Plants and aphids: the chemical ecology of infestation

T. Shepherd, G.W. Robertson, D.W. Griffiths & A.N.E. Birch

Aphids can cause serious damage to a variety of economically-important host plants, either by the direct effects of feeding or by the transmission of pathogenic viruses. Host plant selection involves a complex sequence of events, which include selection in response to visual and olfactory cues, landing on a plant, testing of the leaf surface, probing and penetration by the stylet to locate the phloem tissues, and testing of the phloem contents. Selection of a suitable host plant is followed by feeding and reproduction. In the absence of the appropriate stimuli, the sequence may be interrupted at any stage, and ultimately the insect may leave. Behavioural analysis indicates that, for many of the Aphidae, the nature of the leaf surface is an important determinant factor. Chemicals found within plant cuticular waxes are thought to have a direct involvement in host selection and in many cases are insect-host specific (Table 1). Optimal leaf surface physiochemical characteristics for successful colonisation by aphids include good surface adhesion and minimal impediment to movement, probing and stylet penetration. These traits are often associated with glossy (glabrous) phenotypes, which usually have less wax, a simpler wax microstructure, and altered chemical composition when compared to the normal waxy (glaucous) phenotypes[1].

The raspberry plant – raspberry aphid system At SCRI, we have a long-standing interest in factors that confer resistance to insect infestation, particularly of the *Rubus* genus to the large raspberry aphid, *Amphorophora idaei*, the only vector of significance for several viruses that infect raspberry in Europe[2-4]. Preliminary studies indicated that raspberry leaf wax plays an important role in determining resistance and susceptibility to the insect[5]. We have now conducted a more rigorous comparison between the *A. idaei*-resistant cultivar, Autumn Bliss, which contains the major resistance gene A_{10}, and the *A. idaei*-susceptible cultivar, Malling Jewel[6]. To correlate aphid behaviour with leaf chemistry accurately, bioassays were performed with *A. ideai*, which densely populates Malling Jewel but not Autumn Bliss, immedi-

Plant Species	Aphid or Related Insect	Behaviour Effected	Determinant Factor (R = Resistance or inhibition; S = susceptibility or Stimulation)
Cabbage (*Brassica oleracea* L.)	*Brevicoryne brassicae* L	Feeding	Increased surface wax levels (R)
Sorghum (*Sorghum bicolor* L.)	Green bug, *Schizaphis graminum* (Rondani)	Feeding	Increased surface wax levels (R)
Sorghum	Various aphids	Feeding	Higher levels of α- and β-amyrin (R)
Winter wheat (*Triticum aestivum* L., glossy phenotypes)	English grain aphid, *Sitobion avenae* (F.)	Feeding	Increased surface wax levels (R); Lower levels of secondary alcohols, ketones, diketones and hydroxy ketones (R)
Alfalfa (*Medicago sativa* L.)	Spotted alfalfa aphids, *Therioaphis maculata* (Buckton)	Feeding	Increased wax levels on young leaves (R); Reduced wax levels on mature leaves (S); Triacontanol (C_{30}) in wax (R); Higher abundance of wax esters (R)
Various	Alate green peach-potato aphids, *Myzus persicae* (Sulzer)	Settling	Short chain fatty acids (C_8-C_{13}) (R), Fatty acids of chain length > C_{16} (S)
	M. persicae	Settling/ Crop damage	Application of dodecanoic acid (C_{12}) to leaves (R)
Vicia faba L (host).	Pea aphid *Acyrthosiphon pisum* (Harris)	Probing and feeding	Alkanes from wax (main components C_{27}, C_{29}, C_{31} C_{33}) (S).
Brassica spp (non-host)	*A. pisum*		Alkanes from wax (mainly C_{29}) (R)
V. faba	*A. pisum*	Movement to lower leaf surface (feeding site)	Alkanes (S)

Table 1 Factors influencing behaviour of aphids on host and non-host plants.

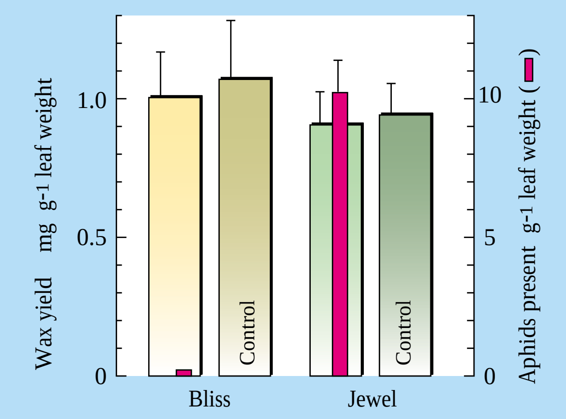

Figure 1 Yields of wax recovered from leaves of red raspberry cultivars Autumn Bliss and Malling Jewel and bioassay results for resistance to *A. idaei*. Values are means and standard deviations for four replicates.

ately prior to collection and chemical analysis of the epicuticular wax (Fig. 1). This confirmed that, at the time of sampling, Autumn Bliss was strongly aphid-resistant and Malling Jewel was highly aphid-susceptible. An aphid-free control group of plants, not subject to aphid bioassay, was analysed to test for any aphid-induced effect on wax composition. The similarity of wax yields from both species suggests that wax thickness was not itself significant. Resistance or susceptibility was more likely related to chemical differences in wax composition.

Wax composition - an invitation to the aphid? Chemical analysis by capillary gas chromatography-mass spectrometry (GC-MS) revealed a broad spectrum of wax components, of which primary alcohols and long-chain esters were most abundant. Sections of the GC-MS Total Ion Chromatogram (TIC) traces obtained for Bliss and Jewel are shown in Figures 2A and 2B. These illustrate the major compositional differences between the waxes.

Studies of other plant-insect systems show that most classes of wax component have potential for biological activity, which may be related to factors such as their abundance in the wax and the distribution of individual compounds, including homologues and positional isomers. Several correlations were made between the observed variation in raspberry wax composition and resistance/susceptibility to *A. idaei*, and are summarised in terms of the major chemical classes as follows.

Fatty acids, primary alcohols and alkanes Resistance may be associated with a narrower chain length distribution for acids and alkanes and a greater abundance of long alcohols in Autumn Bliss, and susceptibility with a wider distribution of acids and alkanes and increased abundance of short alcohols in Malling Jewel.

Alkyl esters Biological activity towards *A. idaei* was unlikely to be related directly to the amounts of alkyl esters present in the wax, which were similar for both raspberry genotypes. However, as the predominant constituent, esters influence wax structure and morphology. For example, esters with long acid-short alcohol combinations were more abundant in Malling

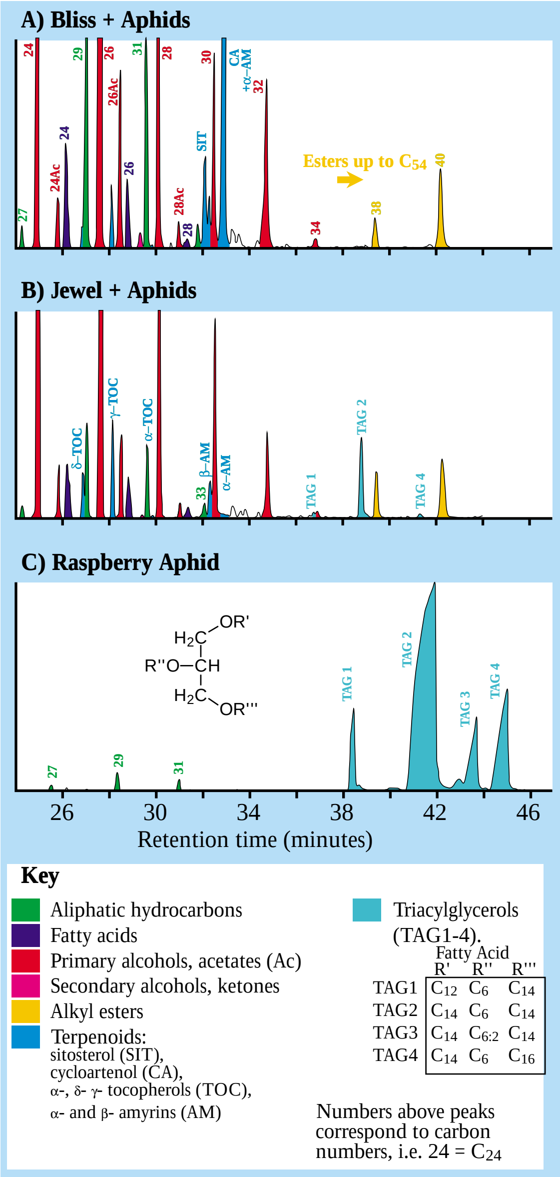

Figure 2 Main components of cuticular waxes from raspberry cultivars Autumn Bliss (A) and Malling Jewel (B) and raspberry aphid (C).

Jewel, and this may effect the distribution and morphology of other wax components and the way in which the aphid perceives specific compounds. Synergism between leaf surface chemicals has been postulated for a number of plant-insect interactions[1].

Secondary alcohols and ketones Susceptibility may be related to the presence of the symmetrical C_{29} secondary alcohol and the equivalent C_{29} ketone, both minor components in wax from Malling Jewel, whereas they were absent from Bliss.

Triterpenoids and terpene esters The greatest difference between the raspberry cultivars was manifest in the distribution of certain terpenoids. Resistance to *A. idaei* may be associated with the much higher levels of sterols, particularly cycloartenol and its alkyl esters, found in wax from Autumn Bliss. There was no correlation between general levels of tocopherols, amyrins and amyrin esters and resistance to *A. idaei*, although there were differences in the distribution of individual members of each class. However, resistance may be associated with the higher abundance of α–amyrin and its alkyl esters in Bliss, since α–amyryl palmitate from suberin wax of the sandal tree, *Santalum album*, is known to affect development of several lepidoptera species[7]. Low levels of sterols in Malling Jewel may be indicative of impaired synthesis from squalene, whereas transformation of squalene to amyrins appears to proceed with equal facility in both genotypes. The high cycloartenol/sterol ratio observed for Autumn Bliss may also indicate reduced transformation of cycloartenol to sterols, since cycloartenol is not usually found in plant leaf waxes.

All these factors identified as possible characteristics of resistance to *A. idaei* in raspberry, appear to be collectively similar to those identified in numerous other plant-aphid studies (Table 1).

Triacylglycerols – the aphid's calling card Small amounts of an unusual group of triacylglycerols were found exclusively in wax from the susceptible genotype, Malling Jewel, that had been subject to bioassay with *A. idaei* (Fig. 2B). These have a short C_6 acid moiety at C-2 of the glycerol backbone and differ from those found in internal plant lipids which usually have three long chain fatty acids. We found the same triacylglycerols as the major surface lipids of *A. idaei*. (Fig. 2C), and clearly the occurrence of these triacylglycerols in the plant wax was due solely to the presence of aphids. Previous reports of similar compounds as wax constituents from various grasses and Canada thistle, *Cirsium arvense* L.[8], can probably now more correctly be attributed to the presence of aphids, since the source plants were grown outdoors and were sampled when flowering, a time when the probability of aphid infestation was high.

These 'marker' triacylglycerols, also found in other species of aphid, occur internally and also externally in defensive secretions produced by the insect's cornicle glands[9]. Interestingly, we found that their characteristic chemical signature was retained by exuviae shed during the insect's growth and development. Leaf surface triacylglycerols are most likely then to arise from the presence of insect exuviae and the direct incorporation of cornicle fluid into leaf wax. The 'marker' compounds were found on leaves of field-grown Malling Jewel, but not Autumn Bliss, at a time when aphids were ubiquitous in the environment of both plants. On widening the study, they were also detected on field-grown plants of other species, including brassica and potato, which were colonised by other aphid species and not *A. idaei*. These findings suggest that measurement of the relative levels of aphid-specific triacylglycerols in plant waxes would provide a chemical index of the degree of aphid-infestation, and hence susceptibility, and should be a useful tool for the field screening of plants for aphid-resistance.

Indeed, these aphids appear to have left their 'chemical fingerprints' all over the scene of the crime, or as Sherlock Holmes put it, "Elementary my dear Watson".

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