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# *cis*-Jasmone as allelopathic agent in inducing plant defence

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## ABSTRACT

The study of plant/insect interactions, particularly in multitrophic systems, it is possible to identify the insect semiochemicals that may induce defence responses in plants. From such work, *cis*-jasmone was identified as having highly specific and persistent effects in regulating the expression of genes associated with plant defence. The molecular genetic mechanisms involved are being investigated in *Arabidopsis thaliana* by microarray analyses, the use of knockout lines and by functional gene expression studies in *A. thaliana* and other systems. In cereals, there are major varietal differences in the level of defence induced by *cis*--jasmone. With some elite cultivars, long term protection against aphids has been established in the field. Chemical studies and investigations with insects using electrophysiological and behavioural assays have shown that 6-methyl-5-hepten-2-one, the production of which is induced by *cis*-jasmone, is highly active in reducing aphid colonisation and increasing foraging by parasitoids. Differential induction between cultivars may provide a useful means to elucidate the associated genetics. In both wheat and barley, there is additionally an induction with *cis*-jasmone of antibiotic effects against aphids. In wheat, this appears to be due to enhanced production of hydroxamic acids. For these secondary metabolites, the associated genes are known and RT-PCR is used to determine the induction of expression. $MS^n$, after derivatisation and chromatography, provides analytical tool to estimate these and unknown antibiotic agents in barley.

**Key words:** Allelopathy, *Arabidopsis*, *cis*-jasmone, defence, induction, insect

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# 1. INTRODUCTION

We now know that the attraction of insects to plants and other organisms involves detection of specific semiochemicals (natural signal chemicals mediating changes in behaviour and development) or specific ratios of semiochemicals (6). We also know that the avoidance of unsuitable hosts involves the detection of specific semiochemicals associated with non-host taxa. During host alternation by many pest aphids, there can be repulsion away from a host that is not suitable for use at that developmental stage. For example, the winter, or primary, hosts of aphids can act as repellents in the spring to aphids on their migration back to their summer, or secondary, hosts. Similar phenomena can be observed during colonisation by a herbivorous insect, because the plant releases signals indicating that it is already infested and therefore unsuitable as a host (7). These signals can repel other incoming insects, but can also increase foraging by predators and parasitic wasps. The first interaction with the semiochemicals involved in these types of non-host recognition is usually on the insect antenna (6). Therefore, by using electroantennography (EAG) or single cell recording (SCR) from individual olfactory neurons, coupled to high resolution gas chromatography (GC), we can identify the compounds involved (23).

# 2. SEMIOCHEMICALS

## 2.1. Stress related semiochemicals

Using plants upon which herbivores are feeding, and investigating, by GC-EAG or GC-SCR, the volatile compounds released, it is possible to identify a range of compounds that are electrophysiologically active and which may subsequently prove to be active in behavioural assays as repellents of insect pests. These compounds can also be active in increasing foraging by predators and parasitoids which attack the pests. The compounds involved come from a wide range of biosynthetic pathways, but prominent in these are the isoprenoid and lipoxygenase pathways (19). For example, monoterpenes such as (*E*)-ocimene, and sesquiterpenes such as (-)-germacrene D, can be produced by plants and cause repellency to herbivores. However, it is difficult to deploy these chemicals in the field as there is no long-lasting effect, and the chemicals themselves are highly volatile and unstable. Heterologous expression of the genes associated with the biosynthesis of these compounds has been attempted, but it is often very difficult to obtain useful expression rates, or at least expression that leads to useful production of these compounds (1). However, recently, we have found that the heterologous expression of an (*E*)-β-farnesene synthase in *Arabidopsis thaliana* can be accomplished so that large amounts of (*E*)-β-farnesene are produced, which can affect aphids and their parasitoids (2).

Methyl salicylate has been identified as a stress-related plant semiochemical and most insects that we have examined, including some haematophagous insects, show strong electrophysiological responses to this compound. The cereal aphids *Rhopalosiphum padi*, *Sitobion avenae* and *Metopolophium dirhodum* have, in an olfactory organ (the primary rhinarium) on the sixth antennal segment, a specific olfactory neuron for methyl salicylate (20). This compound, as predicted, is associated with avoidance of cereal crops treated with a slow release formulation of this material. Thus, in spring field trials, methyl salicylate applied to wheat significantly reduced (by 30-40%) the overall number of aphids colonising

the crop (20). Methyl salicylate is biosynthetically related to salicylic acid, a signal of systemic acquired resistance (14) and salicylic acid and jasmonic acid pathways interact in induced defence (28). This may indicate that the plant is upregulating defence pathways associated with hormonal activity of salicylate and could thereby present difficulties for colonisation by aphids. However, the effect was not long-lived and the formulation needed to continue to release to provide ongoing field activity.

**2.2. Plant semiochemical induction of defence**

Methyl salicylate has been shown, when applied aerially to plants, to induce defence against fungal pathogens (27). However, a great deal of attention has been attached to the jasmonate pathway (Figure 1) (13), which is part of the lipoxygenase pathway referred to above. Again, jasmonic acid can act internally as a plant hormone associated with a damage/stress response but, when methylated (i.e. methyl jasmonate, Figure 1), can be released by the plant and, whether naturally or not, will certainly have an effect on intact plants by upregulating defence related and other genes. Unfortunately, a great number of genes are influenced and this can have a deleterious effect on plant development and yield for agricultural crops (3). When we were studying the host alternation semiochemistry of the lettuce aphid, *Nasonovia ribis-nigri*, we found, as predicted from the above hypothesis, that the spring migrants were repelled by their winter hosts (members of the Saxifragiaceae, e.g. the blackcurrant, *Ribes nigrum*) and that these semiochemicals could act as repellents for such migrants searching for the summer host, lettuce, *Lactuca sativa* (Asteraceae). However, the mixture of semiochemicals contained *cis*-jasmone, which is also involved in the jasmonate pathway (Figure 1). Previously it had been suggested that *cis*-jasmone is a metabolic product of jasmonate and represents a sink for this pathway (12). The response with *N. ribis-nigri* was very pronounced with this compound alone. A specific olfactory neuron was identified which responded exclusively to *cis*-jasmone, with virtually no response from methyl jasmonate at orders of magnitude greater stimulus concentrations, even though *cis*-jasmone and methyl jasmonate have a close structural resemblance (see Figure 1) (3). *cis*-Jasmone was also found to be a repellent for the damson-hop aphid *Phorodon humuli*, taxonomically very different in terms of having a *Prunus* species (Rosaceae) as its primary host and, as a secondary host, the hop *Humulus lupulus* (Cannabiaceae). It was also found that *cis*-jasmone would increase attraction and searching by an aphid predator, the seven-spot ladybird, *Coccinella septempunctata*. Therefore, because of *cis*-jasmone's relationship with the jasmonate pathway, we decided to see if aerial application of *cis*-jasmone could influence the defence of intact plants. This was achieved by placing low levels of *cis*-jasmone over bean plants contained in bell jars. The plants were tested for residual *cis*-jasmone, which was found to be completely absent after 48 h, and these and control plants were then placed in a wind tunnel and the effect on an aphid parasitoid, *Aphidius ervi*, was investigated. In both dual and single choice experiments, there was, respectively, three-fold and two-fold increases in oriented flight to the *cis*-jasmone treated plant, with both results being highly significant statistically (3). One of the compounds showing induced release as a consequence of the *cis*-jasmone treatment was (*E*)-ocimene, which is known to be partly responsible for the response by *A. ervi*. Although this compound was also induced by methyl jasmonate, the effect was short-lived and had disappeared 48 h after the initial treatment. However, the effect with *cis*-jasmone remained for 8 days (3).

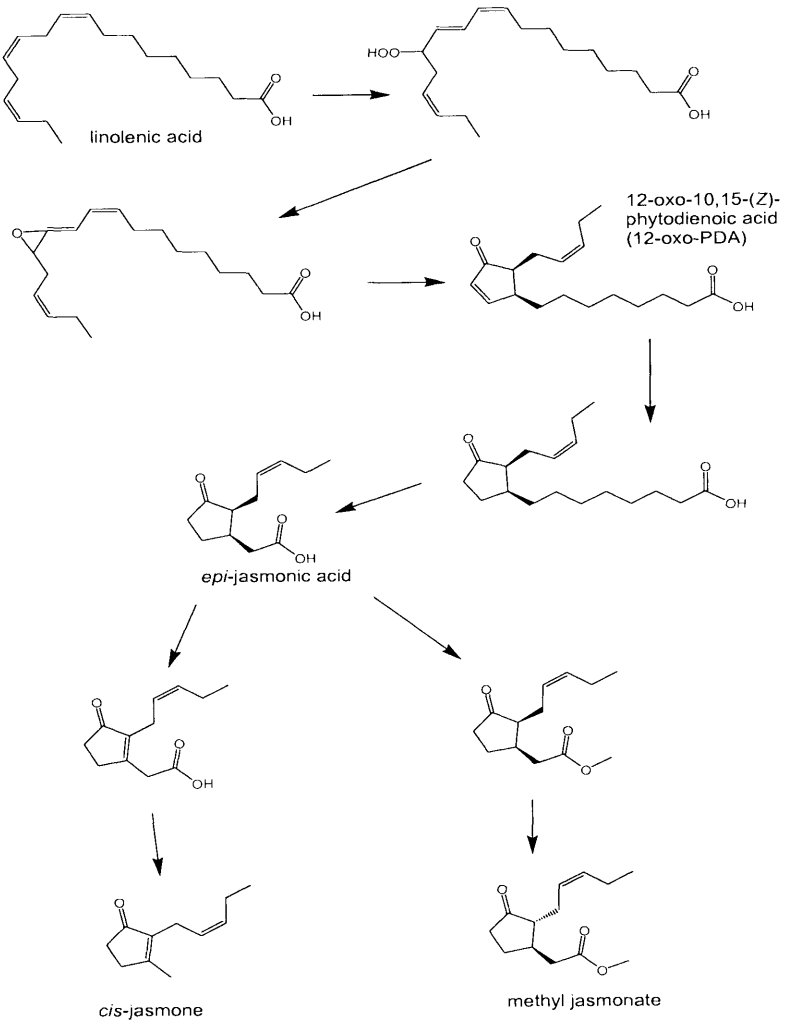


Figure 1. Biosynthesis of methyl jasmonate and putative route to *cis*-jasmone.

## 3. *CIS*-JASMONE

### 3.1. Molecular biological studies

Bean plants treated with *cis*-jasmone, and also with methyl jasmonate as a positive control, were investigated by differential gene display. Although demonstrating clearly that *cis*-jasmone was indeed causing specific gene expression (3), this work did not identify genes apparently associated with either the persistent effect of *cis*-jasmone, or receptor systems by which the plant could respond to this signal. None the less, it was subsequently

shown that *cis*-jasmone affected the acceptability to aphids and the foraging behaviour of *A. ervi* on *A. thaliana*, a model plant for molecular genetics. 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 (EE 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*-jasmone, was upregulated by *cis*-jasmone. However, in this plant, there was no upregulation of the gene for (*E*)-ocimene synthase.

It was decided to investigate the effects of *cis*-jasmone on gene expression in *A. thaliana* by using the Stanford *Arabidopsis* microarray facility, in which the effects of *cis*-jasmone would be tested against a control, comprising plants treated in a similar way with methyl jasmonate. Thus, intact eight-week-old *A. thaliana*, ecotype Columbia, were exposed for 24 h in sealed boxes (3.7 litres) to methyl jasmonate or *cis*-jasmone as a vapour from 1 $\mu$l (*ca* 1 mg) of undiluted material. The extracted messenger RNA was hybridised to the Stanford array, giving the following comparisons: control against *cis*-jasmone, control against methyl jasmonate, *cis*-jasmone against methyl jasmonate. There were about 30 genes upregulated by exposure to *cis*-jasmone. Confirmation of this upregulation was obtained for a subset of the initially recognised genes by differential expression to *cis*-jasmone using Northern blots, and included genes annotated as cytochromes P450, a 4-methyl-5(2-hydroxyethyl)thiazole monophosphate biosynthase and an oxophytodienoic acid reductase gene, *OPR1*. 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 cytochromes P450 may fulfil such a role, but this has not yet been determined. Alternatively, the cytochromes P450 could be involved in the selective discrimination of *cis*-jasmone as the activating signal. It is now known that *OPR3*, rather than *OPR1/2*, is involved in the reduction step in the specific biosynthesis of jasmonic acid from oxophytodienoic acid (25,26). *OPR1* and *OPR2* are considered likely to be involved in the removal of isomers different from the 9*S*,13*S*- isomer of the oxophytodienoic acid, which has the appropriate stereochemistry to be the precursor of *epi*-jasmonic acid (Figure 1). It may be that, since methyl jasmonate was also observed to upregulate *OPR3* and only *cis*-jasmone upregulated *OPR1*, this latter gene might be involved in the direct biosynthesis of *cis*-jasmone as part of a feedback loop (Figure 1). This might account for the persistent effect of *cis*-jasmone and the mode of action is under investigation.

In addition to progress towards uncovering the mechanism by which *cis*-jasmone 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*-jasmone has been cloned and linked to a marker gene encoding a luciferase and then expressed transgenically in *A. thaliana*. Thus, when this transgenic plant is exposed to aerial *cis*-jasmone, the luciferase gene is expressed and the plant emits light when treated with the substrate luciferin, whereas without *cis*-jasmone, the gene remains inactivated. This demonstrates the principle of using *cis*-jasmone with *cis*-jasmone responsive promoters to switch on other genes, not merely acting as markers but of potential value (21). Such genes could relate to other aspects of plant protection; for example, the activator promoter sequence could be linked to insect defence genes or genes associated with valuable agronomic traits. There would also be the possibility of using

*cis*-jasmone responsive promoters associated with light sensitive markers that could act in sentinel plants as indicators of the onset of insect attack or disease development.

### 3.2. Practical use of *cis*-jasmone

While we continue to investigate the molecular basis of *cis*-jasmone plant activation as a means of eventually providing transgenic delivery of these types of crop protection approaches, we have been looking at elite cereal cultivars for high levels of activation with *cis*-jasmone. One of the compounds that is our target for increased expression is 6-methyl-5-hepten-2-one. We have found this to be one of a number of compounds (24) that is produced when *R. padi* attacks cereals and which causes repulsion of this aphid from normally attractive wheat seedlings. We also know that 6-methyl-5-hepten-2-one is an important foraging cue for the aphid parasitoid *A. ervi* (10). The biosynthesis for 6-methyl-5-hepten-2-one has been reported (9) as an oxidation product of isoprenoids by microbes. However, we have found that, in certain elite wheat cultivars, there is upregulation of the production of this compound with *cis*-jasmone. We also find that, as a consequence of this and other effects, there is repellency to the cereal aphid *S. avenae* when the wheat cultivar is treated with *cis*-jasmone (4). This has been followed through in the field where, in three seasons out of four, we have had reduced levels of cereal aphids on winter wheat, one month after *cis*-jasmone was applied (at a low field dose in an emulsifiable concentrate) (4). Although we have not been able to do similar field work on aphid parasitoids, we have shown, in simulated field trials on wheat seedlings treated with *cis*-jasmone, that there is a statistically significant increase in foraging by *A. ervi* (5).

### 3.3. *cis*-jasmone upregulates antibiotic effects

In addition to the behavioural effects induced in cereals, it was observed that there were reductions in aphid development. These involved statistically significant reductions in mean intrinsic rate of population increase and in nymph production by *S. avenae* on certain wheat varieties previously treated with *cis*-jasmone (4). This appears to relate to induction of antibiotic secondary metabolites such as from the hydroxamic acid pathway, including 2,4-dihydroxy-7-methoxy-1,4-benzoxazin-3-one (DIMBOA). The genes involved in this biosynthetic pathway were first identified in maize (11). However, the analogous genes were identified in wheat (16) and shown also to form clusters (17) as a consequence of the original evolution of these genes sequentially from a mutation at the gene beginning the oxidative stages of the pathway. This is an evolutionary trait, perhaps similar to that for the avenacia pathway in oats, *Avena sativa* (18). Thus, RT-PCR, using the published sequences to design primers, is allowing the induction of this process to be investigated, not only to exploit *cis*-jasmone induction for aphid control but also against other pest problems, including weeds, against which the hydroxamic acid pathway products can be allelopathic. However, more advanced analytical methods are needed and $MS^n$, after high resolution GC on trimethylsilylated derivatives, provides such and will be essential in identifying the antibiotic agents involved in barley, which lacks the hydroxamic pathway.

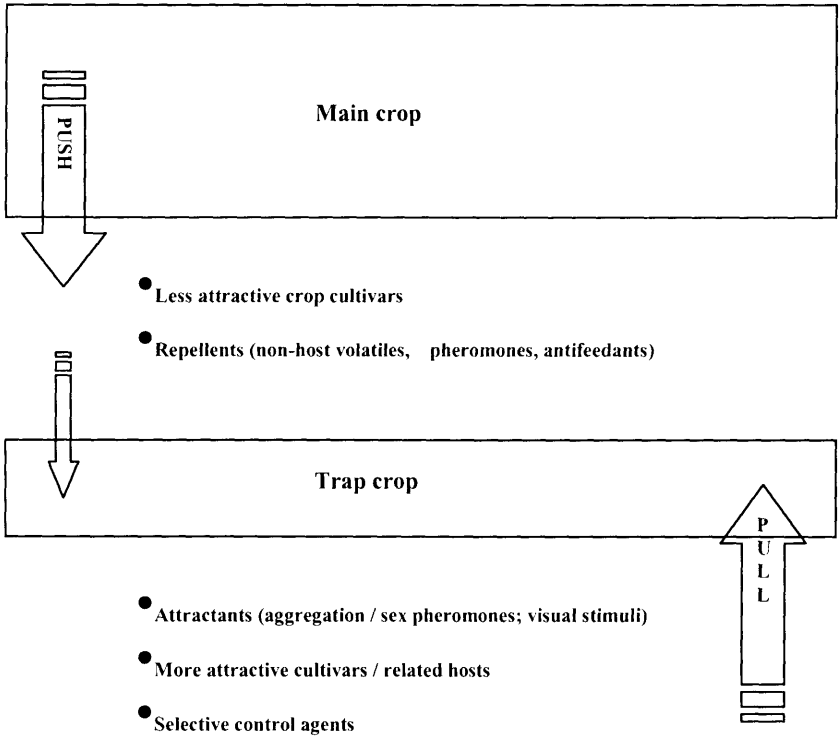


Figure 2. The general principles of a "push-pull" crop protection system.

## 4. THE "PUSH-PULL" STRATEGY

Although delivery of semiochemicals by plants, whether induced or not, provides a means of economically viable delivery, particularly for unstable or highly volatile compounds, the effects may not be sufficient to reduce the pest problem below the economic threshold (22). Also, in an attempt to avoid rapid development of resistance to semiochemical control strategies, we and other groups put together a number of semiochemically based control methods into a stimulo-deterrent, or "push-pull" strategy. This involves creating a "push" effect from the main crop, by using less attractive crop cultivars and by using repellents such as non-host volatiles, or oviposition deterrent pheromones and plant derived antifeedants (see Figure 2). The system also requires a trap crop ("pull") to which the pests are attracted by aggregation or sex pheromones, visual stimuli and more attractive cultivars/related hosts. On the trap crop can also be deployed a highly selective control agent. Economics do not usually allow the use of biological control agents in broad-acre crops, but application to a limited area of trap crop, particularly one in which the best conditions for infectivity with the biological agent can be established, will make the process economically feasible. Into this system also comes the potential to exploit beneficial organisms such as predators and parasitoids of the pests and so, as part of the

"push" strategy, there is also an involvement of foraging cues to ensure that the main crop is visited by predators and parasitoids before the pest population builds up. We have attempted to do this in the UK on the oilseed rape crop, initially using a trap crop comprising turnip rape, which produces both visual cues and volatile semiochemical attractants. Eventually, we hope to "switch on" the effects of "push" and "pull" by means of the kind of plant activators, such as *cis*-jasmone, described above.

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