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Specificity and Variability of Induced Volatile Signalling in Maize Plants

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Specificity and Variability of Induced Volatile Signalling in Maize Plants

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ABSTRACT

Many parasitoids that attack phytophageous insects make use of plant odours to locate the habitat of their host. In maize large amounts of these odours are specifically emitted by a plant after it has been damaged by a herbivore, but not after mechanical damage. The odour emissions occur systematically throughout the plant. Factors in the oral secretion of the herbivores are the main elicitors of the plants' reaction. The induced maize odour blend, which is mainly composed of terpenoid compounds, is a useful cue for the parasitoids and may indicate the presence of a potential host. We have studied different biotic and abiotic factors that determine the specificity and variation of the signal emitted by maize plants in response to caterpillar damage. We found enormous quantitative and qualitative differences among maize genotypes. The absolute amount of volatiles emitted was negatively correlated with plant age, while larval instar appeared to have little or no effect. Light intensity is the most important abiotic factor, but odour emissions were also affected by the soil and air humidity, temperature and the degree of fertilisation. We discuss these results in the context of reliability of plant-induced signals as cues for parasitic wasps to find a suitable host.

RÉSUMÉ

Les interactions entre les plantes et les insectes sont diverses et ont mené à différentes théories évolutives. Ces interactions peuvent être mutualistes comme c'est le cas, par exemple, entre des insectes pollinisateurs et certaines fleurs, ce qui peut améliorer considérablement le succès reproductif de la plante. Toutefois, la plupart des plantes sont abusées par les insectes, leur parties végétatives étant une source importante de nourriture pour les insectes phytophages. Ainsi, ces insectes herbivores ont pu contribuer à la sélection de différentes stratégies de défense de la part des plantes, que ce soit au niveau chimique ou au niveau physique. Il semble malgré tout que les défenses chimiques soient une bonne stratégie de défense, dans la mesure où des changements rapides de la constitution chimique d'une plante sont possibles sans que cela n'induise trop de coût pour cette dernière.

Une des stratégies de défense des plantes a attiré l'attention depuis ces quinze dernières années. Il s'agit de l'émission d'odeurs suite à une attaque par des insectes phytophages. Cette odeur est particulièrement attractive pour les ennemis naturels de ces herbivores. Cette réaction de la plante après une attaque est assez fréquente, elle a été signalée notamment chez différentes plantes importantes en agriculture : le haricot (Fabacea), le chou (Brassicea), le pommier (Rosacea), le concombre (Cucurbitacea), le coton (Malvacea), et le maïs (Poacea). Le système tritrophique impliquant le maïs, des chenilles de *Spodoptera* et leurs parasitoïdes (*Cotesia marginiventris*) a été particulièrement étudié. La figure 1-1 illustre le fait que le maïs sain n'émette quasiment pas d'odeur, mais que cette dernière est produite par le maïs attaqué et non par les chenilles elles-mêmes ou leurs fèces. La réaction consistant à émettre une odeur particulière suite à une attaque par des chenilles est très rapide chez le maïs, déjà 5 ou 6 h après le début de l'endommagement. Chez le coton, ce même type de réaction prend 48 h. En fait, il a été

montré que l'émission d'odeur est induite par un facteur dans la bave des chenilles. Un constituant de la salive de *Spodoptera exigua* a été isolé et identifié, il s'agit de la *N*-[17-hydroxylinolenoyl]-L-glutamine, appelée volicitin. Ce composé, lorsqu'il est appliqué sur une surface préalablement endommagée avec une lame de rasoir induit l'émission d'une odeur identique à celle produite suite à une attaque par des chenilles. Le dégagement de cette odeur est systémique, i.e. même les feuilles de maïs non endommagées exhalent ce parfum.

L'odeur induite du maïs est constituée principalement de composés volatiles d'odeur verte, de terpenoïdes et d'indole. Les composés d'odeur verte inclus des molécules saturées ou insaturées à 6 carbones, telles que des alcools, des aldéhydes et des esters. Ils sont émis suite à l'endommagement lui-même, quand les cellules de la feuille sont broyées, et sont synthétisés par lypoxygenèse. Les terpénoïdes comprennent à la fois les homoterpènes, les monoterpènes et les sesquiterpènes qui contiennent respectivement 10 et 15 atomes de carbone. Deux chemins de biosynthèses permettent la synthèse de ces composés : la synthèse via l'acide mévalonique et la synthèse sans cet acide. L'indole est un composé azoté, qui implique une chaîne de synthèse impliquant l'acide shikimique. Ces différentes chaînes de synthèse sont résumées dans la figure 1-2.

Différentes études semblent indiquer que l'odeur produite suite à une attaque par un insecte herbivore est spécifique soit à l'espèce de l'insecte phytophage, soit au stade larvaire des chenilles. Certaines différences dans l'odeur ont pu être détectées, elles peuvent être quantitatives ou également qualitatives lorsque la composition chimique de l'odeur induite est modifiée, soit par un changement dans les proportions des constituants de l'odeur, soit par l'absence ou la présence de certain composés. Toutefois, d'autres exemples semblent indique qu'au contraire les odeurs produites ne sont pas d'aussi bon indicateur de l'identité de l'herbivore, ni de sa qualité. La question reste donc posée : dans quelle mesure le signal odorant émis à la suite d'une attaque par des insectes phytophages

est-il spécifique et sure pour les ennemis naturels de ces ravageurs potentiels ? Dans l'étude qui suit, mon but a été de mesurer l'importance relative de différents facteurs biotiques et abiotiques dans la caractérisation des variations du signal odorant induit chez le maïs attaqué par des chenilles de *Spodoptera littoralis*.

Les grandes lignes de la thèse.

Dans cette thèse, je me suis concentrée sur les sujets suivant :

1. Dans quelle mesure différents stades larvaires de *Spodoptera littoralis* (Lepidoptera : Noctuidae) affectent-ils l'émission d'odeur induite chez le maïs ?

Une étude a montré que l'odeur induite produite par le maïs peut différer selon le stade larvaire de chenilles de *Pseudatelia separata* (Lepidoptera : Noctuidae) qui l'attaquent. Nous avons testé si dans notre système, composé également du maïs, mais de chenilles de *Spodoptera littoralis* (Lepidoptera : Noctuidae) de telles différences existent. De plus, nous avons également testé si l'endoparasitoïde solitaire, *Microplitis rufiventris* (Hymenoptera : Braconidae), qui ne peut parasiter que de jeunes chenilles de *Spodoptera littoralis*, peut distinguer les plantes attaquées par un stade larvaire adéquat de celles attaquées par un stade larvaire trop avancé. Nous avons déterminé dans un premier temps l'effet de la salive de chenilles de 2^{éme}, 3^{éme} et 5^{éme} stade sur l'induction d'odeur chez du maïs de la variété Delprim. Puis l'effet de l'attaque par des densités variables de chenilles de ces différents stades larvaires a été mesuré. En plus une expérience imitant grossièrement l'endommagement fait par les chenilles a été réalisé et confirme le résultat indiquant que seul la quantité d'endommagement a un effet sur l'émission d'odeur induite (Chapitre 2).

2. Est-ce que l'émission de l'odeur induite du maïs dépend de l'âge et des feuilles des plantes ?

Une attaque par un ravageur comme *Spodoptera littoralis* peut dramatiquement réduire la production de grains. Le parasitisme par certains parasitoïdes peut considérablement réduire cet effet négatif de l'herbivore. Ainsi, il serait profitable pour la plante de se défendre dès ce stade vulnérable. Si l'émission d'odeur suite à l'attaque de phytophages constitue une défense, on pourrait penser qu'elle serait plus importante chez les plantes jeunes et dans les parties jeunes des plantes, qui ont plus à perdre lors d'une attaque par un ravageur que des plantes plus âgées ou des feuilles déjà vieilles. Pour vérifier cela, nous avons analysé l'odeur induite émise par des plantes d'âges variant de 1 à 8 semaines. Les plantes âgées de 2 semaines sont celles qui émettent le plus d'odeurs induites comparées aux plantes plus vieilles. L'odeur émise par les 4 feuilles d'une plante de 2 semaines a été également analysée, en utilisant différentes méthodes. Lorsque les plantes de maïs sont incubées dans une solution contenant de la bave de chenilles de *S. littoralis*, les feuilles le plus jeune ($3^{ème}$) émettent le plus, mais cette différence est essentiellement due à une différence de poids des feuilles. Lorsque la $1^{ère}$ ou $3^{ème}$ feuille a été endommagée mécaniquement, puis que de la salive de *S. littoralis* a été appliquée sur cet endommagement, les résultats divergent. L'attaque de la feuille la plus âgée semble induire une réaction systémique moindre comparée à celle après une attaque d'une feuille plus jeune. La $3^{ème}$ feuille produit plus d'odeur quand elle est endommagée. Cette fois encore, le poids des feuilles jouent un rôle. L'importance de telles variations dans l'émission d'odeurs est discutée dans le chapitre 3.

3. Dans quelle mesure l'odeur induite du maïs varie-t-elle parmi différentes variétés de maïs cultivées et différentes espèces d'un ancêtre du maïs ?

La spécificité des odeurs induites à la suite d'une attaque par des insectes phytophages reste un vaste sujet de discussion. Certaines études semblent indiquer qu'une telle spécificité existe, mais cela n'est pas généralisable à tous les systèmes. Dans certains cas, la variation due au génotype des plantes est plus grande que celle liée à l'espèce du phytophage. Dans le chapitre 4, nous avons comparé onze variétés de maïs cultivées et cinq espèces ou sous-espèces d'un parent sauvage du maïs (teosinte), et également les descendants de huit individus de *Zea mays mexicana*. Cette étude donne une estimation de l'intervalle de variation des odeurs induites entre différents génotypes et au sein d'un seul génotype de plante. Les plantes de maïs ont été induites à émettre ces odeurs en endommageant mécaniquement deux feuilles et en appliquant du regurgitat de chenilles. Les odeurs ont été collectées sur trois périodes consécutives de trois heures, de manière à éviter des biais liés à des émissions différées dans le temps selon les variétés. Les résultats ont révélé des variations considérables, à la fois au niveau quantitatif et qualitatif, parmi les variétés de maïs cultivées ainsi que parmi les teosinte (Chapitre 4).

4. Des changements de conditions environnementales affectent-ils l'émission d'odeur induite chez le maïs ?

La plupart des études sur les odeurs induites chez les plantes sont réalisées en laboratoire et en conditions contrôlées. Peu d'informations sont disponibles sur l'effet de différents facteurs abiotiques sur l'émission d'odeurs induites. Nous avons testé si différents facteurs tels que l'humidité du sol et de l'air, la température, l'intensité lumineuse et le taux de fertilisation, affectent l'émission de volatiles chez le maïs (Chapitre

5). Chaque facteur a été testé séparément sous des conditions constantes pour les autres facteurs. Toutes les expériences ont été réalisées avec du maïs de la variété Delprim. Dans chaque cas, des changements de conditions dues à un seul facteur ont eu un effet sur la quantité d'odeur émise après induction. Les augmentations les plus dramatiques de l'émission de volatiles ont été obtenues en augmentant l'intensité lumineuse et l'apport en nutriments.

CHAPTER 1.

INTRODUCTION AND THESIS OUTLINE

Interactions between insects and plants are diverse and have led to various ecological and evolutionary patterns. The interactions can be mutualistic as is the case with insects that are vectors of pollen for flowering plants and thus positively affect the reproduction of the latter. But most plants also serve as major food sources for phytophagous insects. Thus, insect herbivores may also have shaped selection for diverse chemical and physical defences from plants (Bernays and Chapman, 1994).

Because plants have an extremely limited capacity of movement and thus cannot directly escape from attack, they have to resort to other strategies to avoid the attack by insects. Morphological defences such as thorns, spines, hairs and glandular trichomes serve as deterrents mainly to large herbivores (vertebrates), but can also directly affect the acceptability as visual cues or by influencing the ability of insects to walk on or bite into tissue (Walters *et al.*, 1989; Roessingh and Städler, 1990; Bernays and Chapman, 1994). The strategy of defence using chemicals seems to offer significant advantages over other forms of defence. Chemicals can act at low concentration and thus may need less energy for production than may be required by a change in morphology. Furthermore, the ecological responsiveness of plants through chemicals can be more flexible. A small change in the structure of a particular chemical can affect its biological activity dramatically. The rapid response potential, the various steps in biosynthetic pathways and the multiplicity of regulatory mechanisms allow plants to switch chemical defence options rapidly (Berenbaum and Zangerl, 1999). Chemical defence also represents various costs to plants (Baldwin, 1991; Karban and Baldwin, 1997), which include investment of energy, materials or other resources for the production of chemical defence that could otherwise be

invested in growth and reproduction. A way of optimising defence appears through inducibility of secondary metabolites in plants following a stress or an attack by insects. By producing chemical defence only when necessary, the cost may be minimised (Baldwin, 1994; Karban and Baldwin, 1997; Agrawal and Karban, 1999). Apart from the evolutionary aspects underlying relationships between plants and insects, the development of large scale agriculture has raised the interest in species of insects, which have become major pests and more recently in insects that are useful in the control of these pests.

Constitutive as well as induced defences in plants are often separated into direct or indirect defences (Dicke, 1999). Direct induced defence refers to bitrophic systems, in which herbivore attack leads to a defence response from plants that directly affects the attacker. For example, leaf alkaloid content in tobacco plants increases dramatically after damage, which can reduce the growth rate of herbivores that feed on such plants (Baldwin, 1988). Indirect defence, on the other hand, deals with tritrophic systems, in which plants increase the effectiveness of natural enemies of the herbivore and thus reduce herbivory impact (Price *et al.*, 1980). Recent studies have shown that predators and parasitoids which attack phytophagous insects make use of plant induced odour to locate the habitat of their potential prey or hosts (Dicke and Sabelis, 1988; Tumlinson *et al.*, 1992; Vet and Dicke, 1992; Dicke, 1994; Turlings *et al.*, 1995; Röse *et al.*, 1997). This phenomenon of signalling between plants and natural enemies has been observed for several tritrophic systems, for example with predatory mites and spider mites on Lima beans (Dicke and Sabelis, 1988) or with parasitoids and caterpillar on cabbage (Mattiacci *et al.*, 1994), on cotton (McCall *et al.*, 1994; Röse *et al.*, 1997.) or on maize (Turlings *et al.*, 1990). In cotton, Paré and Tumlinson (1997a,b) showed that the compounds of the induced odour blend are synthesised *de novo* by the plants after caterpillar attack.

One of the most intensively studied tritrophic systems comprises maize plants, lepidopteran caterpillars and parasitic wasps. Feeding damage inflicted on maize plants by

caterpillars triggers the systemic release of specific volatile compounds, which are not emitted when the maize plants are unharmed (Turlings *et al.*, 1990; Turlings and Tumlinson, 1992). This is illustrated in figure 1-1 where I plotted the chromatograms of odours collected from a) healthy plants: maize plants with no damage, b) the complete host-plant-complex, which is comprised of *Spodoptera littoralis* (Lepidoptera: Noctuidae) larvae, maize plants attacked by the larvae and the caterpillars' by-products, c) caterpillar-damaged plants only: the plants were fed on by caterpillars but the larvae and its by-products were removed from damaged plants before collection of odour, d) caterpillar feces only: the feces of larvae, which had fed on a maize plant, e) caterpillars (larvae) only. The baseline odour of healthy maize plants (without any damage) is plotted in the first graph. Except for the monoterpene linalool which seems to be continously emitted by the variety Delprim used in this experiment, no other compounds are emitted by the undamaged maize plant. The caterpillars and its feces produce very little odour. Only pentadecane is present in the odour blend of larvae and this product is actually emitted by the spit of the larvae that they regurgitate when they fight among each other (Turlings *et al.*, 1991). Large quantities of volatiles were collected from the complete host-plant-complex, and is shown to be the source of these volatiles.

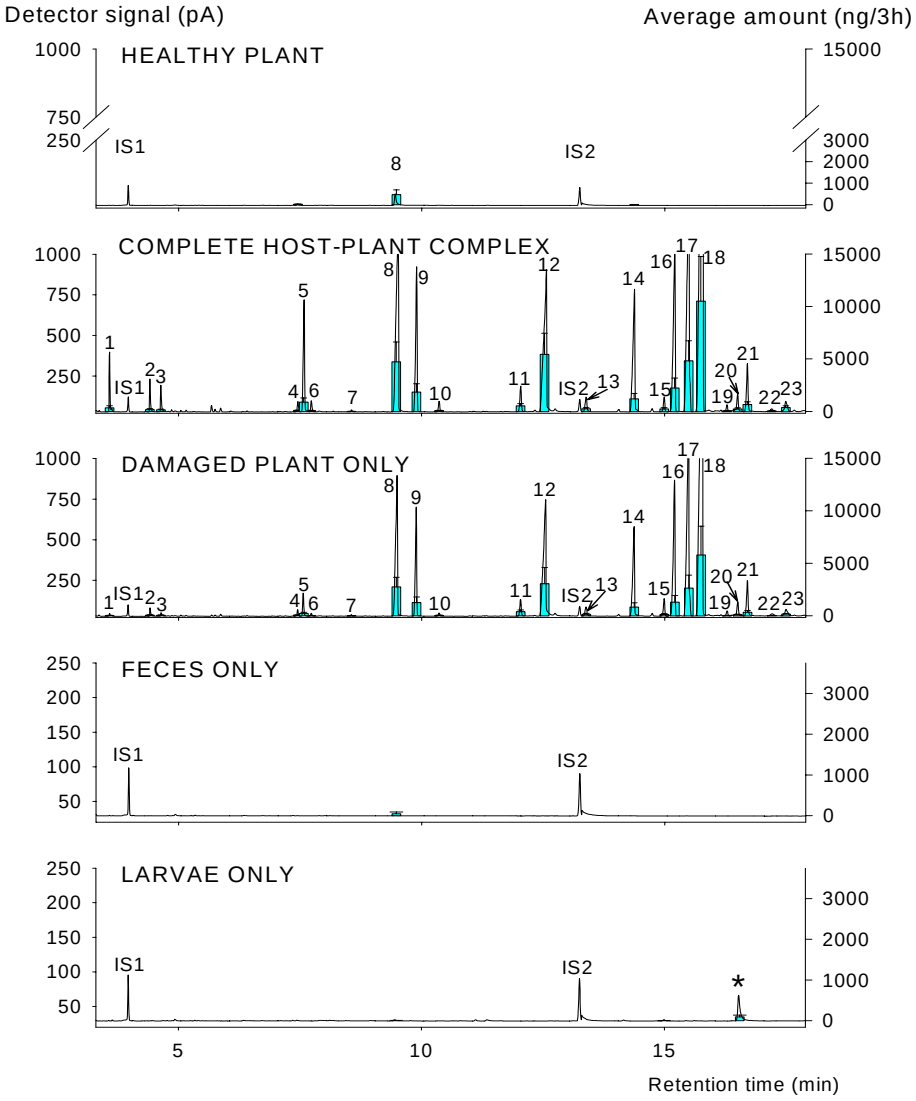

Fig. 1-1. Odour profiles of undamaged plant and of the different parts of the complete host-plant-complex. The two IS peaks are internal standards that were added in the samples before analysis (IS1: n-octane, IS2: nonyl acetate). (1) (*E*)-2-hexenal, (2) (*Z*)-3-hexen-1-ol, (3) (*E*)-2-hexen-1-ol, (4) β-myrcene, (5) (*Z*)-3-hexen-1-yl acetate, (6) hexyl acetate, (7) *E*-β-ocimene (8) linalool, (9) (3*E*)-4,8-dimethyl-1,3,7-nonatriene, (10) benzyl acetate, (11) phenethyl acetate, (12) indole, (13) methyl anthranilate, (14) geranyl acetate (15) unknown, (16) α-caryophyllene, (17) α-(*E*)-bergamotene, (18) (*E*)-β-farnesene, (19) unknown sesquiterpene, (20) β-bisabolene + (*E,E*)-α-farnesene, (21) β-sesquiphellandrene, (22) nerolidol, (23) (3*E*,7*E*)-4,8,12-trimethyl-1,3,7,11-tridecatetraene. Bars represent the mean quantity + SE (ng/ 3h) of the different chemicals emitted.

The odour emitted by maize plants under caterpillar attack is mainly composed of green-leaf volatiles, terpenoids and indole and is highly attractive to parasitic wasps like the generalist *Cotesia marginiventris* (Hymenoptera:Braconidae) (Turlings *et al.*, 1990). Green-leaf odours which consist of a blend of saturated and unsaturated six-carbon alcohols, aldehydes, and esters are produced by autolytic oxidative breakdown of membrane lipids through the lipoxygenase pathway and are generally released when leaves are mechanically damaged (Turlings *et al.*, 1998; Paré and Tumlinson, 1999). Terpenoids comprise homo- and monoterpenes and sesquiterpenes which are respectively ten and fifteen carbon molecules. Two biosynthetic pathways are involved in the synthesis of induced terpenoids, the mevalonate dependent pathway and the mevalonate independant pathway, which both produce isopentenyl pyrophosphate (Rohmer *et al.*, 1996; Lichtenthaler *et al.*, 1997; Arigoni *et al.*, 1997). The nitrogen-containing compound, indole, is produced through the shikimic acid pathway and the tryptophan or non-tryptophan pathway (Donath and Boland, 1994). The different pathways leading to the synthesis of the different compounds that are present in the induced odour from maize plants are summarised in the figure 1-2. The emission of indole and the terpenoids is truly induced and occurs relatively fast in maize. Only 5 to 6 hours after herbivory starts, plants begin releasing these compounds (Turlings *et al.*, 1998). In cotton, the emission of induced volatiles is much slower and occurs only 48h after feeding by lepidopteran larvae, but cotton plants also contain glands with stored terpenoids, which are release directly after mechanical damage (McCall *et al.*, 1994; Loughrin *et al.*, 1995; Röse *et al.*, 1996; Röse *et al.*, 1998). There are no such releases of constitutive terpenoids in maize. The emission of volatiles from maize can be triggered by applying caterpillar oral secretion on the mechanically wounded sites (Turlings *et al.*, 1990) or by incubating the cut stem of a maize seedling in a solution of caterpillar regurgitant (Turlings *et al.*, 1998). With the latter

method, has been confirmed that, as known from previous study (Turlings and Tumlinson, 1992) the plants' response is systemic and occurs in all leaves (Turlings *et al*., 1993).

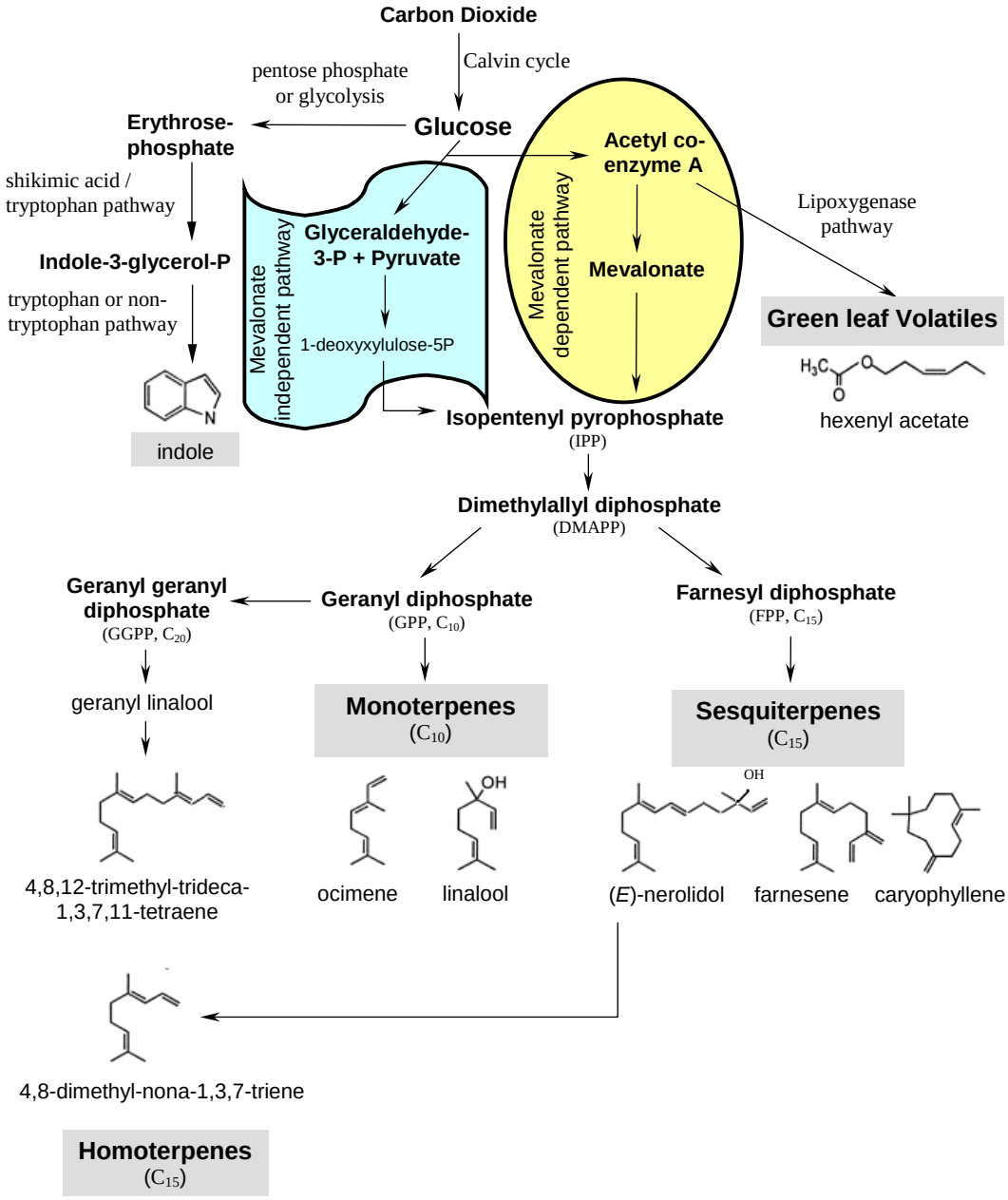

Fig. 1-2. Biosynthetic pathways leading to the release of plant volatiles. It comprises the mevalonate-dependent and the mevalonate-independent biosynthesis pathways of terpenoids, the lipoxygenase pathway leading to green leaf volatiles and the shikimic acid pathway forming indole (modified from Lichtenthaler *et al.*, 1997; Paré and Tumlinson, 1999; Boland *et al.*, 1999).

It been shown that this systemic release of volatiles by maize plants is induced by a factor in the oral secretions of the caterpillar (Turlings *et al.*, 1993). Dicke *et al.* (1993) also found the presence of an elicitor in the exudate from phytoseids mite-infested lima beans leaves. β-Glucosidase in the regurgitant of *Pieris brassicae* larvae was suggested to elicit a response in cabbage resulting in an increased emission of overall cabbage plant odour (Mattiacci *et al.*, 1994; Mattiacci *et al.*, 1995). In the case of induced maize volatiles, the insect elicitors are not enzymes but fatty acid-derived molecules. Such an elicitor was isolated from *Spodoptera exigua* regurgitant, identified as *N*-[17-hydroxylinolenoyl]-L-glutamine and was named volicitin (Alborn *et al.*, 1997; Alborn *et al.*, 2000). The bioactivity of volicitin was found to be identical to the bioactivity of the regurgitant itself (Turlings *et al.*, 2000). Paré and Tumlinson (1998) showed that caterpillars synthesise this elicitor by adding a hydroxyl group and glutamine to linolenic acid, which is obtained directly from the plant on which the caterpillar feeds. However, the specific function of the elicitor in the herbivores' regurgitant remains unclear.

As reviewed by Dicke (1999), induced indirect defences through volatile signals have been demonstrated in twelve plant families, both monocotyledons and dicotyledons, and herbivores causing this signalling in plants have been recorded for twelve families and four insect orders as well as for mites. Natural enemies that use the induced indirect defence from plants to locate potential prey or hosts are as diverse as Hymenoptera, Hemiptera or mites (Dicke, 1999). In all those systems, one question remains: how specific and reliable is the signal emitted by plants to natural enemies? This question was first addressed by Vet and Dicke (1992) through the reliability-detectability problem. In the field, natural enemies are faced with a great variety of olfactory stimuli, if we only consider this sensory modality. Both plants and hosts or prey produce odours that can give some information to the foraging predators or parasitoids. The usefulness of the information was described by Vet and Dicke (1992) as depending on two factors: (1) the

reliability of the odour in indicating herbivore presence, accessibility and suitability and (2) the detectability of the stimuli. Herbivores are generally quite cryptic either in their shape and colour or in the odour they release themselves or through their by-products (Fig. 1-1). In contrast, plants under attack release large amounts of odour (Fig. 1-1), and should therefore be more detectable to natural enemies. Then the question of the specificity and reliability of such a detectable signal comes in to play.

Some studies have shown that the odour released by plants may give specific information on the herbivores suitability for predators or parasitoids. Sabelis and van de Baan (1983) and Takabayashi *et al.* (1991) found that carnivorous mites can distinguish between apple plants infested by two different herbivorous mites species (*Panonychus ulmi* and *Tetranychus urticae*). Differences in the odour emitted by apple trees infested by the two species of mites are mainly qualitative due to variations in the ratio among compounds. Generalist predatory mites (*Amblyseius filandicus* and *A. andersoni*) were attracted to apple foliage on which their preferred prey, the European red spider mite *P. ulmi*, is feeding and not to apple foliage infested with the spider mite *T. urticae*. The specialist predator of *T. urticae*, *Phytoseiulus persimilis*, is more attracted to plants infested by its preferred prey. Parasitoids can also discriminate among plant odours to find their suitable host. *Cardiochiles nigriceps*, a specialist parasitoid of *Heliothis virescens*, is more attracted to plants (tobacco, cotton and corn) fed on by its specific host than by plants fed on by a close related species, *H. zea*. For maize, differences in the chemistry of the signal released after attack by either *H. virescens* or *H. zea* are mainly quantitative (De Moraes *et al.*, 1998). Another striking example comes from Broad bean plants and the pea aphids *Acyrthosiphon pisum*. When attacked, Broad bean release a blend that includes 6-methyl-5-hepten-2-one, this chemical is not released when Broad bean is attacked by another aphid, *Aphis fabae* (Du *et al.*, 1998). Very often, parasitoids can only attack a specific larval instar of their host, in this situation, a parasitoid would benefit if signals from plants also

give information on the herbivore developmental stages. Takabayashi *et al.* (1995) showed that maize plants release induced odour only when attacked by young instar larvae of *Pseudatelia separata* and not when attacked by late larval instars. In this system, the plant's signal indicates the suitability of the larvae as hosts to the parasitoids *Cotesia kariyai*, which can attack the early instars. Such specificity of induced plant odours production is not general to all systems. For example, the parasitoid *Cotesia glomerata* does not distinguish among cabbage plants infested by different instar larvae of its host *Pieris brassicae* (Mattiacci and Dicke, 1995).

Hence there are several examples that show that plants synomones can give specific information on the herbivore feeding on it, but in some other cases the information that herbivore-induced plant odour can provide to natural enemies seem to be not so reliable. The reliability of the plant-provided signal is complicated by the fact that within one species of plant the induced signal can vary considerably (Takabayashi *et al.*, 1991; Turlings *et al.*, 1998). This variation can be in the quantity of induced odour released as well as in the quality of the odour blend. In maize for example, the cultivar Ioana does not release β-caryophyllene while this compound is released by other cultivars (Turlings *et al.*, 1998). In such cases, the odour blends are different, but all indicate the presence of the same herbivore feeding on maize. Only the parasitoids themselves can show us how important such variation is in detecting the presence of potential hosts.

The results from several specificity studies and those that have shown large genetic variability in odour emissions among plants appear to be in conflict and still leave a lot of questions about the reliability of induced plant odour to the third trophic level. In the following studies I aimed to measure the relative importance of various biotic and abiotic factors in determining the variation in induced maize odour when attacked by larvae of *Spodoptera littoralis* (Fig 1-3).

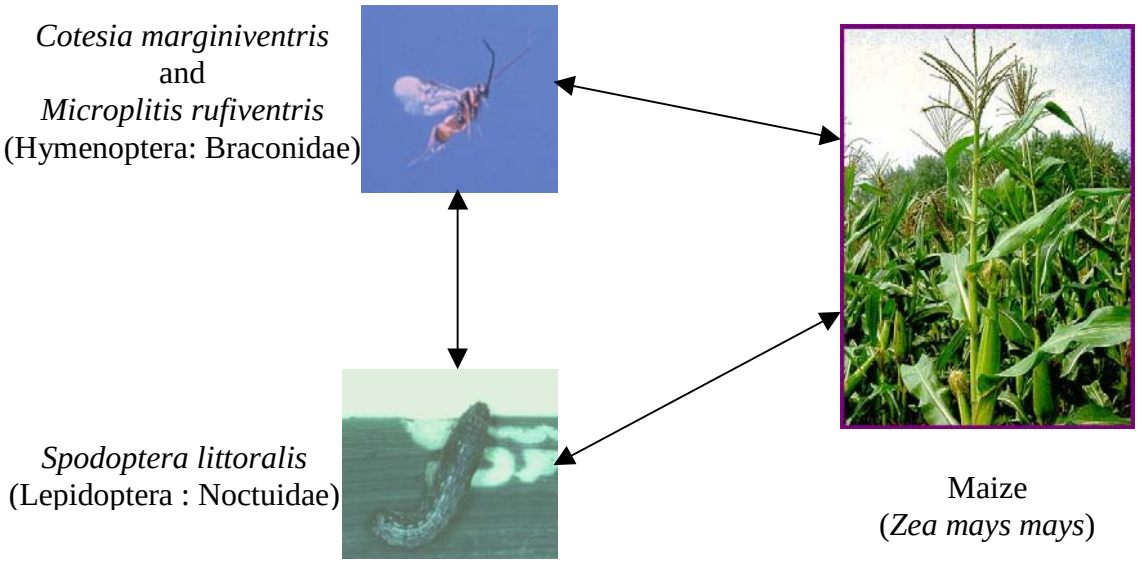

Fig. 1-3. Tritrophic system composed of maize plants, a pest herbivore of maize, the lepidopteran larvae of *Spodoptera littoralis* and two parasitoids of *Spodoptera* species, the solitary endoparasitoids, *Cotesia marginiventris* and *Microplitis rufiventris*.

The maize variety Delprim was chosen as the standard plant in all experiments because the induced odour blend released by this variety comprises the full range of compounds that were previously found in some other maize varieties (Turlings and Tumlinson, 1992; Turlings *et al.*, 1998). A particularity of the variety Delprim is that undamaged plants release linalool in significantly amounts (Fig 1-1). Also only mechanical damage alone causes Delprim to emit induced volatiles, although the application of *S. littoralis* regurgitant significantly increases the release of volatiles (Fig 1-4). The feeding by larvae remains the most effective way to induce plants to release volatiles, but it is difficult to control for the amount of damage done, a factor that is very important in determining the amount of volatiles release (Chapter 2). In order to control for induction of volatiles we used the classical method consisting of scratching two maize leaves on 2 cm^2 and applying regurgitant of *S. littoralis* on the damaged sites (Turlings and Tumlinson, 1992).

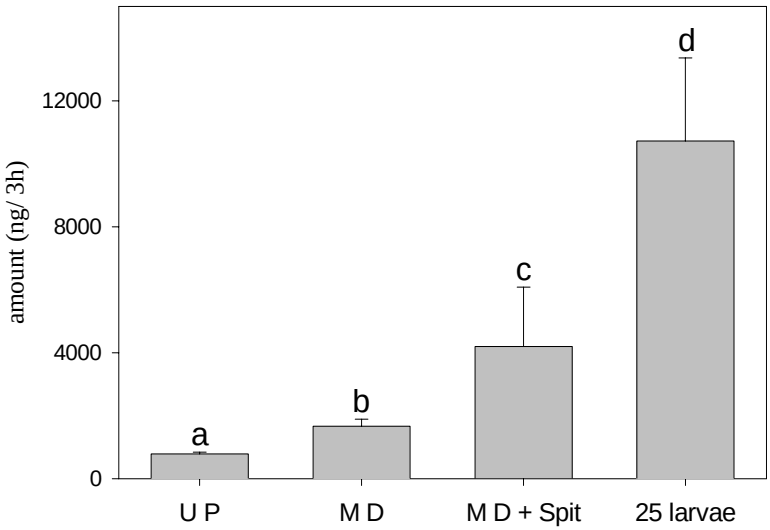

Fig. 1-4. Total amount (mean ± S. E) of induced volatiles released by maize plants of the variety Delprim. Different induction methods were used, plant were either left unharmed (UP : Undamaged plants) as control, or mechanically damage (MD) or mechanically damage with application of *S. littoralis* regurgitant on the wounded sites (MD + Spit) or 25 2nd instar larvae of *S. littoralis* were left feeding on a plant over night. Differences among treatment were determined with one-way anova ($F=31.012$; $p<0.000$). Letters above bars represents significant differences between treatments after Student-Newman-Keuls post hoc test ($\alpha=0.05$).

Factors that can affect the induced odour blend are diverse, we organised them in two groups: (a) biotic factors, like the developmental stage of the herbivore, the age of plants and plant genotype, and (b) abiotic factors, which are environmental factors, as temperature, soil humidity, air humidity, light, and fertilisation rate.

THESIS OUTLINE

In this thesis, I focus on the following questions:

1. How do different larval instars of *Spodoptera littoralis* (Lepidoptera:Noctuidae) affect the emission of induced volatile in maize ?

A study by Takabayashi *et al.* (1995) showed that different larval instars of the caterpillar *Pseudatelia separata* (Lepidoptera: Noctuidae) affect the emission of induced volatiles from maize plants differently. We tested if in our tritrophic system, which also includes maize, but attacked by a different lepidoptera, *Spodoptera littoralis* (Lepidoptera: Noctuidae), the induction of odour is also herbivore instar-dependent. Moreover, we tested if the solitary endoparasitoid *Microplitis rufiventris* (Hymenoptera: Braconidae), which can only parasitise young instar larvae *of S. littoralis*, can differentiate among plants that are attacked by different instars. We first determined the effect of regurgitant of 2^{nd}, 3^{rd}, and 5^{th} instar larvae of *S. littoralis* on the induction of odour in maize seedlings of the variety Delprim. Then, the effect of direct feeding of each instar larvae was assessed by analysing the induced odour blend induced after the feeding of different densities of 2^{nd}, 3^{rd} and 5^{th} instar larvae on maize plants. An additional experiment, in which the feeding damage of the three instars was roughly mimicked, confirmed the overall result that the amount of damage was the most important factor in determining variability in induction (Chapter 2).

2. Is the emission of induced maize volatiles dependent on leaf and plants age?

Attack by *Spodoptera* larvae can dramatically reduce seed production in young maize plants. Parasitisation by certain parasitoids can significantly reduced this negative effect of the herbivores (M-E Fritzsche-Hoballah, unpub. data). Hence, it would be profitable to plants to defend themselves at this vulnerable stage. If herbivore-induced odour emissions constitute a defence, it can be expected to be most pronounced in young plants and in young parts of plants, which appear to have more to lose by caterpillar attack than older plants or leaves. To determine this, the emission of volatiles by maize plants of 5 different ages (1 week, 2, 4, 6 and 8 weeks) in response to standardised induction with artificial damage and application of regurgitant of *S. littoralis* was measured. We found the strongest response in two week old plants. In addition, we measured the release of induced volatiles from each leaf of a 2-week old maize plant. Seedlings were induced either by incubating cut stems in solution of regurgitant or by mechanical damage and applying regurgitant on the oldest leaf (1^{st} leaf) or on a young leaf (3^{rd} leaf). Systemic response of the different leaves was not qualitatively different but higher response was obtained by young leaves (3^{rd}). With the second method, higher response occurred also from the 3^{rd} leaves when they were damaged, and in this case the quality of the induced odour blend changed through leaves ages. (Chapter 3).

3. What is the range of variation in herbivore-induced odour emissions among maize cultivars and their wild ancestors (teosinte) ?

It is still unclear to what extent herbivore-induced plant odours are specific for the herbivore that feeds on the plant. Some of the systems described above provide evidence in

favour of the emission of specific signals by plants. But some other studies suggest that variability is mainly determined by plant genotype rather than by the herbivore. In chapter 4, we compare eleven maize varieties and five species or subspecies of wild relative of maize (teosinte), as well as the offspring of eight mother plants of *Zea mays mexicana*. This study provides an estimate of the range of variation in the induced odour blend between genotypes and within a single genotype of plants. Maize plants were induced to release volatiles with artificial damage and regurgitant treatment. Collection of induced odour occurred in three consecutive 3hr periods to control for any differential timing in the release of volatiles. The results revealed considerable quantitative and qualitative differences among varieties as well as among the teosintes.

4. How do abiotic factor affect the emission of induced volatiles in maize ?

Most of the studies on induced plant odour are tested in controlled laboratory conditions. Little information is available on how various abiotic conditions affect the odour emission. We tested different abiotic factors like the humidity of soil and air, temperature, light intensity and fertilisation rate to assess their individual effects on the release of induced volatiles in maize plants (Chapter 5). Each factor was tested separately under constant conditions for the other factors. In all cases we used two-week old maize plants of the variety Delprim. Changes in each of the abiotic conditions had some effect on the quantity of the volatiles that were emitted after induction. The most dramatic increases were obtained with increases in light intensity and fertilisation.

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CHAPTER 2.

INDUCTION OF VOLATILE EMISSIONS IN MAIZE BY DIFFERENT LARVAL INSTARS OF *SPODOPTERA LITTORALIS* (LEPIDOPTERA: NOCTUIDAE).

Abstract—Maize plants under attack by caterpillars emit a specific blend of volatiles that is highly attractive to parasitic wasps. The release of these odorous signals is induced by elicitors in the caterpillar regurgitant. Recent studies suggest that plants respond differently to different herbivore species and even to different herbivore stages, thus providing parasitoids and predators with specific signals. We tested if this is also the case for different larval instars of the noctuid moth *Spodoptera littoralis* when they feed on maize plants. In a first experiment, cut maize plants were incubated in diluted regurgitant from second, third, or fifth instar caterpillars. The emission of volatiles was not different among the different instars, neither qualitatively nor quantitatively. These results indicate that any possible differences in emission among instars are not due to the activity of their regurgitant. We then tested the effect of the actual feeding by the larvae. Potted plants were infested with caterpillars of one of the three instars and volatiles were collected the following day. The intensity of the volatile emissions was correlated with the number of larvae feeding on a plant, and with the amount of damage they inflicted, but was independent of the larval instar that caused the damage. We also roughly mimicked the manner of feeding of each larval instar to test the importance of physical aspects of damages for the odour emission. The emission was highly variable, but no significant differences were found among the different types of damage. In olfactometer tests, *Microplitis rufiventris*, a parasitoid that parasites second and early third instar *S. littoralis*, did not differentiate among the odours emitted by maize plants attacked by different instar larvae. These results indicate that maize odours induced by *S. littoralis* provide parasitoids

with poor information on the larval developmental stage. We discuss the results in the context of specificity of odorous plant signals for natural enemies.

Key Words — larval instar, induced plant volatiles, specificity-reliability, 6-arm olfactometer, *Zea mays*, *Spodoptera littoralis*, *Microplitis rufiventris*.

INTRODUCTION

Plants subjected to feeding damage by insects respond with the release of specific blends of volatiles that attract parasitoids and predators (Dicke and Sabelis, 1988; Dicke *et al.*, 1990; Agelopoulos and Keller, 1994; Mattiacci *et al.*, 1994; McCall *et al.*, 1993; Röse *et al.*, 1996; Steinberg *et al.*, 1992; Turlings *et al.*, 1990; Du *et al.*, 1998). The release of odours by attacked plants has been found to be triggered by elicitors in oral secretions of the herbivores (Dicke *et al.*, 1993; Turlings *et al.*, 1993a; Mattiacci *et al.*, 1994). In the regurgitant of the caterpillar *Pieris brassicae* (Lepidoptera: Pieridae), the elicitor was identified as the enzyme, β-glucosidase (Mattiacci *et al.*, 1995). Alborn *et al.* (1997) identified a non-protein elicitor from the regurgitant of *Spodoptera exigua* (Lepidoptera: Noctuidae), N-(17-hydroxylinolenoyl)-L-glutamine (named volicitin). In maize plants, volicitin triggers a similar response as is triggered by *S. exigua* feeding (Alborn *et al.*, 1997; Turlings *et al.*, 2000). The induced odour is mainly composed of terpenoids and is highly attractive to the braconid parasitoids *Cotesia marginiventris* (Hymenoptera: Braconidae) and *Microplitis croceipes* (Hymenoptera: Braconidae) (Turlings *et al.*, 1990; McCall *et al.*, 1993).

Interestingly, *M. croceipes* cannot parasites *S. exigua* larvae, but is nevertheless highly attracted to maize odours induced by a non-host (McCall *et al.*, 1993; Turlings *et al.*, 1993a). This potential limitation to the reliability of herbivore-induced plant signals

has been discussed by Vet and Dicke (1992). They argue that the large quantities of the plant-provided cues make them highly detectable, but they may be poor indicators of herbivore identity. However, some recent studies suggest that plant signals can be herbivore specific. De Moraes *et al.* (1998) demonstrate that the parasitoid *Cardiochiles nigriceps* (Hymenoptera: Braconidae) can distinguish odours from plants damaged by its specific host from odours induced by a non-host. Differences in the attractiveness to the wasps were mainly ascribed to quantitative differences in the reaction of the plant. Guerrieri *et al.* (1999) showed that different aphid species elicit different odour emissions in bean plants and that the aphid parasitoid, *Aphidius ervi* (Hymenoptera: Braconidae: Aphidiinae), can use these differences to distinguish plants infested by its host, *Acyrthosiphon pisum* (Homoptera: Aphididae), from those infested by a non-host, *Aphis fabae* (Homoptera: Aphididae). Another intriguing example of specificity is the one reported by Takabayashi *et al.* (1995) who show that the parasitic wasps *Cotesia kariyai* (Hymenoptera: Braconidae) can differentiate between maize plants under attack by young instar larvae and late instar larvae of *Pseudaletia separata* (Lepidoptera: Noctuidae). These studies clearly suggest that herbivores can induce specific signals in plant that give information on the identity and stage of potential hosts for parasitoids, but in some other systems this appear not to be the case (McCall *et al.*, 1993; Turlings *et al.*, 1993a). The specialist parasitoid *M. croceipes* could not distinguish among plants fed on by unsuitable host (McCall et al., 1993). Different herbivore regurgitant induced the release of volatiles by maize plants, which were highly attractive to parasitoids (Turlings *et al.*, 1993a). In the cabbage system, composed of cabbage plants-*Pieris brassicae*-*Cotesia glomerata* (Hymenoptera: Braconidae), the parasitoid does not discriminate between plants infested by first instar larvae and fifth instar larvae (Mattiacci and Dicke, 1995). This was surprising because chemical analyses of collected cabbage odours suggested that the host instars differed in the odour the induced in the plant. The specificity of plant responses

seems to differ for different systems and the reliability of the information provided by the chemical signals varies accordingly.

We wanted to test if in the tritrophic system composed of maize plants, *Spodoptera. littoralis* (Lepidoptera: Noctuidae) caterpillars and the endoparasitoid *Microplitis rufiventris* (Hymenoptera: Braconidae), the induced odour differs with the developmental stage of the herbivore and whether such differences are detected and used by the wasp, which can only attack early instar larvae. For this purpose, volatiles emitted by maize plants that were subjected to various treatments were collected and analysed. In a first experiment, plants were incubated in the regurgitant of second, third and fifth instar larvae. Secondly, plants were subjected to actual feeding damage by these three instars. In a third experiment, artificial damage was used to roughly mimic the damage caused by the three instars. In an additional experiment, we tested if *M. rufiventris* distinguished among the odours from plants attacked by the different instars. In all experiments, the amount of damage was the main factor that determined the intensity of the odour emissions and their attractiveness. No major differences were found in the identities and relatives ratios of the various compounds. The results indicate that in this system odours emitted by the maize plants provide no information on the instar of *S. littoralis* that feeds on it.

METHODS AND MATERIAL

Plants. Maize plants of the variety Delprim were used in all experiments. Seeds were sown in individual plastic pots (360 ml, 10 cm diameter, 7 cm high) filled with regular fertilised soil (Coop, Switzerland). Maize plants were grown in a climate chamber (Type 10'US/+5 DU-PI, Weiss Umwelttechnik GmbH, Switzerland) at 23°C, 60% RH, and under 16L:8D light regime. Plants of 9-10 days old were used for experiments, at which age they carry 3 to 4 leaves.

Insects and S. littoralis *regurgitant collection. S. littoralis* larvae and eggs were received weekly from Novartis (Basle, Switzerland). Batches of *S. littoralis* eggs were placed on moist filter paper in a petri dish. Newly hatched larvae were put on maize leaves (var. Delprim), in transparent plastic boxes (13.5 x 15 x 5 cm). Larvae of 2^{nd}, 3^{rd}, and 5^{th} instar were used in the experiments. Regurgitant of the different instars was collected as described by Turlings *et al.* (1993).

A colony of the parasitoid *M. rufiventris* was started with cocoons provided by Dr. E. Hegazi (University of Alexandria, Egypt). The colony was maintained on *S. littoralis* larvae fed with artificial diet. At emergence, adults parasitoids were sexed and kept in plastic cages (30 x 30 x 30 cm, Bugdorm I, MegaView Science Education Service Co, Ltd, Taiwan) under laboratory conditions (25°C±3°C, 40% RH). Insects were provided with honey and the cages were sprayed with water daily to compensate for the relatively dry lab conditions.

Collection and analysis of induced odour. Two different systems were used to collect induced odours from maize plants. The first collection system was an all glass push-pull odour collection system modified from Turlings *et al.* (1991). It basically consists of 3 cylindrical Pyrex glass pieces. The first tube contains a glass frit, which ensure laminar airflow into the second tube. It is 14 cm long and ends with a 6 cm male ground-glass joint that was connected to a female counterpart of the second tube. The second glass tube holds the odour sources and is about 29 cm long (including joints) with an outside diameter of 7 cm. It ends in a 3 cm long female ground glass joint that fits the inlet of the third tube, which tapers down and ends in a glass screw fitting. An open screw cap with a Teflon rubber ferrule (6 mm ID) was used to connect a collection trap at this upwind end of the third glass tube, while the downwind end was connected with plastic

(Tygon) tubing to flowmeters (5-channel Adjustable Vacuum Flow Volatile Collection System, Model VCS-5ASP-MAN, Analytical Research Systems, Gainesville, Florida, USA), which were connected to a vacuum pump. Humidified and purified air was pushed and pulled through the glass tubes over the plants through a collection trap at a rate of 0.6 ml/min. Collection traps were made according to Heath and Manukian (1994). Before each collection filters were rinsed with 500 µl of pentane, followed by 500 µl of methylene chloride.

The second collection system was designed to collect from growing plants. It has been described in detail by Turlings *et al.* (1998). It consists of 6 vertically placed cylinders (9.5 cm inner diameter, 54 cm high). The aerial part of a plant was placed in a cylinder, while the pot was placed outside, separated from the rest of the plant by a Teflon disk consisting of two halves with a hole in the centre (Turlings *et al.*, 1998). Purified and humidified air was pushed into each cylinder at a rate of 1 l/min. Around the base of each cylinder, just above the Teflon disk, 8 openings served as ports that could hold the collection traps. Only one port was used during an experiment. Collection traps were the same as described above. For each collection air was pulled through a trap at a rate of 0.8 l/min, while the rest of the air vented out through the hole in the bottom, thus preventing outside, impure air from entering. The automated part of the collection system (Analytical Research System, Gainesville, Florida, USA) controlled the flow through the traps and made it possible to switch this flow from one trap to another. The climate chamber (CMP4030, CONVIRON, Winnipeg, Canada) in which the collection cylinders were housed was kept at 17.5°C. Due to the irradiation heat, the temperature inside the cylinders was 23 ± 3°C. During the light cycle, light intensity was about 20000 lm/m^2.

After each collection, traps were extracted with 150µl of methylene chloride (Lichrosolv, Merck, Switzerland) and 200 ng of n-octane and nonyl acetate (Sigma, Switzerland) in 10 µ l methylene chloride were added to the samples as internal standards.

Analyses were done with a Hewlett Packard HP 6890 series gas chromatograph equipped with an automated on-column injection system (HP G1513 A) and a flame ionisation detector. Of each sample, a 3 µl aliquot was injected onto an apolar EC-1 capillary column (30m, 0.25 mm. i.d., 0.25 µm film thickness, Alltech Associates, Inc, USA) preceded by a deactivated retention gap (10 m, 0.25 mm i.d., Connex, USA) and a deactivated pre-column (30 cm, 0.530 mm i.d., Connex, USA). Helium (24 cm/s) was used as carrier gas. Following injection, the column temperature was maintained at 50°C for 3 min., increased to 230°C at 8°C/ min and held at 230°C for 9.5 min. The detector signal was processed with HP GC Chemstation software.

Experiment 1. Effect of S. littoralis *instar regurgitant on the induction of odour in maize plants.* Oral secretion of 2nd, 3rd and 5th instar larvae was tested. For treatment with each category of regurgitant, three plants were used. The cut stem of each maize seedling were incubated into a solution of regurgitant (50 µl in 450 µl of distilled water) during one night (14-15 hours). Control plants were incubated in distilled water only. Before collection, the part of the stem that had been submerged in the regurgitant solution was cut off, and the fresh cut was wrapped in a piece of moist cotton to avoid desiccation. The three plants that were treated with the same spit solution were placed together in one of the glass tubes of the odour collection system. The experiment was replicated thirteen times. Total amounts as well as the composition of the induced odour blend emitted by the maize plants treated with the different regurgitants were compared.

One batch of the regurgitant of each of the three different instar was analysed at the USDA-ARS, Gainesville, Florida, USA. The compositions of the three larval instars were compared in order to detect any variation in elicitors' quality or quantity. Regurgitant samples were collected as described above. To denature enzymes and to eliminate bacterial degradation each sample of oral secretions was diluted with an equal amount of acetonitrile

immediately after collection. The samples were centrifuged at 14,000 RPM (Eppendorf Centrifuge 5415) to remove the solids, and the supernatant filtered through 0.45 and 0.22 μm sterilising membranes (Millipores Millex-HV and Millex-GV, Bedford, MA). For quantitative analyses, 5 μl of *N*-palmitoleoyl-L-glutamine solution (1 μg/μl) in CH_3CN/H_2O (8:2 v/v) was added to each sample (50 μl) as an internal standard. Ten μl of each sample was then analysed by HPLC with UV detection at 200 nm (constaMetric 4100 pump, SpectroMonitor 3200 detector, Spectra System AS 3500 autosampler, Thermo Separation Products, Riviera Beach, FL). A reverse-phase column (YMC-Pack ODS-AMQ, 250 X 4.6 mm ID, YMC, Kyoto, Japan) was eluted (1 ml/min) with a solvent gradient of 20 to 95% CH_3CN (High Purity Solvent, Burdick & Jackson, Muskegon, MI) containing 0.8% acetic acid (Aldrich, Milwaukee, WI), in water (Milli-Q UV PLUS system, Millipore, Bedford, MA) containing 0.5% acetic acid, over 40 min, and then returned to the initial conditions at 45 min. The column temperature was maintained at 60°C. The detector response to the internal standard was used to calculate the amounts of *N*-(17-hydroxylinolenoyl)-L-glutamine (volicitin), *N*-(17-hydroxylinoleoyl) glutamine, 17-hydroxylinolenic acid, 17-hydroxylinoleic acid, *N*-linolenoyl-L-glutamine, *N*-linoleoyl glutamine, linolenic acid, and linoleic acid. These components have earlier been isolated and identified in beet armyworm (*Spodoptera exigua*) oral secretions (Alborn *et al.*, 2000), and the structures were confirmed using methods described in Mori *et al* (in press).

Experiment 2. Density effect of 2nd, 3 rd and 5th instar larvae on the induced emissions. In order to test if larvae of different instars feeding on maize induce different releases of volatiles, we collected odours from plants attacked by different numbers of larvae of the three different instars. For 2nd instar larvae, either 1, 5, 10, 25, 50 or 70 larvae were placed on one maize plant. For 3rd instar larvae, 1, 5, 10, 20, or 50 larvae were placed on a maize seedling. For the 5th instar larvae, lower densities were chosen, due to their size

and feeding rate, only 1, 2, 3, 4, 5, 10 larvae were placed on a plant. A cellulose bag (Celloclair, Liestal, Switzerland) was placed over each plant to avoid escape of caterpillars. Larvae were placed on the plant in the evening, and were allowed to feed all night for 15 h. They were removed from the plant just before an odour collection. After collection the amount of damage on maize plants was estimated and expressed in the percentage of leaf surface removed.

Experiment 3. Effect of different types of damage on the induced emission of volatiles in maize plants. The damage caused by the different instar larvae of *S. littoralis* is rather different. Young instar larvae graze the surface of the leaf, creating "windows" and leaving most of the veins intact, while late instar larvae remove all parts of a leaf. Intermediate instar larvae combine both types of damage, they partly graze on the leaves and remove small parts of tissue. To determine if the different types of damage inflicted by the feeding of the different larvae instar is correlated with differences in odour emissions, we roughly mimicked the damage done by the caterpillars. Second instar mimicry was done as follows, only the surface of the leaf was remove with a razor blade, like shaving the surface in order to leave the veins intact. In each case, 2 cm^2 was damaged per leaf. For 3^{rd} instar mimicry, the same manipulation was done as for 2^{nd} instar mimic, but stronger and some cuts were also inflicted to each leaf. Again, 2 cm^2 was damaged per leaf. For 5^{th} instar mimicry, 10 holes were punched per leaf. Holes were 4 mm diameter, which corresponds to a surface of damage of 5 cm^2 per leaf.

In all treatment, 10 µl of caterpillar regurgitant was applied on the damaged areas on each leaf, for the 5^{th} instar damage, the spit was spread only on the border of the hole. Two plants were used per treatment. The plants were treated in the evening (18:00), and the plants stayed in the dark until the moment of collection (9:00). This was replicated nine times.

Experiment 4: Does the parasitoid M. rufiventris *distinguish among plant fed on by different instar larvae of* S. littoralis*?* Choice test experiments were conducted in a six-armed olfactometer. It is basically a three level system. The bottom level holds the odour sources and consists of six vertically placed glass cylinders that in our case, contained a growing plant. Purified and humidified air entered each cylinder, passed over the plant and was pushed to the upper level of the system via flexible Teflon tubes. The upper part holds the actual olfactometer, which consists of a six arm glass star, each arm is connected with the Teflon tube to a cylinder of the bottom level. The air charged with plant odour passed through each arm to the central chamber of the star. This central chamber is connected to the middle level of the system, where the wasps are released into this chamber. The air from all the arms is pulled through the bottom of the release chamber. After release, the wasps first climb up to the upper central part of the star. They are initially attracted to the upper part by a lamp that is placed above it. If the wasps are attracted to an odour they walk into the arm that carries that odour and are trapped in a glass vessel at the end of the arm.

Three undamaged plants were alternated with plants fed on by each instar of *S. littoralis* caterpillars. Results from experiment 2 showed that the maximum emission of odour occurred at about 60 % of damage on a plant independently of the instar larvae. This amount of damage was correlated to a corresponding number of larvae for each instar tested, thus maize plants were fed on by 60 2^{nd} instar larvae, 30 3^{rd} instar larvae or 3 5^{th} instar larvae. Odour emission from plants fed on by the different instar larvae was collected for three hours during the experiments. Four groups of six female parasitoids were tested within one day. One group of six wasps consisted of naive females, the other trials were conducted with six female wasps that had an oviposition experience with 2^{nd} instar larvae that were placed on plants that been fed upon for one night by either 2^{nd}, 3^{rd} or 5^{th} instar larvae. Such experiences are known to increase the responsiveness of female

parasitoids to the experienced odour (Lewis and Takasu, 1990; Turlings *et al.*, 1993b; Vet *et al.*, 1995). If the odours differ among the 3 instars, it could be expected that this would reflect in the response of the different groups. The position of the plants remained the same on a particular day to avoid an effect of odour contamination in the arms, but the position of treated plants and control plants was changed between days of experiments. The experiments were replicated 10 times (10 days).

Statistical analyses. Experiments 1 and 3: The total amount of induced odour released was compared using one-way anova. Data were ln-transformed to comply with anova assumptions. Student-Newman-Keuls was performed as post hoc test for multiple comparison. Comparison of the amounts of the twenty dominant compounds was performed using a multivariate anova. Dunett T3 post hoc test was used for multiple comparisons.

Experiment 2: Quadratic regressions on ln-transformed data were performed to test for the effect of the quantity of damage on the release of induced volatiles. The analysis was done for each instar separately. Confidence intervals of equation coefficients were calculated and compared for differences. For comparison of the different odour blend among the three instars, data were grouped in five damage classes. Multivariate analysis of variance was performed to compare the effect of the instar and of the class of damage on the release of six dominant compounds. Dunett T3 post hoc test was used for multiple comparison.

Experiment 4 : Chi-square analysis was performed to test for differences among proportion of wasps that choose the different odours provided.

RESULTS

Experiment 1: Effect of S. littoralis *instar regurgitant on the induction of odour in maize plants*. No significant differences were found in the total amount of induced volatiles emitted by maize plants incubated in regurgitant solutions from 2nd, 3rd and 5th instar of S. littoralis (Fig. 2-1). Plants incubated in water only released significantly less odour compared to plants incubated in regurgitant solutions ($F=16.138$, $p<0.001$; Fig. 2-1).

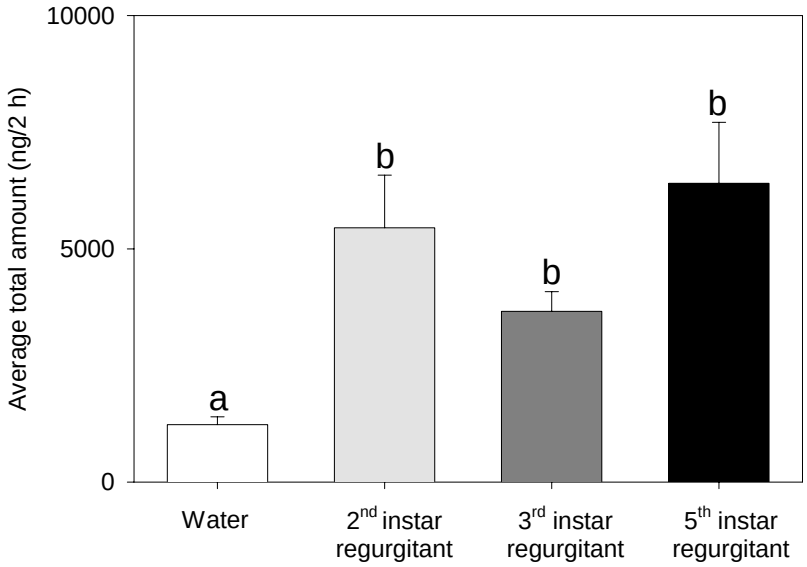

Fig 2-1. Average total amount (ng/2h) + SE of induced odour emitted by maize seedlings after incubation in a solution with regurgitant of a specific larval instar. Incubation in water was used as control. Letters above each bar indicate differences among treatments according to Student-Newman-Keuls post hoc test after one way anova.

Composition of the odour blend induced by the three instar regurgitants was not significantly different (Fig. 2-2) except for the compounds phenethyl acetate and α-humulene, which were released in higher quantities by plants incubated in 5th instar regurgitant solution compared to other treatments.

Analysis of the regurgitant of the three instars tested showed that all of them contained volicitin (Fig. 2-3). In all instars, linolenic acid is dominant in the composition of the regurgitant. The ratios of the different compounds present in the regurgitant are variable among the three instars tested (Fig. 2-3). Because only one sample of 2nd instar regurgitant was available, no statistical comparison was possible, but differences among the regurgitant from the three instars appear marginal.

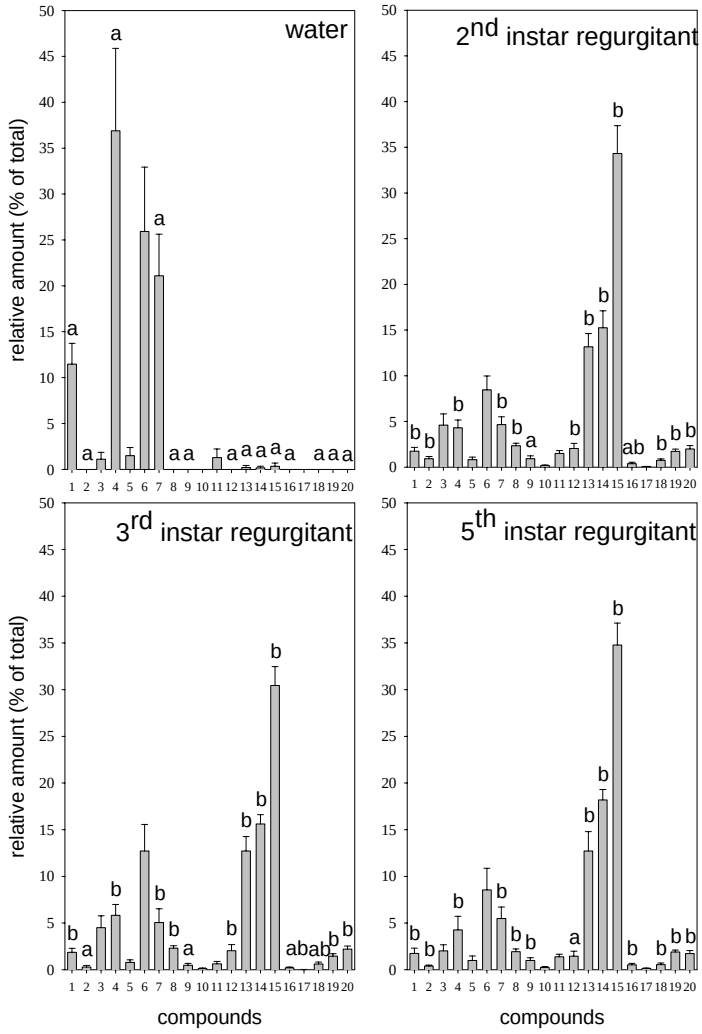

Fig. 2-2. Average relative amount (% of total) of the main compounds (ng/2h) + SE released by maize seedlings after incubation in a solution with regurgitant of a specific larval instar. Incubation in water was used as control. Peak identities: (1) (*Z*)-3-hexenal, (2) (*E*)-2-hexenal, (3) (*Z*)-3-hexen-1-ol, (4) (*E*)-2-hexen-1-ol, (5) β-myrcene, (6) (*Z*)-3-hexen-1-yl acetate, (7) linalool, (8) (3*E*)-4,8-dimethyl-1,3,7-nonatriene, (9) phenethyl acetate, (10) indole, (11) geranyl acetate (12) unknown, (13) β-caryophyllene, (14) α-(*E*)-bergamotene, (15) (*E*)-β-farnesene, (16) α-humulene, (17) unknown sesquiterpene, (18) β-bisabolene+ (*E,E*)-α-farnesene, (19) β-sesquiphellandrene, (20) (3*E*, 7*E*)-4,8,12-trimethyl-1,3,7,11-tridecatetraene

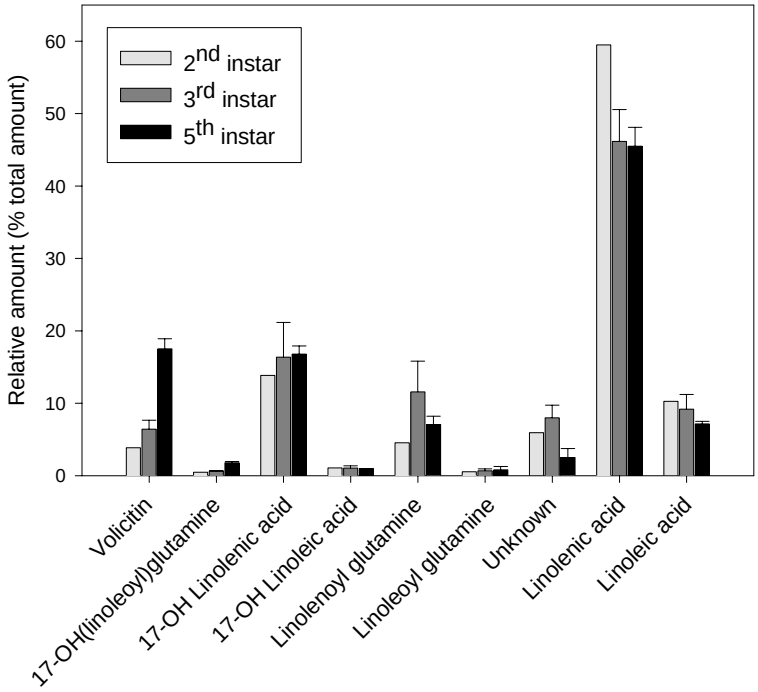

Fig. 2-3. Relative amount (mean of % total amount + SE) of the different components present in the regurgitant of 2nd, 3rd, and 5th instar larvae of *S. littoralis*.

Experiment 2: Density effect of 2nd, 3rd and 5th instar larvae on the induced emission. Percentage of damage done to maize plants was highly correlated with the number of larvae feeding on them. For each larval instar the amount of volatiles released by the maize plants and the amount of damage inflicted by the larvae closely fit a quadratic relationship (Fig. 2-4). The maximum emission of induced odour occurred in plants from which about 60% of damage of the surface had been removed (Fig. 2-4).

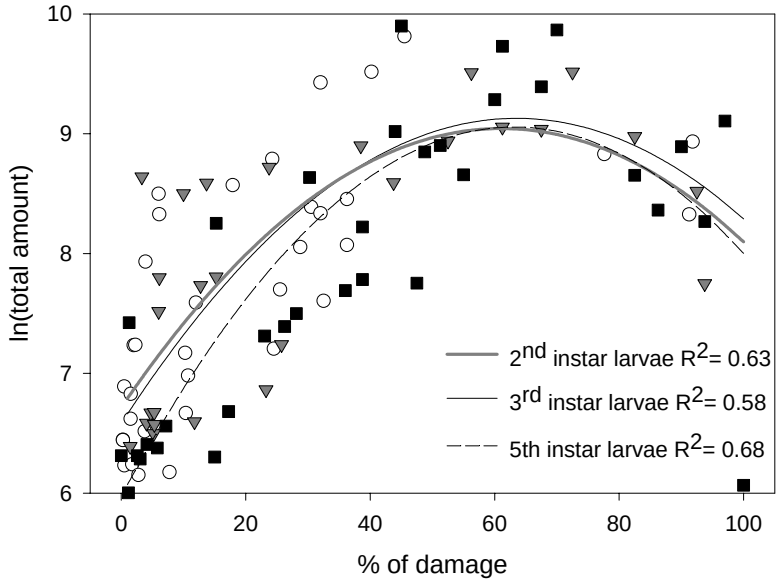

Fig. 2-4. Total amount of induced volatiles emitted by 2nd, 3rd and 5th instar larvae (ln transformed data) vs. percentage of damage on plants. 2nd instar: F=28.586, p<0.001; 3rd instar: F=16.685, p<0.001; 5th instar: F=33.879, p<0.001.

The confidence intervals for each coefficient of the three equations overlapped almost completely (Table 2-1), which means that the amount of induced volatiles released after caterpillar feeding did not differ among the three larval instars.

Table 2-1. Confidence intervals for the regression curves of emission of volatiles vs the % of damage for 2nd instar (A), 3rd instar (B) and 5th instar (C) larvae.

A	value	SE	P value	Confidence intervals	
y0	6.5826	0.1795	<0.0001	6.23078	6.93442
a	0.0801	0.013272	<0.0001	0.05408688	0.10611312
b	-0.0006	0.000154	0.0003	-0.00090184	-0.00029816

B	value	SE	P value	Confidence intervals	
y0	6.717107	0.258239	<0.0001	6.21095856	7.22325544
a	0.07621	0.017826	0.0003	0.04127104	0.11114896
b	-0.000624	0.000198	0.0044	0.00101208	0.00023592

C	value	SE	P value	Confidence intervals	
y0	5.974161	0.273097	<0.0001	5.43889088	6.50943112
a	0.097568	0.013916	0.0003	0.07029264	0.12484336
b	-0.000774	0.000143	0.0044	0.00105428	0.00049372

Two sesquiterpenes, α-bergamotene and (*E*)-β-farnesene, are dominant in the odour blend of all instars. Figure 2-5 illustrated the ratio of the main compounds in induced odour blend for five classes of damage and for the three instars tested. Multivariate analysis of variance indicates that the instar had no significant effect on the amount of the different induced volatiles (F=1.164, p=0.279), while the classes of damage had a significant effect (F=1.676, p=0.004), the intercept between instar and the damage classes was not significant (F=0.896, p=0.755). Among the compounds tested, linalool, indole, β-caryophyllene, α-bergamotene, and (*E*)-β-farnesene were released in significantly different amounts among the different classes of damage. The relative amount of linalool decreased when the amount of damage increased excepted between 40 and 60% of damage in which plants damaged by 2nd instar emitted a large amount of linalool (Fig. 2-5). The ratio of indole increased in the odour blend with the amount of damage done to a plant (Fig. 2-5). The ratio of β-caryophyllene in the induced odour blend increased slightly with the amount of damage (Fig. 2-5). About 20% of the induced odour in maize were composed of α-bergamotene, which was present in a slightly higher proportion when 40 to 60 % of a plant was damaged. The same trend was observed for (*E*)-β-farnesene, which was the main substance in the induced odour blend (about 40%, Fig. 2-5).

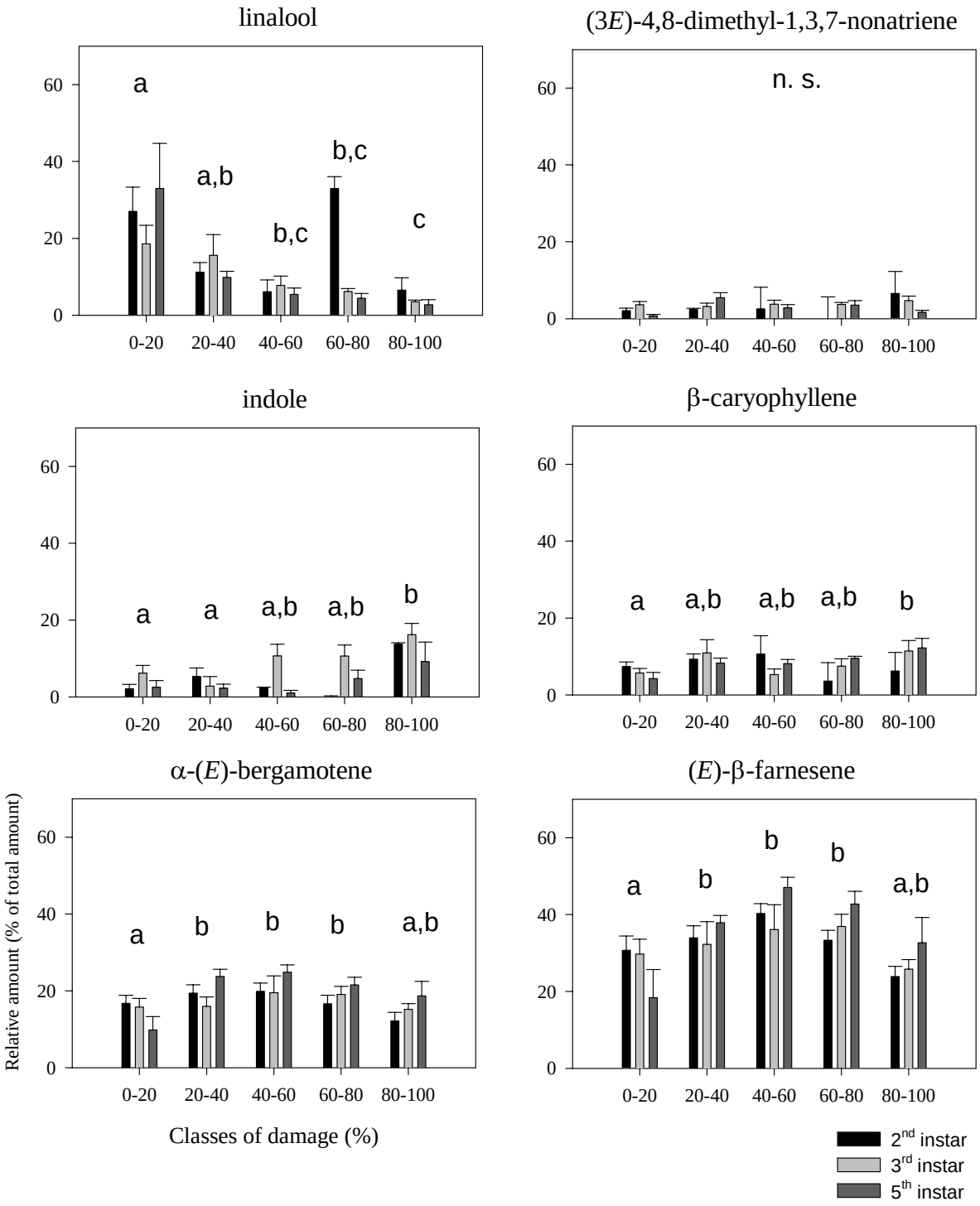

Fig. 2-5. Average relative amount of the six main induced volatiles (mean of % of total amount + SE) emitted for five classes of damage done to the plants. Letters above bars indicate significant differences between classes of damage after Dunnet T3 post hoc test (α=0.05).

Experiment 3: Effect of different types of damage on the induced emission of volatiles in maize plants. Total amounts of induced volatiles for the three types of damage were not significantly different from each other, but were different from the undamaged plant ($F=9.727$, $p<0.001$; Fig. 2-6). Multivariate analysis of variance indicated that the type of damage had no significant effect on the composition of the odour blend ($F=1.728$, $p=0.923$; Fig. 2-7).

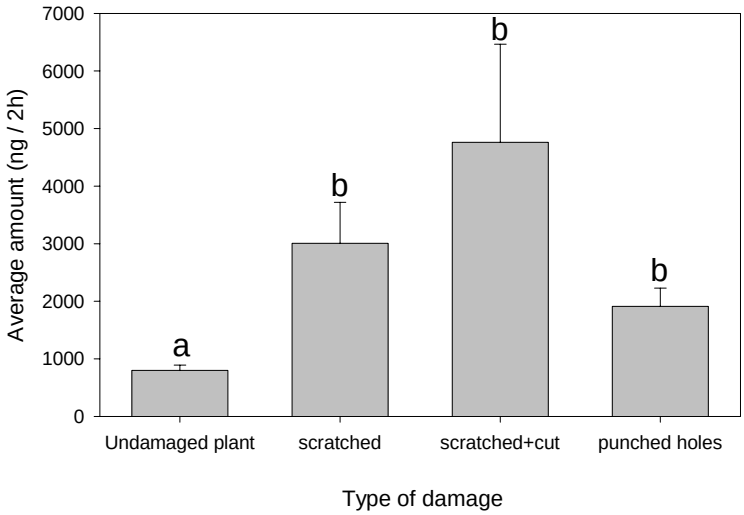

Fig. 2-6. Average total amount (mean + SE) of induced odour emitted by maize plants after three different types of damage. Undamaged plants served as controls. Letters above each bar indicate significant differences after Student-Newman-Keuls post hoc test ($\alpha=0.05$).

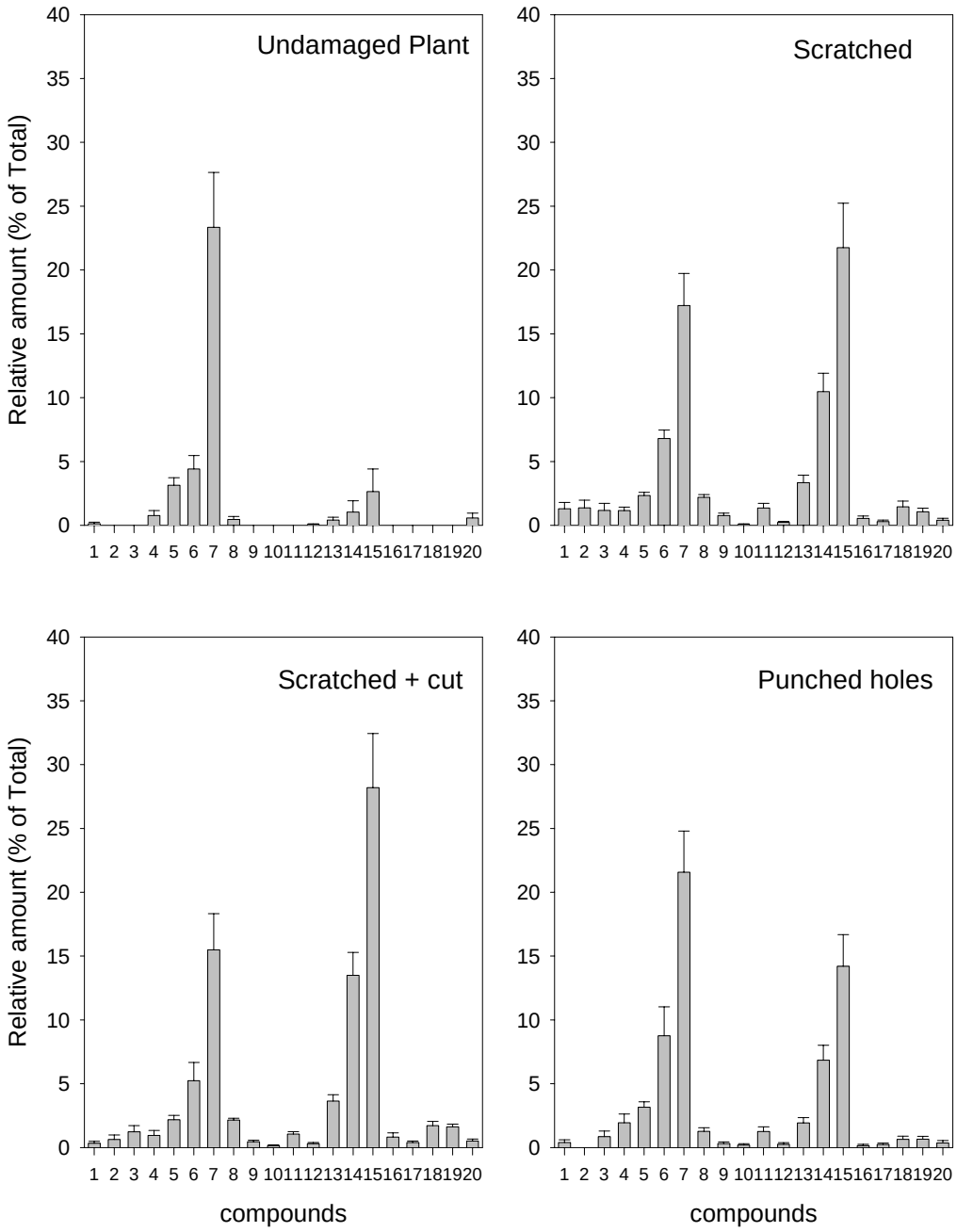

Fig. 2-7. Average relative amount of the different compounds of the induced odour blend (mean of % of total amount ± SE). (1) (*Z*)-3-hexenal, (2) (*E*)-2-hexenal, (3) (*Z*)-3-hexen-1-ol, (4) (*E*)-2-hexen-1-ol, (5) β-myrcene, (6) (*Z*)-3-hexen-1-yl acetate, (7) linalool, (8) (3*E*)-4,8-dimethyl-1,3,7-nonatriene, (9) phenethyl acetate, (10) indole, (11) geranyl acetate (12) unknown, (13) β-caryophyllene, (14) α-(*E*)-bergamotene, (15) (*E*)-β-farnesene, (16) α-humulene, (17) unknown sesquiterpene, (18) β-bisabolene + (*E,E*)-α-farnesene, (19) β-sesquiphellandrene, (20) (3*E*, 7*E*)-4,8,12-trimethyl-1,3,7,11-tridecatetraene

Experiment 4: Does the parasitoid M. rufiventris distinguish among plants fed on by different instar larvae of S. littoralis*?* Female parasitoids significantly preferred plants which were fed on by larvae than undamaged plants. On average 87% of the females made a choice; of those 91% chose for a damaged plant and 9% for an undamaged plant.

When female parasitoids had no previous experience (naive) or had experience with odours of plants fed on by 3rd or 5th instar larvae, no significant preferences were found in their choices for plants fed on by the different instar larvae (Fig. 2-8 A, C, D). Surprisingly, female wasps experienced with odours of plants fed on by 2nd instar showed a preference for the odour of plants fed on by 3rd instar when they were released in the olfactometer (Fig. 2-8 B). Interestingly, less females chose for the odour of plants attacked by 5th instar larvae, but this trend is certainly due to the lower quantities of volatiles emitted by these plants (Fig. 2-9). Over the 10 replicates, 6 were done with plants fed on by 5th instar larvae that had very little damage.

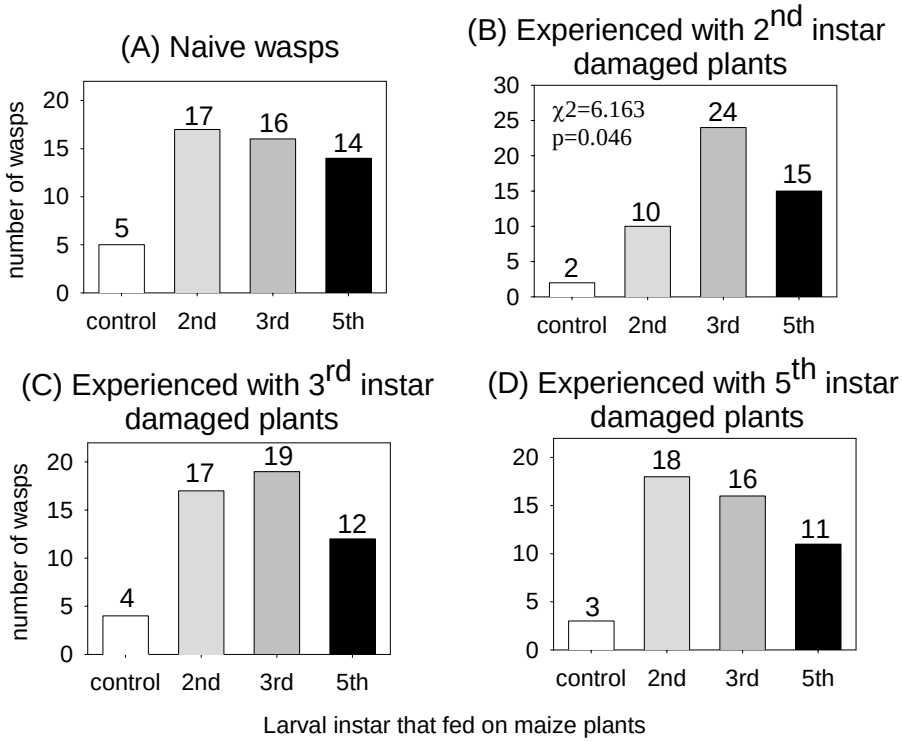

Fig. 2-8. The number of wasps that chose for the odour of maize plants damaged by either 2nd, 3rd, or 5th instar larvae or for undamaged plants (control). The female wasps were either (A) naive, or experienced by allowing them to oviposit in a 2nd instar larvae on a plant that had been damaged by either (B) 2nd, (C) 3rd, or (D) 5th instar larvae.

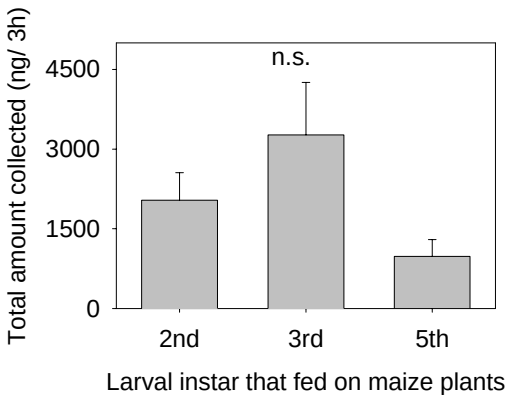

Fig. 2-9. Total amount of induced odour (mean + SE) released by plants fed on by 2nd, 3rd and 5th instar larvae during the 3h of collection in the 6 arms olfactometer collection system. No significant differences were found among the quantity of volatiles emitted by the plants (F=2.793, p=0.079).

DISCUSSION

In all cases, the three different instars of *S. littoralis* larvae induced an emission of volatiles in maize plants. Regurgitant of the three different instars induced very similar volatile blends, both in terms of quantity as well as quality. Analysis of the regurgitants of the three different instars did not reveal any major differences and all contained the known elicitor volicitin. The total amount of volatiles released by maize plants was correlated with the amount of damage done by the larvae of the different instar, also in this respect no differences among larval instars could be found. This result was confirmed by roughly mimicking the damage done by the different developmental stage of larvae. The way the plant had been damaged did not affect the emission of induced volatiles. When larvae had been feeding directly on maize plants, differences in the relative amount of the six dominant compounds were mainly due to differences in the amount of damage inflicted on the plants. The parasitoid *M. rufiventris* did not show any differences in its responses to the odours induced by the different larval instars. There may be some subtle differences in the odour profiles that we could not detect because of the limitation of detection in the analytical methods used, but the responses of the wasps do not provide any evidence for such differences.

In contrast to the results found here for *S. littoralis*, Takabayashi *et al.* (1995) found that the different developmental stages of *Pseudatelia separata* larvae affected the emission of induced volatiles in maize differently and that the parasitic wasp *C. kariya* uses these differences to locate a suitable host. Late instar larvae of *P. separata* do not induce the release of volatiles from maize plants, while early instars do. Takabayashi *et al*. (1995) suggest that the plant releases different blends of induced odour to provide information on herbivore stage and suitability for parasitoids. This idea was not supported in another system studied by Mattiacci and Dicke (1995). The parasitoid *C. glomerata* does

not discriminate among cabbage plants infested by different larval instars of *Pieris brassicae* even though it can attack only young instar larvae. In our study similar results were found, *S. littoralis* larvae induced a similar blend of volatiles independently of the instar that was feeding on the plants. The generalist parasitoid, *M rufiventris* did not distinguish among plants infested by the different instars tested; it is apparently not able to specifically locate plants with suitable early stages of *S. littoralis* larvae. It is known that the foraging behaviour of female wasps is more effective when they have previously encountered a suitable host and are able to learn the surrounding odour at the time that they lay an egg (Vet, 1983; Lewis and Tumlinson, 1988; Turlings *et al.*, 1993b). McCall *et al.* (1993) showed that *M. croceipes* increases its responsiveness after encountering its host, but the increased effectiveness in the foraging behaviour only occurs after repeated experiences that the wasps had. For *M. rufiventris*, even when female wasps had a previous experience with the odour of maize plants fed on by the different developmental stages, they did not distinguish among the odour of plants that had carried the different instars. Female wasps were even more attracted to odour of plants fed on by 3rd instar when they previously experienced the odour from 2nd instar attacked plants. The small differences we observed in the choice of the female parasitoids may be explained by the differences in the quantity of volatiles emitted by the plants. The behaviour of *M. rufiventris* confirmed the results obtained by analytical methods that induced maize odour did not indicate which larval instar had been feeding on the plant.

Specificity of induced signalling in plants has been demonstrated in several tritrophic systems. The aphid parasitoid *Aphidius ervi* can distinguish between plants fed on by its host *Acyrthosiphon pisum* from plants fed on by a non-host aphid. This distinction is suggested to be due to a specific compound, 6-methyl-5-hepten-2-one, the emission of which dramatically increases when the parasitoids' host is feeding on the plant (Du *et al.*, 1998). De Moraes *et al.* (1998) found that the specialist parasitoid, *Cardiochiles*

nigriceps, can use plants volatiles to differentiate among plants infested by its host from plants infested by a closely related non-host species. In this case, the induced odour from plants attacked by the two different host noctuids was somewhat different in the ratios of the compounds within the odour blend. Thus, in some systems, induced odours from plants seem to give parasitoids information on the suitability of the herbivore feeding on the plant.

The volatile signal induced by *S. littoralis*, a generalist herbivore, in maize plants does not seem to provide reliable information on the stage of this herbivore. Other cues than the induced plants may be useful for parasitoids to determine host identity and age. Contact chemicals in the by-products, such as faeces and silk could be such cues. *C. marginiventris* is also attracted by host herbivore frass and moth scales (Locke and Ashley, 1984). Such kairomones, that come directly from the host are more reliable than are plants odours (Vet and Dicke, 1992), but far less detectable or only detectable upon contact. But in the context of high variability of induced plants odour as is described in Chapter 4, it seems not only effective for the parasitoids to maintain some flexibility in their foraging responses to volatiles cues, but also to rely on different cues to find and identify suitable hosts.

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CHAPTER 3.

INDUCED ODOUR EMISSION IN MAIZE DIFFERS FOR PLANTS AND LEAVES OF DIFFERENT AGES.

Abstract— Parasitoids are attracted to maize plants under attack by caterpillars. This attraction is mainly due to a blend of volatiles emitted by plants fed on by caterpillars and this emission of odour is triggered by caterpillar oral secretions. The reaction in maize plants is systemic; even leaves that are not damaged by herbivory emit such induced odours. The attraction of certain parasitoids can benefit a plant by reducing herbivory. In such cases, it would be beneficial for the plants to employ this indirect defence when they have most to lose. In this study we first tested the effect of plant age. Maize plants of 1, 2, 4, 6 and 8 weeks were induced to release volatiles by scratching two leaves and applying regurgitant. The volatiles were collected and analysed, and the quantity and quality of induced odour blend were compared. We also tested if the reaction is the same for all leaves of 2-week old maize by incubating plants in a solution of regurgitant, or by damaging and applying regurgitant on the wounded site of the oldest leaf (1^{st}) or of a young leaf (3^{rd}). Dramatic differences in the amount of induced odour were found among the different ages of plants, with 2-week old plants releasing far higher amounts of volatiles than older plants. The proportions of the dominant compounds also changed with the age of the plants. Systemically induced odour from undamaged leaves did not differ for the different leaves in quantity and composition. But of the damaged leaves, young leaves released higher amount of volatiles than the older leaves. The quality of the odour also depended on the age of the leaves. We discuss these results in terms of the benefits plants may receive from attracting parasitoids.

Key words — Age of leaves, age of plants, induced volatiles, tritrophic interactions, maize, *Spodoptera littoralis*.

INTRODUCTION

Plants have developed a battery of defence against insect herbivores. Among the various strategies available to the plants, chemical changes that occur in a plant in response to herbivore feeding has received most interest recently. Several plants species under insects attack release induced odour, which is highly attractive to parasitoids and predators of the attackers (Dicke *et al.*, 1990b; Turlings *et al.*, 1990; Takabayashi *et al.*, 1991; Geervliet *et al.*, 1994; McCall *et al.*, 1994; Bertschy *et al.*, 1997; Röse *et al.*, 1997; Guerrieri *et al.*, 1999). The volatiles emitted by plants are diverse and vary with plant species. In maize, the induced odour is mainly composed of green leaf volatiles, terpenoids and indole (Turlings *et al.*, 1991). The release of the odour occurs a few hours after the attack by caterpillars, and is actually triggered by factors in caterpillar oral secretions (Turlings *et al.*, 1993; Turlings *et al.*, 1998). One factor has been isolated and identified as the *N*-(17-hydroxylinolenoyl)-L-glutamine (volicitin); it triggers the release of the same odour blend in maize as observed after caterpillar feeding (Alborn *et al.*, 1997; Turlings *et al.*, 2000). In many tritrophic systems, the response of plants to herbivory is systemic: not only the part of the plant that is attacked emits the induced odour, but the entire plant (Dicke *et al.*, 1990a; Takabayashi *et al.*, 1991; Turlings and Tumlinson, 1992; Röse *et al.*, 1996). The mechanisms underlying the systemic response are not well known, but it was suggested that the elicitors are transported from the damage site to the other parts of the plant (Dicke and Dijkman, 1992; Turlings and Tumlinson, 1992; Röse *et al.*, 1996). Some differences can occur in the induced odour emitted by undamaged leaves compared to induced odour released by leaves with actual feeding damage (Röse *et al.*, 1996). In the case of cotton, Röse *et al.* (1996) found that the systemic induced odour blend was missing

isomeric hexenyl butyrate, 2-methylbutyrates and indole compared to the induced odour blend from leaves with direct damage. In addition, the systemic induced odour may be different for leaves of different ages. Takabayashi *et al.* (1994) showed that old leaves of cucumber plants infested with spider mites were less attractive to predatory mites than young infested leaves. This difference in attractiveness must have been due to variations in the induced odour emission by the two leaf ages. One observed difference was that older leaves produced more of two oximes (Takabayashi *et al.*, 1994).

In the current study, we wanted to test if in maize, differences occur in induced emissions from plants and leaves of different ages. Studies on direct defence compounds show such differences. The concentration of the direct defence compound, DIMBOA (2,4-dihydroxy-7-methoxy-1,4-benzoxazin-3-one) varies with the age and plant organ in maize (Cambier *et al.*, 2000). In maize aerial parts, the concentration of DIMBOA-Glc increases with the age until 10 days after germination and then decreases. This was also the case for the age of the leaves. Older leaves showed a decreased concentration of DIMBOA (Cambier *et al.*, 2000). No information is available for maize on the effect of plant age on the emission of induced volatiles. If the observed emissions serve as an indirect defence to attract natural enemies, it can also be expected to be highest in young plants. Young plants are more vulnerable and may never recover from an attack by caterpillars, while the damage inflicted on older plants may have little impact on the plants' fitness. Recently it was found that parasitisation of caterpillars on young maize plants can increase seed production (M-E Fritzsche-Hoballah, unpub. data), confirming our notion that young plants should invest in attracting parasitoids. We tested the effect of plant and leaf age on the emission of induced volatiles in maize under controlled conditions.

MATERIAL AND METHODS

Plants. Maize plants (*Zea mays mays*) of the variety Delprim were grown on regular fertilised soil (Coop, Switzerland). One and two weeks old plants were kept in small plastic pots (360 ml, 10 cm diameter, 7 cm high), while plants of 4, 6 and 8 weeks old were put in larger pots (3 l, 18.5 cm diameter, 14 cm high) after 4 weeks. All plants were grown in a climate chamber (Type 10'US/+5 DU-PI, Weiss Umwelttechnik GmbH, Switzerland) at 23°C±3°C, 60%±10% RH, 16L :8D photoperiod.

Collection of caterpillar regurgitant. The caterpillar oral secretions were collected from 3rd to 4th instar *S. littoralis* larvae (Turlings *et al.*, 1993). Briefly, this consisted of gently squeezing caterpillars with light-weight forceps in the head region. This caused the caterpillars to regurgitate the content of their foregut. *S. littoralis* caterpillars were obtained from Novartis Insect control, Basle (Switzerland), and were kept on maize.

Age of plants. In the experiment testing different ages of maize plants, we induced the release of volatiles by mechanically damaging two leaves on 2cm^2 with a razor blade, and applying 10 µl of *S. littoralis* regurgitant on each wounded site. According to the age of the plant different leaves were treated. One-week old plants were treated on the 1st and 2nd leaves, 2-week old plants were treated on the 2nd and 3rd leaves, 6-week old plants were treated on the 5th and 6th leaves, and 8-week old plants were treated on the 8th and 9th leaves. The plants were induced in the evening and kept in the dark (13 hrs) at laboratory conditions (24°C ± 2°C; 45 % R.H.) until collection. The experiment was repeated seven times.

Age of leaves. Two different methods were used to induce the release of odours by maize plants. The first method consisted of incubating the cut stem of a maize seedling in a solution of *S. littoralis* regurgitant (10% dilution into distilled water) (Turlings *et al.*, 1993). Control plants were incubated in distilled water only. The second method used was already described in the experiment on the age of plants. But in this experiment, we treated either the 1st (old) or the 3rd (young) leaf. For both induction methods, plants were kept in the dark until collection (14-15h) at laboratory conditions (23 ± 2°C, 40 % R:H). Before odour collection, leaves of each plant were carefully cut and separated into four stages, increasing from the base to the top of the plant. Four leaves of the same age were grouped and their cut extremities were wrapped together in moist cotton to avoid desiccation. All experiments were replicated seven times.

Collection of the induced odours. Two different systems of collection were used because of experimental requirements. To collect induced odour emitted by the different leaves of maize plants, we used an all glass push-pull odour collection system modified from Turlings *et al.* (1991). This collection system has been already described in details in Chapter 2. Super Q collection traps were made according to Heath and Manukian (1994). Before each collection filters were rinsed with 500 µl of pentane, followed by 500 µl of methylene chloride.

In the experiment testing different ages of maize plants, collections took place in a climate chamber (CMP4030, CONVIRON, Winnipeg, Canada) at 17.5 °C, 70% RH, and under 20000 lm/m^2. The aerial part of each plant was placed in a clean cooking bag (®Nalophan, Wursthullen, Germany). Bags were tightly closed at the bottom around the stem of the plant and at the top. To collect the odours emitted by the plants, we used an automated collection system (5-channel Adjustable Vacuum Flow Volatile Collection System, Model VCS-5ASP-MAN, Analytical Research Systems, Gainesville, Florida,

USA). Purified and humidified air was pushed into each bag at a rate of 1 l/min, via a flexible Teflon tube. For the attachment of the tube at the upper part of the bag, a glass tube (3cm x 1 cm) was connected to the bag. The glass tube was placed inside the bag and had a screw fitting that was capped with an open screw cap via the outside of the bag. The screw cap contained a Teflon O-ring rubber. The surface of the plastic bag was squeezed between the glass tube and the O-ring. The Teflon tube was attached by piercing the bag through the O-ring and tightening the screw cap. The head space atmosphere in the bag was pulled at 0.8 l/min, the rest of the air vented out through the probable leaks at the closing parts of the bag, thus preventing outside, impure air from entering. The vacuumed air passed through a Super Q filter connected at the bottom site of the bag with the same technique as described for the attachment of the Teflon tube. The odour collection lasted for 2 hours, from 9:00 a.m. to 11:00 a.m. Before each collection filters were rinsed with 500 μl of pentane, followed by 500 μl of methylene chloride.

The odour blend was extracted from the filter with 150μl of methylene chloride (Lichrosolv., Merck, Switzerland), 200 ng of n-octane and nonyl acetate (Sigma, Switzerland) in 10 μl methylene chloride were added to the sample as internal standards.

The samples were analysed by gas chromatography. Of each sample, 3 μl aliquot were analysed on an apolar capillary column (EC-1, 30m, 0.25 mm. i.d., 0.25 μm film thickness, Alltech Associates, Inc, USA) combined with a deactivated retention gap (10 m, 0.25 mm i.d., Connex, USA) and a deactivated pre-column (30 cm, 0.530 mm i.d., Connex, USA). The Hewlett Packard HP 6890 series gas chromatograph was equipped with an automated on-column injection system (HP G1513 A) and a flame ionisation detector. Helium (24 cm/s.) was used as carrier gas. Following injection, the column temperature was maintained at 50°C for 3 min. and then increased to 230°C at 8°C/ min and held at 230°C for 9.5 min. The detector signal was processed with HP GC Chemstation software.

For each replicate size and weights (wet and dry weight) were recorded to be able to correct for any biomass effect.

Statistical analyses. Total amounts of induced odour released by the different ages of plants and by the different leaves were compared using univariate analysis of variance. Comparison of the relative amounts of the dominant compounds was performed using a multivariate anova. Student-Newman-Keuls was performed as post hoc test for multiple comparisons.

RESULTS

Age of the plants. The total amount of induced volatiles emitted by maize plants varied with the age of plants (Fig. 3-1 A and B). Two and four week old plants produced larger quantities of volatiles than older plants. Very young plants of one week old released very little. Figure 3-1 B represents the amount of odour emitted per grams of dried biomass of the plant. With this correction the differences are even more dramatic, with the 2 week old plants emitted much larger quantities of volatile compounds than older plants.

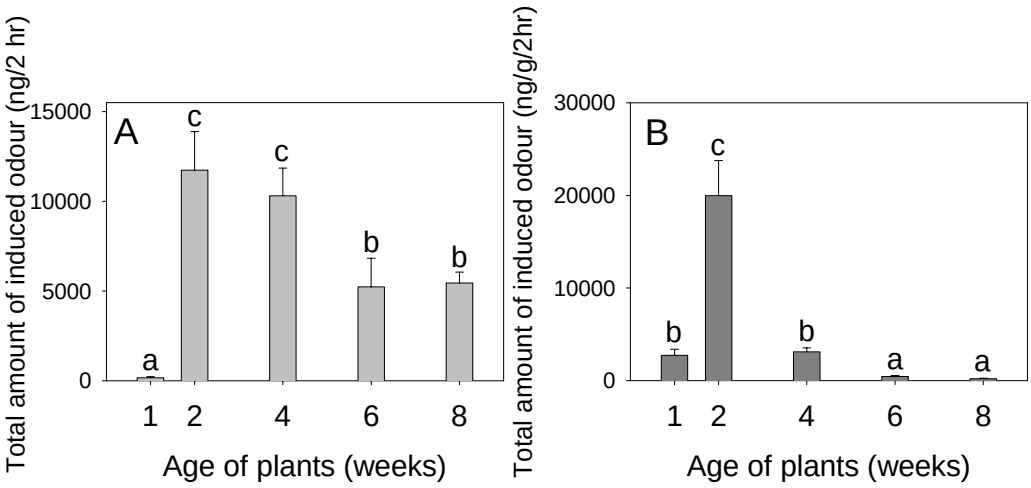

Fig. 3-1. Total amount (mean + SE) of (**A**) raw production of induced volatiles by different ages of maize NOT corrected for biomass and (**B**) production of induced volatiles by different ages of maize after correction for biomass. Letters above bars indicate significant differences among the different ages of plants after Student-Newman-Keuls post hoc test (α=0.05)

The average relative quantities of the twelve dominant compounds for the 6 different ages are plotted in Figure 3-2. The age of maize affected significantly the induced odour blend ($F=2.955$; $p<0.001$). Only, the quantities of the (*E*)-β-farnesene and the combination β-bisabolene + (*E*,*E*)-α-farnesene were not affected by the age of the plants (Fig. 3-2). In most cases young plants released relatively lower amounts of volatiles than older ones, but surprisingly they released more α-bergamotene, and there is a trend for them to release more (*E*)-β-farnesene compared to older plants (Fig. 3-2). In contrast, 8-week old plants released relatively more β-myrcene, (*Z*)-3-hexenyl acetate, and (3*E*)-4,8-dimethyl-1,3,7-nonatriene, but the old plants released relatively less of the sesquiterpenes (Fig. 3-2).

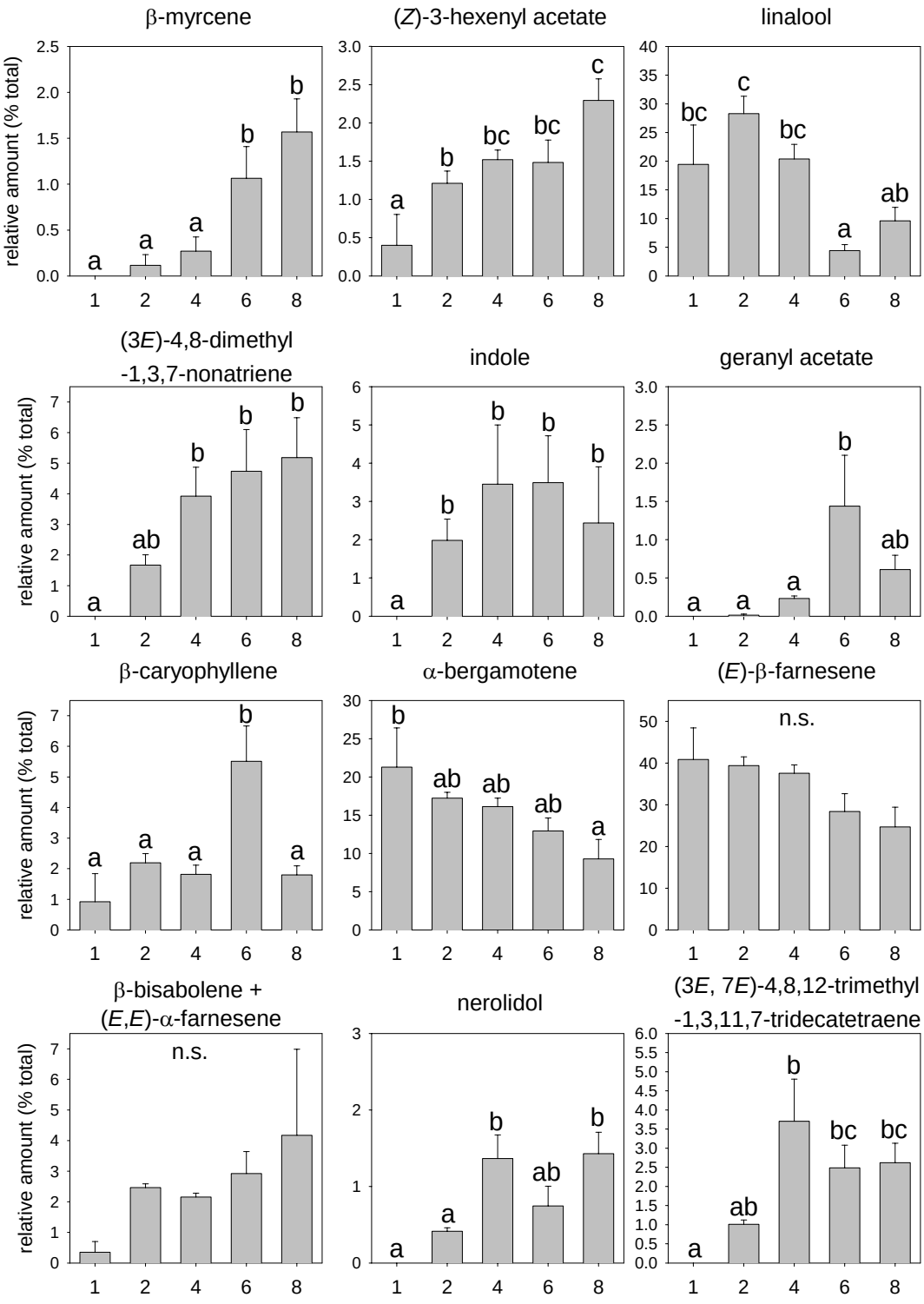

Fig. 3-2. Relative amount (mean of % of total + SE) of the twelve dominant compounds in the induced odour blend of maize plants of different ages. Letters above bars indicate significant differences among the different ages tested, after Student-Newman-Keuls post hoc test (α=0.05)

To summarise, plant age affected the composition of the induced of blend emitted in maize. The relative amount of the green leaf volatiles, the homoterpenes, and the monoterpenes were the most different among the six ages (Fig. 3-3). Plants of 2 and 4 weeks old released less of the green leaf volatiles, while older plants released more of these compounds (Fig. 3-3). A similar trend appeared for the quantities of the homoterpenes. On the other hand, the relative proportions of the monoterpenes were higher in the odour blend of young plants. The sesquiterpenes were released in similar proportions by all ages, with a slight tendency for older plants to release less (Fig. 3-3).

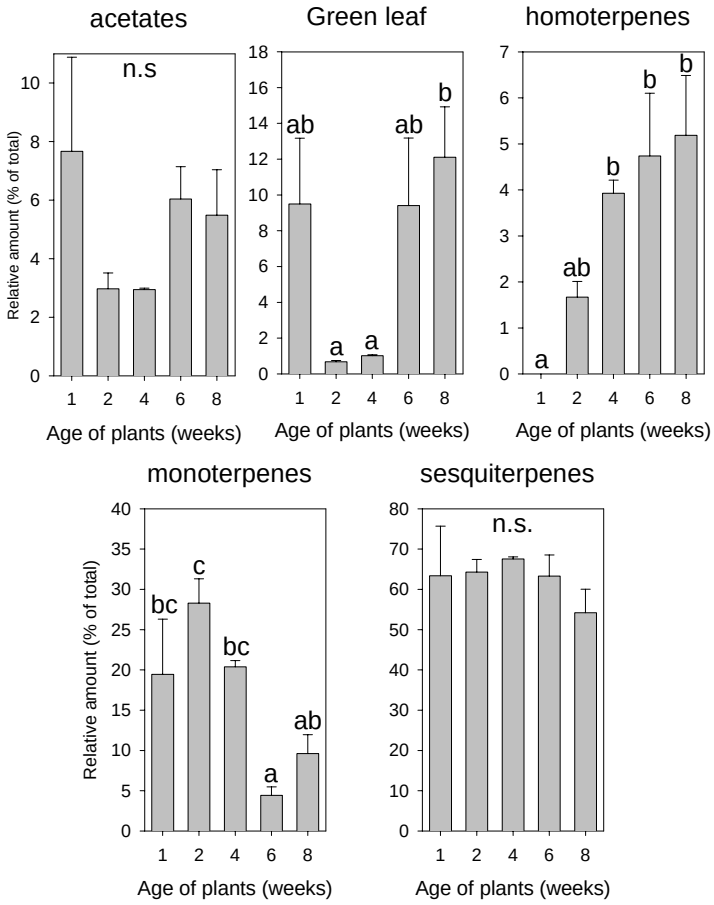

Fig. 3-3. Relative amount (% of total) of the main type of compounds, which are present in the induced blend of different ages of maize plant. Letters above bars indicate significant differences among the relative amount emitted by the different ages of plants after Student-Newman-Keuls post-hoc test (α=0.05)

Leaf stages. All leaves released induced odour after they had been incubated in a solution with caterpillar regurgitant (Fig. 3-4). The amount of induced volatiles increased with the position of the leaf on the plant excepted for the 4th leaf, which released relatively small amounts of volatiles (Fig. 3-4 A). But those results were mainly due to the fact that 3rd leaf was bigger than the other leaves on a plant. After correction for biomass, no significant differences were found anymore among the leaves (Fig. 3-4 B). The quality (relative proportions of the different compounds) of the odour emitted by the different leaves was not different among the different ages of the leaves tested (F=1.035; p=0.46).

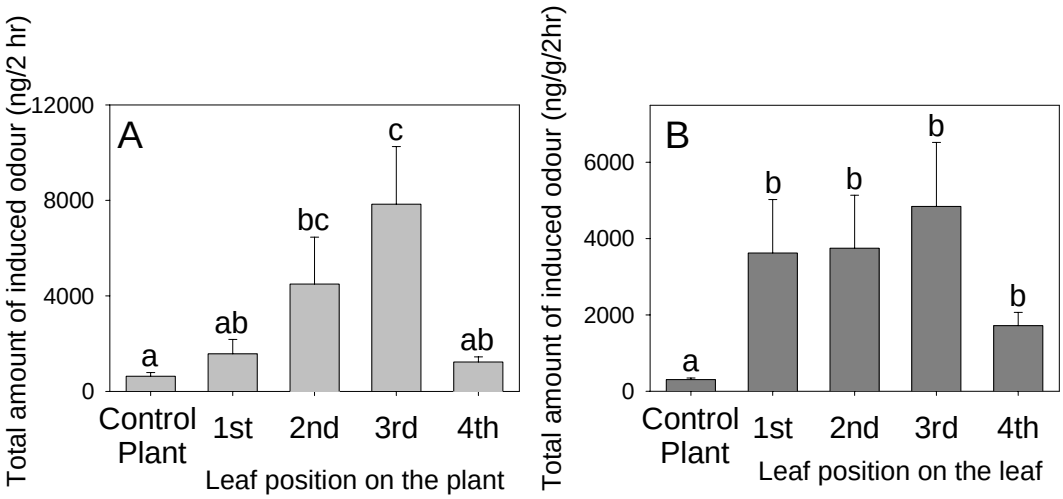

Fig. 3-4. Total amount (mean + SE) of induced volatiles emitted by control plants (plant incubated in water only) and by the different leaves of maize plants. (**A**): Total amount collected without correction for biomass; (**B**): Total amount emitted after correction for biomass of leaves. Letters above bars indicate significant differences among the different leaves and control plant after Student-Newman-Keuls post hoc test (α=0.05)

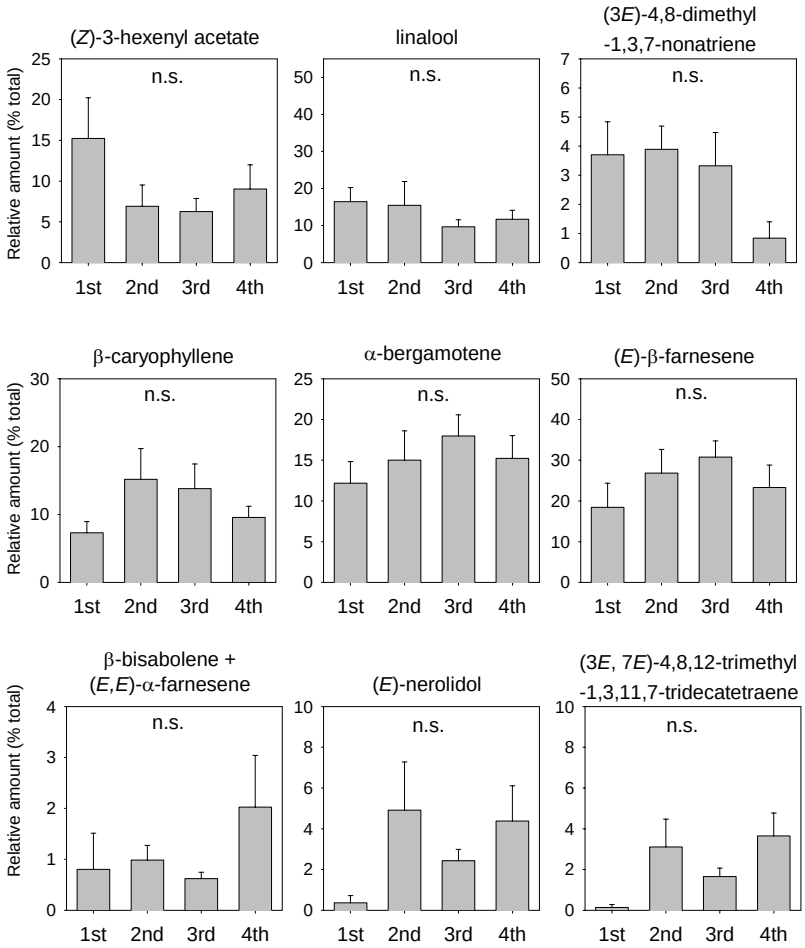

Fig. 3-5. Relative amount (mean of % of total amount + SE) of the nine main induced compound in the odour blend of the different leaves of maize plants. No significant differences (n.s.) were found among the different leaves for the proportion of each compound.

Although the differences were not significant, there is a trend that the 1st leaf emits less (*E*)-nerolidol and (3*E*, 7*E*)-4,8,12-trimethyl-1,3,11,7-tridecatetraene than the other leaves (Fig. 3-5).

When individual leaves (1st or 3rd) were mechanically damaged and regurgitant was applied to the damaged site, the total amount of volatiles released differed for the different leaves depending on which leaf had been damaged. When the older leaf (1st leaf) was treated, no significant differences in the total amount of volatiles released were found among the 4 leaf stages (Fig. 3-6 A). But when the 3rd leaf was treated, the amounts were

significantly different, with the largest amount being released by the 3rd leaf, which was of course the one that had received the damage and the regurgitant (Fig. 3-6 B). The smallest amount was released by the first leaf, which is the older one and the more distant from the treated leaf. (Fig. 3-6 B).

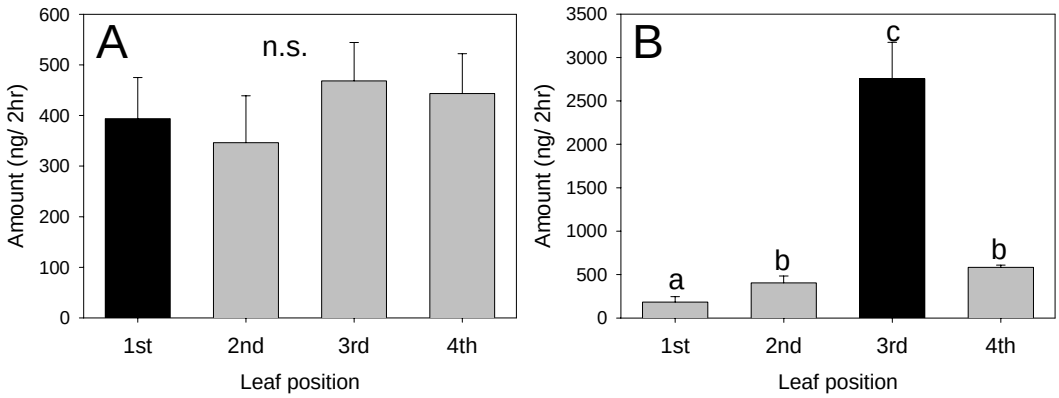

Fig. 3-6. Total amount (mean + SE) of induced volatiles, which was released when (**A**) the 1st leaf was induced (mechanical damage + regurgitant) and when (**B**) the 3rd leaf was induced. Letters above bars indicate significant differences after Student-Newmann-Keuls post hoc test among the different ages of leaves (α=0.05).

It is interesting to note that the overall quantity of induced volatiles produced when the 1st leaf was treated was considerably lower than the quantity emitted when the 3rd leaf was treated (Fig. 3-6 A and B). A different picture emerged when we corrected the emission of volatiles for biomass of each leaf (Fig. 3-7 A and B). In this case, when the first leaf was damaged, it released higher quantities of induced volatiles than the other leaves ($F= 16.375$; $p<0.001$) (Fig. 3-7 A). The differences in quantity of induced odour released by third leaves were less dramatic for the data that were corrected for biomass. Still for the third leaf tended to emit more volatiles than other leaves, but this was not statistically significant ($F= 0.532$; $p=0.665$) (Fig. 3-7 B).

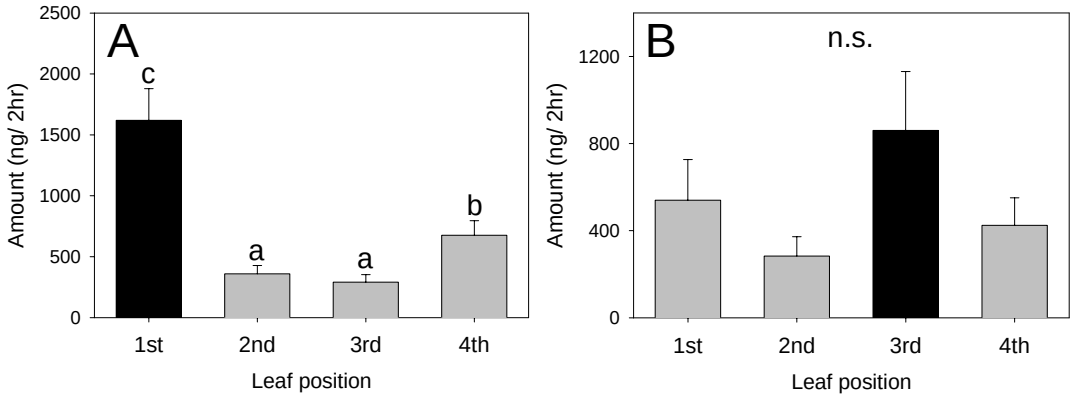

Fig. 3-7. Total amount (mean + SE) of induced volatiles corrected for biomass of the different leaves, which was released when (**A**) the 1st leaf was induced (mechanical damage + regurgitant) and when (**B**) the 3rd leaf was induced. Letters above bars indicate significant differences after Student-Newmann-Keuls post hoc test among the different ages of leaves (α=0.05)

The relative amount of the twelve dominant compounds in the induced odour blend was significantly different among the different leaves when the 1st leaf was mechanically damaged and the regurgitant was applied (F=2.101; p=0.017) (Fig. 3-8). Only three compounds were emitted in significantly different ratios among the leaves (Fig. 3-8). Linalool was released in lower relative quantity in the odour blend of older leaves (Fig. 3-8). Second leaves released significantly more (3*E*)-4,8-dimethyl-1,3,7-nonatriene compared to the other leaves (Fig. 3-8). On the contrary, the combination of β-bisabolene + (*E*,*E*)-α-farnesene was released in significantly higher amounts by the first leaves. For all the other nine compounds, no significant differences in the relative amount were detected.

Mechanical damage and application of regurgitant on the 3rd leaf did also significantly affect the ratio of compounds emitted by the different leaves (F= 3.346; p=0.027) (Fig. 3-8). Six of the twelve dominant compounds were released in significantly different proportions among leaves (Fig. 3-9). β-Myrcene was emitted in lower quantity by

the 3rd leaves, and in higher quantity by 1st leaves (Fig. 3-9). (3*E*)-4,8-Dimethyl-1,3,7-nonatriene was released in higher amounts by 2nd leaves compared to other leaves (Fig. 3-9). The three sesquiterpenes, β-caryophyllene, α-bergamotene, and (*E*)-β-farnesene were released more by 3rd leaves (the treated leaves). Results for (*E*)-nerolidol and (3*E*, 7*E*)-4,8,12-trimethyl-1,3,11,7-tridecatetraene were very variable, but 3rd leaves emitted less (*E*)-nerolidol, while the 4th leaves did not emit any detectable (3*E*,7*E*)-4,8,12-trimethyl-1,3,11,7-tridecatetraene (Fig. 3-9).

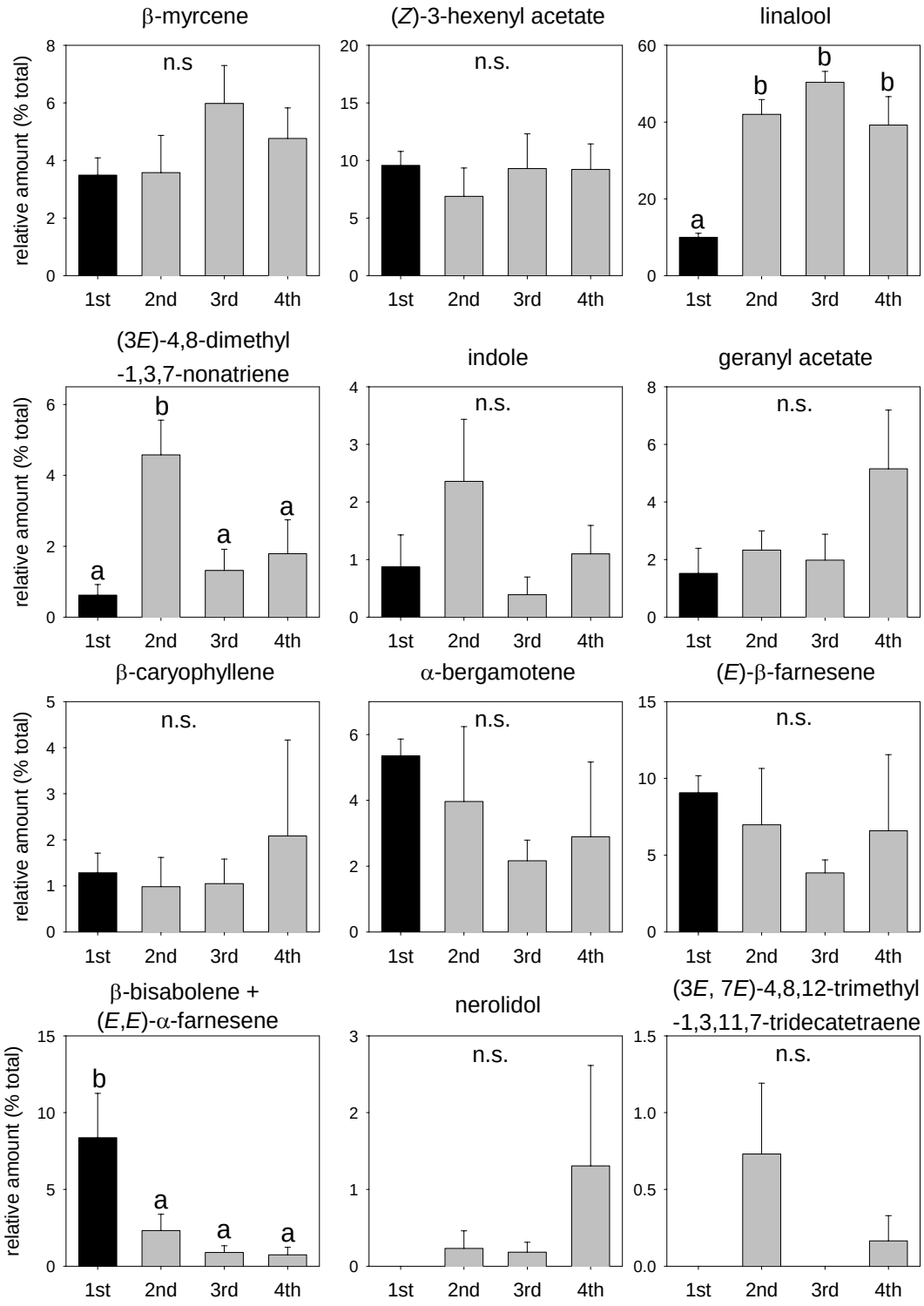

Fig. 3-8. Relative amount (% of total) of the twelve dominant compounds in the induced odour blend release by the different ages of leaves when the 1st leaf was induced. Letters above bars indicate significant differences among the different leaves after Student-Newman-Keuls post hoc test (α=0.05)

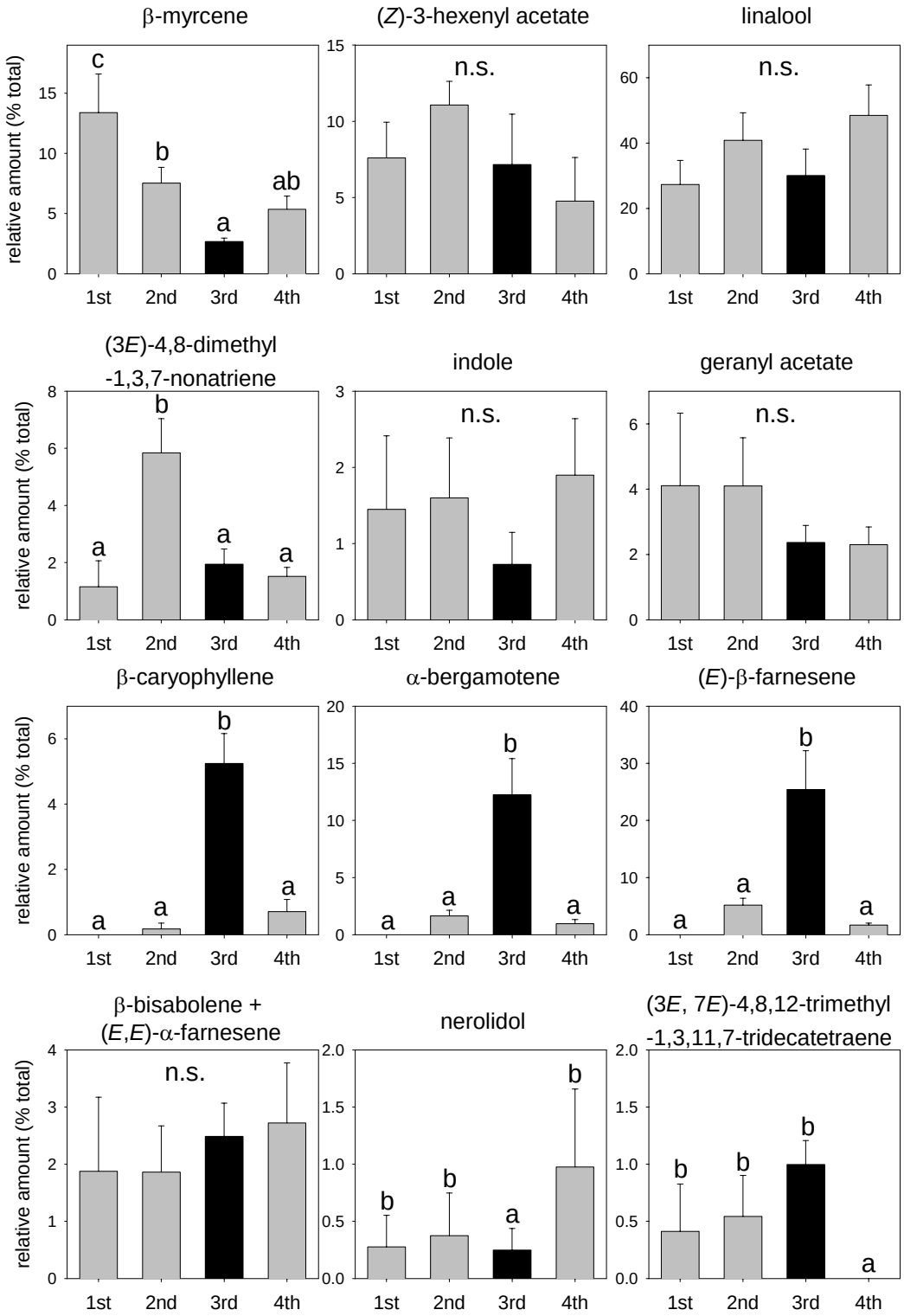

Fig. 3-9. Relative amount (% of total) of the twelve dominant compounds in the induced odour blend release by the different ages of leaves when 3rd leaves was induced. Letters above bars indicate significant differences among the different leaves after Student-Newman-Keuls post hoc test (α=0.05)

DISCUSSION

The emission of induced odour by maize plants was dramatically affected by the age of the plants. Already before correction for weight, plants of 2 weeks ages emitted larger quantities of volatiles than older plants. As the emission of volatiles to attract natural enemies of herbivore may benefit plant fitness (Agrawal and Karban, 1999, Maria Elena Fritzsche-Hoballah, unpub. data), it can be expected that plants defend themselves strongest when they have most to lose. For instance, young maize plants attacked by caterpillars parasitised by *C. marginiventris* had a higher seed production than plants attacked by unparasitised larvae (Maria Elena Fritzsche-Hoballah, unpub. data). Six week and 8 week old plants have almost reached their mature stage and an attack by herbivores will not dramatically change their fitness, while a plant of only 2 weeks old can be completely destroyed by caterpillars. The effect of the age of plants has also been found for the production of chemicals that function in the direct defence of the plant. Induced proteinase inhibitor activity in tomato plants decreases when the plants get older (Wolfson and Murdock, 1990). In contrast, Cipollini and Redman (1999) showed that the activity of peroxidase increases with the age of tomato plants. In maize, DIMBOA (2,4-dihydroxy-7-methoxy-1,4-benzoxazin-3-one) content varies considerably with plant age (Cambier *et al.*, 2000). The amount of DIMBOA increased during the first 10 days after germination, and then decreased. The increase in quantity of DIMBOA is not proportional to the growth rate of maize, since the concentration of this compound decreases already after 2 days following germination (Cambier *et al.*, 2000). In contrast to what is found with DIMBOA, the investment of very young plants in volatile production is very low; additional experiments using a different collection method (data not shown) with 1 week old plants confirmed these results. We also found differences in the quality of the odour blend when comparing the different ages of maize plants. Young plants released less of the green leaf volatiles and homoterpenes, two types of volatiles that are widely emitted by various plant

species. However, they seemed to release relatively more of the sesquiterpenes, which can be more specific for each plant species. These differences may also be due to differences in the timing in which volatiles are released. As young plants are also smaller, the diffusion of the elicitors in the plant may be more rapid in the plant and thus leads to some changes in the ratio in which compounds are released, as it was already shown by Turlings *et al.* (1998). It is hard to speculate on the effect that such differences can have on the behaviour of parasitoids. Further experiments should be done to determine which compounds are most important for the parasitoids in providing host location cues. It may also be important to note that the absolute damage done on plants was the same for each age, it is highly probable that different results would have been observed if an equal proportion of the plant surface had been damaged, as the amount of damage has an effect on the emission of volatiles by maize plants (Chapter 2).

In the study on induced emissions by leaves of different stages, the results differed for the two types of treatment. Maize plants that were induced by incubating their stems in a regurgitant solution showed differences in the absolute amount that was released per leaf. However, after compensation for differences in leaf mass, no difference in quantity were measured, although there was a tendency for the 3rd leaves to release more than the other leaves. These results suggest that the systemic emission of volatiles takes place in all leaves of a young plant, and that no differential investment occurs in terms of the total amount volatiles released per gram of dry weight of each leaf. The proportions of the dominant compounds in the induced odour blend were also not different among the different leaves when plants were incubated in a solution with regurgitant. Apparently, when plants are induced to release volatiles only systemically, the age of leaves on the plant does not affect the odour blend.

Comparison of the quantities of volatiles emitted by the different ages of leaves, when the 1st or 3rd leaves were artificially damaged and treated with regurgitant, showed

that treatment of the 1st leaf resulted in a weaker response by the plant than treatment of the 3rd leaf. Treatment of the 3rd leaf dramatically increased the amount of induced volatiles emitted by this leaf compared to the other leaves; this was not the case when the 1st leaf was damaged. Results were different when the amount of volatiles emitted was expressed per gram of dry weight. These data showed that the 1st (older) leaf produced more in response to treatment than the other leaves, while no difference was found among leaves when the 3rd one was damaged. There was still a trend for higher production of volatiles by the attacked leaves. These quantitative differences in emission of volatiles with the age of leaves are correlated with differences in biomass. Some differences were observed in the quality of the odour blend when comparing between plants with the 1st or 3rd leaf treated. Only significant differences were found in the proportion of linalool and (3*E*)-4,8-dimethyl-1,3,7-nonatriene when the 1st leaf was damaged; the proportion of all the other compounds was not different among the leaves. Linalool was released in higher proportion by the other three leaves than by the 1st leaf. The second leaf emitted relatively high amounts of (3*E*)-4,8-dimethyl-1,3,7-nonatriene compared to the other leaves. When the 3rd leaf was damaged, significant differences in the proportions of β-myrcene, (3*E*)-4,8-dimethyl-1,3,7-nonatriene, β-caryophyllene, α-bergamotene, (*E*)-β-farnesene, nerolidol and (3*E*,7*E*)-4,8,12-trimethyl-1,3,11,7-tridecatetraene were found among the different leaves. The attacked leaves released higher proportions of the three sesquiterpenes, β-caryophyllene, α-bergamotene, (*E*)-β-farnesene, but relatively little of the other compounds. In cotton, differences in the odour profile of damaged leaves and undamaged leaves of treated plants have already been reported (Röse *et al.*, 1996). It was found that some compounds were missing in the odour blend that was systemically released by undamaged cotton leaves compared to the blend emitted by attacked leaves (Loughrin *et al.*, 1994; Röse *et al.*, 1996). Turlings and Tumlinson (1992) found that undamaged leaves of maize plants, which were induced to release volatiles after damage of the 1st and 3rd

leaves, released systemically induced volatiles. But the undamaged leaves did not emit (*E*)-α-bergamotene nor (*E*)-β-farnesene, while the damaged leaves did. However, a systemic release of these two sesquiterpenes can be observed later after treatment (Turlings *et al.*, 1995). In our case, no compounds were missing in the odour blend of systemic induced leaves compared to damaged leaves, but the proportions had changed. Similarly to what was found by Turlings and Tumlinson (1992), we found that when the 3rd leaf was damaged, the emission of the sesquiterpenes was higher from damaged leaves compared to undamaged leaves. This was not the case when the 1st leaves were damaged. It seems that when younger leaves were attacked the reaction triggered was stronger than when old leaves were attacked, this difference was reflected also in the quality of the odour blend. Similar results were obtained with cucumber plants infested by spider mites (*Tetranychus urticae*). Infested younger leaves were more attractive to predatory mites (*Phytoseiulus persimilis*) than old leaves (Takabayashi *et al.*, 1994). It was suggested that differential attractiveness of predators may be adaptive, as growing parts (younger) of plants contribute more to plant fitness than old parts. In the case of maize plants, it seems that when old leaves are attacked, the emission of volatiles is also lower than when younger leaves are damaged. But caterpillars that are feeding on old leaves will eventually also attack younger parts of the plant, so the emission of induced volatiles to attract natural enemies seems adaptive to any young plant under caterpillar attack, independent of where they start to feed.

It seems that the emission of odour by maize plants is dependent on the leaf stage as well as on the age of the plants. In small plants, younger leaves emit more induced odour when attacked than older ones. Similar results are observed for the entire plant; young plants released more volatiles than older plants. These results support the hypothesis that induced plant volatiles constitute an indirect defence that function to attract natural

enemies. It seems to be adaptive that higher emissions occur in plant stages and plant parts that are most vulnerable.

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CHAPTER 4.

VARIABILITY IN HERBIVORE-INDUCED ODOUR EMISSIONS AMONG MAIZE CULTIVARS AND THEIR WILD ANCESTORS (TEOSINTE)

Abstract— Maize plants respond to caterpillar feeding with the release of relatively large amounts of specific volatiles, which are known to serve as cues for parasitoids to locate their host. Little is known about the genetic variability in such herbivore-induced plant signals and about how the emissions in cultivated plants compare to those of their wild relatives. For this reason we compared the total quantity and the qualitative composition of the odour blend among eleven maize cultivars and five wild *Zea* (Poaceae) species (teosinte), as well as among the offspring of eight *Zea mays mexicana* plants from a single population. Young plants were induced to release volatiles by mechanically damaging the leaves and applying oral secretions of *Spodoptera littoralis* (Lepidoptera: Noctuidae) caterpillars to the wounded sites. Volatiles were collected 7 hr after treatment and subsequently analysed by gas chromatography. The total amounts of volatiles released were significantly different among maize cultivars as well as among the teosintes. Moreover, striking differences were found in the composition of the induced odour blends. Caryophyllene, for instance, was released by some, but not all varieties and teosintes, and the ratios among monoterpenes and sesquiterpenes varied considerably. The offspring of different mother plants of the *Z. m. mexicana* population showed some variation in the total amounts that they released, but the composition of the odour blend was very consistent within the population of this teosinte species. We discuss the ecological significance of these findings in terms of specificity and reliability of induced plant signals for parasitoids.

Key words. induced plant volatiles - specificity- reliability - tritrophic interactions - *Zea mays* - maize - teosinte

INTRODUCTION

Odour emissions by plants in response to herbivory have been intensively studied for the past decade. This phenomenon has been demonstrated for several systems, mostly involving cultivated plants like Lima bean (Fabaceae), cabbage (Brassicaceae), cucumber (Cucurbitaceae), apple (Rosaceae), cotton (Malvaceae) and maize (Poaceae) (Dicke *et al.*, 1990; Turlings *et al.*, 1990; Takabayashi *et al.*, 1991; Turlings *et al.*, 1993a; Agelopoulos and Keller, 1994; Mattiacci *et al.*, 1994; McCall *et al.*, 1994; Pallini *et al.*, 1997; Röse *et al.*, 1997). For a variety of natural enemies of herbivores it has been found that they make use of these induced plants odours for long-range prey or host location (Dicke and Sabelis, 1988; Turlings *et al.*, 1990; Agelopoulos and Keller, 1994; Takabayashi *et al.*, 1991; Powell *et al.*, 1998). This reliance on plant-provided cues is thought to be a consequence of the absence of detectable amounts of kairomones, cues that originate directly from the host (Tumlinson *et al.*, 1993; Vet and Dicke, 1992). Plant odours are considered to be less reliable than kairomones, because plant odours appear to provide little information on the identity of the herbivore that causes their release (Vet and Dicke, 1992). Moreover, suitable hosts can occur on different plant species that all release their own odour blend (Turlings *et al.*, 1993b). It appears that even within one species of plant the odours can vary considerably (Takabayashi *et al.*, 1991; Turlings *et al.*, 1998b).

Variability in induced plant odours complicates the reliance of parasitoids on these cues. One way of dealing with variability is through associative learning (Turlings *et al.*, 1993b; Vet *et al.*, 1995), which may allow parasitoids to learn which cues are most likely to lead them to suitable hosts at a particular time in a particular area. Moreover, recent studies suggest that plant odours alone carry specific information on the herbivores by

which they are attacked. For example, predatory mites are able to distinguish between the odours of apple trees infested by two herbivores species (Sabelis and van de Baan, 1983; Sabelis and Dicke, 1985; Takabayashi *et al.*, 1991). Guerrieri *et al.* (1999) showed that different aphid species elicit different odour emissions in bean plants and that the aphid parasitoid, *Aphidius ervi* (Hymenoptera: Braconidae: Aphidiinae), can use these differences to distinguish plants infested by its host, *Aphis pisum* (Homoptera: Aphididae), from those infested by a non-host, *Aphis fabae* (Homoptera: Aphididae). Similarly, De Moraes *et al.* (1998) found that the specialist parasitoid, *Cardiochiles nigriceps* (Hymenoptera: Braconidae), is more attracted by plants on which their specific host, *Heliothis virescens* (Lepidoptera: Noctuidae), has been feeding than by plants attacked by *Helicoverpa zea* (Lepidoptera: Noctuidae). These examples show that the induced signals emitted by plant under herbivore attack can vary depending on the insect species that feed on the plant and could therefore provide parasitoids and predators information on the suitability of the herbivore on the plants.

Differences in the intensity by which the odours are emitted are unlikely to provide specific information. Distinguishable differences would require that some volatiles are released in different proportions relative to each other, which appear to be what *C. nigriceps* is able to detect (De Moraes *et al.* 1998), or that the odour blends contain different volatiles substances, which may be what the aphid parasitoid uses (Du *et al.*, 1998).

Considering that different plant species release entirely different odour blends and that even within one plant species there are clear differences among genotypes (Takabayashi *et al.*, 1991; Turlings *et al.*, 1998b), the possibility for parasitoids and predators to rely on specific cues seems therefore limited. Studies into the specificity of herbivore-induced plant signals should consider this intraspecific variability. The little information that is available on the extent of the variability comes from studies on

cultivated plants (Takabayashi *et al.*, 1991; Turlings *et al.*, 1998b; Krips, 2000). For a better understanding of the ecological relevance and evolutionary history of herbivore-induced plant signalling, it is necessary to study these signals in wild systems. The only such study has been done with a naturalised cotton variety which was found to release much higher quantities of induced volatiles than cultivated varieties (Loughrin *et al.*, 1995).

The current study was conducted to obtain more information on the specificity and variation in the induced responses within the genus *Zea*, including several wild species. Odours from eleven cultivated varieties of maize were collected at different times after leaves were damaged and treated with the oral secretions of *Spodoptera littoralis* (Lepidoptera: Noctuidae) larvae. The same experiment was conducted with five species and subspecies of teosinte, the wild relatives of maize. The obtained variation in induced odour blends was compared to the variation within a population of the teosinte *Zea mays mexicana*. For this, we analysed the volatiles collected from plants grown from seed collected from eight individual plants in a Mexican population.

MATERIALS AND METHODS

Plants. Seed from eleven commercially available varieties of maize (*Zea mays mays* L.) were provided by UFA Semences, Bussigny (Switzerland), except for the varieties Byzance and Pactol, which were obtained from Novartis, St Sauveur (France). In all experiments the variety Delprim was used as a reference. The ten other cultivars were tested in two separate blocks. In an additional experiment, we tested five taxa of teosinte, *Zea perennis, Z. diploperennis, Z. luxurians, Z. mays mexicana,* and *Z. m. parviglumis*. Seeds of these species were provided by the USDA-ARS, North Central Regional Plant Introduction Station, Iowa State University, USA. Teosinte seed were first placed on moist filter paper in a 5.5 cm diameter Petri dish for germination. The same procedure was used

for seed of eight individual plants of *Z. m. mexicana* race Chalco that had been collected in December 1998 from a small population near Texcoco, Mexico. To break dormancy, the seeds were placed at 4°C for a week before they were placed on moist filter paper to germinate. All seeds were eventually planted in pots (360 ml, 10 cm diameter, 7 cm high) filled with soil mixture composed of 80% of regular potting soil (Coop, Switzerland) and 20% of vermiculite (medium size, HK, Switzerland). Plants were kept in a climate chamber (Type 10'US/+5 DU-PI, Weiss Umwelttechnik GmbH, Switzerland) under the following conditions: 23°C±4°C, 60%±10% r. h., and 40000 lm/m^2 with a photoperiod of L16:D8. The plants were watered daily. After 10 days, at which age all cultivars carried three developed leaves with the fourth showing, the plants were used for experiment. The teosintes carried the same number of leaves, but the leaves were longer and thinner than those of cultivated plants.

Treatment of plants. Regurgitant used to elicit odour emission was collected from third and fourth instar *Spodoptera littoralis* larvae as described by Turlings *et al* (1993a). The larvae were provided by Novartis Insect Control, Basle (Switzerland) and fed maize leaves for at least one day before regurgitant collection.

Maize seedlings were induced to emit volatile synomones by scratching 2cm^2 of a leaf surface with a razor blade and applying 10 µl of *S. littoralis* regurgitant to the damaged site. We chose this easily standardised method rather than using actual larvae to avoid differential feeding damage, which may result in high variability in odour emissions (Gouinguené, unpubl. data). For all cultivated varieties and *Z. m. mexicana*, the second and third leaf were treated. Because of considerable differences in leaf size we decided to treat the third and fourth leaves of the other four teosintes. Treatments took placed during the dark period, 7 h before lights were turned on.

Collection and analysis of induced maize volatile. The volatile collection system has been described in detail by Turlings *et al.* (1998). It basically consists of 6 vertically placed cylinders (9.5 cm inner diameter, 54 cm high). The aerial part of a plant was placed in a cylinder, while the pot with the subterranean part remained outside, separated from the shoot by a teflon disk consisting of two halves with a hole in the centre (Turlings *et al.*, 1998b). Purified and humidified air was pushed into each cylinder at a rate of 1 l/min. Around the base of each cylinder, just above the teflon disk, 8 openings served as ports that could hold the collection traps. Three ports were used during an experiment; the others were sealed. Collection traps consisted of 6 mm diameter and 7 cm long glass tube that held 25 mg of Super Q adsorbent (80-100 mesh, Alltech, Deerfield, Illinois, USA) (Heath and Manukian, 1994). During the collections, air was pulled through a trap at a rate of 0.8 l/min, while the rest of the air vented out through the hole in the bottom, thus preventing outside, impure air from entering. The automated part of the collection system (Analytical Research System, Gainesville, Florida, USA) controlled the flow through the traps and made it possible to switch this flow from one trap to another. The climate chamber (CMP4030, CONVIRON, Winnipeg, Canada) in which the collection cylinders were housed was kept at 17.5°C, due to the irradiation heat, the temperature inside the cylinders was 23 ± 3°C. During the light cycle, light intensity was about 20000 lm/m^2.

Immediately when lights went on, i.e. 7 h after treatment, odours were collected during three consecutive 3-h periods. In most cases, the maximum volatile production occurred during the second collection period, therefore collection was only done during this period in the experiment with the *Z. m. mexicana* plants from the Texcoco population.

Traps were extracted with 150µl of methylene chloride (Lichrosolv., Merck, Switzerland) and 200 ng of n-octane and nonyl acetate (Sigma, Switzerland) in 10 µl methylene chloride were added to the samples as internal standards.

Analyses were done with a Hewlett Packard HP 6890 series gas chromatograph equipped with an automated on-column injection system (HP G1513 A) and a flame ionisation detector. Of each sample a 3 µl aliquot was injected onto an apolar EC-1 capillary column (30m, 0.25 mm. i.d., 0.25 µm film thickness, Alltech Associates, Inc, USA) preceded by a deactivated retention gap (10 m, 0.25 mm i.d., Connex, USA) and a deactivated pre-column (30 cm, 0.530 mm i.d., Connex, USA). Helium (24 cm/s) was used as carrier gas. Following injection, the column temperature was maintained at 50°C for 3 min, increased to 230°C at 8°C/ min and held at 230°C for 9.5 min. The detector signal was processed with HP GC Chemstation software. Tentative identification of compounds was based on comparison of retention times with analyses from previous studies (Turlings et a., 1998a). These identities were confirmed with spectra from the Wiley library after mass spectrometry analyses of 3 selected samples with the above column and temperature program in a Hewlett-Packard 5973 mass selective detector (transfer line 230 °C, source 230 °C, quadrupole 150 °C, ionisation potential 70 eV, scan range 50-400 amu).

Statistical analyses. The amounts of the eighteen dominating compounds in the collections were determined based on their relative peak areas and those of the internal standards. The total amounts of these compounds emitted during the three collection periods (9h) were summed. Differences between varieties and teosinte taxa were determined using one way analysis of variance. Each experimental block was analysed separately. The Student-Newman-Keuls test was performed for multiple comparisons. To comply with ANOVA assumptions, all data were ln-transformed. The same analysis was performed for each compound to check for differences between varieties and teosinte.

RESULTS

Quantitative differences. The amount of volatiles varied with time after plant treatment (Fig. 4-1). The maximum emission occurred during the second collection period, 10 to 13h after treatment, except for the varieties Byzance and Pactol, and the teosintes, *Z. m. parviglumis* and *Z. luxurians*, which released more during the third collection period, 13 to 16h after treatment (Fig. 4-1).

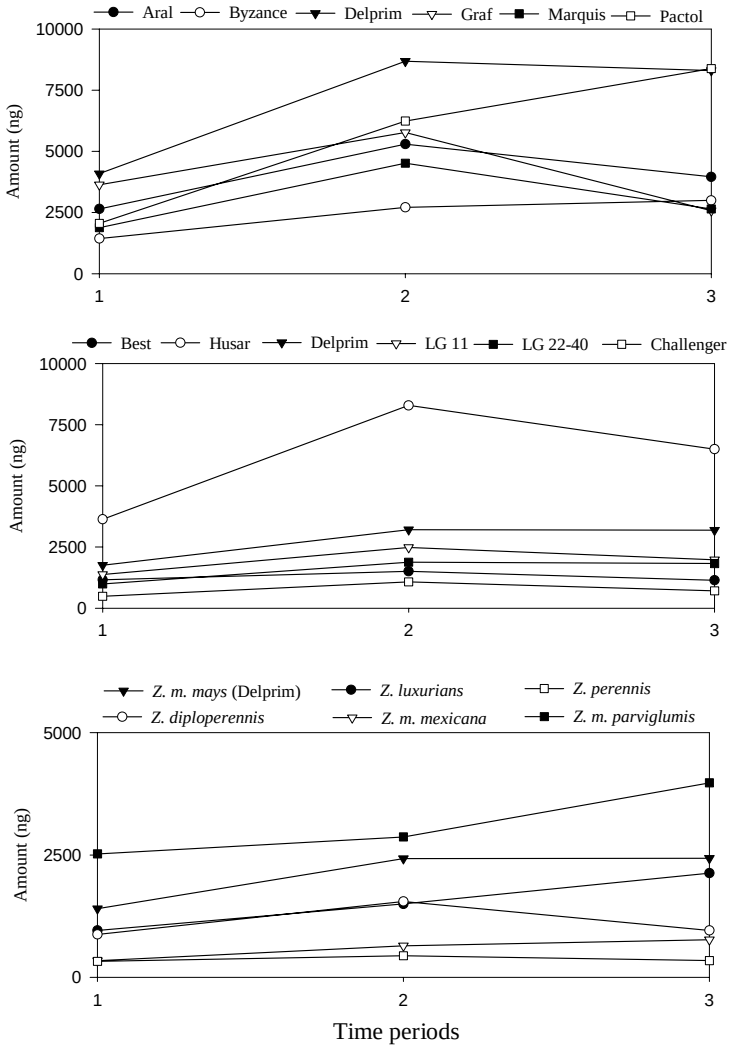

Fig. 4-1. Total amount of induced volatile compounds emitted by maize cultivars and teosinte after damaging and treating them with caterpillar regurgitant (n=6) (period 1: from 7 to 10 h after treatment; period 2: from 10 to 13 h after treatment; period 3: from 13 to 16 h after treatment).

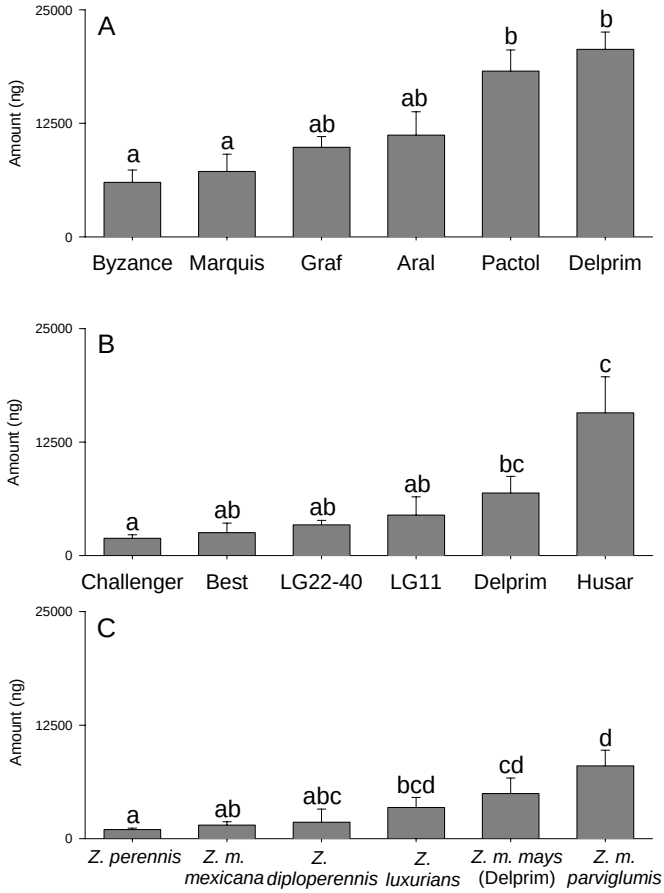

Fig. 4-2. Total amount of induced volatiles emitted by different maize varieties (**A, B**) and teosintes (**C**) in ng summed for three collection periods (9h). Statistical analysis were performed on ln transformed data and the graphs are based on the back transformation of the mean (± SE, n=6). Letters above each bar indicate significant differences after Student-Newman-Keuls post hoc test for α=0.05.

The total amount of induced volatiles emitted during the 9 h of collection varied considerably among the different maize genotypes (Fig. 4-2). For example, the variety Pactol emitted 3 times more than the variety Byzance ($F=3.711$, $p=0.013$; Fig. 4-2 A). Even more dramatic were the differences among the varieties in the second block ($F=8.099$, $p<0.001$; Fig. 4-2 B). The average amount released by the variety Husar was 8 times and 6 times higher than for the varieties Challenger and Best, respectively. Significant differences were also found in the total amount of odour emitted among the teosinte. For example, *Z. m. parviglumis* releases 8 times as much as *Z. perennis* ($F=6.608$,

$p<0.001$; Fig. 4-2 C). The wild relatives of maize emitted lower amount of odour compared to Delprim, except for the subspecies *Z. m. parviglumis*, which emitted more than this reference variety.

The considerable differences in total amounts emitted by Delprim for the different blocks may be due to differences in light quality in the climate chambers over time. The experimental blocks were conducted several months apart.

Qualitative differences. Differences were also found in the quality of the odour blends. The proportion of the principle compounds in the blends appeared characteristic for each genotype (Fig. 4-3). The ratio between the two terpenoids, linalool and (*E*)-4,8-dimethyl-1,3,7-nonatriene was different for several varieties. For example, Delprim emitted more linalool than (*E*)-4,8-dimethyl-1,3,7-nonatriene, but the reverse was found for the variety Byzance and *Z. luxurians*. Also, the amount of the aromatic compound, indole, varied with variety. Byzance was the only variety in which indole was the major compound, representing about 40% of total emission. Differences in compound ratio was most dramatic among the three sesquiterpenes, β-caryophyllene, (*E*)-α- bergamotene and (*E*)-β-farnesene, which together accounted for 22% to 84% of the total emitted by the different genotypes. All the varieties released these three sesquiterpenes, except Pactol, which did not emit β-caryophyllene. Among the teosintes, *Z. diploperennis* and *Z. m. mexicana* released very small quantities of β-caryophyllene (respectively 0.63% and 0.92% of the total blend).

Byzance, Graf and LG22-40 were the varieties in which the sum of the three sesquiterpenes made up less than 30 %. In the species *Z. diploperennis* and *Z. m. mexicana* these compounds dominated and represented 74% and 84% of the entire blend, respectively

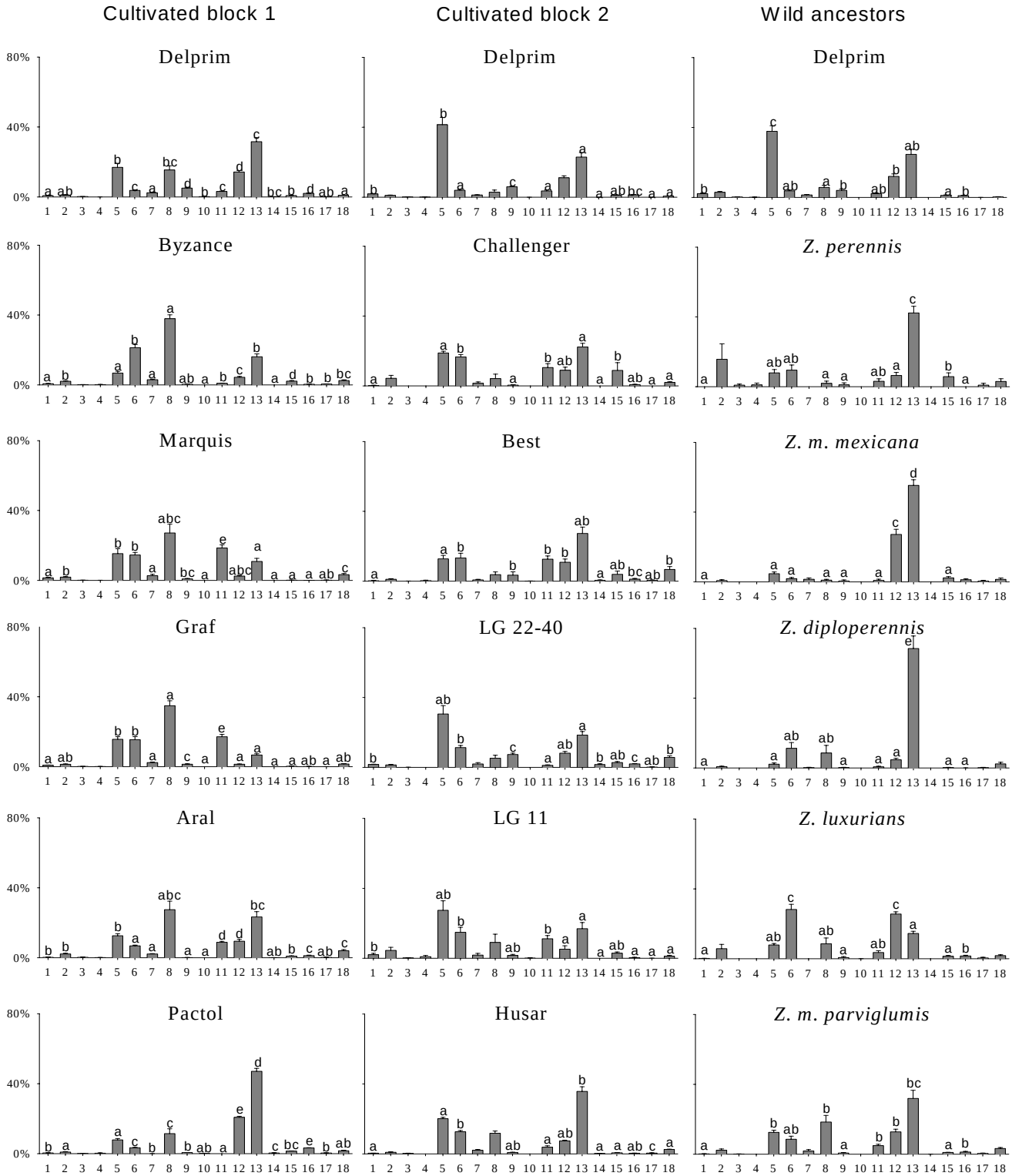

Fig. 4-3. Relative amount (± SE) of the 18 main compounds (% of the total amount of induced odour) emitted by cultivated maize varieties and 5 species of teosinte. One-way anova was performed for each compound and followed by Student-Newman-Keuls test. Letters above the bars represents significant differences in the emission of a particular compound within each of the three columns (experimental blocks). (**1**) β-myrcene; (**2**) (*Z*)-3-hexen-1-yl acetate; (**3**) hexyl acetate; (**4**) (*Z*)-β-ocimene; (**5**) linalool; (**6**) (*E*)-4,8-dimethyl-1,3,7-nonatriene; (**7**) phenethyl acetate; (**8**) indole; (**9**) geranyl acetate; (**10**) unknown; (**11**) β-caryophyllene; (**12**) (*E*)-α-bergamotene; (**13**) (*E*)-β-farnesene; (**14**) α-humulene; (**15**) unknown sesquiterpene; (**16**) (*E*,*E*)-α-farnesene; (**17**) (*E*)-nerolidol; (**18**) (3*E*, 7*E*)-4,8,12-trimethyl-1,3,7,11-tridecatetraene.

Variation within the Z. m. mexicana *population.* The total amount of induced odour emitted by the offspring of eight different *Z. m. mexicana* mother plants is shown in Figure 4-4. Differences between families of this small population were not significant, despite a clear trend that some plants emitted lower amount of induced odour than others did. The composition of the induced odour was very similar from one plant to another, independent of the mother plant (Fig. 4-5). Hence, variability within the *Z. m. mexicana* appears small.

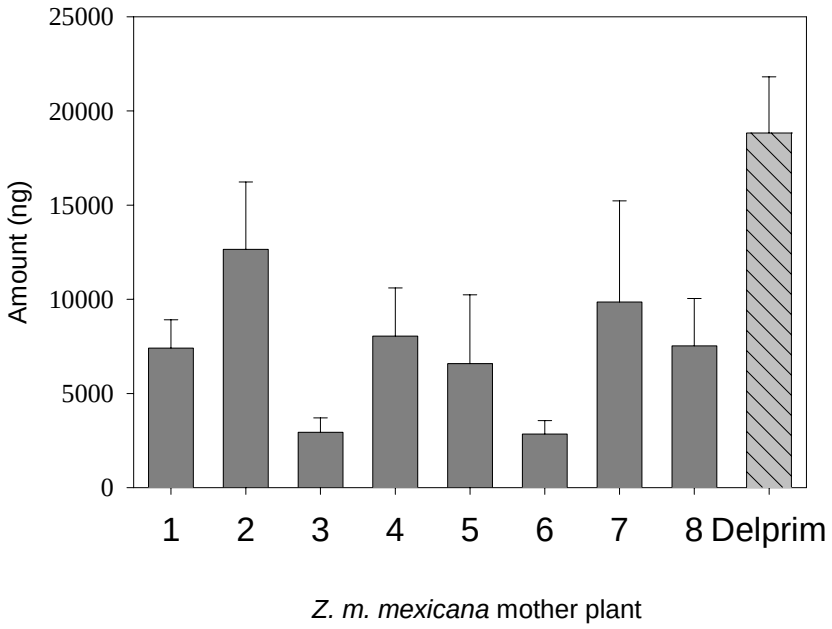

Fig. 4-4. Total amount in ng of volatiles emitted by the offspring of eight *Z. m. mexicana* plants and Delprim. Statistical analysis were performed on ln transformed data and the graphs are based on the back transformation of the mean (± SE, n=8). No significant differences were found among the teosinte (F=1.510, P=0.208).

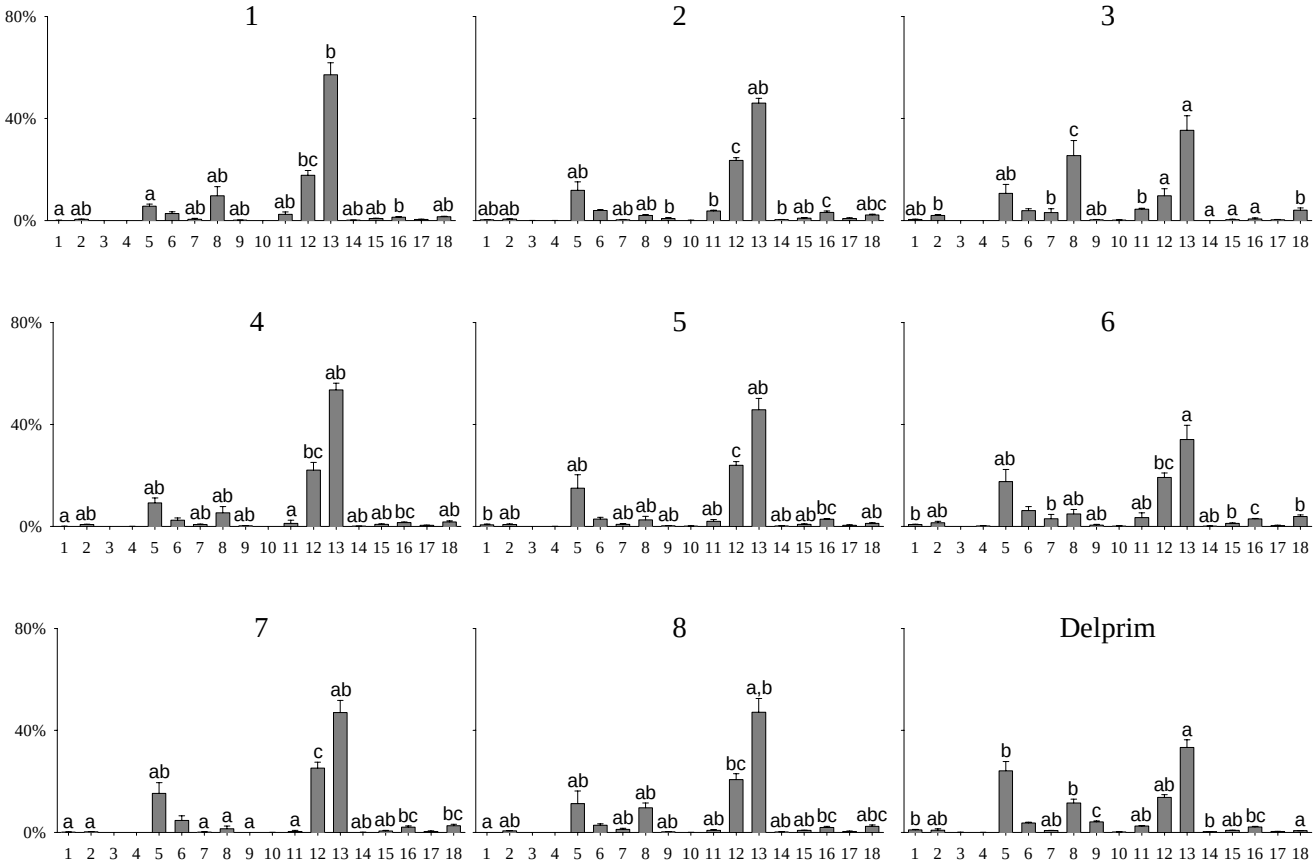

Fig. 4-5. Relative amount of the main compounds (% of the total amount of induced odour + SE) emitted by the offspring (n=8) of eight Z. m. mexicana mother plants obtained from a single population. One-way anova was performed for each compound and followed by Student-Newman-Keuls test in case of significant differences, which are indicated by letters above bars. ((1) β-myrcene; (2) (Z)-3-hexen-1-yl acetate; (3) hexyl acetate; (4) (Z)-β-ocimene; (5) linalool; (6) (E)-4,8-dimethyl-1,3,7-nonatriene; (7) phenethyl acetate; (8) indole; (9) geranyl acetate; (10) unknown; (11) β-caryophyllene; (12) (E)-α-bergamotene; (13) (E)-β-farnesene; (14) α-humulene; (15) unknown sesquiterpene; (16) β-bisabolene + (*E*,*E*)-α-farnesene; (17) (E)-nerolidol; (18) (3E, 7E)-4,8,12-trimethyl-1,3,7,11-tridecatetraene.

DISCUSSION

We found substantial variability in induced volatile signals among maize cultivars, as well as among wild relatives of maize, both in terms of total amount (quantity) and quality. Some genotypes released up to 8 times more than others did. Differences in quality of induced odour blends refer to differences in identity of the compounds within a blend and to differences in ratios among these compounds. Such differences were most apparent for the sesquiterpenes, which showed considerable variability in ratios. As we previously found (Turlings *et al.*, 1998b), β-caryophyllene was not emitted by all genotypes. The comparison of the three collection periods (Fig. 4-1) indicated that maize genotypes also vary in the timing of their response.

The range of variation in the amounts of volatiles emitted by the teosintes is very comparable to the one found for the cultivars. Concerning the quality of the odour blend, the volatile profile appears largely preserved in maize. All compounds in wild relatives can also be found in cultivated varieties. The only other study that compared the odour emissions between cultivars and a wild form was by Loughrin *et al.* (1995) who compared several cultivated cotton (Malvaceae) varieties with a naturalised variety. The wild version released considerably more volatiles. This was not the case in our study, which indicates that the breeding process did not significantly affect this trait in maize. This is contrary to the expectation that breeding for increased yield and palatability will result in a decrease of secondary defence substances in domesticated plants (Benrey *et al.*, 1998). Interestingly, *Z. m. parviglumis*, which is considered the closest relative to cultivated maize (Doebley and Wang, 1997; Kellogg, 1997), produced more than the other teosintes.

Many examples exist on the differences in emission of induced odour by different plant species, on how different herbivore types can affect the induced odour blend, and how natural enemies discriminate between plants infested by different herbivore

types(Dicke and Vet, 1998) Indeed, recent studies suggest that plants provide specific information on the identity of the herbivore by which they are attacked. For instance, Dicke and Takabayashi, 1991) found that mite-induced synomones can be specific for both the herbivore species and the plant species. In fact, predatory mites are able to distinguish between apple foliage infested by different species of spider mites (Sabelis and van de Baan, 1983; Dicke and Takabayashi, 1991). Similarly, the aphid parasitoid *Aphidius ervi* can distinguish between plants infested by its host aphid and plants infested by a non-host aphid. This difference in the attraction of parasitoid is speculated to be due to a compound (6-methyl-5-hepten-2-one), which was only detected in the odour blend of plants that are damaged by the host (Guerrieri *et al.*, 1999; Du *et al.*, 1998). As pointed out by Du *et al.* (1998), the study was done with only one genotype of one plant species, but the host aphid can feed on several other plant species. It would be interesting to see if *A. ervi* is able to distinguish among hosts and non-hosts on different plants and if the same compound or compounds are involved.

De Moraes *et al.* (1998) showed that the specialist endoparasitoid *Cardiochiles nigriceps* is more attracted to tobacco (Solanaceae) attacked by its host (*Heliothis virenscens*) than to tobacco attacked by a related non-host (*Heliothis zea).* Collections of the odour emitted by tobacco attacked by either herbivore revealed consistent differences in the ratios in which several compounds were released (De Moraes *et al.*, 1998).

The induced odour emitted by plants may also give information on the host stage that is feeding on a plant. Takabayashi *et al.* (1995) found that late instar larvae of *Pseudatelia separata* (Lepidoptera: Noctuidae) do not induce an emission of volatiles in maize plants, while plants attacked by early stages of herbivore release large amounts of induced odour and are very attractive to the specialist parasitoid, *Cotesia kariyai* (Hymenoptera: Braconidae).

It seems that signal specificity requires that there is little intraspecific variability in how plants respond to a particular herbivore. The few studies that compare induced odour blends emitted by different cultivars of the same plant species attacked by the same herbivores indicate considerable variation. For example, the odour induced by the mite *Tetranicus urticae* (Acari: Tetranychidae) varies considerably between two apple cultivars and these differences are considerably larger than differences found for one apple cultivar infested by two different mite species (Takabayashi *et al.*, 1991). Krips (2000) compared the odour emissions in four gerbera (Asteraceae) cultivars after spider mite attack and also found large differences in terpenoid emissions. For instance, the variety Sirtaki does not release (*E*)-β-farnesene, while it is present in the odour blend of the other varieties. We previously showed a distinct difference in the induced odour blend of two maize cultivars, LG11 and Iona sweet corn (Turlings *et al.*, 1998b). β-caryophyllene is not released by Ioana sweet corn, as was found here for the variety Pactol.

This comparison of several cultivars has provided additional information on the range of variation between genotypes of the same species. Within the genus *Zea*, variation can be quite dramatic, both in overall quantity as well as in the composition of the induced odour blend. Such genetic variability is likely to be larger than what different herbivores contribute to variation. Yet, herbivores may cause detectable differences through different feeding habits (Turlings *et al.*, 1998a) or through different elicitors that come in contact with leaf tissue while they are feeding (Hopke *et al.*, 1994). The reliability of induced plant signals for the third trophic level remains unclear. As pointed out by Dicke (1999), chemical analyses provide limited information in this respect because of the detection limits of the techniques that we employ. Behavioural assays with parasitoids will have to be carried out to determine what information they exactly obtain from the different odour blends.

The results presented here reveal considerable variation in induced odours among maize genotypes. Parasitoids that will have to deal with this variability may benefit from being flexible in their responses. To make optimal use of the cues that are reliably associated with hosts in a given plant population they probably rely on their ability to learn (Vet and Groenewold, 1990; Vet *et al.*, 1995; Zanen and Cardé, 1991; Turlings *et al.*, 1993b). The observed variability also suggests that maize genotypes will vary in their attractiveness to natural enemies of herbivores. If so, this could be exploited in crop protection by selecting and breeding crop plants that release compounds that are particularly attractive to biological control agents. In maize, there appears to be sufficient genetic variation to facilitate such efforts. An important next step is to determine which of the volatiles are essential in the foraging behaviour of beneficial insects.

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CHAPTER 5.

VARIATION IN INDUCED MAIZE ODOURS CAUSED BY ABIOTIC FACTORS.

Abstract—After herbivore attack, plants respond to this injury by systemically emitting a specific blend of volatiles, which is highly attractive to natural enemies of herbivores. In maize, the odour blend is mainly composed of terpenoids and indole. Specific factors in caterpillar oral secretions trigger the response in maize plant. The emission of the odourous signal can vary with plant species and genotypes, but little is known about the variation of induced odour blends due to abiotic factors. To determine this, we tested the effect of soil humidity, air humidity, temperature, light and fertilisation rate on the emission of induced volatiles in maize. Maize plants of the variety Delprim were induced to release volatiles by mechanically damaging two leaves and applying *Spodoptera littoralis* regurgitant on the wounded site. Each factor was tested separately under constant conditions for the other factors. We found a significant effect of soil humidity, with higher emission occurring when plants were standing in dry soil. Air humidity affected the odour emissions differently, with an optimal release around 60% air humidity. Temperature also affected the release of induced volatiles; temperatures between 22 and 27°C led to a higher emission of induced volatiles than at a higher (37°C) or lower (17°C) temperature. The most dramatic effect was found with light intensity. The emission of volatiles did not occur in the dark and increased steadily with an increase in the light intensity. This release was also photophase dependent, as the emission of induced odour occurred only when light was on and stopped immediately when the light was turned off. Fertilisation also had a dramatic effect on the production of induced volatiles. When plants were grown under low nutrition, the emission of induced volatiles was also very low, even

when results were corrected for biomass. Changes in abiotic factors also had some small but significant effect, on the quality of the induced odour blend, except for the air humidity. These results indicate that environmental conditions can be important factors in determining the intensity and variability in the release of induced volatiles. We discuss these in terms of specificity of induced signal in plants.

Key words. induced plant volatiles – abiotic factors – soil humidity – air humidity – temperature – light – fertilisation - specificity - tritrophic interactions - *Zea mays mays* – *Spodoptera littoralis*

INTRODUCTION

Chemical changes in plants after insect damage have received the attention of plant physiologists and ecologists ever since such changes have been suggested to function as a possible defence against herbivory (Green and Ryan, 1972). The importance of the third trophic level as a part of the battery of plant defences was stressed by Price *et al.* (1980), and has since led to the description of many examples in which plants produce odours after insect feeding that attract parasitoids and predators (Dicke *et al.*, 1990; Turlings *et al.*, 1990; Takabayashi *et al.*, 1991; Agelopoulos and Keller, 1994; Mattiacci *et al.*, 1994; McCall *et al.*, 1994; Pallini *et al.*, 1997; Röse *et al.*, 1997). In lima beans, cabbage and corn, it was shown that the production of volatiles is triggered by an elicitor in insect oral secretions (Dicke *et al.*, 1993; Turlings *et al.*, 1993; Mattiacci *et al.*, 1995). A fatty acid derivative, the N-[17-hydroxylinolenoyl]-L-glutamine was isolated and identified from the regurgitant of *Spodoptera exigua* (Lepidoptera: Noctuidae) and was named volicitin (Alborn *et al.*, 1997). This elicitor triggers the emission of the same odour blend in maize plants as does *Spodoptera* feeding (Turlings *et al.*, 2000).

Variation in induced plant odours has been particularly studied in terms of signal specificity that can give information to parasitoids and predators on the suitability of the host or prey attacking the plant. Induced odour can vary with the herbivore instar that attack the plant (Takabayashi *et al.*, 1995). Also, different herbivores species can trigger the release of different odour blends in plants, such differences can be exploited by parasitoids and predators to locate plants that carry the most suitable hosts or prey (Sabelis and van de Baan, 1983; Sabelis and Dicke, 1985; Takabayashi *et al.*, 1991; Du *et al.*, 1998; Guerrieri *et al.*, 1999; De Moraes *et al.*, 1998). Variability in induced odour emission due to plant genotype has also been reported in few studies (Takabayashi *et al.*, 1991; Loughrin *et al.*, 1995; Turlings *et al.*, 1998; Krips, 2000, Chapter 3).

Variation in induced odour due to various biotic factor is well documented, but little information is available on how various abiotic conditions affect the odour emission. It is known that changes in environmental conditions can largely affect the physiological state of plants; growing plants under different light condition will lead to different growth rates and similar effects can be easily demonstrated with levels of nutrition. Some studies provide information on how environmental factors affect direct chemical defences in plants. For example, Green and Ryan (1973) showed that in young tomato leaves, the accumulation of the proteinase inhibitor factor increases with light intensity and that optimal temperature for the highest accumulation level is about 36°C. The indole alkaloid content in *Catharanthus roseus* (Apocinacae) increases with water availability in soil (Frischknecht *et al.*, 1987). Temperature and day length affected the induction of hydroxamic acid in wheat (*Triticum aestivum* L.) seedlings after cherry-oat aphid, *Rhopalosiphum padi* infestation (Gianoli and Niemeyer, 1996). Studies on emission of fragrance in flowers provide information on the importance of light in the release of odour by plants. Altenburger and Matile (1990) found that the emission of volatiles by flowers can be either regulated by an internal clock (circadian rhythm), or only by a diurnal

phenomenon that completely depends on the photoperiod of the environment. A similar diurnal emission was observed for induced volatiles in cotton plants. Loughrin *et al.* (1994) showed that the induced emission of volatiles in cotton (Malvaceae) plants was higher during the afternoon and significantly decreased at night. Similar results were reported by Takabayashi *et al.* (1994) who found that uninfested leaves of Lima bean (Fabaceae) placed under high light intensity are more attractive to predatory mites than when they are placed under low light conditions. The difference in insect attraction to plants was reported to be due to different volatile emission under the two light regimes. Water stress also had an effect on the attractiveness of lima beans plants to predatory mites. When plants were water-stressed, they released higher amounts of volatiles than non-stressed plants, which could explain the differential attractiveness of plants to predatory mites. Recently, Halitschke *et al.* (2000) reported that the emission of induced odour by tobacco plants was highly dependent on light, and that even when plants were induced to release volatiles, the emission began only with the onset of the next light period. In maize plants, information on the effects of environmental factors on the emission of induced volatiles is very limited. Only the effect of the photophase on the release of induced volatiles has been reported (Turlings *et al.*, 1995; Tumlinson *et al.*, 1999).

The current study was conducted to obtain more information on the importance of various abiotic factors for the production of induced odour in maize. The effects of variations in soil humidity, air humidity, temperature, light intensity and phases and fertilisation rate was tested

METHODS AND MATERIAL

Plants. Maize plants of the variety Delprim were used in all experiments. Seeds were planted individually in plastic pots (360 ml, 10 cm diameter, 7 cm high) filled with fertilised soil (Coop, Switzerland). The seedlings were grown in a climate chamber (Type 10'US/+5 DU-PI, Weiss Umwelttechnik GmbH, Switzerland) at 23°C, 60%RH and 16L:8D light regime. The plants were watered daily in the morning. Plants of 10-11 days old were used for each experiment, at this age seedlings carry 3 to 4 leaves. For the experiment on the effect of fertilisation on volatile emissions, the method for growing plants is described below.

Induction of odour. Caterpillar oral secretion was collected from third and fourth instar *Spodoptera littoralis*, which were provided by Novartis Insect Control, Basle (Switzerland). They were fed with maize leaves for at least one day before their regurgitant was collected as described by Turlings *et al.* (1993). Maize seedlings were induced to emit volatile synomones by scratching $2cm^2$ of the leaf surface with a razor blade and applying 10 µl of *S. littoralis* regurgitant to the damaged site. The second and third leaves were treated this way. An additional induction method was used in the experiments on the effects of soil humidity. For this method, we injected the caterpillar oral secretions directly into the stem of maize plants (T. Degen, unpub data). Two injections of 10 µl were performed, one immediately after each other. All treatments took place during the dark period, 10 h before lights turned on.

Collection of induced odour. The volatile collection system has been described in detail by Turlings *et al.* (1998). It basically consist of 6 vertically placed cylindrical glasses (9.5 cm inner diameter, 54 cm high). A split Teflon plate with a hole in the centre at the

base of a cylinder closed loosely around the stem of a plant, allowing the separation of the aerial part of a plant, which was placed in a glass cylinder, from the pot, which remained outside (Turlings *et al.*, 1998). Purified and humidified air was pushed into each cylinder at a rate of 1 l/min and passed down over the plant. Around the base of each cylinder, just above the teflon disk, 8 openings served as ports that could hold the collection traps. Only 1 port was used during an experiment. For collections, air was pulled (0.8 l/min) through a Super-Q adsorbant trap (Heath and Manukian, 1994), while the rest of the air vented out through the hole in the bottom, thus preventing outside, impure air from entering. The automated part of the collection system (Analytical Research System, Gainesville, Florida, USA) controlled the flow through the trap. The climate chamber (CMP4030, CONVIRON, Winnipeg, Canada) in which the collection cylinders were housed was kept at 17.5°C, due to the irradiation heat, the temperature inside the cylinders was 23 ± 3°C. During the light cycle, light intensity was about 20000 lm/m^2.

Collections started 3hr after lights went on, 10 hrs after treatment. Each collection lasted 3 hr.

After each collection, traps were extracted with 150 µl of methylene chloride (Lichrosolv., Merck, Switzerland) and 200 ng of n-octane and nonyl acetate (Sigma, Switzerland) in 10 µl methylene chloride were added to the samples as internal standards.

Analysis were done with a Hewlett Packard HP 6890 series gas chromatograph equipped with an automated on-column injection system (HP G1513 A) and a flame ionisation detector. Of each sample a 3 µl aliquot was injected onto an apolar EC-1 capillary column (30m, 0.25 mm. i.d., 0.25 µm film thickness, Alltech Associates, Inc, USA) preceded by a deactivated retention gap (5 m, 0.25 mm i.d., Connex, USA) and a deactivated pre-column (30 cm, 0.530 mm i.d., Connex, USA). Helium (24 cm/s) was used as carrier gas. Following injection, the column temperature was maintained at 50°C for 3

min., increased to 230°C at 8°C/ min and held at 230°C for 9.5 min. The detector signal was processed with HP GC Chemstation software.

Abiotic factors tested

Soil humidity. Three plants were each placed in an individual pot that was watered prior to the treatment with regurgitant, while the three other potted plants were not watered for 18 hours. Over 6 plants, 4 were treated with both type of odour induction while 2 plants were left unharmed as controls. Soil humidity was measured after odour collection. This was done by weighing the soil in each pot without maize roots just after an experiment. Then the soil was dried in an oven at 70°C for 3 days and weighed again. The percentage of water in the soil was then calculated. Eleven replications were performed for both types of odour induction.

Air humidity. Air passed through a bubbler just before it entered a glass collection chamber. Two bubblers were filled with 300 ml of silica gel to create a dry atmosphere in the collection glass chamber, two bubblers were left empty and the last two were filled with 300 ml of demineralised water in order to create high humidity in the glass chambers. For each replication and each air treatment, one maize seedling was induced to emit odour while another one was left unharmed as a control. Hygrometers were placed in the collection chamber to measure the relative humidity inside the glass chamber. 12 replications were performed (n=36).

Temperature. Temperature inside the climate chamber with the odour collection system was set at either 10°C, 15°C, 20°C or 30°C, which corresponded to 17°C, 22°C,

27°C or 37°C inside the collection glasses. Light intensity was kept constant was constant during all collections (n= 12 per temperature).

Light intensity. Maize plants of the variety Delprim were grown as described earlier, at a 45000 lm/m^2 16h light regime. Five light intensities were used during odour collections ranging from 0 to 20000 lm/m^2, differing in 5000 lm/m^2 increments. Plants were treated in the evening 10 h before the beginning of collection. Collection lasted for 3h, with 12 replicates for each light intensity.

Induced odour emission in relation to light rhythm. Induced Delprim maize plants were submitted to a dark-light regime of three hours each. Odour collection was performed during the last two hours of each period. A total of 6 collections were done per plant, starting 5 h after treatment while plants were in the dark. Light intensity was 20000 lm/m^2 during the periods of illumination. A total of 12 plants were tested over 4 replicates.

Fertilisation rate. Seeds of the commercial maize variety Delprim were washed carefully with demineralised water and chlorine solution (2%), then the seeds were placed in demineralised water and aerated during one night. Seeds were rolled in wet paper and the roll was put in a beaker covered with a plastic film, the beaker was placed in a dark cabinet under lab conditions. Seeds were left to germinate for 3 days. Then, the root and embryo were excised from the seed with a razor blade and planted in pots (360 ml, 10 cm diameter, 7 cm high) filled with a mixture of vermiculite and non fertilised regular soil (Coop, Switzerland). This way the nutrient reserves of the seeds were removed to avoid any use of these reserves for odour production. Pots were kept in a climate chamber (Type 10'US/+5 DU-PI, Weiss Umwelttechnik GmbH, Switzerland) under the following conditions: 23°C±4°C, 60%±10% r. h., and 40000 lm/m^2 with a photoperiod of L16:D8.

The plants were watered daily either with a complete nutritive solution for the fertilised plants or with demineralised water for the unfertilised plants. A third treatment was obtained by watering certain plants with the complete nutritive solution for 7 days after planting, then plants were divided into two groups for the next 8 days, one fertilised (watered with complete nutritive solution) and one unfertilised (watered with demineralised water only). The plants were used 15 days after planting when they carried four developed leaves. Twelve replicates were performed.

Statistical analyses. Regression analyses were performed for experiments on soil humidity and air humidity. For the other abiotic factors tested, univariate analysis of variance was performed to compare the total amount of induced odour blend. For comparison of odour blend quality, the ratio (amount in ng/ total amount emitted by plant) of the twelve dominant compounds were analysed. For the experiments testing the effect of soil and air humidity, data were grouped into five categories of 20% intervals. Student-Newman-Keuls was done as post hoc test. Repeated measures anova was used to test for differences in induced odour emission between treatments in the experiment on the effect of light cycle.

RESULTS

Soil humidity. In both methods used to induced maize to release volatiles the soil humidity has an effect on the emission of induced odour, but no effect of soil humidity was found for the emission by undamaged plants (Fig. 5-1).

The relation between the humidity of the soil and the amount of induced odour emitted was negative: the higher the humidity the lower the released of induced volatiles (Fig. 5-1). On average, more odour was emitted from maize plants induced by mechanical

damage and applying regurgitant on the wound than by injecting the regurgitant directly in the stem (Fig. 5-1).

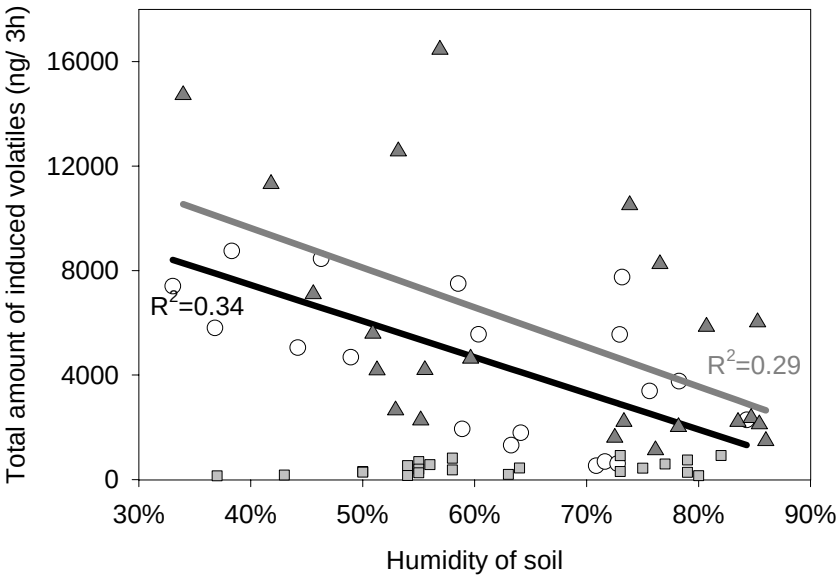

Fig. 5-1. Total amount (ng/ 3hr) of induced volatiles emitted by maize plants submitted to different soil humidity. Triangles represent the amount of volatiles released after induction by mechanical damage +regurgitant on the wounded site, circles represent the amount of volatiles emitted after injection of regurgitant directly in the stem of a maize, and squares represent the odour emitted by healthy undamaged plants. Light grey line represents the linear regression for mechanical damage + regurgitant treated plants (F=8.577, p=0.008) and dark line represents the linear regression for injection method treated plants (F=8.667; p=0.0091).

In the experiment using the mechanical damage plus regurgitant method, the soil humidity also had a significant effect on the quality of the odour blend (F=1.925; p=0.033). The effect of the humidity of soil was not the same for the twelve dominant compounds plotted in Figure 5-2. Ratios among compounds differed for different humidity classes. The relative quantity of eight compounds was significantly different according to the category of soil humidity. The compounds linalool, β-caryophyllene, (*E*)-α-bergamotene showed a tendency to increase with humidity, while the release of (*Z*)-3-hexen-1-yl acetate, (3*E*)-4,8-dimethyl-1,3,7-nonatriene, indole geranyl acetate, and *E*-β-farnesene were very low at high

humidity (Fig. 5-2). When the regurgitant was injected directly in the stem, no significant effect of the humidity of soil on the quality of the induced odour blend was found (F=1.897; p=0.091) (Fig. 5-3).

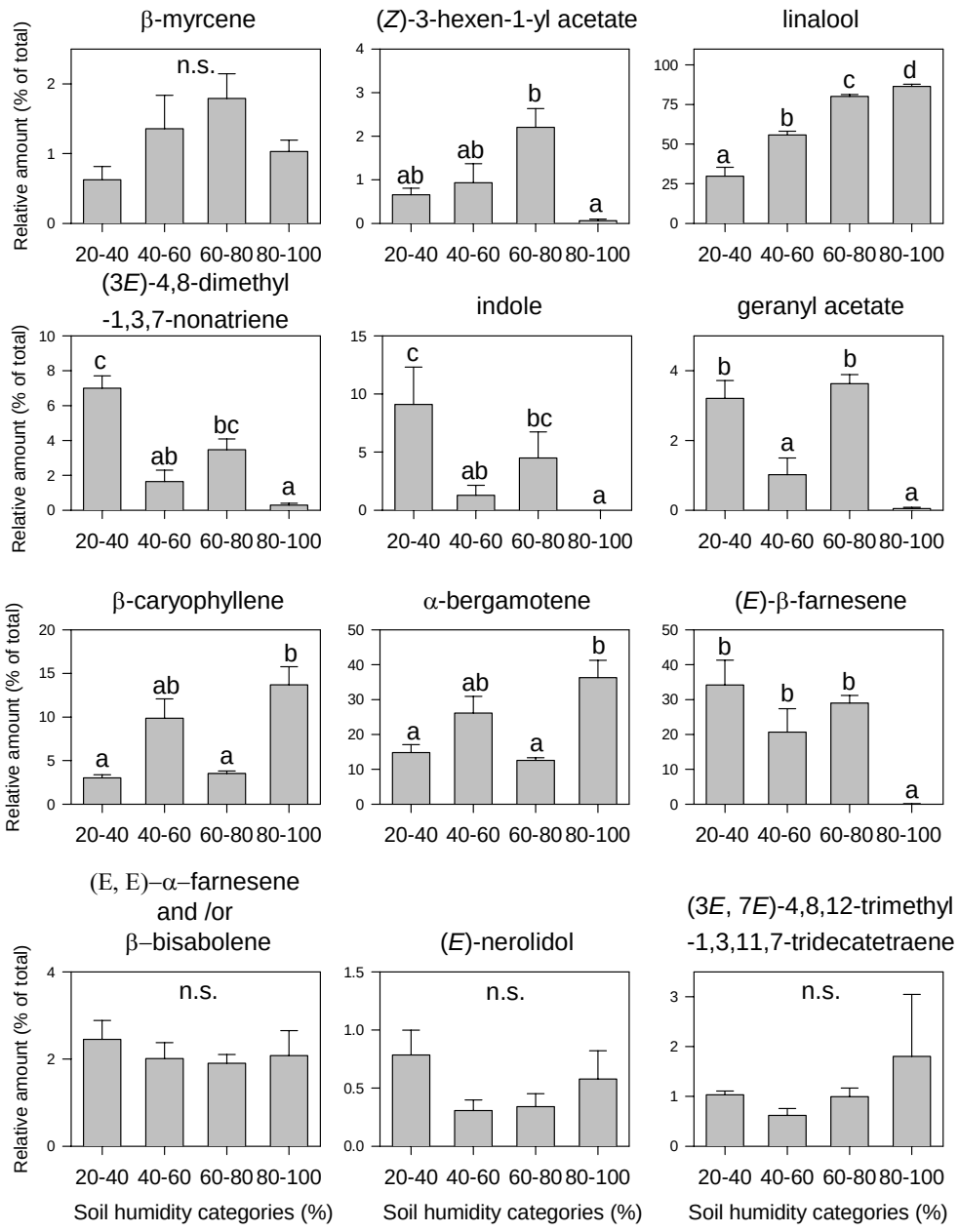

Fig. 5-2. Relative amount (mean of % of total + SE) of the twelve dominant compounds in the odour blend induced after mechanical damage and regurgitant application at different soil humidities. Humidities were divided in four categories of 20% intervals. Letters above bars indicate significant differences among soil humidity categories after Student-Newman-Keuls post hoc test (α=0.05).

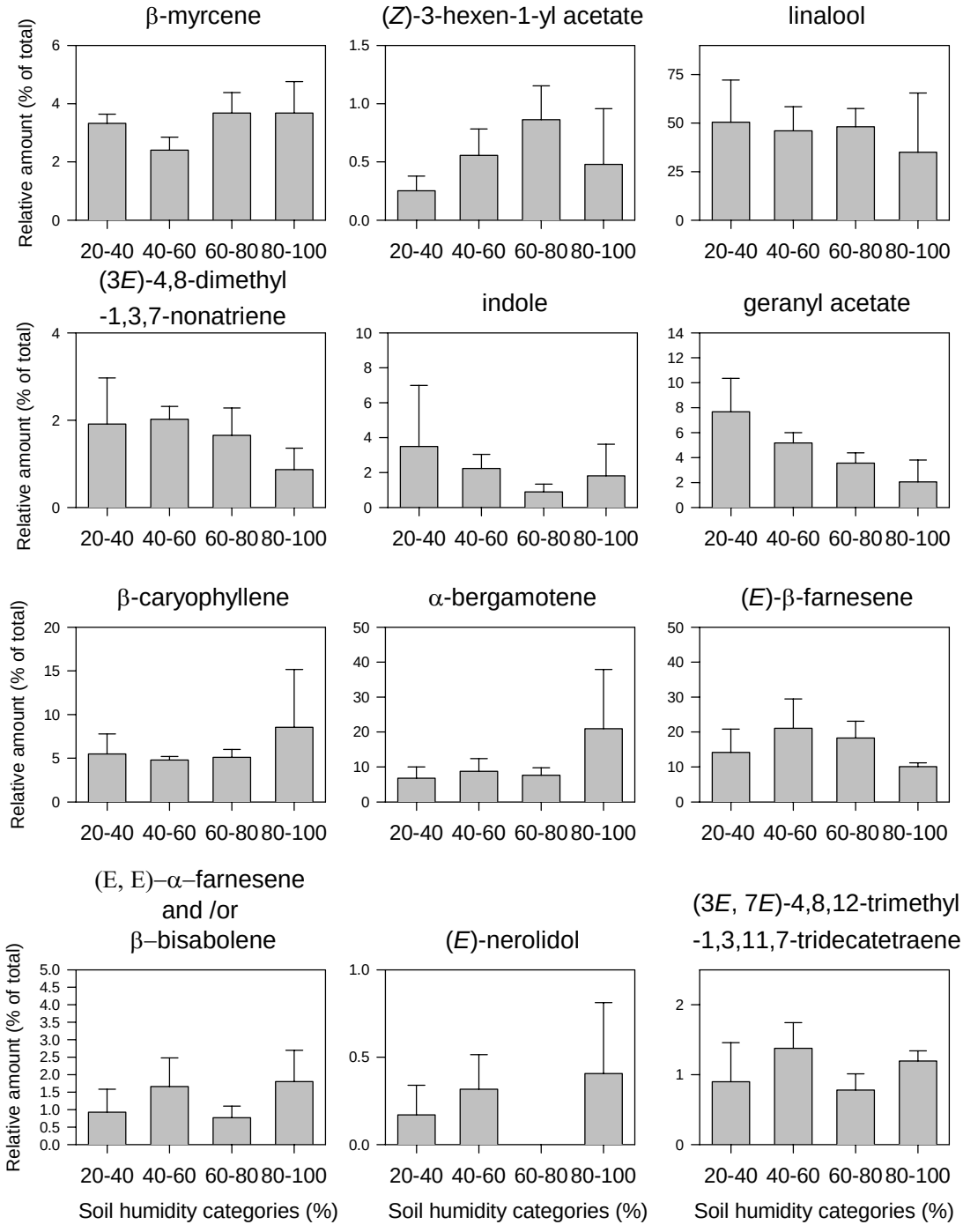

Fig. 5-3. Relative amount (mean of % of total + SE) of the twelve dominant compounds of the odour blend induced by injection of regurgitant at different soil humidities. Humidity was divided in four categories of 20% intervals.

Air humidity. The air humidity inside the collection chamber had a significant effect on the release of induced volatiles by maize plants (Fig. 5-4). When the air in the glass chamber was dry or very humid the release of odour was lower than when humidity was intermediate. It seemed that the optimal air humidity for the release of induced odour occurred between 45 % and 65 % of relative humidity (Fig. 5-4). The quality of the odour was not different over the range of air humidities that the plants were subjected to (F=1.169; p=0.259; Fig. 5-5).

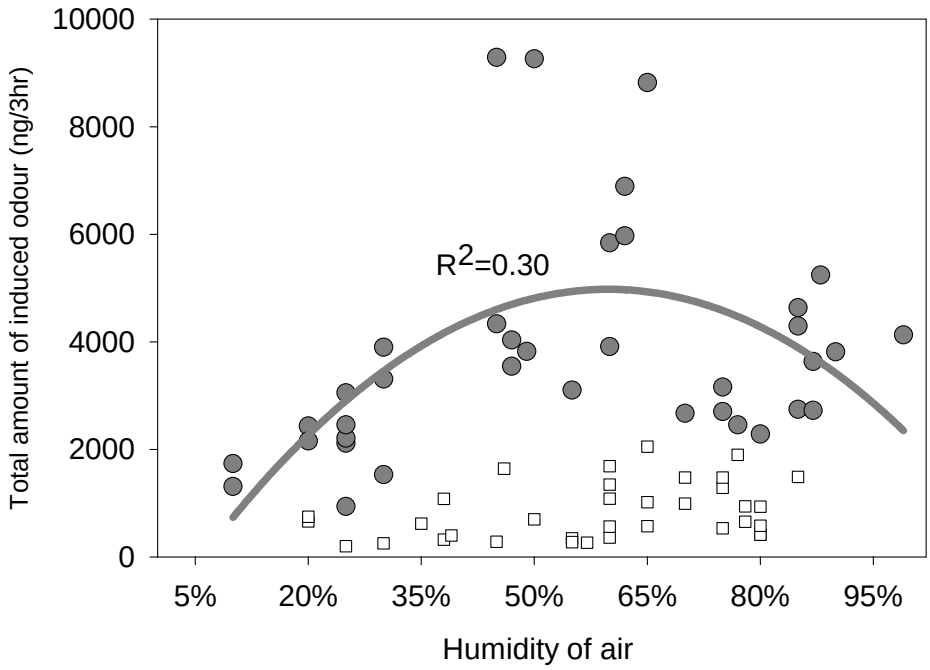

Fig.5-4. Total amount (ng/ 3hr) of induced volatiles emitted by maize plants under different air humidities. Circles represent the amount released by induced maize plants and squares represent the odour released by undamaged plants. Grey curve represents the relation between the amount emitted by induced plants and the air humidity (F=7.09; p=0.0027).

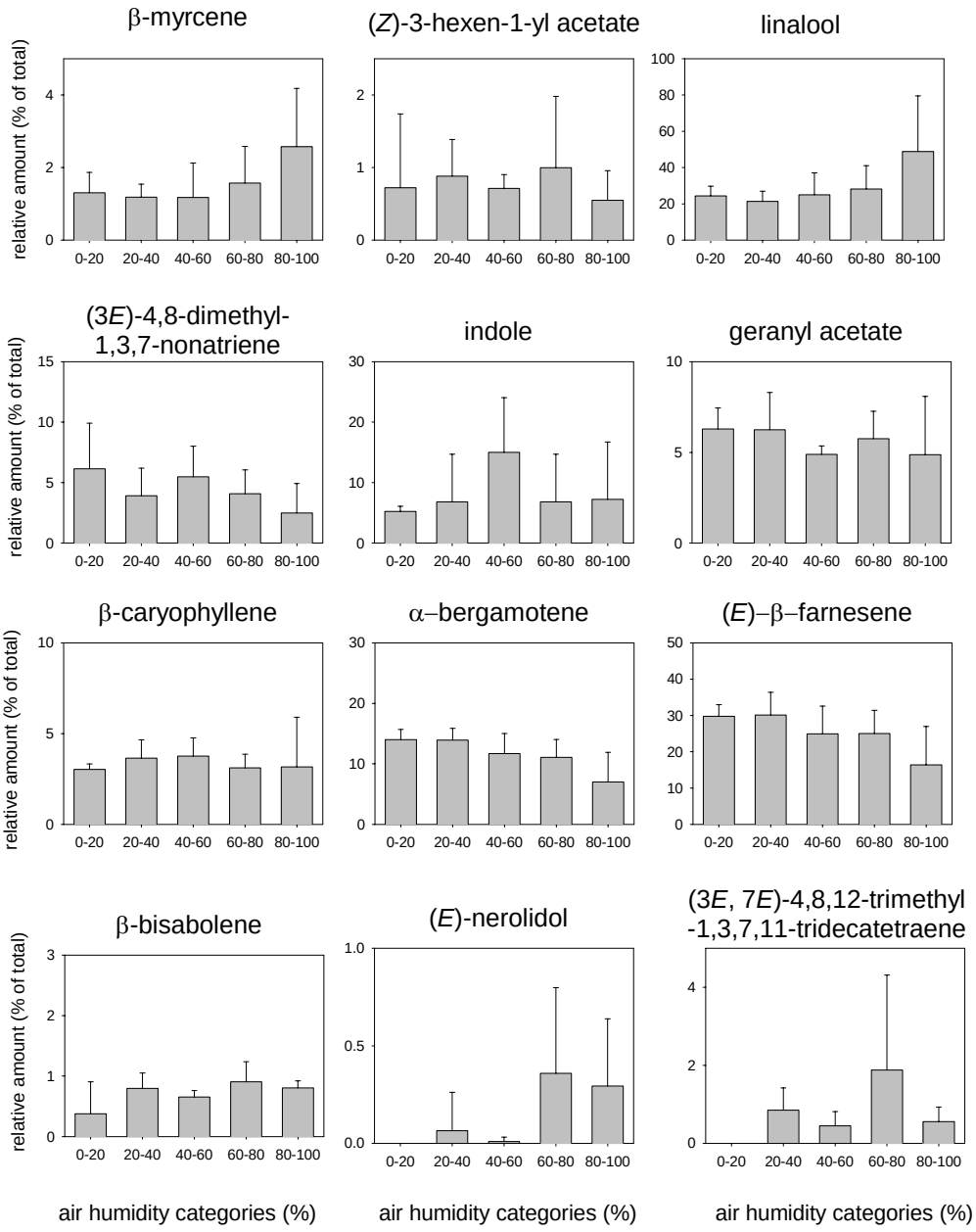

Fig. 5-5. Relative amount (mean of % of total amount + SE) of the twelve dominant compounds of the induced odour blend over five categories of air humidity.

Temperature. For the range of temperatures tested, we found significant differences in the total amount of induced volatiles released by the maize plants (F=3.514; p=0.02; Fig.5-6). Emissions were highest at 22°C and 27°C (Fig. 5-6). The total amount released by undamaged maize plants was significantly different from the amount emitted by

induced plants (F=35.148; p<0.001) and it was not affected for the different temperatures tested (F=1.793; p=0.189).

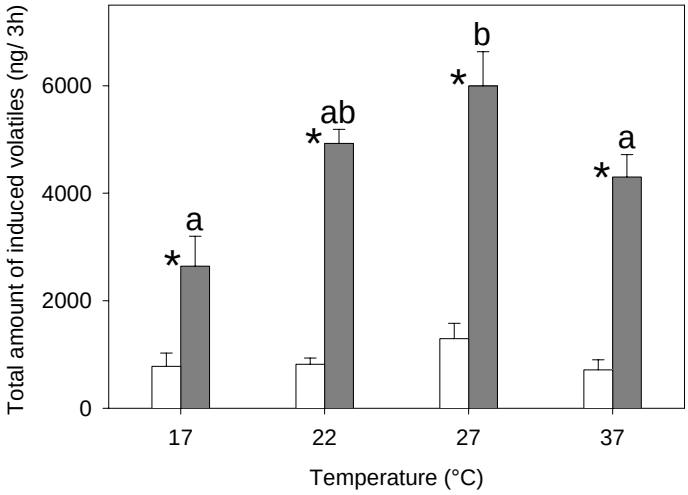

Fig. 5-6. Total amount (mean + SE) of odour emitted by maize plants under different temperatures (°C). Grey bars represent induced plants and white bars represent undamaged plants. Stars indicate significant differences between induced plants and undamaged plants (F=35.148; p<0.001) and letters above grey bars indicate significant differences among the different temperature tested for induced plants by Student-Newman-Keuls post hoc test (α=0.05).

The temperature also had an effect on the quality of the odour (F =2.630; p<0.0001; Fig. 5-7). For example, the amount of β-caryophyllene was highest at the highest temperature (37°C) (Fig. 5-7). The same results were observed for (*Z*)-3-hexenyl acetate, (*E*)-nerolidol and (3*E*, 7*E*)-4,8,12-trimethyl-1,3,7,11-tridecatetraene (Fig. 5-7). The relative amount of β-bisabolene followed the same tendency but the difference was not significant. In contrast the proportion of geranyl acetate was higher at 22°C (Fig. 5-7). Among the other compounds some trends could be observed, for example the relative amount of (3*E*)-4,8-dimethyl-1,3,7-nonatriene was higher at lower temperature, and remained constant at

higher temperatures. The other compounds followed the same trend as for the total amount; their relative amount was highest at 22°C and 27°C (Fig. 5-7).

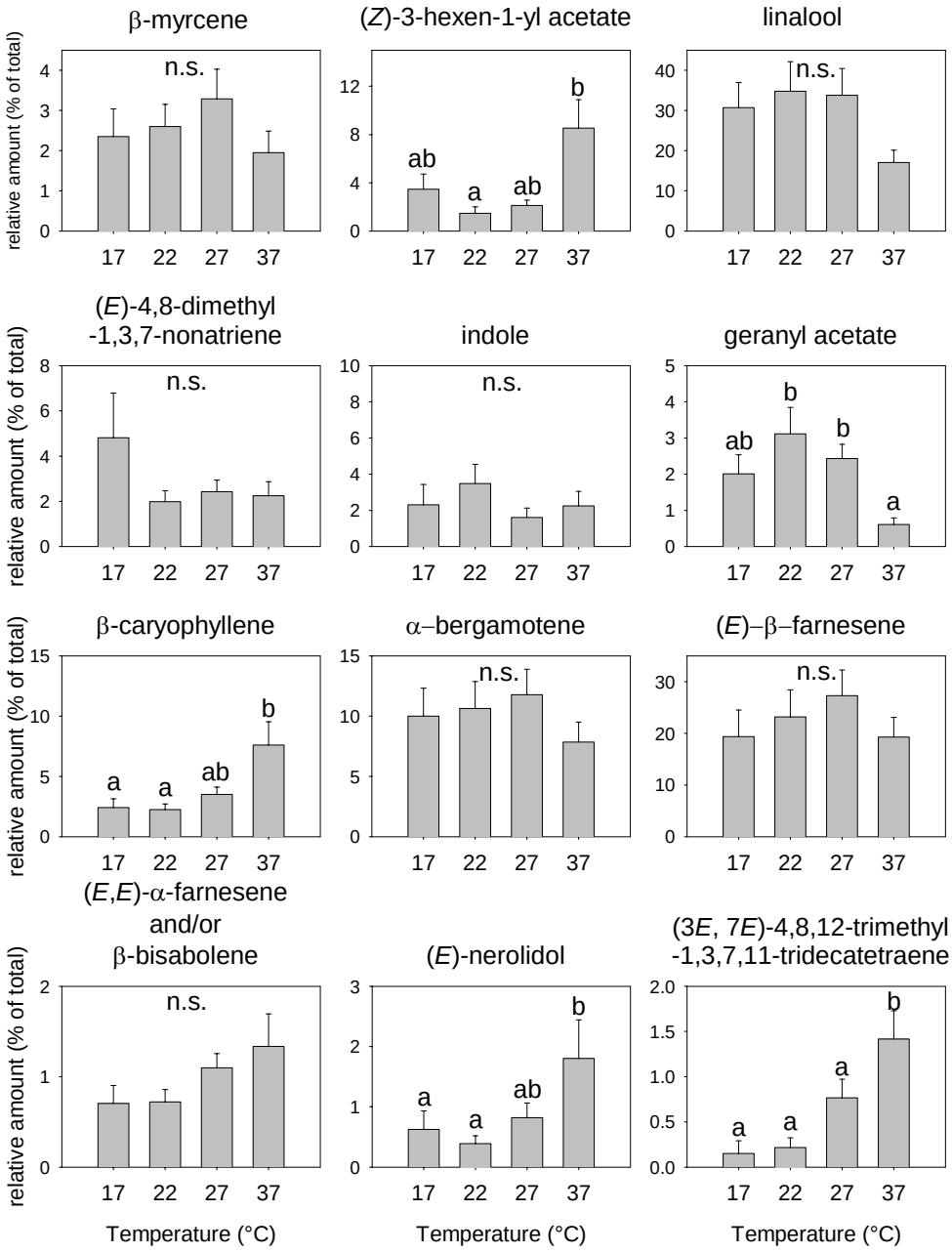

Fig. 5-7. Relative amount (mean of % of total + SE) of the twelve dominant compounds in the induced odour blend. Letters above bars indicate significant differences among soil humidity categories after Student-Newman-Keuls post hoc test (α=0.05).

Light intensity. Light intensity had a dramatic effect on the emission of induced volatiles (F=19.174; p<0.001; Fig. 5-8). The release of volatiles increased with the increase in light intensity (Fig. 5-8). When plants were kept in the dark, very little odour was emitted, and the release from induced plants was not different from the odour of undamaged plants. No significant effect of the light intensity was found for the release by undamaged plants (F=0.755; p=0.577).

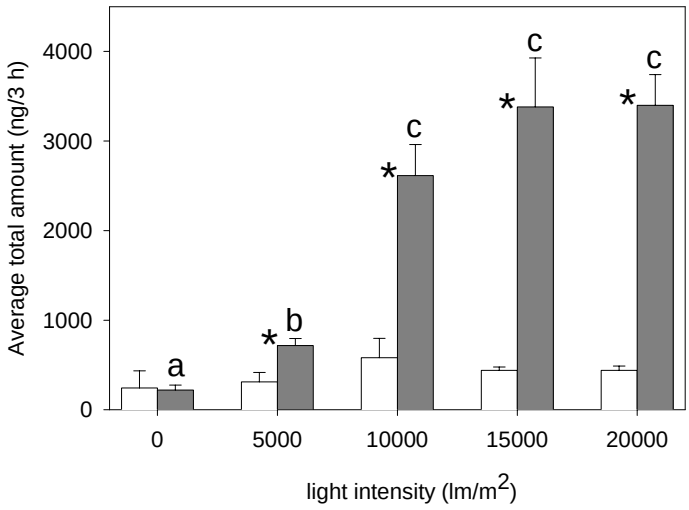

Fig. 5-8. Total amount (mean + SE) of volatiles emitted by maize plants under different light intensities. Grey bars represent induced maize plants, and white bars represent undamaged plants. Stars indicate significant differences between the total amount of odour released by induced plant and undamaged plants (α=0.05), and letters indicate significant differences after Student-Newman-Keuls post hoc test among light intensity for induced plants (α=0.05).

The light intensity had a significant effect on the induced odour blend quality as well (F=3.792; p<0.001). The relative amount of the twelve dominant induced compounds differed among the different light intensities tested (Fig. 5-9). The amount of β-myrcene, (*Z*)-3-hexenyl acetate and β-bisabolene + (*E*,*E*)-α-farnesene significantly decreased with

the increase in light intensity. Most of the other compounds showed a significant increase in their relative proportion when the light was on (Fig. 5-9). Only (3*E*)-4,8-dimethyl-1,3,7-nonatriene, α-bergamotene and (*E*)-β-farnesene did not show a significant change in their proportion among the different light intensity tested even though the release of these compounds significantly increased with light intensity.

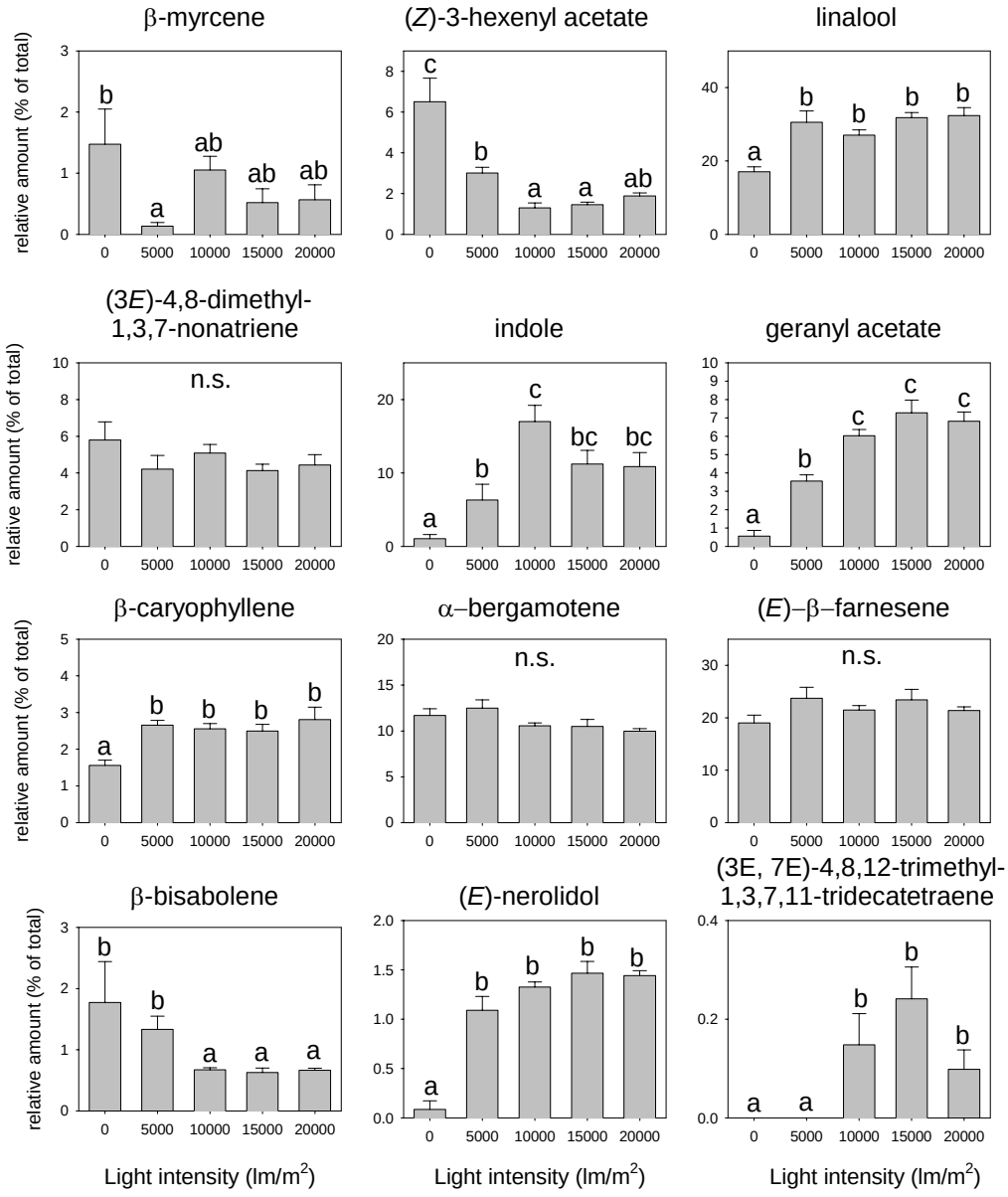

Fig. 5-9. Relative amount (mean of % of total + SE) of the twelve dominant compounds in the induced odour blend. Letters above bars indicate significant differences among light intensities after Student-Newman-Keuls post hoc test (α=0.05).

Light rhythm. As with the previous results plants that were kept in the dark, released no induced volatiles. Five hours after treatment the maize plants did not emit any additional odour while they were in the dark phase, but as soon as the light was on (eight hours after damage), the emission of volatiles dramatically increased (Fig. 5-10). Interestingly, the emission of odour immediately stopped again when the light was switched off (Fig. 5-10).Obviously, significant differences in the amount of induced odour released were found among the different light phases (F=38.405; p=0.001). The odour signal was "switched" on or off with the presence or absence of light, independently of a possible "circadian" internal rhythm of the plants. Total amount of induced volatiles released decreased with time after damage as it was previously shown by Turlings *et al.* (1998).

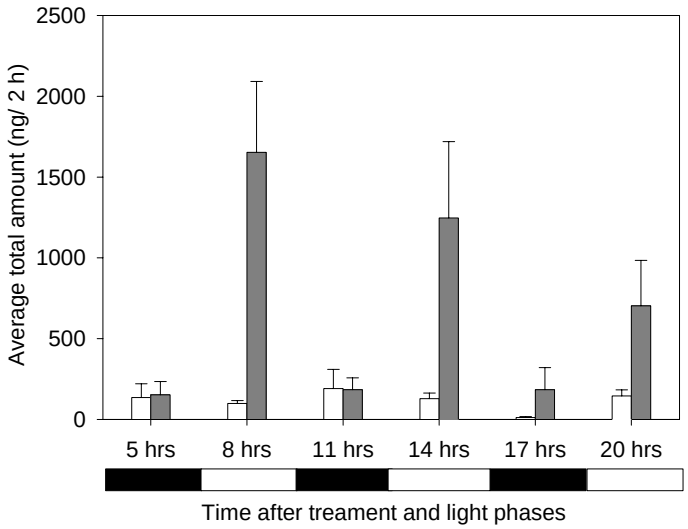

Fig. 5-10. Total amount (mean + SE) of volatiles emitted by maize plants under dark-light phases. Grey bars represent induced maize plants, and white bars represent undamaged plants. The horizontal bar represents the respective dark and light phases.

Fertilisation rate. The fertilisation had an significant effect on the size of plants used for the experiment (Fig. 5-11). The size is represented by the dry weight of plants subjected to the different fertilisation levels; plants watered with only demineralised water were 3.5 time smaller than plants watered with the complete nutritive solution (Fig. 5-11).

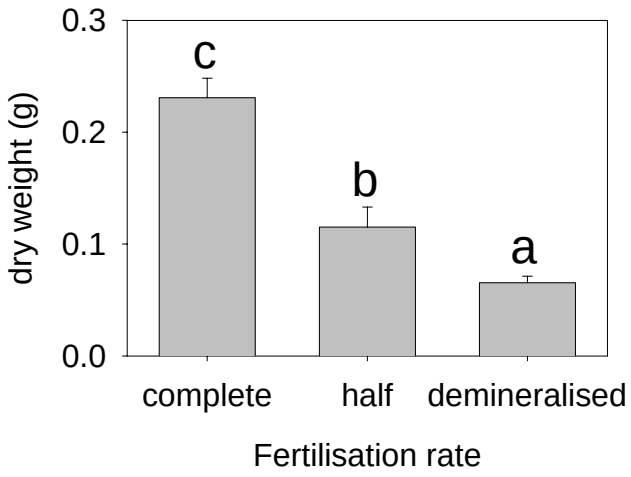

Fig. 5-11. Dry weight (mean + SE) of maize plants grown under three different fertilisation rates (F=40.707; p<0.001). Letters above bars indicate significant differences among the different fertilisation treatments after Student-Newman-Keuls post hoc test (α=0.05)

The fertilisation rate had also a significant effect on the emissions (F=36.160; p<0.001), even when the total amount was corrected for biomass of plants (F=33.490; p<0.001). Plants that received little fertilisation released significantly lower amount of volatiles (Fig. 5-12 A and B). As before, induced plants emitted significantly more than undamaged plants except when maize plants were watered only with demineralised water (Fig. 5-12). Interestingly, undamaged plants also released less when they were not fertilised.

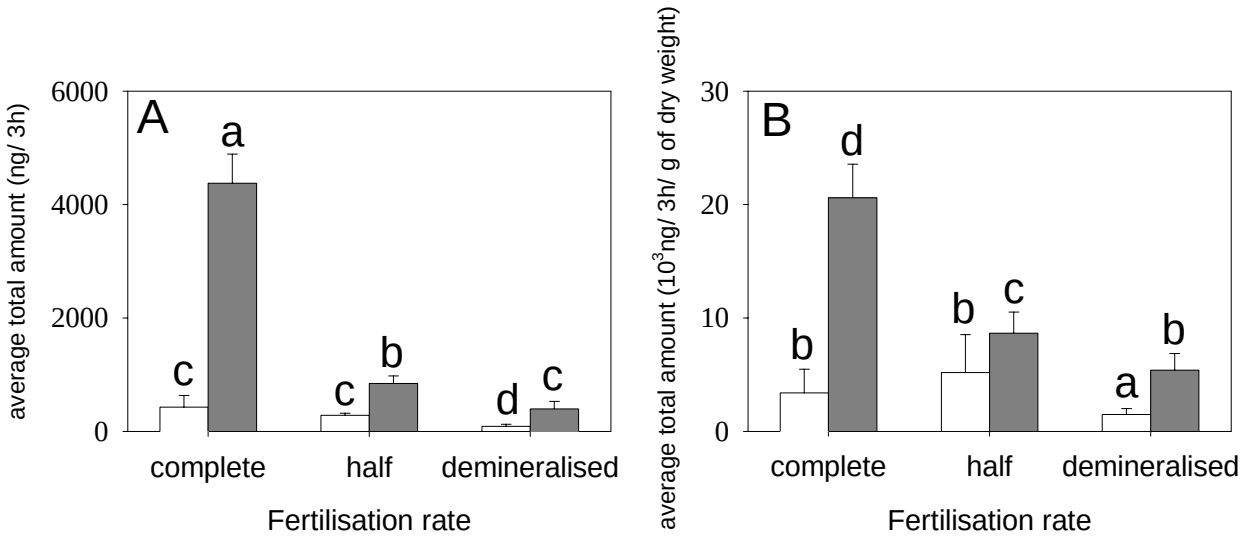

Fig. 5-12. Total amount (mean + SE) of volatiles emitted by maize plants under three different fertilisation rate for. Graph (**A**) represent the amount without correction for biomass and graph (**B**) represent the amount corrected for biomass. Grey bars represent induced plants, and white bars represent undamaged plants. Letters above bars indicate significant difference among fertilisation rates after Student-Newman-Keuls post hoc test (α=0.05).The fertilisation rate also had an effect on the overall odour blend composed of the twelve dominant compounds (F=2.689; p=0.001).

Only the proportions of β-myrcene, α-bergamotene and (*E*)-β-farnesene were not significantly affected by the rate of fertilisation (Fig. 5-13). Most of the other compounds tested were released in a lower proportion when the plants were grown under low fertilisation. Only the proportion of linalool was higher for the half fertilisation treatment than for the two other fertilisation treatments. A surprising result is that the relative amount of linalool did not differ for plants watered with the complete nutritive solution and the ones watered only with demineralised water.

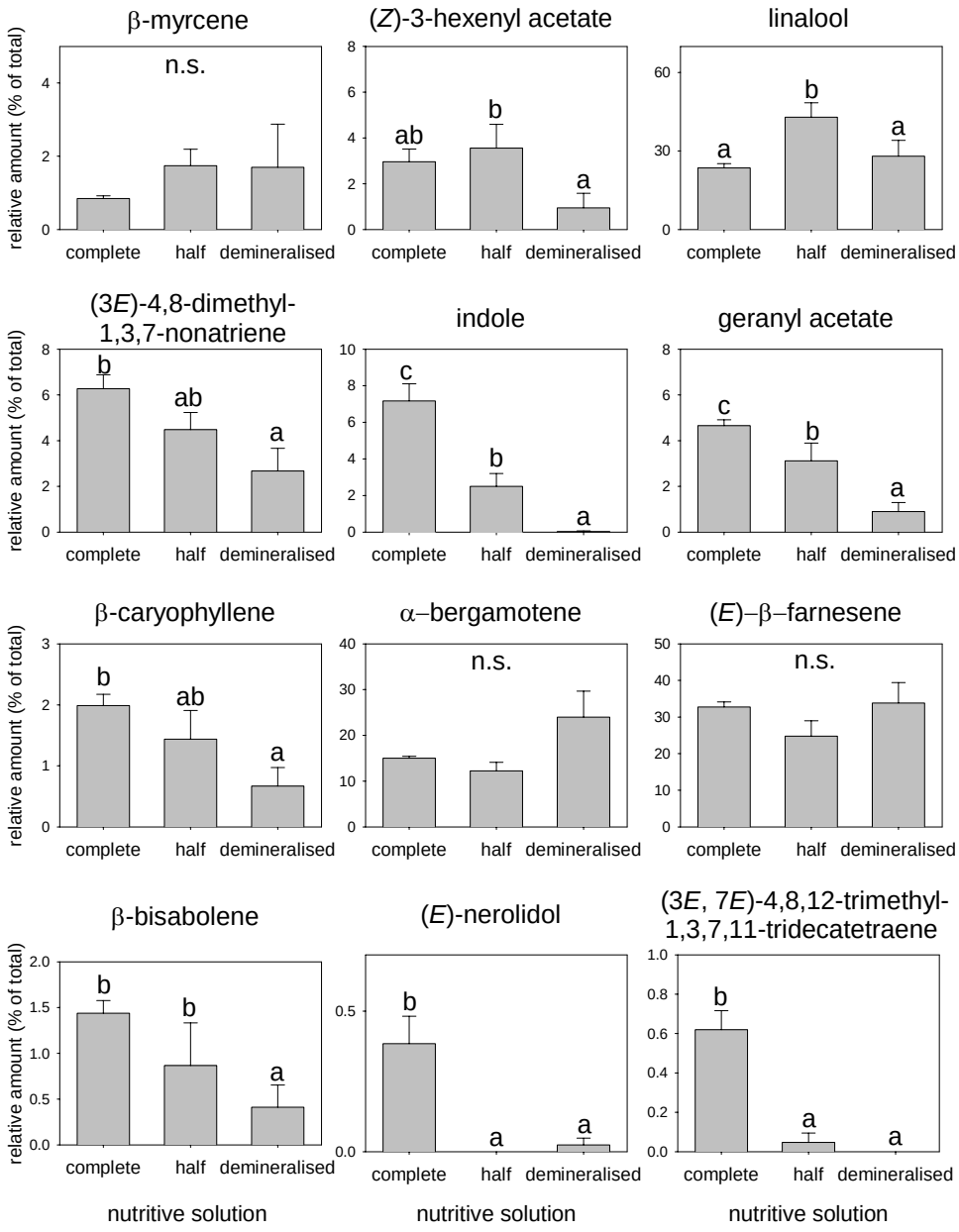

Fig. 5-13. Relative amount (mean of % of total + SE) of the twelve dominant compounds in the induced odour blend. Letters above bars indicate significant differences among fertilisation rates after Student-Newman-Keuls post hoc test (α=0.05).

DISCUSSION

All of the different abiotic factors that were tested had a significant effect on the quantity of induced volatiles emitted by maize plants. The magnitude and direction of this effect was different according to the factor considered. Higher emission of induced volatiles occurred when the soil humidity was low, the relative air humidity was between 45 and 65%, the temperature between 22 and 27 °C, with high light intensity, and with continuous fertilisation of the soil. In many cases, the different abiotic factors also affected, the quality of the odour blend. Soil humidity affected the ratio among the twelve dominant compounds only when plants were mechanically damaged and the regurgitant of *S. littoralis* was applied on the wounded site and not when the regurgitant was directly injected in the stem. We found no evidence that air humidity affects the quality of the odour blend, while temperature, light intensity, and fertilisation rate all had an effect on the composition of the odour blend. Some compounds seem not to be too sensitive to changes in environmental factors. For example the proportion in which (*E*)-β-farnesene is released is very stable while (*E*)-nerolidol and (3*E*, 7*E*)-4,8,12-trimethyl-1,3,7,11-tridecatetraene are released in very variable proportions. Fertilisation rate seems to affect the proportion of most of these particular compounds in the odour blend.

Takabayashi *et al.* (1994) reported that lima bean under water stress were more attractive to spider mites. Chemical analysis confirmed that lima bean plants under water stress produced more of the attractive volatiles than non stressed plants. Their study was done with undamaged plants and they did not report on any effect of water stress on the emission of induced volatiles (Takabayashi *et al.*, 1994). In the case of maize, undamaged plants were not affected by soil humidity but the emission of induced volatiles was considerably higher when plants were kept in dry soil, even if the plants did not show any symptoms of desiccation. This can perhaps be explained by the fact that plants standing in

dry soil have a faster take up and transportation of the regurgitant than when they are grown in wet soil (Taiz and Zeiger, 1998). This must certainly be true for the injection method.

The effect of air humidity on the emission of induced volatiles has not be studied before. The emission of induced volatiles was highest at about 60% relative humidity. A possible explanation for the effect of air humidity is that the aperture of stomata is correlated with the air humidity. Stomata opening increases with the dryness of the air, but only up to a certain limit, after which the stomata close (Taiz and Zeiger, 1998). If we suppose that the emission of induced volatiles occurs through the stomata, it could explain the curve that was obtained (Fig. 5-4).

Temperature has been shown to have an effect on the induced chemical defence in wheat after aphid infestation (Gianoli and Niemeyer, 1996). In this study, lower temperature led to higher production of hydroxamic acids, an induced compound accumulated after aphid infestation. But this effect was mainly due to the growth rate of the plants, they grew faster under higher temperature conditions, and the concentration of hydroxamic acid was negatively correlated with growth rate. In tomato, Green and Ryan (1973) found that both light and temperature have an effect on the induced production of proteinase inhibitors; temperatures below 22°C led to a severe reduction of this defence compound. Loughrin *et al.* (1994) showed that in cotton the induced volatiles follow a diurnal rhythm. They suggested that the increase in the release of volatiles during the day was due to the increase of light intensity, but it could also have been due to an increase in temperature, which occurred simultaneously with the increase of daylight. Here, we show that in maize the temperature under controlled light conditions, can affect the release of induced volatiles. The emission of volatiles was higher at temperatures between 22 and 27°C, but decreased at lower or higher temperature. Again it could have something to do

with the aperture of stomata increase, if this is affected by temperature (Taiz and Zeiger, 1998).

The effect of light on the production of induced defence compounds is better documented. The production of a proteinase inhibitor was shown to be temperature, but also light dependent. In darkness only very low accumulation of this defence factor was detected (Green and Ryan, 1973). In lima bean, spider mites were more attracted to healthy plants that were placed under high intensity light than plants under low light intensity (Takabayashi *et al.*, 1994). Differential attractiveness was correlated with by differential release of the volatiles by uninfested plants (Takabayashi *et al.*, 1994). Loughrin *et al.* (1994) showed that herbivore-injured cotton plants in a greenhouse emit more volatiles during midday, when the light intensity is highest. In Turlings *et al.* (1995), there is some information on maize. These authors showed that the released of induced volatiles was higher during the day than during the night, but again the experiments were done in a greenhouse in which it was not possible to control for temperature variations. Here we show that in maize the emission of induced volatiles increase with the light intensity even under constant temperature conditions. This indicates that the production of induced volatiles is correlated with the photosynthetic activity in plants. This was also indicated by the results demonstrating that emission of induced volatiles occurred only during the photophase, independently of the internal rhythm of the plants. Paré and Tumlinson (1997) indicated that in cotton , the de novo production of induced volatiles is tightly coupled with the photosynthesis.

The level of nutrients available in soil will greatly influence the growth rate of plants (Taiz and Zeiger, 1998). Any lack in nutrients will also affect the production of secondary metabolites as predicted by the carbon-nutrient balance theory (Gershenzon and Croteau, 1991). When nutrient availability is limited, it will reduce the growth rate of plants, but photosynthesis remains constant due to carbon availability. Accumulation of

carbohydrate will lead to the synthesis of terpenoids (Gershenzon, 1994). In maize, the production of induced defence was lower when nutrient availability was low. No fertilisation and half fertilisation did reduce the plants' growth rate. But even after correction for biomass, plants with low level of fertilisation produced less of the induced volatiles. A lack in constitutive elements that are needed for the *de novo* synthesis of induced compounds may explain this observed reduction, which is contradictory to what would be predicted by the carbon-nutrient theory.

Most of the environmental factors tested, had an effect on the total production of induced odour and some of them also on the quality of the odour blend. Further experiments on the interactions between these different factors should reveal more on their relative importance for the production of induced volatiles. The effect of the observed differences in emission on parasitoid behaviour remains to be studied. Some variation in the emission of maize odour is correlated with the activity of most parasitoids. For example, most of them are diurnal and are most active at higher temperatures, under which conditions the releases of induced volatiles are particularly high. This could be coincidence, but perhaps also the result of adaptations by both plant and insect to each other activities.

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CHAPTER 6.

GENERAL DISCUSSION AND CONCLUSIONS

The determination of which factors are important for the emission of induced volatiles for parasitoid host location is an essential step to enhance the attractiveness of maize plants. As summed up in table 6-1, some studies already looked at some of these factors, but the results are sometimes controversial and vary according to the tritrophic system studied. The objective of this study was to evaluate all possible factors that determine the variation and specificity of induced odour in maize. We classified the factors into biotic and abiotic factors. Biotic factors included the effect of herbivore instar, plant genotype, and the age of plants and leaves. Abiotic factors included factors like soil and air humidity, temperature, light and fertilisation.

Price *et al.*, 1980 were the first to suggest that "insect-plant interactions cannot progress realistically without consideration of the third trophic level" and that "the third trophic level must be considered as part of a plant's battery of defence against herbivores". A large number of studies have since showed how plants provide cues that attract natural enemies (see for review Dicke and Vet, 1998; Dicke, 1999a; Turlings and Fritzsche, 1999). It is now well established that plants emit induced odour under herbivory, but there is an on-going discussion about the function and specificity of the induced volatile signal. There are some examples, which indicate that the plants provide a specific signal that give information to the natural enemies on the suitability of the potential hosts or prey, but several other studies showed that such specificity does not always occur (Table 6-1). A good understanding of specificity potential requires an understanding of the mechanisms and the factors that determine variability.

Table 6-1. Examples of specificity or non specificity of induced plant odour to natural enemies of insects herbivores

System	Type of specificity	Literature	System	No specificity	Literature
Plant Apple (*Malus domestica*) Herbivores *Tetranychus urticae, Panonychus ulmi* (Acari: Tetranychidae) Predators *Amblyseius potentillae* *A. finlandicus* (Acari: Phytoseiidae)	Predatory mites attracted to apple leaves attacked by their prefrred prey *P. ulmi*. Different ratios of compounds in the volatile mixture.	Sabelis and van de Baan, 1983 Dicke *et al*., 1990 Takabayashi *et al*., 1991	Plant Maize Herbivores regurgitant *Spodoptera frugiperda*,, *S. exigua*, *Heliothis zea*, *Trichoplusia ni*, *Anticarcia gemmatalis* (Lepidoptera: Noctuidae) Parasitoids *Cotesia marginiventris* (generalist) *Microplitis croceipes* (specialist)	Both parasitoids generally attracted to corn incubated in insect regurgitant, even when the regurgitant is from none hosts Induced odours very similar in all cases	Turlings *et al*., 1993
Plant Broad bean (*Vicia faba*) Herbivore *Acyrthosiphon pisum, Aphis fabae*(Homoptera: Aphididae) Parasitoid *Aphidius ervi* (Hymenoptera: Braconidae: Aphidiinae)	Parasitoid attracted to plant on which specific host (*A. pisum*) is feeding Emission of 6-methyl-5-hepten-2-one only by plants fed on by A. pisum	Guerrieri *et al*., 1999 Du *et al*., 1998	Plant Cabbage Herbivore *Pieris rapae* (Lepidoptera: Pieridae) *Pieris spp* Parasitoids *Cotesia rubecula* (Hymenoptera: Braconidae) (specialist on *P. rapae*) *C. glomerata* (generalist on *Pieris spp* and *Aporia crataegi*)	No distinction between cabbage plants fed on by host and non-host herbivores.	Geervliet *et al.*, 1994
Plants Maize (*Zea mays*) Herbivore P*seudatelia separata* (Lepidoptera: Noctuidae) Parasitoid *Cotesia kariyai* (Hymenoptera: Braconidae)	Parasitoid attracted more by plants with young larvae Different odour profiles induced by different larval instars	Takabayashi *et al*., 1995	Plant Brussel sprout (Brassica oleracea) Herbivore *Pieris brassicae* (Lepidoptera: Pieridae) Parasitoid *Cotesia glomerata* (Hymenoptera. Braconidae)	Parasitoid do not distinguish between plants fed on by young larvae and plants fed on by late instar larvae	Mattiacci and Dicke, 1995
Plants Tobacco (*Nicotania spp*.), cotton (*Gossypium hirsutum*), maize (*Zea mays*) Herbivores *Heliothis virescens, Helicoverpa. zea* (Lepidoptera:Noctuidae) Parasitoid *Cardiochiles nigriceps* (Hymenoptera: Braconidae)	Parasitoid more attracted to plants fed by its specialised host. Different chemical profiles emitted by plants attacked by the different herbivores	De Moraes *et al*., 1998	Plant Cotton (*Gossypium hirsutum*) Herbivores *Spodoptera exigua*, *H. zea* (Lepidoptera: Noctuidae) Parasitoids *Cotesia marginiventris*, *Microplitis croceipes* (Hymenoptera: Braconidae)	*M. croceipes* is attracted to odour released systemically after feeding by the non host, *S. exigua*. No differences in the induced odour blend emitted by plants attacked by host and non host caterpillars	Röse *et al*., 1998

Through out all tests, it was apparent that the volatiles emitted by maize plants after herbivore attack is quite variable (Table 6-2). Changes in temperature, soil and air humidity modify the amount and the quality of the odour released by plants to a limited extent (Table 6-2). The effects of these environmental conditions on the production of plant secondary metabolites have been intensively studied, for plants that are of interest because of their flavouring and pharmaceutical properties. In a review by Gershenzon (1983), the effect of water stress on several secondary plant metabolites are discussed. For example, in mint (*Mentha piperita*), irrigation of the soil decreases the concentration of essential oil per plant. Not only the quantity, but also the composition of the essential oil can be affected by soil humidity. The proportion of monoterpenoids in the essential oil of *Thymus serrulatus* decreases with an increase of the soil humidity (Gershenzon, 1983). Based on studies with lima bean, Takabayashi *et al.* (1994) showed that water stress can increase the attraction of predatory mites to plants. Their study was done with undamaged plants. As suggested by the authors, it would be interesting to look at the effect of water stress on the odours induced by spider mite infestation (Takabayashi *et al.*, 1994).

Table 6-2. Effect of different factors on the variation in induced odour emitted by maize plants.

Factors	Variation in quantity	Variation in quality
Biotic		
Larval instar of *S. littoralis*	-	-
Amount of damage on plant	+++	+
Age of plant	+++	++
Leaf position on plant	+	+
Genotype of maize and wild relative of maize	+++	+++
Abiotic		
Soil humidity	++	+
Air humidity	++	-
Temperature	+	+
Light intensity	+++	+
Light cycle	+++	?
Fertilisation rate	+++	+

Differences in induced volatiles were more dramatic when plants were subjected to different light intensities and varying nutrient levels. The release of induced odour is highly correlated with the presence of light, and the amount of odour increases with an increase in light intensity (Chapter 5). This suggests that the emission of induced volatiles is mainly controlled by the photosynthetic activity in the plant. In cotton, Paré and Tumlinson (1997) stressed the importance of light and photosynthetic activity on the production and emission of induced volatiles. Other studies showed that the release of induced odour by plants was correlated with light (either light intensity or day length), but the effect of temperature, which also increased with the light intensity was never taken into account (Loughrin *et al.*, 1994; Takabayashi *et al.*, 1990; Turlings *et al.*, 1995). Recently, Halitschke *et al.* (2000) showed that the emission of induced volatiles by tobacco after jasmonic acid induction are strongly light-dependent, even under constant temperatures. In maize also, changes in light intensity have a stronger effect on the emission of volatiles than changes in temperature (Chapter 5). The nutrition of the plants also turned out to be an important factor in determining the amount of odour released by the maize plants. Plants that were cultivated under low nutrition level also had low emission rates. It is possible that under such conditions, the available nutrients in the growth of the plant may be a higher priority than investment in its defence, which is not what would be predicted by the carbon-nutrient balance theory (Bryant *et al.*, 1988; Gershenzon, 1994).

Of the biotic factors that were tested, the larval instar of *S. littoralis* feeding on maize appeared to have very little effect on the induced odour (Table 6-2). Chemical analysis revealed only small differences in the total amount of volatiles emitted and in the proportion of the compounds in the blend (Chapter 2). Major differences were the result of differences in the amount of damage that the larvae inflicted. *M. rufiventris*, a parasitoid of *S.littoralis*, can only lay eggs in young larvae, but it did not distinguish among plants fed on by different

instar larvae and was also attracted to plants attacked by larvae of the unsuitable instar. This is not always the case, Takabayashi *et al.* (1995) reported that the odour release by maize varies quantitatively and qualitatively depending on the herbivore instar of *P. separata* feeding on it, and that the attraction of the larval parasitoid *Cotesia kariyai* varies accordingly. Maybe elicitors in *P. separata* oral secretion are different for the different instars and thus change the induction of odour in maize plants. We analysed regurgitants of *S. littoralis* from different instars and found the potent elicitor volicitin in all of them. The inability of parasitoids to recognise plants fed on by suitable hosts was already shown in systems (Mattiacci and Dicke, 1995; Turlings *et al*., 1993a; Röse *et al*., 1998).

Emission of induced volatiles was also affected by the age of plants and leaf position. Plants older than 2 weeks showed a steady decrease in odour emission with age (Chapter 3). Although young leaves released more odour when attacked than older leaves in absolute amount, this difference was solely due to differences in size of the leaves. Apparently there is no differential investment in this type of defence among the leaves. Two weeks old plants are particularly vulnerable to attack than older plants. Parasitisation of herbivores can in some cases reduce their feeding and then benefit the plant under attack (Maria-Elena Fritzsche-Hoballah, unpub. data). Effective attraction of parasitoids may be most beneficial to young plants. For larger plants, this may also be true for young leaves. It remains to be investigated if in older plants investment in induced emission differs with leaf age. It seems plausible that the plants invest more in protecting the growing parts of the plants, than in parts that will contribute less to their overall fitness.

The most dramatic variability in induced odour quantity and quality was observed for the different genotypes of cultivated and wild maize (Chapter 4). In cultivated maize, a 3-fold difference in the total amount of volatiles released was observed among varieties. This was even more dramatic among different species of teosinte (Chapter 4). The genotypic variation

was not only quantitative but also qualitative. The odour profile varied considerably with variety. Some varieties and teosinte did not release β-caryophyllene or only in minute quantities, while in other it was the dominant compound. The proportions of several compounds showed clear differences among the different genotypes. Such differences can lead to completely different odour blends. It appears that the full potential of releasing induced volatiles has been conserved during selection from wild maize to modern cultivated maize. This is the first study to draw such a conclusion. A previous comparison between the induced odour of naturalised and cultivated cotton found that cultivated cotton releases significantly less volatiles (Loughrin *et al.,* 1995). The wide range of variability observed in the induced odour of cultivated maize and its wild ancestors contrasts with the notion that the odours may contain specific information that indicates which herbivores or which stages of larvae are feeding on a plant (Takabayashi *et al.*, 1995; De Moraes *et al.*, 1998; Guerrieri *et al.*, 1999; Dicke, 1999b).

It remains to be determined how this is possible with so much variation in the intensity and quality of the odour signal. Analyses of collected odours have revealed some differences that could explain how natural enemies may be attracted to plants fed on by the right instar larvae or by the right host (Takabayashi *et al.*, 1995; Du *et al.*, 1998), but usually the differences in the odour blend among plants fed on by different herbivores species are not clear (De Moraes *et al.*, 1998; Turlings *et al.*, 1998). The range of variation observed in induced maize odour appears to be much larger than the differences found among different herbivores. The degree of specialisation of parasitoids may play an important role in their

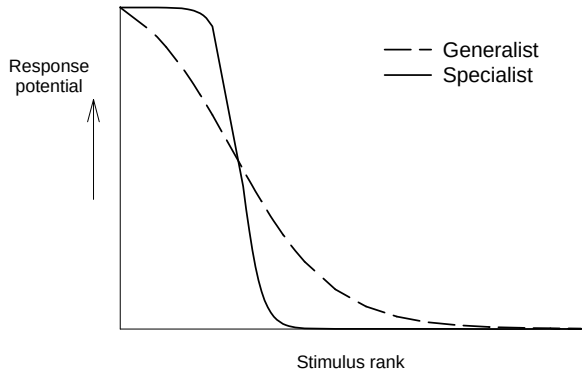

Fig. 6-1. Differences in response potential curves between specialist and generalist parasitoid species (From Vet *et al.*, 1990)

need and ability to distinguish among odours induced by different herbivores (Fig. 6-1). A specialist parasitoid with a reduced host range is likely to use different cues to locate suitable hosts than a generalist parasitoid. Vet and Dicke (1992) suggested that, depending on the degree of specialisation in host plant use of the herbivore and on the degree of specialisation of the natural enemy, different patterns of response can be expected. If the specialisation is strict for the herbivore and for the natural enemy, then the response by natural enemy to herbivore induced synomone should be strongly congenitally fixed (Vet and Dicke, 1992). They further argue that if a natural enemy specialises on a generalist herbivore it has to deal with the variation of induced plant odours produced by different plant species. Here we see that variation within one plant species can already be considerable. Depending on environmental conditions, plant age, host density and particularly plant genotype, plant-provide foraging cues will vary. One way of dealing with this variation may be through associative learning, which allows a flexibility in the foraging behaviour, and may increase the wasps' efficiency by concentrating their efforts on cues that they associate with oviposition experiences (Vet, 1983; Godfray and Waage, 1988; Lewis and Tumlinson, 1988; Vet and Groenewold, 1990; McCall *et al.*, 1993; Turlings *et al.*, 1993b; Cortesero *et al.*, 1995) Cortesero *et al.* (1997) suggest that different parasitoids, like the generalist *C. marginiventris*

and the specialist, *M. croceipes* rely on different cues to find a host. Both parasitoids are attracted by systemic induced odour released by cotton plants attacked by *S. exigua*, which is not a suitable host for *M. croceipes* (Cortesero *et al.*, 1997a; Turlings *et al.*, 1993a). However, after *M. croceipes* detects the presence of host frass on undamaged cotton leaves that release systemically produced odour, they show an increased attraction to these leaves (Cortesero *et al.*, 1997a). For specialist parasitoids, substances from host frass represent a source of specific information allowing discrimination between host and non host species feeding on a plant. Because the frass emits very little odour, the wasp will still have to exploit the less reliable plant odours. The results found by Cortesero *et al.* (1997) fit the predictions suggested by Vet *et al.* (1990) on the reliability-detectability problem, which is illustrated on figure 6-1. Eventually the parasitoids will have to show us what they perceive and what is important in their initial and subsequent responses to plant signals. Probably the distinction that some parasitoids show in their foraging behaviour is the result of differences in detection specificity that natural enemies have evolved rather than a result of adaptations by plants to attract specific natural enemies. The specificity of odour released by plants under attack by herbivore is not general to all systems, and even for one plant species, like maize, different results have been obtained. Table 6-1 lists the studies that are in favour of signal specificity and studies that, on the contrary, show a relatively low degree of specificity. The question on specificity remains open. Studies on genetic mechanisms involved in the production of induced odour may lead to some answers. If plants respond differently to different herbivores, it may well be because of different elicitors such as ones in caterpillar oral secretions, as it was suggested by Paré and Tumlinson (1999).

From an applied point of view, and as was pointed out by Cortesero *et al.* (2000), different factors can enhance the effectiveness of natural enemies on infested plants such as extra food sources, protection via trichomes and domatia. Induced odour is one plant attribute,

which has not been taken into account by breeders in order to increase the efficiency of biological control agents (Cortesero *et al.*, 2000). The observed variation in induced odour among genotypes gives us the opportunity to test what is important in the odour blends for the attraction of parasitoids. This knowledge can lead to further plant manipulations and breeding to create plants highly responsive to herbivore attack that emit an odour that is very attractive to parasitoids. Also, a better understanding of the effect of environmental factors on the odour production can help in determining growing conditions that can lead to higher and more attractive odour emission, and thus enhance the efficacy of natural enemies.

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CURRICULUM VITAE

Sandrine GOUINGUENE

Date of birth : 12.06.72 (28 years old) at Clichy (92), France.

EDUCATION

1996-2000:	PhD student at the University of Neuchâtel, Institute of Zoology, Lab. Of Animal Ecology and Entomology.
1995-1996 :	Master student, Analytical Chemistry and Quality of Bioproducts, at the INAPG (National Institute of Agronomy at Paris-Grignon), in Paris (75), France. Passed degree.
1992-1995:	Engineer student at the ENITIAA (National Engieering School of Food Science Techniques, Higher education), in Nantes (44), France. Passed degree.
1990-1992 :	Student in Bio Maths Sup. and Bio Maths Spé to prepare to competitive exams for entrance in Agronomic Higher Education school, leading to the engineering degree.
1990 :	Baccalaureate D (Maths, Biology).

RESEARCH PROJECTS

Expertise in analytical chemistry (GC, GC-MS), insect behaviour (olfactometer, flight tunnel), collection and extraction of odours, insect rearing (parasitoids, noctuids larvae and adults), electroantennography, computer programming (Pascal, excel macro), statistical analysis (experimental design, classical parametric and non parametric tests, bootstrap, robust statistic, multiple analysis statistic).

2001:	Electrophysiological responses of parasitic wasps to induced plant odours Integrated Approach to Crop Research (IACR), Biological and Ecological chemistry department,Rothamsted, Harpenden, UK.
1996-2000:	Specificity and variation of induced volatile signalling in maize plants. PhD project at the Laboratory of Animal Ecology and Entomology, under the supervision of Dr Ted Turlings, Institute

of Zoology, Neuchâtel, Switzerland.

***1997 (February-April)*:** Effects of associative learning on the olfactory responses of a parasitoid, *Cotesia marginiventris* (Hymenoptera:Braconidae). US Dept. of Agriculture, Charleston, South Carolina, USA.
In collaboration with Dr. Wilant van Giessen (USDA, Charleston, USA), Dr. Ted Turlings (University of Neuchâtel, Switzerland), and Dr. Felix Wäckers (ETH-Zurich, Switzerland).

1996 (January-June:) Thermal modulation of the elution of an odorant compound. Application to the Gas Chromatography-Electroantennography coupling. In order to pass the Master degree. Realised at the Dpt of Phytopharmacy - Laboratory of chemical mediators of the INRA (National Institute of Research in Agronomy), in Versailles (78), France.

1995 (March-September): Gustatory quality and chemical composition of five tomato varieties cultivated in greenhouse. To obtain the engineering degree. Realised at the CTIFL (Interprofessional Technical Centre on Fruits et Vegetable) in Carquefou (44), France.

1994-1995 (October-March): Optimisation of the extraction of a seafood flavour from an algae, the *Fucus serratus*. Laboratory of Food Biochemistry of the ENITIAA in Nantes (44), France.

ATTENDANCE OF CONGRESSES

2000 (August 20-26) XXI International Congress of Entomology.
Poster presentation: "Variation and specificity of herbivore-induced plant odors as foraging cues for parasitoids.". Foz do Iguassu, Brazil.

2000 (August 15-19) 17^{h} annual meeting of the International Society of Chemical Ecology.
Oral presentation : "Factors that determine variation in induced maize volatiles ". Poços de Caldas, Brazil.

2000 (February 18-19) Zoologia et Botanica '00
Oral presentation : " Factors that determine the usefulness and reliability of herbivore-induced plant odours as foraging cues for parasitoids.". Lausanne, Switzerland

1999 (December 12-16): Annual meeting of the Entomological Society of America.
Replacement of Dr. Ted Turlings as invited speaker, oral presentation: " Manipulating herbivore-induced odor emission in maize plants to increase their attractiveness to beneficial insects". Atlanta, Georgia, USA.

1999 (November 13-17): 16th annual meeting of the International Society of Chemical Ecology.
Oral presentation: "Factors that determine variation in induced maize volatiles". Marseilles, France.

1999 (February 17)	Zoologia et Botanica '99 Oral presentation : " How parasitoids of caterpillars find a suitable host?". Zurich, Switzerland.
1998 (July 4-10):	10th International Symposium on Insect-Plant Relationships. Poster presentation: "Induction of odour emissions in maize plants by different larval instars of *Spodoptera littoralis* (Lepidoptera:Noctuidae)". Oxford, UK.
1998 (June 20-24):	International Society of Chemical Ecology, 15th Annual Meeting. Poster presentation: "Induction of odour emissions in maize plants by different larval instars of *Spodoptera littoralis* (Lepidoptera:Noctuidae).", not attended. New York, Ithaca, USA.
1997 (July 12-16):	The XI International Entomophagous Insects Workshop. Oral presentation: "Experience and peripheral sensitivity to plant volatiles in *Cotesia marginiventris",* . Madison, Wisconsin, USA.
1997 (March):	Entomological Society of America- Souteastern branch. Oral presentation: "Thermal modulation of the elution of an odorant compound. Application to the Gas Chromatography-Electroantennography coupling". Asheville, North Carolina, USA.

TEACHING AND TRAINING EXPERIENCES

2000 (January 28):	Invited speaker to the course "ECOFOC", Applied entomology section. 1 h.
1999(May-June):	Training of the lab technician student : hydroponic cultures of maize, collection and extraction of odour, gas chromatography analysis.
1997-2000 (Summer semesters):	Participation to the practical course of Chemical Ecology. Organisation and supervision of small research projects led by students. 6 lessons of 3 h.
1998-2000 (Winter semesters):	Participation to the teaching of the Statistic course (Base of statistic), presentation and training on the use of excel and statistic software (SPSS and Statview). 4 lessons of 3h.

GRANTS OBTAINED

2001 Post doc fellowship for young scientists (Bourse pour chercheurs débutants) to study the electrophysiological responses of parasitic wasps to induced plant odours at Integrated Approach to Crop Research (IACR), Biological and Ecological chemistry department,Rothamsted, Harpenden, UK.

2000 : Student travel grant from the Swiss Zoological Society to attend the 17th congress of the International Society of Chemical Ecology in Brazil.

1997 (February) : Grant from Wutrich and Mathey-Dupraz fundings. This grant supported the project on the effects of associative learning on the olfactory responses of a parasitoid, *Cotesia marginiventris* (Hymenoptera:Braconidae). US Dept. of Agriculture, Charleston, South Carolina, USA.

ORGANISATION OF CONGRESS AND WORKSHOP

2000 (September 25-27): Participation to the organisation of a IIIème Cycle Romand workshop on "Parasitoids: Biology, Ecology and Application", organised by Dr. T. Turlings and Dr. B. Benrey, at University of Neuchâtel, Switzerland.

1998 (September 18-20): Participation to the organisation of a IIIème Cycle Romand workshop on "The coevolutionary process", organised by Dr. B. Benrey, Pr. B. Betschart and Pr. M. Rahier, at Le Peupéquignot, Switzerland.

PROFESSIONAL EXPERIENCES

1998-1999 (October): Wine quality controller for the laboratory of food safety and quality of the canton of Neuchâtel, Switzerland. Control of the sugar in the grape juice for the classification of the wine.

1996 (December): Hotel employee, Novotel, Paris, France. Breakfast preparation and room-service.

1996 (August): Bank employee at the Crédit du Nord, Paris, France. Division management of enterprises.

1995 (July-August-September):	Quality controller at Omospondia, Thessaloniki, Greece. Control of the quality of peaches for peach syrup products and of the microbiological quality of tomato paste.
1993 (August):	Bank employee at the Crédit du Nord, Paris. Division management of the patrimoine.
1992 (July-August):	Leader group of teenager linguistic camp in Ireland. Management of the budgets and organisation of activities (sports, visits).
1989-1991:	Leader group of teenager ski camps or summer camps.

LANGUAGES

- **French** : native language.
- **English** : Fluent, daily use.
- **Spanish** : school level.

PUBLICATION LIST

Gouinguené S. , de Cruz I., van der Pers J., Wadhams L., Marion-Poll F., 1998, A new method to improve olfactory responses to GC effluents. Chemical Senses 23: 647-652.

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Turlings Ted C. J., Gouinguené Sandrine, Degen Thomas and Fritzsche Maria Elena, The chemical ecology of plant-caterpillar-parasitoid interactions *in* Teja Tscharntke & Bradford A. Hawkins (Eds), Multitrophic level interactions, Cambridge University Press. In preparation.

GENERAL INFORMATION

- Member of the Swiss Society of Zoology.
- Member of the International Society of Chemical Ecology.