Session 8 Novel Chemistry

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EXPLOITING CHEMICAL ECOLOGY FOR SUSTAINABLE PEST CONTROL

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ABSTRACT

The study of chemical ecology, particularly involving pheromones and other semiochemicals that influence insect behaviour, promises methods of pest control as alternatives to the exclusive use of broad-spectrum toxicants. However, if the potential of semiochemicals in crop protection is to be realised, a greater understanding of insect/insect/plant interactions and insect chemical ecology generally is essential. Semiochemicals, when employed alone, often give ineffective or insufficiently robust pest control. Use of semiochemicals should therefore be combined with other approaches in integrated management strategies. The main components of such strategies are pest monitoring, to allow accurate timing of pesticide treatments, combined use of semiochemicals, host plant resistance and trap crops, to manipulate pest behaviour, and selective insecticides or biological control agents, to reduce pest populations. The objective is to draw together these approaches into a push-pull or stimulo-deterrent diversionary strategy (SDDS). In an SDDS, the harvestable crop is protected by host-masking agents, repellents, antifeedants or oviposition deterrents. At the same time, aggregative semiochemicals, including host plant attractants and sex pheromones, stimulate colonisation of pests on trap crops or entry into traps where pathogens can be deployed. Because the individual components of the SDDS are not in themselves highly efficient, they do not select for resistance as strongly as conventional toxicant pesticides, thereby making the SDDS intrinsically more sustainable.

Semiochemicals in pest control

The semiochemicals which have been used most successfully in pest control are the sex pheromones of Lepidoptera and the aggregation pheromones of Coleoptera (Howse et al., 1995). Many commercially developed systems exist for using the sex pheromones of Lepidoptera in slow-release formulations to disrupt normal mate location. In the control of forest pests, aggregation pheromones of bark beetles are used in trap-out procedures. However, in dealing with the main pests of arable agriculture in Northern Europe, which principally comprise the aphids, alternative types of semiochemicals have to be employed, not only to control aphid pests directly, but also to reduce their transmission of plant virus diseases (Pickett et al., 1994).

Antifeedants against aphid pests

In efforts directed towards control of aphids, strategies involving more

sophisticated use of semiochemicals have been developed, including integration with population-reducing components such as biological agents. A number of antifeedant compounds, principally derived from plants, have been identified as potentially useful against aphid colonisation and feeding (Griffiths et al., 1989). Some of these act sufficiently quickly to reduce virus transmission, even when the viruses are transmitted in the non-persistent or semi-persistent modes. In the laboratory, the drimane sesquiterpenoid antifeedant (-)-polygodial, extracted from the water-pepper plant, Polygonum hydropiper, reduced transmission of potato virus Y by the peach-potato aphid, Myzus persicae, by over 70%, thus demonstrating activity against a nonpersistently transmitted virus disease which can be passed on to the plant after very limited contact with the aphid. In the field, the target was the bird-cherry-oat aphid, Rhopalosiphum padi, as a vector of the persistently transmitted barley yellow dwarf virus. Although polygodial can now be synthesised on a large scale using a method modified from Hollinshead et al. (1983), this procedure gives the racemic mixture and although the unnatural (+)-polygodial has similar antifeedant activity to the natural isomer (Asakawa et al., 1988), a nature-identical material was considered more appropriate for field use. To this end, a quantity of P. hydropiper was cultivated and after liquefied carbon dioxide extraction of the plant material on an industrial scale, the product contained a high concentration of (-)-polygodial. This was applied to cereals at 50 g/ha on three occasions in the autumn and, compared with untreated plots, gave over a t/ha improved yield, equivalent to the yields obtained using cypermethrin, a broad-spectrum synthetic pyrethroid insecticide (Pickett et al., 1987). The pesticide was employed at a similar application rate, but with only one treatment because of its longer half-life compared to the relatively unstable (-)-polygodial. However, such approaches, based on only one semiochemical type, were considered to be insufficiently robust for general farming practice and a direct population-reducing component was added.

Integrated use of the aphid alarm pheromone

When aphids are attacked, they release an alarm pheromone which causes other aphids in the area to disperse. The pheromone comprises the sesquiterpene hydrocarbon (E)- β -farnesene, and the synthetic product has been developed for use against aphid pests together with other agents causing direct reductions in population. Thus, in glasshouse trials using a hand-held electrostatic application system, the aphid alarm pheromone combined with spores of *Verticillium lecanii* gave a substantial improvement in control of the cotton aphid, *Aphis gossypii*, as compared to unsprayed chrysanthemums or the two treatments alone (Pickett *et al.*, 1986). In the field, a similar approach was adopted with a tractor-mounted electrostatic system, but with the contact pyrethroid permethrin as the population-reducing agent. Again, a highly significant improvement was obtained with the combined pheromone and pesticide treatment, as compared to the single treatments or unsprayed plots (Dawson *et al.*, 1990).

Integrated use of antifeedants

In addition to aphids, there are many coleopterous pests of arable agriculture in Northern Europe, but the drimane antifeedants, described above, are not as active

against these insects. However, it was found that antifeedants in the clerodane class of diterpenoids, e.g. the ajugarins, were extremely effective against coleopterous pests, particularly in the family Chrysomelidae. Against adults of the mustard beetle, Phaedon cochleariae, a concentration of 0.00001% applied to leaves showed significant antifeedant activity, whereas with the diamondback moth, Plutella xylostella, a concentration of 0.01%, similar to the field rate of polygodial used against aphids, was necessary (Griffiths et al., 1988). The ajugarins had virtually no activity against aphids. This high selectivity against Coleoptera, and particularly the Chrysomelids, is also demonstrated with the Colorado potato beetle, Leptinotarsa decemlineata. In simulated field trials, electrostatic spraying of an ajugarin protected the top parts of mustard plants, Brassica nigra (Griffiths et al., 1991). Here, it was essential to combine use of the antifeedant with a population-controlling agent since, as the plants grew through the applied antifeedant, the insects would feed even more avidly on the growing tips. Thus, when the insect growth regulant teflubenzuron was simultaneously applied to the lower parts of the plants, the population of insects was reduced within 24 h to less than 1% and the top parts of the plants, where the flowers and seed would subsequently develop, were completely protected. Currently, this approach is being applied to oilseed rape, Brassica napus, with the insect growth regulant replaced by various species of fungal pathogen active against Chrysomelidae and other coleopterous pests (Pickett et al., in press).

A new range of antifeedants is now being developed comprising the β -acids or lupulones (I) of the hop, *Humulus lupulus*. These compounds are produced as waste

products during hop extraction as they have no role in the brewing process. Nonetheless, they show strong antifeedant activity against a range of insect pests, including mites (Sopp et al., 1990), and are being used to protect the very crop from which they are extracted (Jones et al., submitted).

The push-pull or stimulo-deterrent diversionary strategy

It has been demonstrated that, for best use of semiochemicals, certainly against Northern European arable crop pests, more sophisticated regimes combining semiochemicals with population-reducing components should be adopted. Such approaches are encapsulated in the push-pull or stimulo-deterrent diversionary strategy (SDDS) (Pyke et al., 1987; Miller and Cowles, 1990) (Figure 1), in which

semiochemicals are deployed to "push" colonising insects away from the harvestable crop and also to attract predators or parasitoids into the area. At the same time, the pests are aggregated on a sacrificial or trap crop so that a selective control agent, e.g. a fungal pathogen, can be used directly to reduce the pest population.

"PUSH" (away from the crop)	"PULL" (into traps or trap crops)
Kairomone inhibition	Kairomones
Repellents, antifeedants, oviposition deterrents	Aggregation, sex and oviposition pheromones
	Visual cues
Attractants for parasitoids and predators	Selective control agents (e.g. pathogens)

Figure 1. The push-pull or stimulo-deterrent diversionary strategy (SDDS)

The model chosen for initial demonstration of the SDDS was the pea and bean weevil, Sitona lineatus, on field beans, Vicia faba. The "pull" component of the SDDS was the aggregation pheromone of S. lineatus, identified previously as a simple 1,3-diketone (II). The activity of this compound as an attractant was shown to be enhanced

by plant components including (Z)-3-hexen-1-ol, (Z)-3-hexen-1-yl acetate and (R+S)-linalool. The identification of these compounds, particularly the aggregation pheromone, relied heavily on electrophysiological preparations from the antennae of S. lineatus, both the electroantennogram (EAG) and single-cell recordings (SCR), directly coupled with high resolution gas chromatography (GC) (Blight et al., 1984, 1991). Although the plant components identified as synergising the attractiveness of the aggregation pheromone are ubiquitous in the plant kingdom, the insect nonetheless employs highly specific receptors for their detection. For example, the olfactory cells specifically responding to (Z)-3-hexen-1-ol are relatively insensitive to (Z)-3-hexen-1-yl acetate and (R+S)-linalool. These compounds together, or the aggregation pheromone alone, can be used as lures in yellow-coloured traps similar to those employed in cotton against the boll weevil, Anthonomus grandis (Blight et al., 1991).

Traps baited with the aggregation pheromone should be commercially available for monitoring of S. lineatus within the near future.

The "push" component of the SDDS against *S. lineatus* involved use of a commercially available antifeedant based on an extract of the Indian neem tree, *Azadirachta indica*. Although there are many claims for the effectiveness of neem extracts in the general scientific literature, in arable agriculture such materials do not compare favourably with conventional pesticides. However, against *S. lineatus*, sufficient antifeedancy was observed to allow further investigation within the SDDS. Thus, in field trials comprising the "push" and "pull" components described, significantly fewer *S. lineatus* were found on the "push" plots and more on the "pull" plots relative to untreated. Although the "push" plots were insufficiently well protected for high input agriculture, these trials nonetheless demonstrated the principle of the SDDS (Smart *et al.*, 1994).

SDDS components of aphid control

The recent identification of aphid sex pheromones (Dawson et al., 1987, 1990; Guldemond et al., 1993) allowed the demonstration that these insects could make oriented flight to a distant source of semiochemical (Campbell et al., 1990). This stimulated the search for plant-derived semiochemicals that might be involved in longrange selection of host plants.

The primary rhinaria on the fifth and sixth segments of the aphid antenna are implicated in detection of host plant chemicals. Single-cell recordings from olfactory nerve cells within these organs, made using electrolytically sharpened tungsten electrodes, allowed identification of a range of host plant attractants for these pests (Wadhams, 1990; Nottingham et al., 1991). However, certain cells appeared to have no function in interactions with host plants, nor did they seem to be involved in insect/insect communication. Other groups working on larger insects have also noticed such apparently redundant cells. By investigating a number of non-host plants, it appeared that such cells did in fact have a role in detecting chemicals typical of plants upon which the aphid could not feed. Again using coupled GC-SCR, accomplished for the first time with aphids in connection with this work, it was possible to identify compounds from non-host plants to which the apparently redundant cells responded. Thus, the black bean aphid, Aphis fabae, which feeds on many plants but seldom on members of the Cruciferae (= Brassicaceae), detects specific isothiocyanates which are typical of these plants. It was shown that such compounds act as repellents for this aphid and also as masking agents for the normal attractancy of bean volatiles (Nottingham et al., 1991). Similarly, when members of the Labiate family (= Lamiaceae) were investigated, other compounds having a similar role were identified, including the monoterpene oxidation product (-)-(1R,5S)-myrtenal, which again significantly reduced attractiveness of host plant volatiles (Hardie et al., 1994a).

R. padi colonises Prunus padus, the bird-cherry, as its primary host for sexual reproduction in the autumn. However, in the spring, it must migrate to the summer or secondary host, cereal crops. One of the compounds shown to be highly active in GC-SCR work, using volatiles from the primary host, was methyl salicylate, which therefore

became a candidate for repellent activity against the spring migratory morphs. In 1992, in field work conducted on barley in Sweden, over 50% reduction in population of *R. padi* was obtained using methyl salicylate, either released from an emulsifiable concentrate sprayed onto the crop or using slow-release vials (Pettersson *et al.*, 1994). Subsequently, high repellent activity was found with other species of cereal aphids, including the grain aphid, *Sitobion avenae*. Although *S. avenae* does not normally host-alternate, its employment of methyl salicylate as a repellent may indicate the role of this compound as a plant stress signal because of its relationship with the damage-inducible phenylalanine ammonia lyase pathway (Ward *et al.*, 1991). Thus, in 1993 and 1994, approximately 50% reduction of cereal aphid population was again achieved with release rates of 1-5 mg/plot/day, with plot sizes of 10 m² (unpublished results).

Parasitoid attraction in an aphid SDDS

Another important component of the SDDS against aphids is the attraction of parasitoids into the crop at an early stage of population development. This can be achieved by use of synomones, released by plants on feeding damage, which attract aphid parasitoids and stimulate foraging behaviour. Such synomones represent a learned response and it is not always possible to attract parasitoids into crops when they have been foraging on non-crops with non-pest aphids as a host source. However, GC-coupled electrophysiological studies, performed for the first time on braconid wasps such as the general aphid parasitoid *Praon volucre*, have shown that components of aphid sex pheromones, e.g. the nepetalactone isomer III, can act as potent attractants

(kairomones) in an unlearned situation (Hardie et al., 1993, 1994b). In field trials using potted plants, a four-fold increase in attack and egg-laying by P. volucre on S. avenae was obtained with this compound. For the more specific parasitoid Aphidius ervi, an important control agent for the pea aphid, Acyrthosiphon pisum, no attraction or increase in parasitism was observed in these trials. However, the sex pheromone of A. pisum comprises largely the nepetalactol isomer IV and when this compound was employed in field pot trials, there was a three-fold increase in parasitism (Pickett et al., 1994). The exploitation of natural populations of aphid parasitoids in reducing aphid populations on a number of crops is now being investigated with combined Levy Board funding.

SDDS and sustainability

It has been shown that the SDDS comprises a number of components affecting different aspects of the behaviour and development of pests. Although each

component, when compared to conventional broad-spectrum toxicants, is relatively ineffective, e.g. the plant-derived aphid repellents which reduce populations by only 50%, this has the advantage of not selecting efficiently for resistance and thus contributes to the sustainability of the SDDS.

Sustainability and transgenic crop plants

The defence chemistry of many modern crops is relatively inefficient, largely because this has been removed by long-term plant breeding programmes in the interests of high yield and nutritional value for human consumers. With recombinant DNA technology, it is now feasible to produce metabolites of value in crop protection within the parts of the plant that are not consumed (Hallahan et al., 1992). Sustainability is again likely to be greater where the targets involve semiochemicals because, even if constitutively expressed, these agents would not select strongly for resistance as do the potent biological toxins, such as that from Bacillus thuringiensis, currently under commercial development. Two general strategies can be adopted: one is to modify existing secondary metabolism pathways for defence by altering the level of expression of endogenous genes. The alternative is to insert alien genes, preferably from other higher plants and particularly wild species, that have retained defences based on secondary metabolism, so as to augment an existing biosynthetic route. approaches are being adopted for oilseed rape (Pickett et al., 1995), where the objective is to reduce the production of glucosinolate precursors for specific pest attractants and, at the same time, to produce highly attractive cultivars for use as trap crops. Such crops could also have industrial value in terms of the oil produced. With regard to the insertion of alien genes from higher plants, a number of terpenoids have been targeted, particularly components produced by plants in the Nepeta genus, including compounds III-IV (Hallahan et al., 1995), which are related to the aphid sex pheromones. In the long term, it is intended to investigate modification and augmentation of phenolic production related to the phenylalanine ammonia lyase pathway, which would involve target semiochemicals including the methyl salicylate discussed earlier. Such work has a major world significance in that compounds, e.g. veratrole (1,2-dimethoxybenzene), found to be highly active to the rice pest Nilaparvata lugens, the brown planthopper, are also produced via an extension of this particular pathway (Cocking et al., 1994).

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REFERENCES

Asakawa, Y.; Dawson, G.W.; Griffiths, D.C.; Lallemand, J-Y.; Ley, S.V.; Mori, K.; Mudd, A.; Pezechk-Leclaire, M.; Pickett, J.A.; Watanabe, H.; Woodcock, C.M.;

- Zhang, Z-n. (1988) Activity of drimane antifeedants and related compounds against aphids, and comparative biological effects and chemical reactivity of (-)-and (+)-polygodial. *Journal of Chemical Ecology* 14, 1845-1855.
- Blight, M.M.; Pickett, J.A.; Smith, M.C.; Wadhams, L.J. (1984) An aggregation pheromone of Sitona lineatus. Naturwissenschaften 71, S.480.
- Blight, M.M.; Dawson, G.W.; Pickett, J.A.; Wadhams, L.J. (1991) The identification and biological activity of the aggregation pheromone of *Sitona lineatus*. Aspects of Applied Biology 27, 137-142.
- Campbell, C.A.M.; Dawson, G.W.; Griffiths, D.C.; Pettersson, J.; Pickett, J.A.; Wadhams, L.J.; Woodcock, C.M. (1990) Sex attractant pheromone of damson-hop aphid *Phorodon humuli* (Homoptera, Aphididae). *Journal of Chemical Ecology*, 16, 3455-3465.
- Cocking, E.C.; Blackhall, N.W.; Brar, D.S.; Davey, M.R.; Khush, G.S.; Ladha, J.K.; Pickett, J.A.; Power, J.B.; Shewry, P.R. (1994) Biotechnological approaches to rice genetic improvement. *Proceedings, Food Security in Asia, The Royal Society, London, 1st November, 1994*, pp. 23-26.
- Dawson, G.W.; Griffiths, D.C.; Janes, N.F.; Mudd, A.; Pickett, J.A.; Wadhams, L.J.; Woodcock, C.M. (1987) Identification of an aphid sex pheromone. *Nature*, 325, 614-616.
- Dawson, G.W.; Griffiths, D.C.; Merritt, L.A.; Mudd, A.; Pickett, J.A.; Wadhams, L.J.; Woodcock, C.M. (1990) Aphid semiochemicals a review, and recent advances on the sex pheromone. *Journal of Chemical Ecology*, 16, 3019-3030.
- Griffiths, D.C.; Hassanali, A.; Merritt, L.A.; Mudd, A.; Pickett, J.A.; Shah, S.J.; Smart, L.E.; Wadhams, L.J.; Woodcock, C.M. (1988) Highly active antifeedants against coleopteran pests. Proceedings of the Brighton Crop Protection Conference Pests and Diseases, 1041-1046.
- Griffiths, D.C.; Pickett, J.A.; Smart. L.E.; Woodcock, C.M. (1989) Use of insect antifeedants against aphid vectors of plant virus disease. *Pesticide Science* 27, 269-276.
- Griffiths, D.C.; Maniar, S.P.; Merritt, L.A.; Mudd, A.; Pickett, J.A.; Pye, B.J.; Smart, L.E.; Wadhams, L.J. (1991) Laboratory evaluation of pest management strategies combining antifeedants with insect growth regulator insecticides. *Crop Protection*, 10, 145-151.
- Guldemond, J.A.; Dixon, A.F.G.; Pickett, J.A.; Wadhams, L.J.; Woodcock, C.M. (1993) Specificity of sex pheromones, the role of host plant odour in the olfactory attraction of males, and mate recognition in the aphid *Cryptomyzus*. *Physiological Entomology*, 18, 137-143.
- Hallahan, D.L.; Pickett, J.A.; Wadhams, L.J.; Wallsgrove, R.M.; Woodcock, C.M.
 (1992) Potential of secondary metabolites in genetic engineering of crops for resistance. In: Plant Genetic Manipulation for Crop Protection, A.M.R.
 Gatehouse, V.A. Hilder and D. Boulter (Eds.), Wallingford: C.A.B.
 International, pp. 215-248.
- Hallahan, D.L.; West, J.M.; Wallsgrove, R.M.; Smiley, D.W.M.; Dawson, G.W.; Pickett, J.A.; Hamilton, J.G.C. (1995) Purification and characterization of an acyclic monoterpene primary alcohol:NADP⁺ oxidoreductase from catmint (Nepeta racemosa). Archives of Biochemistry and Biophysics 318, 105-112.
- Hardie, J.; Isaacs, R.; Nazzi, F.; Powell, W.; Wadhams, L.J.; Woodcock, C.M. (1993) Electroantennogram and olfactometer responses of aphid parasitoids to

- nepetalactone, a component of aphid sex pheromones. Behavioural ecology, augmentation and enhancement of aphidophaga. Proceedings, 5th International Symposium of the Global IOBC Working Group: Ecology of Aphidophaga.
- Hardie, J.; Isaacs, R.; Pickett, J.A.; Wadhams, L.J.; Woodcock, C.M. (1994a) Methyl salicylate and (-)-(1R,5S)-myrtenal are plant-derived repellents for black bean aphid, Aphis fabae Scop. (Homoptera: Aphididae). Journal of Chemical Ecology 20, 2847-2855.
- Hardie, J.; Hick, A.J.; Höller, C.; Mann, J.; Merritt, L.A.; Nottingham, S.F.; Powell, W.; Wadhams, L.J.; Witthinrich, J.; Wright, A.F. (1994b) The responses of *Praon* spp. parasitoids to aphid sex pheromone components in the field. *Entomologia Experimentalis et Applicata*, 71, 95-99.
- Hollinshead, D.M.; Howell, S.C.; Ley, S.V.; Mahon, M.; Ratcliffe, N.M.; Worthington, P.A. (1983) The Diels-Alder route to drimane related sesquiterpenes; synthesis of cinnamolide, polygodial, isodrimeninol, drimenin and warburganal. *Journal of the Chemical Society, Perkin Transactions 1*, 1579-1589.
- Howse, P.; Stevens, I.; Jones, O. (1995) Insect Pheromones and Their Use in Pest Management, London: Chapman & Hall, 256 pp.
- Jones, G.; Campbell, C.A.M.; Pye, B.J.; Maniar, S.P., Mudd, A. Repellent and oviposition-deterring effects of hop beta-acids on the two-spotted spider mite *Tetranychus urticae*. *Pesticide Science* (submitted).
- Miller, J.R.; Cowles, R.S. (1990) Stimulo-deterrent diversionary cropping: a concept and its possible application to onion maggot control. Symposium on Semiochemicals and Pest Control, Wageningen, October 1989. Journal of Chemical Ecology, 16, 3197-3212.
- Nottingham, S.F.; Hardie, J.; Dawson, G.W.; Hick, A.J.; Pickett, J.A.; Wadhams, L.J.; Woodcock, C.M. (1991) Behavioral and electrophysiological responses of aphids to host and nonhost plant volatiles. *Journal of Chemical Ecology* 17, 1231-1242.
- Pettersson, J.; Pickett, J.A.; Pye, B.J.; Quiroz, A.; Smart, L.E.; Wadhams, L.J.; Woodcock, C.M. (1994) Winter host component reduces colonization by the bird-cherry-oat aphid, *Rhopalosiphum padi* (L.) (Homoptera, Aphididae), and other aphids in cereal fields. *Journal of Chemical Ecology*, 20, 2565-2574.
- Pickett, J.A.; Cayley, G.R.; Dawson, G.W.; Griffiths, D.C.; Hockland, S.H.; Marples, B.; Plumb, R.T.; Woodcock, C.M. (1986) Use of the alarm pheromone and derivatives against aphid-mediated damage. Abstracts 6th International Congress Pesticide Chemistry, IUPAC, Ottawa, 1986, 2C-08.
- Pickett, J.A.; Dawson, G.W.; Griffiths, D.C.; Hassanali, A.; Merritt, L.A.; Mudd, A.; Smith, M.C.; Wadhams, L.J.; Woodcock, C.M.; Zhang, Z-n. (1987) Development of plant-derived antifeedants for crop protection. In: *Pesticide Science and Biotechnology*, R. Greenhalgh and T.R. Roberts (Eds.), Blackwell Scientific Publications, pp. 125-128.
- Pickett, J.A.; Wadhams, L.J.; Woodcock, C.M. (1994) Attempts to control aphid pests by integrated use of semiochemicals. *Brighton Crop Protection Conference Pests and Diseases -1994*, 1239-1246.
- Pickett, J.A.; Butt, T.M.; Doughty, K.J.; Wallsgrove, R.M.; Williams, I.H. Minimising pesticide input in oilseed rape by exploiting natural regulatory processes. *Proceedings of the 9th International Rapeseed Congress 1995, Cambridge, UK*, in press.

- Pyke, B.; Rice, M.; Sabine, B.; Zalucki, M. (1987) The push-pull strategy behavioural control of *Heliothis*. Australian Cotton Grower, May-July 1987, 7-9.
- Smart, L.E.; Blight, M.M.; Pickett, J.A.; Pye, B.J. (1994) Development of field strategies incorporating semiochemicals for the control of the pea and bean weevil, Sitona lineatus L. Crop Protection 13, 127-135.
- Sopp, P.I.; Palmer A.; Pickett, J.A. (1990) The effect of a plant-derived antifeedant on Tetranychus urticae and Phytoseiulus persimilis: "A first look". SROP/WPRS Bulletin XIII/5 (1990), 198-201.
- Wadhams, L.J. (1990) The use of coupled gas chromatography: electrophysiological techniques in the identification of insect pheromones. In: *Chromatography and Isolation of Insect Hormones and Pheromones*, A.R. McCaffery and I.D. Wilson (Eds), New York: Plenum, pp. 289-298.
- Ward, E.R.; Uknes, S.J.; Williams, S.C.; Dincher, S.S.; Wiederhol, D.L.; Alexander, D.C.; Ahl-Goy, P.; Métraux, J.-P.; Ryals, J.A. (1991) Coordinate gene activity in response to agents that induce systemic acquired resistance. *Plant Cell* 3, 1085-1094.

THE EFFECT OF FOLIAR APPLIED POTASSIUM CHLORIDE ON ERYSIPHE GRAMINIS INFECTING WHEAT.

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ABSTRACT

Potassium chloride fertiliser applied as a foliar spray in field and glasshouse experiments significantly reduced the percentage leaf area of wheat affected by powdery mildew (Erysiphe graminis) compared to an application of fertiliser The response was dependent upon the to the soil. concentration of the solution applied. The optimum concentration in glasshouse conditions was approximately 10% w/v. The foliar applied potassium chloride reduced the germination of powdery mildew spores on the leaf surface and also inhibited the The inhibition of establishment of infections. infection was associated with increases in the leaf water potential. Experiments using polyethylene glycol to produce biologically inert solutions of equivalent osmotic potential to potassium chloride revealed that both the inhibition of germination and the reduction of the disease symptoms may be due to the physico-chemical properties of the fertiliser rather than metabolic toxicity or nutritional effects on the host.

INTRODUCTION.

Work carried out in the late 1980's to investigate the response of cereal yields to foliar application of potassium chloride revealed that these applications could also reduce the leaf area affected by Septoria spp. and powdery mildew (Kettlewell et al., 1990). It was decided that foliar applied potassium chloride may be a potentially inexpensive disease control agent. If the potassium chloride fertiliser applied to the crop could also reduce the severity of disease to below the threshold at which conventional fungicide control methods would be used it could potentially reduce the total resources used in crop production with economic and environmental benefits. The object of this work was to evaluate foliar applied potassium chloride for the control of powdery mildew and investigate the mode of activity.

FIELD WORK.

Initially field experiments were carried out, on wheat, to evaluate the effect of potassium chloride fertilisers on a range of pathogens by sequential applications throughout the season. Potassium chloride was applied at different timings throughout the growing season to identify pathogens against which foliar applied potassium chloride was effective. Potassium chloride fertiliser was applied as soil dressings or foliar sprays to investigate whether the control was due to the nutritional properties of the fertiliser or some factor intrinsic to the foliar application.

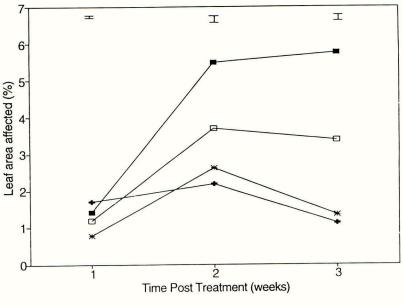
The field experiments were conducted at sites in Shropshire during 1992 on cultivar Mercia and 1993 on cultivars Riband and Apollo. The sites had soil potassium indices of one and zero respectively. The experiments were factorial designs with three factors which were growth stages at application of 32, 39 and 51 each with two levels which were potassium chloride applied as a foliar spray or a solid powder. Each level comprised 33.3 kg/ha potassium chloride applied as a solid powder or a foliar spray of 15.136% w/v solution at 220 1/ha. For comparative purposes two treatments were included outside the factorial design. These were standard practice which comprised 100 kg/ha soil applied potassium chloride at growth stage 32 and standard practice plus a three spray prophylactic fungicide programme. The experiments were assessed by visual observation weekly after treatment for three weeks using A.D.A.S. disease charts.

There were no visible disease symptoms before treatments were applied in either year. The application of potassium chloride as a foliar spray at growth stage 32 consistently reduced the leaf area affected by powdery mildew compared to the solid fertiliser and standard practice treatments for three weeks after treatment in both years. This is illustrated for 1992 (fig.1). In both years the leaf area affected by powdery mildew was not significantly different between the foliar treatment and the standard practice plus fungicides. The leaf area of the flag leaf affected by powdery mildew was very small in both years. There was no interaction between cultivar and fertiliser application method.

GLASSHOUSE AND LABORATORY STUDIES.

Having established the effectiveness of foliar applied potassium chloride as an agent for the control of powdery mildew on wheat, glasshouse and laboratory work was conducted to investigate its mode of action.

Figure 1. The effect of potassium chloride applied to the foliage or soil at growth stage 32 on the percentage leaf area of winter wheat (cv. Mercia) affected by powdery mildew in 1992.



-■ 33.3 kg/ha soli → 220 l/ha 15.1% → Std. practice + → Std. practice

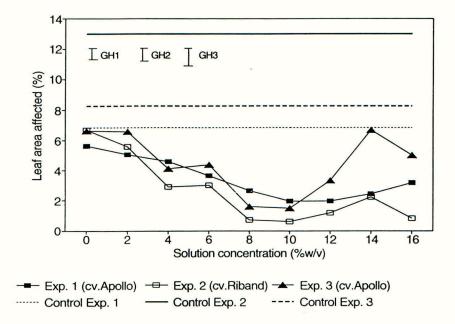
Rate responses.

Three initial experiments examined the effect of different concentrations of potassium chloride solution applied to the leaves of unvernalised winter wheat plants in order to establish the optimum concentration for the control of powdery mildew.

The plants were sprayed with a precision pot sprayer at a rate equivalent to 200 l/ha and inoculated by shaking pots of artificially inoculated seedlings over the plants.

In three experiments there was a linear decline in the leaf area affected by mildew up to 10% w/v (fig.2). Above this concentration the leaf area affected increased. This increase probably reflected a decline in plant resistance to the pathogen due to osmotic stress.

Figure 2. The effect of increasing the concentration of potassium chloride solution on percentage leaf area of wheat affected by powdery mildew.



Timing Responses.

An experiment was conducted in which unvernalised wheat plants (cv. Apollo) were treated with a foliar spray of water at 200 l/ha or a 10% w/v potassium chloride solution at 200 l/ha either as a foliar spray or a soil applied solution. The treatments were applied at four timings which were seven or three days before or after inoculation with $\it E. graminis$).

The foliar applied potassium chloride resulted in a lower percentage area of the wheat leaves being affected by powdery mildew than soil applied potassium chloride or water applied to the leaves. This indicated that the control of powdery mildew achieved by foliar applied potassium chloride was due to some intrinsic factor concerning the solution and application to the leaf rather than a property of the potassium chloride itself. There was no significant difference between the percentage area of the leaves affected by powdery mildew with regard to timing of application. This indicated that the foliar applied potassium chloride had both protective and curative properties if applied within seven days of inoculation.

Spore germination in vitro.

In view of the linear relationship between percentage leaf area affected by powdery mildew and increasing concentration of the fertiliser solution it was proposed that potassium chloride solution might act on the spores of *E. graminis* by creating an osmotic potential and causing an eflux of water from the spores so reducing their viability rather than direct metabolic toxicity.

An experiment was carried out to examine the effect of solution osmotic potential on spore viability in vitro. Solutions of potassium chloride or polyethylene glycol with osmotic potentials of 0, 12.8, 25.6, 38.4, 51.3 and 64.1 bar were created. These were equivalent to potassium chloride solutions of 0,2,4,6,8, and 10% w/v. Polyethylene glycol was used as a control for the potassium chloride because it is a biologically inert osmotica. Spores were incubated in these solutions on glass slides. A minimum of thirty spores per slide were monitored at each assessment. The plasmolysis of spores and the production of germ tubes were recorded.

The spores did not collapse in distilled water but retained their integrity as described elsewhere (Corner, 1935; Manners and Housain, 1963). A proportion also retained the viability to produce germ tubes. All patterns in the response to osmotic potential were essentially evident after six hours. Increasing solution osmotic potential resulted in a negative linear response in spore germination (P=0.001) and a linear increase in plasmolysis (P=0.001). No consistent differences were detected between the effects of the two osmotica. Overall it appeared that the critical factor affecting spore germination was the osmotic potential of the solutions rather than the osmotica.

Spore Germination in vitro.

Since the wax cuticle interacts with surface solutions and spray deposition is uneven across the leaf it was difficult to draw direct comparisons between solutions of the same concentration on the leaf surface and *in vitro* results as the conditions are so different. Therefore an experiment was conducted *in vivo* examining the effect of solution osmotic potential on the germination of *E. graminis* spores.

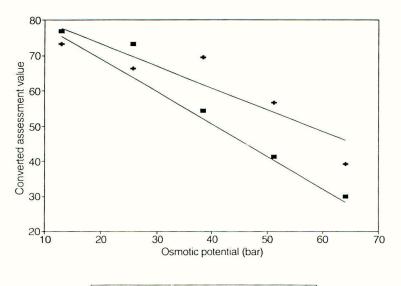
The experiment was conducted using unvernalised wheat plants (cv. Apollo) with three fully expanded leaves. The plants were grown in a low potassium compost in a spore free propagator with an ambient air source. The plants were inoculated and sprayed with polyethylene glycol and potassium chloride solutions as detailed for the *in vitro* experiment at a rate equivalent to 200 l/ha. After twenty

four hours the upper fully expanded leaf of certain plants were detached and cleared with a 3:1 mixture of acetic acid and ethanol followed by rehydration with an alcohol series. The leaves were stained with trypan blue and observed. The remaining plants were visually assessed for percentage area of the upper leaf affected by powdery mildew after two weeks.

The area of the upper fully expanded leaf affected by $E.\ graminis$ declined in a linear manner (P=0.001) as the osmotic potential of the solutions increased with no significant difference between the two osmotica. Microscopic examination revealed that the percentage germination of the spores declined in a linear manner with increasing osmotic potential of the solution applied (P=0.001) with no difference between osmotica. However virtually all spores which produced germ tubes continued to develop and produce infection.

The means of percentage leaf area affected and spore germination were expressed as percentages of their controls and plotted together (fig.3). A parallel regression was carried out revealing that both data sets had similar slopes.

FIGURE 3. The reduction in spore germination and percentage area affected by *E. graminis in vivo* expressed as a percentage of control.



leaf area affected + spore germination

Leaf Water Potential.

Percentage germination was much higher than the equivalent percentage leaf area affected. This suggested that some other factor may have an influence upon leaf area affected by interfering with the establishment of the Preliminary experiments had indicated that pathogen. foliar applied potassium chloride was rapidly taken up by the leaves of wheat plants and that it also resulted in an increase in the leaf water potential. This elevation of the leaf water potential lasted over thirteen days and therefore such an effect could conceivably have been responsible for the control of powdery mildew observed when potassium chloride was applied seven days before inoculation.

An experiment was conducted to investigate the relationship between the osmotic potential of a foliar applied solution, the leaf water potential and the percentage leaf area affected. Unvernalised wheat (cv. Apollo) plants with four fully expanded leaves were treated with either polyethylene glycol or potassium chloride solutions and inoculated with spores from stock pots of the The solutions had a range of osmotic same wheat cultivar. Two plants per block received each treatment. potentials. One plant was destructively sampled after twenty four hours. The upper fully expanded leaf was detached and the leaf water potential determined using a pressure bomb. The other plant was visually assessed for the percentage area of the upper fully expanded leaf affected with powdery mildew after The leaf water potential was positively fourteen days. correlated with the osmotic potential of the solution The leaf area affected was negatively correlated applied. with the solution osmotic potential. This suggested that the leaf water potential increase could be responsible for the reduction in disease symptoms. Linear modelling revealed a possible correlation between leaf water potential and the leaf area affected after the removal of block effects. However the relationship was different with respect to the two osmotica. It was thought that the effect of solution osmotic potential on spore germination was obscuring the responses to changes in leaf water potential. Therefore the experiment was repeated using three plants per treatment per block. The upper fully expanded leaf of the third plant was detached, cleared and the percentage germination of the spores determined. multiple regression model was then used to test the correlation of the leaf water potential and the percentage leaf area affected by powdery mildew after accounting for the differences in spore germination. This experiment confirmed the previous experient.

Microscopic examination of leaves four days after inoculation revealed that very few germinated spores

developed haustoria on leaves treated with a foliar applied potassium chloride or polyethylene glycol of 64 bar osmotic potential. Development was normal on untreated leaves and those sprayed with water.

CONCLUSION.

The application of potassium chloride fertiliser as a foliar spray may be an effective method for the control of powdery mildew disease on wheat. The effect could be the result of the inhibition of spore germination on the leaf surface and partly by induced changes in the leaf physiology. The most likely change to affect pathogen establishment is a rise in the leaf water potential following application. The effect appears to be non nutritional and purely due to the physico-chemical properties of the solution. This suggests that other osmotically active fertiliser solutions could have a similar effect.

The control of powdery mildew by foliar applied potassium chloride was comparable with that provided by conventional fungicides. This suggests that with further development alternative fertiliser strategies using foliar potassium chloride could be developed leading to a reduction in fungicide usage. This would considerably reduce the costs of cereal production and the use of non renewable resources in agriculture leading to more sustainable crop production.

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REFERENCES

- Corner, E.J.H. (1935). Observations on resistance to powdery mildews. New Phytologist, 34, 180-200.
- Kettlewell, P.S., Bayley, G.L., and Domleo, R., (1990).
 Evaluation of late season foliar application of potassium chloride for disease control in winter wheat.
 Journal of Fertiliser Issues. 7 (1), 17-23.
- Manners, J.G. and Housain, S.M.M. (1963). Effects of temperature and humidity on conidial germination in Erysiphe graminis. Transactions of the British Mycological Society. **46**(2), 225-234.