MINIMISING PESTICIDE INPUT IN OILSEED RAPE BY EXPLOITING NATURAL REGULATORY PROCESSES

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ABSTRACT

There is continuing demand for the agriculture industry to reduce reliance on pest control agents with toxic modes of action and there may be significant problems of resistance to pesticides and of inappropriate redistribution in the environment. Research and development on alternatives to pesticides are essential but those currently available require critical appraisal. Pest control based on chemicals with non-toxic modes of action (e.g. pheromones and host plant semiochemicals), although now showing great promise, must be integrated with the use of biological agents, which themselves lack reliability for arable crops when employed alone. The oilseed rape agro-ecosystem represents a useful model for exploring such an integration of approaches. As well as having a high profile in arable farming, it possesses a range of natural processes with regulatory roles in pest and disease development which can potentially be exploited in this crop. Also, techniques for genetic transformation of the crop are already advanced, giving the opportunity to control semiochemical production by molecular genetics. Results from work at the Institute of Arable Crops Research, Rothamsted, are discussed.

INTRODUCTION

Oilseed rape, *Brassica napus* (Brassicaceae = Cruciferae) is now an ubiquitous crop in Europe and North America. In Britain, around 400,000 ha are sown each year. It is attacked by a range of insect pests and fungal pathogens. During initial colonisation and larval development on the crop, insects interact with their host by means of chemical cues (semiochemicals). The development of fungal pathogens within the plant may also be determined by chemical components.

Semiochemicals are studied to develop new methods of pest control without recourse to toxic modes of action. The primary objective is to reduce colonisation and development on the harvestable crop by inhibiting attractants and other stimulants, for example by interfering with normal insect behaviour on the crop using antifeedants or oviposition deterrents. As this push away from the crop is created, a sacrificial or "trap" crop is used towards which attraction is maximised by visual cues, such as early flowering, and enhancement of plant-derived attractants, and also by the deployment of insect-derived attractant and aggregation pheromones. At the same time, attempts are made to attract predators and parasitoids of the pests. Having created an initial protection for the harvestable crop and a pull onto the trap crop, highly selective control agents, particularly entomogenous fungal pathogens, can be employed to reduce insect populations to below the economic threshold. This approach is economical in its use of the biological control agent and allows better growth and conditions for infection of the pests on the trap crop. This general strategy is termed a "push-pull" or "stimulo-deterrent diversionary strategy" (SDDS) (Miller and Cowles, 1990; Pickett *et al.*, 1991). In the longer term, the secondary metabolism of the different varieties used as the harvestable and trap crops will be manipulated using plant molecular genetics. Initially, attempts are being made to modify endogenous gene expression for key enzymes relating to existing pathways, but as the project develops, genes within the harvestable crop will be modified by augmentation from alien genes so as to produce a wider range of metabolites for reducing pest and disease development (Dawson *et al.*, 1989; Hallahan *et al.*, 1992).

As a member of the Brassicaceae, oilseed rape is part of a family with a well characterised chemical defence based on various secondary plant metabolites, the most studied of which are the glucosinolates. These generate the biocidal organic isothiocyanates, or mustard oils, which are rendered innocuous for storage within the plant by being present as β-thioglucoside precursors. When the plant is damaged, glucose is cleaved from the precursors by myrosinase, a thioglucosidase enzyme (Fenwick *et al.*, 1983) and after desulphation, a chemical rearrangement gives rise to the organic isothiocyanate or related products, although the indolylglucosinolates do not form stable isothiocyanates (Hanley and Parsley, 1990; Hanley *et al.*, 1990). Stable organic isothiocyanates slow the development of unspecialised diseases and herbivores on these plants but act as semiochemicals that attract and stimulate the development of adapted pests (Kjaer, 1976; Daxenbichler *et al.*, 1991). Although oilseed rape is grown principally for its oil, the protein-rich meal remaining after oil pressing is used for animal feed. Farm animals are poorly adapted to feed on brassicaceous plants. In the case of progoitrin (2-hydroxy-3-butenylglucosinolate), which gives rise to the toxic goitrin ([*S*]-5-ethenyloxazolidine-2-thione) by thioglucosidase activity, there can be a reduction in the growth rate of cattle when meal is presented at a high ratio in their feed (Bjerg *et al.*, 1989). As a result, plant breeders have developed the so-called double-low cultivars of oilseed rape with reduced levels of glucosinolates in their seed. Early double-lows were susceptible to attack by non-specialist herbivores and fungal pathogens (Kimber, 1984; Von Kries, 1988), but modern double-lows have better resistance (Williams *et al.*, 1991). For example, the double-low cultivar Cobra is more susceptible to the polyphagous peach-potato aphid, *Myzus persicae* (Aphididae), than the normal glucosinolate-containing cultivar Bienvenu (Porter *et al.*, 1990). Other groups of insects, and slugs, can also show improved development on the double-low cultivars (Milford *et al.*, 1989; Glen *et al.*, 1989). A wide range of pests has adapted to brassicaceous hosts (Traynier and Truscott, 1991; Renwick and Radke, 1990; Blight *et al.*, 1989, 1992) and can employ glucosinolates or their catabolites as cues for colonisation and development (Dawson *et al.*, 1993a; Read *et al.*, 1970; Titayavan and Altieri, 1990). Some of the insects are attacked by predators and parasitoids which have, in turn, evolved to locate their prey using specific semiochemicals released from the host plant during feeding damage.

DISCUSSION

Identification of semiochemicals of insect pests

The main insect pests of oilseed rape are the cabbage stem flea beetle, *Psylliodes chrysocephala* (Chrysomelidae), the cabbage seed weevil, *Ceutorhynchus assimilis* (Curculionidae), the Brassica pod midge, *Dasineura brassicae* (Cecidomyiidae) and the pollen beetles, *Meligethes* spp. (Nitidulidae). The midge may be considered as a secondary pest since it can only colonise the pods after they have been pierced by *C. assimilis* during feeding or oviposition (Alford *et al.*, 1991).

Initial colonisation of oilseed rape by insects is mediated by visual and olfactory cues. The main organ for detection of olfactory stimuli is the antenna. The electroantennogram (EAG), a summated recording from the whole antenna, and the single cell recording (SCR) techniques have been used to investigate physiological responses of oilseed rape pests to olfactory cues (Wadhams, 1990; Blight *et al.*, 1989, 1995c; Evans and Allen-Williams, 1992). When directly coupled to a high resolution capillary gas chromatograph (GC), the simultaneous monitoring of both the physiological and chemical properties of related compounds provides an extremely powerful tool in the identification of active semiochemicals in complex natural product mixtures (Wadhams, 1990). In such studies, it is important that samples of volatile plant components are isolated under similar conditions to those in which the insects and plants would naturally interact. By simply presenting high levels of synthetic compounds to the insect antenna, quite incorrect impressions of their importance may be suggested. However, by entraining the volatiles released from intact plants onto adsorbents such as the polymer Porapak Q, the re-eluted volatiles have a true relationship to the materials that the insect would normally encounter and employ in pre-colonisation behaviour (Blight, 1990). Several hundred individual compounds can be collected from intact oilseed rape plants by this means. When the plants are fed upon by an insect pest, the profile of volatiles alters as damage causes a change in the relative proportions or the release of different compounds. To this are added other metabolites, including any semiochemicals such as pheromones, generated by the insect itself. By GC-EAG, a number of active peaks were located for *P. chrysocephala* (Blight *et al.*, 1989) and using this, and the more sensitive SCR technique, 22 active compounds have been identified for *C. assimilis* (Blight *et al.*, 1992). It is likely that these compounds constitute the main semiochemicals used by these insects.

The identified compounds fall into three groups: amino acid metabolites, which include the isothiocyanates since they are generated from glucosinolates, in turn biosynthesised from amino acids;

fatty acid derivatives, including the leafy alcohol (*Z*)-3-hexen-1-ol; terpenoids, for example (*E*,*E*)-α-farnesene. The olfactory cells responding to isothiocyanates show a high degree of specificity. One particular cell type is extremely sensitive to 3-butenyl and 4-pentenyl isothiocyanates, responds hardly at all to 2-phenylethyl isothiocyanate and not at all to allyl isothiocyanates at normal physiological concentrations. However, another cell type responds specifically to the 2-phenylethyl homologue (Blight *et al.*, 1992). A high compound or functional group specificity is also well exemplified by cells responding to 1-octen-3-ol. Here, very weak responses are obtained for the analogues octan-3-ol, (*Z*)-3-octen-1-ol, octan-3-one and 1-octen-3-one. In a number of instances, recordings showed consistent pairings of certain cell types. In most cases, the amplitudes of the two cells could be discriminated, for example with cells responding to methyl salicylate (small amplitude cell) and the isothiocyanates (large amplitude cell), and to 1,8-cineole (large amplitude cell) and 2-phenylethanol (small amplitude cell) (Blight *et al.*, submitted).

Insect behaviour

The linear track olfactometer has proved to be an invaluable device in investigating the behavioural role of the semiochemicals identified by chemical and electrophysiological techniques (Sakuma and Fukami, 1985). This olfactometer involves insects walking on a wire which leads to a junction where olfactory stimuli arriving from two directions present a choice. Highly significant percentages of *C. assimilis* turned towards volatiles from flowering rape compared to a control of moist filter paper in the olfactometer. Similar effects were found with the isothiocyanates, found to be active in earlier studies (Bartlet *et al.*, 1992, 1993).

Field trapping of insect pests

A range of trap designs have been field tested, showing yellow sticky traps and water traps to be equally effective, but when baited with a mixture of the active isothiocyanates, both caught significantly more *C. assimilis* and *Meligethes* spp. than unbaited traps (Smart *et al.*, 1993). Both insects responded to traps baited with individual isothiocyanates (Blight *et al.*, 1995b; Smart *et al.*, 1995), and this system is now being used to test the response to other groups of active compounds in the field. Attempts are also being made to entrain volatiles from a field crop to obtain a more accurate estimation of the amounts and ratios of compounds present above the crop canopy.

Feeding behaviour

The insect pests involved with this crop show distinct preferences for members of the Brassicaceae and for a few other plants also containing glucosinolates, such as *Reseda alba* (Resedaceae). This has been well exemplified with *P. chrysocephala* (Bartlet and Williams, 1989, 1991). The role of the glucosinolates themselves has been investigated by using the synthetic products presented to the insect in agar jelly, thereby allowing a quantitative assessment of feeding (Bartlet *et al.*, 1994).

Pheromones of insect pests

Pheromones are thought to affect the behaviour of certain pests of oilseed rape (Evans and Bergeron, 1994) and the oviposition deterrent employed by *C. assimilis* to prevent multiple-oviposition into pods is studied because of its potential for reducing oviposition by this pest (Ferguson and Williams, 1989). *D. brassicae* females produce a sex pheromone that attracts mates and recently the glandular site at which this pheromone is produced within the female insect has been located by morphological investigations.

Beneficial insects

When herbivorous pests damage crop plants, they may release semiochemicals, termed "synomones", that attract parasitoids. Indeed, interactions have been found between the parasitoids of *D. brassicae* and isothiocyanates and it has been possible to attract these potentially valuable natural enemies into a trap using 2-phenylethyl isothiocyanate (Murchie *et al.*, 1995). Attempts are being made to use natural populations of the parasitoids and other agents in the SDDS to improve control of pests.

Pest insect pathology

In searching for pathogens for reducing populations of pest insects, attention has focused on entomogenous fungi because they, unlike bacterial or viral biocontrol agents, penetrate directly and do not need to be ingested to cause infection. In preliminary bioassays, isolates of the entomogenous hyphomycete fungi *Metarhizium anisopliae*, *Beauveria bassiana* and *Paecilomyces fumosoroseus* have been identified which are pathogenic to *P. chrysocephala*, *C. assimilis*, and *Meligethes* spp., respectively (Butt, unpublished). Since then, more aggressive isolates of *M. anisopliae* have been identified (Butt *et al.*, 1992, 1994). These are highly pathogenic not only to *P. chrysocephala* but also to other crucifer pests, including mustard beetle, *Phaedon cochleariae* (Chrysomelidae), *M. persicae* and turnip aphid, *Lipaphis erysimi* (Aphididae), but only weakly pathogenic to honey bees (Butt *et al.*, 1994). Studies on invasive and developmental processes have shown that this specificity can depend on the responses of the pathogens to cuticular cues (Butt *et al.*, 1995). So far, two years of field trials with these isolates have shown encouraging reductions in pest populations (Butt, unpublished). An electrostatic spraying system, developed at Rothamsted (Arnold *et al.*, 1984), has been extremely effective in applying spores of these pathogens to crops (Butt and Pye, unpublished). A recent advance has been to identify molecular markers that can be used to follow the distribution and survival of individual isolates in the field from inoculation to infection of insects (Leal *et al.*, 1994).

Plant pathology

The possibility that glucosinolates are involved in resistance to fungal pathogens is suggested by a number of findings. Firstly, pathogens are susceptible to the toxic effects of pure isothiocyanates *in vitro* (e.g. Dawson *et al.*, 1993a); secondly, infected oilseed rape tissues accumulate glucosinolates, although the responsiveness of cultivars differs (Doughty *et al.*, 1991); thirdly, fungitoxic isothiocyanates have been identified among the volatiles released in greater amounts by *Brassica rapa* seedlings infected by the necrotrophic pathogen *Alternaria brassicae*, confirming that conditions in infected tissues are suitable for the degradation of glucosinolates to their fungitoxic hydrolysis products (Blight *et al.*, 1995a). Nevertheless, a number of pathogens can infect oilseed rape cultivars, despite the presence of glucosinolates in their tissues. Work is in progress to investigate the possibility that enhancing the concentration of glucosinolates in the tissues of these cultivars may hinder the development of specialist pathogens. Given that glucosinolates are inducible, the approach is to simulate the natural response to infection, using elicitors of glucosinolates. For example, two compounds (salicylic acid and methyl jasmonate) that selectively induce the accumulation of particular types of glucosinolates (Kiddle *et al.*, 1994; Doughty *et al.*, 1995a) also decrease the susceptibility of oilseed rape seedlings to infection by particular pathogens (Doughty *et al.*, 1995b). Future work will involve these and other selective elicitors to investigate the relative contribution of glucosinolates to this induced resistance.

Biochemistry and plant molecular biology of glucosinolates

The glucosinolates are biosynthesised from chain-elongated or homoamino acids. In the case of the alkenylglucosinolates, elimination of methanethiol, almost certainly as an oxidation product, takes place, presumably after initial incorporation of homomethionines into the pathway. The route to the glucosinolates was thought to be analogous with the biosynthesis of the cyanogenic glycosides, involving mixed function oxidative decarboxylation of the amino acid to the aldoxime and then further elaboration to the glucosinolate. Radiolabelled homoamino acids, dihomomethionine and homophenylalanine, have allowed the pathway to be investigated, specifically in oilseed rape. In the case of the biosynthesis of gluconasturtiin (2-phenylethylglucosinolate) from homophenylalanine, the aldoxime has been isolated and unequivocally characterised (Dawson *et al.*, 1993b). However, the enzyme system responsible for the oxidative decarboxylation, in this case and in the case of dihomomethionine, is not a mixed function oxidase or cytochrome P450 type, but instead is a flavin-linked mono-oxygenase with many properties similar to those of animal flavin-linked mono-oxygenases (Bennett *et al.*, 1993).

CONCLUSIONS

Considerable progress has been made in defining the chemicals that influence the development of oilseed rape pests and diseases. Field work has demonstrated the possibility of exploiting this knowledge in devising new protection strategies for this crop. Further work on defining the role and nature of semiochemicals is necessary, especially where more benign natural compounds than the organic isothiocyanates are encountered. For the longer term, it is essential to exploit this information by use of plant molecular biology and the chemical, biochemical, entomological and plant and insect pathological studies will continue in concert in order to approach the objective of minimising pesticide input into oilseed rape.

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