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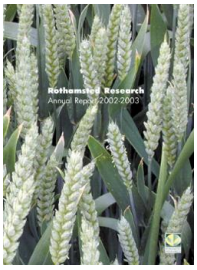
Rothamsted Research Annual Report 2002-2003



Full Table of Content

Plant Activators

Rothamsted Research

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Activating defence mechanisms and other useful traits in crop plants by means of benign chemical signals offers a new approach to pest control and other aspects of plant production. We have recently shown that the common plant volatile *cis*-jasmone can activate plants to become less attractive to herbivorous pests, and more attractive to pest natural enemies such as parasitic wasps. We have now demonstrated use of *cis*-jasmone in the field to reduce populations of cereal aphids. This opens up other practical and scientific prospects for using *cis*-jasmone as a plant activator.

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Developing plant activators for the field

Toby J Bruce, John A Pickett and Lesley E Smart

Background

The so-called "plant activators" that have been developed thus far by industry are non-volatile, persistent synthetic organic compounds. However, the natural products that provided the lead for these synthetic molecules act externally to the plant and often involve volatile products such as methyl salicylate and methyl jasmonate. *Cis*-Jasmone, although related biosynthetically to methyl jasmonate, had, until our recent work, been overlooked as a potential activator of plant defence.

Methyl jasmonate is formed initially as the *epi*-jasmonate isomer by oxidation and cyclisation of linolenic acid via 12-oxo-PDA (Figure 1). This biosynthetic pathway can be activated during damage caused by herbivory and other biotic agents, or by mechanical wounding. Methylation of the *epi*-jasmonic acid produces the volatile methyl ester, which can be emitted from the plant and used by animals searching for damaged plants (either because they wish to feed on the plant or on other animals feeding on the plant). Methyl jasmonate released from the essential oil of plants such as the sage brush, *Artemisia tridentata*, or from commercial sources, has been used to stimulate elevated defence responses in plants. For example, we have previously released methyl jasmonate above oilseed rape plants to enhance production of certain defensive glucosinolates.

Recently, we encountered *cis*-jasmone as an aphid repellent, and investigated it because of its relationship to methyl jasmonate (Figure 1). In these studies, we showed that two different aphid predators, a ladybird, and the parasitoid wasp *Aphidius ervi*, were attracted by *cis*-jasmone. This work was conducted initially on the bean plant, *Vicia faba*. We tested the hypothesis that *cis*-jasmone alters plant metabolism such that the plant becomes less attractive to herbivores and more attractive to parasitic wasps (Figure 2). Chemical analysis showed that *cis*-jasmone was rapidly taken up by the bean plants. After 24 hours, none could

The aphid parasitoid, *Aphidius ervi*, attacking the cereal aphid, *Sitobion avenae*. (inset left)

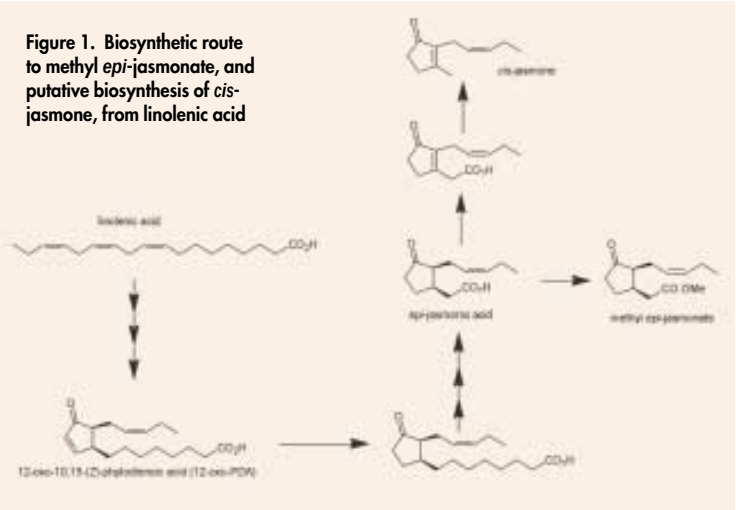

Figure 1. Biosynthetic route to methyl *epi*-jasmonate, and putative biosynthesis of *cis*-jasmone, from linolenic acid

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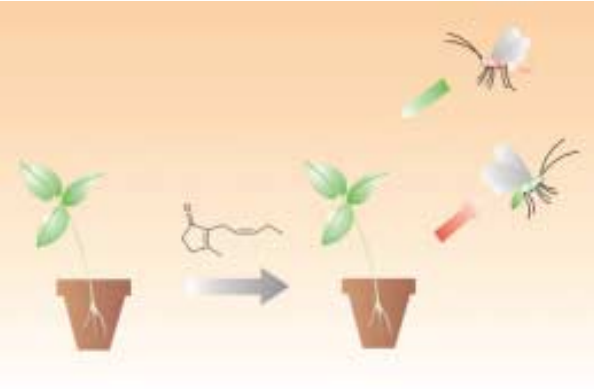

Table 1. Development of *Sitobion avenae* on treated wheat seedlings

	Treatment			
	Ethylan BV	*cis* -Jasmone	S.E.D.	*P* value
7 day wt	0.461 a	0.329 b	0.0371	<0.001
MRGR[1]	0.362 a	0.302 b	0.015	<0.001
D^2	11.53	11.90	0.231	0.113
PD^3	39.82	36.65	1.569	0.068
r_m[4]	0.237 a	0.223 b	0.005	0.013

Values followed by different letters in a row are significantly different (Least Significant Difference test). [1] Mean Relative Growth Rate. [2] Time (days) from birth to production of first nymph. [3] Total nymphs produced over equivalent time. [4] Intrinsic rate of population increase.

Figure 2. Plants placed in air containing a small amount of the volatile plant-derived chemical *cis*-jasmone can become less attractive to herbivores, e.g. aphids, and more attractive to beneficial insects such as aphid parasitoids

be detected, either in the air above the plant, from which the *cis*-jasmone had been absorbed, or on the plant surface itself. The plants were retained for another 24 hours to ensure that no *cis*-jasmone remained and were then tested in a wind tunnel, and found to be significantly more attractive to the aphid parasitoid *A. ervi*.

From beans to cereals

In behavioural tests using a Pettersson olfactometer, *cis*-jasmone was highly repellent, even at low levels, to the cereal aphid *Sitobion avenae*. Winter wheat seedlings treated with low levels of *cis*-jasmone were investigated in field simulation experiments. Large numbers of *S. avenae* were released downwind of a tray of either *cis*-jasmone treated or control wheat seedlings in no-choice tests. After 24 hours, the proportion of *S. avenae* settling on the plants had been significantly reduced, from a mean of 60% on control plants to 38% on treated plants ($P = 0.012$). However, the time spent foraging by the parasitoid *A. ervi* on *cis*-jasmone treated wheat seedlings was significantly increased, from 6.6 minutes on control plants to an average of 17.6 minutes on the treated plants ($P = 0.045$). We also noted a phenomenon not observed with bean plants; the intrinsic rate of aphid population increase in repeated experiments was significantly reduced on *cis*-jasmone treated plants (Table 1).

Field trials on winter wheat

Cis-jasmone was formulated with the non-ionic surfactant Ethylan BV, and applied by spraying the emulsion through a hydraulic nozzle (Figure 3). Successful reductions in aphid populations have now been obtained for four seasons. Results from a representative season are given in Figure 4. The principle of using *cis*-jasmone as a plant activator in this way is clearly demonstrated by these field trials. The overall effect on aphid populations may be due to a combination of reduced settling and slower population development. The approach of applying an aqueous emulsion is possible, even for a highly volatile compound such as *cis*-jasmone, since the effect clearly persists after the initial contact. This also means that the dose received by the plants is substantially lower than the applied field rate. Indeed, further studies will be made on improving the application of materials such as *cis*-jasmone, so that a higher activity level than implied here can be exploited.

In the earlier studies with beans, it was noted that the release of the parasitoid foraging stimulant (*E*)-ocimene, induced by *cis*-jasmone, persisted for over 8 days, whereas the same induction by methyl jasmonate only lasted for the first 48 hours after exposure. Induction of

Figure 3. Application of *cis*-jasmone using a hydraulic spray boom in field plot trials

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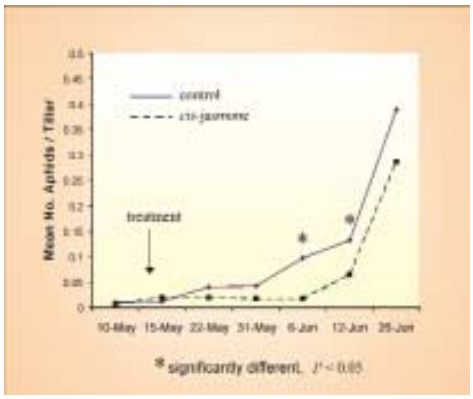

Table 2. Mean proportion of *Rhopalosiphum padi*, in no-choice test, settled on barley exposed to solutions containing *Elytrigia repens* compounds

Treatment	Proportion settled: Treated	Proportion settled: Control	P
E. repens root exudate	0.67	0.86	<0.01
Mixture*	0.72	0.84	<0.001
Carboline (0.1 mg/ml)	0.62	0.89	<0.0001

*Mixture = hydroxytryptophan, hydroxytryptophan hydrate, indoleacetic acid, carboline (1, 1, 1, 0.2 mg/ml respectively)

Figure 4. Field results of treating winter wheat with an emulsifiable concentration of *cis*-jasmone in May with statistically significant reduction in aphid counts in June

wheat plants with *cis*-jasmone also caused an increase in release of (*E*)-ocimene. It was observed that foraging *A. ervi* spent significantly longer on treated wheat plants in the laboratory, resulting in more aphid mummies being formed. However, in field experiments, natural populations of parasitoids were too low to observe statistically significant effects on numbers of aphid mummies.

Opportunities for identifying further plant activators

In collaboration with the Swedish University of Agricultural Sciences at Uppsala, it has been shown that certain plant species can release chemicals that stimulate defence in neighbouring plants, even without the initial plant being damaged. These plants include thistle, *Cirsium* spp., and couch grass, *Elytrigia repens*, neither of which would receive favour from farmers as an intercrop and source of plant-activating chemistry. However, it was also found that certain cultivars of barley could stimulate the defences of other cultivars when grown in close proximity. So far, a number of volatile compounds have been identified which contribute to the induction of defence, but none are as active or persistent as *cis*-jasmone. Some of these interactions, as well as taking place through aerial contact, can involve communication via the rhizosphere. Couch grass, grown adjacent to barley plants, can significantly reduce aphid colonisation of the barley plants, where there is contact through the rhizosphere. A carboline, 6-hydroxy-tetrahydro-β-carboline-3-carboxylic acid, one of the compounds exuded from the roots of couch grass, has been shown to account for a substantial part of this effect (Table 2). We have now made a larger scale synthesis of the carboline, for field trials here and with our collaborators at Uppsala.

Exploitation

Although the results obtained in these field trials would not compete in terms of efficacy with broad-spectrum eradicant pesticides, the effect of *cis*-jasmone could be exploited by selecting cultivars that are genetically particularly responsive to defence induction by this signal molecule. Indeed, such traits could be bred into plants as a new strategy for exploiting plant activators in crop protection. Also, if the mechanism by which the persistent effect induced by *cis*-jasmone were better understood, alternative strategies to exploit this knowledge could be envisaged. There are reports in the literature that the putative biosynthetic pathway (Figure 1) allows the plant to regulate the effects of activity through a volatile sink (*cis*-jasmone), without there being a role for this component of the pathway. The different context of our discoveries allowed patenting and commercialisation of *cis*-jasmone as a plant activator.

It has been demonstrated that *cis*-jasmone treated *Arabidopsis thaliana* is less attractive to aphids. Johnathan Napier and Michaela Matthes of the Crop Performance and Improvement Division have demonstrated the up regulation of a specific set of genes, different from those affected by methyl jasmonate. These genes are involved in various functions including the metabolism of 12-oxo-PDA (Figure 1). It is hoped that these studies will enable elucidation of the mechanisms by which *cis*-jasmone upregulates genes involved in the biosynthesis of other volatile signals as well as the physiological effects, which reduce the development of pest insects. Promoter sequences that are responsive to *cis*-jasmone have been identified and could provide the means of activating other valuable biosynthetic pathways such as those determining drought tolerance, nutritional composition or crop development.