SESSION 3C

RECENT ADVANCES IN BIOPESTICIDES AND PLANT BREEDING

SESSION ORGANISER DR K. D. SUNDERLAND

POSTERS 3C-1 to 3C-17

FEEDING BEHAVIOUR OF THE WHITEBACKED PLANTHOPPER *SOGATELLA FURCIFERA* (HOMOPTERA: DELPHACIDAE) ON RESISTANT AND SUSCEPTIBLE RICE PLANTS AND THEIR EXTRACTS

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ABSTRACT

Feeding behaviour of *Sogatella furcifera* on the plants of resistant and susceptible rice varieties and on their extracts was investigated by measuring honeydew excretion. The insect excreted significantly less honeydew on resistant varieties than on susceptible TN1, being least on Rathu Heenati. The resistant plants were more effective against the brachypters than the macropters. The levels of resistance to *S. furcifera* in the varieties Rathu Heenati and ADR 52 were relatively high and stable when tested on both insect morphs. The insect fed significantly less from the water extract of resistant Rathu Heenati than from that of susceptible TN1 when incorporated with 15% (wt/V) of sucrose and 1% (wt/V) of aspartic acid, suggesting that reduction in feeding on the resistant plants could be attributed to the presence of certain water-soluble inhibitors in the plant.

INTRODUCTION

The whitebacked planthopper, *Sogatella furcifera*, is emerging as a serious pest of rice in several Asian countries, particularly in areas where varieties resistant to the brown planthopper, *Nilaparvata lugens*, have been grown successfully (Heinrichs & Rapusas, 1983). It attacks rice plants directly by sucking the phloem sap (Auclair & Baldos, 1982; Khan & Saxena, 1984a, b) resulting in slow growth, delayed tillering, reduction in grain formation, plant mortality and poor yields. Unlike *N. lugens*, *S. furcifera* is not known to transmit rice viruses.

More than 60,000 rices from all over the world have been screened at IRRI for resistance to *S. furcifera* and nearly 400 of them were found to be resistant (Romena *et al.*, 1986). Various behavioural and physiological responses of *S. furcifera* to different resistant varieties have been investigated (Heinrichs & Rapusas, 1983; Khan & Saxena, 1985; Gunathilagaraj & Chelliah, 1985a, b; Liu & Wilkins, 1988). Volatiles from resistant rice plants were found to have a negative effect on the preference of *S. furcifera* for orientation and settling on its host plants (Pablo, 1977; Khan & Saxena, 1985; Liu *et al.*, 1988).

There may be several defensive barriers for a resistant variety to prevent the attack by insects. *S. furcifera* thrives on susceptible rice

varieties, but fails to feed, grow, survive and reproduce adequately on resistant ones (Heinrichs & Rapusas, 1983). No mechanical barriers to *S. furcifera* feeding have been identified in resistant plants; therefore, reduced feeding on them may be attributed to absence of phagostimulants or presence of deterrents in the plant surface or in the phloem. Water-soluble silicic acid and oxalic acid in the rice plant were reported to inhibit *N. lugens* feeding (Yoshihara *et al*., 1979, 1980). It was soon found that both acids occurred in resistant and susceptible rice plants (Yoshihara and Sogawa, 1979). However, resistant rice plants mights contain other water-soluble deterrents. The work reported here was designed to test whether the water-soluble compounds present in the plant extracts of a particular resistant variety inhibited *S. furcifera* feeding. The macropters of *S. furcifera* were used as test insects at the University of Newcastle upon Tyne, U.K. and the brachypters at IRRI, Philippines.

MATERIALS AND METHODS

Feeding response of *S. furcifera* to different rice varieties

Macropters

The 6-week-old plants of rice varieties N22, ARC 10239, ADR 52, Podiwi A8, Rathu Heenati and TN1 were used. Honeydew excretion was collected in a feeding chamber. The chamber was constructed as follows: a main tiller with soil was placed in a pot (8 x 9 cm) which was then covered by a medially-perforated, 8 cm diameter plastic dish through which the tiller emerged. A 7 cm medially-perforated Whatman No. 1 filter paper disc was placed over the dish around the base of the tiller and then covered by an inverted plastic cup, attached to the dish with adhesive tape. Five females, starved but water-satiated for 3-4 h, were introduced into each feeding chamber through the hole which was then plugged with cotton wool. One chamber represented a replicate with 3 replicates per variety. After 24 h feeding, the filter paper discs were removed and briefly immersed in 0.1% ninhydrin solution in acetone and dried over the flame of a Bunsen burner. The honeydew spots on the discs appeared purple or violet. Quantitative determination of the honeydew spots was carried out following the method of Gunathilagaraj and Chelliah (1985a). The quantity of amino acids in honeydew was expressed in terms of the glutamic acid standard, which converted linearly with the area of the corresponding honeydew spots.

Brachypters

Varieties N22, ARC 10239, ADR 52, Podiwi A8, N'Diang Marie, Rathu Heenati and TN1 (4 week old) were used. Feeding chambers were the same as described above, except filter paper discs pretreated with 1% bromocresol green in ethanol were used. Five females, starved but water-satiated for 3-4 h, were released into a feeding chamber which represented a replicate with 5 replicates for each variety. The females were allowed to feed on the plants for 24 h and then the filter paper discs were removed. The areas of honeydew spots on the discs were measured using a transparent sheet marked in 1 mm hatching.

Reductions in honeydew excretion by *S. furcifera* on resistant varieties were calculated as follows:

$$\text{Reduction in honeydew (\%)} = \frac{A - B}{B} \times 100$$

where, A = honeydew excreted by the insect on susceptible TN1,
B = honeydew excreted by the insect on a resistant variety.

Effect of plant extracts on *S. furcifera* feeding

Extraction of plant materials

Rathu Heenati and TN1 rice plants were harvested at 50 days after sowing. Based upon the extraction procedure described by Renwick and Radke (1987), about 1 kg of fresh leaves and leafsheaths of rice plants of each variety was chopped into short pieces and immediately dropped into boiling ethanol to minimize enzyme degradation of plant constituents. After cooling, the tissue plus ethanol was ground. The resulting macerate was squeezed as much as possible to remove ethanol and dried at room temperature. The dried plant tissue weighing 34 g (equivalent to 200 g fresh weight) as a sample was extracted with 400 ml of hexane and the plant tissue dried again at room temperature. The dried tissue was extracted with 600 ml of distilled water and about 300 ml of the water extract (equivalent to approximately 0.7 g original fresh weight/ml) were collected. Half of this water extract (150 ml) was thoroughly shaken with 1-butanol (water extract:butanol, 2:1, V/V) together in a separatory funnel for 5 min. The butanol settled above the water layer in the funnel and was discarded. The water layer was kept in an ice-water-cooled open container in which the temperature was maintained at 0-2°C for 4 days in a fume hood to allow the excess of butanol to evaporate completely. The water extracts before (W_1) and after (W_2) partitioning with butanol were stored at -20°C.

Bioassays

Both water extracts were used to prepare test solutions which contained 15% (wt/V) of sucrose and 1% (wt/V) of aspartic acid. A solution containing the same percentages of sucrose and aspartic acid served as control. The pH of the solutions was adjusted to the range between 7 and 7.2 with an appropriate solution of potassium hydroxide (KOH). Honeydew excretion was assayed using feeding chambers which were made of glass rings (25 mm outer diameter, 25 mm high). One end of a chamber was covered with a small piece of aluminium foil. After introducing 5 brachypters into the chamber, the opposite end was closed with a stretched parafilm membrane on to which was pipetted 0.1-0.2 ml of a test solution. The solution was then sealed with another piece of parafilm membrane. Insects were allowed to feed for 24 h during which the excreted honeydew was collected on the aluminium foil bottoms of the chamber. The foil bottoms were individually placed in test tubes (16 x 100 mm) and then extracted with 2 ml of distilled water. The solution in each tube was transferred to another to which was added 0.1 ml of 97.1% phenol and 2 ml of concentrated sulphuric acid. The intensity of the yellow-orange colour reached a maximum rapidly and was then quite stable. The mixture was allowed to cool to room temperature and shaken well. Absorbance was read on a Spectronic 20 spectrophotometer at 490 nm and the quantity of sucrose in the excretion was calculated based upon the standard curve. One feeding chamber represented a replicate with 5 replicates per treatment.

RESULTS AND DISCUSSION

Feeding response of *S. furcifera* to different rice varieties

In general, *S. furcifera* females excreted less honeydew on resistant

plants than on susceptible TN1 plants; the selected resistant varieties were more effective against the brachypters than the macropters (Figure 1). The high percentages of reduction in honeydew excreted by both the macropters and brachypters on Rathu Heenati and ADR 52 revealed that these two varieties were highly resistant to *S. furcifera* on both insect morphs (Table 1). Honeydew production of the macropters and brachypters on the susceptible TN1 was similar but differed on the remaining resistant varieties. This is possibly due to the variation of environmental conditions (temperature, photoperiod, soil condition and fertilizer application) for culturing test rice plants and insects; the macropters being more sensitive to the variations. Wu and Saxena (unpublished) demonstrated that environmental temperature affected the growth of rice plants and *S. furcifera* females, and varietal resistance expression in turn.

Effect of plant extracts on *S. furcifera* feeding

S. furcifera fed significantly less from W_1 than from W_2 of resistant Rathu Heenati plants when incorporated with 15% of sucrose and 1% of aspartic acid (Figure 2), indicating that fractionation of the polar extract of the test plant by partitioning between water and butanol resulted in transfer of the insect's feeding inhibitor(s) into the butanol. Moreover, both water extracts from these two varieties induced a reduction in *S. furcifera* feeding compared to the control. Partitioning the water extract of TN1 into butanol did not alter the feeding deterrency. Thus, it appears that some of the factors causing feeding deterrency are transformed in this process or, alternatively, that there are a number of deterrent components with different polarities. Das (1976) also reported that the larvae of rice stemborer, *Chilo suppressalis*, showed a definite preference for artificial media incorporated with fresh-plant water extract of susceptible Rexoro plants to that of resistant TKM6 plants.

TABLE 1. Areas of honeydew spots and percent reductions in honeydew excretion by the macropters and brachypters of *S. furcifera* on plants of resistant and susceptible rice varieties.

Variety	Resistance gene	Honeydew excretion (mm^2/female in 24 h)		Reduction in honeydew excretion (%)	
		Macrop.[1]	Brachyp.[2]	Macrop.	Brachyp.
N22	*Wbph*1	117.9 a	2.0 a	6	98
ARC 10239	*Wbph*2	100.8 b	17.3 b	22	81
ADR 52	*Wbph*3	54.4 c	12.2 ab	68	87
Podiwi A8	*wbph*4	117.9 a	32.8 c	6	65
N'Diang Marie	*Wbph*5	--	29.7 cd	--	68
Rathu Heenati	?	52.7 c	19.4 bd	70	79
TN1 (susceptible)	none	123.1 a	92.9 e	--	--

In a column, means followed by the same letters are not significantly different at the 5% level by Duncan's multiple range test (DMRT). [1]Average of 3 replicates. In transformed value (y = 26.948 + 1716.977x, y = areas of honeydew spots, x = absorbance at 490 nm). [2]Average of 10 replicates.

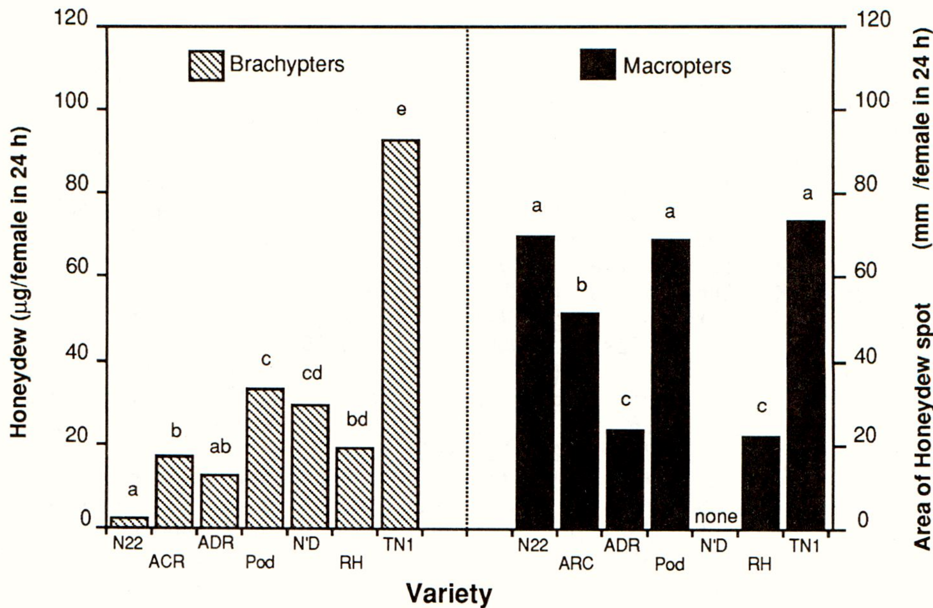

Fig. 1. Honeydew excreted by the brachypters and macropters of *S. furcifera* on 6-week-old rice plants of N22, ARC 10239 (ARC), ADR 52 (ADR), Podiwi A8 (Pod), N'Diang Marie (N'D), Rathu Heenati (RH) and TN1. Columns with the same letters in a test are not significantly different at the 5% level by DMRT.

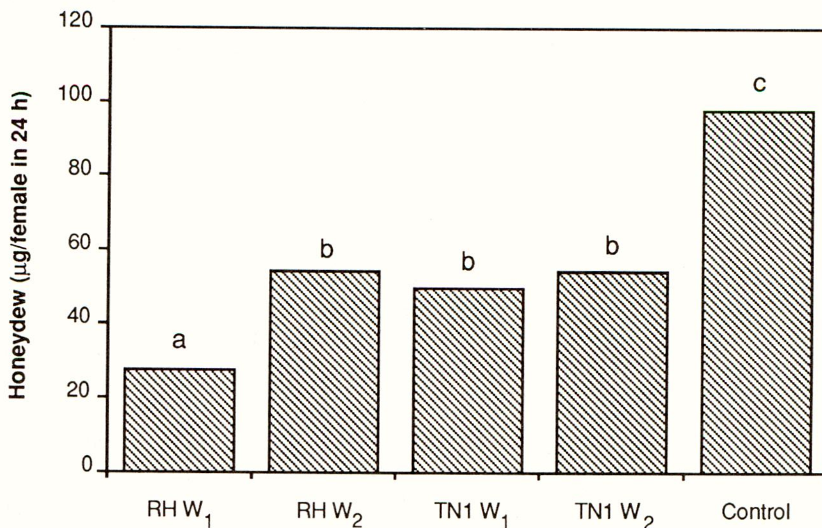

Fig. 2. Honeydew excreted by the brachypters of *S. furcifera* on the water extracts of resistant Rathu Heenati (RH) and susceptible TN1 plants before (W_1) and after (W_2) partitioning with butanol. Columns with the same letters are not significantly different at the 5% level by DMRT.

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RESISTANCE IN GROUNDNUT AND ITS WILD RELATIVES TO APHIS CRACCIVORA, AND ITS RELEVANCE TO GROUNDNUT ROSETTE DISEASE MANAGEMENT.

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ABSTRACT

In the Third World, groundnuts (Arachis hypogaea)are a prominent source of dietary protein and lipid, and often also provide a cash income. However, virus infections transmitted by Aphis craccivora can be a major constraint to production. Resistance to the vector has been identified in the cultivar EC36892. Field and laboratory behaviour studies showed that this resistance takes effect only after the insect has fed for about 2 h. Electrical studies of probe penetration showed that phloem location is almost as successful in the resistant cultivar EC36892 as in the susceptible cultivar TMV2, but the mean feeding duration on the resistant cultivar was only half that on the susceptible cultivar. Chemical analysis suggests that in the resistant EC36892 an isoflavonoid-like substance may confer limited resistance at the probing stage while a high concentration of the condensed tannin procyanidin in the phloem sap may be a major constraint to prolonged ingestion. Wild Arachis species also show evidence of resistance.

INTRODUCTION

Groundnuts (peanuts) (Arachis hypogaea L.) are a prominent source of dietary protein and lipid, and are a cash crop throughout much of southern Asia and sub-Sahelian Africa. Factors causing yield loss are therefore of major importance to farmers in the Third World (Feakin, 1973; Wightman & Amin, 1988). Among these biotic constraints is groundnut rosette virus (GRV) (Storey & Bottomley, 1928). Resistance to GRV has been identified in germplasm from Burkino Faso and Cote d'Ivoire (De Berchoux, 1958). However, these cultivars have some unacceptable agronomic characters and breeding from them is difficult because the resistance factor is double recessive (Nigam & Bock, 1990). The Southern Africa Development Co-ordination Conference (SADCC)/ICRISAT Malawi breeding programme has made progress in developing more suitable GRV-resistant lines but an

alternative approach which may prove valuable is resistance to the aphid vector, Aphis craccivora.

GROUNDNUT ROSETTE DISEASE

ICRISAT screening trials in Malawi (Table 1) showed that, even under the high aphid infestation pressure of the groundnut rosette disease screening nursery the cultivar EC36892 showed significantly fewer diseased plants than any other cultivar.

TABLE 1 The incidence of groundnut rosette disease at Chitedze Agricultural Research Station Malawi: 1987 (Wightman et al, 1989).

	% rosette incidence			
Variety	29 Jan	13 Feb	12 Mar	13 Apr
Local	49.9	69.9	81.3	99.3
EC36892	8.1	13.7	31.8	43.9

Symptoms of rosette disease are associated with infection by GRV (Storey & Bottomley, 1928). This virus is transmitted by Aphis craccivora but only from plants that are also infected with a second virus, groundnut rosette assistor virus (GRAV; Hull & Adams, 1968), which itself causes no symptoms in groundnut. The two viruses therefore spread in nature as a complex. A. craccivora transmits the GRV/GRAV complex in the persistent (circulative) manner, remaining able to inoculate plants for many days after completing the acquisition feed (at least 4.5 h; Dubern, 1980). EC36892 is susceptible to both GRV and GRAV when inoculated by viruliferous A. craccivora in greenhouse experiments (Murant; unpublished data). This suggests that the field resistance of EC36892 to rosette is based on resistance to the aphid vector, which causes a concomitant decrease in the efficiency with which the aphids acquire and inoculate the rosette viruses.

VECTOR BEHAVIOUR

There are three points at which a plant can exert resistance to aphids:

1. At a distance; a volatile chemical and/or a visual characteristic of the plant deter winged females from landing.

2. At the plant surface; landing could occur on all varieties but a surface feature influences acceptability.

3. Within the plant tissues; some chemical or physical factor within the plant inhibits feeding or reduces survival.

Alate arrival

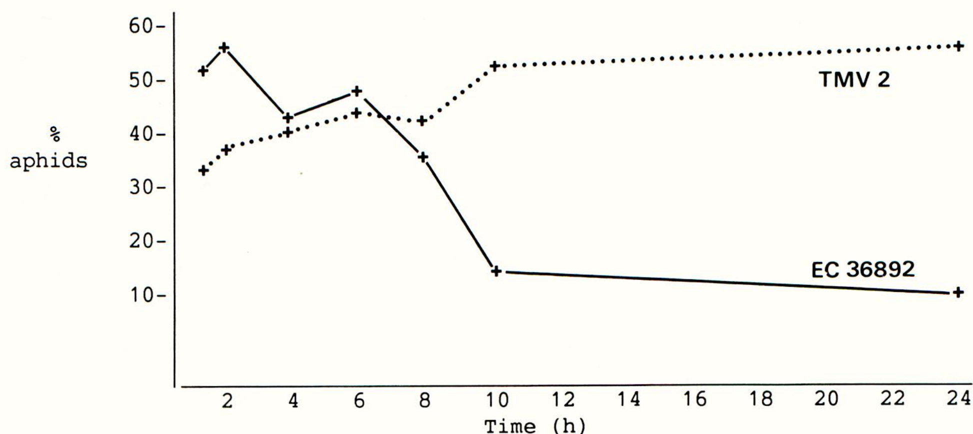
Two cultivars, TMV2 (susceptible) and EC36892 (resistant) were planted in a randomised block and, from germination onwards, examined for

A. craccivora every 3 days. There was no significant difference in the rate of alate arrival between the two varieties and [in strictly numerical terms] the population development was only moderately reduced on the resistant variety (peak values: TMV2, 2.8 aphids per plant; EC36892, 2.0 aphids per plant). However, immediately before the last count heavy rain reduced the population on TMV2 by 60% and on EC36892 by 86%. The reduced survival under adverse conditions suggests that the aphids were less vigorous on EC36892 than on TMV 2.

Apterae host selection

To examine the behaviour of the insect at the plant surface, 50 apterae were placed in the centre of an arena of alternate EC36892 and TMV2. Their distribution was counted periodically over 24h (Fig 1).

Figure 1. The distribution of aphids between the susceptible (TMV2) and resistant (EC36892) cultivars over 24h (n = 1600). NB: Where total no. of aphids <100%, the missing aphids were not dead but were on the soil or had left the arena.



As with the field results there was no initial rejection of the resistant variety, in fact a significant preference in the first 2h ($p = 0.001$; Chi square). However, by 4 h there was no significant difference in the distribution between the two varieties and from 10h onwards there was a significant rejection of the resistant variety. Thus the point at which rejection occurred was at a point after 2h from the start of the experiment, i.e. after significant examination of the internal composition of the plant by the insects (Padgham et al, 1990).

The development of the insect on the two varieties was compared by enclosing individual first instar nymphs in cages attached to young leaves. On EC36892 the mean time taken to reach the reproductive stage was significantly longer ($p = 0.01$; t test) and the mean number of offspring was significantly less than on TMV2 ($p = 0.001$).

Host tissue exploration

When an aphid feeds it pushes its stylet into the plant tissues in an attempt to find the phloem. A protective sheath of coagulated saliva

is formed round the stylet and, in histological sections, this can be stained to determine its track. Saliva sheaths can be branched or unbranched and this can indicate the route taken by the stylets to find a feeding site and the ease with which it is found. With TMV2, only approximately 1% of probes (sample size: 1919 sheaths) were branched whereas with EC36892approximately 50% (sample size: 3458 sheaths) were branched. It is not possible to say whether the phloem had been located before branching of the sheaths occurred but the results imply that either the aphids were less able to locate the phloem in the resistant cultivar or the phloem was found but rejected so that stylets were partially withdrawn and pushed in another direction - thus making branches.

Thus resistance in EC36892 is an internal feature and possible mechanisms were investigated using the Electrical Penetration Graph (EPG) (Tjallingii, 1978). The insect and plant are connected as components of a sensitive electrical circuit which monitors changes in electrical resistance as the insect penetrates the plant as well as neural activity. Different and characteristic signals are generated by different activities and it is possible to identify with great accuracy when plant tissue penetration begins, when the phloem is located and for how long phloem-stylet contact persists. Thus results from experiments using EC36892 and TMV2 can be summarised:

1. The overall success in finding the phloem is slightly reduced with EC36892, 70% compared with 80% with TMV2.

2. There is no significant difference in phloem location time: EC36892, 96 min; TMV2, 94 min.

3. The time spent feeding in the phloem is half as long in EC36892 (57 min) as in TMV2 (112.3 min). (Kimmins; unpublished data.)

While the EPG system provides an indication of stylet tip position and the uniformity of the wave pattern suggests constant passive ingestion, the EPG system does not provide information on rates of ingestion. Thus it is feasible that whilst ingestion time is less on EC36892, the rate of ingestion might be faster than on TMV2 to compensate. However, honeydew excretion rates, measured using a honeydew clock (Banks & Macaulay, 1964), were proportional to the length of phloem-stylet contact and it was concluded that ingestion rates are similar on the two cultivars (TMV2 1.1; EC36892 0.55 honeydew droplets h^{-1}).

The observations indicate that:

1. From the reduced success in phloem finding and the greatly increased probe branching there is good evidence of some resistance to phloem location in EC36892.

2. From the much reduced phloem ingestion, as indicated by reduced honeydew excretion and the reduced fecundity and rate of population development on EC36892, there is a major resistance factor in the phloem.

3. The observed 50% reduction in the phloem feeding time on EC36892 will probably reduce the acquisition of viruses like those of the GRV/GRAV complex which have a minimum acquisition time of approximately 4.5 h (Dubern, 1980).

PLANT CHEMISTRY

Analysis of whole plant

To investigate further the minor resistance encountered during parenchyma penetration, chemical extracts were made of whole leaves and petioles of EC36892 and TMV2. Ethanolic/aqueous extracts were prepared and investigated for phenolic and other ultraviolet light absorbing compounds by means of two-dimensional paper chromatography (2-D PC) and high performance liquid chromatography (HPLC). All four types of extract showed very similar patterns of phenolic substances. However, one substance found in extracts of EC36892 (in larger concentrations in the petiole extract than in the leaf extract) was absent from the TMV2 extracts. The identity of this compound is under investigation (Grayer; unpublished data), but its properties indicate that it may be an isoflavonoid.

Phloem sap analysis

Samples of phloem sap, extracted into ethylene-diamine-tetra-acetic acid (EDTA) (King & Zeevaart, 1974), were also examined by means of 2-D PC and HPLC. No qualitative differences between TMV2 and EC36892 were found. The isoflavonoid-like substance detected in the petiole and leaf extracts of EC36892 but not in those of TMV2, was not present in phloem sap of either variety of groundnut. Boiling samples of the phloem sap exudate with dilute HCl made the extracts turn bright red. This indicates the presence of a condensed tannin, which was later identified as procyanidin (Grayer; unpublished data). Preliminary investigations showed that phloem sap of EC36892 contained on average 1.8 times more procyanidin than the phloem sap of TMV2. Thus the minor resistance may be due to an isoflavonoid-like compound, and the major resistance to a high concentration of the condensed tannin procyanidin. Further studies using bioassay techniques are under way to test this hypothesis.

WILD *ARACHIS* SPECIES

We have also used the EPG system to examine the nature of plant resistance in wild *Arachis* species. Significant differences were found between species (Table 2).

TABLE 2. Mean EPG data for aphids feeding on wild and cultivated *Arachis* species (10 replicates per species, each for a duration of 4 h).

Species	Number of probes	Time to find phloem (min)	Phloem ingestion time (min)	Extracellular ingestion time (min)	Xylem ingestion time (min)
A. hypogaea TMV2	22.0	94.2	112.3	27.4	0
A. hypogaea EC36892	21.6	96.6	57.4	33.3	0
A. chacoensis	19.5	94.6	81.2	33.4	0
A. correntina	23.3	19.6	72.6	37.6	0
A. cardenasii	38.7	95.9	5.1	53.6	4.1
A. villosa	22.4	192.6	11.1	74.6	0
A. glabrata	22.0	-	0	22.2	0
A. stenophylla	28.4	-	0	76.7	0

There was little difference in the number of probes made but phloem ingestion time was significantly reduced in all species compared with TMV2. The data suggest a resistance comparable to that of EC36892 in A. chacoensis, a very strong phloem resistance in A. correntina and A. cardenasii, both resistance to phloem location and in the phloem in A.villosa and total resistance to phloem location in A. glabrata and A. stenophylla (Kimmins; unpublished data).

It is envisaged that this information would be useful not only to entomologists in screening and identifying resistant genotypes but also to plant breeders and cytogeneticists for producing new genotypes with a range of resistance mechanisms.

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PLANT RESISTANCE AND A BIOPESTICIDE FROM LEMONGRASS OIL FOR SUPPRESSION OF *SPODOPTERA LITURA* ON PEANUT

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(NO WRITTEN SUBMISSION)

WILD UMBELLIFEROUS SPECIES AS SOURCES OF RESISTANCE TO CARROT FLY

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ABSTRACT

Techniques for screening wild umbelliferous species of plants for their resistance to carrot fly (*Psila rosae*) have been developed at Wellesbourne. Using these techniques, 132 species have been tested in field experiments over a period of 15 years and a wide range of responses to the insect have been observed. Several species of plants failed to support any insects in repeated testing and many others produced small numbers of insects. The most promising species discovered were those closely related to the cultivated carrot (*Daucus carota*); these species present the least problems in exploitation of the resistance in breeding programmes. Several wild species of *Daucus* in particular are of interest and offer possibilities for widening the gene pool for future breeding. Attempts have begun to transfer genes for resistance to carrot fly from *Daucus* species to commercially-acceptable carrot cultivars.

INTRODUCTION

Carrot fly, *Psila rosae* is the most serious pest of carrots in temperate regions of the world despite 100 years of concerted effort to eliminate the insect from fields, allotments and gardens. The current range of insecticides approved for use on crops of carrot and other related species in the UK does not provide a satisfactory answer to the carrot fly problem for a number of reasons. For example, insecticides are insufficiently persistent to provide season-long protection for main- and overwintered-crops, they may destroy natural enemies of the pest and other wildlife, and their repeated use can induce enhanced degradation of the chemicals in the soil (Suett, 1989). Some growers in the UK exacerbate the problem by failing to rotate their crops sufficiently, spatially and temporaly, and this leads to a build-up of the pest population; some growers also keep carrots in the field over the autumn and winter as a means of storage and this enables carrot fly larvae to continue feeding on the roots. In addition, wild hosts of the carrot fly are common and widespread in carrot-growing districts and therefore, even in the absence of a crop, populations of carrot fly are maintained on the reservoir of hosts in hedgerows or field margins.

One of the alternative control methods to insecticides which is being investigated at this Institute is the exploitation of plants resistant to carrot fly. In this type of work, the research effort concentrates initially on a search for resistance in cultivated crop plants as future breeding work with close relatives is likely to be more straightforward than if distantly related species are used. Partial levels of resistance to carrot fly have been discovered in carrot cultivars and these have been shown to be able to make a significant contribution to integrated

programmes of carrot fly control (Ellis & Hardman, 1988). Certain of these cultivars have been the subject of a breeding programme to develop lines possessing enhanced levels of resistance (Ellis, Hardman & Saw, 1990). However, these sources of resistance provide only a 50% reduction in damage and a 50% reduction in the numbers of insects remaining in the field after the crop is harvested. Ideally, higher levels of pest reduction are desired and, therefore, the search for resistance has extended more widely to wild relatives of the cultivated carrot in the Umbelliferae. Early studies on Umbelliferae as hosts of carrot fly were not aimed at determining which species possessed resistance to the pest but more to determining the insect's host range. These records were reviewed recently (Hardman & Ellis, 1982; Hardman, Ellis & Saw, 1990). A concerted effort to search for sources of resistance in wild species began at Wellesbourne in 1972 when accessions of *Daucus carota* from Turkey and Persia were evaluated in field trials; this programme was extended and continued until recently (Hardman & Ellis, 1982 ; Hardman, Ellis & Saw, 1990). Wild accessions of *Daucus carota* collected from botanic gardens and during expeditions in The Netherlands and Israel were screened for resistance to carrot fly at Wageningen. Levels of resistance to the pest were surprisingly low and, on average, no higher than that of cultivated carrot lines grown alongside them in the same experiments (de Ponti, Freriks, Steenhuis & Inggamer, 1981); selections were made of seven of the wild carrot lines but they have not provided material which could form the basis of a breeding programme to develop resistant varieties.

In this paper, we report on the investigations at Wellesbourne which have identified many promising sources of resistance.

MATERIALS AND METHODS

Umbelliferous species were collected by the authors from wild habitats or obtained from gene banks, botanic gardens or plant breeders working at research establishments. In collecting material, a special effort was made to obtain those species which are related most closely to the cultivated carrot (*Daucus carota carota*). A list of the genera classified in the Umbelliferae is given in Table 1. Seed was multiplied in polyethylene tunnels at Wellesbourne. All species were tested against carrot fly in randomised block field experiments at Wellesbourne using 3 replicates of 10-plant plots of each treatment. Full details of the methods used to evaluate species are given in Hardman, Ellis & Saw (1990). Plants were raised either in pots in a glasshouse and frame or sown directly in the field. Depending on growth characteristics, the different species were tested in the spring against first generation carrot fly attack or in late summer, autumn and winter against late generations of the insect. The carrot cultivar 'Danvers Half Long 126', which had been shown to be highly susceptible to the carrot fly in earlier work (Ellis, Freeman & Hardman, 1984), was used as a standard for comparative purposes in all experiments. In all cases, attempts were made to test plants in a vegetative stage of growth, rather than in a reproductive stage, to maximise the chances of species being colonised by the insect. The numbers of adult flies produced per plant were chosen as an estimate of the resistance of the plant species. Carrot plants were collected from plots, lifted, bagged and maintained in a tygan frame house or caged *in situ* in the field. Insects emerging from infested roots were caught in yellow water traps (Finch & Skinner, 1974) placed on the soil in the bag or cage and counted regularly.

TABLE 1. Classification of the family Umbelliferae listing selected genera (based on Heywood, 1978a)

Subfamily	Tribe	Genus
Hydrocotyloideae	Hydrocotyleae	*Hydrocotyle*
	Mulineae	*Azorella*
Saniculoideae	Saniculeae	*Eryngium*, *Astrantia*, *Sanicula*
	Lagoecieae	*Lagoecia*, *Petagnia*
Apioideae	Echinophoreae	*Echinophora*
	Scandiceae	*Scandix*, *Chaerophyllum*, *Anthriscus*, *Myrrhis*
	Coriandreae	*Coriandium*
	Smyrnieae	*Smyrnium*, *Conium*, *Cachrys*, *Scaligeria*
	Apiieae (Ammieae)	*Bupleurum*, *Pimpinella*, *Apium*, *Seseli*, *Oenanthe*, *Ligusticum*, *Foeniculum*
	Peucedaneae	*Angelica*, *Ferula*, *Heracleum*, *Pastinaca*
	Laserpiteae	*Laserpitium*, *Thapsia*
	Dauceae (Caucalideae)	*Daucus*, *Torilis*, *Caucalis*

The production of carrot flies on each species was related to the numbers produced on the standard cultivar 'Danvers Half Long 126' by calculating a 'susceptibility index'. This expressed the maximum numbers of flies produced on the test species as a percentage of the numbers on the susceptible standard. Certain umbelliferous species which supported few or no flies were tested repeatedly to confirm their resistance.

RESULTS AND DISCUSSION

An understanding of the development and life cycle of umbellifers is critical in a study of this kind. The natural dormancy of many species ensures that seed will germinate only in late autumn or winter. For these species, it is impossible to achieve a plant stand from seed sown in the spring. Therefore experiments designed to coincide with first generation carrot fly attack have to be sown in the previous autumn. It is also important to ascertain whether a species is an annual, biennial or perennial so that it can be exposed to the pest at an appropriate stage in its development. Thus, annual species need to be tested against first generation carrot fly before they flower, whereas biennials are tested best against late generations of the insect. Many perennials can be tested early or late in the season. Our experience has shown that wild umbellifers are tested best when they are in the rosette stage of vegetative growth.

Between 1981 and 1989, a total of 132 umbelliferous species and sub-species was tested against carrot fly in the field experiments. There was great variability in the numbers of insects produced on plants of the standard cultivar between seasons and a 1200-fold range between the numbers of carrot flies produced on the most and least productive species. The maximum number of insects produced on 'Danvers Half Long 126' was 13.0 flies per plant; the numbers of insects produced on the different species of umbellifer were related to this figure. Two species, *Aethusa cynapium* (fool's parsley), and *Daucus muricatus*, yielded more flies than the standard (16.4 and 17.0 flies per plant respectively). Twenty seven species failed to support the pest, despite repeated exposure of some of them (Table 2) and a further thirty eight supported up to 0.5 insects per plant which represents < 5% of those produced on 'Danvers' (Table 3). There was no indication that resistance to the pest was associated with a plant species' life cycle, that is whether they were annuals, biennials or perennials. There was also no suggestion that the resistance was related to factors used in the taxonomy of the family, for example grouping of species into particular tribes or genera. The cultivated carrot (*Daucus carota* ssp. *carota*) belongs to the tribe Caucalideae, the spiny-fruited umbellifers (Table 1). This tribe is largely Mediterranean in its distribution and has about 20-25 genera and 60-100 species (Heywood, 1978b). We tested numerous species of Caucalideae against carrot fly and discovered a wide range of susceptibilities but no indication of an association with this tribe and greater or lesser susceptibility towards the pest. Within the genus *Daucus*, the productivity of insects ranged from nil to a level which exceeded the standard susceptible cultivar. The *Daucus* genus itself is a highly complex group of species and subspecies whose taxonomy is critical and much disputed (Heywood, 1983). The genus is divided into seven sections based on a wide range of characters. Most attempts to hybridise different *Daucus* species has been unsuccessful, due to the failure of pollen to germinate or failure of the pollen tubes to

TABLE 2. Umbelliferous species tested between 1981-1989 at Wellesbourne which failed to support carrot fly

Species	Nos. experiments	Nos. of plants exposed to carrot fly attack
Annuals		
Bifora radians Bieb.	2	18
Bupleurum asperuloides Heldr. ex Boiss.	1	30
Bupleurum subovatum Link, false thorow-wax	6	503
**Cuminum cyminum* L., cumin	2	11
**Orlaya grandiflora* (L.) Hoffm.	1	30
Scandix balansae Reut.	1	30
Scandix pecten-veneris L., shepherd's-needle	6	769
Scandix stellata Banks & Solander in A. Russell	5	208
**Torilis arvensis* ssp. *arvensis* (Hudson) Link, spreading hedge-parsley	4	171
**Torilis tenella* (Delile) Reichenb. fil. in Reichenb. & Reichenb.	3	151
Biennials		
**Daucus broteri* Ten.	2	40
Smyrnium olusatrum L., Alexanders	4	120
Perennials		
Astrantia carniolica Jacq. 'Ruby'	1	30
Bupleurum barceloi Cosson ex Willk.	1	30
Bupleurum rigidum L.	1	30
Carum verticillatum (L.) Koch, whorled caraway	2	42
Eryngium alpinum L.	2	48
Eryngium variifolium	1	30
Laser trilobum (L.) Borkh.	2	167
Ligusticum lucidum Miller	1	27
Molopospermum peloponnesiacum (L.) Koch	1	30
Oenanthe silaifolia Bieb.	2	33
Peucedanum longifolium Waldst. & Kit.	1	30
Pimpinella peregrina L.	1	30
Portenschlagiella ramosissima (Portenschl.) Tutin	1	30
Sanicula europaea L., sanicle	1	30
Thapsia villosa L.	3	33

* species classified in the tribe Caucalideae

TABLE 3. Umbelliferous species tested between 1981-1989 at Wellesbourne and which supported < 5% of the insects produced on the standard 'Danvers Half Long 126'

Species	Nos. experiments	Susceptibility index
Annuals		
Bupleurum praealtum L.	6	0.2
Bupleurum rotundifolium L., thorow-wax	7	0.2
Chaerophyllum coloratum L.	2	2
Lagoecia cuminoides L.	1	0.8
Ridolfia segetum Moris	3	0.4
Scandix pecten-veneris ssp. *brachycarpa* (Guss.) Thell. in Hegi	2	2
**Torilis arvensis* ssp. *neglecta* (Schultes) Thell. in Hegi	1	2
**Torilis elongata* (Hoffmans. & Link) Samp.	4	0.5
Biennials		
Apium graveolens L., wild celery	2	0.5
Chaerophyllum bulbosum L.	2	1
Carum carvi L., caraway	1	4
**Daucus capillifolius* Gilli	4	2
**Daucus glochidiatus* (Labill.) Fisch. Mey et Ave-Lall.	4	4
**Daucus gracilis* Steinh.	3	4
**Daucus involucratus* Sibth. & Sm.	5	3
**Daucus littoralis* Sibth. & Sm.	4	0.6
**Daucus pusillus* Michaux	8	2
Ferula communis L., giant fennel	3	2
Perennials		
Aegopodium podagraria L., ground-elder	3	2
Angelica laevis Gay ex Ave-Lall.	2	2
Astrantia major L., astrantia	1	0.2
Bupleurum falcatum L.	2	4
Chaerophyllum aureum L., golden chervil	1	2
Cicuta virosa L., cowbane	1	3
Crithmum maritimum L.	3	0.4
Eryngium agavifolium Griseb.	3	2
Eryngium campestre L. field eryngo	2	0.5
Eryngium maritimum L., sea holly	2	4
Foeniculum vulgare var. *azoricum* (Miller) Thell., finnochio	1	2
Grafia golaka (Hacq.) Reichenb.	5	0.8
Meum athamanticum Jacq.	2	0.8
Myrrhis odorata (L.) Scop., sweet cicely	3	2
Oenanthe lachenalii L., parsley water-dropwort	1	3
Oenanthe peucendanifolia Pollich	4	0.5
Peucedanum baicalense Koch	2	4
Pimpinella major (L.) Huds., greater burnet-saxifrage	3	0.2
Pimpinella saxifraga L., burnet saxifrage	5	0.2
Trinia glauca (L.) Dumort, honewort	1	3

* species classified in the tribe Caucalideae

penetrate down into the stigma tissues. McCollum (1975; 1977) widened the gene pool by obtaining crosses between the Libyan wild carrot (*Daucus capillifolius*) and *D. carota*. *D. capillifolius* supported very few insects in our experiments and therefore would appear to offer great promise for breeding as it is highly resistant to carrot fly and yet can be crossed with cultivated carrot. Our work on breeding lines derived from crosses between these two species will be reported elsewhere (Ellis, Hardman, Saw, Dowker & Crowther, in prep.).

Daucus carota exists in the wild state over a very wide geographical area and it is difficult to establish where its cultivation as a crop began (Heywood, 1983). The picture is complicated by the fact that the various subspecies and forms hybridise with one another, carrots are generally cross-fertilised and there has been frequent introgression between wild populations and cultivated crops. Under these circumstances, it is very difficult to establish the true nature of the first cultivated carrot. The main types in cultivation today stem from the western or carotene carrot and the eastern or anthocyanin carrot. In the past, we have tried to demonstrate a link between the origin of carrot material and the distribution of the carrot fly in the wild but lack of detailed information on the insect and host prevents us from drawing positive conclusions. Thus, the few anthocyanin-rich carrot cultivars obtained from the Middle and Far East and screened at Wellesbourne possessed no more resistance than carotene-rich carrot material obtained from Western Europe. However, it is possible that the levels of partial resistance discovered in a number of Nantes-type carrots developed by French plant breeders in the Normandy region of France may have developed in response to selection pressure from the consistently high populations of the pest in this region. The existence of high levels of resistance in various wild *Daucus* species cannot always be linked to the same selection pressures as many of the species have their origins in climates and countries where the pest does not exist or where it has been introduced recently. Thus, resistant *D. capillifolius* from north Africa, *D. pusillus* from southern America and western north America and *D. glochidiatus* from Australia and New Zealand do not appear to overlap with indigenous populations of carrot fly. Carrot fly is believed to have been introduced relatively recently into New Zealand. However, genes for resistance have been known to accumulate in plants in the absence of selection pressure from pests, so this may explain why they fail to support appreciable numbers of carrot flies.

There does not appear to be a shortage of sources of resistance to the carrot fly in the Umbelliferae. Clearly certain sources can be exploited using conventional breeding technniques. Other sources in distantly-related species will be more difficult to use. However, with new biotechnological techniques of gene-transfer and rapid breeding of plants, the future looks bright for the development of many different forms of resistance to this very important pest.

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THE USE OF POLYACRYLAMIDE GRADIENT GEL ELECTROPHORESIS TO IDENTIFY GENETICALLY INHERITED VARIATIONS IN THE OCCURRENCE OF ISOZYMES AS MARKERS FOR THE RESISTANCE OF *LACTUCA* SPECIES TO THE LETTUCE ROOT APHID

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ABSTRACT

Pest and disease resistance has been incorporated into cultivated lettuce (*Lactuca sativa*) from some close wild relatives (*L. saligna, L. serriola, L. virosa*) and primitive forms of *L. sativa*. Isozymes have potential for use as markers for resistance in practical plant breeding programmes.

Transparent polyacrylamide gels give better resolution than starch gels of isozyme bands and there is also more opportunity to optimize the resolution, by using discontinuous pH and variation in pore size. This paper describes simple, routine polyacrylamide gradient gel electrophoresis of isozyme patterns surveyed in wild populations of four *Lactuca* species. Using multivariate statistical techniques, these isozyme patterns were related to the resistance of accessions of the four species to the lettuce root aphid.

INTRODUCTION

Many uses of isozyme electrophoresis in starch gels in basic research on plant genetics and in practical plant breeding have been reviewed extensively (Tanksley & Orton, 1983). For example, variations in isozyme patterns can be used to delineate phylogenic relationships (Kesseli & Michelmore, 1986) but the lack of clarity and the relatively poor resolution associated with starch gel electrophoresis may hinder the interpretation of zymograms, especially if the differences between isozymes are small. In contrast, polyacrylamide gels are transparent, giving sharper resolution of bands. Also, by using discontinuous pH and pore size to 'stack' the enzymes before their resolution in the resolving gel, they offer possibilities for optimizing the resolution of bands. The incorporation of a continuous gradient pore size within a single gel is of particular value when subtle differences in migrational behavior of isozyme variants are being investigated.

In the study reported in this paper, wild populations of *Lactuca* spp. obtained from the Vegetable Gene Bank at the Institute of Horticultural Research-Wellesbourne were surveyed for variations in isozymes, using

polyacrylamide gradient gel electrphoresis (PAGGE). Eight accessions of each of *L. virosa*, *L. serriola*, *L. saligna* and primitive *L. sativa* were used, covering a wide regional variation in source. The resistance of each accession to the lettuce root aphid (*Pemphigus bursarius*) was also evaluated to determine the potential of using variations in isozymes as markers for resistance to this pest.

METHODS

Sample extraction

Fifty seeds from each accession (Table 1) were germinated on slopes in an incubator at 25±1°C for 5 days. The seedcoats were then removed and the seedlings were dried of excess moisture before they were ground in liquid nitrogen in a pestle and mortar. Isozymes were extracted at 0°C using a modification of the protein extraction buffer described by Manganaris & Alston (1987). Each extract was centrifuged at 14000 g for 15 min. and 300 μl of the supernatant from each were pipetted into Eppendorf tubes for storage at -20°C in a deep freeze. Before electrophoresis, 10 μl 0.5% (w/v) bromophenol blue tracking dye were added to each extract and, after mixing, samples (10 μl) were loaded into 10 slots in the stacking gel. Each gel contained 8 samples of *Lactuca* spp and one sample from each of the cultivated cultivars Webbs Wonderful and Avoncrisp, used as standards for comparison.

Gel preparation

Four 5-20% polyacrylamide gels (0.75 mm) were formed (Davis, 1964), using an LKB 2050-200 Midget multicast gel casting stand and gradient maker. The tops of the gels were covered with primary butanol, saturated with distilled water and left overnight at 5±1°C. Immediately before use, a 10 mm 5% polyacrylamide stacking gel was poured on top of the gradient gel and 10 sample wells were formed by the slot former, which was removed after 30 min.

Electrophoresis

Electrophoresis was carried out using an LKB 2050 Midget Electrophoresis unit at 0°C with a constant voltage of 100 V until the tracking dye had entered the gradient gel. The voltage was then raised to 150 V. A 0.025 M tris-glycine buffer (pH 8.8) was used as the electrode buffer. The electrophoresis was terminated after ca. 1.5 h, when the tracking dye had migrated to the bottom of the gel.

TABLE 1. Origins of accessions of *Lactuca* species.

Ref. No.	Species/Origin	Ref. No.	Species/Origin
	L. virosa		*L. saligna*
(1)	Kew (United Kingdom)	(3)	Portugal
(8)	Cluj Romania (Romania)	(6)	Israel
(11)	Stuttgart (Germany)	(9)	Uppsala (Sweden)
(14)	Brussels (Belgium)	(16)	Unknown
(17)	Amsterdam (Netherlands)	(19)	Budapest (Hungary)
(24)	Paris (France)	(22)	Nijmegen (Netherlands)
(27)	Bucharest (Romania)	(25)	Nantes (France)
(30)	Bonn (Germany)	(25)	Rotterdam (Netherlands)
	L. serriola		*L. sativa*
(2)	Uppsala (Sweden)	(4)	Pakistan
(5)	Wageningen (Netherlands)	(7)	Turkey
(12)	Guttenberg (Germany)	(10)	Lotz (Poland)
(15)	Ogrod (Poland)	(13)	China
(18)	Montreal (Canada)	(20)	Italy
(21)	Algarve (Portugal)	(23)	El Brahma (Egypt)
(28)	Versailles (France)	(26)	Guttenberg (Germany)
(31)	Komensky (Czechoslovakia)	(29)	Nijmegen (Netherlands)

Staining for isozyme activity

Six enzymes were assayed: glutamate oxaloacetate transaminase, alcohol dehydrogenase, leucine aminopeptidase, esterase, malate dehydrogenase and diphorase. Before the gels were incubated in staining solutions (Vallejos, 1983) for 1 h at 30 ± 1°C in the dark, they were soaked for 1 min in the staining buffer; after staining, the gels were fixed in methanol: glacial acetic acid: distilled water (4:1:15) for 5 min and photographed with Ilford XP 1400, 6 cm x 6 cm film.

Statistical analysis of isozyme patterns

The isozyme patterns were recorded as presence/absence matrices, with a row for each accession and a column for each isozyme locus. For each pair of accessions, a measure of similarity was calculated by counting the numbers of common bands and dividing these by the total numbers of isozyme bands. A dissimilarity matrix for the 32 accessions was constructed by subtracting the measure of similarity from unity. This matrix was subjected to average linked heirarchical cluster analysis, using a Genstat 5 program, to determine whether the 32 accessions formed natural groups. The original presence/absence matrices were also subjected to principal component analysis, using a Genstat 5 program, to indicate which combinations of isozyme bands discriminated best between the accessions. The relative susceptibility of the individual accessions to the lettuce

root aphid was regressed against the principal component analysis to determine which isozymes might be useful as markers for root aphid resistance.

Evaluation of the resistance of accessions to the lettuce root aphid

The resistance of the accessions of *Lactuca* to the lettuce root aphid was evaluated using a modification of the method described by Dunn (1974). Individual lettuce plants were grown in 50-mm pots embedded in trays of Levington compost, with six rows of four replicate plants in each tray. Each tray contained, in randomly selected positions, one row each of Webbs Wonderful (susceptible to the lettuce root aphid) and Avoncrisp (resistant to the lettuce root aphid), used as standards for comparison, and one row of each *Lactuca* species. In total, 192 plants were grown in 8 trays in an air-conditioned glasshouse compartment maintained at 20±5°C. Four weeks after the lettuce seeds germinated, two apterae from a laboratory culture of the aphid were placed on the roots of each plant and, after a further two weeks, the numbers of aphids in the resulting colonies were recorded.

RESULTS

Two accessions, *L. virosa* ( ) and *L. sativa* ( ) did not germinate sufficiently to provide seedlings for extraction. The six enzymes showed 41 zones of enzyme activity that could be considered as loci (Table 2).

TABLE 2. Relative mobilities of allozymes: glutamate oxaloacetate transaminase (GOT), alcohol dehydrogenase (ADH), leucine aminopeptidase (LAP), esterase (EST), malate dehydrogenase (MDH) and diphorase (DIA) in accessions of *Lactuca* spp.

Isozymes	Relative mobility	Isozymes	Relative mobility
GOT 1	32,33,34	ADH	15,null
GOT 2	21,22,25	GOT 3	6,7
LAP	37,41	EST 1	58,59
MDH 1	5	EST 2	55,56,57
MDH 2	28,31,35	EST 3	50,52
EST 4	47,48	DIA 1	32,34,36
EST 5	38,34	DIA 2	23,24,27,28,30
EST 6	30,31,33,35,37	DIA 3	14,18,20
EST 7	24,25	EST 8	17,22

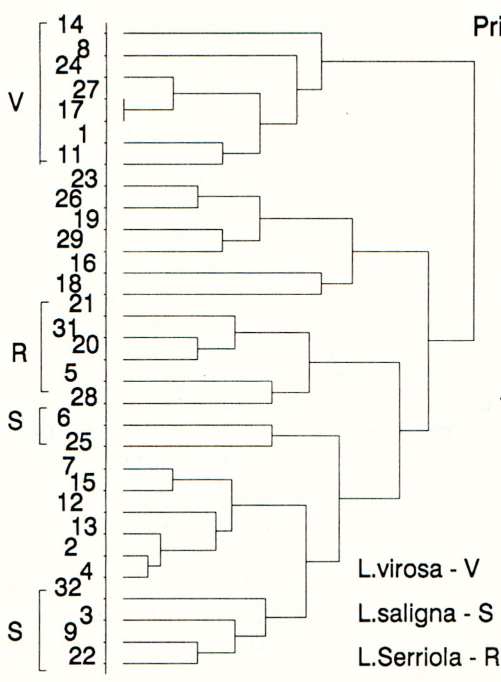

Fig.1 Dendrogram showing genetic relationships among Lactuca spp.

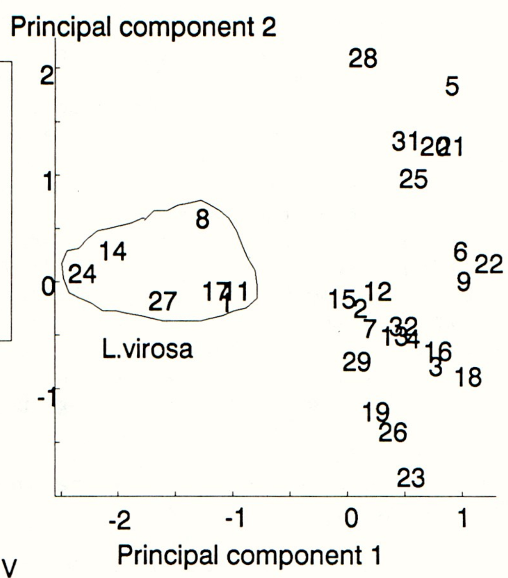

Fig.2 Plot of the isozyme-generated similarity matrix relative to the first two principal components

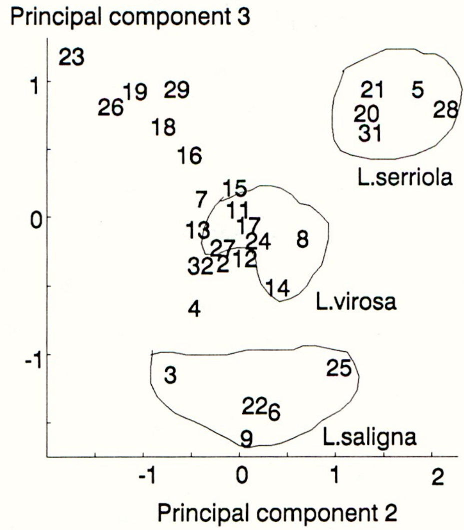

Fig.3 Plot of isozyme-generated similarity matrix for principal components 2 and 3

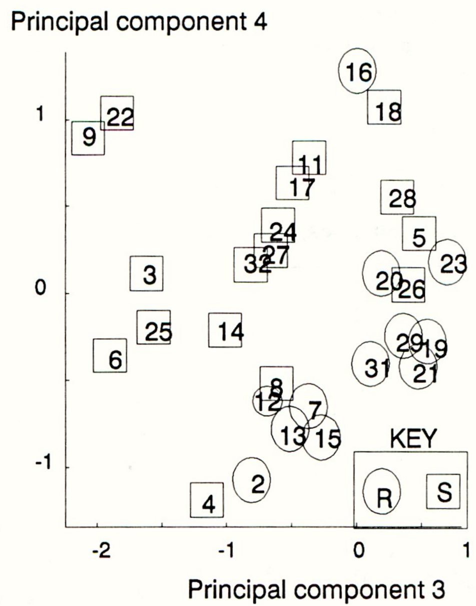

Fig. 4 Plot of principal components 3 and 4 relative to numbers of lettuce root aphid

Multivariate analysis of the isozyme patterns was partially successful in differentiating between the four *Lactuca* species (Fig. 1). The seven *L. virosa* accessions which germinated formed a linked group in the cluster analysis. *L. saligna* accessions formed two linked groups, with close similarities to some *L. serriola* and *L. sativa* accessions which were generally difficult to separate with the enzyme systems used in this experiment. The first principal component in the principal component analysis reflected the contributions from GOT 2 (25), EST 8 (17), EST 7 (25), DIA 1 (36), and ADH (50) and clearly separated *L. virosa* from the three other species (Fig. 2). The second principal component reflected contributions from GOT 2 (21), GOT 2 (22), EST 5 (38), DIA 2 (28) and DIA 1 (32) and separated some *L. serriola* accessions from *L. saligna* and *L. sativa*. However, this separation was less clear than with the first principal component and showed some possibly-missassigned species. The third principal component showed large negative loadings for EST 6 (33),(35) and these separated *L. saligna* from the other three species (Fig. 3). A three-dimensional plot of the first three principal components would provide an adequate separation of the *Lactuca* species, using only four isozyme systems.

Aphid numbers were estimated as geometric means of the grades from four plants. Table 3 shows that all *L. virosa* and most *L. saligna* accessions were susceptible to colonisation by lettuce root aphid. When aphid numbers were regressed on principal component scores, principal components 1,3 and 4 accounted for most of the variation in aphid numbers. The first principal component was significant as it clearly separated the *L. virosa* species from the other three species on susceptibility to root aphid but was unable to predict resistance to this pest. A plot of principal components 3 [Est 6 (33) and (35)]and principal component 4 [Dia 1 (34) and Dia 3 (18) and (20)] (Fig. 4) revealed a grouping of accessions into root aphid resistant and susceptible areas.

TABLE 3. Relative numbers of lettuce root aphids colonizing *Lactuca* species.

Ref. No.	Species/Mean grade*	Ref. No.	Species/Mean grade*
	L. virosa		*L. saligna*
(1)	3.6	(3)	2.9
(8)	4.0	(6)	1.3
(11)	3.4	(9)	3.1
(14)	3.5	(16)	1.3
(17)	3.1	(19)	2.9
(24)	2.5	(22)	3.7
(27)	3.5	(25)	2.7
(30)	4.0	(25)	1.5
Mean grade	3.45	Mean grade	2.42

	L. serriola		*L. sativa*
(2)	1.1	(4)	2.0
(5)	3.7	(7)	1.7
(12)	1.0	(10)	4.0
(15)	0.8	(13)	1.1
(18)	2.1	(20)	0.0
(21)	1.2	(23)	1.0
(28)	2.9	(26)	3.2
(31)	1.9	(29)	0.3
Mean grade	1.84	Mean grade	1.66

* Geometric means of aphid grades from four plants: 1 - <2 aphids per plant; 2 - 2-5 aphids per plant; 3 - 6-20 aphids per plant; 4 - >20

DISCUSSION

The gradient polyacrylamide mini gels were very transparent and showed clearly-reproducible differences in the zymograms, including the numbers of isozyme bands, isozyme mobility and activity. Because of their transparency, enzymes such as alcohol dehydrogenase with low activity were detected readily. Gradient gels produced very sharp bands which enabled variations in isozymes with small differences in migrational capacity to be detected. The disadvantages of polyacrylamide gels include the toxicity of the monomer. This was reduced in these experiments by the use of a ready-mixed precursor, 'Protogel' (National diagnostics). The expense of the acrylamide monomer was offset by using mini-cells having the added advantage of requiring small sample volumes. This permitted analyses with small quantities of plant material.

The system chosen for scoring the zymograms was the presence or absence of all bands treating all alleles equally. Alternatively, each isozyme may be scored as homozygote or heterozygote for each allele, thus producing more genetically-useful information. This alternative will be considered when the numbers of alleles per locus have been determined. Principal component analysis of the six enzyme systems used in these experiments were able to cluster the four *Lactuca spp.* into related groups with a degree of accuracy. A regression of aphid numbers on principal components 3 and 4 was also able to predict some markers for lettuce root aphid resistance among the six enzyme systems investigated. Although most commercial lettuce cultivars are susceptible to lettuce root aphid colonization these studies suggest that resistance to lettuce root aphid is readily available in a number of primative *L. sativa* and closely related *L. serriola* species. Further investigation of additional enzymes should increase the accuracy of species segregation and possibly provide futher markers for resistance to lettuce root aphid colonisation.

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HYDROXAMIC ACIDS - POTENTIAL RESISTANCE FACTORS IN WHEAT AGAINST THE CEREAL APHIDS *SITOBION AVENAE* AND *RHOPALOSIPHUM PADI*

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ABSTRACT

A significant negative correlation had previously been found between the intrinsic rate of increase (r_m) of *Sitobion avenae* and concentrations of hydroxamic acid ([Hx]) of 20 lines of tetraploid and hexaploid *Triticum*. The effects of aphid feeding, artificial damage and of varying environmental conditions on subsequent [Hx] and mean relative growth rates (RGR) of aphids were examined in an attempt to explain the residual variation in the above relationship. Re-assessment of six cultivars under carefully controlled conditions showed a strong correlation between *S. avenae* mean RGR and [Hx] in the youngest leaf (leaf one) of seedlings. A significant correlation was also found when cultivars were assessed as mature plants.

INTRODUCTION

The progress of plant breeders in their search for host-plant resistance in wheat to aphids is restrained by a number of factors. These include the absence of a reliable, rapid and convenient assay for resistance and the lack of information on the mechanisms of resistance when it is found. Possibly as a result, aphid resistance has not been deliberately bred into any UK wheat variety. This is despite the substantial contribution which partial resistance could make in the control of aphids (Acreman, 1984).

DIMBOA (2,4-dihydroxy-7-methoxy-1,4-benzoxazin-3-one) is the most abundant compound in a group of cyclic hydroxamic acids (Hx) which have been isolated in wheat, maize and several wild Gramineae. These compounds are found in the plant as glucosides which are enzymically hydrolysed to the corresponding aglucones when the plant tissue is injured (Hofman & Hofmanova, 1969). Hydroxamic acids have been shown to be involved in the resistance of cereals against bacteria, fungi and several insects including aphids (Niemeyer, 1988). Bohidar *et al.*(1986) showed that 96% of the variation in the resistance of seedlings of six wheat cultivars to *S. avenae* was explained by [Hx]. Thackray *et al.* (1990) found Hx explained 35% of the resistance to *S. avenae* (measured as r_m) when the genetic range was increased to 20 seedling tetraploid and hexaploid wheats. Leszczynski *et al.* (1989) have recently shown strong correlations between r_m of *S. avenae* and [Hx] in flag leaves of *Triticum* during anthesis (GS 60 - 69).

The objectives of the present investigation were to attempt to explain the residual variation in the relationship between resistance to *S. avenae* and [Hx] found in the previous study of a wide genetic range of *Triticum* material (Thackray *et al.*, 1990). The effects of artificial damage and aphid feeding and of manipulating water and nutrient availability on [Hx] were studied and a small group of *Triticum* taxa were re-assessed under very carefully controlled conditions using a method based on a shorter period of assessment of aphid performance (mean relative growth rate) than that needed for r_m. This assessment examined both seedling and mature plants.

MATERIALS AND METHODS

Plant and insect material

Triticum aestivum cv. Avalon was used in experiments examining the effects of aphid infestations, of artificial damage and of nutrient availability on [Hx]. This is a modern cultivar grown commercially in the UK, with relatively high [Hx] in the seedling and available in large quantities of seed material. Six cultivars were selected for further assessment of the relationship between aphid performance and [Hx]. These were *T. aestivum* cvs. Avalon, Armada, Apostle, Naofen and Likay and *Triticum durum* cv. SNA3. As seedlings, these cultivars had previously been shown to represent Hx concentrations ranging from 3.52 to 15.41 m mole/kg dry weight (Thackray *et al.*, 1990). Seeds were sown in John Innes No. 2 compost, given a drench of the fungicide Milstem (ethirimol) to discourage mildew and kept in a culture box as described in Bohidar *et al.* (1986).

The method of Hx extraction followed that of Bohidar *et al.* (1986) and was based on the colorimetric absorption of a hydroxamic acid-ferric chloride complex. This procedure does not differentiate between the different hydroxamates present in extracts and an appraisal of the technique may be found in Thackray *et al.* (1990).

Stock cultures of *S. avenae* and *Rhopalosiphum padi* were clonal, originating from single parthenogenetic females and were maintained on barley (cv. Golden Promise), which is Hx lacking, in the culture room as above. All experiments were conducted in the culture room, unless otherwise indicated, at 20 ℃ with a 2 ℃ range, 60-70% relative humidity and a 16 hour photoperiod.

Effects of aphid infestation and of artificial damage on [Hx]

9-day-old plants were infested with about 50 3rd-instar *S. avenae* per seedling and measurements of [Hx] made after 24, 48 and 96 hours of infestation. P.V.C. cylinders with terylene mesh tops were placed over seedlings to restrict aphid movement. They were also placed over control plants which were kept free of aphids. Plants were grown in groups of seven to a pot with ten replicates per treatment. All aphids were removed by gentle brushing before plants were dissected into leaf one and leaf two (leaving leaf sheaths intact with each leaf) and weighed into four 1g samples for each treatment which were stored at -20 ℃ for later anlysis.

80 grit carborundum powder was gently rubbed over a 4cm-long area of the lower surface of leaf one of ten-day-old plants. Control plants received no damage. Plants were grown in groups of seven to a pot with twenty replicates per treatment. Plant material was harvested 48 and 96 hours after damage and four 1g pooled samples were taken from each of leaf one, leaf two and leafsheaths for storage.

Effects of manipulating nutrient availability on [Hx] and mean RGR

Plastic flower pots containing John Innes No. 2 and planted with groups of 10 seedlings for Hx analysis or with single seedlings for mean RGR study, were placed in individual Petri dishes to avoid cross contamination of water sources and then put on trays in the culturing facilities previously mentioned. Treatments were arranged alternately:
Control - Plants watered as usual with no added fertiliser.
Fertilised - Seeds watered upon sowing with 25 ml/seedling of a solution of I.C.I. Liquid Garden Plus (14 ml/4.5 l water; contains 7% nitrogen, 6% soluble phosphoric acid, 5% potash), and watered with the same solution (25 ml/seedling) when five days old.

When the plants were 6 days old (GS 11) 3rd-instar nymphs of approximately the same weight (180 - 230 g) were removed from the stock culture of *S. avenae*, weighed on a micro balance and placed individually on the centre leaf section of leaf one of each of 20 replicate plants for each treatment. A P.V.C. tube placed over each plant prevented movement onto neighbouring plants. Clip cages were not used since these might cause damage to the plant and thus interfere with the results.

Aphids were re-weighed after two days and the mean relative growth rate (RGR) calculated as:

$$\frac{\log_e \text{final weight} - \log_e \text{initial weight}}{\text{no. of days over which weight increase measured}} + 0.5$$

Occasionally an aphid showed a decrease in weight over the measurement period and the weighting of 0.5 was used in all calculations to avoid negative values which complicate statistical analyses.

Relationship between *S. avenae* mean RGR and [Hx] for six selected cultivars examined at GS 11 and GS 47

Seedlings

Seedlings were harvested for Hx analysis seven days after germination, at growth stage 11/12, dissected into leaf one and two and weighed into 1g samples for storage at -20 °C. Mean RGR was measured over 72 hours, 2nd-instar aphids having been placed on 7-day-old plants (GS 11) and confined with P.V.C. tubes. Most aphids remained on leaf one, where they had been placed, throughout the experiment. Mean RGR for both *S. avenae* and the bird cherry oat aphid *R. padi* were measured, with 25 replicates each. All pots were watered with Hewitt's Long Ashton Nutrient Solution every two days to ensure no depletion in essential nutrients.

Mature plants

Previously vernalised seeds of all cultivars were sown and maintained in a glasshouse at a temperature of 20 °C with a 5 °C range and a minimum 16 hour photoperiod. All pots were regularly watered with Hewitt's solution until the plants were at growth stage 21, sprayed with the fungicide Tilt Turbo (propiconazole + tridemorph) and transferred to a field site at Chilworth, Hampshire. 32 plants of each cultivar were placed in each of six 2m x 2m x 2m field cages which were covered in Tygan (1mm mesh) to prevent natural infestations of aphids and also as a barrier against rabbit grazing. The plants were watered daily until roots were well established and then twice a week during the following dry period. Flag leaves were analysed for [Hx] at GS 47 and mean RGR (with 24 replicates) of *S. avenae* measured over 72 hours as before. Individual aphids were confined on the flag leaf with clip cages.

RESULTS

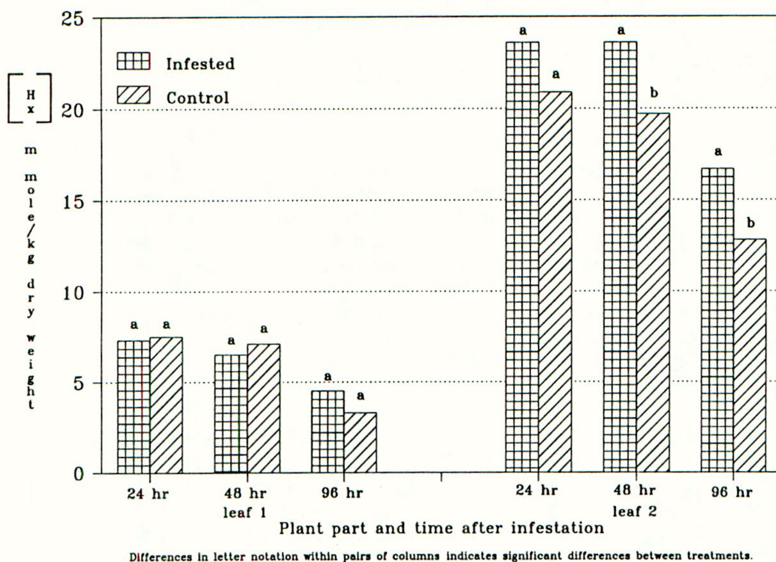
Figure 1 shows the effects of aphid infestation on the Hx concentrations of different leaves of the plant. Over the 96-hour period of infestation, Hx concentrations in the leaves of all seedlings declined but were always highest in leaf two (the youngest leaf) of the infested plants. There were no significant differences in Hx concentrations between treatments for leaf one, but Hx concentrations were significantly higher in leaf two of plants subjected to 48 and 96 hours of infestation. Differences in the letter notation within pairs of columns indicates significant differences in [Hx] between control and infested plants.

Carborundum damage also affected [Hx]. At 48 hours after damage the Hx levels in sheaths, leaf one and leaf two of damaged plants were

significantly higher than in those of undamaged plants ($F = 25.56$; d.f. = 1,36; $P < 0.001$; $F = 20.77$; $P < 0.001$; $F = 211.6$; $P < 0.001$; respectively). At 96 hours there were no significant differences in Hx concentrations between treatments.

The investigation into the effects of nutrient availability on [Hx] showed no significant difference between treatments for total plant [Hx] in 6-day-old plants but the mean [Hx] in 8-day-old plants was slightly higher in fertilised plants than in control plants ($F = 24.32$; d.f. = 1,12; $P < 0.005$). The mean RGR for aphids on fertilised plants was significantly lower than on control plants ($F = 9.8$; d.f. = 1,31; $P < 0.05$).

FIGURE ONE The [Hx] of aphid infested and un-infested seedlings of Avalon after 24, 48 and 96 hours of infestation



Differences in letter notation within pairs of columns indicates significant differences between treatments.

Values for mean relative growth rate (RGR) of *S. avenae* and *R. padi*, and [Hx] obtained in the screening of plant material at GS 11 and GS 47 are given in Table 1.

TABLE 1 Mean RGR of 2nd instar aphids and [Hx] for six Chilean and UK cultivars at GS 11 and GS 47.

Cultivar	[Hx] m mole/kg dry wt			Mean RGR		
	Leaf 1	Leaf 2	Flag leaf	*S. avenae* GS 11	*S. avenae* GS 47	*R. padi* GS 11
SNA3	25.07	39.51	4.77	0.74	0.732	0.89
Avalon	10.01	31.70	1.51	0.79	0.792	0.91
Naofen	7.21	21.14	2.79	0.78	0.752	0.86
Apostle	7.13	18.40	1.61	0.79	0.849	0.88
Likay	6.92	17.79	2.78	0.82	0.766	0.92
Armada	4.16	11.06	1.82	0.89	0.786	0.92

The relationship between mean RGR of *S. avenae* and [Hx] in leaf two of seedlings of the six cultivars studied is shown in Figure 2. The strongest correlation (69%) was found between mean RGR for *S. avenae* and [Hx] in leaf two ($r = -0.83$; $P < 0.05$); 55% of the variation between mean RGR for *S. avenae* was explained by Hx concentrations in leaf one but this relationship was not significant ($r = -0.74$). The correlation between mean RGR of *R. padi* and [Hx] in either leaf was not significant (leaf one, $r = -0.17$; leaf two, $r = -0.20$). The variation in mean RGR was very small for *R. padi* within the six cultivars studied.

A significant relationship was also shown between mean RGR of *S. avenae* and [Hx] of the flag leaf of six cultivars at GS 47. 63% of the variation in mean RGR between cultivars was explained by [Hx] ($r = -0.80$; $P < 0.05$). The relationship between mean RGR of *S. avenae* on flag leaves and the [Hx] of leaf one of seedlings at GS 11 of the same cultivar was not significant ($r = -0.54$; $r^2 = 30\%$).

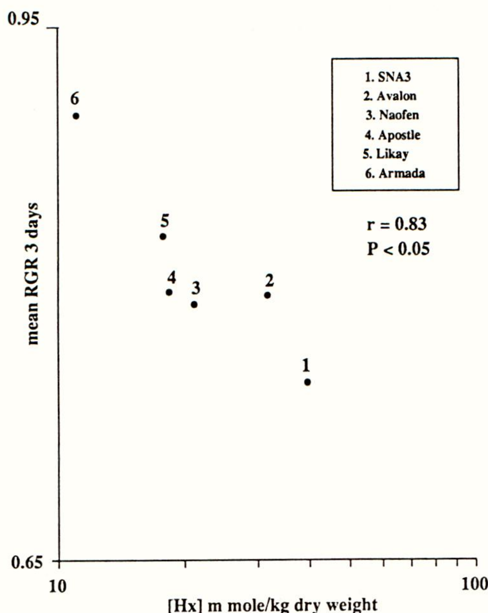


FIGURE TWO

The relationship between mean RGR of *Sitobion avenae* and the [Hx] in leaf two of seedlings of six cultivars at growth stage 11.

DISCUSSION

The aims of this study were to take an applied approach in attempting to elucidate and validate the possible role of hydroxamic acids as resistance factors in wheat and to establish the viability of using [Hx] as an indicator of resistance in wheat to aphids. The experimental work reported here along with similar studies conducted at Southampton have demonstrated the existence of an induced response to aphid feeding and to artificial damage although changes in [Hx] were not very large and appeared short-lived. Changes in external environmental factors such as water and nutrient availability have been shown to affect [Hx] and aphid mean RGR and thereby emphasise the need for carefully controlled and constant working conditions. Hx concentrations appear to be readily affected by many factors, in particular the age and growth stage of plant material and concentrations within certain tissues may be constantly changing, possibly influenced by translocation of the glucoside within the phloem, though there is as yet no proof for this hypothesis. A greater knowledge of the behaviour of Hx in localised areas of the plant within the different tissues would be a considerable aid to interpretation of effects on aphids.

The conclusions from this study and those of others working on resistance would appear to encourage further assessments of the potential of these compounds, particularly DIMBOA, as resistance factors in modern wheat to aphids. The use of high performance liquid chromatography (HPLC) would greatly facilitate further study by offering a technique which requires only small amounts of material and is specific for individual hydroxamic acids. The significant relationships found between the performance of *S. avenae* (measured as r_m or mean RGR) and [Hx] of seedling plants should encourage the continued screening of seedling and mature plant taxa for suitable germplasm with high levels of DIMBOA in particular, for use in wheat breeding programmes. Whilst a significant relationship was not demonstrated between *R. padi* mean RGR and seedling [Hx] this may be attributable to the feeding sites of this aphid which are confined mainly to the leaf sheaths. Total leaf analysis may have masked the [Hx] which these aphids were actually encountering. Recent work by Wratten *et al.* (1990) using HPLC has indicated a strong negative correlation ($r^2 = 0.75$) between the mean RGR of *S. avenae* and [Hx] of a selection of cultivars previously examined by Lowe (eg. Lowe, 1981) for mature plant resistance. Furthermore, a strong negative relationship ($r^2 = 0.80$) between mean RGR of *R. padi* and [Hx] of leaf sheaths of a number of *Triticum* seedlings has also been demonstrated (Wratten *et al.*, 1990). Given these encouraging developments, the use of HPLC by plant breeders to assess for [Hx] as a measure of resistance in new lines looks a promising technique for the future.

ACKNOWLEDGEMENTS

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MYCOSTOP - A NOVEL BIOFUNGICIDE BASED ON *STREPTOMYCES GRISEOVIRIDIS*

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ABSTRACT

Mycostop is a biofungicide based on a selected *Streptomyces griseoviridis* isolate from Finnish *Sphagnum* peat. Introduced into the soil by seed dressing or soil treatment, the organism secretes antibiotic substances which inhibit seed- and soil-borne fungal pathogens. Efficacy has been shown against *Alternaria brassicicola* and other seed-borne pathogens. In glasshouse tests and controlled temperature cabinet studies *S. griseoviridis* controlled *A. brassicicola* on cauliflower and cabbage. Results which have been reported elsewhere are summarised. These include work on the control of *Fusarium* spp. on wheat, of *Fusarium oxysporum* f. sp. *dianthi* on carnation, and *Botrytis cinerea* on lettuce.

INTRODUCTION

Isolates of *Streptomyces* spp. have been assessed in Finland for 10 years for their ability to control fungal pathogens. Many isolates from light-coloured *Sphagnum* peat suppress seed- and soil-borne pathogens. A selected *Streptomyces griseoviridis* isolate has been developed as a biofungicide by Kemira OY and tested on a wide range of organisms on glasshouse and field crops (Lahdenpera, 1987; Tahvonen, 1988; Tahvonen & Avikainen, 1987; Tahvonen & Lahdenpera, 1988). The present paper describes work from Finland and the UK on the control of *Alternaria brassicicola* on brassicas, it also summarises some results with other crop/pathogen combinations.

MATERIALS AND METHODS

The commercial formulation from Kemira OY based on *S. griseoviridis* is produced by fermentation and freeze drying of the organism and contains 10^8-10^9 colony forming units/g of product.

Finnish experiment

Cauliflower (*Brassica oleracea* var. *botrytis*) cv. Menovi SV seed was artificially infected with *A. brassicicola*. The seed was first surface sterilised with 0.5% sodium hypochlorite for 3 min and then rinsed three times in sterile water. A 2 week old culture of the fungus was suspended in 100 ml distilled water, the seeds were immersed in the suspension for

1.5 min before drying between sheets of filter paper. Seed was treated with the *S. griseoviridis* preparation at 2, 5 or 8 g/kg by shaking in a glass jar. Untreated and treated seeds were sown in steam-sterilised *Sphagnum* peat with basic fertiliser of 10 g dolomite limestone/l and 1.5 g Peat Y nutrient/l; there being three replicates of 40 seeds of each treatment. The experiments were done in a glasshouse with temperatures of 18-24°C. Emerged seedlings were counted 7, 10 and 28 days after sowing.

UK experiment

Cabbage (*Brassica oleracea* var. *capitata*) cv. Celtic seed was assessed for seed-borne infection with *A. brassicicola* by plating 200 seeds, either non-surface sterilised or surface-sterilised (30 seconds in 25% Chloros) on prune lactose yeast agar containing streptomycin and erythromycin (Maude & Humpherson-Jones, 1980). Seed was treated with *S. griseoviridis* preparation at 5 g/kg as described above, a sample was also treated with iprodione (Rovral, 50% a.i. w.p.; Rhone-Poulenc) at 2.5 g a.i./kg. Seeds were sown in sterile soil which was subsequently incubated at 10, 15, 20 or 25°C, there were 4 replicates of 50 seeds sown/treatment. Emerged seedlings were counted at 4, 5, 6, 7, 14 and 21 days after sowing. At the final count seedlings were scored as disease-free, infected (evidence of damage by *A. brassicicola*) or abnormal. Emergence data was analysed by Genstat 5 programs which determined differences in percentage emergence, time to 50% of final emergence, and differences in the percentage of disease-free seedlings at the final assessment. Seedlings classed as infected were plated out on prune lactose yeast agar as described above, and the plates were incubated at 20°C for 7 days before examination for the presence of *A. brassicicola*.

RESULTS

Finnish experiment

The effects of the *S. griseoviridis* preparation on damping-off of cauliflower cv. Menovi SV are shown in Table 1. For most treatment rate/fermentation batch combinations, the *S. griseoviridis* preparation gave emergence equal to, or higher than that in the uninoculated control. With one fermentation batch at the lower rates of treatment the degree of control was lower. In other similar experiments reported elsewhere it appeared that the 2 g/kg rate might be marginal, particularly if seeds were sown some time after treatment.

TABLE 1. The effect of *Streptomyces griseoviridis* preparation on damping-off of cauliflower caused by *Alternaria brassicicola*

Percentage of seedlings emerged*

Healthy control	Infected control	Seed treatment rates g/kg 2	5	8
91.7	38.3	94.1	93.3	92.2
91.7	45.8	83.0	88.5	87.2
89.2	43.3	69.2	77.1	83.4
94.2	69.2	94.2	96.3	94.6

*Data summarise four experiments where different fermentation batches of *S. griseoviridis* were tested, the different rates being tested with 12 separate batches.

UK experiment

Contamination of the cabbage Celtic was shown to be 69.0% of seeds having detectable *A. brassicicola*, with internal infection with the same organism at 8.5%. No other fungi or bacteria were detected. The results obtained in the experiment with cabbage are shown in Figs. 1a and b. Emergence from seed treated with *S. griseoviridis* preparation or iprodione was generally higher than that from untreated seed, the differences being significant (P = 0.05) at 10 and 20°C. Neither the *S. griseoviridis* preparation nor iprodione significantly affected time to 50% of final emergence. At all temperatures both iprodione and *S. griseoviridis* preparation significantly (P = 0.05) increased the percentage of seedlings which were disease-free at the final assessment. *S. griseoviridis* was significantly (P = 0.05) less effective than iprodione at 10 and 15°C. In the untreated controls, *c.* 9.0% of seedlings were dead at the final assessment.

OTHER CROP/DISEASE COMBINATIONS (KEMIRA OY WORK)

Wheat/*Fusarium* spp.

Both artificial and natural infection with *Fusarium* spp. were used in wheat trials in Finland and Hungary (Lahdenpera, Simon & Uoti, 1990). The main application of *S. griseoviridis* preparation was as a seed treatment at 1 or 2 g/kg. Seedling blight was controlled and yield significantly increased. Treatment at flowering with *S. griseoviridis* preparation as a spray at 1-2 kg/ha gave good control of internal infection of wheat grains by *Fusarium* spp.

Carnation/*F. oxysporum* f. sp. *dianthi*

In carnation pot experiments the substrate was artificially inoculated with *F. oxysporum* f. sp. *dianthi* before planting. In large scale trials in commercial glasshouses the disease occurred naturally. Treatments were by

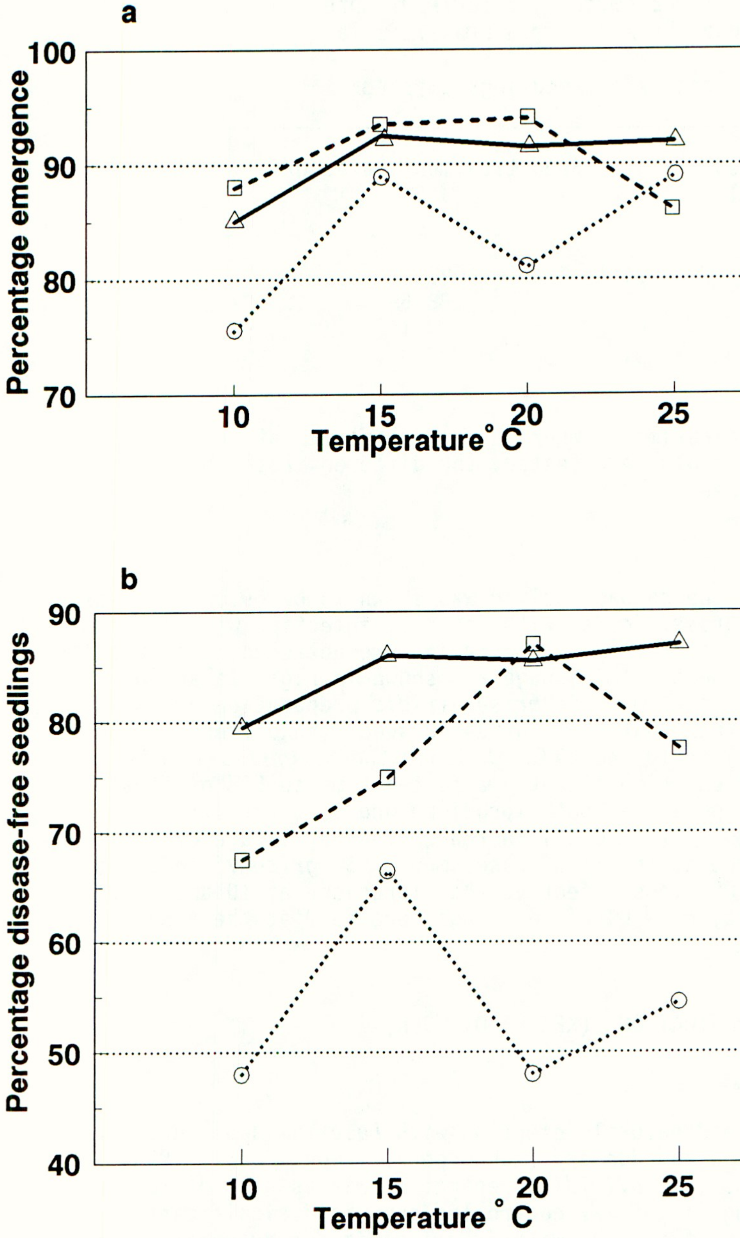

Fig 1. The effect of Streptomyces griseoviridis preparation on :-

(a) percentage emergence of cabbage cv. Celtic

(b) percentage of disease-free seedlings of cabbage cv. Celtic

○······○ Untreated seed

□------□ Seed treated with S. griseoviridis preparation

△——△ Seed treated with iprodione

dipping the roots of cuttings and/or by spraying the substrate with a 0.001-0.1% suspension of *S. griseoviridis* preparation. The surface of the peat was sprayed with the suspension at 10 l/100 m^2. The first application was immediately after planting and the treatment was repeated at 1-3 month intervals during the growing season depending on the severity of the disease (Lahdenpera, 1987).

In several commercial trials *S. griseoviridis* preparation gave consistent protection against carnation wilt caused by *F. oxysporum* f. sp. *dianthi*. The most effective treatment was a combination of root dipping and repeated soil spraying with a 0.01% suspension (Lahdenpera, Simon & Uoti, 1990) .

Lettuce/*Botrytis cinerea*

In experiments with *B. cinerea* on lettuce it was found that spraying seedlings with a 0.01% suspension of *S. griseoviridis* preparation before planting, with a further application at that concentration of 100 ml/m^2 reduced disease incidence and significantly increased yield (Tahvonen & Lahdenpera, 1988).

DISCUSSION

Streptomyces griseoviridis preparation gave good control of damping-off of cauliflower caused by artificial inoculum of *A. brassicicola*, and was effective in controlling natural infection on cabbage. In the latter case there were indications of reduced efficacy at 10 and 15°C. However, results were generally consistent with those of Tahvonen & Avikainen (1987) who worked with a range of brassica crops. In the UK experiment it was found that the *S. griseoviridis* preparation reduced the number of seedlings having viable *A. brassicicola* lesions, indicating a degree of control of transmitted disease in tissue of the seedlings, but in the Copenhagen Tank test and the soil tests there were indications that internal infection of seeds was not controlled.

The *S. griseoviridis* preparation can provide an alternative to chemical fungicides. Registration has been completed to allow sale of the material for use on carnation in Finland. Environmental toxicological analyses are in progress to facilitate use on edible crops. In 1991 registration is expected in Bulgaria, Hungary, Switzerland and the USA.

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BIOLOGICAL CONTROL OF SOIL-BORNE PLANT PATHOGENS

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ABSTRACT

The selection of biocontrol agents (BCAs) for the control of soil-borne plant pathogens was enhanced by using *in vivo* screens which incorporated realistic environmental parameters.

INTRODUCTION

The biological control of plant pathogens is not yet a commercial reality (Powell *et al.*, 1990). The major problem facing the commercialisation of biocontrol agents is unreliable activity (Jutsum, 1988). This is frequently seen when a BCA has demonstrated biocontrol activity in growthroom screens but not in the field, or when activity is demonstrated at one field site but not at a second, or when disease control is lost in a subsequent season. If biocontrol is to succeed commercially then it must be reliable, safe and cost-effective.

Changes or fluctuations of environmental parameters are frequently cited as major limitations to the reproducibility of biocontrol activity. However, rarely do we see selection programmes which have included a range of environmental parameters in their screens. More often the initial screens are to select microbes which inhibit the pathogen *in vitro*, usually in agar plates. The relevance of such screens has been questioned (Merriman & Russell, 1990). Such *in vitro* screens are capable of a high throughput of potential BCAs, but how much do the conditions in these tests reflect the environment into which the microbes will be finally introduced? The production of secondary metabolites or fungal cell-wall degrading enzymes may be important modes of action to a BCA once it has reached the site of action, but the successful establishment of a competitive BCA requires a range of other, less easily defined microbial qualities.

In practice the lead BCAs will be formulated, possibly as a dry powder or a slurry which will then be applied to seeds. The microbes will have to

go through a process of de-hydration to be formulated, packaged, transported and stored. When used they will be applied to seeds or incorporated into the soil; they must then re-hydrate, be released from the formulation matrix, multiply and compete for the available nutrients, reach the site of action on the roots, seed and/or soil and then antagonise the pathogen. All of these processes will be sensitive to environmental limitations, mainly the availability of water and nutrients. It is therefore of the upmost importance that microorganisms selected for disease control are capable of actively growing and competing for nutrients in the presence of an indigenous soil microflora under a range of agronomically realistic environmental conditions.

The aim of this research report is to illustrate the isolation and screening procedures developed by ICI Agrochemicals and ICI Seeds for the selection of BCAs to control soil-borne plant pathogens, taking into account the environmental criteria highlighted above.

STRATEGY FOR THE SELECTION OF BCAs

Figure 1 illustrates a typical screening cascade for the selection of BCAs for the control of plant pathogens. The cascade is analogous to the selection of pesticides. As with pesticides the costs of research and development increases as the cascade descends. However, BCA technology is still in its infancy. At present all lead BCAs require individual optimisation for fermentation and formulation; this requires dedicated research effort. It is therefore very important that the selection procedure during the initial and less expensive stages of the programme does select the best potential BCAs prior to formulation optimisation and field testing.

Isolation of microbial antagonists

The aim of the isolation procedure was to obtain bacteria and fungi which have the potential to outcompete selected soil-borne plant pathogens in a range of field soils. This approach required a good knowledge of the etiology of each pathogen to enable the establishment of model systems (microcosms), which incorporated sensitive crop host and appropriate environmental conditions for disease development. For example, selection of potential BCAs for the control of *Pythium* spp. would be by isolation from seeds and root tips of susceptible crops grown under warm/wet and cold/wet conditions. In practice over 7000 microbes were isolated from the roots and seeds of crops grown in over 100 soils obtained from the USA and Europe.

Screening for microbial antagonism

The overall objective of this screening programme was to ensure the detection of 'robust' microbial antagonists which would reproducibly control selected soil-borne diseases under a range of field conditions. The first aim was to design and establish a series of *in vivo* tests where several thousand potential BCAs could be screened quickly (Table 1). A range of plant pathogens and susceptible crops were selected. All screens were run under environmental conditions conducive to disease development. For example the *Fusarium culmorum*/wheat screen was run under warm/dry conditions, while the *Pythium ultimum*/pea screen was run under cold/wet

FIGURE 1. Screening cascade for the selection of a BCA.

Fusarium

Rhizoctonia

Pythium

Target Pathogen

↓

Isolation

↓ crops/soils/environmental factors

Screening
(growthroom)

lead characterisation
fermentation
formulation
mode of action

↓

Selection for field

↓

Screening

Patenting

large scale bulking up

↓

crops
environmental conditions
cultural/agronomic conditions

Development

conditions. The screens were set up in a series of commercial composts and/or field soils. The field soils allowed the test microorganisms, applied to both seeds and soil, to be evaluated for competitive ability versus an indigenous microflora.

TABLE 1. Screens used for the selection of microbial antagonists for the control of soil-borne plant pathogens.

Crop	Pathogen	Temp (°C)	Moisture	Soil
1. Sugar beet	complex	12 - 15	Wet	F
2. Sugar beet	*Aphanomyces cochlioides*	12 - 15	Wet	F
3. Peas	*Pythium ultimum*	12 - 15	Wet	C/F
4. Peas	*Pythium ultimum*	24 - 27	Wet	C/F
5. Peas	*Rhizoctonia solani*	24 - 27	Wet/Dry	C/F
6. Wheat	*Fusarium culmorum*	18 - 21	Dry	C/F
7. Soyabean	*Phytophthora megasperma*	20 - 24	Wet	F
8. Soil	*Pythium* spp.	10 - 12	Wet	C
9. Potato slice	*F. moniliforme*	20 - 24	NA	NA
10. Potato slice	*F. sambusinum*	20 - 24	NA	NA
11. Sugar beet	*Phoma* spp.	15 - 19	NA	NA

C = compost
F = field soil
NA = not applicable

The biocontrol activity of the 7000 microbes was determined for each screen. To obtain an overall score for activity across the range of screens, the activities of individual strains were compared to that of the standard fungicide control.

The activity scale used for this evaluation was:

0	< 59% control	cf standard chemical
1	60 - 79% control	cf standard chemical
2	80 -100% control	cf standard chemical
3	>100% control	cf standard chemical

The results for all the potential BCAs were tabulated and the scores were added for the eleven screens, the maximum score possible was 33 (11 x 3). Table 2 shows the results of five isolates, two microbes (1 and 2) of low activity against the range of pathogens and three more active microbes (3, 4 and 5). Those microorganisms having the maximum scores from all of the screens were classified as the lead BCAs and were taken forward for further evaluation. Some of the more active BCAs could match and even better the control of pathogens given by the chemical standards.

TABLE 2. Activity of five potential BCAs in the eleven screens described in TABLE 1. (The scale 0 - 3 is described in the text).

Screens	1	2	3	4	5	6	7	8	9	10	11	T
Microbes												
1	0	0	0	0	0	0	0	1	0	1	0	2
2	0	0	0	0	0	0	0	0	1	1	0	2
3.	3	2	0	2	0	2	1	3	1	0	1	15
4.	3	2	3	3	0	2	1	2	1	0	2	19
5.	2	0	3	2	3	3	0	3	0	0	0	16

T = Totals

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