PLANT VOLATILE SIGNALS IN RESPONSE TO HERBIVORE FEEDING

PAUL W. PARÉ AND JAMES H. TUMLINSON
Insect Attractants, Behavior, and Basic Biology Research Laboratory,
Agricultural Research Service, United States Department of Agriculture
Gainesville, Florida 32604

ABSTRACT

A surge in release of volatiles by several plant species can be observed in response to insect feeding. Oral secretions from these feeding herbivores provide the initial chemical signal that triggers the release of plant volatiles; one or more elicitors from the oral secretion allow the plant to identify and differentiate herbivore feeding from mere mechanical wounding. Elicitor(s), in combination with mechanical wounding, trigger the release of compounds both locally and systemically. These volatiles, which may be a blend of constitutive and induced compounds, vary in their relative and absolute concentration over time. They serve as easily detectable and distinctive chemical cues for predators and parasitoids of the herbivores feeding on the plants. Volatile compounds released from herbivore infested plants include the monoterpenes and sesquiterpenes of the isoprenoid pathway, green leaf volatiles of the fatty acid/lipoxygenase pathway and aromatic metabolites, such as indole and methyl salicylate, of the shikimic acid/tryptophan pathway.

Key Words: Plant defenses, herbivores, plant volatiles, chemical cues, predators, parasitoids.

RESUMEN

La liberación de volátiles puede ser observada en varias especies de plantas cuando los insectos se alimentan de ellas. Las secreciones orales de los herbívoros mientras se alimentan de las plantas provee la señal química inicial que dispara la liberación de los volátiles. Una o más sustancias estimulantes de las secreciones orales permiten a la planta identificar y diferenciar el efecto de los herbívoros al alimentarse del efecto de las heridas mecánicas. La(s) sustancia(s) estimulante(s), en combinación con las heridas mecánicas, disparan la liberación de compuestos local y sistémicamente. Estos volátiles, que pueden ser una mezcla de compuestos constitutivos e inducidos, varían en su concentración absoluta y relativa en el tiempo. Ellos funcionan como señal química fácilmente detectable y distintiva para los depredadores y parasitoides de los herbívoros. Los compuestos volátiles liberados de plantas infestadas con herbívoros incluyen monoterpenos y sesquiterpenos de la vía de los isoprenoides, volátiles de hoja verde de la vía del ácido graso/lipoxygenase y metabolitos aromáticos, tales como el indol y el methyl salicilato de la vía del ácido shikimic/tryptophan.

When plants are subjected to pathogen or herbivore attack, they activate biochemical defenses, such as phytoalexins (Grayer & Harborne 1994) or proteinase inhibitors (Nelson et al. 1983, Pearce et al. 1993), that directly target their natural enemies. Additionally, the release of plant volatiles can cue insect parasitoids and/or predators that act as another line of plant defense (Tumlinson et al. 1993, Stowe et al. 1995). It appears that blends of volatile plant terpenoids, which are released in response to insect feeding and not to mechanical damage alone (Turlings et al. 1990, Korth et al. 1995), allow natural enemies of insect herbivores, such as parasitic wasps, to distinguish between infested and non-infested plants, thus aiding in the location of hosts or prey (Turlings et al. 1995). These phytodistress signals, which result in an active interaction between herbivore-damaged plants and a third trophic level, have been described for several plant species. Examples include (1) lima beans that produce volatiles that attract the predatory mite *Phytoseiulus persimilis* when damaged by the spider mite *Tetranychus urticae* (Dicke et al. 1993) or (2) corn plants that produce volatiles that attract the *Hymenopterous* larval parasitoids *Cotesia marginiventris* and *Microplitis croceipes* when under attack by *Spodoptera exigua* caterpillars (Turlings et al. 1991, Turlings & Tumlinson 1991).

There are several reports which describe the chemical cues of particular plant/herbivore/carnivore interactions. In this review, examples will be drawn from several such tritrophic interactions to examine how plants identify herbivore feeding and what role chemical storage and *de novo* synthesis may play in the blend of volatiles released, both locally and systemically from the plant.

VOLATILES RELEASED BY PLANTS

With or without insect feeding, plants usually release a variety of hydrocarbons during periods of high temperatures (Sharkey & Singsaas 1995). Recent evidence suggests that this release is a strategy for coping with high temperatures. It is suspected that fat soluble hydrocarbons dissolve into the thylakoid membrane which surrounds chlorophyll molecules and serve as a glue to keep the chloroplast from melting when temperatures exceed the plant's biological optimum. Because these molecules are so volatile, they quickly evaporate as the temperature rises, prompting the plant to release more molecules (Mlot 1995). A large number of compounds have been identified in the collection of head space volatiles from different plant species. Included in the list are fatty acid derived aldehydes and alcohols (Croft et al. 1993), terpenes derived from mevalonic acid, and aromatic metabolites such as indole and methyl salicylate derived from shikimic acid (Mann 1987). Clearly, more than one biosynthetic pathway is responsible for this phytochemical release. The total energy channelled into the synthesis and release of volatiles is uncertain, although it is estimated that isoprene production alone typically siphons off 2% of the carbon fixed through photosynthesis (Mlot 1995).

In response to herbivore damage, this blend of volatiles can radically change with the surge of individual constituents. For example, in cucumber plants infested with spider mites, there is an increase in release of the terpenes (*E*)-β-ocimene and (*E*)-4,8-dimethyl-1,3,7-nonatriene from undetectable levels to 25 and 54%, respectively, of the total volatiles. This causes the relative amounts of (*Z*)-3-hexen-1-yl-acetate and (*Z*)-3-hexen-1-ol to drop from 35 to 50% of the total volatiles released from uninfested plants to 1% from infested plants (Takabayashi et al. 1994a). In the apple cultivar Summer Red, a similar shift in component ratios is observed with a surge of the terpenes (*E*,*E*)-α-farnesene and (3*E*,7*E*)-4,8,12-trimethyl-1,3,7,11-tridecatetraene after spider mite infestation. This results in a relative drop of volatiles such as *n*-hexanal and (*E*)-2-hexenal compared with uninfested plants (Takabayashi et al. 1994b). It has not yet been established whether the increased amount of volatile compounds released is caused by *de novo* biosythesis due to herbivore feeding, or whether such compounds are present constitutively in the plant and their release from storage is triggered by this feeding.

Loughrin et al. (1994) suggest that in cotton some monoterpenes and sesquiterpenes are constitutive, providing built-in protection against invading organisms, while others are induced after feeding begins. The constitutive compounds are identified by their almost immediate release after feeding starts, their relatively constant release over time, and their rapid wane after feeding stops. Such metabolites can be synthesized and stored in external epidermal hairs called trichomes or in cells embedded in the leaf, such as mesophyll pigment glands (Gershenzon et al. 1989, Croteau & Johnson 1984). In either case, these volatiles can be released by the simple breaking of the glands as a result of herbivore feeding. In contrast, the induced compounds show a delay between the time feeding starts and the release of volatiles. For example, in cotton this delay of compound release is between 12 and 24 hours after herbivore feeding begins (Loughrin et al. 1994). In addition, these induced compounds show a diurnal cycling of release which continues after herbivore feeding has ceased. Examples of proposed constitutive compounds in cotton include α-pinene and caryophyllene; induced metabolites include (*E,E*)-α-farnesene and (*E*)-β-farnesene, (*E*)-β-ocimene and *(E)*-4,8-dimethyl nonatriene (Loughrin et al. 1994). Thus far, there has been no determination of induced versus constitutive compounds with labeling experiments using radioactive or stable isotope precursors to establish the time sequence of synthesis with respect to the release of volatiles.

In addition to the release of volatiles at the site of herbivore feeding, analysis of volatile emissions from unharmed leaves of insect damaged plants has established that there is a systemic response. In both corn and cotton, leaves distal to the site of herbivore feeding showed an increase in the release of volatiles. The chemical blend of volatiles from undamaged leaves differs from the volatiles collected from the entire plant (Turlings & Tumlinson 1992). One set of compounds not systemically released are the leafy green volatiles of the lipoxygenase pathway, which are usually detected in freshly cut or damaged tissue. In addition, at least in cotton, some of the monoterpenes and sesquiterpenes, as well as indole, are only released locally.

Signal Activation in Response to Feeding

The systemic release of specific volatiles by damaged as well as undamaged leaves suggests a mobile inducer which can travel from the wounded leaf to the undamaged portions of the plant. The signalling mechanism responsible for the systemic release of predator and parasitoid attractants by plants is under intense investigation in several laboratories (Turlings et al. 1993, Boland et al. 1992). One of the proposed models of signal transduction is that the plant stores volatile metabolites as glycosides; when cells are broken by herbivore feeding and exposed to β-glucosidase from the saliva of the feeding herbivore, stored glucoside molecules are hydrolyzed and the volatile compounds are released. The presence of β-glucosidase activity in saliva from the larvae *Pieris brassicae* which feed on cabbage and induce the release of volatiles was reported recently (Mattiacci et al. 1995). In addition, the same laboratory group observed the release of volatiles from cabbage plants when commercially purified almond β-glucosidase was exogenously applied to mechanically wounded leaves. Radiochemical-labeling studies are needed to establish whether such an enzyme inducer moves through the plant and accounts for the systemic release of volatiles that is observed.

A second model of signal transduction leading to plant volatile release is the lipid-based lipoxygenase pathway which has already been shown to activate *de novo* synthesis of defense related proteins. In a series of experiments, Farmer & Ryan (1992) demonstrated that intermediates of the lipoxygenase pathway are potent elicitors causing the synthesis and accumulation of proteinase inhibitors. They have also shown that compounds such as *p*-chloromecuribenzene sulfonic acid and phenidone, which channel intermediates out of the pathway, are effective inhibitors of protein synthesis associated with plant defense responses (Narvaez-Vasquez et al. 1994 and information within Cucurou et al. 1991). In their model, the interaction of signal molecules with plasma membrane receptors leads to the activation of lipase and the release of linolenic acid into the cytoplasm. Linolenic acid is then converted through phytodienoic acid to jasmonic acid which interacts with receptors to activate proteinase inhibitor gene expression.

In tomato, the earliest signal that has been identified with herbivore wounding is the release of an 18 amino acid polypeptide, systemin. Radiolabeling studies have shown that this signal moves throughout the plant via the phloem (Pearce et al. 1991) and inhibitor studies suggest that systemin is loaded apoplastically into the vascular tissue (Narvaez-Vasquez et al. 1994). In other words, the polypeptide is loaded into the sieve tube/companion cell complex from the free space of the apoplast and not via the plasmodesmata.

In this laboratory, an elicitor has been isolated, purified and partially characterized from the oral secretions of beet armyworms, *Spodoptera exigua* Hübner. The elicitor is a low molecular weight compound, too small to be an enzyme. When this partially purified factor is supplied to young corn plants through cut stems at approximately 100 nM level, volatiles which are specific to insect feeding are released throughout the plant (Turlings et al. 1993). Experiments will be carried out to determine whether such an elicitor may activate the lipoxygenase pathway, as has been implicated with the plant elicitor systemin. The procedures for a large scale purification of this saliva factor have been established so that a complete chemical analysis will be possible.

VOLATILE SIGNAL VARIATIONS BETWEEN AND WITHIN PLANT SPECIES

At the species level, head space volatiles of several crop plants have been analyzed with and without insect damage. Though the chemical blend, as well as the total volatile release varies, there are some compounds that many species have in common. Repeatedly, the acyclic C_{11} homoterpene (*E*)-4,8-dimethyl-1,3,7-nonatriene and the C_{16} homoterpene (3*E*,7*E*)-4,8,12-trimethyl-1,3,7,11-tridecatetraene have been identified in the head space of herbivore infested plants including lima bean, apple, cowpea, cucumber, corn and cotton (Dicke 1994, Loughrin et al. 1994, Turlings et al. 1991).

Data on variation in volatiles released at the cultivar level is limited. In cotton, no significant differences were reported in the volatiles released among commercial cotton varieties, though a naturalized cotton variety did emit substantially greater quantities of volatiles per plant than any of the commercial hybrids (Loughrin et al. 1995). Five varieties of corn exposed to herbivore injury differed significantly in the quantity and variety of terpenes released. The genotypes showed variation as well in their resistance to the important corn pest, fall armyworm. However, these differences did not appear to affect the ability of the parasitoids *Cotesia marginiventris* and *Microplitis croceipes*, to locate their host, the fall armyworm (Turlings et al. 1995). Clearly some variation in the volatile blend released by an individual plant does not completely eliminate chemical signalling.

Leaves from different parts of an individual plant also can show variation in the release of herbivore-induced volatiles. In cucumber, although young and old leaves do not differ much with regard to the total volatiles released from spider mite induced plants, a greater variety of compounds are released from young than older leaves (Takabayashi et al. 1994a). Young and mature leaves on beet army worm-infested cotton plants differ in the blend of volatiles that are released (Röse & Tumlinson unpublished data).

Differences in the amount of volatiles released between individual plants are often subtle and can easily be masked by variations in environmental conditions that influence the plants' physiology. Several species, including corn, cotton (Loughrin & Tumlinson unpublished data) and lima bean (Takabayashi et al. 1994b) respond to reduced light, either from 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 be directly related to volatiles released. With less water available for the plant, elevated levels of volatiles are released from infested individuals relative to non-water stressed controls. 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).

The variation between species in herbivore-induced volatile release and the generality of their response in the plant kingdom can not yet be satisfactorily addressed because only a limited number of crop plants have been studied.

BIOSYNTHESIS OF PLANT VOLATILES

Though there are numerous reports on the release of volatiles with insect feeding as well as the effect of these volatiles on herbivore parasitoids, there is little information on the way in which plants regulate the blend of compounds emitted. Are the released parasitoid attractants synthesized *de novo*? If so, what are the biosynthetic pathways that are activated under such regulation? An outline of the biosynthetic route to plant volatiles is shown in Fig. 1. At least three biosynthetic pathways are expected to be responsible for the blend of volatiles that are released; the isoprenoid pathway which produces monoterpenes and sesquiterpenes, the fatty acid/lipoxygenase pathway which generates green leaf volatiles and jasmone, and the shikimic acid/tryptophan pathway which results in several compounds, including indole (Mann 1987). From data reported on the timing of volatile release with insect feeding in cotton, it appears that the terpene pathway is responsible for the release of most of the inducible compounds (Loughrin et al. 1994).

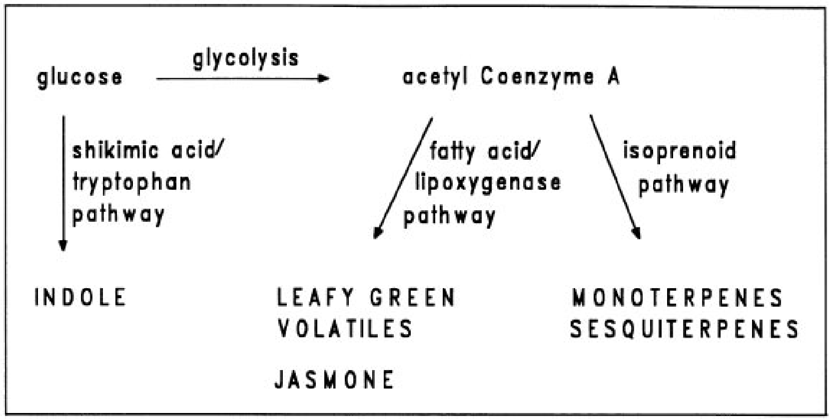

Figure 1. Primary and secondary metabolic pathways leading to volatile compounds released in herbivore damaged plants.

A key intermediate in the isoprenoid pathway is mevalonic acid which is formed by the condensation of three acetyl-CoA molecules to (3S)-3-hydroxy-3-methylglutaryl CoA and a two step reduction to mevalonic acid (Goodwin & Mercer 1990). As shown in Fig. 2, isopentenyl pyrophosphate (IPP), the five carbon building block of terpenes, is then formed via decarboxylation of mevalonic acid pyrophosphate (Gershenzon & Croteau, 1989). Half of the IPP is enzymatically converted to the isomer dimethylallyl pyrophosphate and the two isomers condense via prenyltransferases to form geranyl pyrophosphate (GPP). GPP can be channelled into monoterpene biosynthesis catalyzed by cyclase enzymes (Alonso & Croteau 1993) resulting in metabolites including the acyclic structures linalool and (*E*)-β-ocimene, the monocyclic structure limonene, and the bicyclic structure α-pinene, all of which are released with insect feeding in cotton. GPP can also be condensed with another IPP molecule to form (1) the C_{15} molecule, farnesyl pyrophosphate, the precursor of sesquiterpenes such as the acyclic compounds nerolidol, (2) α- and β-farnesene, the monocyclic compounds humulene and λ-bisabolene and often (3) the bicyclic compound caryophyllene. All of these are also released from cotton after insect feeding.

Almost all of the identified volatiles systemically released in response to herbivore feeding are terpenes. At least in cotton, these systemic terpenes are early biosynthetic products of the isoprenoid pathway. Farnesene and (*E*)-β-ocimene are likely formed via an ionization-isomerization-elimination reaction, analogous to a monoterpene syntheses proposed mechanism (Savage et al. 1994), with farnesyl- and geranyl-pyrophosphate as respective precursors. Linalool, another systemically released metabolite, is structurally similar to ocimene except that the former compound undergoes an additional reaction with the loss of the pyrophosphate moiety to form the tertiary al-

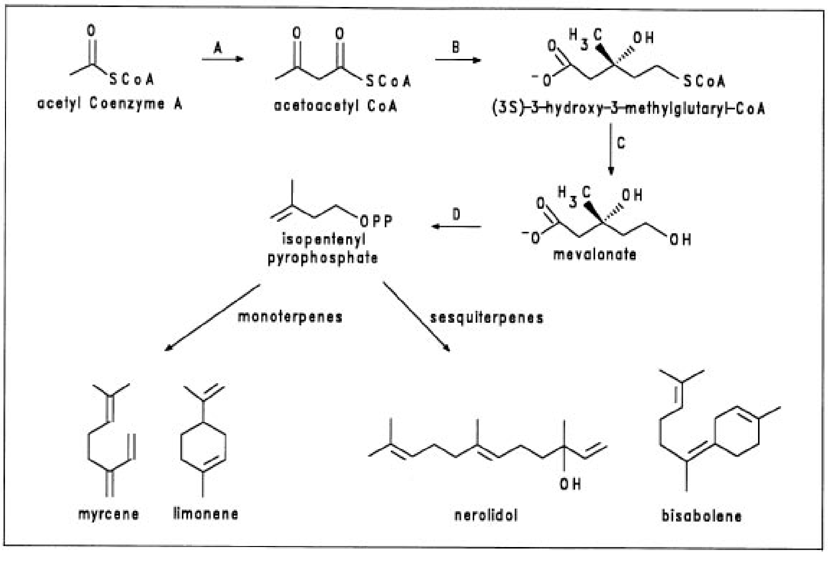

Figure 2. Biosynthetic steps in the isoprenoid pathway leading to volatile monoterpene and sesquiterpene formation from acetyl-CoA. Enzymes in the pathway include: acetyl-CoA acetyl-CoA transferase (A), hydroxymethylglutaryl-CoA syntheses (B), hydroxymethylglutaryl-CoA reductase (C), mevalonate kinase, phosphomevalonate kinase and pyrophosphomevalonate decarboxylase (D).

cohol instead of proton elimination to form an additional double bond (see Fig. 3). In the case of the systemically released C_{11} homomonoterpene, (*E*)-4,8-dimethyl-1,3,7-nonatriene and C_{16} homosesquiterpene (3*E*,7*E*)-4,8,12-trimethyl-1,3,7,11-tridecatetraene appear to be formed by a series of degradations with an overall loss of four carbons from the sesquiterpernoid nerolidol or from the diterpenoid geranyllinalool, respectively.

Why acyclic terpenes are released throughout the plant while more biosynthetically complex derivatives are released only locally is not clear. One explanation is that unequal enzyme activation increases the flux of terpenoid biosynthesis at the level of mevalonic acid; however, insufficient cyclase activity limits formation of the cyclic terpenes.

Perhaps not surprisingly, in cotton, the systemically released terpenes are the same compounds proposed by Loughrin et al. (1994) to be induced, and which have a diurnal cycling pattern in their release. A pulse chase experiment using a labeled substrate, such as glucose or carbon dioxide, is needed to establish whether the release of volatiles can be divided into two chemical responses: (1) the release of stored metabolites at the site of wounding and (2) *de novo* synthesis of particular metabolites throughout the plant.

The pathway responsible for the synthesis of the leafy green volatiles is the fatty acid/lipoxygenase pathway. For fatty acid synthesis, C_{16} palmitic acid is assembled by a series of condensation steps with malonyl-CoA serving as substrate as shown in Fig. 4. This intermediate length C_{16} acid can then undergo elongation and/or desaturation reactions to form long chain fatty acids (C_{18}-C_{22}). The widely distributed fatty acids in plants are monocarboxylic acids with unbranched, even-numbered carbon chains. The unsaturated fatty acids have *cis* double bonds; in polyunsaturated acids, these cis double bonds are arranged in a methylene-interrupted system precluding conjugation.

In contrast, the lipoxygenase pathway catalyzes the breakdown of lipids resulting in short chain volatile compounds. The C_6 volatiles and jasmone are formed from the polyunsaturated octadecenoid fatty acids (*Z*,*Z*)-9,12-octadecadienoic acid (linoleic

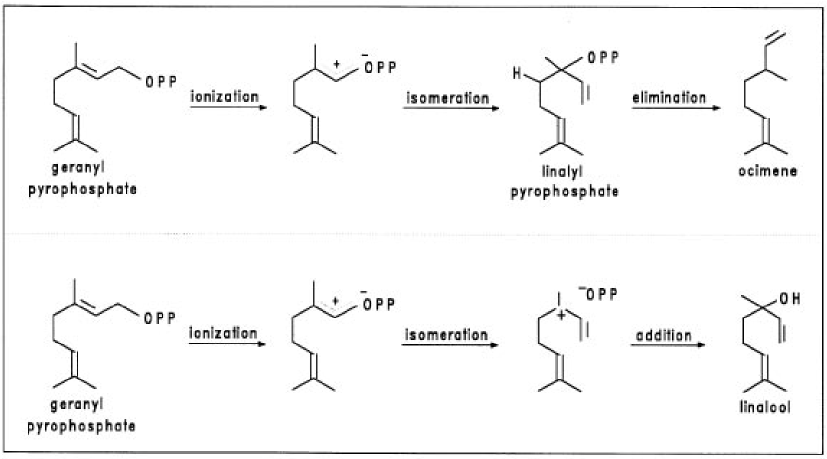

Figure 3. A biosynthetic mechanism for the formation of ocimene and linalool from geranyl pyrophosphate (OPP= pyrophosphate).

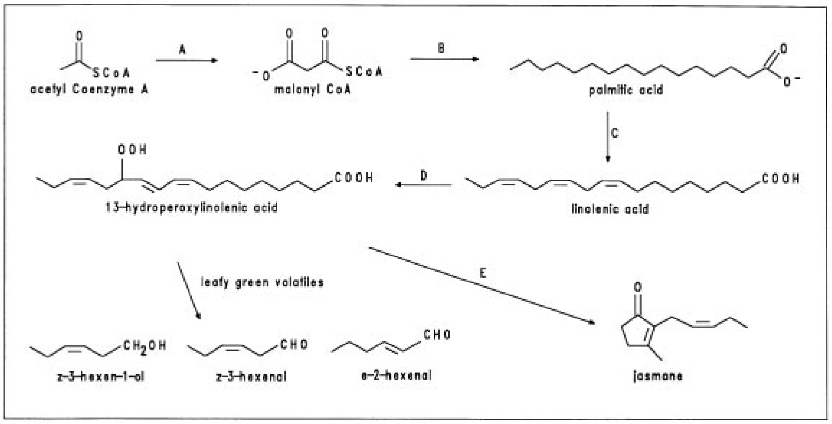

Figure 4. Biosynthetic steps in the fatty acid (A-C) and lipoxygenase pathway (D-E). Enzymes in the pathways include: acetyl-CoA carboxylase (A), fatty acid synthase (B), fatty acid elongation and desaturation enzymes (C), lipoxygenase (D), hydroperoxide dehydrase, reductase, and β-oxidation enzymes (E).

acid) or (*Z*,*Z*,*Z*) 9,12,15-octadecatrienoic acid (linolenic acid). The substrates for lipoxygenase, linoleic acid and linolenic acid are major components in plant membranes, but are not readily acted upon by the enzyme lipoxygenase until in the free acid form (Croft et al. 1993). In the formation of the C_6 volatiles, or green leaf volatiles, linolenic acid is oxidized to either 9- or 13-hydroperoxylinolenic acid or a mixture of both. Hydroperoxide lyase then catalyzes the formation of the C_6 *Z*-3-hexenal and the C_{12} 12-oxo-*Z*-9-dodecenoic acid. Rearrangement, reduction and/or esterification of the C_6 product result in induced volatiles such as (*Z*)-3-hexenal, (*E*)-2-hexenal, (*Z*)-3-hexenol, and (*Z*)-3-hexenyl acetate, all of which have been identified in the head space collections of insect damaged cotton (Rose et al. 1996).

Indole, the only volatile detected in the tryptophan pathway from plants attacked by insects, is the penultimate intermediate in the biosynthesis of tryptophan. The shikimic acid pathway provides the substrate for tryptophan synthesis as well as other aromatic plant volatiles that have been identified in association with herbivore feeding such as methyl salicylate and phenylacetonitrile (Loughrin et al. 1995, Takabayashi et al. 1994a). Figure 5 includes branch point metabolites which have been established as intermediates in the shikimic acid and tryptophan pathway leading to the formation of indole (Crawford 1989).

In vivo labeling experiments can establish whether herbivore feeding results in the release of stored metabolites, the synthesis of new volatile compounds, or more likely some combination of the two. A comparison of activity for enzymes in the terpene pathway before and after herbivore feeding may provide insight into why particular terpenes are or are not induced and/or systemically released from the plant.

CONCLUSIONS

In several crop plants, feeding by herbivores results in a time and concentration controlled release of volatile metabolites; such volatiles can attract parasitic and predatory arthropods which may prevent the plant from sustaining severe damage. We propose that the combination of a signal strong enough to be detected by the par-

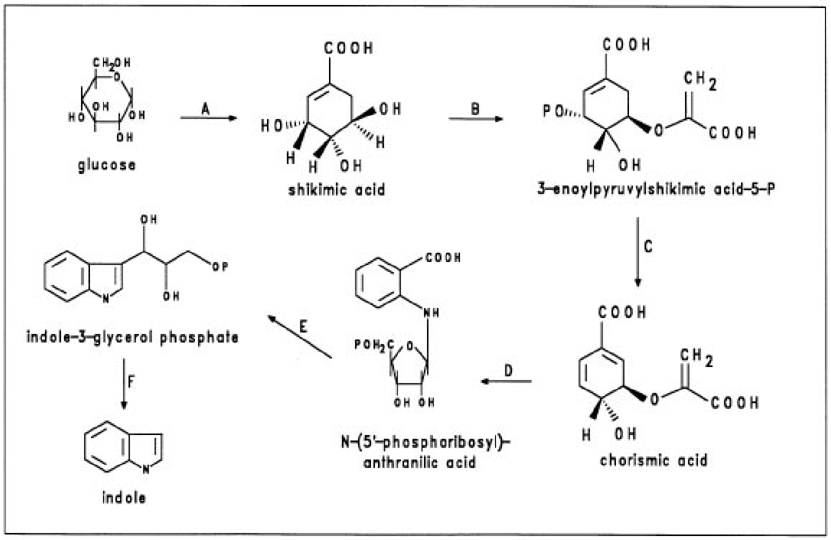

Figure 5. Biosynthetic steps in the shikimic acid/tryptophan pathway. Enzymes in the pathway include: shikimic acid biosynthetic enzymes (A), shikimate kinase and 3-enoylpyruvylshikimic acid 5-phosphate synthase (B), chorismate syntheses (C) anthranilate synthase and anthranilate phosphribosyl transferase (D), N-5'-phosphoribosyl-anthranilate ketol-isomerase and indole-3-glycerol-phosphate synthase (E), tryptophan synthase F.

asitoid, a signal distinctive enough to be uniquely associated with feeding by herbivores, and a signal rhythmic enough to be emitted at the time of day when the parasitoids forage has resulted in an effective chemical cue for parasitoids and predators. The biochemical responses by plants involved in this tritrophic interaction are only now being questioned and addressed. Many of the protocols used to study other inducible plant responses can readily be adapted to investigate plant signalling involving the release of volatiles triggered by herbivore feeding.

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