Can we attract parasitoids by chewing on a plant? Phytochemical induction in the *Cotesia-Pieris-Brassica* system

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**INTRODUCTION**

Parasitoids use stimuli from different odour sources to locate their hosts. These stimuli are many and varied, and the behaviours that the stimuli elicit in various species of parasitoids are equally varied. In the last 20 years research has been focused on the modalities and nature of the stimuli involved, and the behaviours that these stimuli elicit (reviewed e.g. by Vinson, 1991 and Godfray, 1994).

Already at the beginning of the 1980s, Price and coworkers (1980) emphasized that all terrestrial communities are based on at least three interacting trophic levels: plants, herbivores and natural enemies of herbivores. They argued that ecological research could not progress realistically without consideration of the interactions among all levels involved.

In this framework an impressive amount of experimental evidence has been provided in the last few years to demonstrate that parasitoids use plant volatiles to locate their herbivore hosts (for reviews see Dicke et al, 1990b, Vet & Dicke, 1992, Turlings et al, 1993b, Dicke, 1994). Foraging parasitoids can even discriminate between the volatiles emitted by mechanically-damaged plants and those of a herbivore-damaged plants. Recently the concept of semiochemically mediated host-location in a tritrophic context has been extended to ecosystems based on stored products such as wheat grain and clothes. For example *Lariophagus distinguendus*, a parasitoid of the granary weevil *Sitophilus granarius*, can discriminate between the volatiles emitted by infested grains vs. non infested grains (Steidle and Schöller, 1997). Tackács et al (1997) demonstrated that *Apanteles carpatus*, a braconid parasitoid of Tineid larvae locates the host through chemicals emitted by host-infested beaver or rabbit pelts which are preferred to non-infested pelts.

In contrast with the wealth of knowledge available on the behavioural and semiochemical aspects of the tritrophic interactions, little is known about the...
biochemical factor(s) eliciting volatiles production and emission in the host-food (plant or non-living substrate).

In this paper we discuss the function of a digestive enzyme of a herbivore as elicitor that affects the production of parasitoid attractants by the herbivores food plant. The investigation was conducted on a system of Brussels sprouts leaves (*Brassica oleracea* var. *gemmafera*), caterpillars of the large cabbage white butterfly, *Pieris brassicae*, and the parasitoid *Cotesia glomerata*. Furthermore we present data suggesting that the enzyme is a widely distributed and general elicitor of these type of plant responses, exploited by insect carnivores.

**The tritrophic system**

*Cotesia glomerata* is a gregarious larval parasitoid of several pierid species such as the cabbage white caterpillars *Pieris brassicae* (L.) and *Pieris rapae* (L.). In a green-house flight chamber, female *C. glomerata* discriminate among undamaged, mechanically-damaged and caterpillar-infested cabbage plants (Steinberg et al. 1992, 1993). The plant-herbivore complex (PHC), where host larvae are actively feeding, is most attractive. Yet, after removing the host larvae the herbivore-damaged plant (HD) remains very attractive to the parasitoids for at least several hours. In contrast, the attractiveness of mechanically-damaged cabbage plants quickly wanes after the infliction of the damage is stopped (Steinberg et al. 1993). Apparently herbivore damage results in a different response from the plant than mechanical damage (Steinberg et al. 1993). Cabbage plants respond to caterpillar (*Pieris brassicae*) herbivory by releasing a mixture of volatiles which makes them highly attractive to parasitic wasps (*Cotesia glomerata*) that attack the herbivores. Furthermore *Cotesia glomerata* is clearly able to distinguish between different herbivore infested plant species and cultivars. Infested plants are always preferred to non-infested ones, even when infested by non-host larvae (Geervliet et al. 1996).

Infested cabbage leaves that are mechanically damaged and subsequently treated with gut regurgitate of *P. brassicae* caterpillars, release a volatile blend similar to that of herbivore damaged plants. Flight chamber dual choice tests showed that mechanically damaged cabbage leaves were less attractive than herbivore damaged leaves and mechanically damaged leaves treated with larval regurgitate. Chemical analysis of the headspace of undamaged, mechanically damaged, caterpillar infested and caterpillar regurgitate-treated mechanically-damaged leaves, showed that the plant responds to damage with an increased release of volatiles. Green leaf volatiles and several terpenoids are the major components of cabbage leaves headspace. Terpenoids are emitted in analogous amounts in all treatments, including undamaged leaves. On the other hand, if the plant is infested by caterpillars or if caterpillar regurgitate is applied to damaged leaves, the emission of green leaf volatiles is highly enhanced (Mattiacci et al, 1994).
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Phytochemical induction

It is well-known that plants may react to herbivory or to pathogen infestation by phytochemical responses (reviewed in Dixon 1986, Tallamy & Raupp 1991, Dicke 1994). The first step in such responses is the recognition of the attack by the plant. How plants recognise infestation of a pathogen has been intensively studied and many pathogen-derived exogenous elicitors of phytoalexins have been identified (Ryan & Farmer, 1991). In contrast, knowledge on the recognition of herbivorous arthropods by plants is scarce, being mostly restricted to the involvement of herbivore secretions (e.g. Miles 1969, Lin et al. 1990, Hartley & Lawton 1991). Yet, a wealth of knowledge is available on endogenous elicitors that originate from mechanical damage (Farmer & Ryan 1992) and on subsequent steps in the signal transduction pathway (Farmer & Ryan, 1992, Enyedi et al. 1992) in responses of plants to herbivores.

A well known phytochemical response, studied in the past decade, is the production of volatiles that attract carnivorous arthropods such as predators and parasitoids of herbivore insects (reviewed by Dicke 1994). For instance, lima bean plants respond to infestation by the spider mite *Tetranychus urticae* by producing volatile terpenoids and methyl salicylate that attract a predator (*Phytoseiulus persimilis*) of these herbivores (Dicke & Sabelis 1988, Dicke et al. 1990a, Dicke et al., 1990b, Dicke 1994). Recently, studies have been initiated on exogenous herbivore elicitors of this plant response. For instance, the response of corn plants to herbivory by fall armyworm caterpillars is similar to the response to administration of caterpillar regurgitate into a mechanical wound or fed through the petiole of an intact corn leaf (Turlings et al. 1990, 1993). These treatments result in the production of volatile terpenoids and indole that attract the parasitoid *Cotesia marginiventris*. The elicitor in the armyworm regurgitate has been recently identified (Alborn et al., in prep.). A very interesting clue on the identity of the elicitor of herbivore-induced plant odors came from a study of the biosynthesis of 2 homoterpenes that are emitted by spider-mite infested lima bean and by regurgitate-treated corn plants. Boland and co-workers (Boland et al., 1992, Hopke et al., 1994) showed that application of β-glucosidase onto mechanically damaged lima bean leaves resulted in the production of one of the carnivore attractants (Dicke et al., 1990a). Application of β-glucosidase onto undamaged leaves had no effect, neither did mechanical damage without application of β-glucosidase. This reflects the data on spider-mite damaged, mechanically damaged and undamaged lima bean leaves (Dicke et al., 1990a). Other enzymes such as lipases were not effective. This suggested that β-glucosidase may be an herbivore-related elicitor. However, this could not be concluded until its existence in herbivore secretions, its effect on the total volatile blend emitted, and the attraction of carnivores towards β-glucosidase-treated leaves has been demonstrated.
β-glucosidase as an elicitor of phytochemical induction

Based on the above-mentioned studies, we decided to investigate if salivary enzymes could play a major role in phytochemical induction in the cabbage system. In fact, we were able to demonstrate (Mattiacci et al., 1995) that β-glucosidase applied onto cabbage plants has the same effect as the regurgitate of the caterpillar *Pieris brassicae*: it results in the emission of a similar bouquet of volatiles and in the attraction of the parasitoid *Cotesia glomerata*. Moreover, we showed that β-glucosidase is present in caterpillar regurgitate.

In a flight bioassay, leaves treated with almond β-glucosidase (Sigma) were highly attractive to the parasitic wasp *C. glomerata*, and parasitoids did not discriminate between leaves treated with β-Glucosidase and leaves treated with caterpillar regurgitate (Fig. 1, β-GLU vs. AD and β-GLU vs. REG). However, the application of β-glucosidase was only effective when the leaf surface was mechanically damaged. β-Glucosidase was also recorded in cabbage leaf extract, but this was not as effective as caterpillar β-glucosidase in eliciting the volatile production. Caterpillars that feed on a β-glucosidase-free diet, secrete the enzyme and their regurgitate is an effective elicitor of the plant response. These findings showed that β-glucosidase is secreted by *P. brassicae* and it is an elicitor of the defence response of cabbage plants to herbivore injury, inducing the emission of volatiles that are used by parasitoids of the herbivore to locate their victims.

Is β-glucosidase a generalised elicitor?

Specificity of volatile induction by herbivores varies among different tritrophic systems and depending on the mechanisms and elements (e.g. species or age of the herbivore) inducing the phytochemical response (Takabayashi et al., 1991 and 1995, Turlings et al., 1995). *Cotesia glomerata* is attracted to cabbage plants damaged by various herbivores, including non-hosts (Geervliet et al., 1996). Such an unspecific response could be due to an unspecific, but widespread elicitor such as β-glucosidase. In order to test this hypothesis, we decided to apply a non-host-related type of “regurgitate” to cabbage leaves, human saliva, and to test if the pattern of response of plant and parasitoid would be similar to that of caterpillar damaged plants.

MATERIAL AND METHODS

Behavioural observations were performed in a flight chamber set-up consisting of a "tent" made of white cotton sheets inside a greenhouse compartment, at 22 ± 2 °C and 60 ± 10 % RH. Cabbage leaves, with the petiole in a glass vial with water, were placed on a table over which two electrical fans generated an airstream of 30-40 cm/sec. The leaves formed an equilateral triangle
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with 40 cm sides with the release point, from where the wasps were individually released. In order to minimize visual stimuli a white screened cloth (20 mesh) was placed between the release point and the test leaves. One ml human saliva was applied on mechanically damaged leaves 18 h prior to the bioassay. As a control artificially damaged leaves (AD) were treated with distilled water only (more details on the bioassays in Steinberg et al., 1992, Mattiacci et al., 1994).

Volatile emitted by excised and treated cabbage leaves were collected on Tenax-TA traps for 1 hour and analysed in a gas chromatograph-mass spectrometer as described in Mattiacci et al. (1994).

Presence of β-glucosidase activity in the test material was determined through incubation with 5 mM 4-nitrophenyl-β-D-glucopyranoside (Boehringer) in 1 ml 0.1 M NaOH-citrate buffer at pH 6.0. Absorbance of the samples was measured in a Zeiss PMQ3 spectrophotometer (Mattiacci et al., 1995).

RESULTS

In the two-choice flight chamber bioassay, we observed that mechanically damaged cabbage leaves treated with human saliva exerted a significant attraction on the parasitoids over mechanically damaged leaves (Fig. 1, HS vs. AD). Cabbage leaves treated with human saliva release a volatile blend that is very similar to the blend emitted by regurgitate or β-glucosidase treated cabbage leaves (Fig. 2). For a comparison with undamaged and mechanically damaged leaves, see Mattiacci et al., 1994. β-glucosidase was detected in human saliva with an activity of 0.10 ± 0.04 units/ml (mean ± SD, n=3) with 4-nitrophenyl-β-D-glucopyranoside as substrate. This activity is similar to the activity of about 5 larval equivalents of caterpillar regurgitate (=25 ml: 0.074 ± 0.012 units/25 ml, Mattiacci et al., 1995).

DISCUSSION

In recent work (Turlings et al., 1993, Mattiacci et al., 1994, Potting et al., 1995) it has been shown that the induction of plant volatiles by herbivores is based on the interaction of components from the salivary secretions of the feeding herbivore with the endogenous signal-transduction pathways of the plant’s defence response. For the lima bean and the cabbage systems, it has been demonstrated that β-glucosidase is an elicitor of the plant volatiles mediating the interaction among plants, herbivore and carnivore insects (Boland et al., 1992, Hopke et al, 1994, Mattiacci et al., 1995). So far only for the cabbage system it has been shown that parasitoid response to β-glucosidase treated plants is similar to that one to caterpillar damaged plants (Mattiacci et al., 1995).
Figure 1: Flight response of female C. glomerata to cabbage leaves treated with β-glucosidase (β-GLU), with caterpillar regurgitate (REG), with human saliva (HS) and artificially damaged (AD). Bars represent parasitoid choices for the treatment indicated on the side of the bar. Number of replicates = 50. Numbers next to bars indicate percentage of parasitoids making a choice for one of the two odor sources, or not making a choice at all (% no response). Asterisks indicate significant differences within the choice test *** P<0.001. Chi square for Goodness-of-fit (Sokal and Rohlf, 1981).
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Figure 2: Total Ion Chromatograms of headspace collections of cabbage plants that underwent different treatments. Treatments and mean total peak area (between brackets) are as follows: A βglu= β-glucosidase (from almonds) treated leaves (56,923), B REG= caterpillar regurgitate treated leaves (50,475), C HS= leaves treated with human saliva (26,638). Peaks are labelled with a number corresponding to the identified chemicals. 1= hexanal; 2= (E)-2-hexenal; 27= 3-methyl-3-buten-1-yl acetate plus (E)-2-hexenal; 3= nonanal; 4= decanal; 5= 3-pentanone; 6= 1-penten-3-ol; 7= 1-hexanol; 8= (E)-2-hexen-1-ol; 9= (Z)-3-hexen-1-ol; 10= 1-hexen-1-yl acetate; 11= (E)-2-hexenyl acetate; 12= (Z)-3-hexen-1-yl acetate; 13= (Z)-3-hexen-1-yl butyrate; 14= (Z)-3-hexen-1-yl isovalerate; 15= β-pinene; 16= α-thujene; 17= sabinene; 18= myrcene; 19= limonene; 20= 1,8-cineol; 21= β-elemene. All treatments were made 20 h prior to collection of headspace volatile. Unlabelled peaks eluting after peak 21 are system impurities. Other unlabelled peaks are not common to the treatments.
Glucosidases have been found in several insect orders among which Lepidoptera, where they are usually reported in the caterpillar gut (Yu 1989; Ahmad & Hopkins 1992). Almond β-glucosidase is known to be a mixture of β-glucosidases with a broad degree of substrate specificity (Conn, 1993). Plants treated with human saliva emitted a volatile bouquet similar to that of caterpillar damaged plants and they are highly attractive to C. glomerata females. We detected β-glucosidase activity also in human saliva (probably from micro-organisms). It seems likely that an unspecific β-glucosidase present in human saliva elicits the phytochemical response in cabbage and therefore affects the parasitoid response.

β-glucosidase is widespread in animal oral secretions (e.g. Yu, 1989), and it is possible that this or similar digestive enzymes, play a major role in the induction of volatile chemicals used by parasitoids to localize host infested areas.

It would be interesting to see if glucosidases are responsible for the induced responses in other tritrophic systems and if the specificity of response observed in some (Takabayashi et al., 1991, 1995) tritrophic systems is modulated by other subsequent signals within the plant. The new findings on tritrophic interactions involving insect feeding not on the vegetative parts of a plant but on substrates with different biochemical characteristics like dry seeds and animal pelts, offer interesting possibilities for further investigations.

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