The use of gas chromatography-mass spectrometry in the study of plant and insect defence compounds

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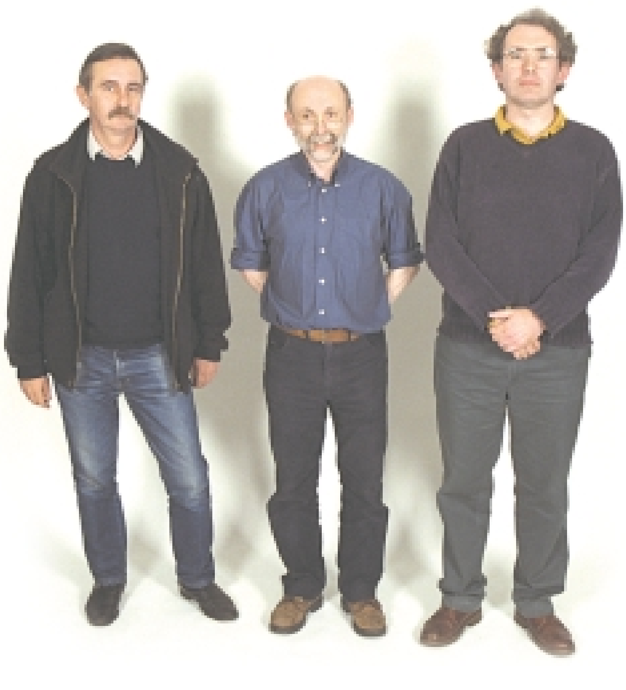
In the natural world, we are surrounded by a myriad of chemicals, some of which we can detect by our senses of taste and smell. Plants contribute much to this olfactory environment with distinctive vegetative and floral aromas and, indeed, many plants, such as the sweet pea (*Lathyrus oderatus*), are cultivated almost solely for their fragrance. Until comparatively recently, the chemical constituents of this complex environment remained unidentified and their underlying biological significance was largely ignored. The advent of sophisticated sampling and analytical techniques is changing this situation rapidly.

The involvement of chemicals in the external interactions between individual organisms is now beginning to be recognised. While most people are familiar with the concept of intra-specific (within species) communication, typified by insect sex-attractants or aggregation pheromones, it is only recently that the subtle complexities of inter-specific (between species) communication have been recognised. Plant 'info'-chemicals can affect not only neighbouring plants of the same species, but can also act indirectly on other organisms, such as insect pests, by eliciting changes in physiological and/or behavioural responses at some distance from the plant.

It is apparent that compounds that convey information over a distance between organisms must be reasonably volatile and capable of inducing a response at very low concentrations. These characteristics, together with their structural diversity, have made gas chromatography-mass spectrometry (GC-MS) an ideal technique to characterise, identify and quantify such chemicals. The advent of capillary columns with high resolving capacities has also aided in their separation from the complex mixture of compounds frequently released by insects and plants.



Part of the Phytochemistry Unit's research remit is to explore the nature of the chemical interface of various plant and insect species with their immediate biological environment, and in the course of these investigations, several examples of these 'info'-chemicals have been detected and characterised by mass spectrometry.

Pheromones released by insects can act to promote aggregation, as an oviposition stimulus, or as an alarm signal. A commonly cited example is the aphid alarm pheromone (E)-β-farnesene, a sesquiterpene hydrocarbon. Some aphid species release secretions containing this compound from their cornicles when alarmed or irritated, causing other individuals to disperse [1]. Consequently, plants that either continually release this compound or do so in response to damage, may confuse the aphids and thus reduce levels of infestation.

Plants under attack by pathogens or insects often produce elevated levels of methyl jasmonate, which appears to activate genes responsible for the production of a range of defensive compounds. The external application of this compound to plants has been shown to induce the synthesis of protease inhibitors and lycopene in tomatoes, glucosinolates in brassicas and glycoalkaloids in potatoes. This would suggest that this volatile compound may be an important means of communicating impending attack from

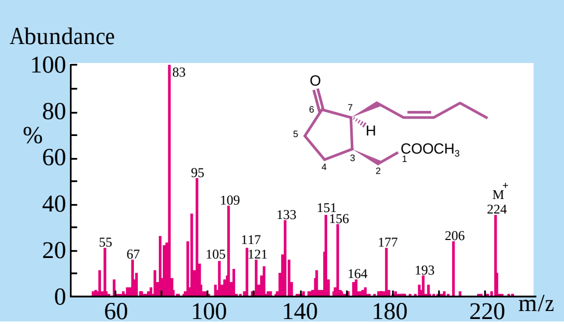


Figure 1 Mass spectrum of (+)-(3R,7S)-epi-methyl jasmonate, $C_{13}H_{20}O_3$.

plants already under threat to nearby uninfected plants, thus allowing the latter to induce their natural defence mechanisms prior to actual attack. The configuration of chiral centres in the structure of jasmonic acid and methyl jasmonate molecule at C-3 and C-7 are believed to be critical for this activity and naturally occurring jasmonates from plants are reported to have the R stereochemistry at C-3 and either the R or S at C-7. The initial isomer formed in the plant is believed to be the (+)-(3R, 7S)-form with *cis* side chains, (+)-epi-jasmonic acid, but it has a tendency to epimerize to the more thermodynamically stable (-)-(3R,7R)-form (-)-jasmonic acid with *trans* side chains [2]. In a recent study of the surface chemistry of blackcurrant (*Ribes nigrum*) leaves at SCRI [3], both epimers of methyl jasmonate were identified on the basis of their mass spectra. However, the relative concentration of the two epimers was dependant on the extraction technique employed. Solvent extraction at room temperature produced more (+)-epi-methyl jasmonate (Fig. 1), whilst steam distillation resulted in a greater proportion of (-)-methyl jasmonate, thus emphasising the importance of developing suitable sampling procedures in such investigations.

Plants have other allies in their struggle against predators and among these are the coccinellid beetles, more familiarly known as ladybirds. Ladybirds are known to consume large numbers of aphids in a day and are a significant factor in reducing plant damage. They are themselves the subject of attack and have developed powerful chemical defensive weaponry of their own. The primary line of defence is a process known as reflex bleeding, where in response to the attentions of a predator, a defence fluid is exuded from the insect's knee joints. Bitter-tasting alkaloids present in the fluid make the ladybird unpalatable, and will often induce the predator to release its intended victim. The seven spotted ladybird, *Coccinella septempunctata*, produces precoccinelline and its N-oxide, coccinelline (Fig. 2), which are particularly distasteful to birds. The smaller two-spotted ladybird, *Adalia bipunctata*, produces a different alkaloid, adaline (Fig. 3), which is based on a completely different ring structure [4]. Adaline is much less unpleasant than the coccinellines, and therefore the beetle may have to exude up to seven times more alkaloid in its reflex fluid than its seven spot-

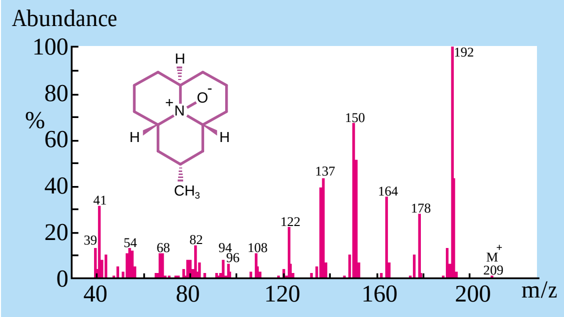


Figure 2 Mass spectrum of a mixture of precoccinelline, $C_{13}H_{23}N$ and coccinelline, $C_{13}H_{23}NO$.

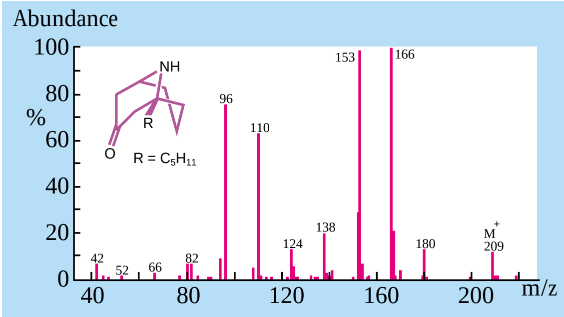


Figure 3 Mass spectrum of adaline, $C_{13}H_{23}NO$.

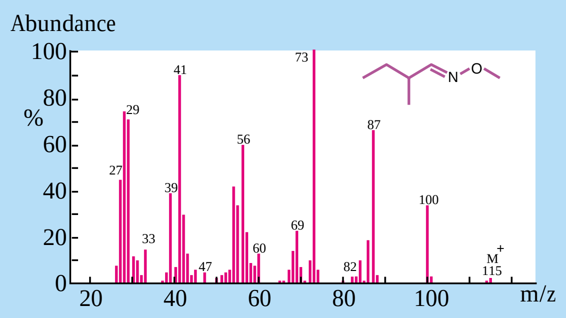


Figure 4 Mass spectrum of (E)-2 methyl butanal-O-methyl oxime, $C_6H_{13}NO$.

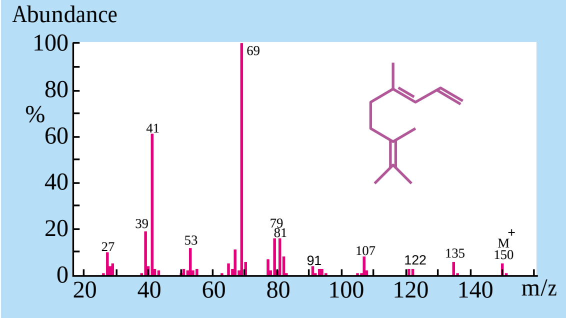


Figure 5 Mass spectrum of (E)-4,8-dimethyl-1,3,7-nonatriene, $C_{11}H_{18}$.

ted relatives, to provide an effective defence. Both types of alkaloid are aliphatic nitrogen-containing heterocycles, and are probably derived from amino acids. They were detected at SCRI in a study of lipid mobilization during parasite infestation. Production and excretion of defensive fluids is energy demanding, and the ladybirds have evolved additional defensive mechanisms. One is the bright aposematic (warning) coloration for which the beetles are renowned. Another is the emission of volatile repellent compounds, 2-methoxy-3-alkylpyrazines, based on an aromatic nitrogen-containing heterocyclic ring system.

The complexity of the (interspecific) interaction between plants and pests is well illustrated by the action of the herbivore-induced synomone given off by cucumber leaves under attack by the herbivorous spider-mite, *Tetranychus urticae* [5]. The volatile compounds given off included the homo-monoterpene (3E)-4,8-dimethyl -1,3,7-nonatriene, the acyclic monoterpene (E)-β-ocimene and (E)-2-methylbutanal, O-methyloxime. The interaction of these compounds remains unclear but (3E)-4,8-dimethyl -1,3,7-nonatriene acts as attractant to the predatory mite, *Phytoseiulus persimiliis*, which in turn reduces the herbivore mite population.

In conjunction with the Department of Soft Fruit and Perennial Crops, gas chromatography-mass spectrometry is currently being used to study raspberry (*Rubus idaeus*) flower volatiles. The larvae of the raspberry beetle, *Byturus tomentosus*, can cause significant economic damage to developing raspberry fruit, with adult beetles attracted to the flowers in early summer to congregate, feed and mate. In an endeavour to characterise the chemicals acting as attractants, flower volatiles from four commercial raspberry varieties were adsorbed onto Tenax-TA and analysed by thermal-desorption GC-MS. All four varieties were found to contain the (Z) and (E) isomers of 2-methylbutanal, O-methyloxime (Fig. 4) and 4,8-dimethyl-1,3,7-nonatriene (Fig. 5) with the (E) form predominating in each case [6]. Polyenes similar to the nonatriene have been identified as aggregation pheromones for the Australian sap beetle *Carpophilus davidsoni* [7]. The true ‘info’-chemical significance of the presence of these compounds in raspberries is currently under investigation at SCRI using both an electro-antennogram linked to a GC and complementary behavioural assays.

It is clear from the examples presented that capillary gas chromatography combined with mass spectrometry is a powerful tool in revealing the complexity of the chemical environment in which both plants and insects co-exist.

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