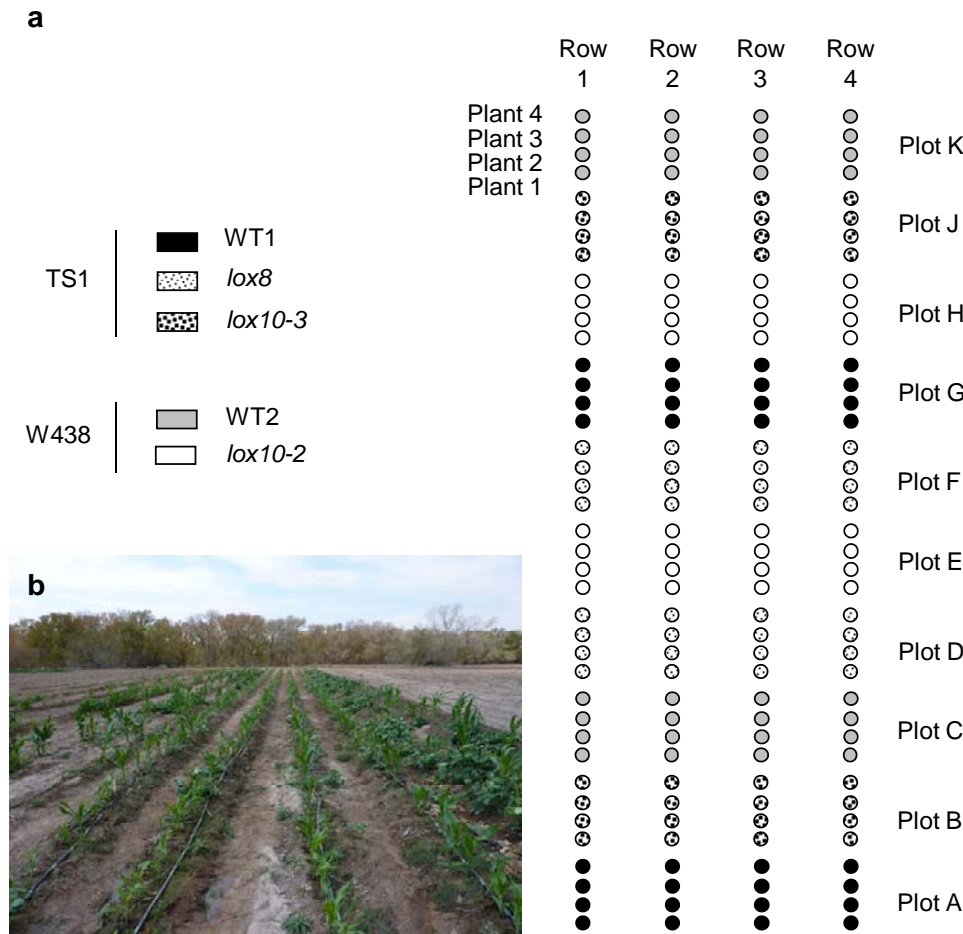


**Fig. 6** Approximate field location. The field was located in Texas, USA, near Navasota

*Measures of Plant Growth and Herbivory* Six days after larval infestation, all plants were measured from the stem base to the tip of the longest leaf. Herbivore damage was visually estimated on a 0-3 scale (with 0 being no or very minor damage and 3 major damage). Afterwards, every second plant in each row was harvested, placed in a paper bag, and transported to the laboratory. While recovering the larvae, plant material was separated into leaf and stem and placed in individual paper bags. The bags were left to dry and the content weighed to determine dry shoot biomass (stem + leaf biomass).

*Measures of Larval Parasitism* In the laboratory, *S. frugiperda* larvae were retrieved from the harvested shoots. The number of larvae feeding on each plant was counted and individual larvae were weighed. The larvae were subsequently placed in individual wells of a 24-well ELISA plate, supplied with artificial diet and reared at room temperature until adult emergence or, in the case of parasitism, the emergence of parasitoids (Hoballah et al., 2004). The adult parasitoids, dead parasitoid larvae and cocoons were individually preserved in 70% ethanol in Eppendorf tubes. The wasps were visually examined to identify the species (Cave, 1995). Parasitoid pupae were identified based on the adults that emerged from identical pupae.

*Measures of Final Shoot Dry Biomass* On November 8, 2011, frost killed the remaining plants and ended the growing season before the ears were mature. In addition, *lox8* mutants produce feminized tassels (Fig. 5; Acosta et al., 2009), rendering fitness determination in terms of seed production unreliable. As seed production could not be taken as a measure of plant fitness, we determined final shoot biomass, a factor highly correlated to plant fitness (Hoballah and Turlings, 2001). Aboveground plant tissue was harvested in paper bags and stem and leaf material was separated for every plant. All material was left to dry, after which dry shoot biomass (stem + leaf biomass) was determined.

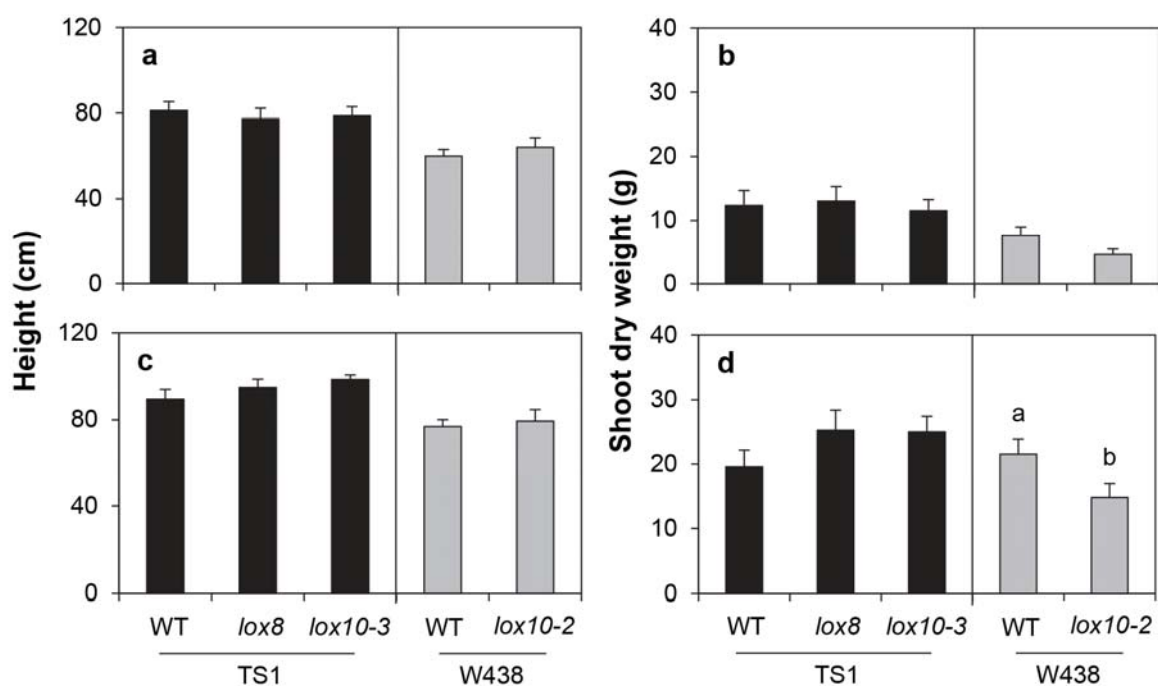


**Fig. 7** Schematic representation of the field set-up. (a) The field was divided into four rows and ten ranges. In every range, 16 seeds of every plant genotype were planted, with a total of 32 plants per genotype. There was 30 cm distance between plants in a row and 60 cm between rows. Three experimental blocks were planted, and sweetcorn was planted around the experimental field. (b) An overview of the experimental field

**Statistical Analysis** Data of wildtype plants and *lox* mutants in TS1 and in W438 genetic background were analysed separately. Differences in height, biomass, larval weight and herbivory between plants in TS1 background were compared using one-way analysis of variance, post-hoc Tukey test (when normally distributed) or Kruskal-Wallis one-way analysis of variance on ranks, post-hoc Dunn's method (when normality test failed). For plants in W438 background, the above-mentioned differences were compared using Student's t-test (when normally distributed) or Mann-Whitney rank sum test (when normality test failed). Differences in parasitism were compared using chi-square tests. All analyses were performed with SigmaStat version 2.0 (Systat Software, San Jose, CA, USA).

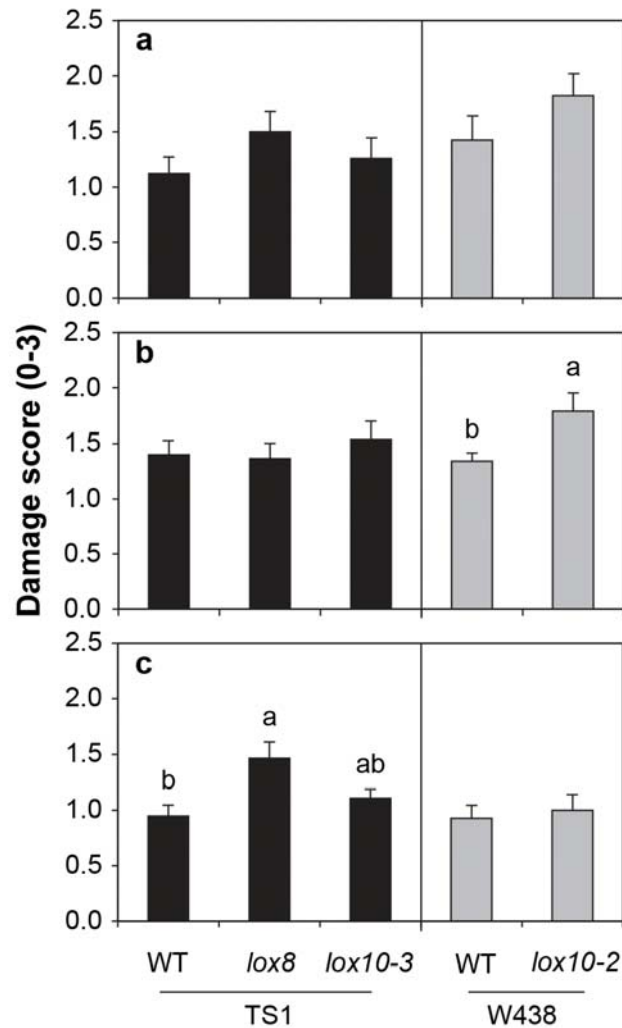
## Results

**Plant Growth** Six days after larval infestations, no differences in plant height were observed between *lox10* and *lox8* mutants and their corresponding wildtypes ( $P > 0.05$ ; Fig. 8a,c). In TS1 background, there was no difference in shoot biomass between wildtype and mutant plants ( $P > 0.05$ ; Fig. 8b,d). In W438 background, there was a trend towards a lower biomass for the *lox10-2* mutant in the second trial ( $P = 0.091$ ; Fig. 8b). The *lox10-2* mutant had a significantly lower biomass than its corresponding wildtype in the third trial ( $P = 0.045$ ; Fig. 8d). These differences were mainly due to variations in leaf biomass ( $P = 0.05$  and  $P = 0.011$  in the 2<sup>nd</sup> and the 3<sup>rd</sup> trial, respectively); the amount of stem biomass was similar for wildtype and *lox10-2* mutants ( $P > 0.05$ ; data not shown).



**Fig. 8** Plant growth characteristics. Plant height in the second (a) and third (b) trial and dry shoot biomass (stem + leaves) in the second (c) and third (d) trial. Measurements were taken six days after infestation with *Spodoptera frugiperda* larvae. Different letters indicate significant differences between plant genotypes in W438 background (grey bars) ( $P < 0.05$ )

**Herbivory** In the first trial, where plants were not manually infested with larvae, there were no significant differences in herbivore damage between *lox10* and *lox8* mutants and their corresponding wildtypes ( $P > 0.05$ ; Fig. 9a). In the second and third trials, where plants were manually infested with *S. frugiperda* larvae, non-consistent differences in herbivory were observed. In the W438 background, *lox10-2* manifested a higher damage score than its corresponding wildtype in the second trial ( $P = 0.014$ ; Fig. 9b), but not in the third trial ( $P > 0.05$ ; Fig. 9c). In TS1 background, no differences were observed in the second trial ( $P > 0.05$ ; Fig. 9b), while *lox8* manifested a higher damage score than its corresponding wildtype in the third trial ( $P = 0.013$ ; Fig. 9c).

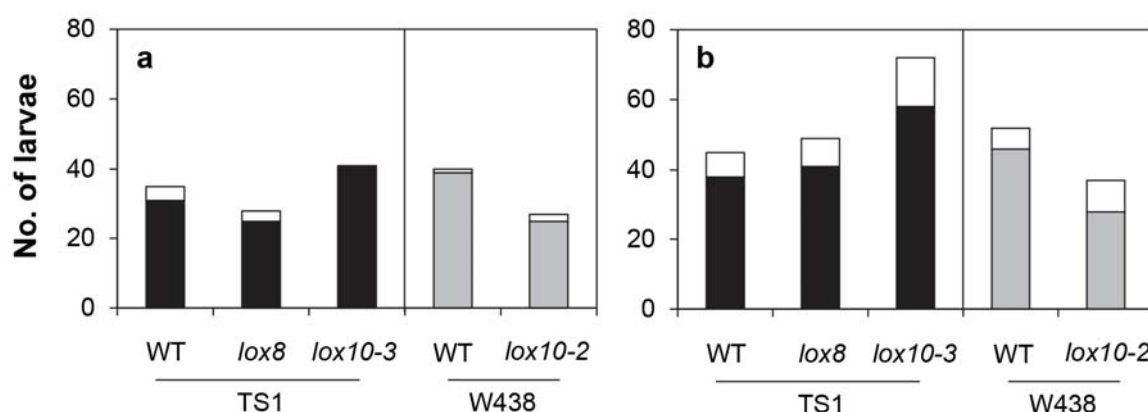


**Fig. 9** Herbivory. Estimated damage in the first trial, where plants were not artificially infested by *Spodoptera frugiperda* larvae (a) and in the second (b) and third (c) trial, one week after manual infestation with 25 second-instar larvae per plant. Damage was estimated on a 0-3 scale. Different letters indicate significant differences between plant genotypes in TS1 background (black bars) or W438 background (grey bars) ( $P < 0.05$ )

**Parasitism** We retrieved 14% and 11% of the larvae that were released in the second and the third trial, respectively, approximately corresponding to 3 larvae per plant. Average larval weight was not significantly different when larvae were retrieved from wildtype or mutant plants ( $P > 0.05$ ; data not shown). When rearing the retrieved larvae on artificial diet, on average 5% and 16% in the second and the third trial, respectively, turned out to be parasitized. There were no significant differences in parasitism of larvae retrieved from wildtype and mutant plants, neither in the second (Sample size too low to allow for statistical testing; Fig. 10a) nor in the third ( $P > 0.05$ ; Fig. 10b) trial.

The main parasitoid in the second trial was the generalist egg-larval endoparasitoid *Chelonus* spp. (Hymenoptera: Braconidae), probably *Chelonus insularis* Cresson and *Chelonus sonorensis* Cameron. In the third trial, the most frequent parasitoid was the

generalist larval endoparasitoid *C. marginiventris* (Table 1), for which it is known that it is strongly attracted to herbivore-induced maize volatiles (Turlings et al., 1990).



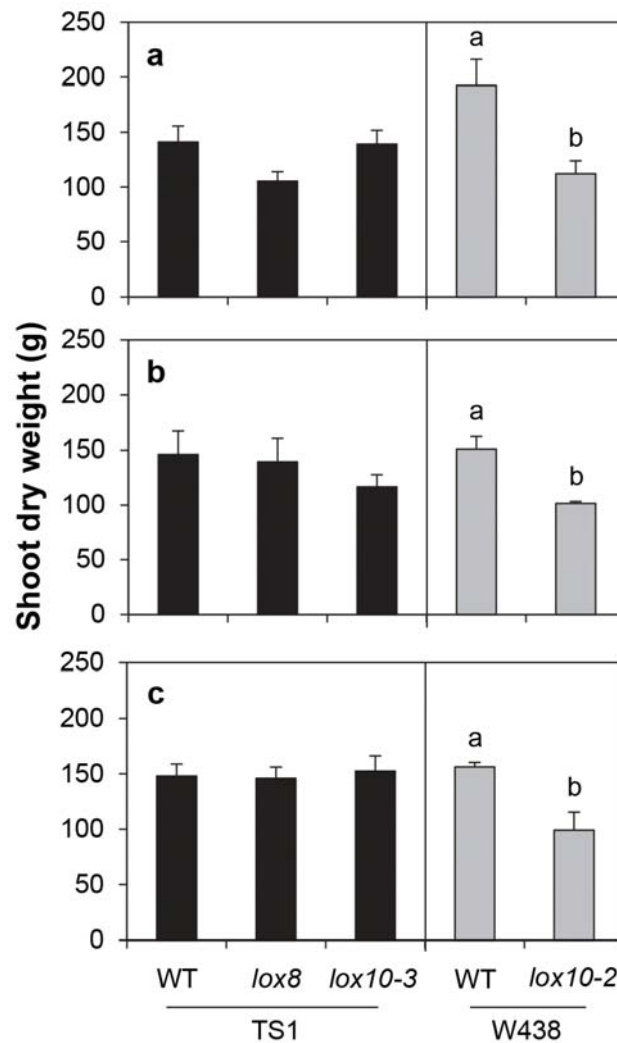
**Fig. 10** Parasitism. Total amount of *Spodoptera frugiperda* larvae recaptured from plants in the second (a) and the third (b) trial. Filled bars indicate non-parasitized larvae, while blank bars indicate parasitized larvae. Sample size in (a) was too low to allow statistical testing and no significant differences were observed in (b) ( $P > 0.05$ )

**Table 1** Total number of parasitoids that emerged from collected *S. frugiperda* larvae

Trial	Species	TS1			W438	
		WT	<i>lox8</i>	<i>lox10-3</i>	WT	<i>lox10-2</i>
2	<b>Total parasitism</b>	<b>4</b>	<b>3</b>	<b>0</b>	<b>1</b>	<b>2</b>
	<i>Cotesia marginiventris</i> *	1	0	0	0	0
	<i>Chelonus</i> spp.*	3	1	0	1	1
	Unknown	0	2	0	0	1
3	<b>Total parasitism</b>	<b>7</b>	<b>8</b>	<b>14</b>	<b>6</b>	<b>9</b>
	<i>Cotesia marginiventris</i> *	6	8	13	3	9
	<i>Chelonus</i> spp.*	0	0	0	1	0
	Unknown	1	0	1	2	0

\*Hymenoptera: Braconidae

**Final Shoot Dry Biomass** Near the end of their life cycle, plant shoots were harvested and final shoot dry biomass was taken as a measure for plant fitness (Hoballah and Turlings, 2001). In TS1 background, no significant differences in dry weight were observed between wildtype plants and *lox* mutants, neither in the first trial for which plants were not infested with larvae ( $P > 0.05$ ; Fig. 11a), nor in the second and third trials for which plants had been manually infested with larvae ( $P > 0.05$ ; Fig. 11b,c). In W438 background, *lox10-2* plants had a lower biomass than its corresponding wildtype, in the first ( $P = 0.012$ ; Fig. 11a), second ( $P = 0.027$ ; Fig. 11b) and third ( $P = 0.001$ ; Fig. 11c) trials. These differences were mainly due to variations in leaf biomass; ( $P = 0.009$ ,  $P = 0.018$  and  $P < 0.001$  in the 1<sup>st</sup>, 2<sup>nd</sup> and the 3<sup>rd</sup> trial, respectively); the amount of stem biomass was similar for wildtype and *lox10-2* mutants ( $P > 0.05$ ; data not shown).



**Fig. 11** Final shoot dry weight. Dry shoot biomass (stem + leaves) of plants in the first trial, not artificially infested by *Spodoptera frugiperda* larvae (a), and in the second (b) and third (c) trial, where plants were manually infested with larvae when five weeks old. Measurements were taken near the end of plant development, when plants were approximately 3 months old, after all plants died of freezing. Different letters indicates significant differences between plant genotypes in W438 background (grey bars) ( $P < 0.05$ )

## Discussion

Our results did not provide any evidence for reduced parasitoid attraction by GLV-impaired maize mutants, contrary to what has previously been observed in a laboratory experiment (Christensen et al., 2013). On the other hand, our results are in accordance with the findings by Von Mérey et al. (2011), who observed no enhanced parasitism of lepidopteran larvae on maize plants supplemented with synthetic GLVs. However, a recent study on wild tobacco plants, modified to be impaired in GLV production, reported that herbivory on these plants increased due to reduced predation. This also resulted in a reduction in flower production (Schuman et al., 2012). As the numbers of parasitoid wasps collected from the plants in our study were very low, we will have to repeat the experiment in order to confirm the apparent insignificance of GLVs as attractants for parasitoids under the tested field conditions.

*S. frugiperda* is a major pest of maize in the Americas (Luginbill, 1928; Kranz et al., 1977). In Texas, populations are especially abundant in September and October, by the time the experiment was performed (Pair et al., 1986b). We therefore expected that the natural enemies of this species would be present in the experimental field. Indeed, we encountered parasitoid wasps: *C. marginiventris* and *Chelonus* spp. (Pair et al., 1986a). As *Chelonus* spp. mainly parasitizes insect eggs, it might not be specifically attracted to herbivore-induced GLVs. However, *C. marginiventris*, which infests small larvae, preferred wildtype plants over GLV-impaired mutants in laboratory olfactometer experiments (Christensen et al., 2013), raising the expectation that this species would be preferentially attracted to wildtype plants in a field setting.

It should be noted that the laboratory tests were performed with naïve wasps that did not have any experience with a plant or a host before. Under field conditions, it can be reasonably assumed that wasps are not naïve, but rather have encountered a broad range of plant odors and possibly hosts. *C. marginiventris* is able to learn to associate a certain odor to the presence of a suitable host (Tamò et al., 2006a). Although the species is innately attracted to an odor blend high in GLVs, an experience with a suitable host can change its preference towards plants emitting a different odor blend (Hoballah and Turlings, 2005). It would therefore be worthwhile to test the attractiveness of *lox10* mutants to experienced wasps in an olfactometer, in order to shed light on the importance of GLVs for parasitoid host localization.

Our experimental field was an irrigated green oasis in an otherwise dry and empty landscape, which attracted a variety of different arthropods (Elvira S. de Lange and Mickaël D.P. Gaillard, personal observations). As plant volatile emission varies with environmental conditions, biotic (Rasman and Turlings, 2007) as well as abiotic (Gouinguéné and Turlings, 2002), it is not unthinkable that neither wildtype nor mutant plants emitted high amounts of parasitoid-attracting compounds under the given conditions, which could explain the absence of high numbers of parasitoids. Although this scenario is highly unlikely, measuring plant volatile emission could have provided a clue as to the amounts of odors that were emitted in response to *S. frugiperda* attack.

The field experiment has been performed with two maize *lox10* mutants in different genetic backgrounds. No consistent phenotype has been observed for the *lox10* mutants compared to their respective wildtypes in terms of plant growth, resistance to herbivory, and final biomass accumulation (Fig. 8,9,11), indicating that the strength of the mutation depends considerably on the genetic background.

Furthermore, the fact that the *lox10* mutants were not only impaired in GLV biosynthesis, but also in JA biosynthesis, presumably had a confounding effect on plant growth characteristics. Plants with a mutation in the HPL gene would allow for testing of the effects of GLVs alone, but, to date, such mutants are only available in *Arabidopsis* (Shiojiri et al., 2006a) and tobacco (Kessler et al., 2004), but not maize. Previous field studies with maize *lox10* and *lox8* mutants (Christensen et al., 2013), as well as studies with transformed tobacco plants (Kessler et al., 2004) have reported a remarkable decrease in herbivore

resistance in plants with a mutation in the JA signaling pathway. Although *lox10-2* mutants had a higher damage score than wildtype plants in the second trial and *lox8* mutants had a higher damage score than their corresponding wildtype in the third trial, results that correspond with these previous findings, we did not find consistent evidence for a JA-mediated reduction of herbivory (Fig. 9). It is interesting to note, however, that a considerable quantity of *lox10-2* mutant plants suffered natural mortality ahead of manual larval infestations, indicating that the lack of JA-dependent defenses severely impairs plant fitness (Elvira S. de Lange and Mickaël D.P. Gaillard, personal observations).

In addition, the *lox10-2* mutant had a lower biomass than its corresponding wildtype, both after 6 weeks of growing (Fig. 6) and near the end of its development (Fig. 11). Unfortunately, plant fitness in terms of seed production could not be determined as all plants had frozen to death before the kernels were mature. Furthermore, *lox8* mutants develop deviant sexual structures; the tassel or male inflorescence develops female features (*tasselseed1* or *ts1* mutation; Acosta et al., 2009) with an expected fitness cost. However, final biomass accumulation is an adequate measure for plant fitness in maize (Hoballah and Turlings, 2001). A difference in final shoot biomass between *lox10-2* and wildtype plants was observed both in the presence (Fig. 11a) and the absence (Fig. 11b,c) of extensive *S. frugiperda* herbivory, indicating that there might be a fitness cost associated with the mutation, although this phenomenon has not been observed during earlier field seasons (Shawn A. Christensen, personal communication). As the summer and fall of 2011 have been remarkably hot, a fitness cost of the *lox10-2* mutation might only present itself under challenging conditions. These observations stress the need to grow wildtype and mutant plants under controlled conditions to evaluate the impact of a mutation in the GLV pathway on plant growth and development.

In conclusion, we did not find evidence for reduced parasitoid wasp attraction of GLV-impaired maize plants in the field, although parasitoid sample size was too low to completely exclude this possibility. It has been proposed that genetically modifying crops to enhance GLV-emissions is a potential way to combat insect pests (Shiojiri et al., 2006a), although the authors recognize the necessity of tests on a large spatial scale under natural conditions. We concur a need for further field experiments to establish the importance of GLVs for parasitoid attraction under natural circumstances and to fully determine their potential use in biological control.

*Acknowledgements* - We thank Eli Borrego, Ramadhika Damarwinasis, Gerald DelaFuente, Travis Gordon, Sydney Hermione, Sarah Keith, Chase Perry, Gretta Sharp and Cassie Warren for technical assistance. We are grateful to Benzon Research (Carlisle, PA, USA) for the shipments of *S. frugiperda* eggs. This work was supported by a grant from the Swiss National Science Foundation (grant 31003A-122132).



## General discussion

Plants are subject to attack by a myriad of herbivores, which has prompted the question – why is the world still green? Plant physical and chemical defenses (“bottom up” regulation), as well as predators and parasitoids (“top down” regulation) are important in keeping the herbivore population in check (Ode, 2006). Plants can emit VOCs in response to an herbivore attack, which is regarded as an indirect defense trait, as these VOCs can provide parasitoids and predators with cues to find and kill herbivorous hosts and prey (Turlings et al., 1990; Turlings and Wäckers, 2004). Maize plants are especially responsive to herbivory and when attacked by caterpillars, they emit copious amounts of odors that are highly attractive for various species of parasitoid wasps (e.g. Turlings et al., 1990; Tamò et al., 2006a). Presently, maize is cultivated all over the world, but its domestication started in subtropical Mexico, where its wild ancestor, teosinte, still grows nowadays (Sánchez González and Ruiz Corral, 1995; Matsuoka et al., 2002). Reportedly, man’s objective to domesticate plants to improve nutritive quality and yield has had consequences for the defenses of crop plants against pathogens and pests (Wink, 1988; Rosenthal and Dirzo, 1997; Dávila-Flores et al., 2013). In this thesis, the importance of herbivore-induced plant volatiles was explored for the attraction of parasitoid wasps, using maize and teosinte as model plants. The capacity of various maizes and teosintes was compared to emit VOCs and attract parasitoids, and the potential benefits of VOC emission for plant growth and survival were studied.

### Do maize and teosinte differentially attract parasitoid wasps?

Despite the fact that we did not identify a large difference in the VOC emission profiles of maize and teosinte upon herbivory by lepidopteran herbivores, important pests of maize, the parasitoid wasps *Cotesia marginiventris* and *Campoletis sonorensis* responded in a distinct way to the odor blends (Chapters 3, 4). *C. marginiventris* preferred the odors of teosinte over those of maize, while *C. sonorensis* did not discriminate between the odor blends. This indicates that the two wasp species orient towards different compounds in the odor blends, but also suggests that maize and teosinte differ in the release of behaviorally active compounds. For *C. marginiventris*, this could involve so-called “key compounds” that yet have to be identified (D’Alessandro et al., 2009). A “key compound” for the attraction of insect-killing nematodes in the soil in response to feeding by a root herbivore is (*E*)- $\beta$ -caryophyllene (Rasmann et al., 2006). Most American maize varieties have lost the ability to emit this compound (Köllner et al., 2008). Similarly, during domestication, modern maize might have lost part of its capacity to optimally attract *C. marginiventris* wasps (Chapters 3, 4). However, we have assessed the wasp attractiveness of solely one European maize variety and two teosintes. It is known that VOC emissions can be highly variable among maize varieties or inbred lines, as well as different teosintes, both in the total amounts released and in the pattern of individual compounds in the whole odor blend, which is reported for shoots (Gouinguéné et al., 2001; Degen et al., 2004; Erb et al., 2011a) and for roots

(Rasmann et al., 2005; Köllner et al., 2008). For other plants, like the ornamental crop *Gerbera jamesonii* Bolus (Asteraceae) (Krips et al., 2001) and cucumber (*Cucumis sativus* L., Cucurbitaceae) (Kappers et al., 2010, 2011), considerable variability has been identified between varieties in herbivore-induced odor emission, which translated into differential attraction of predatory mites. Also, inbreeding has severely influenced VOC emission and attraction of natural enemies in horsenettle plants (*Solanum carolinense* L., Solanaceae) (Kariyat et al., 2012). The attractiveness of VOCs emitted by a broader range of teosintes and maize varieties should be assessed in order to draw more robust conclusions about the behavioral activity of VOCs in the crop and its wild ancestor.

Although herbivore-induced plant odors, as reliable cue for the presence of hosts, were attractive to parasitoid wasps in olfactometer bioassays (Chapters 3, 4), host finding is no guarantee for host acceptance and successful development of a parasitoid wasp (Vinson 1976). Caterpillars may employ various ways to defend themselves against parasitoid wasps. Behavioral adaptations include fending off the wasp by head jerking, secreting saliva and attempting to bite (Gross, 1993), as well as “bungee-jumping” off the plant in order to escape (Luginbill, 1928; Perović et al., 2008). Lepidopteran hosts, including *Spodoptera frugiperda* and *S. littoralis*, can also successfully encapsulate parasitoid eggs or larvae, thereby preventing the wasps from developing (Prévost et al., 1990; Hegazi et al., 1991; Stettler et al., 1998). To avoid this, wasps like *C. sonorensis* can inject a virus with the egg when ovipositing in order to reduce the larval immune system (Edson et al., 1981; Li and Webb, 1994; Luckhart and Webb, 1996). Interestingly, some insects can cure themselves of a parasite by “medicating” themselves with toxic plant material (de Roode et al., 2013). Woolly bear caterpillars, *Grammia incorrupta* Edwards (Lepidoptera: Arctiidae), actively increase their ingestion of plant toxins when infested by parasitoid flies (Singer et al., 2009), which increased caterpillar survival. Similarly, larvae of the fruit fly *Drosophila melanogaster* Meigen (Diptera: Drosophilidae) selectively consume rotting fruit with a higher ethanol content when parasitized, which serves as an effective medicine (Milan et al., 2012). The other way around, certain parasitoid flies may affect the food choice of their lepidopteran hosts, which potentially improves the flies’ fitness (Karbon and English-Loeb, 1997). We observed that the host plant could have a slight, but significant, influence on wasp development; weight of *S. frugiperda* larvae parasitized by *C. sonorensis* was higher when feeding on maize than when feeding on teosinte, and *C. sonorensis* cocoons were larger when their host had fed on maize (Chapter 4). It would therefore be interesting to test for host plant effects on the behavior and encapsulation capacity of the herbivore larvae. Maize and teosinte commonly co-occur in Central America. It would also be worthwhile to assess the preference of parasitized and healthy larvae for maize or teosinte as a food source in a choice experiment.

### **Do plants benefit from VOC emission?**

In a semi-field experiment with large tents in Mexico, we assessed the growth and survival of teosinte plants in the presence or absence of *S. frugiperda* herbivores and *C. sonorensis*

wasps, three organisms that have a long evolutionary history together and nowadays still co-occur (Chapter 2). The notion that plants may benefit from recruiting parasitoid wasps is highly controversial, as there has thus far been limited evidence from field experiments for a beneficial effect of the presence of natural enemies, especially in the light of parasitoid wasps, which do not immediately kill their hosts (Allison and Hare, 2009; Agrawal, 2011; Hare, 2011; Kessler and Heil, 2011). We found new evidence that the presence of parasitoid wasps is beneficial for young teosinte plants (Chapter 5), which may help to settle the discussion on the defensive function of herbivore-induced volatiles, which has received considerable attention lately (Kaplan, 2012b; Schuman et al., 2012).

To assess the benefits of individual VOCs for plant growth, we also studied maize *lox10* mutants, compromised in the emission of GLVs, in an open field experiment in Texas, USA. While laboratory experiments convincingly showed that a lack of GLVs diminishes plant attractiveness to the parasitoid wasp *C. marginiventris* (Christensen et al., 2013), we did not observe this phenomenon in the field, nor could we see an effect of herbivore-induced GLV emission on final plant biomass (Chapter 6). We recognize a need for further field experiments to establish the importance of GLVs for parasitoid attraction under natural circumstances, as well as the importance of other individual VOCs and VOC blends. Herbivore-induced VOCs may not only attract parasitoids, they may also attract other herbivores, with potential negative effects for the emitting plant (Carroll et al., 2006; Von Mérey et al., 2013). To make matters even more complex, it was recently discovered that hyperparasitoids, the natural enemies of parasitoids, use herbivore-induced VOCs to locate their hosts (Poelman et al., 2012). These members of the fourth trophic level may be an important mortality factor for parasitoids, so attracting hyperparasitoids may counteract the potential beneficial effects of VOC emission for plants. Additional studies of the plant-associated community, at multiple trophic levels, are necessary to establish the importance of VOC emission for plants. Such studies may advance the use of parasitoids as biological control agents.

### **Manipulating plant VOC emission to enhance biological control**

Use of parasitoids, predatory insects, predatory mites and entomopathogenic nematodes as biological control agents is a key aspect of integrated pest management (IPM), a strategy of sustainable pest control (Ehi-Eromosele et al., 2013). As mentioned in the introduction, strengthening natural plant immunity is considered highly valuable for sustainable crop protection (e.g. Turlings and Ton, 2006; Bruce, 2010; Gurr et al., 2012). Manipulating plant VOC emission is a promising way to increase the effectiveness of natural enemies as biological control agents (e.g. Pickett et al., 2006; Khan et al., 2008; Szendrei and Rodriguez-Saona, 2010; War et al., 2011; Peñafior and Bento, 2013; Rodriguez-Saona et al., 2013; Sobhy et al., 2013, in press).

Spraying VOCs on plants or releasing them in dispensers could potentially increase the attractiveness of plants to natural enemies, although this is not guaranteed (Szendrei and Rodriguez-Saona, 2010). Furthermore, this strategy poses a certain risk. When constitutively

presenting parasitoids with odors of herbivore-infested plants, even in the absence of the pest, they might learn to no longer associate these odors with the presence of a suitable host (Kaplan, 2012a). At an ecological level, since VOCs share certain compounds with floral scent, application of herbivore-induced VOCs could potentially distract pollinators (Lucas-Barbosa et al., 2011; Kaplan, 2012a).

Novel ways of habitat management, such as planting a flower border next to a crop field, have been suggested to increase plant diversity and thereby create a source of food and alternative hosts, as well as shelter, for parasitoids (Landis et al., 2000). Also, a practice of intercropping with plants repellent for herbivores could be adopted (Parker et al., 2013). A very successful example of biological control has been developed in eastern Africa and is based on the “push-pull” strategy (Khan et al., 1997a,b). Maize and sorghum fields in Kenya suffer severely from stemborer infestation. By planting “push” crops in the field that repel the larval herbivores, as well as attract parasitoids, and planting “pull” crops on the edges of the field that attract the ovipositing moths and further increase the efficiency of natural enemies, the pest could be significantly reduced (Khan et al., 1997a,b). Implementation of the “push-pull” strategy has led to remarkable increases in crop yield (Khan et al., 2010).

To increase crop VOC emission in response to herbivore attack, those varieties with the desired traits could be specifically selected (e.g. Krips et al., 2001). It has also been proposed to genetically modify crops in order to enhance the emission of various odors (e.g. Degenhardt et al., 2003; Shiojiri et al., 2006a; Kos et al., 2009). As mentioned above, most American maize lines have lost the ability to emit (*E*)- $\beta$ -caryophyllene upon an attack by root-feeding *Diabrotica virgifera virgifera* larvae, a compound that is attractive to insect-killing nematodes (Rasmann et al., 2005; Köllner et al., 2008). By introducing a gene from oregano, the emission of this compound could be restored, leading to increased resistance to the pest insect in the field (Degenhardt et al., 2009). These results are promising in the light of enhancing or restoring plant defenses in a way that is compatible with biological control. Again, however, constitutive emission of herbivore-inducible odors could prove to be less efficient on the long term (Kaplan, 2012a). Engineering plants that respond more quickly and strongly to herbivory with the emission of VOCs, rather than emit VOCs constantly, remains a challenge for the future.

Most commercially available biological control agents are used in a greenhouse setting (Van Lenteren, 2003). Indeed, when the natural enemies are confined in a greenhouse, they can be rather effective in eradicating a target pest (Van Lenteren and Woets, 1988). In the field, they may easily disperse, maintaining insufficient levels to have an effect on the pest population. Nevertheless, there are numerous examples of successful biological control of pests, by means of parasitoids, in the field. In Mexico, for instance, citrus blackfly (*Aleurocanthus woglumi* Ashby) (Hemiptera: Aleyrodidae) and Rhodesgrass mealybug (*Antonina graminis* Maskell) (Hemiptera: Pseudococcidae) have been locally almost eradicated (Williams et al., 2013). When attracting natural enemies, it is important to not deplete surrounding areas of their natural biological control agents (Kaplan, 2012a). Furthermore, the host range of the target natural enemies has to be carefully studied. Release or

conservation of native natural enemies would pose fewer risks than the importation of exotic ones, a practice that is frequently used to combat invasive pests. A well-known example of a biological control agent that causes severe non-target effects on local fauna is the Asian lady beetle, *Harmonia axyridis* Pallas (Hymenoptera: Coccinellidae). Not only does it predate on aphid pests, it is eradicating native ladybeetles as well (e.g. Roy et al., 2012). In general, the success of biological control is dependent on the quality of the natural enemies, which are mainly mass-reared by commercial companies (Van Lenteren, 2003).

In nature, a plant is hardly ever attacked by one herbivore alone. Other herbivores could potentially influence VOC emission and therefore natural enemy attraction in unexpected ways (Dicke et al., 2009; Soler et al., 2012). Also, beneficial soil-borne microbes could have an impact on plant aboveground VOC emission (Pineda et al., 2010). Further dedicated field experiments are needed to determine the potential of herbivore-inducible VOCs for use in effective biological control strategies.

### **Parasitoid wasps as biological control agents of *S. frugiperda***

In Mexico, *S. frugiperda* is mainly combatted with highly toxic chemical products, although recently, there has been a change towards slightly less noxious products with fewer harmful effects on beneficial fauna (Fernando Bahena-Juárez, personal communication). Natural compounds like neem, an extract of the tree *Azadirachta indica* A. Juss. (Gutiérrez-García et al., 2010) and Bt, the gram-positive bacterium *Bacillus thuringiensis* itself or an extract of the Cry toxin it produces (Nava C. et al., 2004), could be rather effective pesticides. However, because the spraying costs per hectare are much higher than for conventional chemicals, these natural compounds are scarcely used (Fernando Bahena-Juárez, personal communication). In various regions, *Trichogramma* (Hymenoptera: Trichogrammatidae) and *Telenomus* (Hymenoptera: Scelionidae) egg-parasitoids have been mass-reared and released, which resulted in substantial control of *S. frugiperda* (García Roa et al., 1999; Cave, 2000; Flores G. and Domínguez M., 2003). The study of other potential biocontrol species is actively encouraged (Ruíz-Cancino et al., 2012). *Chelonus insularis*, as well as *C. sonorensis*, are currently being evaluated as promising biological control agents of *S. frugiperda* (Fernando Bahena-Juárez, personal communication). Specific attraction of these wasps could potentially increase their effectiveness as natural enemies. However, for maize it remains to be determined which compounds in the herbivore-induced odor blend are behaviorally active as attractants of parasitoid wasps (D'Alessandro and Turlings, 2006). The research described in this thesis suggests that teosinte odors could be used as starting material for identification of these key compounds (Chapters 3, 4).

### **The various functions of herbivore-induced VOCs**

Vegetative plant volatiles have been assigned four distinct functions: attracting natural enemies, repelling herbivores, plant-plant communication and within-plant communication (Rodríguez-Saona and Frost, 2010). Thus far, we have merely assessed the effectiveness of maize and teosinte odors in attracting natural enemies. Herbivore-induced maize VOCs have

been reported to repel the corn leaf aphid (Bernasconi et al., 1998). However, attractiveness of these VOCs to herbivores, for instance larvae of *S. frugiperda* and *S. littoralis*, has also been reported (Carroll et al., 2006; Von Mérey et al., 2013). Plants can perceive the VOCs emitted by their herbivore-attacked neighbors and use the containing information to prepare for a possible attack, a phenomenon known as priming. Upon an actual attack, a primed plant responds with a faster and stronger induction of defenses than a non-primed plant (Frost et al., 2008a). Priming occurs within species, as shown for a number of species, including maize (Engelberth et al., 2004; Ton et al., 2007), but also between species, as proven for sagebrush and tobacco (Kessler et al., 2006). Relatively recently, it has been discovered that VOCs serve as internal plant signals, preceding slower vascular signals, for plants like lima bean (Heil and Silva Bueno, 2007) and trees like poplar (Frost et al., 2007). It would be highly interesting to assess the differences and similarities between maize and teosinte odors with respect to these other putative functions of plant odors as well.

### **Teosinte and the improvement of cultivated maize**

There is a promising potential for the use of teosinte in the improvement of modern maize (e.g. Wilkes, 1977; Flint-Garcia 2013). Reportedly, teosinte genes could potentially augment maize productivity (Cohen & Galinat 1984; Magoja & Pischedda 1994; Wang et al., 2008b) and nutritional quality of the kernels (Flint-Garcia et al., 2009; Swarup et al., 1995; Wang et al., 2008a). Although teosinte has been substantially studied with regard to maize evolution (e.g. Dorweiler et al., 1993; Doebley et al., 1995), it has been rather ignored in research on pathogen and insect resistance (reviewed in Chapter 1). The research described in this thesis suggests that studying teosinte odors might be promising in the light of improved defenses of the maize crop (Chapters 3, 4). Furthermore, teosinte could be a model for studying the highly debated defensive function of these odors (Chapter 5).

Unfortunately, teosinte is threatened with extinction due to deforestation, urbanization and growth of non-traditional crops (Wilkes, 2007). A large effort is undertaken to preserve its genetic resources, for instance at the CIMMYT (Centro Internacional de Mejoramiento de Maíz y Trigo) Wellhausen-Anderson Plant Genetic Resources Center, which has a storage capacity of 450.000 seed samples of not only different maize varieties, but also wild teosintes (Taba et al., 2004). Also, INIFAP (Instituto Nacional de Investigaciones Forestales, Agrícolas y Pecuarias) has a germplasm bank containing teosinte seeds (Sánchez González and Ruiz Corral, 1995). Importantly, maize is the first crop plant to have one of its closest wild relatives protected in a natural reserve (*Z. diploperennis* in Sierra de Manatlán Biosphere Reserve, Colima, Mexico; Wilkes, 2007), but continuous efforts are needed to maintain and be able to study teosinte in its natural environment.

## References

- ACOSTA, I. F., LAPARRA, H., ROMERO, S. P., SCHMELZ, E., HAMBERG, M., MOTTINGER, J. P., MORENO, M. A., and DELLAPORTA, S. L. 2009. *tasselseed1* Is a lipoxygenase affecting jasmonic acid signaling in sex determination of maize. *Science* 323:262-265.
- AGRAWAL, A. A. 2000. Overcompensation of plants in response to herbivory and the by-product benefits of mutualism. *Trends in Plant Science* 5:309-313.
- AGRAWAL, A. A. 2011. Current trends in the evolutionary ecology of plant defence. *Functional Ecology* 25:420-432.
- AGRAWAL, A. A. and RUTTER, M. T. 1998. Dynamic anti-herbivore defense in ant-plants: The role of induced responses. *Oikos* 83:227-236.
- AHARONI, A., GIRI, A. P., VERSTAPPEN, F. W. A., BERTEA, C. M., SEVENIER, R., SUN, Z., JONGSMA, M. A., SCHWAB, W., and BOUWMEESTER, H. J. 2004. Gain and loss of fruit flavor compounds produced by wild and cultivated strawberry species. *The Plant Cell* 16:3110-3131.
- AHMAD, S., VEYRAT, N., GORDON-WEEKS, R., ZHANG, Y., MARTIN, J., SMART, L., GLAUSER, G., ERB, M., FLORS, V., FREY, M., and TON, J. 2011. Benzoxazinoid metabolites regulate innate immunity against aphids and fungi in maize. *Plant Physiology* 157:317-327.
- ALBORN, H. T., HANSEN, T. V., JONES, T. H., BENNETT, D. C., TUMLINSON, J. H., SCHMELZ, E. A., and TEAL, P. E. A. 2007. Disulfoxy fatty acids from the American bird grasshopper *Schistocerca americana*, elicitors of plant volatiles. *Proceedings of the National Academy of Sciences of the United States of America* 104:12976-12981.
- ALBORN, H. T., TURLINGS, T. C. J., JONES, T. H., STENHAGEN, G., LOUGHRIN, J. H., and TUMLINSON, J. H. 1997. An elicitor of plant volatiles from beet armyworm oral secretions. *Science* 276:945-949.
- ALI, J., ALBORN, H., and STELINSKI, L. 2010. Subterranean herbivore-induced volatiles released by citrus roots upon feeding by *Diaprepes abbreviatus* recruit entomopathogenic nematodes. *Journal of Chemical Ecology* 36:361-368.
- ALLISON, J. D. and HARE, J. D. 2009. Learned and naïve natural enemy responses and the interpretation of volatile organic compounds as cues or signals. *New Phytologist* 184:768-782.
- ALLMANN, S. and BALDWIN, I. T. 2010. Insects betray themselves in nature to predators by rapid isomerization of green leaf volatiles. *Science* 329:1075-1078.
- ALLMANN, S., SPÄTHE, A., BISCH-KNADEN, S., KALLENBACH, M., REINECKE, A., SACHSE, S., BALDWIN, I. T., and HANSSON, B. S. 2013. Feeding-induced rearrangement of green leaf volatiles reduces moth oviposition. *eLife* 2: e00421.
- ANDREWS, K. L. 1988. Latin American research on *Spodoptera frugiperda* (Lepidoptera: Noctuidae). *The Florida Entomologist* 71:630-653.
- ARIMURA, G., OZAWA, R., HORIUCHI, J., NISHIOKA, T., and TAKABAYASHI, J. 2001. Plant-plant interactions mediated by volatiles emitted from plants infested by spider mites. *Biochemical Systematics and Ecology* 29:1049-1061.
- AWMACK, C. S. and LEATHER, S. R. 2002. Host plant quality and fecundity in herbivorous insects. *Annual Review of Entomology* 47:817-844.
- BAHENA-JUÁREZ, F. 2008. Enemigos naturales de las plagas agrícolas del maíz y otros cultivos. Uruapan, Mexico: SAGARPA - INIFAP.
- BAHENA-JUÁREZ, F., DE LANGE, E., FARNIER, K., CORTEZ-MONDACA, E., SÁNCHEZ-MARTÍNEZ, R., GARCÍA-PÉREZ, F., MIRANDA-SALCEDO, M., DEGEN, T., GAUDILLAT, B., and AGUILAR-ROMERO, R. 2010. Parasitismo en gusano cogollero del maíz *Spodoptera frugiperda* (J. E. Smith) (Lepidoptera: Noctuidae) en el centro de México. Memorias del XXXIII Congreso Nacional de Control Biológico. Mexico. p 204-209.
- BALLHORN, D. J., KAUTZ, S., LION, U., and HEIL, M. 2008. Trade-offs between direct and indirect defences of lima bean (*Phaseolus lunatus*). *Journal of Ecology* 96:971-980.
- BALMER, D., FLORS, V., GLAUSER, G., AND MAUCH-MANI, B. 2013a. Metabolomics of cereals under biotic stress: Current knowledge and techniques. *Frontiers of Plant Sciences* 4:82

## References

---

- BALMER, D., PLANCHAMP, C., and MAUCH-MANI, B. 2013b. On the move: Induced resistance in monocots. *Journal of Experimental Botany* 64:1249-1261.
- BANUETT, F. 1995. Genetics of *Ustilago Maydis*, a fungal pathogen that induces tumors in maize. *Annual Review of Genetics* 29:179-208.
- BARBOSA, P., LETOURNEAU, D., and AGRAWAL, A. 2012. Insect outbreaks revisited. Chichester, UK: John Wiley & Sons, Ltd.
- BARBOSA, P. 1998. Conservational biological control. San Diego, USA: Academic Press.
- BARRY, D., WIDSTROM, N. W., DARRAH, L. L., McMILLIAN, W. W., RILEY, T. J., SCOTT, G. E., and LILLEHOJ, E. B. 1992. Maize ear damage by insects in relation to genotype and aflatoxin contamination in preharvest maize grain. *Journal of Economic Entomology* 85:2492-2495.
- BASANDRAI, A. K. and SINGH, A. 2002. Fungal diseases of maize. In: Gupta VK, Paul YS, editors. Diseases of field crops. New Delhi, India: Indus Publishing Company.
- BEADLE, G. W. 1939. Teosinte and the origin of maize. *Journal of Heredity* 30:248-247.
- BEADLE, G. W. 1972. The mystery of maize. *Field Museum of Natural History Bulletin (Chicago)* 43:2-11.
- BEADLE, G. W. 1980. The ancestry of corn. *Scientific American* 242:112-119.
- BELLOTA, E., MEDINA, R. F., and BERNAL, J. S. 2013. Physical leaf defenses – altered by Zea life-history evolution, domestication, and breeding – mediate oviposition preference of a specialist leafhopper. *Entomologia Experimentalis et Applicata* 149:185-195.
- BENNETT, R. N. and WALLSGROVE, R. M. 1994. Secondary metabolites in plant defence mechanisms. *New Phytologist* 127:617-633.
- BENNETZEN, J., BUCKLER, E., CHANDLER, V., DOEBLEY, J., DORWEILER, J., BRANDON, G., FREELING, M., HAKE, S., KELLOGG, E., POETHIG, R. S., WALBOT, V., and WESSLER, S. 2001. Genetic evidence and the origin of maize. *Latin American Antiquity* 12:84-86.
- BENNETZEN, J. L. and HAKE, S. C. 2009. Handbook of maize: Genetics and genomics. New York, USA: Springer Science + Business Media, LCC.
- BENREY, B., CALLEJAS, A., RIOS, L., OYAMA, K., and DENNO, R. F. 1998. The effects of domestication of *Brassica* and *Phaseolus* on the interaction between phytophagous insects and parasitoids. *Biological Control* 11:130-140.
- BERNASCONI, M. L., TURLINGS, T. C. J., AMBROSETTI, L., BASSETTI, P., and DORN, S. 1998. Herbivore-induced emissions of maize volatiles repel the corn leaf aphid, *Rhopalosiphum maidis*. *Entomologia Experimentalis et Applicata* 87:133-142.
- BERNASCONI, M. L., TURLINGS, T. C. J., EDWARDS, P. J., FRITZSCHE-HOBALLAH, M. E., AMBROSETTI, L., BASSETTI, P., and DORN, S. 2001. Response of natural populations of predators and parasitoids to artificially induced volatile emissions in maize plants (*Zea mays* L.). *Agricultural and Forest Entomology* 3:201-209.
- BIRKETT, M. A., CHAMBERLAIN, K., GUERRIERI, E., PICKETT, J. A., WADHAMS, L. J., and YASUDA, T. 2003. Volatiles from whitefly-infested plants elicit a host-locating response in the parasitoid, *Encarsia formosa*. *Journal of Chemical Ecology* 29:1589-1600.
- BORLAUG, N. E. 1946. Diseases of teosinte in Mexico. *Phytopathology* 36:395.
- BOTTRELL, D. G., BARBOSA, P., and GOULD, F. 1998. Manipulating natural enemies by plant variety selection and modification: A realistic strategy? *Annual Review of Entomology* 43:347-367.
- BRANSON, T. F. 1982. Olfactory response of larvae of *Diabrotica virgifera virgifera* to plant roots. *Entomologia Experimentalis et Applicata* 31:303-307.
- BRANSON, T. F. and REYES R., J. 1983. The Association of *Diabrotica* spp. with *Zea diploperennis*. *Journal of the Kansas Entomological Society* 56:97-99.
- BREWBAKER, J. 1979. Diseases of maize in the wet lowland tropics and the collapse of the Classic Maya civilization. *Economic Botany* 33:101-118.
- BRONSTEIN, J. L., ALARCON, R., and GEBER, M. 2006. The evolution of plant-insect mutualisms. *New Phytologist* 172:412-428.
- BRUCE, T. A. 2010. Tackling the threat to food security caused by crop pests in the new millennium. *Food Security* 2:133-141.



- BRUINSMA, M., VAN BROEKHOVEN, S., POELMAN, E., POSTHUMUS, M., MÜLLER, M., VAN LOON, J., and DICKE, M. 2010a. Inhibition of lipoxygenase affects induction of both direct and indirect plant defences against herbivorous insects. *Oecologia* 162:393-404.
- BRUINSMA, M., VAN LOON, J. J. A., and DICKE, M. 2010b. Increasing insight into induced plant defense mechanisms using elicitors and inhibitors. *Plant Signaling & Behavior* 5:271-274.
- BUCKLER, E. S., GAUT, B. S., and MCMULLEN, M. D. 2006. Molecular and functional diversity of maize. *Current Opinion in Plant Biology* 9:172-176.
- BUKOVINSZKY, T., POELMAN, E., GOLS, R., PREKATSAKIS, G., VET, L. E. M., HARVEY, J. A., and DICKE, M. 2009. Consequences of constitutive and induced variation in plant nutritional quality for immune defence of a herbivore against parasitism. *Oecologia* 160:299-308.
- BUKOVINSZKY, T., VAN VEEN, F. J. F., JONGEMA, Y., and DICKE, M. 2008. Direct and indirect effects of resource quality on food web structure. *Science* 319:804-807.
- BUSHING, R. W. and BURTON, V. E. 1974. Leafhopper damage to silage corn in California. *Journal of Economic Entomology* 67:656-658.
- CAFFREY, D. J. and WORTHLEY, L. H. 1927. A progress report on the investigations of the European corn borer: US Government Printing Office.
- CAMACHO VILLA, T. C., MAXTED, N., SCHOLTEN, M. A., and FORD-LLOYD, B. V. 2005. Defining and identifying crop landraces. *Plant Genetic Resources: Characterization and Utilization* 3:373-384.
- CAMPBELL, B. C. and DUFFEY, S. S. 1979. Tomatine and parasitic wasps: Potential incompatibility of plant antibiosis with biological control. *Science* 205:700-702.
- CARROLL, M., SCHMELZ, E., MEAGHER, R., and TEAL, P. 2006. Attraction of *Spodoptera frugiperda* larvae to volatiles from herbivore-damaged maize seedlings. *Journal of Chemical Ecology* 32:1911-1924.
- CARSON, R. 1962. Silent Spring. Boston, USA: Houghton Mifflin.
- CAVE, R. D. 1995. Manual para el reconocimiento de parasitoides de plagas agrícolas en América Central. El Zamorano, Honduras: Zamorano Academic Press.
- CAVE, R. D. 2000. Biology, ecology and use in pest management of *Telenomus remus*. *Biocontrol news and information* 21:21N-26N.
- CHEN, Y. H., LANGELLOTTO, G. A., BARRION, A. T., and CUONG, N. L. 2013. Cultivation of Domesticated Rice Alters Arthropod Biodiversity and Community Composition. *Annals of the Entomological Society of America* 106:100-110.
- CHEN, Y. H. and WELTER, S. C. 2002. Abundance of a native moth *Homoeosoma electellum* (Lepidoptera: Pyralidae) and activity of indigenous parasitoids in native and agricultural sunflower habitats. *Environmental Entomology* 31:626-636.
- CHEN, Y. H. and WELTER, S. C. 2003. Confused by domestication: Incongruent behavioral responses of the sunflower moth, *Homoeosoma electellum* (Lepidoptera: Pyralidae) and its parasitoid, *Dolichogenidea homoeosomae* (Hymenoptera: Braconidae), towards wild and domesticated sunflowers. *Biological Control* 28:180-190.
- CHEN, Y. H. and WELTER, S. C. 2005. Crop domestication disrupts a native tritrophic interaction associated with the sunflower, *Helianthus annuus* (Asterales: Asteraceae). *Ecological Entomology* 30:673-683.
- CHEN, Y. H. and WELTER, S. C. 2007. Crop domestication creates a refuge from parasitism for a native moth. *Journal of Applied Ecology* 44:238-245.
- CHRISTENSEN, S. A. and KOLOMIETS, M. V. 2011. The lipid language of plant-fungal interactions. *Fungal Genetics and Biology* 48:4-14.
- CHRISTENSEN, S. A., NEMCHENKO, A., BORREGO, E., MURRAY, I., SOBHY, I. S., BOSAK, L., DEBLASIO, S., ERB, M., ROBERT, C. A. M., VAUGHN, K. A., HERRFURTH, C., TUMLINSON, J., FEUSSNER, I., JACKSON, D., TURLINGS, T. C. J., ENGELBERTH, J., NANSEN, C., MEELEY, R., and KOLOMIETS, M. V. 2013. The maize lipoxygenase, ZmLOX10, mediates green leaf volatile, jasmonate and herbivore-induced plant volatile production for defense against insect attack. *The Plant Journal* 74:59-73.
- CLC BIO, A QIAGEN COMPANY. 2013. CLC Sequence Viewer. Aarhus, Denmark: CLC Bio. <http://www.clcbio.com/products/clc-sequence-viewer/>.

- COHEN, J. I. and GALINAT, W. C. 1984. Potential use of alien germplasm for maize improvement. *Crop Science* 24:1011-1015.
- COLAZZA, S., McELFRESH, J. S., and MILLAR, J. 2004. Identification of volatile synomones, induced by *Nezara viridula* feeding and oviposition on bean spp., that attract the egg parasitoid *Trissolcus basalis*. *Journal of Chemical Ecology* 30:945-964.
- COLEMAN, R. A., BARKER, A. M., and FENNER, M. 1999. Parasitism of the herbivore *Pieris brassicae* L. (Lep., Pieridae) by *Cotesia glomerata* L. (Hym., Braconidae) does not benefit the host plant by reduction of herbivory. *Journal of Applied Entomology* 123:171-177.
- COMMONWEALTH INSTITUTE OF ENTOMOLOGY. 1985. Distribution maps of insect pests. Series A (Agricultural), Map no. 68, *Spodoptera frugiperda* (J.E. Smith).
- CÓRDOVA-CAMPOS, O., ADAME-ÁLVAREZ, R., ACOSTA-GALLEGOS, J., and HEIL, M. 2012. Domestication affected the basal and induced disease resistance in common bean (*Phaseolus vulgaris*). *European Journal of Plant Pathology* 134:367-379.
- CORTESERO, A. M., STAPEL, J. O., and LEWIS, W. J. 2000. Understanding and manipulating plant attributes to enhance biological control. *Biological Control* 17:35-49.
- CROFT, K. P. C., JUTTNER, F., and SLUSARENKO, A. J. 1993. Volatile products of the lipoxygenase pathway evolved from *Phaseolus vulgaris* (L.) leaves inoculated with *Pseudomonas syringae* pv phaseolicola. *Plant Physiology* 101:13-24.
- D'ALESSANDRO, M. and TURLINGS, T. C. J. 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.
- D'ALESSANDRO, M. and TURLINGS, T. C. J. 2006. Advances and challenges in the identification of volatiles that mediate interactions among plants and arthropods. *The Analyst* 131:24-32.
- D'ALESSANDRO, M., BRUNNER, V., VON MÉREY, G., and TURLINGS, T. C. J. 2009. Strong attraction of the parasitoid *Cotesia marginiventris* towards minor volatile compounds of maize. *Journal of Chemical Ecology* 35:999-1008.
- D'ALESSANDRO, M., HELD, M., TRIPONEZ, Y., and 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* 32:2733-2748.
- DARWIN, C. 1868. The variation of animals and plants under domestication, vols. 1 and 2. London, UK: John Murray.
- DARWIN, C. 1859. On the origin of species by means of natural selection. London, UK: John Murray.
- DÁVILA-FLORES, A., DEWITT, T., and BERNAL, J. 2013. Facilitated by nature and agriculture: Performance of a specialist herbivore improves with host-plant life history evolution, domestication, and breeding. *Oecologia*:1-13.
- DE BOER, J. G. and DICKE, M. 2004. The role of methyl salicylate in prey searching behavior of the predatory mite *Phytoseiulus persimilis*. *Journal of Chemical Ecology* 30:255-271.
- DE BOER, J. G., POSTHUMUS, M. A., and DICKE, M. 2004. Identification of volatiles that are used in discrimination between plants infested with prey or nonprey herbivores by a predatory mite. *Journal of Chemical Ecology* 30:2215-2230.
- DE LANGE, E. S. 2008. Suppression of maize indirect defenses by a specialist lepidopteran herbivore. MSc thesis, University of Neuchâtel, Neuchâtel, Switzerland.
- DE LA PAZ-GUTIÉRREZ, S. 2008. Diversidad de insectos en maíz y teocintle y efecto de factores agroclimáticos en plagas rizófagas en México. PhD thesis, University of Guadalajara, Guadalajara, Mexico.
- DE MORAES, C. M., LEWIS, W. J., PARÉ, P. W., ALBORN, H. T., and TUMLINSON, J. H. 1998. Herbivore-infested plants selectively attract parasitoids. *Nature* 393:570-573.
- DE MORAES, C. M., MESCHER, M. C., and TUMLINSON, J. H. 2001. Caterpillar-induced nocturnal plant volatiles repel conspecific females. *Nature* 410:577-580.
- DE ROODE, J. C., LEFÈVRE, T., and HUNTER, M. D. 2013. Self-medication in animals. *Science* 340:150-151.

- DEGEN, T., BAKALOVIC, N., BERGVINSON, D., and TURLINGS, T. C. J. 2012. Differential performance and parasitism of caterpillars on maize inbred lines with distinctly different herbivore-induced volatile emissions. *PLoS ONE* 7:e47589.
- DEGEN, T., DILLMANN, C., MARION-POLL, F., and TURLINGS, T. C. J. 2004. High genetic variability of herbivore-induced volatile emission within a broad range of maize inbred lines. *Plant Physiology* 135:1928-1938.
- DEGENHARDT, J. 2009. Indirect defense responses to herbivory in grasses. *Plant Physiology* 149:96-102.
- DEGENHARDT, J., GERSHENZON, J., BALDWIN, I. T., and KESSLER, A. 2003. Attracting friends to feast on foes: Engineering terpene emission to make crop plants more attractive to herbivore enemies. *Current Opinion in Biotechnology* 14:169-176.
- DEGENHARDT, J., HILTPOLD, I., KÖLLNER, T. G., FREY, M., GIERL, A., GERSHENZON, J., HIBBARD, B. E., ELLERSIECK, M. R., and 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* 106:13213-13218.
- DENHOLM, I., DEVINE, G. J., and WILLIAMSON, M. S. 2002. Insecticide resistance on the move. *Science* 297:2222-2223.
- DIEZEL, C., ALLMANN, S., and BALDWIN, I. T. 2011. Mechanisms of optimal defense patterns in *Nicotiana attenuata*: Flowering attenuates herbivory-elicited ethylene and jasmonate signaling. *Journal of Integrative Plant Biology* 53:971-983.
- DICKE, M. and BALDWIN, I. T. 2010. The evolutionary context for herbivore-induced plant volatiles: Beyond the 'cry for help'. *Trends in Plant Science* 15:167-175.
- DICKE, M., GOLS, R., LUDEKING, D., and POSTHUMUS, M. A. 1999. Jasmonic acid and herbivory differentially induce carnivore-attracting plant volatiles in lima bean plants. *Journal of Chemical Ecology* 25:1907-1922.
- DICKE, M. and SABELIS, M. W. 1988. How plants obtain predatory mites as bodyguards. *Netherlands Journal of Zoology* 38:148-165.
- DICKE, M. and VAN LOON, J. J. A. 2000. Multitrophic effects of herbivore-induced plant volatiles in an evolutionary context. *Entomologia Experimentalis et Applicata* 97:237-249.
- DICKE, M., VAN LOON, J. J. A., and SOLER, R. 2009. Chemical complexity of volatiles from plants induced by multiple attack. *Nature Chemical Biology* 5:317-324.
- DOEBLEY, J. 1992. Mapping the genes that made maize. *Trends in Genetics* 8:302-307.
- DOEBLEY, J. 2001. George Beadle's other hypothesis: One-gene, one-trait. *Genetics* 158:487-493.
- DOEBLEY, J. 2004. The genetics of maize evolution. *Annual Review of Genetics* 38:37-59.
- DOEBLEY, J. and STEC, A. 1991. Genetic analysis of the morphological differences between maize and teosinte. *Genetics* 129:285-295.
- DOEBLEY, J. and STEC, A. 1993. Inheritance of the morphological differences between maize and teosinte: Comparison of results for two F<sub>2</sub> populations. *Genetics* 134:559-570.
- DOEBLEY, J., STEC, A., and GUSTUS, C. 1995. *teosinte branched1* and the origin of maize: Evidence for epistasis and the evolution of dominance. *Genetics* 141:333-346.
- DOEBLEY, J., STEC, A., and HUBBARD, L. 1997. The evolution of apical dominance in maize. *Nature* 386:485-488.
- DOEBLEY, J., STEC, A., WENDEL, J., and EDWARDS, M. 1990. Genetic and morphological analysis of a maize-teosinte F<sub>2</sub> population: Implications for the origin of maize. *Proceedings of the National Academy of Sciences of the United States of America* 87:9888-9892.
- DOEBLEY, J. F., GAUT, B. S., and SMITH, B. D. 2006. The molecular genetics of crop domestication. *Cell* 127:1309-1321.
- DORWEILER, J., STEC, A., KERMICLE, J., and DOEBLEY, J. 1993. *Teosinte glume architecture 1*: A genetic locus controlling a key step in maize evolution. *Science* 262:233-235.
- DU, Y., POPPY, G. M., POWELL, W., PICKETT, J. A., WADHAMS, L. J., and WOODCOCK, C. M. 1998. Identification of semiochemicals released during aphid feeding that attract parasitoid *Aphidius ervi*. *Journal of Chemical Ecology* 24:1355-1368.
- DUDAREVA, N., KLEMPIEN, A., MUHLEMANN, J. K., and KAPLAN, I. 2013. Biosynthesis, function and metabolic engineering of plant volatile organic compounds. *New Phytologist* 198:16-32.

## References

---

- DUVICK, D. N. 2001. Biotechnology in the 1930s: The development of hybrid maize. *Nature Reviews Genetics* 2:69-74.
- DUVICK, D. N. and DONALD, L. S. 2005. The contribution of breeding to yield advances in maize (*Zea mays* L.). *Advances in Agronomy* 86: 83-145.
- EDDINS, A. H. 1933. Infection of corn plants by *Physoderma zae-maydis* Shaw. *Journal of Agricultural Research* 46:241-253.
- EDSON, K., VINSON, S., STOLTZ, D., and SUMMERS, M. 1981. Virus in a parasitoid wasp: Suppression of the cellular immune response in the parasitoid's host. *Science* 211:582-583.
- EHI-EROMOSELE, C. O., NWINYI, O. C., and AJANI, O. O. 2013. Integrated pest management. In: Soloneski S, Larramendy M, editors. *Weed and Pest Control - Conventional and New Challenges*. Rijeka, Croatia: InTech.
- ENGELBERTH, J., ALBORN, H. T., SCHMELZ, E. A., and TURLINSON, J. H. 2004. Airborne signals prime plants against insect herbivore attack. *Proceedings of the National Academy of Sciences of the United States of America* 101:1781-1785.
- ENNEKING, D. and WINK, M. 2000. Towards the elimination of anti-nutritional factors in grain legumes. In: Knight R, editor. *Linking research and marketing opportunities for pulses in the 21<sup>st</sup> century*. The Netherlands: Springer. p 671-683.
- ERB, M., BALMER, D., DE LANGE, E. S., VON MÉREY, G., PLANCHAMP, C., ROBERT, C. A. M., RÖDER, G., SOBHY, I., ZWAHLEN, C., MAUCH-MANI, B., and TURLINGS, T. C. J. 2011a. Synergies and trade-offs between insect and pathogen resistance in maize leaves and roots. *Plant, Cell & Environment* 34:1088-1103.
- ERB, M., FLORS, V., KARLEN, D., DE LANGE, E., PLANCHAMP, C., D'ALESSANDRO, M., TURLINGS, T. C. J., and TON, J. 2009a. Signal signature of aboveground-induced resistance upon belowground herbivory in maize. *The Plant Journal* 59:292-302.
- ERB, M., FORESTI, N., and TURLINGS, T. 2010. A tritrophic signal that attracts parasitoids to host-damaged plants withstands disruption by non-host herbivores. *BMC Plant Biology* 10:247.
- ERB, M., GORDON-WEEKS, R., FLORS, V., CAMAÑES, G., TURLINGS, T. C. J., and TON, J. 2009b. Belowground ABA boosts aboveground production of DIMBOA and primes induction of chlorogenic acid in maize. *Plant Signaling & Behavior* 4:636-638.
- ERB, M., ROBERT, C. A. M., HIBBARD, B. E., and TURLINGS, T. C. J. 2011b. Sequence of arrival determines plant-mediated interactions between herbivores. *Journal of Ecology* 99:7-15.
- EUBANKS, M. 1995. A cross between two maize relatives: *Tripsacum dactyloides* and *Zea diploperennis* (Poaceae). *Economic Botany* 49:172-182.
- EUROPEAN UNION. 2013. Commission Implementing Regulation No 485/2013. Brussels, Belgium: European Union.
- EVANS, L. T. 1996. Crop evolution, adaptation and yield. Cambridge, UK: Cambridge University Press.
- FAEGRI, K. and VAN DER PIJL, L. 1979. The principles of pollination ecology. Oxford, UK: Pergamon Press.
- FALK, B. W. and TSAI, J. H. 1985. Serological detection and evidence for multiplication of maize mosaic virus in the planthopper, *Peregrinus maidis*. *Phytopathology* 75:852-855.
- FAO. 1992. Maize in human nutrition. Rome, Italy: FAO.
- FAO. 2013. Food and agricultural commodities production. From <http://faostat.fao.org/site/339/default.aspx>.
- FITT, G. P. 1989. The ecology of *Heliothis* species in relation to agroecosystems. *Annual Review of Entomology* 34:17-53.
- FLINT-GARCIA, S. A. 2013. Genetics and consequences of crop domestication. *Journal of Agricultural and Food Chemistry* 61:8267-8276.
- FLINT-GARCIA, S., BODNAR, A., and SCOTT, M. P. 2009. Wide variability in kernel composition, seed characteristics, and zein profiles among diverse maize inbreds, landraces, and teosinte. *Theoretical and Applied Genetics* 119:1129-1142.
- FLORES G., A. G. and DOMÍNGUEZ M., V. M. 2003. Eficiencia de *Trichogramma pretiosum* (Hymenoptera: Trichogrammatidae) en el parasitismo de gusano cogollero *Spodoptera frugiperda* (Lepidoptera: Noctuidae) en maíz. *Memorias del XXXVIII Congreso Nacional de Entomología*. Mexico. p 373-375.
- FONSECA, S., CHICO, J. M., and SOLANO, R. 2009. The jasmonate pathway: The ligand, the receptor and the core signalling module. *Current Opinion in Plant Biology* 12:539-547.

- FREY, M., SCHULLEHNER, K., DICK, R., FIESSLMANN, A., and GIERL, A. 2009. Benzoxazinoid biosynthesis, a model for evolution of secondary metabolic pathways in plants. *Phytochemistry* 70:1645-1651.
- FRITZSCHE HOBALLAH, M. E., TAMÒ, C., and 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* 28:951-968.
- FRITZSCHE HOBALLAH, M. E. and TURLINGS, T. C. J. 2001. Experimental evidence that plants under caterpillar attack may benefit from attracting parasitoids. *Evolutionary Ecology Research* 3:553-565.
- FROST, C. J., APPEL, H. M., CARLSON, J. E., DE MORAES, C. M., MESCHER, M. C., and SCHULTZ, J. C. 2007. Within-plant signalling via volatiles overcomes vascular constraints on systemic signalling and primes responses against herbivores. *Ecology Letters* 10:490-498.
- FROST, C. J., MESCHER, M. C., CARLSON, J. E., and DE MORAES, C. M. 2008a. Plant defense priming against herbivores: Getting ready for a different battle. *Plant Physiology* 146:818-824.
- FROST, C. J., MESCHER, M. C., DERVINIS, C., DAVIS, J. M., CARLSON, J. E., and DE MORAES, C. M. 2008b. Priming defense genes and metabolites in hybrid poplar by the green leaf volatile *cis*-3-hexenyl acetate. *New Phytologist* 180:722-734.
- FUENTES, S. F., LOURDES DE LA ISLA, M., ULLSTRUP, A. J., and RODRIGUEZ, A. E. 1964. *Claviceps gigantea*, a new pathogen of maize in Mexico. *Phytopathology* 54:379-381.
- FUKUNAGA, K., HILL, J., VIGOUROUX, Y., MATSUOKA, Y., SANCHEZ G., J., LIU, K., BUCKLER, E. S., and DOEBLEY, J. 2005. Genetic diversity and population structure of teosinte. *Genetics* 169:2241-2254.
- GÁMEZ, R. and LEÓN, P. 1988. *Maize rayado fino* and related viruses. In: Koenig, R., editor. *The Plant Viruses*. New York, USA: Plenum Press. p 213-233.
- GAO, X., SHIM, W.-B., GÖBEL, C., KUNZE, S., FEUSSNER, I., MEELEY, R., BALINT-KURTI, P., and KOLOMIETS, M. 2007. Disruption of a maize 9-lipoxygenase results in increased resistance to fungal pathogens and reduced levels of contamination with mycotoxin fumonisin. *Molecular Plant-Microbe Interactions* 20:922-933.
- GARCÍA ROA, F., MOSQUERA, A. T., VARGAS S., C. A., and ROJAS A., L. 1999. Manejo integrado del gusano cogollero del maíz *Spodoptera frugiperda* (J.E. Smith). *Corpoica Boletín Tecnico* 7.
- GARDINER, M. M., BARBOUR, J. D., and JOHNSON, J. B. 2003. Arthropod diversity and abundance on feral and cultivated *Humulus lupulus* (Urticales: Cannabaceae) in Idaho. *Environmental Entomology* 32:564-574.
- GARDNER, W. A. and FUXA, J. R. 1980. Pathogens for the suppression of the fall armyworm. *Florida Entomologist* 63:439-447.
- GEERVLIT, J. B. F., VET, L. E. M., and DICKE, M. 1996. Innate responses of the parasitoids *Cotesia glomerata* and *C. rubecula* (Hymenoptera: Braconidae) to volatiles from different plant-herbivore complexes. *Journal of Insect Behavior* 9:525-538.
- GERNERT, W. B. 1917. Aphis Immunity of teosinte-corn hybrids. *Science* 46:390-392.
- GILL, R. J., RAMOS-RODRIGUEZ, O., and RAINE, N. E. 2012. Combined pesticide exposure severely affects individual- and colony-level traits in bees. *Nature* 491:105-108.
- GLAUSER, G., MARTI, G., VILLARD, N., DOYEN, G. A., WOLFENDER, J.-L., TURLINGS, T. C. J., and ERB, M. 2011. Induction and detoxification of maize 1,4-benzoxazin-3-ones by insect herbivores. *The Plant Journal* 68:901-911.
- GLAZEBROOK, J. 2005. Contrasting mechanisms of defense against biotrophic and necrotrophic pathogens. *Annual Review of Phytopathology* 43:205-227.
- GODFRAY, H. C. J. 1994. Parasitoids: Behavioral and evolutionary ecology. Princeton, USA: Princeton University Press.
- GOFF, S. A. and KLEE, H. J. 2006. Plant volatile compounds: Sensory cues for health and nutritional value? *Science* 311:815-819.
- GOLS, R., BUKOVINSZKY, T., VAN DAM, N., DICKE, M., BULLOCK, J., and HARVEY, J. 2008. Performance of generalist and specialist herbivores and their endoparasitoids differs on cultivated and wild brassica populations. *Journal of Chemical Ecology* 34:132-143.
- GOLS, R., BULLOCK, J., DICKE, M., BUKOVINSZKY, T., and HARVEY, J. 2011. Smelling the wood from the trees: Non-linear parasitoid responses to volatile attractants produced by wild and cultivated cabbage. *Journal of Chemical Ecology* 37:795-807.

- GOLS, R. and HARVEY, J. 2009. Plant-mediated effects in the Brassicaceae on the performance and behaviour of parasitoids. *Phytochemistry Reviews* 8:187-206.
- GOMEZ, J. M. and ZAMORA, R. 1994. Top-down effects in a tritrophic system: Parasitoids enhance plant fitness. *Ecology* 75:1023-1030.
- GOINGUENÉ, S. 2000. Specificity and variability of induced volatile signalling in maize plants. PhD thesis, University of Neuchâtel, Neuchâtel, Switzerland.
- GOINGUENÉ, S., ALBORN, H., and TURLINGS, T. C. J. 2003. Induction of volatile emissions in maize by different larval instars of *Spodoptera littoralis*. *Journal of Chemical Ecology* 29:145-162.
- GOINGUENÉ, S., DEGEN, T., and TURLINGS, T. C. J. 2001. Variability in herbivore-induced odour emissions among maize cultivars and their wild ancestors (teosinte). *Chemoecology* 11:9-16.
- GOINGUENÉ, S., PICKETT, J. A., WADHAMS, L. J., BIRKETT, M. A., and 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* 31:1023-1038.
- GOINGUENÉ, S. P. and TURLINGS, T. C. J. 2002. The effects of abiotic factors on induced volatile emissions in corn plants. *Plant Physiology* 129:1296-1307.
- GROSS, P. 1993. Insect behavioral and morphological defenses against parasitoids. *Annual Review of Entomology* 38:251-273.
- GUELDER, R. C., SNOOK MAURICE, E., WISEMAN, B. R., WIDSTROM, N. W., HIMMELSBACH, D. S., and COSTELLO, C. E. 1991. Maysin in corn, teosinte, and centipede grass. In: Hedin, PA, editor. Naturally occurring pest bioregulators. USA: American Chemical Society. p 251-263.
- GURR, G. M., VRATTEN, S. D., SNYDER, W. E., and READ, W. D. M. Y. 2012. Biodiversity and insect pests: Key issues for sustainable management. Chichester, UK: John Wiley & Sons, Ltd.
- GUTIÉRREZ-GARCÍA, S. D. C., SÁNCHEZ-ESCUADERO, J., PÉREZ-DOMÍNGUEZ, J. F., CARBALLO-CARBALLO, A., BERGVINSON, D., and AGUILERA-PEÑA, M. M. 2010. Efecto del nim en el daño ocasionado por el gusano cogollero *Spodoptera frugiperda* (Smith) (Lepidoptera: Noctuidae) en tres variables agronómicas de maíz resistente y susceptible. *Acta Zoológica Mexicana* 26:1-16.
- HARE, J. D. 2010. Ontogeny and season constrain the production of herbivore-inducible plant volatiles in the field. *Journal of Chemical Ecology* 36:1363-1374.
- HARE, J. D. 2011. Ecological role of volatiles produced by plants in response to damage by herbivorous insects. *Annual Review of Entomology* 56:161-180.
- HARFOUCHE, A. L., SHIVAJI, R., STOCKER, R., WILLIAMS, P. W., and LUTHE, D. S. 2006. Ethylene signaling mediates a maize defense response to insect herbivory. *Molecular Plant-Microbe Interactions* 19:189-199.
- HARVEY, J. A. 2005. Factors affecting the evolution of development strategies in parasitoid wasps: The importance of functional constraints and incorporating complexity. *Entomologia Experimentalis et Applicata* 117:1-13.
- HARVEY, J. A., VAN DAM, N. M., WITJES, L. M. A., SOLER, R., and GOLS, R. 2007. Effects of dietary nicotine on the development of an insect herbivore, its parasitoid and secondary hyperparasitoid over four trophic levels. *Ecological Entomology* 32:15-23.
- HARVEY, J. A., VAN NOUHUYS, S., and BIERE, A. 2005. Effects of quantitative variation in allelochemicals in *Plantago lanceolata* on development of a generalist and a specialist herbivore and their endoparasitoids. *Journal of Chemical Ecology* 31:287-302.
- HATANAKA, A. 1993. The biogenesis of green odour by green leaves. *Phytochemistry* 34:1201-1218.
- HEGAZI, E. M., SHAABAN, M. A., and EL-SINGABY, N. R. 1991. Development of *Microplitis rufiventris* (Hymenoptera: Braconidae) in superparasitized *Spodoptera littoralis* (Lepidoptera: Noctuidae). *Annals of the Entomological Society of America* 84:571-574.
- HEIL, M. 2008. Indirect defence via tritrophic interactions. *New Phytologist* 178:41-61.
- HEIL, M. 2009. Damaged-self recognition in plant herbivore defence. *Trends in Plant Science* 14:356-363.
- HEIL, M. and MCKEY, D. 2003. Protective ant-plant interactions as model systems in ecological and evolutionary research. *Annual Review of Ecology, Evolution, and Systematics* 34:425-453.

- HEIL, M. and SILVA BUENO, J. C. 2007. Within-plant signaling by volatiles leads to induction and priming of an indirect plant defense in nature. *Proceedings of the National Academy of Sciences of the United States of America* 104:5467-5472.
- HENRY, M., BÉGUIN, M., REQUIER, F., ROLLIN, O., ODOUX, J.-F., AUPINEL, P., APTEL, J., TCHAMITCHIAN, S., and DECOURTYE, A. 2012. A common pesticide decreases foraging success and survival in honey bees. *Science* 336:348-350.
- HESSELTINE, C. W. and ELLIS, J. J. 1971. *Helminthosporium*: Secondary metabolites, southern leaf blight of corn, and biology. *Journal of Agricultural and Food Chemistry* 19:707-717.
- HILDEBRAND, D. F., BROWN, G. C., JACKSON, D. M., and HAMILTON-KEMP, T. R. 1993. Effects of some leaf-emitted volatile compounds on aphid population increase. *Journal of Chemical Ecology* 19:1875-1887.
- HILL, D. S. 1987. Agricultural insect pests of temperate regions and their control. New York, USA: Cambridge University Press.
- HIURA, M. 1930. Further note on the downy mildew of *Setaria italica*. *Japanese Journal of Plant Protection* 17:7.
- HOBALLAH, M. E., DEGEN, T., BERGVINSON, D., SAVIDAN, A., TAMÒ, C., and TURLINGS, T. C. J. 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. and 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* 31:2003-2018.
- HONEK, A. 1993. Intraspecific variation in body size and fecundity in insects: A general relationship. *Oikos* 66:483-492.
- HOWE, G. A. and JANDER, G. 2008. Plant immunity to insect herbivores. *Annual Review of Plant Biology* 59:41-66.
- HUANG, C.-H., YAN, F.-M., BYERS, J. A., WANG, R.-J., and 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* 16:311-320.
- HUFFAKER, A., DAFOE, N. J., and SCHMELZ, E. A. 2011a. ZmPep1, an ortholog of Arabidopsis elicitor peptide 1, regulates maize innate immunity and enhances disease resistance. *Plant Physiology* 155:1325-1338.
- HUFFAKER, A., KAPLAN, F., VAUGHAN, M. M., DAFOE, N. J., NI, X., ROCCA, J. R., ALBORN, H. T., TEAL, P. E. A., and SCHMELZ, E. A. 2011b. Novel acidic sesquiterpenoids constitute a dominant class of pathogen-induced phytoalexins in maize. *Plant Physiology* 156:2082-2097.
- HUFFAKER, A., PEARCE, G., VEYRAT, N., ERB, M., TURLINGS, T. C. J., SARTOR, R., SHEN, Z., BRIGGS, S. P., VAUGHAN, M. M., ALBORN, H. T., TEAL, P. E. A., and SCHMELZ, E. A. 2013. Plant elicitor peptides are conserved signals regulating direct and indirect antiherbivore defense. *Proceedings of the National Academy of Sciences of the United States of America* 110:5707-5712.
- HUFFORD, M. B., XU, X., VAN HEERWAARDEN, J., PYHÄJÄRVI, T., CHIA, J.-M., CARTWRIGHT, R. A., ELSHIRE, R. J., GLAUBITZ, J. C., GUILL, K. E., KAEPLER, S. M., LAI, J., MORRELL, P. L., SHANNON, L. M., SONG, C., SPRINGER, N. M., SWANSON-WAGNER, R. A., TIFFIN, P., WANG, J., ZHANG, G., DOEBLEY, J., MCMULLEN, M. D., WARE, D., BUCKLER, E. S., SHUANG, Y., and ROSS-IBARRA, J. 2012. Comparative population genomics of maize domestication and improvement. *Nature Genetics* 44:808-811.
- HUNTER, M. D. 2002. A breath of fresh air: Beyond laboratory studies of plant volatile–natural enemy interactions. *Agricultural and Forest Entomology* 4:81-86.
- ILTIS, H. 2000. Homeotic sexual translocations and the origin of maize (*Zea Mays*, Poaceae): A new look at an old problem. *Economic Botany* 54:7-42.
- ILTIS, H. H. 1983. From teosinte to maize: The catastrophic sexual transmutation. *Science* 222:886-894.
- ILTIS, H. H. and BENZ, B. F. 2000. *Zea nicaraguensis* (Poaceae), a new teosinte from Pacific coastal Nicaragua. *Novon* 10:382-390.
- JALALI, S. K., SINGH, S. P., and BALLAL, C. R. 1987. Studies on host age preference and biology of exotic parasite, *Cotesia marginiventris* (Cresson) (Hymenoptera: Braconidae). *Entomon* 12:59-62.
- JAMES, D. and GRASSWITZ, T. 2005. Synthetic herbivore-induced plant volatiles increase field captures of parasitic wasps. *BioControl* 50:871-880.
- JAMES, D. G. 2003. Synthetic herbivore-induced plant volatiles as field attractants for beneficial insects. *Environmental Entomology* 32:977-982.

- JOFRE Y GARFIAS, A. E., ARTAVIA MATA, M., IBARRA RENDÓN, J. E., and ÁLVAREZ MORALES, R. A. 2010. Maíz y teocintle. Compartiendo insectos en Lázaro Cárdenas, Erongarícuaro. In: Seefoó Luján JL, Keilbach Baer NM, editors. Ciencia y paciencia campesina. El maíz en Michoacán. Zamora, Mexico: El Colegio de Michoacán: Gobierno del Estado de Michoacán - Secretaría de Desarrollo Rural.
- JOHNS, T. and ALONSO, J. 1990. Glycoalkaloid change during the domestication of the potato, *Solanum* Section *Petota*. *Euphytica* 50:203-210.
- JOSEFSSON, E. 1967. Distribution of thioglucosides in different parts of *Brassica* plants. *Phytochemistry* 6:1617-1627.
- JOURDIE, V. 2008. Parasitoid communities and genetic structure: Host plant does not matter. PhD thesis, University of Neuchâtel, Neuchâtel, Switzerland.
- JOURDIE, V., ALVAREZ, N., and 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* 10:129-136.
- KAPLAN, I. 2012a. Attracting carnivorous arthropods with plant volatiles: The future of biocontrol or playing with fire? *Biological Control* 60:77-89.
- KAPLAN, I. 2012b. Trophic complexity and the adaptive value of damage-induced plant volatiles. *PLoS Biology* 10:e1001437.
- KAPLAN, L. 1965. Archeology and domestication in American *Phaseolus* (beans). *Economic Botany* 19:358-368.
- KAPPERS, I., HOOGERBRUGGE, H., BOUWMEESTER, H., and DICKE, M. 2011. Variation in herbivory-induced volatiles among cucumber (*Cucumis sativus* L.) varieties has consequences for the attraction of carnivorous natural enemies. *Journal of Chemical Ecology* 37:150-160.
- KAPPERS, I., VERSTAPPEN, F. A., LUCKERHOFF, L. P., BOUWMEESTER, H., and DICKE, M. 2010. Genetic variation in jasmonic Acid- and spider mite-induced plant volatile emission of cucumber accessions and attraction of the predator *Phytoseiulus persimilis*. *Journal of Chemical Ecology* 36:500-512.
- KARBAN, R. and BALWIN, I. T. 1997. Induced responses to herbivory. Chicago, USA: Chicago University Press.
- KARBAN, R. and ENGLISH-LOEB, G. 1997. Tachinid parasitoids affect host plant choice by caterpillars to increase caterpillar survival. *Ecology* 78:603-611.
- KARIYAT, R. R., MAUCK, K. E., DE MORAES, C. M., STEPHENSON, A. G., and MESCHER, M. C. 2012. Inbreeding alters volatile signalling phenotypes and influences tri-trophic interactions in horsenettle (*Solanum carolinense* L.). *Ecology Letters* 15:301-309.
- KENNETH, R. 1981. Downy mildews of graminaceous crops. In: Kenneth, RG, editor. The Downy Mildews. London, UK: Academic Press. p 367-394.
- KESSLER, A. and BALDWIN, I. T. 2001. Defensive function of herbivore-induced plant volatile emissions in nature. *Science* 291:2141-2144.
- KESSLER, A., HALITSCHKE, R., and BALDWIN, I. T. 2004. Silencing the jasmonate cascade: Induced plant defenses and insect populations. *Science* 305:665-668.
- KESSLER, A., HALITSCHKE, R., DIEZEL, C., and BALDWIN, I. T. 2006. Priming of plant defense responses in nature by airborne signaling between *Artemisia tridentata* and *Nicotiana attenuata*. *Oecologia* 148:280-292.
- KESSLER, A. and HEIL, M. 2011. The multiple faces of indirect defences and their agents of natural selection. *Functional Ecology* 25:348-357.
- KESSLER, D., GASE, K., and BALDWIN, I. T. 2008. Field experiments with transformed plants reveal the sense of floral scents. *Science* 321:1200-1202.
- KHAN, Z. R., AMPONG-NYARKO, K., CHILISWA, P., HASSANALI, A., KIMANI, S., LWANDE, W., OVERHOLT, W. A., PICKETT, J. A., SMART, L. E., WADHAMS, L. J., and WOODCOCK, C. M. 1997a. Intercropping increases parasitism of pests. *Nature* 388:631-632.
- KHAN, Z. R., CHILISWA, P., AMPONG-NYARKO, K., SMART, L. E., POLASZEK, A., WANDERA, J., and MULAA, M. A. 1997b. Utilisation of wild gramineous plants for management of cereal stemborers in Africa. *International Journal of Tropical Insect Science* 17:143-150.
- KHAN, Z. R., JAMES, D. G., MIDEGA, C. A. O., and PICKETT, J.A. 2008. Chemical ecology and conservation biological control. *Biological Control* 45:210-224.



- KHAN, Z. R., MIDEGA, C. A. O., BRUCE, T. J. A., HOOPER, A., M., and PICKETT, J. A. 2010. Exploiting phytochemicals for developing a 'push-pull' crop protection strategy for cereal farmers in Africa. *Journal of Experimental Botany* 61:4185-4196.
- KHUSH, G. S. 2001. Green revolution: The way forward. *Nature Reviews Genetics* 2:815-822.
- KÖLLNER, T. G., HELD, M., LENK, C., HILTPOLD, I., TURLINGS, T. C. J., GERSHENZON, J., and DEGENHARDT, J. 2008. A maize (*E*)- $\beta$ -caryophyllene synthase implicated in indirect defense responses against herbivores is not expressed in most American maize varieties. *Plant Cell* 20:482-494.
- KÖLLNER, T. G., SCHNEE, C., GERSHENZON, J., and DEGENHARDT, J. 2004. The sesquiterpene hydrocarbons of maize (*Zea mays*) form five groups with distinct developmental and organ-specific distributions. *Phytochemistry* 65:1895-1902.
- KOS, M., VAN LOON, J. J. A., DICKE, M., and VET, L. E. M. 2009. Transgenic plants as vital components of integrated pest management. *Trends in Biotechnology* 27:621-627.
- KOST, C. and HEIL, M. 2006. Herbivore-induced plant volatiles induce an indirect defence in neighbouring plants. *Journal of Ecology* 94:619-628.
- KRANZ, J., SCHMUTTERER, H., and KOCH, W. 1977. Diseases, pests and weeds in tropical crops. Berlin, Germany: Verlag Paul Parey.
- KRIPS, O. E., WILLEMS, P. E. L., GOLS, R., POSTHUMUS, M. A., GORT, G., and 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* 27:1355-1372.
- LANDIS, D. A., WRATTEN, S. D., and GURR, G. M. 2000. Habitat management to conserve natural enemies of arthropod pests in agriculture. *Annual Review of Entomology* 45:175-201.
- LE GUIGO, P., MAINGENEAU, A., and LE CORFF, J. 2011a. Performance of an aphid *Myzus persicae* and its parasitoid *Diaeretiella rapae* on wild and cultivated Brassicaceae. *Journal of Plant Interactions* 7:326-332.
- LE GUIGO, P., QU, Y., and LE CORFF, J. 2011b. Plant-mediated effects on a toxin-sequestering aphid and its endoparasitoid. *Basic and Applied Ecology* 12:72-79.
- LEISS, K. A., CRISTOFORI, G., VAN STEENIS, R., VERPOORTE, R., and KLINKHAMER, P. G. L. 2013. An eco-metabolomic study of host plant resistance to Western flower thrips in cultivated, biofortified and wild carrots. *Phytochemistry* 93:63-70.
- LI, X. and WEBB, B. A. 1994. Apparent functional role for a cysteine-rich polydnavirus protein in suppression of the insect cellular immune response. *Journal of Virology* 68:7482-7489.
- LINDIG-CISNEROS, R., BENREY, B., and ESPINOSA-GARCÍA, F. 1997. Phytoalexins, resistance traits, and domestication status in *Phaseolus coccineus* and *Phaseolus lunatus*. *Journal of Chemical Ecology* 23:1997-2011.
- LINDIG-CISNEROS, R., DIRZO, R., and ESPINOSA-GARCÍA, F. J. 2002. Effects of domestication and agronomic selection on phytoalexin antifungal defense in *Phaseolus* beans. *Ecological Research* 17:315-321.
- LOBELL, D. B. and ASNER, G. P. 2003. Climate and management contributions to recent trends in U.S. agricultural yields. *Science* 299:1032.
- LOPEZ, L., CAMAS, A., SHIVAJI, R., ANKALA, A., WILLIAMS, P., and LUTHE, D. 2007. Mir1-CP, a novel defense cysteine protease accumulates in maize vascular tissues in response to herbivory. *Planta* 226:517-527.
- LOUGHRIN, J., MANUKIAN, A., HEATH, R., and TUMLINSON, J. 1995. Volatiles emitted by different cotton varieties damaged by feeding beet armyworm larvae. *Journal of Chemical Ecology* 21:1217-1227.
- LOUGHRIN, J. H., MANUKIAN, A., HEATH, R. R., TURLINGS, T. C. J., and TUMLINSON, J. H. 1994. Diurnal cycle of emission of induced volatile terpenoids by herbivore-injured cotton plant. *Proceedings of the National Academy of Sciences of the United States of America* 91:11836-11840.
- LUCAS-BARBOSA, D., VAN LOON, J. J. A., and DICKE, M. 2011. The effects of herbivore-induced plant volatiles on interactions between plants and flower-visiting insects. *Phytochemistry* 72:1647-1654.
- LUCKHART, S. and WEBB, B. A. 1996. Interaction of a wasp ovarian protein and polydnavirus in host immune suppression. *Developmental & Comparative Immunology* 20:1-21.
- LUGINBILL, P. 1928. The fall armyworm. *USDA Technical Bulletin* 34:1-91.
- LYONS, R., MANNERS, J., and KAZAN, K. 2013. Jasmonate biosynthesis and signaling in monocots: A comparative overview. *Plant Cell Reports* 32:815-827.

- MACFADYEN, S. and BOHAN, D. A. 2010. Crop domestication and the disruption of species interactions. *Basic and Applied Ecology* 11:116-125.
- MACIAS, F. A., MARIN, D., OLIVEROS-BASTIDAS, A., and MOLINILLO, J. M. G. 2009. Rediscovering the bioactivity and ecological role of 1,4-benzoxazinones. *Natural Product Reports* 26:478-489.
- MAFFEI, M. E. 2010. Sites of synthesis, biochemistry and functional role of plant volatiles. *South African Journal of Botany* 76:612-631.
- MAGOJA, J. L. and PISCHEDDA, G. 1994. Maize x teosinte hybridization. In: Y.P.S. B, editor. *Biotechnology in agricultura and forestry*. Berlin, Germany: Springer-Verlag. p 84-101.
- MAILAFIYA, D. M., LE RU, B. P., KAIRU, E. W., CALATAYUD, P. A., and DUPAS, S. 2009. Species diversity of lepidopteran stem borer parasitoids in cultivated and natural habitats in Kenya. *Journal of Applied Entomology* 133:416-429.
- MAJETIC, C. J., RAGUSO, R. A., and ASHMAN, T.-L. 2009. The sweet smell of success: Floral scent affects pollinator attraction and seed fitness in *Hesperis matronalis*. *Functional Ecology* 23:480-487.
- MANGELSDORF, P. C. and REEVES, R. G. 1939. The origin of Indian corn and its relatives. *Texas Agricultural Experiment Station Bulletin* 574:1-315.
- MANO, Y. and OMORI, F. 2007. Breeding for flooding tolerant maize using "teosinte" as a germplasm resource. *Plant Root* 1:17-21.
- MARTÍNEZ-SORIANO, J. and AVINA-PADILLA, K. 2009. *Ustilago* and the accidental domestication of maize. *Journal of Cereal Science* 50:302-303.
- MASSEI, G. and HARTLEY, S. E. 2000. Disarmed by domestication? Induced responses to browsing in wild and cultivated olive. *Oecologia* 122:225-231.
- MATSUI, K. 2006. Green leaf volatiles: Hydroperoxide lyase pathway of oxylipin metabolism. *Current Opinion in Plant Biology* 9:274-280.
- MATSUOKA, Y., VIGOUROUX, Y., GOODMAN, M. M., SANCHEZ G., J., BUCKLER, E., and DOEBLEY, J. 2002. A single domestication for maize shown by multilocus microsatellite genotyping. *Proceedings of the National Academy of Sciences of the United States of America* 99:6080-6084.
- MCAUSLANE, H. J., BRADLEIGH VINSON, S., and WILLIAMS, H. J. 1991. Influence of adult experience on host microhabitat location by the generalist parasitoid, *Campoletis sonorensis* (Hymenoptera: Ichneumonidae). *Journal of Insect Behavior* 4:101-113.
- MEDINA, R. F., REYNA, S. M., and BERNAL, J. S. 2012. Population genetic structure of a specialist leafhopper on *Zea*: likely anthropogenic and ecological determinants of gene flow. *Entomologia Experimentalis et Applicata* 142:223-235.
- MELHUS, I. E. 1953. A preliminary study of diseases of corn and some related hosts in Guatemala. *Iowa State College Journal of Science* 27:519-536.
- METCALF, R. L. 1986. Foreword. In: Krysan JL, Miller TA, editors. *Methods for the study of pest Diabrotica*. New York, USA: Springer. p viii-xv.
- MEYER, R. S., DUVAL, A. E., and JENSEN, H. R. 2012. Patterns and processes in crop domestication: An historical review and quantitative analysis of 203 global food crops. *New Phytologist* 196:29-48.
- MILAN, NEIL F., KACSOH, BALINT Z., and SCHLENKE, TODD A. 2012. Alcohol consumption as self-medication against blood-borne parasites in the fruit fly. *Current Biology* 22:488-493.
- MOELLER, D. A. and TIFFIN, P. 2005. Genetic diversity and the evolutionary history of plant immunity genes in two Species of *Zea*. *Molecular Biology and Evolution* 22:2480-2490.
- MOLINA-OCHOA, J., CARPENTER, J. E., LEZAMA-GUTIERREZ, R., FOSTER, J. E., GONZALEZ-RAMIREZ, M., ANGEL-SAHAGUN, C. A., and FARIAS-LARIOS, J. 2004. Natural distribution of hymenopteran parasitoids of *Spodoptera frugiperda* (Lepidoptera: Noctuidae) larvae in Mexico. *Florida Entomologist* 87:461-472.
- MONDRAGÓN-PICHARDO, J. and VIBRANS, H. 2005. Ethnobotany of the Balsas teosinte. *Maydica* 50:123-128.
- MORRIS, S. W., VERNOOIJ, B., TITATARN, S., STARRETT, M., THOMAS, S., WILTSE, C. C., FREDERIKSEN, R. A., BHANDHUFALCK, A., HULBERT, S., and UKNES, S. 1998. Induced resistance responses in maize. *Molecular Plant-Microbe Interactions* 11:643-658.

- MOYA-RAYGOZA, G. 1987. Evaluación de la presencia y daño de los insectos rizofagos de *Zea mays* sobre *Zea diploperennis* en la Sierra de Manatlán. MSc thesis, University of Guadalajara, Guadalajara, Mexico.
- MOYA-RAYGOZA, G. 1994. Diversity of leafhoppers and their hymenopterous parasitoids in maize, teosinte and gamagrass related ecosystems. *Maydica* 39:225-230.
- MOYA-RAYGOZA, G., BEDOY V., V., and SANTANA C., E. 1990. Seasonal patterns of insect abundance in natural patches of *Zea diploperennis*. *Maydica* 35:177-182.
- MOYA-RAYGOZA, G. and TRUJILLO-ARRIAGA, J. 1993. Dryinid (Hym.: Dryinidae) parasitoids of *Dalbulus* leafhopper (Hom.: Cicadellidae) in Mexico. *Entomophaga* 38:41-49.
- MUMM, R., and DICKE, M. 2010. Variation in natural plant products and the attraction of bodyguards involved in indirect plant defense. *Canadian Journal of Zoology* 88:628-667.
- MUSSER, R. O., HUM-MUSSER, S. M., EICHENSEER, H., PEIFFER, M., ERVIN, G., MURPHY, J. B., and FELTON, G. W. 2002. Herbivory: Caterpillar saliva beats plant defences. *Nature* 416:599-600.
- NAULT, L. R. 1980. Maize bushy stunt and corn stunt: A comparison of disease symptoms, pathogen and host ranges, and vectors. *Phytopathology* 70:659-662.
- NAULT, L. R. 1990. Evolution of an insect pest: Maize and the corn leafhopper, a case study. *Maydica* 35:165-175.
- NAULT, L. R. and DELONG, D. M. 1980. Evidence for co-evolution of leafhoppers in the genus *Dalbulus* (Cicadellidae: Homoptera) with maize and its ancestors. *Annals of the Entomological Society of America* 73:349-353.
- NAULT, L. R., GINGERY, R. E., and GORDON, D. T. 1980. Leafhopper transmission and host range of maize rayado fino virus. *Phytopathology* 70:709-712.
- NAULT, L. R. and GORDON, D. T. 1988. Multiplication of maize stripe virus in *Peregrinus maidis*. *Phytopathology* 78:991-995.
- NAULT, L. R., GORDON, D. T., DAMSTEEGT, V. D., and ILLIS, H. H. 1982. Response of annual and perennial teosintes (*Zea*) to six maize viruses. *Plant Disease* 66:61-62.
- NAULT, L. R. and MADDEN, L. V. 1985. Ecological strategies of *Dalbulus* leafhoppers. *Ecological Entomology* 10:57-63.
- NAVA C., U., OORALES O., E., and ESCOBEDO A., M. L. 2004. Efectividad de insecticidas microbiales y convencionales para el control de gusano cogollero (*Spodoptera frugiperda*) y su impacto en las poblaciones de insectos depredadores en maíz forrajero en la comarca lagunera. Memorias del XXVII Congreso Nacional de Control Biológico. Mexico. p 344-347.
- NGUMBI, E., CHEN, L., and FADAMIRO, H. 2009. Comparative GC-EAD responses of a specialist (*Microplitis croceipes*) and a generalist (*Cotesia marginiventris*) parasitoid to cotton volatiles induced by two caterpillar species. *Journal of Chemical Ecology* 35:1009-1020.
- NIEMEYER, H. M. 2009. Hydroxamic acids derived from 2-hydroxy-2H-1,4-benzoxazin-3(4H)-one: Key defense chemicals of cereals. *Journal of Agricultural and Food Chemistry* 57:1677-1696.
- ODE, P. J. 2006. Plant chemistry and natural enemy fitness: Effects on herbivore and natural enemy interactions. *Annual Review of Entomology* 51:163-185.
- OERKE, E. C. 2006. Crop losses to pests. *Journal of Agricultural Science* 144:31-43.
- ORTEGA C., A. 1987. Insect pests of maize: A guide for field identification. Mexico City, Mexico: CIMMYT.
- PAINTER, R. H. 1955. Insects on corn and teosinte in Guatemala. *Journal of Economic Entomology* 48:36-42.
- PAIR, S. D., RAULSTON, J. R., SPARKS, A. N., and MARTIN, P. B. 1986a. Fall armyworm (Lepidoptera, Noctuidae) parasitoids: Differential spring distribution and incidence on corn and sorghum in the Southern United States and Northeastern Mexico. *Environmental Entomology* 15:342-348.
- PAIR, S. D., RAULSTON, J. R., SPARKS, A. N., WESTBROOK, J. K., and DOUCE, G. K. 1986b. Fall armyworm distribution and population dynamics in the southeastern states. *Florida Entomologist* 69:468-487.
- PALAIAS, K. A., MORGANTE, M., WILLIAMS, M., and RAFALSKI, A. 2003. Contrasting effects of selection on sequence diversity and linkage disequilibrium at two phytoene synthase loci. *The Plant Cell* 15:1795-1806.
- PARÉ, P. W. and TUMLINSON, J. H. 1997. De novo biosynthesis of volatiles induced by insect herbivory in cotton plants. *Plant Physiology* 114:1161-1167.

- PARKER, J. E., SNYDER, W. E., HAMILTON, G. C., and RODRIGUEZ-SAONA, C. 2013. Companion planting and insect pest control. In: Soloneski S, Larramendy M, editors. Weed and pest control - Conventional and new challenges. Rijeka, Croatia: InTech.
- PEÑAFLO, M. and BENTO, J. M. S. 2013. Herbivore-induced plant volatiles to enhance biological control in agriculture. *Neotropical Entomology* 42:331-343.
- PEROVIĆ, D. J., JOHNSON, M.-L., SCHOLZ, B., and ZALUCKI, M. P. 2008. The mortality of *Helicoverpa armigera* (Hübner) (Lepidoptera: Noctuidae) neonate larvae in relation to drop-off and soil surface temperature: The dangers of bungy jumping. *Australian Journal of Entomology* 47:289-296.
- PICHERSKY, E. and GERSHENZON, J. 2002. The formation and function of plant volatiles: Perfumes for pollinator attraction and defense. *Current Opinion in Plant Biology* 5:237-243.
- PICKETT, J. A., BRUCE, T. J. A., CHAMBERLAIN, K., HASSANNALI, A., and WOODCOCK, C. M. 2006. Plant volatiles yielding new ways to exploit plant defence. In: Dicke M, Takken W, editors. Chemical ecology: From gene to ecosystem. The Netherlands: Springer. p 161-173.
- PIESIK, D., ROCHAT, D., DELANEY, K. J., and MARION-POLL, F. 2013. Orientation of European corn borer first instar larvae to synthetic green leaf volatiles. *Journal of Applied Entomology* 137:234-240.
- PINEDA, A., ZHENG, S.-J., VAN LOON, J. J. A., PIETERSE, C. M. J., and DICKE, M. 2010. Helping plants to deal with insects: The role of beneficial soil-borne microbes. *Trends in Plant Science* 15:507-514.
- PITRE, H. N., MULROONEY, J. E., and HOGG, D. B. 1983. Fall armyworm (Lepidoptera: Noctuidae) oviposition: Crop preferences and egg distribution on plants. *Journal of Economic Entomology* 76:463-466.
- POELMAN, E. H., BRUINSMA, M., ZHU, F., WELDEGERGIS, B. T., BOURSALT, A. E. JONGEMA, Y., VAN LOON, J. J. A., VET, L. E. M., HARVEY, J. A., and DICKE, M. 2012. Hyperparasitoids use herbivore-induced plant volatiles to locate their parasitoid host. *PLoS Biology* 10:e1001435.
- PORTA, H. and ROCHA-SOSA, M. 2002. Plant lipoxygenases. Physiological and molecular features. *Plant Physiology* 130:15-21.
- POWELL, W., PENNACCHIO, F., POPPY, G. M., and TREMBLAY, E. 1998. Strategies involved in the location of hosts by the parasitoid *Aphidius ervi* Haliday (Hymenoptera: Braconidae: Aphidiinae). *Biological Control* 11:104-112.
- PRÉVOST, G., DAVIES, D. H., and VINSON, S. B. 1990. Evasion of encapsulation by parasitoid correlated with the extent of host hemocyte pathology. *Entomologia Experimentalis et Applicata* 55:1-10.
- PRICE, P. W., BOUTON, C. E., GROSS, P., MCPHERON, B. A., THOMPSON, J. N., and WEIS, A. E. 1980. Interactions among three trophic levels: Influence of plants on interactions between insect herbivores and natural enemies. *Annual Review of Ecology and Systematics* 11:41-65.
- RASBAND, W. S. 1997-2012. ImageJ. Bethesda, Maryland, USA: U. S. National Institutes of Health. <http://imagej.nih.gov/ij/>.
- RASMANN, S., KÖLLNER, T. G., DEGENHARDT, J., HILTPOLD, I., TOEPFER, S., KUHLMANN, U., GERSHENZON, J., and TURLINGS, T. C. J. 2005. Recruitment of entomopathogenic nematodes by insect-damaged maize roots. *Nature* 434:732-737.
- RASMANN, S. and TURLINGS, T. C. J. 2007. Simultaneous feeding by aboveground and belowground herbivores attenuates plant-mediated attraction of their respective natural enemies. *Ecology Letters* 10:926-936.
- R CORE TEAM. 2013. R: A language and environment for statistical computing. Vienna, Austria: R Foundation for Statistical Computing. <http://www.r-project.org/>.
- REDDY, G. V. P. and GUERRERO, A. 2000. Behavioral responses of the diamondback moth, *Plutella xylostella*, to green leaf volatiles of *Brassica oleracea* subsp. *capitata*. *Journal of Agricultural and Food Chemistry* 48:6025-6029.
- RICH, P. J. and EJETA, G. 2008. Towards effective resistance to *Striga* in African maize. *Plant Signaling & Behavior* 3:618-621.
- ROBERT, A. M. 1962. Host ranges and races of the corn rusts. *Phytopathology* 52:1010-1012.
- ROBERT, C. A. M., VEYRAT, N., GLAUSER, G., MARTI, G., DOYEN, G. R., VILLARD, N., GAILLARD, M. D. P., KÖLLNER, T. G., GIRON, D., BODY, M., BABST, B. A., FERRIERI, R. A., TURLINGS, T. C. J., and ERB, M. 2012. A specialist root herbivore exploits defensive metabolites to locate nutritious tissues. *Ecology Letters* 15:55-64.

- RODRIGUEZ-SAONA, C., BLAAUW, B. R., and ISAACS, R. 2013. Manipulation of natural enemies in agroecosystems: Habitat and semiochemicals for sustainable insect pest control. In: Larramendy ML, Soloneski S, editors. Integrated pest management and pest control - Current and future tactics. Rijeka, Croatia: InTech.
- RODRIGUEZ-SAONA, C., VORSA, N., SINGH, A. P., JOHNSON-CICALESE, J., SZENDREI, Z., MESCHER, M. C., and FROST, C. J. 2011. Tracing the history of plant traits under domestication in cranberries: Potential consequences on anti-herbivore defences. *Journal of Experimental Botany* 62:2633-2644.
- RODRIGUEZ-SAONA, C. R. and FROST, C. J. 2010. New evidence for a multi-functional role of herbivore-induced plant volatiles in defense against herbivores. *Plant Signaling & Behavior* 5:58-60.
- ROSENTHAL, J. P. and DIRZO, R. 1997. Effects of life history, domestication and agronomic selection on plant defence against insects: Evidence from maizes and wild relatives. *Evolutionary Ecology* 11:337-355.
- ROSENTHAL, J. P. and WELTER, S. C. 1995. Tolerance to herbivory by a stem-boring caterpillar in architecturally distinct maizes and wild relatives. *Oecologia* 102:146-155.
- ROSTÁS, M., TON, J., MAUCH-MANI, B., and TURLINGS, T. C. J. 2006. Fungal infection reduces herbivore-induced plant volatiles of maize but does not affect naïve parasitoids. *Journal of Chemical Ecology* 32:1897-1909.
- ROY, H. E., ADRIAENS, T., ISAAC, N. J. B., KENIS, M., ONKELINX, T., MARTIN, G. S., BROWN, P. M. J., HAUTIER, L., POLAND, R., ROY, D. B., COMONT, R., ESCHEN, R., FROST, R., ZINDEL, R., VAN VLAENDEREN, J., NEDVĚD, O., RAVN, H. P., GRÉGOIRE, J.-C., DE BISEAU, J.-C., and MAES, D. 2012. Invasive alien predator causes rapid declines of native European ladybirds. *Diversity and Distributions* 18:717-725.
- RUÍZ-CANCINO, E., CORONADO-BLANCO, J. M., and MYARTSEVA, S. N. 2012. Himenópteros parasitoides como prospectos de control biológico en México. In: Ruíz-Cancino E, Coronado-Blanco JM, editors. Recursos naturales. Cd. Victoria, Mexico: Universidad Autónoma de Tamaulipas.
- RUTHER, J. and 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* 31:2217-2222.
- SALVO, A., FENOGLIO, M. S., and VIDELA, M. 2005. Parasitism of a leafminer in managed versus natural habitats. *Agriculture, Ecosystems & Environment* 109:213-220.
- SÁNCHEZ G., J. J., DE LA CRUZ L., L., VIDAL M., V. A., RON P., J., TABA, S., SANTACRUZ-RUVALCABA, F., SOOD, S., HOLLAND, J. B., RUÍZ C., J. A., CARVAJAL, S., ARAGÓN C., F., CHÁVEZ T., V. H., MORALES R., M. M., and BARBA-GONZÁLEZ, R. 2011. Three new teosintes (*Zea* spp., Poaceae) from México. *American Journal of Botany* 98:1537-1548.
- SÁNCHEZ GONZÁLEZ, J. J. and RUIZ CORRAL, J. A. 1995. Teosinte distribution in Mexico. In: Serratos JA, Willcox MC, Castillo F, editors. PROCEEDINGS OF A FORUM Gene flow among maize landraces, improved maize varieties, and teosinte: Implications for transgenic maize. Mexico City, Mexico: CIMMYT. p 18-39.
- SCALA, A., ALLMANN, S., MIRABELLA, R., HARING, M., and SCHUURINK, R. 2013. Green leaf volatiles: A plant's multifunctional weapon against herbivores and pathogens. *International Journal of Molecular Sciences* 14:17781-17811.
- SCHMELZ, E. A., ALBORN, H. T., and TUMLINSON, J. H. 2003. Synergistic interactions between volicitin, jasmonic acid and ethylene mediate insect-induced volatile emission in *Zea mays*. *Physiologia Plantarum* 117:403-412.
- SCHMELZ, E. A., CARROLL, M. J., LECLERE, S., PHIPPS, S. M., MEREDITH, J., CHOUREY, P. S., ALBORN, H. T., and TEAL, P. E. A. 2006. Fragments of ATP synthase mediate plant perception of insect attack. *Proceedings of the National Academy of Sciences of the United States of America* 103:8894-8899.
- SCHMELZ, E. A., KAPLAN, F., HUFFAKER, A., DAFOE, N. J., VAUGHAN, M. M., NI, X., ROCCA, J. R., ALBORN, H. T., and TEAL, P. E. 2011. Identity, regulation, and activity of inducible diterpenoid phytoalexins in maize. *Proceedings of the National Academy of Sciences of the United States of America* 108:5455-5460.
- SCHOONHOVEN, L. M., VAN LOON, J. J. A., and DICKE, M. 2005. Insect-plant biology. Oxford, UK: Oxford University Press.
- SCHUMAN, M. C., BARTHEL, K., and BALDWIN, I. T. 2012. Herbivory-induced volatiles function as defenses increasing fitness of the native plant *Nicotiana attenuata* in nature. *eLife* 1:e00007.
- SCHÜTZ, S., WEIBBECKER, B., KLEIN, A., and HUMMEL, H. E. 1997. Host plant selection of the Colorado potato beetle as influenced by damage induced volatiles of the potato plant. *Naturwissenschaften* 84:212-217.
- SHAHID, M. and ESEN, A. 1998. DIMBOA-glu concentration in different taxa of teosinte. *Maize Genetics Cooperation Newsletter* 72:23-24.

- SHIOJIRI, K., KISHIMOTO, K., OZAWA, R., KUGIMIYA, S., URASHIMO, S., ARIMURA, G., HORIUCHI, J., NISHIOKA, T., MATSUI, K., and TAKABAYASHI, J. 2006a. 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 of the United States of America* 103:16672-16676.
- SHIOJIRI, K., OZAWA, R., MATSUI, K., KISHIMOTO, K., KUGIMIYA, S., and TAKABAYASHI, J. 2006b. Role of the Lipooxygenase/lyase pathway of host-food plants in the host searching behavior of two parasitoid species, *Cotesia glomerata* and *Cotesia plutellae*. *Journal of Chemical Ecology* 32:969-979.
- SHIOJIRI, K., OZAWA, R., MATSUI, K., SABELIS, M. W., and TAKABAYASHI, J. 2012. Intermittent exposure to traces of green leaf volatiles triggers a plant response. *Scientific Reports* 2:378.
- SHIVAJI, R., CAMAS, A., ANKALA, A., ENGELBERTH, J., TUMLINSON, J., WILLIAMS, W., WILKINSON, J., and LUTHE, D. 2010. Plants on constant alert: Elevated levels of jasmonic acid and jasmonate-induced transcripts in caterpillar-resistant maize. *Journal of Chemical Ecology* 36:179-191.
- SIMPSON, M., GURR, G. M., SIMMONS, A. T., WRATTEN, S. D., JAMES, D. G., LEESON, G., and NICOL, H. I. 2011. Insect attraction to synthetic herbivore-induced plant volatile-treated field crops. *Agricultural and Forest Entomology* 13:45-57.
- SINGER, M. S., MACE, K. C., and BERNAYS, E. A. 2009. Self-medication as adaptive plasticity: Increased ingestion of plant toxins by parasitized caterpillars. *PLoS ONE* 4:e4796.
- SMALLEGANGE, R. C., VAN LOON, J. J. A., BLATT, S. E., HARVEY, J. A., and DICKE, M. 2008. Parasitoid load affects plant fitness in a tritrophic system. *Entomologia Experimentalis et Applicata* 128:172-183.
- SMITH, D. R. and WHITE, D. G. 1988. Diseases of corn. In: Sprague GF, Dudley JW, editors. Corn and corn improvement. USA: American Society of Agronomy. p 687-766.
- SOBHY, I., ERB, M., LOU, Y., and TURLINGS, T. C. J. 2013, in press. The prospect of applying chemical elicitors and plant strengtheners to enhance the biological control of crop pests. *Philosophical Transactions of the Royal Society of London. Series B, Biological Sciences*.
- SOBHY, I., ERB, M., SARHAN, A., EL-HUSSEINI, M., MANDOUR, N., and TURLINGS, T. J. 2012. Less is more: Treatment with BTH and Laminarin reduces herbivore-induced volatile emissions in maize but increases parasitoid attraction. *Journal of Chemical Ecology* 38:348-360.
- SOLER, R., Van der PUTTEN, W., HARVEY, J. A., VET, L. E. M., DICKE, M., and BEZEMER, T. M. 2012. Root herbivore effects on aboveground multitrophic interactions: Patterns, processes and mechanisms. *Journal of Chemical Ecology* 38:755-767.
- SPARKS, A. N. 1979. A review of the biology of the fall armyworm. *Florida Entomologist* 62:82-87.
- STAUDT, M., JACKSON, B., EL-AOUNI, H., BUATOIS, B., LACROZE, J.-P., POËSSEL, J.-L., and SAUGE, M.-H. 2010. Volatile organic compound emissions induced by the aphid *Myzus persicae* differ among resistant and susceptible peach cultivars and a wild relative. *Tree Physiology* 30:1320-1334.
- STEENKAMP, E. T., COUTINHO, T. A., DESJARDINS, A. E., WINGFIELD, B. D., MARASAS, W. F. O., and WINGFIELD, M. J. 2001. *Gibberella fujikuroi* mating population E is associated with maize and teosinte. *Molecular Plant Pathology* 2:215-221.
- STEFFEY, K. L., RICE, M. E., ALL, J., ANDOW, D. A., GRAY, M. E., and VAN DUYN, J. W. 1999. Handbook of corn insects. Lanham, USA: Entomological Society of America.
- STEIGER, S., SCHMITT, T., and SCHAEFER, H. M. 2011. The origin and dynamic evolution of chemical information transfer. *Proceedings of the Royal Society B* 278:970-979.
- STEINER, S., STEIDLE, J. L. M., and RUTHER, J. 2007. Host-associated kairomones used for habitat orientation in the parasitoid *Lariophagus distinguendus* (Hymenoptera: Pteromalidae). *Journal of Stored Products Research* 43:587-593.
- STETTLER, P., TRENCZEK, T., WYLER, T., PFISTER-WILHELM, R., and LANZREIN, B. 1998. Overview of parasitism associated effects on host haemocytes in larval parasitoids and comparison with effects of the egg-larval parasitoid *Chelonus inanitus* on its host *Spodoptera littoralis*. *Journal of Insect Physiology* 44:817-831.
- SUMA, S. and MAGAREY, R. C. 2000. Downy mildew. In: Rott P, Bailey RA, Comstock JC, Croft BJ, Saumtally AS, editors. A guide to sugarcane diseases. France: Cirad. p 90-95.

- SWANSON-WAGNER, R., BRISKINE, R., SCHAEFER, R., HUFFORD, M. B., ROSS-IBARRA, J., MYERS, C. L., TIFFIN, P., and SPRINGER, N. M. 2012. Reshaping of the maize transcriptome by domestication. *Proceedings of the National Academy of Sciences of the United States of America* 109:11878-11883.
- SWANSON-WAGNER, R. A., EICHTEIN, S. R., KUMARI, S., TIFFIN, P., STEIN, J. C., WARE, D., and SPRINGER, N. M. 2010. Pervasive gene content variation and copy number variation in maize and its undomesticated progenitor. *Genome Research* 20:1689-1699.
- SWARUP, S., TIMMERMANS, M. C. P., CHAUDHURI, S., and MESSING, J. 1995. Determinants of the high-methionine trait in wild and exotic germplasm may have escaped selection during early cultivation of maize. *The Plant Journal* 8:359-368.
- SZCZEPANIEC, A., WIDNEY, S. E., BERNAL, J. S., and EUBANKS, M. D. 2013. Higher expression of induced defenses in teosintes (*Zea* spp.) is correlated with greater resistance to fall armyworm, *Spodoptera frugiperda*. *Entomologia Experimentalis et Applicata* 146:242-251.
- SZENDREI, Z. and RODRIGUEZ-SAONA, C. 2010. A meta-analysis of insect pest behavioral manipulation with plant volatiles. *Entomologia Experimentalis et Applicata* 134:201-210.
- SZNAJDER, B. and HARVEY, J. A. 2003. Second and third trophic level effects of differences in plant species reflect dietary specialisation of herbivores and their endoparasitoids. *Entomologia Experimentalis et Applicata* 109:73-82.
- TABA, S., VAN GINKEL, M., HOISINGTON, D., AND POLAND, D. 2004. Wellhausen-Anderson Plant Genetic Resources Center: Operations manual, 2004. El Batan, Mexico: CIMMYT.
- TAKABAYASHI, J., DICKE, M., and POSTHUMUS, M. A. 1994. Volatile herbivore-induced terpenoids in plant-mite interactions: variation caused by biotic and abiotic factors. *Journal of Chemical Ecology* 20:1329-1354.
- TAKAHASHI, C. G., KALNS, L. L., and BERNAL, J. S. 2012. Plant defense against fall armyworm in micro-sympatric maize (*Zea mays* ssp. *mays*) and Balsas teosinte (*Zea mays* ssp. *parviglumis*). *Entomologia Experimentalis et Applicata* 145:191-200.
- TAMIRU, A., BRUCE, T. J. A., WOODCOCK, C. M., CAULFIELD, J. C., MIDEGA, C. A. O., OGOL, C. K. P. O., MAYON, P., BIRKETT, M. A., PICKETT, J. A., and KHAN, Z. R. 2011. Maize landraces recruit egg and larval parasitoids in response to egg deposition by a herbivore. *Ecology Letters* 14:1075-1083.
- TAMÒ, C., RICARD, I., HELD, M., DAVISON, A. C., and TURLINGS, T. C. J. 2006a. A comparison of naïve and conditioned responses of three generalist endoparasitoids of lepidopteran larvae to host-induced plant odours. *Animal Biology* 56:205-220.
- TAMÒ, C., ROELFSTRA, L.-L., GUILLAUME, S., and TURLINGS, T. C. J. 2006b. Odour-mediated long-range avoidance of interspecific competition by a solitary endoparasitoid: A time-saving foraging strategy. *Journal of Animal Ecology* 75:1091-1099.
- THALER, J. S. 1999. Jasmonate-inducible plant defences cause increased parasitism of herbivores. *Nature* 399:686-688.
- THOLL, D., SOHRABI, R., HUH, J.-H., and LEE, S. 2011. The biochemistry of homoterpenes – Common constituents of floral and herbivore-induced plant volatile bouquets. *Phytochemistry* 72:1635-1646.
- TON, J., D'ALESSANDRO, M., JOURDIE, V., JAKAB, G., KARLEN, D., HELD, M., MAUCH-MANI, B., and TURLINGS, T. C. J. 2007. Priming by air-borne signals boosts direct and indirect resistance in maize. *The Plant Journal* 49:16-26.
- TURLINGS, T. C. J. and BENREY, B. 1998. Effects of plant metabolites on the behavior and development of parasitic wasps. *Ecoscience* 5:321-333.
- TURLINGS, T. C. J., BERNASCONI, M., BERTOSSA, R., BIGLER, F., CALOZ, G., and DORN, S. 1998a. 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., DAVISON, A. C., and TAMÒ, C. 2004. A six-arm olfactometer permitting simultaneous observation of insect attraction and odour trapping. *Physiological Entomology* 29:45-55.
- TURLINGS, T. C. J. and FRITZSCHE, M. E. 1999b. Attraction of parasitic wasps by caterpillar-damaged plants. In: Chadwick DJ, Goode JA, editors. Insect-plant interactions and induced plant defence. Chichester, UK: John Wiley & Sons, Ltd. p 21-38.

## References

---

- TURLINGS, T. C. J., LENGWILER, U. B., BERNASCONI, M. L., and WECHSLER, D. 1998b. Timing of induced volatile emissions in maize seedlings. *Planta* 207:146-152.
- TURLINGS, T. C. J., LOUGHRIN, J. H., MCCALL, P. J., ROSE, U. S. R., LEWIS, W. J., and TUMLINSON, J. H. 1995b. How caterpillar-damaged plants protect themselves by attracting parasitic wasps. *Proceedings of the National Academy of Sciences of the United States of America* 92:4169-4174.
- TURLINGS, T. C. J., MCCALL, P. J., ALBORN, H. T., and TUMLINSON, J. H. 1993. An elicitor in caterpillar oral secretions that induces corn seedlings to emit chemical signals attractive to parasitic wasps. *Journal of Chemical Ecology* 19:411-425.
- TURLINGS, T. C. J. and 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. J., TUMLINSON, J. H., HEATH, R. R., PROVEAUX, A. T., and DOOLITTLE, R. E. 1991. Isolation and identification of allelochemicals that attract the larval parasitoid, *Cotesia marginiventris* (Cresson), to the microhabitat of one of its hosts. *Journal of Chemical Ecology* 17:2235-2251.
- TURLINGS, T. C. J., TUMLINSON, J. H., and LEWIS, W. J. 1990. Exploitation of herbivore-induced plant odors by host-seeking parasitic wasps. *Science* 250:1251-1253.
- TURLINGS, T. C. J. and WÄCKERS, F. 2004. Recruitment of predators and parasitoids by herbivore-injured plants. In: Cardé RT, Millar JG, editors. *Advances in insect chemical ecology*. Cambridge, UK: Cambridge University Press. p 21-75.
- UNITED NATIONS. 2001. Stockholm convention on persistent organic pollutants. Stockholm, Sweden: United Nations.
- UNITED NATIONS. 2004. World population to 2300. New York, USA: United Nations.
- VAN LENTEREN, J. C. 2003. Commercial availability of biological control agents. In: Van Lenteren JC, editor. *Quality control and production of biological control agents: Theory and testing procedures*. Oxon, UK: CABI Publishing. p 167-179.
- VAN LENTEREN, J. C., COCK, M. J. W., HOFFMEISTER, T. S., and SANDS, D. P. A. 2006. Host specificity in arthropod biological control, methods for testing and interpretation of the data. In: Bigler F, Babendreier D, Kuhlmann U, editors. *Environmental impact of invertebrates for biological control of arthropods*. Wallingford, UK: CABI Publishing. p 38-63.
- VAN LENTEREN, J. C. and WOETS, J. 1988. Biological and integrated pest control in greenhouses. *Annual Review of Entomology* 33:239-269.
- VAN LOON, J. J. A., DE BOER, J. G., and DICKE, M. 2000. Parasitoid-plant mutualism: Parasitoid attack of herbivore increases plant reproduction. *Entomologia Experimentalis et Applicata* 97:219-227.
- VAN POECKE, R. M. P. and DICKE, M. 2003. Signal transduction downstream of salicylic and jasmonic acid in herbivory-induced parasitoid attraction by *Arabidopsis* is independent of JAR1 and NPR1. *Plant, Cell & Environment* 26:1541-1548.
- VAN POECKE, R. M. P., POSTHUMUS, M. A., and DICKE, M. 2001. Herbivore-induced volatile production by *Arabidopsis thaliana* leads to attraction of the parasitoid *Cotesia rubecula*: Chemical, behavioral, and gene-expression analysis. *Journal of Chemical Ecology* 27:1911-1928.
- VAN TOL, R. W. H. M., VAN DER SOMMEN, A. T. C., BOFF, M. I. C., VAN BEZOOIJEN, J., SABELIS, M. W., and SMITS, P. H. 2001. Plants protect their roots by alerting the enemies of grubs. *Ecology Letters* 4:292-294.
- VANCANNEYT, G., SANZ, C., FARMAKI, T., PANEQUE, M., ORTEGO, F., CASTAÑERA, P., and SÁNCHEZ-SERRANO, J. J. 2001. Hydroperoxide lyase depletion in transgenic potato plants leads to an increase in aphid performance. *Proceedings of the National Academy of Sciences of the United States of America* 98:8139-8144.
- VIBRANS, H. and ESTRADA FLORES, J. G. 1998. Annual teosinte is a common weed in the Valley of Toluca, Mexico [*Zea mays* spp. *mexicana* (Schrader) Iltis]. *Maydica* 43:45-48.
- VICKERY, R. A. 1929. Studies on the fall armyworm in the Gulf coast district of Texas. *Technical Bulletin* 138:1-63.
- VIDAVER, A. M. and CARLSON, R. R. 1987. Leaf spot of field corn caused by *Pseudomonas andropogonis*. *Plant disease reporter* 62:213-216.
- VINSON, S. B. 1976. Host selection by insect parasitoids. *Annual Review of Entomology* 21:109-133.
- VISSER, J. H. 1986. Host odor perception in phytophagous insects. *Annual Review of Entomology* 31:121-144.



- VON MÉREY, G. 2010. Manipulating maize volatiles to improve the biological control of insect pests. PhD thesis, University of Neuchâtel, Neuchâtel, Switzerland.
- VON MÉREY, G., VEYRAT, N., MAHUKU, G., LÓPEZ VALDEZ, R., TURLINGS, T. C. J., and D'ALESSANDRO, M. 2011. Dispensing synthetic green leaf volatiles in maize fields increases the release of sesquiterpenes by the plants, but has little effect on the attraction of pest and beneficial insects. *Phytochemistry* 72:1838-1847.
- VON MÉREY, G. E., VEYRAT, N., D'ALESSANDRO, M., and TURLINGS, T. C. J. 2013. Herbivore-induced maize leaf volatiles affect attraction and feeding behaviour of *Spodoptera littoralis* caterpillars. *Frontiers in Plant Science* 4:209.
- VON MÉREY, G. E., VEYRAT, N., DE LANGE, E., DEGEN, T., MAHUKU, G., LÓPEZ VALDEZ, R., TURLINGS, T. C. J., and D'ALESSANDRO, M. 2012. Minor effects of two elicitors of insect and pathogen resistance on volatile emissions and parasitism of *Spodoptera frugiperda* in Mexican maize fields. *Biological Control* 60:7-15.
- WALTON, W. R. and LUGINBILL, P. 1916. The fall army worm, or "grass worm," and its control. *USDA Farmers' Bulletin* 752:1-16.
- WANG, H., NUSSBAUM-WAGLER, T., LI, B., ZHAO, Q., VIGOUROUX, Y., FALLER, M., BOMBLIES, K., LUKENS, L., and DOEBLEY, J. F. 2005. The origin of the naked grains of maize. *Nature* 436:714-719.
- WANG, L., XU, C., QU, M., and ZHANG, J. 2008a. Kernel amino acid composition and protein content of introgression lines from *Zea mays* ssp. *mexicana* into cultivated maize. *Journal of Cereal Science* 48:387-393.
- WANG, L., YANG, A., HE, C., QU, M., and ZHANG, J. 2008b. Creation of new maize germplasm using alien introgression from *Zea mays* ssp. *mexicana*. *Euphytica* 164:789-801.
- WANG, X.-G., NADEL, H., JOHNSON, M. W., DAANE, K. M., HOELMER, K., WALTON, V. M., PICKETT, C. H., and SIME, K. R. 2009. Crop domestication relaxes both top-down and bottom-up effects on a specialist herbivore. *Basic and Applied Ecology* 10:216-227.
- WAR, A. R., SHARMA, H. C., PAULRAJ, M. G., WAR, M. Y., AND IGNACIMUTHU, S. 2011. Herbivore induced plant volatiles: Their role in plant defense for pest management. *Plant Signaling & Behavior* 6:1973-1978.
- WASTERNAK, C. and KOMBRINK, E. 2010. Jasmonates: Structural requirements for lipid-derived signals active in plant stress responses and development. *ACS Chemical Biology* 5:63-77.
- WEI, J., WANG, L., ZHU, J., ZHANG, S., NANDI, O. I., and KANG, L. 2007. Plants attract parasitic wasps to defend themselves against insect pests by releasing hexenol. *PLoS ONE* 2:e852.
- WELTER, S. C. and STEGGALL, J. W. 1993. Contrasting the tolerance of wild and domesticated tomatoes to herbivory: Agroecological implications. *Ecological Applications*. 3:271-278.
- WHALON, M. E., MOTA-SANCHEZ, D., and HOLLINGWORTH, R. M. 2008. Global pesticide resistance in arthropods. Oxford, UK: Oxford University Press.
- WHITEHORN, P. R., O'CONNOR, S., WACKERS, F. L., and GOULSON, D. 2012. Neonicotinoid pesticide reduces bumble bee colony growth and queen production. *Science* 336:351-352.
- WHITMAN, D. W. and ELLER, F. J. 1990. Parasitic wasps orient to green leaf volatiles. *Chemoecology* 1:69-76.
- WILKES, G. 2007. Urgent notice to all maize researchers: Disappearance and extinction of the last wild teosinte population is more than half completed. A modest proposal for teosinte evolution and conservation *in situ*: The Balsas, Guerrero, Mexico. *Maydica* 52:49-58.
- WILKES, H. G. 1977. Hybridization of maize and teosinte in Mexico and Guatemala and the improvement of maize. *Economic Botany* 31:254-293.
- WILKES, H. G. 1995. Teosinte in Mexico: Personal retrospective and assessment. In: Serratos JA, Willcox MC, Castillo F, editors. PROCEEDINGS OF A FORUM Gene flow among maize landraces, improved maize varieties, and teosinte: Implications for transgenic maize. Mexico City, Mexico: CIMMYT. p 10-17.
- WILLIAMS, T., ARREDONDO-BERNAL, H. C., and RODRÍGUEZ-DEL-BOSQUE, L. A. 2013. Biological pest control in Mexico. *Annual Review of Entomology* 58:119-140.
- WILSON, D. D. and RIDGWAY, R. L. 1975. Morphology, development, and behavior of the immature stages of the parasitoid, *Campoletis sonorensis* (Hymenoptera: Ichneumonidae). *Annals of the Entomological Society of America* 68:191-196.

## References

---

- WINK, M. 1988. Plant breeding: Importance of plant secondary metabolites for protection against pathogens and herbivores. *Theoretical and Applied Genetics* 75:225-233.
- WRIGHT, S. I., BI, I. V., SCHROEDER, S. G., YAMASAKI, M., DOEBLEY, J. F., MCMULLEN, M. D., and GAUT, B. S. 2005. The effects of artificial selection on the maize genome. *Science* 308:1310-1314.
- YU, H., ZHANG, Y., WU, K., GAO, X. W., and GUO, Y. Y. 2008. Field-testing of synthetic herbivore-induced plant volatiles as attractants for beneficial insects. *Environmental Entomology* 37:1410-1415.
- ZHANG, L., PEEK, A. S., DUNAMS, D., and GAUT, B. S. 2002. Population genetics of duplicated disease-defense genes, *hm1* and *hm2*, in maize (*Zea mays* ssp. *mays* L.) and its wild ancestor (*Zea mays* ssp. *parviglumis*). *Genetics* 162:851-860.

## Acknowledgements

It's done! I can hardly believe it, but it's true, and there are many people I'd like to thank for supporting me until the end.

Great thanks to my supervisor, Ted Turlings, for giving me the opportunity to do my PhD in your laboratory. Thanks for your trust in me, even when I spent prolonged periods of time on the other side of the world. Thomas, thanks for your support during our many field trips. Without you I still wouldn't be able to distinguish a wasp from a fly. Thanks as well for your support in the lab, and for drawing and improving a number of figures in this thesis.

I'd like to thank Prof. Ken Oyama for providing a field site at UNAM in Morelia, Mexico. Rafa, ¡muchísimas gracias por tu ayuda! Fernando, mil gracias por tu valiosa ayuda y claro gracias por mostrarnos Teotihuacán y las mariposas monarcas. Algo que jamás olvidaré. Great thanks to Víctor Rocha-Ramírez for his valuable support in the field, to Prof. Francisco Espinosa-García for the use of the gas chromatograph, and to Yolanda García-Rodríguez for assistance with the chromatographic analysis. Selene Ramos-Ortiz, thanks a lot for your help with the photosynthesis measurements and Samuel Pineda, thanks for your help with the beet armyworm rearing. Yola, Alicia, John and other members of CieCO, thanks for making my stay so memorable. Benjamin, merci beaucoup pour ton aide sur le terrain et au labo. Kevin, thanks a lot for your support, your patience and your honesty. Good luck finishing your own thesis, you can do it, no doubt about it.

Thanks to Dr. George Mahuku, Carlos Muñoz, Emiliano Gutiérrez and Dr. Suketoshi Taba at CIMMYT in El Batán, Mexico, for your assistance with my field studies. Ricardo, thanks for your help in Toluca and Germán, thanks for taking me to the field station in Tlaltizapán. Ricardo, Kate, Germán, Vanessa, Yuna and the others, thanks for taking me out to Texcoco and to Querétaro, we had a great time. Thanks to Dr. Jorge Ibarra at CINVESTAV in Irapuato, Mexico, for your help identifying the insects that occur on teosinte. Thanks also to Dr. Martin Heil at CINVESTAV and to Prof. Gustavo Moya-Raygoza at the University of Guadalajara in Jalisco, Mexico, for sharing your indispensable tips and tricks for doing field work.

Thank you Dr. Mike Kolomiets for allowing me to stay in your lab at Texas A&M in College Station, USA. Thanks Dr. Shawn Christensen, Dr. Yan, Mickaël Gaillard and the students in the lab for harvesting carloads full of plants and weighing endless numbers of caterpillars. And of course for taking me out to the Texas Roadhouse and many other places, to show me that everything is bigger in Texas!

At LaTrobe University in Melbourne, Australia, thanks to Martin Steinbauer for our discussions.

I would like to thank the jury of my thesis: Prof. Ted Turlings, Prof. Florian Schiestl, Dr. Christophe Praz and Dr. Alisa Huffaker. Prof. Ted Turlings, Prof. Anne-Marie Cortesero, Dr. Gwen Shlichta and Dr. Gaylord Desurmont were kind enough to be part of my mid-thesis committee.

Jorge Cham, thanks so much for drawing a PhD comic of my research, the video is awesome!

Thanks to my past and present colleagues in the FARCE and E-VOL laboratories for bearing with me these past five (!) years. Six, actually, if you count my Masters as well. And a memorable six years it has been. I will miss our Pubquiz nights, poker nights, werewolf nights, hiking tours, skiing trips and apéros. And our scientific discussions, too, you guys taught me a lot.

I owe thanks to many of you for your help with the abstracts: Gaylord, Gwen, Maryse and Thomas, thanks for your comments on the English version; Maryse and Mickaël, thanks for correcting the French version; Johnattan, Josefina and Nataly, thanks for improving the Spanish version; Angela and Daniel, thanks for writing the German version and Tom, thanks for reading through the Dutch version. Also, many of you have been kind enough to read and comment on one or even several thesis chapters. Angela and Thomas, thanks a lot for your comments on the general introduction. Thomas, thanks for your input on Chapters 3 and 4. Gaétan and Gaylord, thanks for helping to improve Chapter 5. Angela and Thomas, thanks for your feedback on the general discussion. I can't thank you guys enough!

Gaétan, thanks for sharing the office with me. I hope you won't stay alone there for too long! Matthias Held, thanks for your support on everything concerning statistics and computers, and for the awesome pictures that are included in my thesis. Michèle, thanks for showing me how to make beautiful SEM pictures. Georg, thanks for taking me along on your last field trip to Mexico, and for teaching me how to set-up my own field study. Georg, Nathalie and Thomas, it was great to do fieldwork with you, and I really enjoyed our weekend trips. Diane, thanks a lot for continuing to do experiments. Science never stops! Matthias Erb, thanks for your advice. Angela, Daniel, Noémie, Dirk, Victoria, it was really nice to go to the meeting in Avignon with you. I've had the opportunity to go to quite a few meetings, thanks a lot to the people with whom I had a great time.

I already started to entwine colleagues in my lab, in other labs at the University of Neuchâtel, in other labs in Switzerland, and beyond. Jo-Anne, Nathalie, Anahí, Sandrine and Christiane, I'll really miss our classes in aquafitness and zumba (but maybe not so much those in condition physique ☺). Livia, I was honoured to be at your wedding, and I wish you all the happiness in the world. Yosra, it is great to have you as a friend. Mickaël, come over any time for some (astronaut) food. Victoria, we only met a short while ago, but it was great to spend time with you and thanks so much for your help moving out. And thanks to everyone who spontaneously showed up on my doorstep the day I had to empty my flat. When sorting out my things, selling my furniture and getting ready for my Postdoc, but also before, you guys really helped me a lot. Angela, your name popped up already many times. Thanks for everything. Gwen, John & Vee, I don't know how to thank you, but thanks for everything.

To Albert, Alfonso, Ashok, Betty, Chantal, Christophe, Jesse & Mila, Claudia, Cyril, Gemma, Geoffrey, Greg, Hao, Igor, Ivan, Jinwon, Jonas, Léonore, Lily, Makiala, Marco d'Alessandro, Marco Fracassetti, Marianne, Max, Meryl, Neil, Pieter, Pip, Raquel, Ruben,

Vincent, Yann, Yury and many others, thanks a lot for your help in the lab and/or the good time we spent together.

Thanks to Yves, Angela, Christelle, Fernanda, Gaylord, Sandrine and the ever-changing group of students at the University of Neuchâtel for the rearing of caterpillars and wasps. Syngenta (Stein, Switzerland) supplied *S. littoralis* eggs and ARS-USDA (USA) provided teosinte seeds.

Thanks to the Doctoral Program in Organismal Biology and Service de l'égalité des chances at the University of Neuchâtel and to the Swiss Zoological Society for providing mobility grants, enabling me to present my work at various scientific conferences. My field studies were financially supported by the Swiss National Science Foundation (grant 31003A-122132).

Christiane, thanks a lot for coordinating the Doctoral Program and Sandrine, thanks for organising the summer schools. Brigitte et Natacha, merci pour votre support. Carol, un grand merci et tout de bon à Paris!

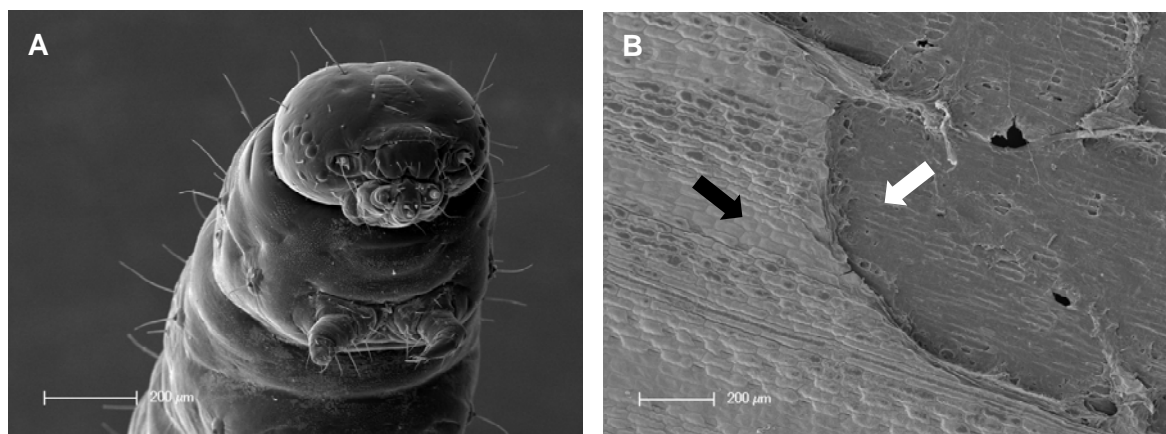
Queridas Josefina y Diana, mil gracias por sus enseñanzas y por ayudarme a hablar español. Wieke, Martin & Quinn, Eva, Kajan, Franciska & Jaco, Anna, Elle Bartha, Esther, Marjolein, Judith, Marijn, Dirk, Mark en Peter, bedankt voor jullie bezoeken aan Zwitserland, het was super gezellig. En natuurlijk ook bedankt aan m'n familieleden en vrienden in Nederland die me niet vergeten zijn, ook al was ik ver weg. Bedankt voor alle pakjes met Nederlandse spullen, van dropjes tot pepernoten. Jullie houden me op de been, ik vind het super dat we nog steeds contact houden en het is altijd gezellig wanneer ik weer terug in Nederland ben. Postcrossers, we'll sure keep writing! Jo-Anne, merci pour une belle amitié, je me réjouis de te revoir aux États-Unis. Aan m'n ouders, jullie zijn me het vaakst komen opzoeken in Zwitserland en ik hoop dat de VS als vakantie-adres ook gaat bevallen. Bedankt voor jullie (over)bezorgdheid en onvoorwaardelijke steun.



## Annex 1







**Fig. S1** Scanning electron microscopy (SEM) images of a *Spodoptera frugiperda* larva and the damage this larva inflicted on maize plants. (A) Second-instar larva. (B) Herbivory. A black arrow indicates undamaged leaf tissue, while a white arrow indicates damaged leaf tissue. Early-instar *S. frugiperda* larvae inflict so-called windowpane-damage, consuming the epidermis and mesophyll from one side of the leaf, while leaving the cuticle and the epidermis of the other side of the leaf intact. Pictures: Elvira de Lange and Michèle Vlimant



## Annex 2



## Chapter 5

### Supplementary data

#### Parasitic wasps can reduce mortality in caterpillar-infested teosinte plants

Elvira S. de Lange, Kevin Farnier, Thomas Degen, Benjamin Gaudillat, Rafael Aguilar-Romero, Fernando Bahena-Juárez, Ken Oyama, and Ted C.J. Turlings

#### Material and methods

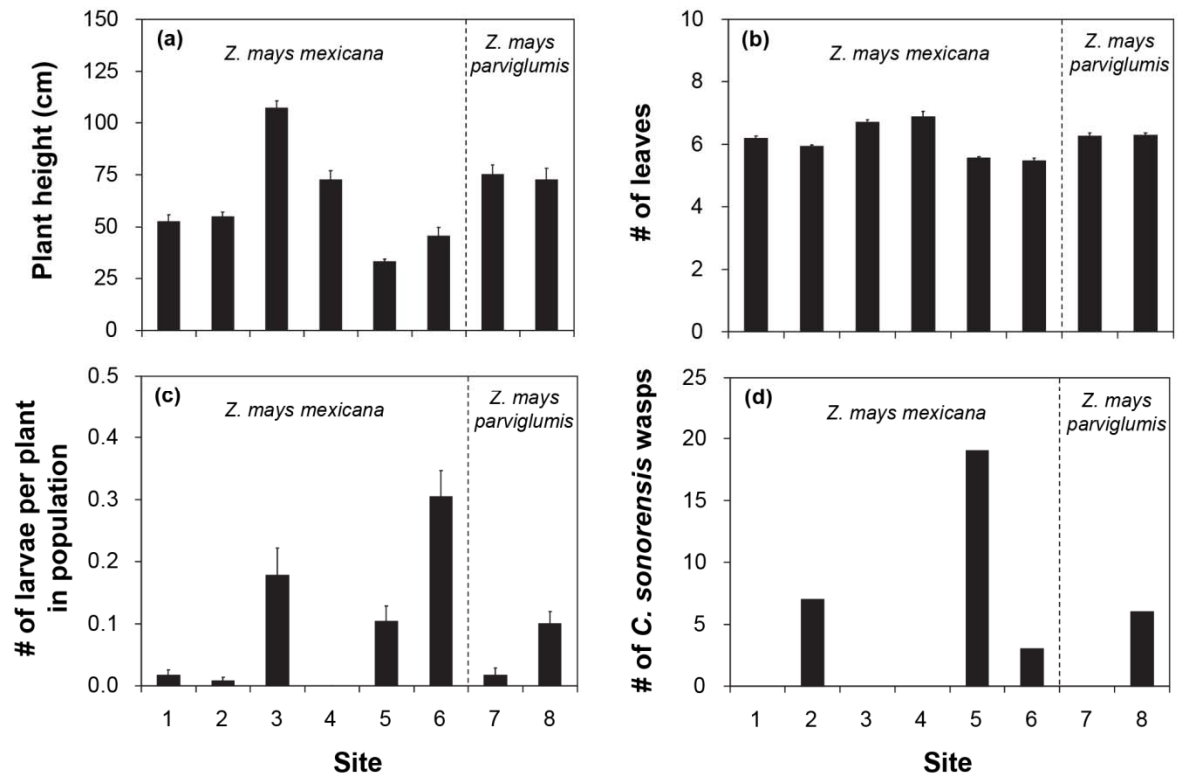
*Survey of Teosinte, Herbivores and Parasitoids* To evaluate plant fitness consequences mediated by parasitoids in an evolutionary context, the study organisms must co-occur. To assess if this criterion was met for our study organisms, we conducted a survey of teosinte populations in Mexico in 2009. We visited teosinte populations at eight different locations in three different Mexican states (Table S1), where we assessed plant height, occurrence of *Spodoptera frugiperda* (Lepidoptera: Noctuidae) and occurrence of its parasitoids. At each site, we measured the height of 10-20 randomly selected plants from the stem base to the tip of the longest leaf. We also counted the total number of developing leaves, including the small expanding ones, of 69-247 plants per site. On these plants, we subsequently recorded the presence or absence of *S. frugiperda* larvae. When present, the larvae were counted, collected and placed in individual compartments of a 24-well ELISA plate. Larvae were supplied with an artificial diet and reared at room temperature until adult emergence or, in the case of parasitism, until parasitoid emergence occurred (Hoballah *et al.*, 2004). The adult parasitoids, dead parasitoid larvae and cocoons were individually preserved in 70% ethanol in Eppendorf tubes. The wasps were visually examined to identify the species (Cave, 1995).

#### Results

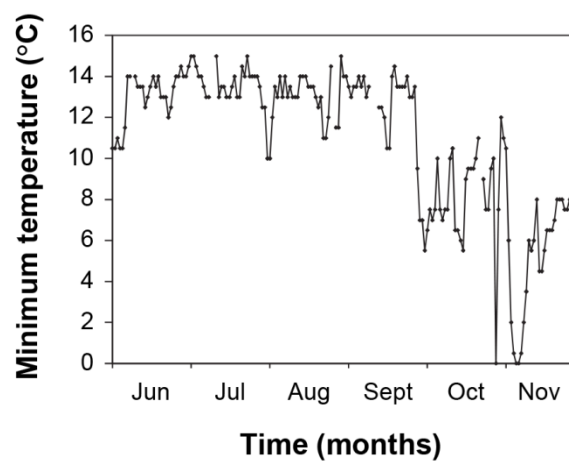
*Survey of Teosinte, Herbivores and Parasitoids* The teosinte plants that were surveyed in 2009 were on average 30-110 cm tall (Fig. S1A) and had 6 developing leaves (Fig. S1B). *S. frugiperda* larvae were present at most locations (Fig. S1C) and were frequently found to be parasitized by *Campoletis sonorensis* wasps (Fig. S1D). When a plant contained *S. frugiperda* larvae, on average, a single larva was present, but two or three larvae on a plant could be observed as well. These data served as a basis for the experimental set-up used in the 2010 field study. Seeds from two surveyed sites ("Tzitzio" and "Cuitzeo") were used for the field experiments in 2010.

#### References

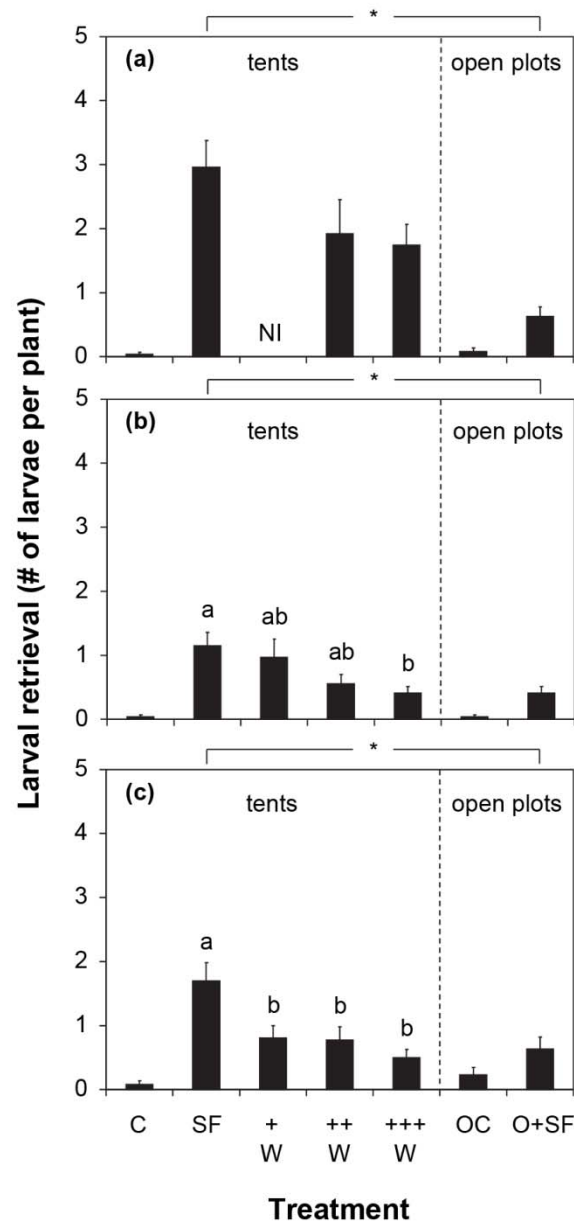
- CAVE, R. D. 1995. Manual para el reconocimiento de parasitoides de plagas agrícolas en América Central. El Zamorano, Honduras: Zamorano Academic Press.
- HOBALLAH, M. E., DEGEN, T., BERGVINSON, D., SAVIDAN, A., TAMÒ, C., and TURLINGS, T. C. J. 2004. Occurrence and direct control potential of parasitoids and predators of the fall armyworm (Lepidoptera: Noctuidae) on maize in the subtropical lowlands of Mexico. *Agricultural and Forest Entomology* 6:83-88.



**Fig. S1** Plant height, herbivore and parasitoid presence in eight teosinte populations in Mexico. (A) Plant height (+SE) at the time of the survey. (B) Average number of leaves (+SE) per plant, including small expanding leaves. (C) Average number of *Spodoptera frugiperda* larvae (+SE) found per plant in each population. (D) Total number of *Campoletis sonorensis* wasps that emerged from the *S. frugiperda* larvae that were collected. For an overview of the sites, see Table S1. Seeds from two of the surveyed sites were used for the field experiments in 2010: site 6 corresponds to “Cuitzeo” and site 7 corresponds to “Tzitzio”

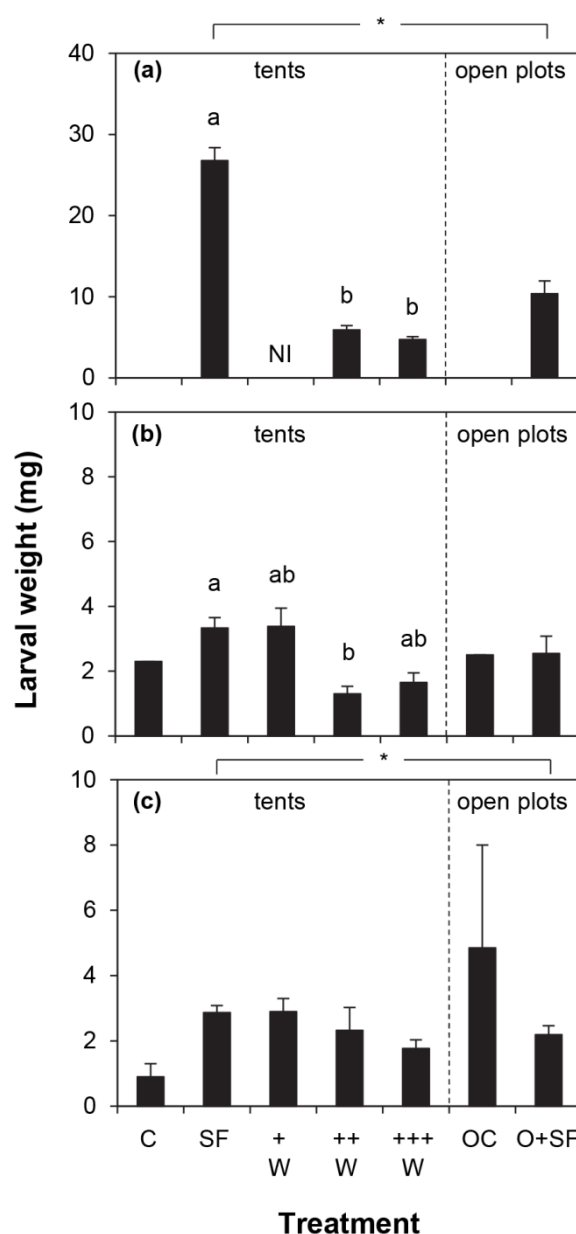


**Fig. S2** Minimum temperatures measured at a weather station nearby the experimental field. The weather station was localized in Cointzio, Michoacán, Mexico, at about 5 km distance of the experimental field. The date the temperature reached zero degrees correlates exactly to the date the plants were lost to freezing

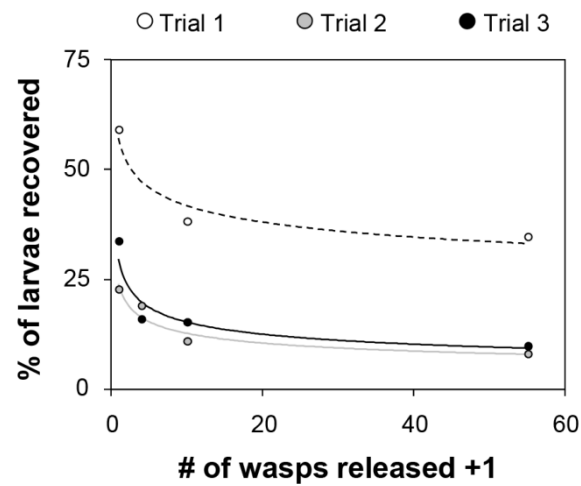


**Fig. S3** Recovery of *Spodoptera frugiperda* larvae. Average number of *Spodoptera frugiperda* larvae retrieved per plant (+SE) in tents in the absence (SF) and in the presence of increasing densities of parasitoid wasps (+W, ++W, +++W), and in plots not covered by a tent (OC, O+SF). (A) First trial. (B) Second trial. (C) Third trial. Initially, five second-instar larvae were placed on each plant in treatments SF, +W, ++W, +++W and O+SF. Different letters indicate significant differences between treatments in tents (GLM, trial 1:  $P_{\text{treatment}} = 0.113$ ,  $P_{\text{population}} = 0.614$ ,  $P_{\text{interaction}} = 0.706$ , trial 2:  $P_{\text{treatment}} = 0.023$ ,  $P_{\text{population}} = 0.021$ ,  $P_{\text{interaction}} = 0.115$ , trial 3:  $P_{\text{treatment}} < 0.001$ ,  $P_{\text{population}} = 0.109$ ,  $P_{\text{interaction}} = 0.902$ ). In trial two, significantly fewer larvae were retrieved from “Tzitzio” plants than from plants of the other varieties. An asterisk indicates significant differences between corresponding treatments in tents and open plots (GLM, trial 1:  $P_{\text{treatment}} < 0.001$ ,  $P_{\text{population}} = 0.544$ ,  $P_{\text{interaction}} = 0.910$ , trial 2:  $P_{\text{treatment}} = 0.002$ ,  $P_{\text{population}} = 0.090$ ,  $P_{\text{interaction}} = 0.071$ , trial 3:  $P_{\text{treatment}} = 0.005$ ,  $P_{\text{population}} = 0.266$ ,  $P_{\text{interaction}} = 0.915$ ). Only plots where larvae were released were included in the statistical analyses. NI = not included. For a more detailed explanation of the insect treatments, see Materials and methods

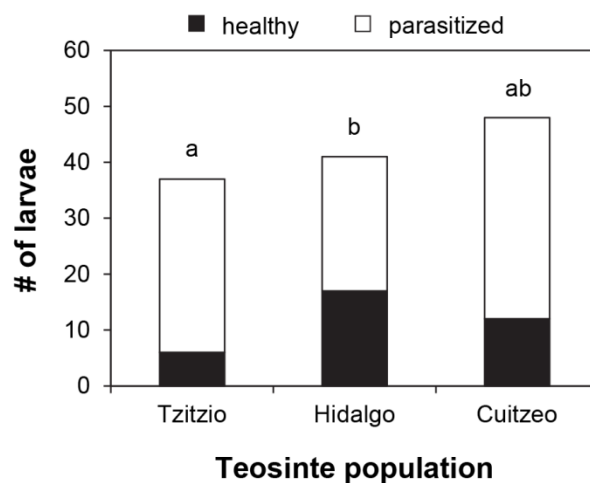




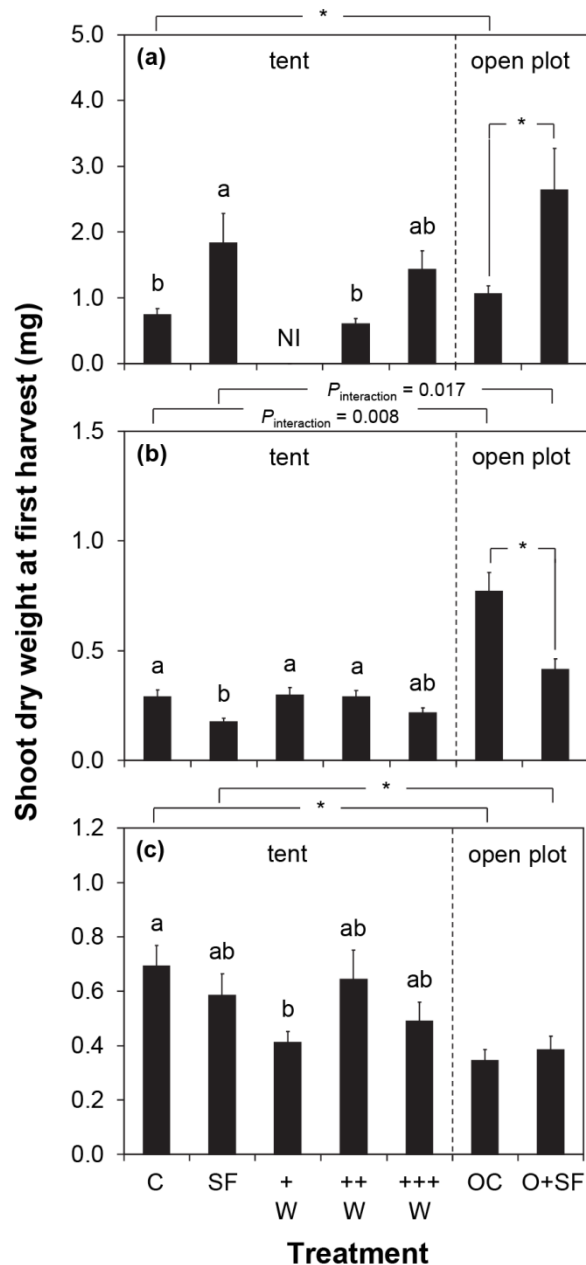
**Fig. S4** *Spodoptera frugiperda* larval weight. Weight of larvae (+SE) retrieved from plants in tents in the absence (SF) and in the presence of increasing densities of parasitoid wasps wasps (+W, ++W, +++W), and in plots not covered by a tent (OC, O+SF). (A) First trial. (B) Second trial. (C) Third trial. Initially, five second-instar larvae were placed on each plant in treatments SF, +W, ++W, +++W and O+SF. Different letters indicate significant differences between treatments in tents (GLM, trial 1:  $P_{\text{treatment}} < 0.001$ ,  $P_{\text{population}} = 0.832$ ,  $P_{\text{interaction}} = 0.714$ , trial 2:  $P_{\text{treatment}} = 0.017$ ,  $P_{\text{population}} = 0.787$ ,  $P_{\text{interaction}} = 0.323$ , trial 3:  $P_{\text{treatment}} = 0.187$ ,  $P_{\text{population}} = 0.358$ ,  $P_{\text{interaction}} = 0.949$ ). An asterisk indicates significant differences between corresponding treatments in tents and open plots (GLM, trial 1:  $P_{\text{treatment}} < 0.001$ ,  $P_{\text{population}} = 0.464$ ,  $P_{\text{interaction}} = 0.899$ , trial 2:  $P_{\text{treatment}} = 0.219$ ,  $P_{\text{population}} = 0.683$ ,  $P_{\text{interaction}} = 0.960$ , trial 3:  $P_{\text{treatment}} = 0.040$ ,  $P_{\text{population}} = 0.128$ ). Only plots where larvae were released were included in the statistical analyses. NI = not included. For a more detailed explanation of the insect treatments, see Materials and methods



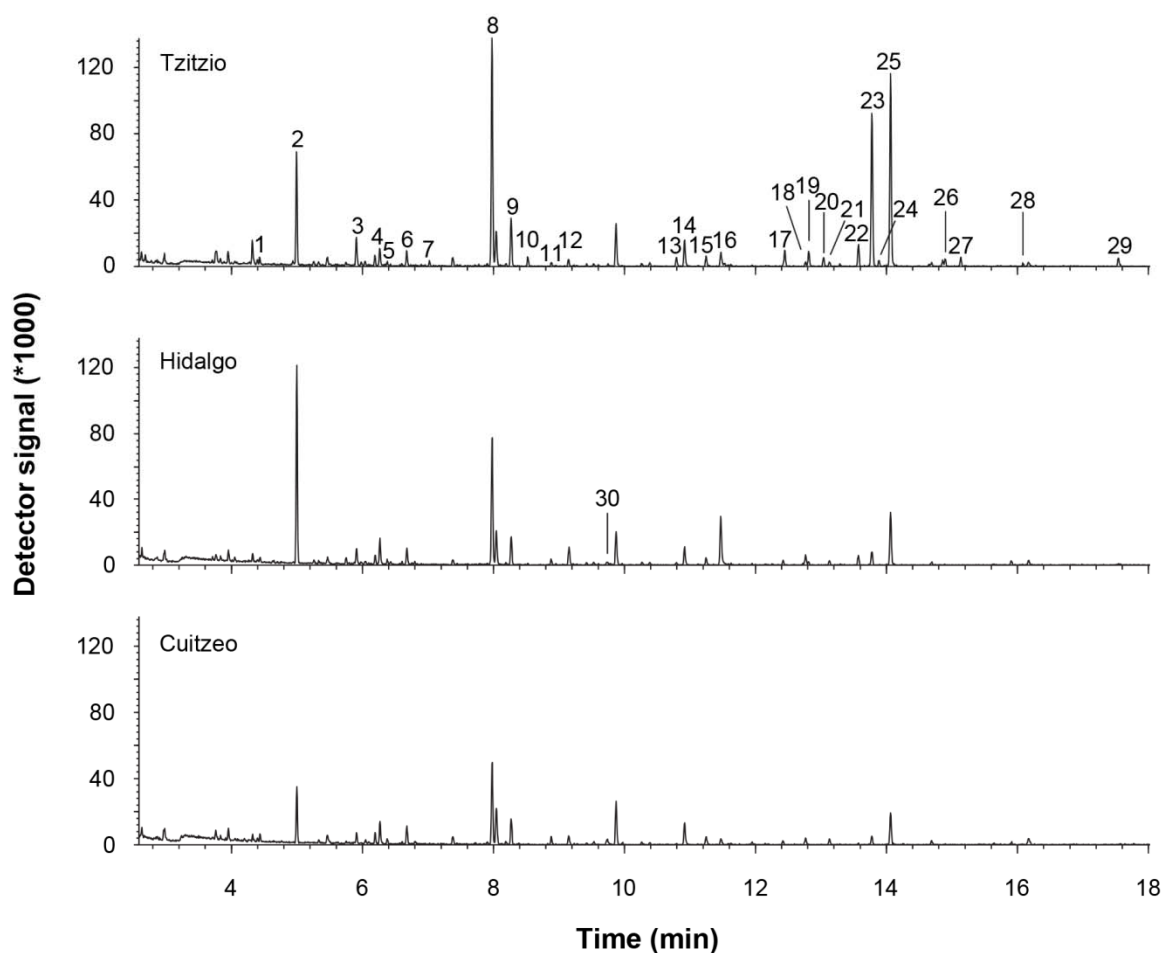
**Fig. S5** Correlation between wasp release and larval recovery. A total number of zero, three, nine or 54 female *Campoletis sonorensis* wasps were released in different tents where all plants were infested with five second-instar *Spodoptera frugiperda* larvae. The experiment was repeated three times; in the first trial, the treatment with the release of three wasps was not included. The dotted line corresponds to the trend curve for the first trial, the solid grey line corresponds to the trend curve for the second trial and the solid black line corresponds to the trend curve for the third trial. The correlation was significant for trials two and three (Linear regression on log-transformed data,  $R^2_{\text{trial1}} = 0.932$ ,  $P_{\text{trial1}} = 0.168$ ;  $R^2_{\text{trial2}} = 0.933$ ,  $P_{\text{trial2}} = 0.034$ ;  $R^2_{\text{trial3}} = 0.914$ ,  $P_{\text{trial3}} = 0.044$ )



**Fig. S6** Parasitism of *Spodoptera frugiperda* larvae, retrieved from the three different teosinte populations, in the presence of *Campoletis sonorensis* wasps. Data were combined for all three trials, from all treatments in tents in the presence of wasps. There was an overall significant difference (chi-square test,  $P = 0.039$ )



**Fig. S7** Shoot dry weight of plants at first harvest, one week after larval infestations. Average shoot dry weight (+SE) of plants in tents not infested with herbivores (treatment “C”) or infested with *Spodoptera frugiperda* larvae in the absence (SF) and in the presence of increasing densities of parasitoid wasps (+W, ++W, +++W), and in plots not covered by a tent (OC, O+SF). (A) First trial. (B) Second trial. (C) Third trial. Different letters indicate significant differences between treatments in tents (GLM, trial 1:  $P_{\text{treatment}} = 0.006$ ,  $P_{\text{population}} = 0.085$ ,  $P_{\text{interaction}} = 0.342$ , trial 2:  $P_{\text{treatment}} = 0.001$ ,  $P_{\text{population}} < 0.001$ ,  $P_{\text{interaction}} = 0.752$ , trial 3:  $P_{\text{treatment}} = 0.008$ ,  $P_{\text{population}} < 0.001$ ,  $P_{\text{interaction}} = 0.583$ ). An asterisk indicates significant differences between treatments in open plots (GLM, trial 1:  $P_{\text{treatment}} = 0.017$ ,  $P_{\text{population}} = 0.060$ ,  $P_{\text{interaction}} = 0.390$ , trial 2:  $P_{\text{treatment}} < 0.001$ ,  $P_{\text{population}} < 0.001$ ,  $P_{\text{interaction}} = 0.147$ , trial 3:  $P_{\text{treatment}} = 0.248$ ,  $P_{\text{population}} = 0.021$ ,  $P_{\text{interaction}} = 0.311$ ), between corresponding control treatments in tents and open plots (GLM, trial 1:  $P_{\text{treatment}} = 0.035$ ,  $P_{\text{population}} < 0.001$ ,  $P_{\text{interaction}} = 0.202$ , two way ANOVA, trial 2:  $P_{\text{interaction}} = 0.008$ , trial 3:  $P_{\text{treatment}} < 0.001$ ,  $P_{\text{population}} < 0.001$ ,  $P_{\text{interaction}} = 0.163$ ), or between corresponding herbivore-infestation treatments in tents and open plots (GLM, trial 1:  $P_{\text{treatment}} = 0.330$ ,  $P_{\text{population}} = 0.194$ ,  $P_{\text{interaction}} = 0.238$ , trial 2:  $P_{\text{interaction}} = 0.017$ , trial 3:  $P_{\text{treatment}} = 0.025$ ,  $P_{\text{population}} = 0.015$ ,  $P_{\text{interaction}} = 0.175$ ). Where significant population effects were found, “Hidalgo” had a higher biomass than “Tzitzio” and the value of “Cuitzeo” was in between. NI = not included. For a more detailed explanation of the insect treatments, see Materials and methods



**Fig. S8** Chromatograms of volatiles collected from herbivore-damaged teosinte plants. Tzitzio, Hidalgo and Cuitzeo represent three teosinte varieties. The labelled compounds are among the most abundant volatiles: (1) heptanal; (2)  $\alpha$ -pinene; (3) 2-methyl-2-hepten-6-one; (4) (Z)-3-hexenyl acetate; (5) hexyl acetate\*; (6) limonene; (7) (Z)- $\beta$ -ocimene; (8) linalool; (9) (3E)-4,8-dimethyl-1,3,7-nonatriene; (10) unidentified; (11) 2-ethylhexyl acetate\*; (12) phenylmethyl acetate\*; (13) 2-phenylethyl acetate; (14) unidentified; (15) unidentified; (16) indole; (17) unidentified; (18) unidentified; (19) unidentified; (20) unidentified; (21) unidentified; (22) (E)- $\beta$ -caryophyllene; (23) (E)- $\alpha$ -bergamotene; (24)  $\beta$ -cedrene\*; (25) (E)- $\beta$ -farnesene; (26) unidentified; (27)  $\beta$ -sesquiphellandrene\*; (28) caryophyllene-oxide\*; (29) unidentified; (30) methyl salicylate. \* = tentative identification

**Table S1** Survey of eight teosinte populations in Mexico in 2009

Site	State	Altitude (m)	GPS coordinates	Species
1	Estado de México	2246	N 19°32.075' W 098°55.422'	<i>Zea mays</i> ssp. <i>mexicana</i>
2	Estado de México	2295	N 19°31.614' W 098°50.144'	<i>Zea mays</i> ssp. <i>mexicana</i>
3	Estado de México	2581	N 19°13.664' W 099°32.790'	<i>Zea mays</i> ssp. <i>mexicana</i>
4	Michoacán	1837	N 20°02.776' W 101°04.573'	<i>Zea mays</i> ssp. <i>mexicana</i>
5	Michoacán	2012	N 19°41.117' W 101°04.841'	<i>Zea mays</i> ssp. <i>mexicana</i>
<b>6</b>	<b>Michoacán</b>	<b>1848</b>	<b>N 19°57.851' W 101°11.485'</b>	<b><i>Zea mays</i> ssp. <i>mexicana</i></b>
<b>7</b>	<b>Michoacán</b>	<b>1316</b>	<b>N 19°32.994' W 100°55.161'</b>	<b><i>Zea mays</i> ssp. <i>parviglumis</i></b>
8	Jalisco	1311	N 20°37.973' W 104°26.192'	<i>Zea mays</i> ssp. <i>parviglumis</i>

Seeds from the two sites displayed in bold were used for the field experiments in 2010. Site 6 corresponds to “Cuitzeo” and site 7 corresponds to “Tzitzio”

**Table S2** Absolute amounts of volatiles collected from herbivore-damaged teosinte plants (in peak area  $\pm$  SE)

VOCs	Trial 1				Trial 2				Trial 3			
	Tzitzio (n=5)	Hidalgo (n=4)	Cuitzeo (n=5)	<i>p</i> -value	Tzitzio (n=5)	Hidalgo (n=5)	Cuitzeo (n=5)	<i>p</i> -value	Tzitzio (n=4)	Hidalgo (n=6)	Cuitzeo (n=6)	<i>p</i> -value
Alcohols	Tr	731 $\pm$ 337.0	Tr	0.437	509 $\pm$ 226.6	Nd	Nd	<b>0.032</b>	288 $\pm$ 190.0	Nd	Nd	<b>0.041</b>
Acyclic hydrocarbons	670 $\pm$ 420.2	Tr	Tr	0.742	469 $\pm$ 152.7	408 $\pm$ 98.4	Tr	0.257	992 $\pm$ 452.0	347 $\pm$ 110.3	Tr	<b>0.047</b>
Aldehydes	Tr	Nd	Tr	0.587	543 $\pm$ 362.3	Nd	262 $\pm$ 185.1	<b>0.045</b>	309 $\pm$ 236.3	180 $\pm$ 81.2	Tr	0.219
Esters	4926 $\pm$ 2014.9	3742 $\pm$ 991.5	3994 $\pm$ 903.9	0.835	1671 $\pm$ 247.2	1309 $\pm$ 248.1	1487 $\pm$ 435.9	0.736	2387 $\pm$ 518.1	2403 $\pm$ 927.7	788 $\pm$ 172.7	0.161
Aromatics	2929 $\pm$ 1017.4	1667 $\pm$ 1047.4	685 $\pm$ 400.0	0.120	1317 $\pm$ 469.4	1100 $\pm$ 479.6	Tr	0.133	1030 $\pm$ 289.8	683 $\pm$ 333.5	109 $\pm$ 108.9	<b>0.046</b>
Homoterpenes	4748 $\pm$ 943.4	1895 $\pm$ 1151.6	2174 $\pm$ 549.9	0.077	1944 $\pm$ 574.4	463 $\pm$ 206.5	602 $\pm$ 162.6	<b>0.026</b>	4334 $\pm$ 1074.0	994 $\pm$ 303.4	645 $\pm$ 370.3	<b>0.001</b>
Monoterpenes	7702 $\pm$ 2198.9	2212 $\pm$ 709.9	2748 $\pm$ 812.3	0.197	8444 $\pm$ 1594.4	6572 $\pm$ 1988.3	4247 $\pm$ 462.7	0.181	4352 $\pm$ 782.3	2630 $\pm$ 787.2	588 $\pm$ 293.7	<b>0.005</b>
Sesquiterpenes	65110 $\pm$ 24507.5	13896 $\pm$ 5543.9	10680 $\pm$ 3713.9	0.051	6100 $\pm$ 2581.0	1400 $\pm$ 871.9	Tr	0.055	14045 $\pm$ 4671.4	3081 $\pm$ 1170.3	107 $\pm$ 70.5	<b>0.002</b>
Unidentified compounds	15739 $\pm$ 5693.6	4147 $\pm$ 1345.8	4000 $\pm$ 950.0	0.067	2623 $\pm$ 805.7	574 $\pm$ 293.7	926 $\pm$ 59.7	<b>0.028</b>	2415 $\pm$ 949.8	1290 $\pm$ 621.0	124 $\pm$ 78.1	0.054
Total VOCs	102844 $\pm$ 29881.5	28512 $\pm$ 8791.3	24853 $\pm$ 5812.3	<b>0.024</b>	23619 $\pm$ 5051.6	11825 $\pm$ 3782.4	8398 $\pm$ 854.5	<b>0.031</b>	30151 $\pm$ 8947.4	11607 $\pm$ 2965.6	2471 $\pm$ 799.0	<b>0.003</b>

Tzitzio, Hidalgo and Cuitzeo represent three different teosinte varieties. Alcohols: (*Z*)-3-hexenol, 1-octanol; Acyclic hydrocarbons: (*E*)-2-octene, 1-nonene, 2-methyl-2-hepten-6-one; Aldehydes: heptanal, benzaldehyde, octanal, decanal; Esters: 1-butanol-3-methyl acetate, (*Z*)-3-hexenyl acetate, phenylmethyl acetate\*, hexyl acetate\*, 2-ethylhexyl acetate\*, methyl salicylate, 2-phenylethyl acetate. Aromatics: *p*-cymene\*, acetophenone\*, indole. Homoterpenes: (3*E*)-4,8-dimethyl-1,3,7-nonatriene, (3*E*, 7*E*)-4,8,12-trimethyl-1,3,7,11-tridecatetraene. Monoterpenes:  $\alpha$ -pinene, camphene\*,  $\beta$ -pinene,  $\beta$ -myrcene, limonene, (*Z*)- $\beta$ -ocimene, linalool. Sesquiterpenes:  $\beta$ -bisabolene,  $\beta$ -ylangene\*,  $\alpha$ -copaene\*,  $\alpha$ -zingiberene\*, (*E*)- $\beta$ -caryophyllene, (*E*)- $\alpha$ -bergamotene,  $\beta$ -cedrene\*,  $\alpha$ -humulene\*,  $\beta$ -selinene, thujopsene\*,  $\beta$ -sesquiphellandrene\*, caryophyllene-oxide\*, (*E*)- $\beta$ -farnesene, farnesol\*. Unidentified compounds: 15 different compounds. \* = tentative identification. Tr = compound found in trace amounts (in less than half of the samples). Nd = compound not detected during the 6 h sampling period. The number of replicates is given in parenthesis. *p*-Values in bold represent a significant difference (one-way ANOVA or Kruskal-Wallis test,  $P < 0.05$ )





## Curriculum vitae





# Elvira de Lange

## CONTACT DETAILS

---

Address: Philip E. Marucci Center for Blueberry and Cranberry Research,  
125a Lake Oswego Rd., Chatsworth, NJ 08019, USA  
Telephone: +1 609 726 1590 Ext. 4444  
E-mail: [elvira.delange@rutgers.edu](mailto:elvira.delange@rutgers.edu)  
Website: <http://about.me/elvirasimone>

## RESEARCH SKILLS

---

Bioassays	Parasitoid wasp behavioral tests in a six-arm olfactometer Insect, pathogen and plant virus performance tests
Field work	Various projects on plant-insect interactions (12 months in total)
Insect rearing	Herbivores: <i>Spodoptera littoralis</i> (Lepidoptera: Noctuidae) <i>Spodoptera frugiperda</i> (Lepidoptera: Noctuidae) <i>Spodoptera exigua</i> (Lepidoptera: Noctuidae) Parasitoids: <i>Campoletis sonorensis</i> (Hymenoptera: Ichneumonidae) <i>Cotesia marginiventris</i> (Hymenoptera: Braconidae)
Chemical analysis	Plant odor collection in lab and field, GC/MS, GC/FID, SPME
Molecular techniques	DNA and RNA extraction from plant tissue, northern blot, PCR and qPCR, gel electrophoresis
Microscopy	Light microscopy, scanning electron microscopy
Informatics	SigmaPlot, Canoco, R, ImageJ, EndNote, Chemstation, XCMS online, Office

## PROFESSIONAL EXPERIENCE

---

Oct 2013 - now	Postdoc Philip E. Marucci Center for Blueberry and Cranberry Research Rutgers, The State University of New Jersey, USA <i>Insect resistance of cranberry</i>
----------------	---

## EDUCATION

---

Sept 2008 - Oct 2013	PhD in Chemical Ecology Laboratory of Fundamental and Applied Research in Chemical Ecology (FARCE) University of Neuchâtel, Switzerland Supervisor: Prof. Ted C.J. Turlings <i>Tritrophic interactions on cultivated maize and its wild ancestor, teosinte</i>
Feb 2006 - Aug 2008	Master of Science in Plant Biology ( <i>cum laude</i> ) Utrecht University, The Netherlands <i>Specialisation: Plant-Microbe Interactions, Chemical Ecology</i>
Sept 2001 - Feb 2006	Bachelor of Science in Biology ( <i>cum laude</i> ) Utrecht University, The Netherlands



## ORGANIZATION EXPERIENCE

---

Jan 2013	Member of the organization committee for the scientific meeting "Plant survival: A story of sex, violence and light" held in Neuchâtel, Switzerland, on January 23-24
Aug 2012	Member of the organization committee for the 9 <sup>th</sup> Solanaceae conference held in Neuchâtel, Switzerland, on August 26-30
Aug 2009	Member of the organization committee for the 25 <sup>th</sup> Annual Meeting of the International Society of Chemical Ecology (ISCE) held in Neuchâtel, Switzerland, on August 23-27
Apr 2009	Fellow organizer of the Annual PhD Students Meeting at the University of Neuchâtel, Switzerland, on April 28

## TEACHING EXPERIENCE

---

Nov 2012 / 2011 2009 / 2008	Practical classes in the Papiliorama butterfly garden (Bachelors) University of Neuchâtel, Switzerland
Feb - Mar 2012	Applied Biology practical classes (Bachelors) University of Neuchâtel, Switzerland
Feb - Aug 2010	Supervision of a Masters student from the University of Burgundy University of Neuchâtel, Switzerland
Oct 2008 / 2009 / 2012	Advances in Chemical Ecology practical classes (Masters) University of Neuchâtel, Switzerland
Aug 2005 - May 2006	Junior College Utrecht practical classes (an honors program for high school pupils) Utrecht University, The Netherlands
May 2003 / 2005	Phytopathology practical classes (Bachelors) Utrecht University, The Netherlands

## MEMBERSHIPS

---

2013 - now	Entomological Society of America (ESA)
2013 - now	International Organisation for Biological Control (IOBC-WPRS)
2012 - now	The Society for Experimental Biology (SEB)
2012 - now	The Society of Plant Signaling and Behavior
2011 - now	International Society of Chemical Ecology (ISCE)
2011 - now	Swiss Zoological Society (SZS)
2003 - now	Netherlands Institute of Biology (NIBI)

## LANGUAGES

---

Dutch (mother tongue), English (fluent), French (fluent), Spanish (basics), German (basics)

## OTHER QUALIFICATIONS

---

International driving license, category B



## GRANTS

---

May 2013	Mobility grant, University of Neuchâtel, Switzerland, 250 EUR
May 2012	Mobility grant, University of Neuchâtel, Switzerland, 260 EUR
Feb 2012	Mobility grant, Swiss Zoological Society, 460 CHF
May 2011	Mobility grant, University of Neuchâtel, Switzerland, 300 EUR
Feb 2010	Mobility grant, Doctoral Program in Organismal Biology, University of Neuchâtel, Switzerland, 940 USD
Jan 2007	Mobility grant, Jo Kolk Studiefonds, the Netherlands, 1000 EUR
Jan 2007	Mobility grant, Vreedefonds, the Netherlands, 3000 EUR

## HONORS AND AWARDS

---

June 2013	Presentation prize 6 <sup>th</sup> meeting of the IOBC-WPRS working group "Induced resistance in plants against insects and diseases", Avignon, France
Feb 2013	Presentation prize Biology13 – Yearly joint congress of the Swiss Zoological Society, the Swiss Botanical Society and the Swiss Systematics Society, Basel, Switzerland
Jan 2013	Poster prize Plant survival: A story of sex, violence and light - Final scientific meeting of the NCCR Plant Survival, Neuchâtel, Switzerland
Sept 2012	Presentation prize Annual Retreat, Doctoral Program in Molecular Plant Sciences, Avenches, Switzerland
Sept 2012	Winner of the "Two-Minute Thesis Contest" "About plants, attacks, and bodyguards" Participants had to describe their research in 2 minutes; winning submissions have been converted to animations by Jorge Cham. My entry is available at <a href="http://www.phdcomics.com/caterpillar">www.phdcomics.com/caterpillar</a>
May 2012	Presentation prize Annual PhD Students Meeting, Neuchâtel, Switzerland
May 2012	Poster prize PhD Summerschool Swiss Plant Science Web, Mürren, Switzerland
May 2011	Poster prize Annual PhD Students Meeting, Neuchâtel, Switzerland
Aug 2009	Poster prize International Society of Chemical Ecology, 25 <sup>th</sup> Annual Meeting, Neuchâtel, Switzerland
June 2002	"Excellent Tracé" certificate An honors program for first-year Bachelors students



## PUBLICATIONS IN PEER-REVIEWED JOURNALS

---

- Von Mérey, G.E., Veyrat, N., **De Lange, E.**, Degen, T., Mahuku, G., López-Valdez, R., Turlings, T.C.J., and D'Alessandro, M. (2012) Minor effects of two elicitors of insect and pathogen resistance on volatile emissions and parasitism of *Spodoptera frugiperda* in Mexican maize fields. *Biological Control* 60: 7-15
- Erb, M., Balmer, D., **De Lange, E.S.**, Von Mérey, G., Planchamp, C., Robert, C.A.M., Röder, G., Sobhy, I., Zwahlen, C., Mauch-Mani, B., and Turlings, T.C.J. (2011) Synergies and trade-offs between insect and pathogen resistance in maize leaves and roots. *Plant, Cell & Environment* 34: 1088-1103
- Leon-Reyes, A., Van der Does, D., **De Lange, E.S.**, Delker, C., Wasternack, C., Van Wees, S.C.M., Ritsema, T., and Pieterse, C.M.J. (2010) Salicylate-mediated suppression of jasmonate responsive gene expression in *Arabidopsis* is targeted downstream of the jasmonate biosynthesis pathway. *Planta* 232:1423-1432
- Erb, M., Flors, V., Karlen, D., **De Lange, E.**, Planchamp, C., D'Alessandro, M., Turlings, T.C.J., and Ton, J. (2009) Signal signature of aboveground-induced resistance upon belowground herbivory in maize. *The Plant Journal* 59: 292-302
- Leon-Reyes, A., Spoel, S.H., **De Lange, E.S.**, Abe, H., Kobayashi, M., Tsuda, S., Millenaar, F.F., Welschen, R.A.M., Ritsema, T., and Pieterse, C.M.J. (2009) Ethylene modulates the role of NONEXPRESSOR OF PATHOGENESIS-RELATED GENES1 in cross talk between salicylate and jasmonate signaling. *Plant Physiology* 149: 1797-1809
- De Vries, R.P., **De Lange, E.S.**, Wösten, H.A.B., and Stalpers, J.A. (2008) Control and possible applications of a novel carrot-spoilage basidiomycete, *Fibulorhizoctonia psychrophila*. *Antonie Van Leeuwenhoek* 93: 407-413

## OTHER PUBLICATIONS

---

- **De Lange, E.S.**, Gaillard, M.D.P., Yan, Y., Turlings, T.C.J., Kolomiets, M.V. and Christensen, S.A. (2013) Are green leaf volatiles important for parasitoid wasp attraction in the field? *IOBC-WPRS Bulletin* Vol. 89, p. 291-294
- **De Lange, E.**, Erb, M., Turlings, T. and Ton, J. (2010) *Spodoptera frugiperda* and *Spodoptera exigua* differentially influence herbivore-induced emission of maize volatiles. *Memorias del XXXIII Congreso Nacional de Control Biológico*. Uruapan, Michoacán, México. p. 469-473
- Bahena-Juárez, F., **De Lange, E.**, Farnier, K., Cortez-Mondaca, E., Sánchez-Martínez, R., García-Pérez, F., Miranda-Salcedo, M., Degen, T., Gaudillat, B., Aguilar-Romero, R. (2012). Parasitismo en gusano cogollero del maíz *Spodoptera frugiperda* (J.E. Smith) (Lepidoptera: Noctuidae) en el centro de México. *Memorias del XXXIII Congreso Nacional de Control Biológico*. Uruapan, Michoacán, México. p. 204-209





## ORAL PRESENTATIONS

---

- 6<sup>th</sup> Meeting of the IOBC-WPRS Working Group “Induced resistance in plants against insects and diseases”, Avignon, France, June 2013
- Biology13 (Yearly joint congress of the Swiss Zoological Society, the Swiss Botanical Society and the Swiss Systematics Society), Basel, Switzerland, February 2013
- Annual Retreat, Doctoral Program in Molecular Plant Sciences, Avenches, Switzerland, September 2012
- PhD Students Meeting, Doctoral Program in Organismal Biology, Neuchâtel, Switzerland, May 2012
- D.Day (Annual Meeting of PhD students in Biology and Medicine), Lausanne, Switzerland, April 2012
- Symposium of Ecology and Evolution Doctoral Students (SEEDS), Neuchâtel, Switzerland, November 2011
- 14<sup>th</sup> Symposium on Insect-Plant Interactions (SIP14), Wageningen, The Netherlands, August 2011
- Symposium for Biology Students of Europe, Coimbra and Aveiro, Portugal, July - August 2008
- Netherlands Annual Ecology Meeting, Lunteren, The Netherlands, February 2008

## POSTER PRESENTATIONS

---

- PhD and postdoc Summerschool EuroVOL “Plant volatiles: From lab bench to application”, Les Diablerets, Switzerland, September 2013
- PhD Students Meeting, Doctoral Program in Organismal Biology, Neuchâtel, Switzerland, May 2013
- Plant survival: A story of sex, violence and light – Final scientific meeting of the NCCR Plant Survival (12-year grant by the Swiss National Science Foundation), Neuchâtel, Switzerland, January 2013
- 9<sup>th</sup> Solanaceae Conference, Neuchâtel, Switzerland, August 2012
- International Society of Chemical Ecology (ISCE), 28<sup>th</sup> Annual Meeting, Vilnius, Lithuania, July 2012
- Gordon Research Conferences – Plant Volatiles, Ventura, California, USA, February 2012
- PR-Proteins & Induced Resistance against Pathogens and Insects (PR-IR), Neuchâtel, Switzerland, September 2011
- 6<sup>th</sup> Utrecht PhD Summerschool on Environmental Signaling, Utrecht, The Netherlands, August 2011
- PhD Summerschool Swiss Plant Science Web “Terrestrial ecosystem dynamics in a changing world”, Mürren, Switzerland, June 2011
- PhD Students Meeting, Doctoral Program in Organismal Biology, Neuchâtel, Switzerland, May 2011
- XXXIII Congreso Nacional de Control Biológico, Uruapan, Michoacán, Mexico, November 2010
- PhD Students Meeting, Doctoral Program in Organismal Biology, Neuchâtel, Switzerland, April 2010
- Gordon Research Conferences – Plant-Herbivore Interactions, Galveston, Texas, USA, February 2010
- Biology10 (Yearly joint congress of the Swiss Zoological Society, the Swiss Botanical Society and the Swiss Systematics Society), Neuchâtel, Switzerland, February 2010
- International Society of Chemical Ecology (ISCE), 25<sup>th</sup> Annual Meeting, Neuchâtel, Switzerland, August 2009
- PhD Students Meeting, Doctoral Program in Organismal Biology, Neuchâtel, Switzerland, April 2009