

Chemically Mediated Tritrophic Plant-Insect Interactions

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Plants respond to insect feeding damage by releasing volatile organic compounds that allow natural enemies of the herbivores, such as insect predators and parasitoids, to distinguish between infested and non-infested plants and thus aid in location of prey or hosts. The biosynthesis and/or release of these volatiles is elicited by interaction of compounds in the saliva or oral secretion of the herbivores with damaged plant tissues. Such chemically mediated tritrophic plant-insect interactions have been documented for several crop plants, including lima beans (Dicke et al., 1990), cucumbers (Takabayashi et al., 1994), corn (Turlings et al., 1990, 1993), and cotton (DeMoraes et al., 1998). Tritrophic systems involving natural enemies of insect herbivores are of great interest because of the need to develop effective methods for biological control of insect pests of agricultural crops.

Elicitors of Plant Volatiles from Insect Herbivores

In cotton and other plants, volatiles are released in greater quantities as a result of caterpillar feeding than with mechanical damage alone (Paré and Tumlinson, 1997). This points to the presence of a substance or substances in the saliva or oral secretions of the herbivores that interact with damaged plant tissues and induce biosynthetic processes leading to the synthesis and/or release of volatiles. Thus far elicitors of plant volatiles have been identified in the oral secretions of two species of insect herbivores.

Mattiacci, et al. (1995) reported that a β -glucosidase in the saliva of *Pieris brassicae* caterpillars elicits the release of volatiles from cabbage leaves.

Alborn et al. (1997) identified volicitin (*N*-[17-hydroxylinolenoyl]-L-glutamine) (Fig. 1) as the

major active elicitor in the oral secretion of beet armyworm, *Spodoptera exigua*, larvae. Synthesized and natural volicitin induce corn (*Zea mays* L.) seedlings to release the same blend of volatile

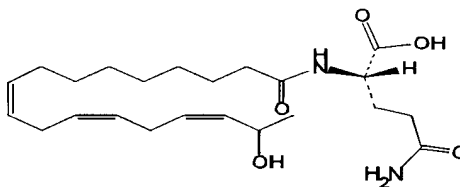


Figure 1. Volicitin

terpenoids and indole released when they are damaged by caterpillar feeding (Alborn *et al.*, 1997). More recently Alborn *et al.* (1999) reported that the oral secretion of beet armyworm larvae contains several compounds analogous to volicitin, including *N*-linolenoyl-L-glutamine, 17-hydroxylinolenic acid, linolenic acid and the linoleic acid analogs of all these compounds.

We have recently demonstrated that beet armyworm caterpillars synthesize volicitin by adding a hydroxyl group and glutamine to linolenic acid obtained directly from the plant on which the caterpillar feeds (Paré *et al.*, 1999). This strongly suggests that these molecules play an important, but still unknown, role either in the metabolism or some other process critical to the life of the herbivorous insects. It is also interesting to note that the plant is providing the linolenic acid, which is essential for most lepidopteran larvae (Stanley-Samuelson 1994, and references therein), with which the insect makes an elicitor of plant chemical defenses, seemingly detrimental to the insect. The full implications of this are not yet understood.

Plant Response to Insect Herbivory

An undamaged plant maintains a baseline level of volatile metabolites that are released from the surface of the leaf and/or from accumulated storage sites in the leaf. These constitutive chemical reserves, which often include monoterpenes, sesquiterpenes and aromatics, accumulate to high levels in specialized glands or trichomes (Paré and Tumlinson, 1997). In addition, green leaf odors, consisting of blends of saturated and unsaturated six carbon alcohols, aldehydes and esters, are produced by autolytic oxidative breakdown of membrane lipids and released when leaves are mechanically damaged. Plants respond to insect feeding damage by releasing volatiles from the damaged site that are markedly different from those released from undamaged or mechanically damaged plants. In cotton, breakage of leaf glands causes stored terpenes to be released in much higher levels and the emissions of lipoxygenase pathway green leaf volatiles also climbs. While the release of these metabolites correlates closely with leaf damage from insect feeding (Loughrin *et al.*, 1994), a subset of terpenes, a nitrogen containing compound indole, and hexenyl acetate are also released in much higher levels with insect feeding, but in a diurnal cycle that is decoupled from short term insect damage. These compounds, linalool and (*E*) β -ocimene (monoterpenes), (*E,E*) α -farnesene, and (*E*) β -farnesene (sesquiterpenes), nonatriene and tridecatetraene (homoterpenes), indole and (*Z*) 3-hexenyl acetate, have an emissions profile more similar to the light cycle with low emissions at night and high levels during the periods of maximal photosynthesis.

Chemical labeling studies have established that the compounds released in much greater quantities during the day from cotton plants damaged by

feeding insects are synthesized *de novo* and are not stored in the plant (Paré and Tumlinson, 1997b). These induced compounds rapidly incorporated a high level of label when plants damaged by feeding caterpillars were held in volatile collection chambers under an atmosphere containing ^{13}C carbon dioxide. The high incorporation of ^{13}C , detected by mass spectral analysis, and the rapid turnover of the label in experiments where short pulses of ^{13}C carbon dioxide were used indicated that the production is tightly coupled with photosynthesis. A consistent several hour delay between when insect feeding begins and the emissions of the induced volatiles supports the hypothesis that a series of biochemical reactions that includes gene expression, protein assembly and/or enzyme induction is required for the synthesis and release of these compounds.

In addition to the release of volatiles at the site of herbivore feeding, in both corn (Turlings and Tumlinson, 1992) and cotton (Rose et al., 1996), leaves distal to the site of herbivore feeding show an increase in the release of volatiles. The chemical blend of volatiles from undamaged cotton leaves differs from the volatiles collected from the entire plant (Rose et al., 1996). The products of the lipoxygenase pathway, including the hexenals and hexenols, which are released from freshly cut or damaged tissue, are not detected in the systemically released volatiles, except for (*Z*)-3-hexenyl acetate. In cotton, some of the monoterpenes and sesquiterpenes, as well as indole and isomeric hexenyl butyrates and 2-methyl butyrates, are only released from damaged leaves (Rose et al., 1996). The terpenoids that are synthesized *de novo* in cotton leaves in response to herbivore damage (Paré and Tumlinson, 1997) are also released systemically from undamaged leaves of an herbivore injured plant.

The volatiles released by several species of crop plants have been analyzed with and without insect damage. The chemical blend, as well as the total volatile release, varies among plant species, but some compounds like (*E*)-4,8-dimethyl-1,3,7-nonatriene and (*3E,7E*)-4,8,12-trimethyl-1,3,7,11-tridecatetraene have been identified in the head space of many herbivore infested plants including lima bean, apple, cowpea, cucumber, corn and cotton (Dicke 1994, Loughrin *et al.* 1994, Turlings *et al.* 1999). Also, the quantity and quality of volatiles released may vary among individual plants of the same species and can easily be influenced by variations in environmental conditions that affect the plants' physiology. Several species including corn, cotton (Loughrin & Tumlinson unpublished data) and lima bean (Takabayashi *et al.* 1994b) respond to reduced light, due to either lower light intensity or shorter day length, with a decline in the release of herbivore induced volatiles. Based on studies with lima bean, water stress seems to elevate levels of volatiles released from infested plants. Correlating this with insect preference showed that predatory mites selected plants which were infested and water stressed over infested but not water stressed plants (Takabayashi *et al.* 1994a). These results, and the fact that, in wind tunnel experiments, the parasitoid *Microplitis croceipes* is

strongly attracted to corn seedlings incubated in beet armyworm oral secretion, although it cannot parasitize beet armyworms (Turlings et al., 1993, 1999), suggested that the blend of volatiles released by a plant was influenced more by environmental and other conditions than by the species of herbivore feeding on it. However, in field trials *Cardiochiles nigriceps* females clearly distinguished between tobacco plants damaged by their host, *Heliothis virescens*, and those infested by *Helicoverpa zea*, which is not a host (DeMoraes et al., 1998). This preference of the parasitoids for plants damaged by their hosts persisted even when all caterpillars and damaged leaves were removed from the plants, leaving only the undamaged leaves from which volatiles are released systemically. Also, the wasps were able to distinguish between cotton plants damaged by their hosts and those damaged by non-hosts. Furthermore, GC-MS analysis of volatiles released by plants damaged by the two herbivore species clearly showed differences in proportions of compounds in blends induced by *H. virescens* and those induced by *H. zea* feeding on both cotton and tobacco. Thus plants do respond differently to damage by different species of herbivores and release signals that can be distinguished by specialist parasitoids. This suggests that different herbivore species produce different elicitors.

Insect Parasitoid Response to Plant Volatiles

It has been clearly demonstrated in many systems that insect parasitoids and predators use chemical cues released by damaged plants to locate their hosts or prey (Dicke et al., 1990; Takabayashi et al., 1991, 1994; Turlings et al., 1990, 1993). Wind tunnel experiments as well as field tests have shown that plants damaged by insect feeding are more attractive to parasitoids or predators than undamaged or mechanically damaged plants. For example, in field experiments with cotton and tobacco plants, 95% of native female wasps (*Cardiochiles nigriceps*) landed on plants damaged by their host, *Heliothis virescens* larvae, versus undamaged plants (DeMoraes et al., 1998). Similar results were obtained even with the damaged leaves removed, so that only systemically produced volatiles from undamaged leaves were available to serve as cues. Furthermore, as indicated earlier, this specialist wasp can distinguish plants damaged by her host from those damaged by non-host larvae, based on plant volatiles alone.

Although the volatile compounds released by insect herbivore damage are similar among the several plant species studied thus far, the specific blends are quite distinct, varying in both the number of compounds and the actual structures produced. Thus, the task of finding a host is more complicated for the parasitoid when the host feeds on several different plant species. The wasps can overcome this obstacle, because they have developed the ability to learn chemical cues associated with the presence of a host (Lewis and Tumlinson, 1988). The chemicals to which a female wasp is exposed during her interactions with her host familiarize her with

particular host location cues. A successful host experience increases the wasp's responsiveness to host-associated chemicals.

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