transcription factors: the nuclear hormone receptors. These receptors are characterised by a set of conserved structural features including a highly conserved DNA binding domain, a fairly well conserved hormone binding domain and a large hypervariable region (Fig. 4). These receptors directly affect gene expression, binding as homo- or heterodimeric complexes. In the intensively studied insect *Drosophila melanogaster*, the EcR affects gene expression as a heterodimer with another steroid receptor; the gene product of the *ultraspiracle* locus which has been identified as a homolog of the vertebrate retinoid X receptor (RXR). The genes coding for the EcR and RXR have been cloned and sequenced from a number of insects; we have used primers designed against the region coding for the conserved DNA binding domain of these molecules to search for genes coding for homologues of these molecules in PCN. We have successfully amplified and cloned a number of gene fragments from PCN whose sequence suggests they code for molecules similar to insect RXRs (Fig. 5). These fragments are now being used as probes to identify other members of this superfamily of receptors in nematodes. Future studies on the timing and localisation of their expression will be used to determine their role in the control of nematode moulting. Other work to examine the nature of the genes whose expression is controlled by these genes will also be made possible by this approach. Although identification of these molecules does not prove that insect and nematode moulting are controlled by the same mechanisms, it does suggest that similar molecules are available to the nematode. The functional role of these molecules remains to be assessed.

Responses to flower volatiles by the raspberry beetle, *Byturus tomentosus* and field evaluation of white traps for monitoring flight

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Introduction The raspberry beetle, *Byturus tomentosus*, is a major pest of cultivated and wild *Rubus* species (raspberries and blackberries, and hybrid berries) in Europe. A closely related species, *B. unicolor*, (raspberry fruitworm) causes similar damage in N. America. *B. tomentosus* larvae normally inflict more damage than adults, by tunnelling into the developing fruit, allowing entry of fungal pathogens (e.g. *Botrytis*) or directly contaminating harvested fruit. Adult beetles can also damage unopened buds, unfolding leaves of first year canes, and opened flowers during feeding. Adults usually start to emerge from the soil in late April to mid-May, and in warm weather migrate to flowers of other Rosaceous plants, including hawthorn. They are then attracted back to early flowering raspberry cultivars where they feed, mate and females lay eggs, mainly on the stamens of raspberry flowers. It is thought that raspberry beetles follow long-distance volatile cues (e.g. flower odours) and also use visual cues (e.g. flower colour) to find suitable host plants. Larvae emerge from eggs after a few days and start to feed on the basal drupelets, before tunnelling into the fruit's central plug.

Limitations of current pest control method At present insecticides are routinely applied to the ripening fruit ("green/pink" stage) close to harvest, targeted against eggs and recently emerged larvae, before feeding larvae have caused fruit damage. The most commonly used products are short-persistence organophosphorus insecticides such as fenitrothion. Although there is no evidence that maximum residue limits (MRLs) have been exceeded for these insecticides on raspberries, there is growing public concern about applying organophosphates, particularly close to fruit harvest. The threat of future removal of these insecticides from approved product lists means that it is highly desirable for the soft fruit industry to develop pest control methods which reduce the use of pesticides.

New, environmentally-sensitive approaches at SCRI As part of a general strategy at SCRI to develop more environmentally-benign control methods for soft fruit, we are investigating the fundamental biology and behaviour of raspberry beetles and other important soft fruit pests. Our main emphasis is on the role

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Figure 1 White sticky traps are hung at 0.6m and 1.2m from the ground in raspberry plantations. The design of the traps (two interlocking sticky surfaces at right angles) provides information on the direction and timing of raspberry beetle flights.

of host plant volatiles in attraction to and recognition of host plants by insects, linked to targeted breeding for pest resistance. Sources of resistance to raspberry beetle in wild *Rubus* species, including *R. coreanus*, *R. crataegifolius*, *R. occidentalis* and *R. phoenicolaisus*, have been reported in the past and have been used in breeding programmes, but little is known about the resistance mechanisms involved. Our approach should enable us to develop selectable markers (chemical and/or molecular) for pest resistance traits, and also to identify plant-derived attractants, repellents and deterrents which complement host plant resistance in sustainable Integrated Crop Management programmes.

White sticky traps to monitor raspberry beetle activity in plantations Ongoing field trials and scanning electron microscope studies indicate that visual and contact stimuli are important behavioural cues for raspberry beetles. In applied studies white, non-U.V. reflective traps, developed in Switzerland, have been tested in raspberry plantations in eastern Scotland (Fig. 1). The aim is to determine when raspberry beetles are active in plantations, so that insecticides can be more carefully timed and spraying thresholds developed. Results show that adult beetles tend to fly at low levels in Scotland, with considerably more adults caught at 0.6m above ground level than at 1.2m. The traps at SCRI also showed that many beetles were flying in a south-westerly direction (i.e. against the prevailing wind) when caught. Further trials at nine commercial plantations in Scotland have shown that numbers and times of peak beetle activity are very variable, so localised monitoring should be advantageous for timing insecticide sprays more effectively.

Screening tests on beetle-resistant *Rubus* species *R. phoenicolasius* (Japanese wineberry; Fig. 2) was selected as a known beetle-resistant, wild *Rubus* species for behavioural and chemical studies. In previous sleeve inoculation tests under field conditions, raspberry beetles laid very few eggs on flowers of *R. phoenicolasius* and the fruit were virtually free from

Figure 2 The Japanese wineberry (*Rubus phoenicolasius*) is resistant to raspberry beetles. Odours emitted from the flowers and glandular hairs on sepals repel beetles when they are searching for egg laying sites.

larval infestation, indicating antixenosis (resulting in non-preference during egg laying) as an important component of resistance. In confirmatory olfactometer tests, giving female beetles the choice of flower volatiles from resistant *R. phoenicolasius* and the susceptible red raspberry cv Glen Prosen, beetles showed a strong preference for red raspberry flower volatiles. *R. phoenicolasius* is covered in glandular hairs which emit a distinctive aromatic odour. These volatiles appear to repel or confuse raspberry beetles during the early stages of host recognition involving flower volatile cues. In more recent "sleeve" inoculation tests at SCRI, resistance was also noted in a purple raspberry breeding line, indicating that the source of resistance may be from a *R. occidentalis* (black raspberry) parent. The *R. occidentalis* cv. Munger has previously been reported to be highly resistant to raspberry beetle. The mechanism of this resistance in purple raspberry selections to raspberry beetle is currently being investigated.

Testing for volatile attractant and repellent odours from host and non-host flowers A linear track olfactometer is used to quantify behavioural responses of male and female raspberry beetles to flower odours and to identified volatile components[1,2]. These tests show that flower volatiles from raspberry or from other Rosaceous hosts are highly attractive in a choice test against a control (moist air with no flower volatiles). In a similar choice test between volatiles from a non-host (oilseed rape flower) and the moist air control, no clear preference was observed. Further choice tests between flower volatiles from the host (raspberry) and a non-host (oilseed rape) again showed a clear preference for the raspberry flower volatiles. Interestingly, raspberry beetles that emerged early in the 1991 season, before raspberries had flowered in the field, showed a distinct preference for hawthorn flower volatiles (an early season "temporary host" for adult aggregation and feeding) over raspberry volatiles (normal host for mating, oviposition and larval feeding). However, this preference for hawthorn flower volatiles was not seen in later tests over three subsequent years. These and other studies suggest that the phenological stage of the host (raspberry) flower alters the chemical composition of volatiles released, and hence the relative attractiveness of raspberry flowers to raspberry beetles during host finding.

Flower volatile chemistry Flower volatiles from four raspberry cultivars and from wild hawthorn were trapped on the porous polymers Haysep Q and Tenax TA. These volatile flower odours were analysed on an automated thermal desorption (ATD)-gas chromatography (GC)-mass spectrometry (MS) system at SCRI and by GC-MS analyses of ether extracts in a collaborative study with IACR, Rothamsted. The major classes of compounds (> 100 detected in each entrainment) included aliphatic and aromatic hydrocarbons, aldehydes, ketones, alcohols and esters, monoterpenes and sesquiterpenes and a number of unusual nitrogen compounds. The volatile profiles of the four raspberry cultivars at full flowering were complex but exhibited only minor differences between them. Volatiles of hawthorn flowers, although similar to raspberry, contained elevated levels of several compounds not found in raspberry cultivars. The volatile profile of the resistant wild species *R. phoenicolasius* contained elevated levels of terpenoid and other compounds, in addition to the typical volatile profile of red raspberry The chemical identities of possible repellent compounds in this and other wild *Rubus* species are currently being investigated at SCRI using ATD-GC (Automated Thermal desorption-Gas Chromatography) linked to an Electroantennogram (EAG) system. This records electrical signals from insects' antennae, generated by exposure to stimulatory volatiles, separated on the GC.

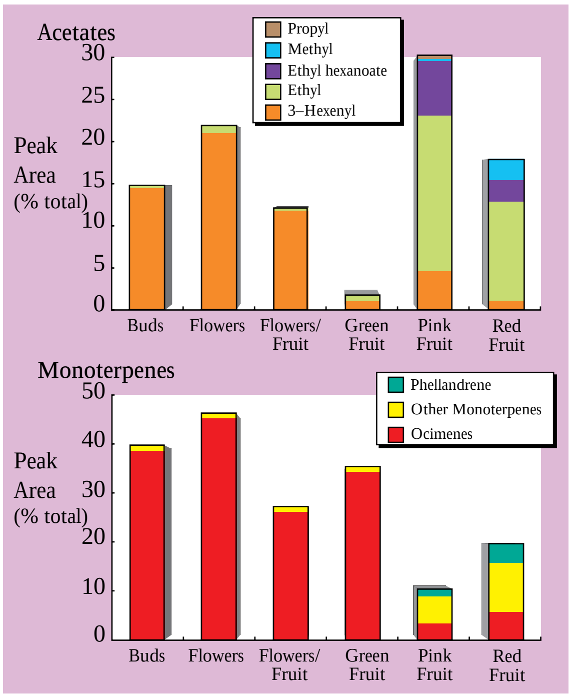

Figure 3 Several groups of plant volatiles are emitted from raspberries change during flower and fruit development (examples shown are acetates and monoterpenes). Raspberry beetles respond differently to these changing chemical signals when selecting suitable sites for egg laying.

Field observations from a joint AFRC (now BBSRC) Link programme with St Andrews University and confirmatory olfactometer tests at SCRI showed that the female's preference for floral oviposition sites is strongly influenced by the phenological stage of the raspberry flowers[3]. In a second series of entrainment experiments, we investigated changes in volatile profiles during flower development of raspberry cv. Glen Prosen. ATD-GC-MS analyses of volatiles from green buds, flowers, green fruit, pink fruit and ripe red fruit showed major changes in the composition of the odour profile. As the flowers matured, levels of "green leaf" volatiles declined whilst several monoterpenes increased (Fig. 3). During fruit ripening several additional compounds appeared, which are highly characteristic of raspberries, followed by the production of higher levels of several types of acetates[4]. Further experiments are now in progress to identify the key, behaviourally-active compounds which convey information on the development state of the flower and fruit to raspberry beetles.

EAG studies to target chemical identification of flower volatiles perceived by raspberry beetle Our studies have revealed the complexity and dynamic nature of volatile profiles emitted from host and non-host flowers. In most GC-MS analyses >100 compounds are detected, many of which are minor components more characteristic of the host than some of the major components (e.g. more ubiquitous "green leaf" volatiles). A further complexity in obtaining "biologically relevant" chemical data for plant-insect studies is that each volatile extraction or entrainment method and GC-MS system used, will produce different sets of chemical data, both in terms of relative proportions of components detected and in the classes of compounds trapped. Because of this we have adopted a strategy of using EAG to identify compounds which the insect's antennae can detect. This then provides a "short-list" of candidate volatiles which must be characterised in terms of their behavioural effects (e.g. attraction, repulsion) over a range of naturally-occurring concentrations.

In collaborative studies at SCRI and IACR Rothamsted, components of raspberry and hawthorn flower extracts separated by GC, were passed over a raspberry beetle antenna. Initially, electrophysiological recordings were made from the whole antenna (EAG; Fig. 4) and later from individual olfactory receptors (single cell recordings) of raspberry beetles at IACR. Several volatile GC peaks which showed electrophysiological activity were identified by GC-MS[5] and are currently undergoing further EAG and behaviour bioassays. Recent results using olfactometers, a wind tunnel and EAG dose-response studies, indicate that both qualitative (number of chemical components in the odour blend) and quantitative (ratios of volatile components) characteristics of the flower profile are important to elicit a normal behavioural response (equivalent to raspberry flowers) by raspberry beetles. Raspberry beetles also appear to obtain extra information on the phenological stage and physiological condition of the developing fruit from characteristic volatile profiles emitted from buds, opened flowers, green and fully ripened fruits. This olfactory informa-

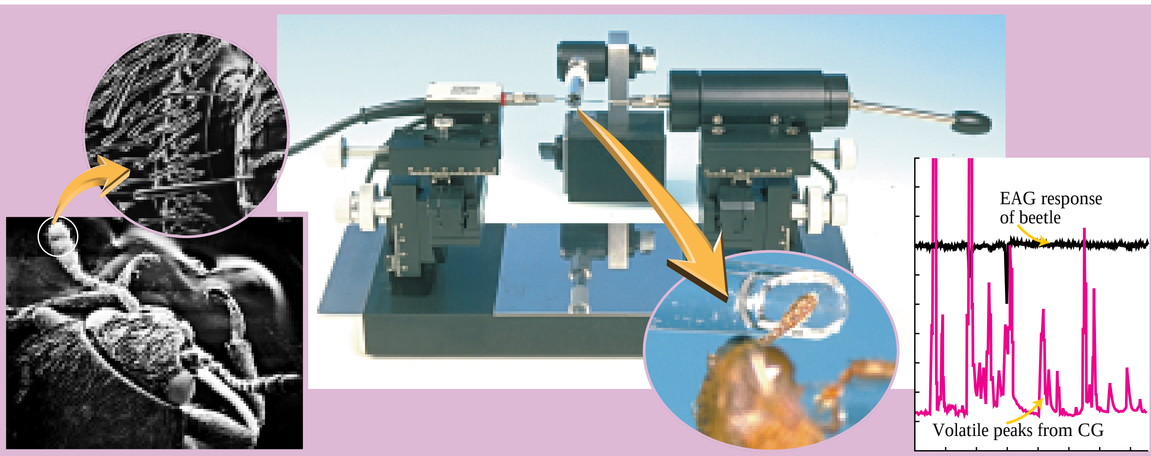

Figure 4 Raspberry beetle's antennae are covered by olfactory receptors which respond to plant volatiles. Electrical responses to volatiles can be recorded from the antenna using an EAG recording system. A typical EAG is shown; the change in DC voltage is measured in millivolts and is proportional to the odour intensity and number of antennal receptors stimulated by the volatile chemical.

tion modifies beetle behaviour in host selection for feeding and egg laying sites.

Conclusions These approaches have led to the successful identification of a number of candidate flower volatiles for evaluation as attractants or repellents. Although this list of EAG-active compounds is not yet complete, it has enabled us to show that both qualitative (the number of chemical components) and quantitative (ratios of components in the odour blend) characteristics appear to be important in order to elicit a strong behavioural response. Similar observations, indicating the importance of the "optimal blend" of volatile components for attracting insects to baited traps, have been reported for other insects. This information is now being used to design improved traps for monitoring raspberry beetle activity in the field and so reduce unnecessary pesticide usage. We will also be able to target plant breeding for resistance to raspberry beetle and other important pests and diseases. These control strategies are currently being combined into an Integrated Crop Management system, which it is hoped will be to the ultimate benefit of soft fruit growers and consumers.

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Effects of plant natural compounds on nematodes

W.M. Robertson, A.N.E. Birch & I.E. Geoghegan

Many existing synthetic nematicides are broad-spectrum compounds and have residues which are highly toxic and persistent. They can contaminate ground water thereby posing an additional threat. The search for environmentally-benign methods for controlling plant parasitic nematodes has led to the exploration of several strategies for replacing commercially available nematicides. As many plant species have evolved pathways for producing defence compounds against a wide variety of pathogens, one possible alternative to synthetic nematicides is to identify and use plant-derived compounds which are effective against nematodes. Although these compounds may also have a broad spectrum of toxicity they are biodegradable and will not persist in the soil for long periods or leave toxic break-down products.

Plant defence compounds can act in subtle ways. Some deter attack by acting as repellents, whereas others inhibit hatching of nematode eggs rather than directly killing nematodes. Such activity would not be detected by the standard test that is used, where invasive stage nematodes are immersed in a series of aqueous solutions of a test compound to determine the lethal dose. Furthermore, compounds that have been found to be toxic in immersion tests have been found subsequently to have no effect when applied to soil.

Members of the *Leguminosae* appear to have particularly well developed chemical defence strategies and we have worked with compounds from several species. Jack bean (*Canavalia ensiformis*) contains at least four