

SESSION 9C

NEW APPROACHES TO CROP PROTECTION BY EXPLOITING STRESS-RELATED SIGNALLING IN PLANTS

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Wageningen, The Netherlands*

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Papers: 9C-1 to 9C-4

Plant defense-inducing *N*-acylglutamines from insect guts: structural diversity and microbe-assisted biosynthesis

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ABSTRACT

N-Acylglutamines are ubiquitously present in the regurgitants of herbivorous insects. The compounds comprise a larger family containing fatty acids of different degrees of unsaturation and chain length. Further modifications occur in the fatty acid moiety by oxidative transformations to secondary alcohols and epoxides. By hydrolysis (epoxide), acylation or phosphorylation of the alcohols the diversity of the compounds is further increased. Screening of cultivable gut bacteria from several Lepidoptera revealed that many of the commensal microorganisms are principally able to synthesize the *N*-acylglutamines. From *Microbacterium arborescens* the first pure *N*-acyl synthase was isolated and characterized as an homooligomeric enzyme with a protomer size of 17.2 kDa. The enzyme displays a pH optimum at pH 8 and second plateau of even higher activity between pH 9-12 corresponding to the strongly alkaline conditions in the insect gut. The enzyme catalyses amide formation between a wide range of free fatty acid and most proteinogenic amino acids. Preceding activation of the acyl moiety is not required.

INTRODUCTION

Plants under attack by a herbivorous insect often release a blend of *de novo* synthesised volatiles that may attract the natural enemies of the attacking insect (Dicke *et al.* 1990; Turlings *et al.* 1993). While the mechanical damage of the feeding process only effects the release of pre-formed volatiles and that from rapid degradation processes, low- and high-molecular weight components (elicitors) from the simultaneously introduced salivary secretions modify the plant's gene expression activating a *de novo* biosynthesis of defensive compounds (Mattiacci *et al.*, 1995; Paré *et al.*, 1997). These secretions may be composed of glandular components from the mouth area and enteric fluids from the foregut from regurgitation. In 1997 Alborn *et al.* isolated in a bioassay guided fractionation of an elicitor-active regurgitate from *Spodoptera exigua* larvae *N*-(17-hydroxylinolenoyl)-glutamine (volicitin) as the first member of a family of *N*-acyl glutamines which was able to trigger volatile emission from corn plants (*Zea mays*). In some insects, for example in the oral secretions of the tobacco hornworm (*Manduca sexta*) the amino acid glutamine is replaced by glutamate (Halitschke *et al.*, 2001).

Structural diversity of *N*-acylglutamines

Besides the simple conjugates of glutamine with saturated or unsaturated fatty acids (Figure 1, Type I), there exist more complex structures with functionalized fatty acid moieties that were isolated recently from regurgitates of lepidopteran larvae (*S. frugiperda*, *S. exigua* and *Heliothis virescens*) (Paré *et al.*, 1998; Pohnert *et al.*, 1999).

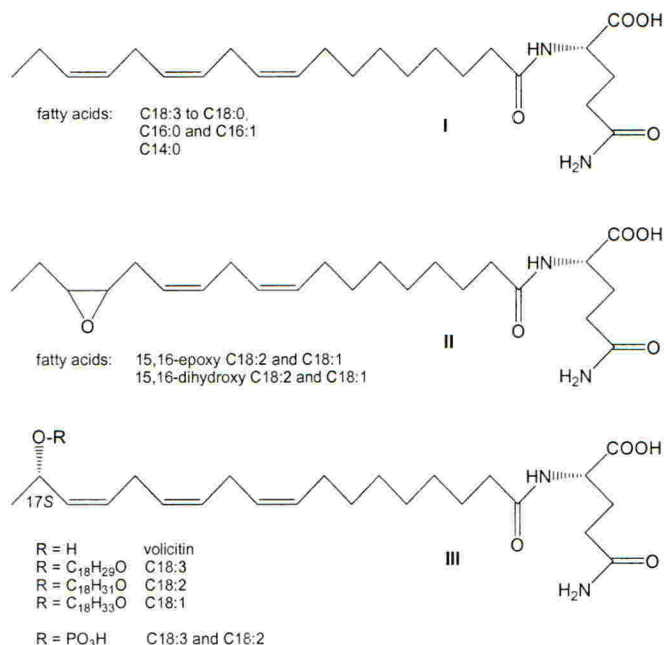


Figure 1. Structural diversity of *N*-acyl glutamines isolated from regurgitants of various lepidopteran larvae.

Additional oxidative transformations of either the intact *N*-acyl-glutamines or their precursor fatty acids lead to *N*-(15,16-epoxy-linoleoyl)-glutamine and *N*-(15,16-dihydroxy-linoleoyl)-glutamine (Figure 1, Type II) (Spiteller & Boland, 2003a). A nother family of conjugates is generated by acylation of volicitin-type precursors which result in very unpolar and rather unstable compounds such as *N*-(17-linolenoxy-linolenoyl)-glutamine (Figure 1, Type III) (Spiteller & Boland, 2003b). The latter exhibit striking structural similarities to the mayolenes previously isolated from defensive secretions of the Lepidopteran *Pieris rapae* (Smedley *et al.*, 2002). Most surprising was the recent discovery of a C(17)-phosphorylated fatty acid conjugate (Figure 1, Type III), which represents the first alkyl-chain phosphorylated fatty acid derivative in nature (Spiteller & Boland, unpublished results). The chiral centre of the functionalized linolenic acid moiety of volicitin was determined as (17*S*, >94% ee) (Spiteller *et al.*, 2001). In case, that volicitin serves as a common precursor *en route* to the more complex structures (Figure 1, compounds of type III), the same configuration is to be expected for the other conjugates.

Biosynthesis of *N*-acyl conjugates by commensal gut bacteria of insects

N-acylamino acid conjugates such as *N*-acylornithines or *N*-acylserines are long known as metabolites of microorganisms (Asselineau, 1991) and, in fact, in a recent study we presented first evidence that microorganisms may be also involved in the biosynthesis of *N*-acylglutamines in insect guts (Spiteller *et al.*, 2001). From gut segments of *Spodoptera exigua*, *Mamestra brassicae* and *Agrotis segetum* c. 30 bacterial strains could be isolated, cultured and identified by sequencing of their 16S rDNA. Many of them (c. 50%) were able to synthesise the typical lepidopteran *N*-acylamino acids from externally added precursors (Spiteller, 2002). Moreover,

the extent of conjugate biosynthesis in the insect could be suppressed to a certain extent by pre-treatment of the food with antibiotics. The effect was, however, in general not very pronounced, but in the case of larvae of *Spodoptera frugiperda* with the antibiotic fosmidomycin and lima bean as the host plant a significant reduction of conjugate biosynthesis was observed (c. 90% reduction).

Isolation of a novel *N*-acyl synthase from *Microbacterium arborescens*

Three non-related microorganisms representing diverse bacterial groups were selected to study the biosynthesis of *N*-acyl glutamines in detail. *Providencia rettgeri* is a Gram negative pathogen causing nosocomial infections. It has been also isolated from oil fly (Kadavy *et al.*, 2000). *Microbacterium arborescens* is a free living Gram positive species with a high GC content (Rainey *et al.*, 1994), and the third species, *Bacillus pumilus* is a low GC content Gram positive soil bacterium (Hallmann *et al.*, 1999). Owing to the wide distribution of the two Gram positive bacteria (for example on leaf surfaces), they will, upon feeding, opportunistically colonize the insect gut. Although the three microorganisms displayed different growth kinetics, amide formation consistently increased at the early stationary phase. At that time, nutrient depletion occurs. Such stringent conditions appear to be general for bacteria colonizing higher organisms, and a number of bacterial genes, especially those responsible for colonization and pathogenicity, are known to be regulated by lag phase signaling systems (Parsek & Greenberg, 2000). Hence, the precise controlled time window of this biotransformation (Figure 2) may suggest a defined interaction between bacteria and their insect hosts.

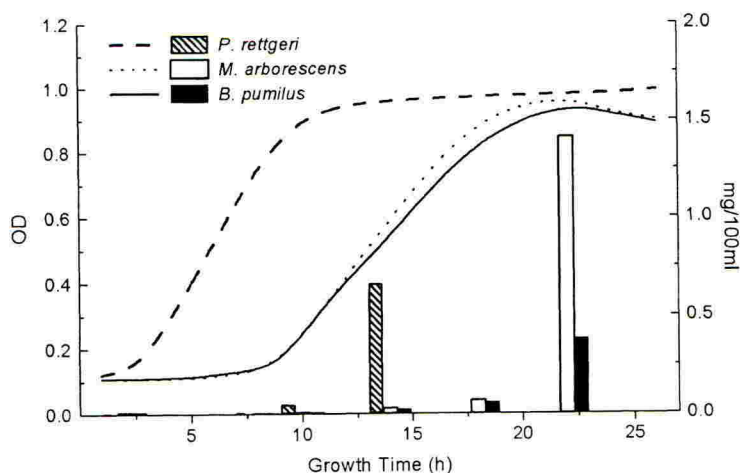


Figure 2. Time course of *N*-acylglutamine biosynthesis in growing microbial cultures. Bacteria were grown in BHI medium at 37°C. Cell density was measured at 600 nm (left axis). Linolenic acid and glutamine were added into the growing culture and samples were taken in four hour intervals and analysed by HPLC. Bars indicate the amount of *N*-acyl glutamine formed (right axis).

M. arborescens, isolated from the regurgitate of *Spodoptera exigua*, was recognized as an efficient producer of *N*-linolenoyl-glutamine and was therefore chosen as a source for the isolation of the catalytically active protein(s). Bacterial cells were harvested at the early stationary phase and yielded an active homogenate after cell rupture. Obviously, the enzyme is not secreted into the medium, since control experiments with the medium indicated much lower catalytic activity. Owing to the high stability and the large molecular weight (> 100 kDa) of the protein ultrafiltration through Vivaspin 20 columns (100 kDa cut off) allowed a very simple removal of lower-molecular weight compounds; c. 90% of active protein was retrieved in the supernatant (Figure 3a). Subsequent treatment with ammonium sulphate resulted in precipitation of the protein between 25-65% (Figure 3b) suggesting that protein surface is not highly charged. Accordingly, an attempt to purify the biocatalyst by chromatography on hydrophobic columns failed due to very tight binding. Further purification was achieved by anion exchange (ResourceQ) and chromatography on Superdex 200 HR. Based on the purification procedure, we conclude that there is only a single active protein present in *M. arborescens* (Ping *et al.*, unpublished results).

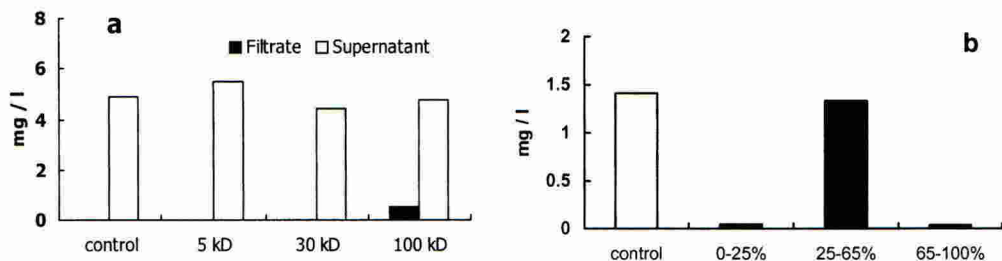


Figure 3. Effect of major purification steps; Figure 3a: Vivaspin 20 passage, Figure 3b: ammonium sulphate precipitation. After each step, the fraction was desalted. The catalytic activity was determined by addition of aliquots to a standardized solution of linolenic acid and glutamine followed by HPLC-analysis of the products.

Enzyme characteristics

First studies with the pure enzyme revealed unusual properties of the biocatalyst. The enzyme displays two pH-optima; one around pH 8 and another as an activity plateau between pH 9-12. Since the foregut of lepidopteran larvae also exhibits a strongly basic milieu in the range of pH 9-11, the bacterial enzyme is perfectly adapted to this particular environment. Characterization of the protein by MALDI-TOF or ESI-MS demonstrated the presence of a protomer of 17.2 kDa, while size exclusion chromatography indicated an apparent molecular weight of c. 310 kDa suggesting a high degree of association of the protomers.

The protein catalyses amide formation between free fatty acids and glutamine without the need for previous activation. Activated derivatives such as acyl-CoA esters, acylglycerides, or phospholipids were not converted into glutamine conjugates. On the other hand, all fatty acids and almost all proteinogenic amino acids (except of Glc, Asp, and Pro) were accepted as sub-

strates. Thus, a single enzyme is able to produce a very large number of different *N*-acyl amino acids. Interestingly, similar observations were made with growing cultures of *M. arborescens*. Since there is no evidence from the purification protocol for additional enzymes of this kind in *M. arborescens*, apparently a single enzyme is sufficient to generate the broad product spectrum that can be generated by the microorganisms from a mixture of precursors.

The physiological function of the *N*-acyl conjugates in the insect and in the herbivore-damaged plants remains to be clarified. It has been previously postulated that the compounds might serve the insect as biosurfactants to aid digestions of plant-derived lipids (Collatz & Mommsen, 1974). This assumption appears to be justified by the very broad distribution of *N*-acyl amino acid conjugates in many different arthropods and even beyond (e.g. spiders and crabs) (Pohnert *et al.*, 1999). Although their mode of action as elicitors is not yet known, the surface active *N*-acylamides act on plant tissue by causing a massive influx of extracellular Ca^{2+} into the cells (unpublished results). Whether or not this is the major signalling pathway leading to the up-regulation of plant defence responses remains to be clarified.

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Synthetic herbivore-induced plant volatiles as field attractants for beneficial insects

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ABSTRACT

Evidence for field attraction by beneficial insects to synthetic herbivore-induced plant volatiles (HIPV's) is presented. Three synthetic HIPV's (methyl salicylate, (Z)-3-hexenyl acetate and (E)-4,8-dimethyl-1,3,7-nonatriene) were evaluated in a Washington State hop yard during April-October 2002, for attractiveness to beneficial insects. The predatory mirid, *Deraeocoris brevis* and the anthocorid, *Orius tristicolor* were attracted to sticky cards baited with (E)-3-hexenyl acetate, while the geocorid, *Geocoris pallens* and hover flies (Syrphidae) were attracted to methyl salicylate-baited cards. The coccinellid, *Stethorus punctum picipes* was attracted to both HIPV's in July and September. (E)-4,8-dimethyl-1,3,7-nonatriene did not attract any beneficial insects. *Lygus hesperus*, *Leptothrips mali*, *Anagrus* spp., other Miridae, Coccinellidae and parasitic Hymenoptera were not attracted to the three HIPV's tested. The possible exploitation of HIPV's in enhancing spring populations of beneficial insects, and conservation biological control in cropping systems is discussed.

INTRODUCTION

Some plants respond to herbivore damage by producing volatiles that attract natural enemies of the herbivores responsible for the damage (Dicke *et al.*, 1990a). The qualitative and quantitative characteristics of herbivore-induced plant volatiles (HIPV's) can vary according to the herbivore involved, the plant species and even the genotype. The phenolic compound, methyl salicylate, has been identified in the HIPV blends from at least 10 plant species including lima bean damaged by spider mites, *Tetranychus urticae* (Dicke *et al.*, 1990a), cucumber [*T. urticae*] (Agrawal *et al.*, 2002), cabbage [caterpillars, *Pieris* sp.] (Geervliet *et al.*, 1997), pear [psyllids] (Scutareanu *et al.*, 1997), hops [aphids, *Phorodon humuli*] (Campbell *et al.*, 1993), bird cherry [aphids, *Rhopalosiphum padi*] (Glinwood & Petterson, 2000), potato [beetles, *Leptinotarsa decemlineata*] (Bolter *et al.*, 1997), *Nicotiana attenuata* [caterpillars, *Manduca quinquemaculata*, leaf bugs, *Dicyphus minmus*, flea beetles, *Epitrix hirtipennis*] (Kessler & Baldwin, 2001), and *Lotus japonicus* [*T. urticae*] (Ozawa *et al.*, 2000). Tobacco plants inoculated with tobacco mosaic virus also produced methyl salicylate (Shulaev *et al.*, 1997). Two other common HIPV's identified from a range of arthropod-damaged plants (e.g. corn, cabbage, beans, pear, cotton) are the terpene, (E)-4,8-dimethyl-1,3,7-nonatriene and the ester (Z)-3-hexenyl acetate (Dicke *et al.*, 1990a).

Laboratory studies have demonstrated that methyl salicylate is attractive to the predatory mite, *Phytoseiulus persimilis* (Dicke *et al.*, 1990a, b) and the predatory bug, *Anthocoris nemoralis* (Drukker *et al.*, 2000). In contrast, olfactometer studies showed methyl salicylate to be repellent to the aphid pests, *Aphis fabae* and *Sitobion avenae* (Hardie *et al.*, 1994).

Similarly, field experiments showed methyl salicylate reduced trap catches of the aphid, *P. humuli*, during spring colonization of hop yards (Losel *et al.*, 1996). Ninkovic *et al.* (2003) demonstrated that methyl salicylate significantly delayed establishment and reduced infestation of the bird cherry oat aphid (*R. padi*) in oats treated with a pellet formulation of the semiochemical. James (2003) in hop yard experiments in Washington State, showed synthetic methyl salicylate to be an attractant for the green lacewing, *Chrysopa nigricornis*.

(*Z*)-3-hexenyl acetate, a 'green leaf volatile' emitted by many plants following herbivore or artificial mechanical damage, is attractive to a number of natural enemies including the aphid parasitoid, *Aphidius ervi* (Du *et al.*, 1996). (*E*)-4,8-dimethyl-1,3,7-nonatriene is a common HIPV resulting from spider mite feeding and is attractive to *P. persimilis* (Dicke *et al.*, 1990a, b).

Most of the research to date on HIPV's and the response by natural enemies has been conducted under laboratory conditions. If these semiochemicals are to realize their potential in applied entomology, then more studies on HIPV's and natural enemy responses in the field environment need to be conducted. In Washington hop yards, effective conservation biological control of mites and aphids appears to depend on spring recruitment of winged predators (James *et al.*, 2003). The use of synthetic HIPVs to enhance the recruitment of beneficial insects in early season hops might provide a useful pest management tool.

MATERIALS AND METHODS

A trapping experiment was conducted in an unsprayed 1 ha hop yard at WSU-Prosser from 11 April-24 October 2002 to determine whether three synthetic HIPV's were attractive to beneficial arthropods. Yellow sticky cards (23 X 18 cm) baited with 2 ml glass vials (containing 1 ml of HIPV solutions or left unbaited) were tied to wooden poles ~ 2 m above the ground. The glass vials were 3.5 cm in length and 1 cm wide with a 5 mm internal diameter of the opening. HIPV's tested were methyl salicylate (99%), (*Z*)-3-hexenyl acetate (98%), and (*E*)-4,8-dimethyl-1,3,7-nonatriene (10 mg in 2 ml hexane). (*E*)-4,8-dimethyl-1,3,7-nonatriene was replaced weekly; the other two chemicals were replaced fortnightly. The release rates for methyl salicylate and (*Z*)-3-hexenyl acetate were similar (~ 0.5 ml/fortnight) while 1 ml of (*E*)-4,8-dimethyl-1,3,7-nonatriene evaporated within 4-6 days. Bait vials were taped vertically to the lower edge of the cards which were placed in a 3 X 4 grid with at least 10 m between the randomized treatments. All treatments were replicated three times and the treatment area occupied about half of the 1 ha hop yard. Sticky cards were replaced weekly and examined under a stereomicroscope for beneficial arthropods. Eleven groups or species of predators or parasitoids were sufficiently numerous to allow assessment of attraction: *Deraeocoris brevis* (Hemiptera: Miridae), *Geocoris pallens* (Hemiptera: Geocoridae), *Orius tristicolor* (Hemiptera: Anthocoridae), *Lygus hesperus* (Hemiptera: Miridae), *Stethorus punctum picipes* (Coleoptera: Coccinellidae), *Leptothrips mali* (Thysanoptera: Phlaeothripidae), *Anagrus* spp. (Hymenoptera: Mymaridae), Miridae (excluding *D. brevis* and *L. hesperus*), Syrphidae, Coccinellidae (excluding *S. punctum picipes*) and Hymenoptera (parasitic families including Ichneumonidae, Scelionidae, Encyrtidae and Mymaridae (excluding *Anagrus* spp.)). Miridae were considered to be beneficial arthropods in this study because most species have at least some carnivorous behavior. Coccinellidae were comprised mostly of the three aphidophagous species, *Coccinella transversoguttata*, *Hippodamia convergens* and *Harmonia axyridis*. *Anagrus* spp were evaluated separately because of our

experience with this genus. Data were $\log(x + 1)$ transformed to equalize variance and analyzed using analysis of variance and Fisher's least significant difference procedure.

RESULTS

The predatory mirid, *D. brevis*, and the anthocorid, *O. tristicolor*, were trapped in significantly higher numbers on (Z)-3-hexenyl acetate-baited cards than on unbaited, methyl salicylate, or (E)-4,8-dimethyl-1,3,7-nonatriene-baited cards (*D. brevis*: $F = 3.36$, $df = 3$, 68 , $P = 0.03$; *O. tristicolor*: $F = 4.20$, $df = 3$, 68 , $P = 0.0088$) (Table 1). The difference for *D. brevis* was most pronounced in August when populations were largest (Figure 1). For both species, attraction to (Z)-3-hexenyl acetate did not occur when populations were small. The geocorid predator, *G. pallens*, and hover flies (Syrphidae) were significantly attracted to methyl salicylate-baited cards compared to unbaited, (Z)-3-hexenyl acetate or (E)-4,8-dimethyl-1,3,7-nonatriene-baited cards (*G. pallens*: $F = 3.71$, $df = 3$, 68 , $P = 0.0156$; Syrphidae: $F = 16.1$, $df = 3$, 68 , $P = 0.0000$) (Table 1). Once again, attraction was most pronounced when populations were large, during June-August for *G. pallens* and in July, September and October for syrphids (Fig.1). No attempt was made to identify syrphid species. Although *S. punctum picipes* was not significantly attracted to any of the HIPV's when analyzed over the entire trapping period (Table 1), significantly greater numbers of this coccinellid were caught on methyl salicylate and (Z)-3-hexenyl acetate-baited cards in July and September (July: $F = 13.2$, $df = 3$, 12 , $P = 0.0004$; September: $F = 3.37$, $df = 3$, 12 , $P = 0.05$) (Fig.1). No attraction to the HIPV's was detected for *L. hesperus*, *L. mali*, *Anagrus* spp., other Miridae, other Coccinellidae or parasitic Hymenoptera ($P > 0.05$) (Table 1). No attraction by any insect species or group was noted for (E)-4,8-dimethyl-1,3,7-nonatriene.

Table 1. Season means (\pm SE) for beneficial insects trapped on sticky cards baited with methyl salicylate, (Z)-3-hexenyl acetate, (E)-4,8-dimethyl-1,3,7-nonatriene or left unbaited. Experiment conducted in a Washington State hop yard during April-October 2002. For each species or group, means followed by a different letter are significantly different ($P < 0.05$)

beneficial insect (species, genus, family or order)	methyl salicylate	(Z)-3- hexenyl acetate	(E)-4,8- dimethyl - 1,3,7- nonatriene	unbaited
<i>Deraeocoris brevis</i>	2.2 (0.6)a	5.2 (1.6)b	1.7 (0.6)a	1.0 (0.5)a
<i>Orius tristicolor</i>	2.3 (0.6)a	6.9 (0.4)b	2.0 (0.4)a	1.4 (0.5)a
<i>Geocoris pallens</i>	5.7 (0.7)b	1.4 (0.7)a	0.8 (0.4)a	0.8 (0.4)a
Syrphidae	2.2 (0.3)b	0.7 (0.3)a	0.4 (0.2)a	0.5 (0.2)a
<i>Stethorus p. picipes</i>	2.8 (0.7)a	2.6 (0.7)a	2.8 (0.6)a	1.7 (0.4)a
<i>Lygus hesperus</i>	1.4 (0.5)a	1.3 (0.3)a	2.5 (0.8)a	1.7 (0.5)a
<i>Leptothrips mali</i>	5.0 (1.5)a	7.5 (2.6)a	4.7 (1.0)a	8.1 (2.1)a
<i>Anagrus</i> spp.	2.0 (1.3)a	5.2 (2.9)a	2.8 (1.3)a	3.1 (1.5)a
Miridae	6.0 (1.7)a	5.2 (1.4)a	6.0 (1.9)a	4.2 (1.4)a
Coccinellidae	1.1 (0.2)a	0.7 (0.2)a	0.5 (0.1)a	1.0 (0.2)a
Hymenoptera	8.6 (1.6)a	9.0 (0.8)a	9.7 (0.8)a	8.2 (0.9)a

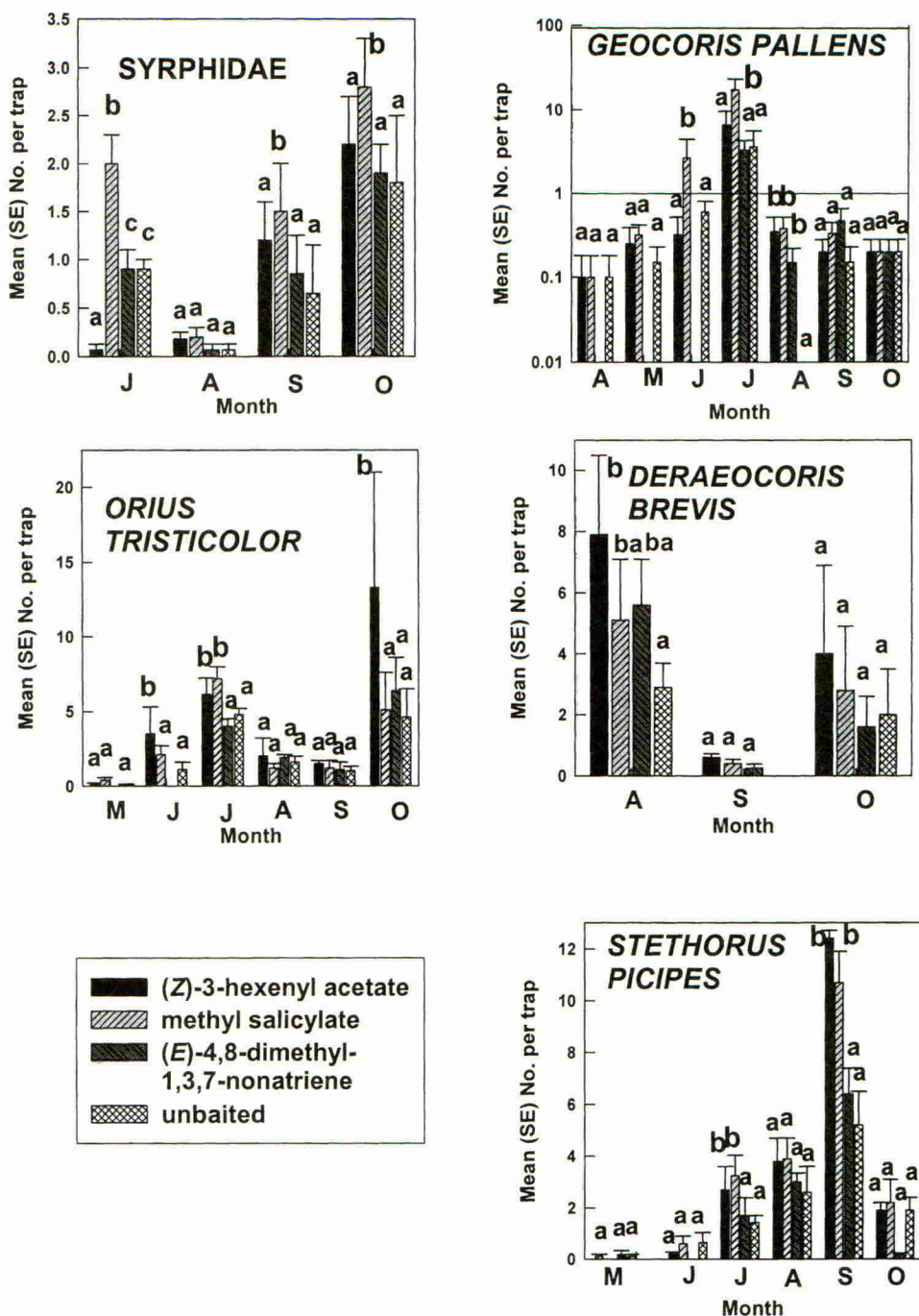


Figure 1. Monthly mean trapping frequency of Syrphidae, *Geocoris pallens*, *Orius tristicolor*, *Deraeocoris brevis*, and *Stethorus punctum picipes* on yellow sticky cards baited with HIPVs or left unbaited in a Washington hop yard in 2002. Error bars represent standard errors with different letters on columns within months indicating significant differences ($P < 0.05$).

DISCUSSION

Although laboratory evidence for plant volatiles manipulating the foraging of natural enemies is now overwhelming, very few studies have been published showing natural enemy responses to plant volatiles under field conditions. In addition, the few outdoor studies that have been published were often correlative (higher densities of predators around pest-infested plants than uninfested plants, or involved inducing HIPV's from plants (without other predator-associated cues) and measuring responses by natural enemies. Field attraction of beneficial insect species to synthetic HIPV's was shown in studies by Flint *et al.* (1979), Zhu *et al.* (1999), and more recently by James (2003).

The present study showed that methyl salicylate is an attractant for the bigeyed bug, *G. pallens* and hover flies and (*Z*)-3-hexenyl acetate is an attractant for the mirid, *D. brevis* and the minute pirate bug, *O. tristicolor*. In addition, both chemicals appeared to have some attractiveness to the mite-eating ladybeetle, *S. punctum picipes*. Previously, methyl salicylate was demonstrated to be attractive to the predatory mite, *P. persimilis*, the pirate bug, *A. nemoralis* and the green lacewing, *C. nigricornis* (Dicke *et al.*, 1990a, b, Ozawa *et al.*, 2000, James, 2003). Demonstration of field attraction of species of predatory true bugs, beetles, flies and lacewings to these two HIPV's suggests that synthetic HIPV formulations might be used in integrated pest management programs to aid conservation biological control.

The results reported here indicate the potential of HIPV's as field attractants for beneficial arthropods. This research is being undertaken in an effort to increase early season recruitment of winged beneficial arthropods to hop yards. Conservation biological control has been shown to aid in the management of the principal pests of hop, twospotted spider mite, *T. urticae*, and hop aphid, *P. humuli* in Washington (James *et al.*, 2003). A key component in the success of this strategy is spring colonization of hop yards by winged, generalist predators of mites and aphids. The current study suggests that deployment of methyl salicylate and (*Z*)-3-hexenyl acetate dispensers in spring hops may enhance recruitment of key natural enemies like *O. tristicolor*, *G. pallens* and *D. brevis*. Methyl salicylate was shown by Losel *et al.* (1996) to reduce trap catches of *P. humuli* in a hop yard. These authors concluded that methyl salicylate was repellent to *P. humuli*, thus the use of this semiochemical in hop yards may serve two benefits to pest management; enhanced recruitment of beneficial insects and repellency of the pest, *P. humuli*.

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Evaluation of the plant defence booster, acibenzolar-S-methyl, for use in Australian agriculture

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ABSTRACT

Acibenzolar-S-methyl was screened for its efficacy against a range of diseases in Australian crops. Crop phytotoxicity was also assessed. Trial results in tomatoes, lettuce, cucurbits, ornamentals and various other crops indicated that acibenzolar-S-methyl has potential as an alternative crop protection tool. In tomatoes, acibenzolar-S-methyl applied at 50 g a.i./ha was significantly more effective than copper hydroxide for management of *Xanthomonas campestris* pv. *vesicatoria* (bacterial spot). In watermelon, acibenzolar-S-methyl was as effective as the conventional copper treatment for control of the bacterium *Acidovorax avenae* subsp. *citrulli* (bacterial blotch).

There was a tendency for increased disease control when lower rates of acibenzolar-S-methyl were used in combination with conventional chemicals. Higher rates alone often resulted in disease control equivalent to the lower rates used in conjunction with conventional chemistry. However, in a number of trials, the higher rates of acibenzolar-S-methyl resulted in crop phytotoxicity.

INTRODUCTION

Plant defence boosters act by triggering plants' natural defence mechanisms. Acibenzolar-S-methyl (Bion) has been shown to induce disease resistance in a number of monocotyledon and dicotyledon plants (e.g. Friedrich *et al.*, 1996; Graves & Alexander, 2002; Romero *et al.*, 2001). The mode of action is through initiation of the systemic acquired resistance (SAR) pathway in plants (Kessmann *et al.*, 1996) which leads to specific plant defence genes being activated. Pathogenesis-related proteins (PR proteins) are synthesised, and accumulate in intercellular spaces, where pathogenic microbes grow before they attack cells. PR proteins include beta-1,3-glucanases and chitinases, enzymes which can degrade fungal and bacterial cell walls.

MATERIALS AND METHODS

Sixteen trials were conducted across Australia as part of a three-year project to evaluate acibenzolar-S-methyl for disease control efficacy and crop phytotoxicity.

In all trials, a range of product rates was used, typically including 12.5, 25, and 50 grams active ingredient/hectare (g a.i./ha). These rates of acibenzolar-S-methyl were also evaluated in combination with conventional crop protection products applied at recommended crop rates.

Treatments were applied to replicated plots (minimum four replicates) in commercial crops using appropriate nozzles. With the exception of three glasshouse trials, all trials were conducted in the field, generally using commercial crops, although three trials were established on governmental research stations. Applications were made using appropriate cone jet spray nozzles, with recommended water rates. Three trials required disease inoculation, while the remainder had sufficient natural disease pressure. Eight trials were conducted in tomatoes, five trials in lettuce and two in cucumbers. Additional trials were conducted in roses, spring onions, grapes (Semillon Blanc) and strawberries.

RESULTS

Results were highly variable between crops, with acibenzolar-S-methyl causing different disease efficacy and phytotoxic responses, depending on the target crop. Results were particularly promising in tomatoes, with the finding that acibenzolar-S-methyl applied at 50 g a.i./ha was significantly more effective than copper hydroxide for management of *Xanthomonas campestris* pv. *vesicatoria* (bacterial spot) (Figure 1). This trial was conducted in the field and the trial was inoculated with disease.

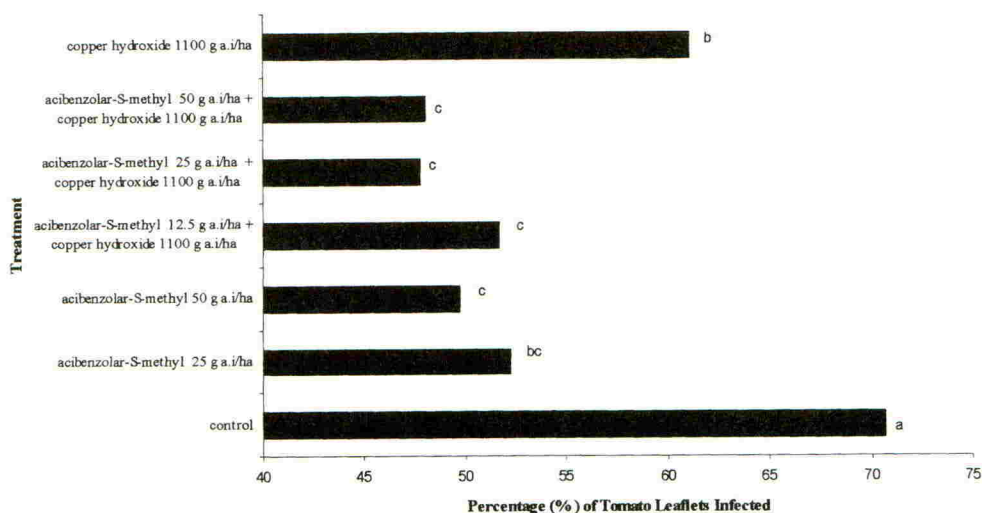


Figure 1. Incidence of *Xanthomonas campestris* pv. *vesicatoria* (bacterial spot) infection in tomato plants.

Good results were also obtained for control of *Acidovorax avenae* subsp. *citrulli* (bacterial blotch) in watermelon, with acibenzolar-S-methyl being as effective as the conventional copper treatment (Table 1). This trial was conducted in a glasshouse, and the trial plants were inoculated with disease. The implications of these results in tomatoes and watermelons are significant because sole reliance on copper as a tool for managing bacteria can lead to pathogen resistance, and copper toxicity problems.

Table 1. Mean disease severity of *Acidovorax avenae* subsp. *citrulli* (bacterial blotch) in watermelon plants receiving weekly sprays of acibenzolar-S-methyl and copper hydroxide. Disease assessments made at 7 and 10 days after inoculation. 0-6 disease severity rating scale was as follows: 0: no disease symptoms, 1: <1% leaf area affected (laa), 2: 1-<5% laa, 3: 5-<10% laa, 4: 10-<25% laa, 5: 25-<50% laa, 6: >50% leaf area affected. Means followed by the same letter were not significantly different ($P < 0.05$).

Treatment	Bacterial blotch severity (0-6)			
	7 days		10 days	
acibenzolar-S-methyl 10 g a.i./100L	0.03	e	0.00	e
acibenzolar-S-methyl 5 g a.i./100L	0.09	e	0.19	de
acibenzolar-S-methyl 2.5 g a.i./100L	0.15	e	0.31	de
acibenzolar-S-methyl 1.0 g a.i./100L	0.40	de	0.78	cd
acibenzolar-S-methyl 0.5 g a.i./100L	0.81	cd	1.19	bc
acibenzolar-S-methyl 0.1 g a.i./100L	1.06	bc	1.75	b
acibenzolar-S-methyl 0.01 g a.i./100L	1.57	a	2.49	a
copper hydroxide 120 g a.i./100L	0.56	c	0.56	d
control	1.87	a	2.75	a
<i>lsd</i> ($P < 0.05$)	0.55		0.62	

Acibenzolar-S-methyl was also effective against a range of fungal diseases including *Pseudoperonospora cubensis* (downy mildew) and *Sphaerotheca fuliginea* (powdery mildew) in cucumbers, *Stemphylium solani* (grey leaf mould) in tomatoes and *Marssonina rosae* (black spot) in roses.

There was a general trend for increased disease control when lower rates of acibenzolar-S-methyl were used in combination with conventional chemicals. Higher rates of acibenzolar-S-methyl alone often resulted in disease control equivalent to the lower rates used in conjunction with conventional chemistry.

Phytotoxicity appeared to be a concern in some crops, particularly at the higher dose rates, although no phytotoxicity was reported in any of the tomato trials. Acibenzolar-S-methyl-induced phytotoxicity was more obvious in the glasshouse trials, where plants were stressed and phytotoxic responses such as stunting and leaf curling were more common. Cumulative phytotoxicity in some crops may be an issue, and additional research is required to refine product rates and timings to avoid plant damage.

DISCUSSION

Acibenzolar-S-methyl represents a new approach to plant disease management, and such a product will require excellent product stewardship. There are practical issues such as timing of product application, which dramatically influence efficacy. For example, acibenzolar-S-methyl must be applied before any disease symptoms appear, in order to allow time for stimulation of plant defence mechanisms before pathogen development.

Currently, the most fundamental limitation to the development of acibenzolar-S-methyl in Australia is a lack of management and responsibility for product development. Product ownership is uncertain, and this issue must be resolved in order to progress preparation of data submissions to relevant government agencies such as the Australian Pesticides and Veterinary Medicines Authority and the Therapeutic Goods Administration.

Specific recommendations for prioritising development of acibenzolar-S-methyl to commercial availability in Australia are listed below.

- Complete the development of acibenzolar-S-methyl for control of bacterial spot and *Stemphyllium* in tomatoes to enable an application for registration to be made as soon as practical.
- Generate data on the other major diseases of tomatoes with a view of including them in the first, or failing that, a subsequent submission.
- Once a maximum residue limit - temporary or otherwise - is established, measure the contribution a schedule of acibenzolar-S-methyl sprays makes to a tomato crop when applied in conjunction with a standard programme.
- Test the efficacy of acibenzolar-S-methyl on as wide a range of bacterial and viral diseases of vegetables as possible to determine where else it brings benefits to vegetable growers.
- Conduct field phytotoxicity work on all cucurbit crops to determine if acibenzolar-S-methyl can be safely developed for use in all or some cucurbits.

In order to progress these recommendations, significant corporate investment in the product will be required.

ACKNOWLEDGEMENTS

Syngenta Crop Protection Pty Ltd, IHD PTY LTD and Horticulture Australia Ltd provided funding for this project. We thank all growers who kindly provided trial sites. We also thank all researchers who undertook trials as part of this project. Researchers included R O'Brien, K Lewis, E Minchinton, N Kita and P Sadler.

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New chemical signals in plant protection against herbivores and weeds

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ABSTRACT

Studies leading to the identification of *cis*-jasmone as a plant derived signal of potential value in protection of cereals against aphids are described. Unpublished work on understanding the molecular mechanisms by which *cis*-jasmone exerts a persistent effect is reviewed, in particular the potential role of *cis*-jasmone in upregulating expression, in *Arabidopsis thaliana*, of genes potentially associated with its own biosynthesis and response by plants. The possibility for similar approaches to weed control is evidenced, using the example of allelopathic control of the witchweed *Striga hermonthica* in subsistence cereals by intercropping with the forage legume *Desmodium uncinatum*.

INTRODUCTION

There are still many new opportunities for the design of novel molecular structures for development as pesticides and herbicides. However, the selection of pests and weeds for resistance to these agents remains an attendant problem to commercialisation, even of the most novel molecular structures. Some examples now exist of cross-resistance to compounds, even before commercialisation (G D Moores, personal communication). These problems are exacerbated by continually growing demands for food to be produced without detectable pesticide residues, even though health or environmental risks are minimal. This continues to inflate the cost of registration, where cost recovery for anything but major pest targets is impossible. Thus, we see a loss of recognised and established pesticides from smaller niche markets and a reluctance to develop new pesticides unless these have a wide range of applications, or at least one major economic pest target. The early promise of using genetically modified plants that express resistance genes and their products has yielded relatively few commercial successes. These are almost entirely based on expression of genes associated with the *Bacillus thuringiensis* endotoxins for insect control, or to a relatively limited range of genes giving herbicide tolerance in crop plants. In addition to the reluctance of sections of the world community to accept food produced by genetically modified crops, there is an over-concern regarding human health and possible environmental impacts which delay commercial introduction, restrict use of genetically modified crop plants, and require increased resources directed at safety and away from technological improvement or innovation in this area.

Fuelled by these problems, but also because of recent scientific advances, induced response to pest attack is seen as offering opportunities for novel approaches to the protection of crop plants against pests and weeds. Thus, we now see attempts worldwide to exploit the responses of plants to attack by herbivores and other antagonistic organisms, including pathogens and competing plants or weeds. In reviewing induced responses to herbivory,

Karban & Baldwin (1997) listed over 20 field and orchard crops upon which one organism could induce resistance against a pest species. Another advantage of induced responses to pest attack is that organisms antagonistic to the pest, either as predators or parasites, can be exploited to a greater extent than normally occurs in monocrop agriculture. In considering the evolution of induced indirect defence of plants, Dicke (1999) referenced over 50 plant/herbivore interactions in which a species antagonistic to the herbivore was attracted on damage by the herbivore. There can also be useful crossovers between organisms from completely different taxa. Thus, the silverleaf whitefly, *Bemisia argentifolii*, alters host plant physiology during feeding to reduce development of phytopathogens (Mayer, *et al.*, 2002).

Natural products have long been viewed as having potential for weed management and include inducible phytotoxins, often exuded from one plant to affect another (Duke, *et al.*, 2002). However, there is now real potential for using induced or constitutive chemical signals to switch on effects in weeds that are deleterious to their competitive development (Birkett, *et al.*, 2001). Such plant/plant interactions occur aerially and in the rhizosphere. They provide chemicals for switching on plant defence, and also an opportunity to use the plants themselves as a means of releasing signals which benefit plants with which they are intercropped. The first notable example (Farmer & Ryan, 1990) involved methyl jasmonate released from one plant and which induces synthesis of proteinase inhibitors active against herbivores in a neighbouring plant. Methyl jasmonate is formed from linolenic acid via jasmonic acid (Figure 1). Jasmonic acid itself is created by epimerisation of the original natural product, *epi*-jasmonic acid, during storage and release. However, if the emitting plant, in the case of this first study the sagebrush, *Artemisia tridentata*, is freshly clipped, then methyl *epi*-jasmonate is released, which has a stronger effect on, for example, wild tobacco, *Nicotiana attenuata*, in terms of inducing defence (Karban, *et al.*, 2000; Preston, *et al.*, 2002) Such induction can take place in other plant taxa, for example in the brassicaceous plant oilseed rape, *Brassica napus*. Here, methyl jasmonate aerially applied above intact plants causes induction of certain defensive secondary metabolites, the glucosinolates (Doughty, *et al.*, 1995).

Defence chemistry is not limited to the lipoxygenase pathway, and methyl salicylate, produced either by the inducible phenylalanine ammonia-lyase pathway or, as more recently suggested, via isochorismate synthase (Wildermuth, *et al.*, 2001), also aerially applied, can cause induction of PR proteins and associated pathogen resistance (Shulaev *et al.*, 1997). Other compounds have been implicated, but methyl jasmonate and methyl salicylate have attracted most attention. Both are methyl esters, thereby deriving volatility for aerial transport compared with the free acids, jasmonic acid and salicylic acid, already established as internally acting plant hormones in their own right. As such, these two esters may also exert deleterious effects on plants treated for protection against pests and diseases. None the less, it is clear that damaged plants, and even intact plants, can transfer other signals, beyond methyl jasmonate and methyl salicylate, that can be beneficial to the recipient plants. Aggressive weeds such as couch grass, *Elytrigia repens*, and thistles, *Cirsium* spp., can also induce repellency against aphids in neighbouring barley plants, although the weeds themselves are not intrinsically repellent (Glinwood, *et al.*, unpublished data). Furthermore, even certain intact barley cultivars can, by aerial transmission of signals, induce defence in neighbouring barley plants of different cultivars (Ninkovic, *et al.*, 2002).

Thus, the scene is set for the identification of new externally acting chemical signals. Although it might initially be considered that, where such signals are volatile, they may be

too ephemeral to use practically, the role in switching on defence, rather than needing to be present continuously, obviates this problem. By use of synthetic compound screening from the original lead of salicylate, the compound acibenzolar-S-methyl has been developed for the induction of plant defence and demonstrates the possibility for commercialisation. Furthermore, by understanding the effects of the natural plant-activating signals themselves on gene expression, it may be possible to exploit these processes more extensively. It is already known that methyl jasmonate influences a range of pathways, for example polyamine metabolism and the induction of systemic protection against powdery mildew in barley plants (Walters, *et al.*, 2002). Also, attempts at profiling the response of plants to herbivorous insects by microarray techniques is set to provide new opportunities in inducible defence gene expression (Schenk, *et al.*, 2000; Korth, 2003).

THE IDENTIFICATION OF NEW CHEMICAL SIGNALS

Chemical signals (semiochemicals) active at the sensory nervous system of animals are identified, in the case of non-sentient taxa such as insects and other arthropods, by means of electrophysiological preparations, involving recording from whole sensory organs or even from individual olfactory neurons (Pickett *et al.*, 1998). Semiochemicals released by plants under pest attack, and which influence defence metabolism in neighbouring plants, are difficult to identify since they are produced, as are most semiochemicals, in very small amounts, but in this case do not interact with as yet identifiable receptor systems. Bioassay linked fractionation could lead to the identification of active compounds, but it is clear that a large number of different molecular structures are involved in the profile of damaged plants. It was noticed that the plant semiochemical methyl salicylate is also used by herbivores, often as a repellent since it denotes plant damage, and at the higher trophic level by organisms that are parasitic on the herbivores as an attractant or foraging cue (Hardie, *et al.*, 1994; Pettersson, *et al.*, 1994). Many insects studied, from five insect orders, had olfactory neurons responding specifically to methyl salicylate or at least evidence of a highly specific response to this compound (Pickett, *et al.*, 1999; Chamberlain, *et al.*, 2000). Thus, it was suggested that insect electrophysiology might also provide a means of identifying semiochemicals that were plant activators of defence.

In the ensuing investigations a number of candidate compounds were found, with the most interesting to date proving to be *cis*-jasmone. *cis*-Jasmone is considered to be biosynthesised by the same pathway that gives rise to jasmonic acid (Figure 1), but in a route that leads off from *epi*-jasmonic acid prior to methylation (Koch, *et al.*, 1997). Indeed, by decarboxylation, *cis*-jasmone represents a more volatile metabolite than methyl jasmonate and so could be a more useful external measure of plant defence, based on the activation of this biosynthetic pathway, compared with methyl jasmonate. Initially, the lettuce aphid, *Nasonovia ribis-nigri*, was found to be repelled by *cis*-jasmone. This was based on the aphid's response via olfactory neurons selectively responsive to *cis*-jasmone, with only a very weak response to the structurally and biosynthetically related methyl jasmonate (Birkett, *et al.*, 2000). It was subsequently shown that other herbivores were repelled, including the hop aphid, *Phorodon humuli*. At the same time, *cis*-jasmone itself was found to attract ladybirds, which are predators of aphids, and parasitoids that also attack these pests (Birkett, *et al.*, 2000). Thus, in addition to the relationship between *cis*-jasmone and methyl jasmonate, and its putative biosynthesis by a stress-related pathway, *cis*-jasmone appeared to be a potent insect

semiochemical, antagonistic to herbivores but giving a positive response with their antagonists (Birkett, *et al.*, 2000).

When bean plants, *Vicia faba*, were treated with *cis*-jasmone from air held above intact plants, these plants remained repellent to herbivores, but attractive to parasitoids, long after the *cis*-jasmone had disappeared. It was therefore decided to investigate the potential field use of *cis*-jasmone in inducing resistance against herbivorous pests, and also to investigate the molecular biological mechanisms by which *cis*-jasmone caused this plant activation.

It is anticipated that other plant signals having better properties than *cis*-jasmone will be identified, particularly by use of insect electrophysiology and new postgenomic approaches to exploiting insect olfaction. However, it was felt that the field demonstration of *cis*-jasmone in controlling pests by inducing plant defence would demonstrate the principle of using essentially ephemeral plant volatiles, as opposed to purely synthetic, relatively persistent compounds such as acibenzolar-S-methyl. Furthermore, by understanding the processes by which *cis*-jasmone is detected by plants at the molecular level and by which it derives its persistent activity, this would pave the way to exploiting these mechanisms more widely.

FIELD APPLICATION OF *cis*-JASMONE AGAINST CEREAL APHIDS

Initial laboratory studies (Birkett, *et al.*, 2000) were made by placing bean plants, *V. faba*, in a bell jar into which a small dose of *cis*-jasmone had been evaporated. This was selectively absorbed by the plant, leaving behind contaminants comprising compounds with closely related structures to that of *cis*-jasmone (Chamberlain, *et al.*, 2001). For field work, it was decided to investigate the protection of winter wheat against cereal aphids, comprising the grain aphid, *Sitobion avenae*, the rose-grain aphid, *Metopolophium dirhodum*, and the bird-cherry-oat aphid, *Rhopalosiphum padi*. In this crop, application via the air could not be made in the field, so treatment was achieved by using an emulsifiable concentrate formulated using the surfactant Ethylan BV, with *cis*-jasmone being applied at the rate of 50 g/ha. Clearly, a large percentage of this application would be lost to the atmosphere, with only a small proportion entering the plant compared to the situation of the bell jar application to bean plants. However, it was intended that any success in the field with this simple emulsifiable concentrate approach would be followed by further studies on more economic and effective application systems (Bruce, *et al.*, 2003a).

Field trials have been made in each year between 1999-2002 and, with the exception of 2001, there was a statistically significant reduction in aphid populations, with, for example in 2000, a 50% reduction in aphid populations one month after application of the *cis*-jasmone. Unfortunately, throughout, there were insufficient aphid parasitoids to determine if there was an associated effect on parasitoid populations. However, in the laboratory, the aphid parasitoid *Aphidius ervi* spent significantly longer foraging on wheat seedlings previously treated with *cis*-jasmone (Bruce, *et al.*, 2003b).

INVESTIGATIONS INTO THE MOLECULAR MECHANISM OF *cis*-JASMONE AS A PLANT ACTIVATOR

Initially, the technique of differential display of genes caused to be expressed by *cis*-jasmonone in *V. faba* was investigated. Although demonstrating clearly that *cis*-jasmonone was indeed causing specific gene expression (Birkett, *et al.*, 2000), this work did not identify genes apparently associated with either the persistent effect of *cis*-jasmonone, or receptor systems by which the plant could respond to this signal. None the less, it was subsequently shown that *cis*-jasmonone affected the acceptability to aphids of a plant more useful for molecular genetic studies. This was the thale cress, *Arabidopsis thaliana* (T J Bruce, personal communication). Information was first acquired from *A. thaliana* gene expression by means of a limited microarray, involving genes already known to be associated with plant stress (E E Farmer, personal communication). This demonstrated that the gene responsible for reduction of the oxophytodienoic acid (Figure 1) in the biosynthetic pathway to jasmonic acid, and the putative pathway to *cis*-jasmonone, was upregulated by *cis*-jasmonone.

It was therefore decided to investigate the effects of *cis*-jasmonone on gene expression in *A. thaliana* by using the Stanford *Arabidopsis* microarray facility, and that the effects of *cis*-jasmonone would be tested against a control comprising plants treated in the same way with methyl jasmonate. Thus, intact eight-week old *A. thaliana*, ecotype Columbia, were exposed for 24 h in sealed Plexiglass boxes, 3.7 litres, to methyl jasmonate or *cis*-jasmonone as a vapour from 1 µl released from a cotton wick. Following combinations and the respective technical replicates, the extracted messenger RNA was hybridised to the Stanford array giving the following comparisons: control against *cis*-jasmonone, control against methyl jasmonate, *cis*-jasmonone against methyl jasmonate.

There were about 30 genes upregulated by exposure to *cis*-jasmonone. Confirmation of this upregulation was obtained for a subset of the initially recognised genes by differential expression to *cis*-jasmonone using Northern blots, and included genes annotated as a cytochrome P450, a 4-methyl-5-(2-hydroxyethyl)thiazole monophosphate biosynthase and an oxophytodienoic acid reductase gene, OPR1/2. It would be expected that products from genes upregulated by plant activators would be enzymes involved in the generation of herbivore repellents and foraging stimulants for predators and parasitoids. The cytochrome P450 may fulfil such a role, but this has not yet been determined. Alternatively, the cytochrome P450 could be involved in the selective discrimination of *cis*-jasmonone as the activating signal. It is now known that OPR3, rather than OPR1/2, is responsible for the reduction step in the specific biosynthesis of jasmonic acid from oxophytodienoic acid (Schaller, *et al.*, 2000; Schaller, 2001). OPR1 and OPR2 are considered likely to be involved in the removal of isomers different from the 9S,13S- isomer of the oxophytodienoic acid, which has the appropriate stereochemistry to be the precursor of *epi*-jasmonic acid. It may be that, since methyl jasmonate was also observed to upregulate OPR3 and only *cis*-jasmonone upregulated OPR1/2, this latter gene might be involved in the specific biosynthesis of *cis*-jasmonone as part of a feedback loop which might account for the persistent effect of *cis*-jasmonone. However, this mode of action is still under investigation.

In addition to progress towards uncovering the mechanism by which *cis*-jasmonone is detected and gives a persistent response, the molecular biological studies have also demonstrated another principle of potential practical value. The promoter sequence for one of the genes upregulated by *cis*-jasmonone has been cloned and linked to a marker gene encoding a

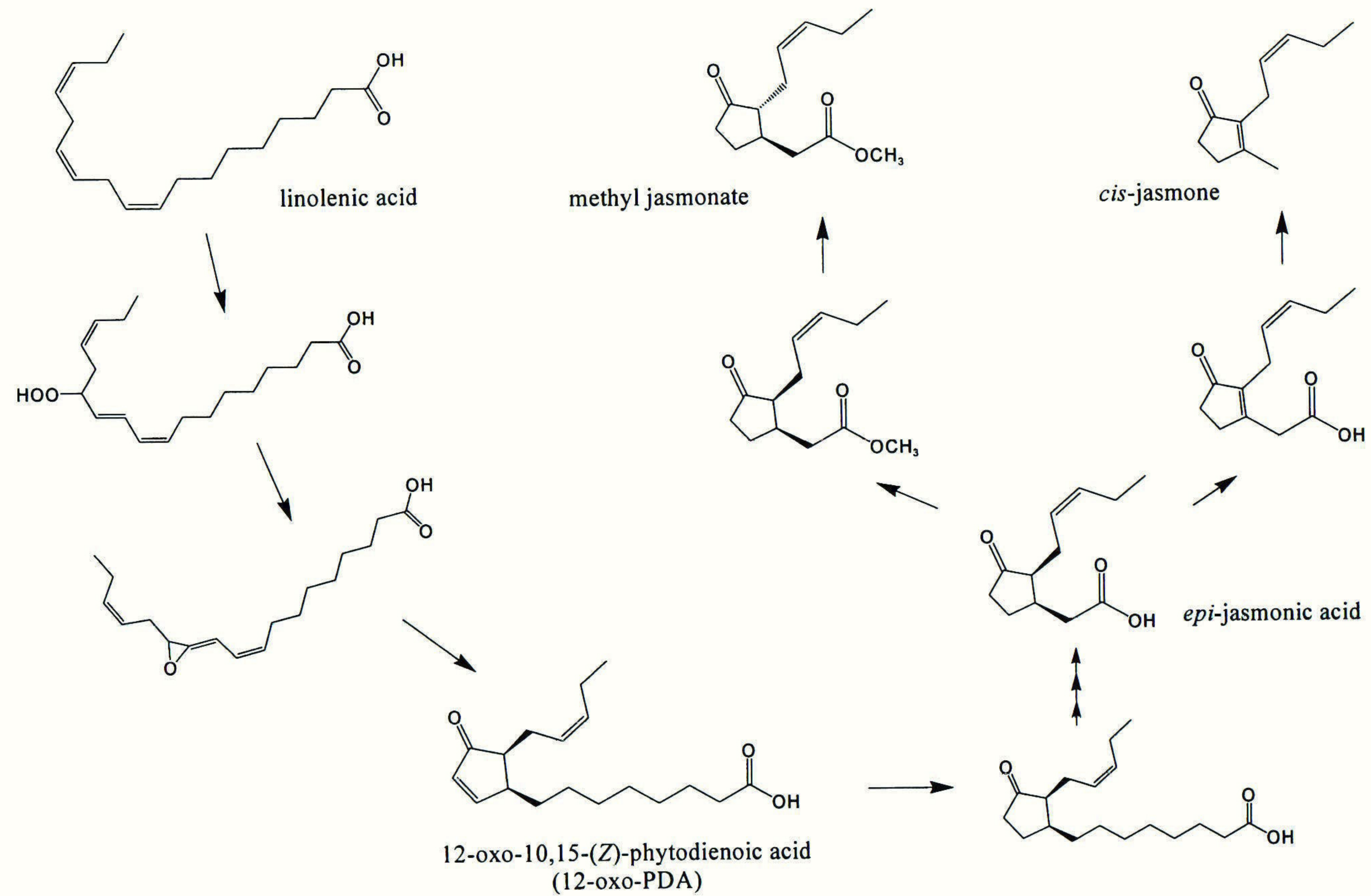


Figure 1. Putative biosynthetic pathway to methyl jasmonate and *cis*-jasmone.

luciferase and then expressed transgenically in *A. thaliana*. Thus, when this transgenic plant is exposed to *cis*-jasmonate from the air, the luciferase gene is expressed and the plant emits light when treated with the substrate luciferin, whereas without *cis*-jasmonate, the gene remains inactivated. This demonstrates the principle of using *cis*-jasmonate with *cis*-jasmonate responsive promoters to switch on other genes, not merely acting as markers but of potential value. Such genes could relate to other aspects of plant protection; for example, the activator promoter sequence could be linked to *Bacillus thuringiensis* insect defence genes, or even genes associated with agronomic traits such as water stress, whereby plant respiration could be shut down during drought (Matthes, *et al.*, 2002).

POTENTIAL FOR WEED CONTROL

Although work in this direction is not as advanced as the development of aerial plant activators, discoveries have been made in the control of the parasitic African witchweed, *Striga hermonthica*, that demonstrate the principle of using plant signals. As part of a programme in East Africa against witchweeds and stem borer pests damaging subsistence maize and sorghum crops, it has been found that forage legumes, e.g. *Desmodium uncinatum*, when intercropped with maize or sorghum, gives very effective control of *S. hermonthica*. The mechanism involves an allelopathic signalling system in which there is initially a stimulated germination of *S. hermonthica* seeds. However, the seeds do not develop to colonise the cereal crop effectively, and there are overall reductions in the seed bank and in the amount of infestation of *S. hermonthica* within the crop (Khan, *et al.*, 2002). Although the chemical identification of active components is still continuing, the compound named, uncinone C, having the novel isoflavanone structure given (Figure 2), moderately inhibited the development of the radicle after germination of *S. hermonthica* (Tsanuo, *et al.*, in press).

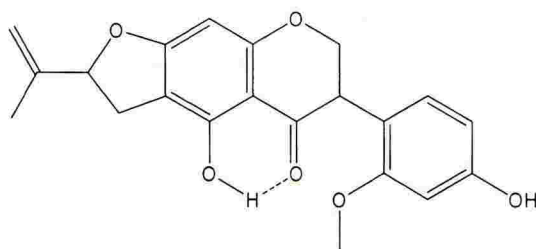


Figure 2. Structure of uncinone C, a novel isoflavanone from *Desmodium uncinatum* which controls witchweed in cereals.

Since the activity of the natural system has not yet been accounted for in the identification work, it would be premature to consider how this signal chemistry can be developed in detail. None the less, knowledge of the chemical structures involved will allow wider screening of legumes to find more economically useful cultivars and more effective plants with respect to

control of *S. hermonthica*, and particularly that can deal with the varied agricultural ecosystems present in sub-Saharan Africa, where the main problem with witchweeds occurs.

CONCLUSIONS

New chemical signals, exemplified by *cis*-jasmone, can be identified which cause natural gene expression conferring pest resistance in agricultural crops. The novel role observed for *cis*-jasmone in causing defence gene expression allows the intellectual property embodied therein to be protected by patenting, and shows commercial potential for natural products as new plant activators for plant protection. Initial investigations into new signal compounds, and the molecular mechanisms by which *cis*-jasmone acts, show considerable promise for further developments in this area. Initial studies in the control of the parasitic weed *S. hermonthica* suggest that plant signals may also find use, beyond protection of plants against pests, to weed control.

ACKNOWLEDGEMENTS

Rothamsted Research receives grant-aided support from the Biotechnology and Biological Sciences Research Council of the United Kingdom. This work was in part supported by the United Kingdom Department for Environment, Food and Rural Affairs.

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