IOBC / WPRS

Working Group "Breeding for Resistance to Insects and Mites"

OILB / SROP

Groupe de Travail "Amélioration des Plantes pour la Résistance contre les Insectes et les Acariens"

In association with EUCARPIA

PROCEEDINGS OF A MEETING

at

Arnhem, The Netherlands
17 - 21 September 1995

Edited by
P.R. Ellis & J. Freuler

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INTRODUCTION

Seventh Triennial Meeting

The seventh triennial meeting of the Working Group was organised by Chris Mollema from the Centre for Plant Breeding and Reproduction Research (CPRO-DLO) and held from 17 to 20 September 1995 at the Conference Centre "Alteveer", Arnhem, The Netherlands. The meeting was opened by Dr Orlando de Ponti of Nunhems Seeds. Dr de Ponti welcomed the participants to The Netherlands and hoped that they would have an interesting and productive conference. He stressed the importance of host plant resistance as an important basis for integrated pest control programmes, particularly in these times of necessity to develop environmentally-acceptable methods of control. He was pleased to see so many countries represented at the meeting and believed it indicated great support for the IOBC.

The scientific programme was followed by an evening excursion by boat along the River Rhine. An excursion took place on Wednesday 20 September 1995 to The Centre for Biological Agriculture at Lelystad followed by a visit to the Planetarium of Eise Eisinga at Franeker.

The meeting was largely sponsored by the IOBC with contributions from Eucarpia (the European Association for Research on Plant Breeding) and Dutch seed companies. Grants were made to participants from eastern Europe who were facing financial difficulties attending the conference.

A total of 55 participants from 9 countries attended the meeting. Most of these were scientists from research institutes and universities but it was most encouraging that nine seed companies were also represented, an indication of the strong support for the Working Group's activities from industry. Twenty-five papers and a poster session were presented during the conference. Most of these papers appear in this Bulletin, the proceedings of the conference.

Further Activities of the Working Group

After the presentation of papers at the meeting, time was set aside for a discussion of the future activities of the Working Group. The main points and decisions arising from these discussions are reported below:

1. Triennial Meetings

This discussion centred around the size, content, participants and frequency of the Working Group's meetings. It was unanimously agreed that the present format of meetings held every three years involving about 45 participants from many different countries and offering two full days for scientific presentations was satisfactory and should be continued in 1998, the proposed date for the next meeting. It was agreed that papers should be presented and published before the next meeting so that maximum time should be given to discussion of research findings.

2. Venue for the Next Meeting

It was agreed that the meeting in 1998 would be held in Scotland and that the local organiser would be Nick Birch.

3. Convenor of the Working Group

Bob Ellis was prepared to continue as Convenor for the next three years. Nick Birch, from the Scottish Crop Research Institute will take up office as Convenor when Bob Ellis retires.

4. Project Groups

A number of Project Groups have existed in the past or are currently active within the Working Group. The reason for setting up Project Groups is to encourage and formalise close collaboration between scientists and/or commercial plant breeders in order that the objectives of the Working Group can be realised more effectively.

The Project Group on western flower thrips held a workshop in Arnhem, The Netherlands, 17 to 19 October 1994 attended by 10 active workers in the field of thrips. A report was submitted to the IOBC. The Project Group on Plant Surface Chemistry/Root Flies has held several meetings. The collaboration research has continued and results are presented in the Bulletin. The Project Group on EPG studies is planning to organise another workshop, this time in France.

5. Publications

a) Aphid Resistance Newsletter. Maarten van Helden agreed to continue producing this Newsletter. All the participants working on aphid/plant relationships agreed that this publication had been very valuable in their work and they hoped that it would continue being published.

b) Bulletin. Bob Ellis will edit the Bulletin and Jost Freuler kindly agreed to write French summaries for each paper.

Finally, we should like to record our sincere thanks to Ruth Gez for all the help in preparing the manuscripts.

P.R. Ellis, February 1996

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Wild brassica species as sources of resistance to *Brevicoryne brassicae* and *Aleyrodes proletella*

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Summary

Thirty accessions of wild *Brassica* species were obtained from gene banks and multiplied to provide seed for use in experiments designed to study resistance to *Brevicoryne brassicae* and *Aleyrodes proletella.* Various aspects of antixenosis, antibiosis and tolerance were studied in laboratory and field experiments in 1992 and 1993. Several species were preferred less for colonisation by both insects indicating the presence of antixenosis. Certain of these species were shown to possess antibiosis resistance which was expressed as reduced survival and reduced progeny production by the insects. There was a strong correlation between the levels of resistance found in the *Brassica* species to *B. brassicae* with that to *A. proletella.* Three species, *Brassica fruticulosa, B. insularis* and *B. spinescens* were shown to possess high levels of resistance to both pest species and have been chosen for a breeding programme to develop resistant brassica varieties.

Introduction

Over 250,000 ha of horticultural brassicas are grown within the European Union and the approximate annual value of these crops is 1000 million ECU. Production of brassicas is dependent on regular and frequent use of agrochemicals to control pests and diseases. There are great incentives for growers to use less pesticides and one possible component of integrated pest management programmes is to use varieties of brassica resistant to pests and disease (Ellis *et al.*, 1996). This paper reports investigations of the resistance in wild *Brassica* species to the cabbage aphid, *Brevicoryne brassicae* (L.), and the cabbage whitefly, *Aleyrodes proletella (L.).*

Brevicoryne brassicae is one of the most widespread and important pests of brassicas in many parts of the world. It is a particularly severe pest of horticultural brassicas and repeated applications of insecticide are required to produce quality crops. The aphid sucks sap from plants and this feeding activity leads to discolouration and malformation of tissues. The mere presence of aphids, their cast skins or honeydew can render crops unmarketable. The insect also transmits viruses in the crop.

In contrast, *A. proletella* causes little or no direct damage to brassica crops by its feeding activity. It is a `cosmetic' pest and its colonies with the associated honeydew reduce the marketability of crops, particularly Brussels sprouts and kales.

At Horticulture Research International (HRI), and other centres, concerted efforts have been made to search for resistance to these two pests but only partial to moderate levels of

resistance have been discovered (Ellis *et al.*, 1996). It was decided to investigate a wider range of *Brassica* material as part of a search for higher levels of resistance.

Materials and Methods

Plant material

Accessions of *Brassica* species were obtained from the gene bank in Madrid, Spain, and multiplied in glasshouses at HRI Wellesbourne. Plants for experiments were raised in John Innes potting compost in black plastic pots maintained in a glasshouse at 20°C. Supplementary illumination was provided from sodium lamps during the period Novemeber to March inclusive.

Brevicoryne brassicae and *Aleyrodes proletella* cultures

The cultures of the two insect species were kept in controlled environment rooms in the Insect Rearing Unit at HRI, Wellesbourne at 20°C, 70% R.H. with 16h day: 8h night. The cultures originated from field-collected insects at Wellesbourne. The aphids and whiteflies were reared in 38 x 38 x 38 cm perspex cages on Brussels sprouts, *Brassica oleracea* var. *gemmifera*, `Oliver', a host which had been found to support large populations of both insects. Stocks of insects were sub-cultured at 14-day intervals on to fresh plants to provide large populations for experiments.

Laboratory experiments

The preferences for *A. proletella* colonisation were evaluated by exposing plants of the different *Brassica* species to whitefly adults in an experimental chamber (Ellis & Hardman, 1975). The standard susceptible Brussels sprouts cv. `Oliver' was the susceptible control in all experiments. Four lots of 250 whiteflies were released into the corners of the chamber and the number of adults, egg-laying circles and eggs were counted on each plant 72 hours later.

Antibiosis resistance to *A. proletella* was investigated by introducing 60 adult females into 38 x 38 x 38 cm cages containing test plants of *Brassica* species. After 21 days the number of pupae produced on each plant was counted and expressed as a percentage of the number produced on the standard susceptible control.

Antibiosis resistance to *B. brassicae* was determined by confining aphids in clip cages attached to two leaves on each test plant (Singh *et al.*, 1994). The development of nymphs was observed and numbers of progeny recorded. Brussels sprouts `Oliver' served as a control.

Field experiments

The *Brassica* species were evaluated in randomised block experiments at HRI Wellesbourne. Two experiments were timed to coincide with the migrations of aphids and whiteflies in the field. Sixty four plants of each accession constituted a plot replicated four times. To supplement the population of *A. proletella*, infested plants were planted down the centre of each block. This was unnecessary for *B. brassicae* as the natural field population at Wellesbourne is always very large. Every plant in the experiment was examined for the presence or absence of *B. brassicae* one week after planting. From this record it was possible to calculate % infestation, a measure of antixenosis resistance. Later in the season the number of colonies of *B. brassicae* on each plant was counted and damage was scored using a grading system (none, slight, moderate or severe). These records provided information on the antibiosis resistance of the different accessions.

Results

Resistance to *A. proletella*

There were highly significant differences between *Brassica* species in their antixenosis resistance. In the laboratory accessions of certain wild *B. oleracea* subsp. *oleracea* were infested significantly more than the susceptible control `Oliver' while three species, *B. fruticulosa. B. repanda* and *B. spinescens,* were significantly less preferred for colonisation than `Oliver'. A comparison of the mortality of *A. proletella* on the different species in the laboratory showed that there were significant differences between the accessions tested. The mortality was highest on *B. fruticulosa* and *B. spinescens* and significantly higher than on `Oliver'. Mortality occurred earlier in insect development on *B. spinescens* than on `Oliver' through the death of crawlers and second instar larvae. Mortality of *A. proletella* on *B. fruticulosa* was spread uniformly between all life stages and very few insects were present at the end of the experiments. Several other *Brassica* species supported fewer pupae than `Oliver', including *B. cretica, B. incana, B. insularis, B. rupestris* and *B. villosa* (Fig. 1).

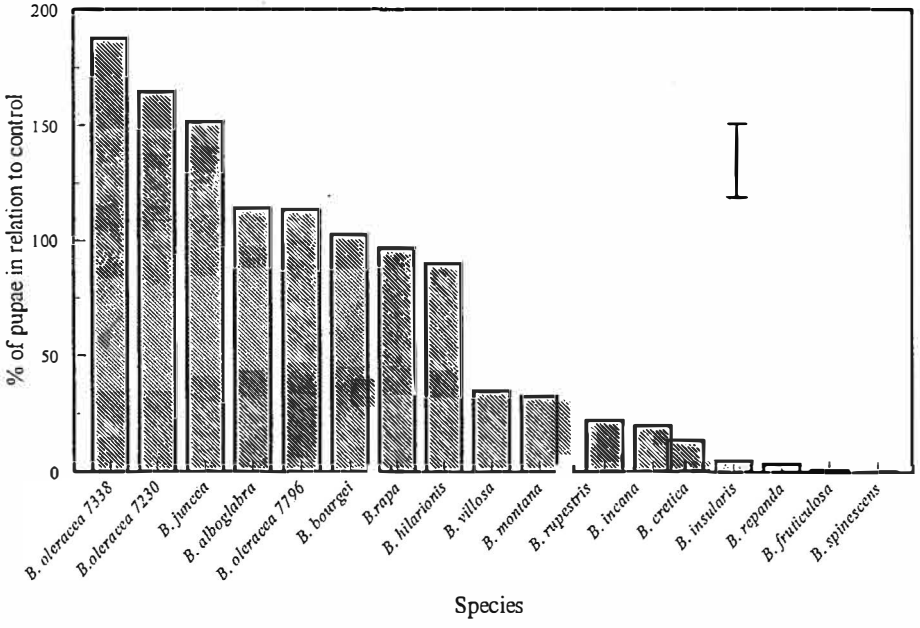

Fig. 1: Relative numbers of *Aleyrodes proletella* pupae on wild brassica species in the laboratory as a percentage of the control. Vertical bar=LSD (P=0.05).

In the field the pattern of resistance to *A. proletella* amongst the *Brassica* species tested was very similar to that observed in the laboratory experiments (Fig.2).

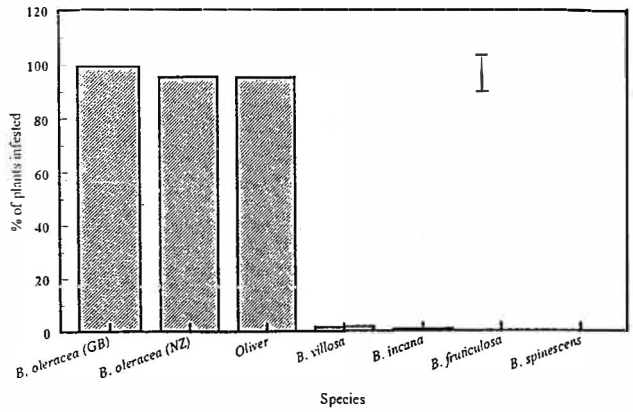

Fig. 2: Percentage of plants infested with *Aleyrodes proletella* in the field in 1992. Vertical bar=LSD (P=0.05).

Evidence of antibiosis resistance was provided from the records of insect development on plants. High levels of resistance were found in *B. fruticulosa*, *B. insularis* and *B. spinescens.*

Resistance to *B. brassicae*

There were significant differences in the development and survival of *B. brassicae* on different *Brassica* species in the laboratory based on the number of progeny produced in clip cage experiments. Significantly fewer progeny were produced and mortality was also higher on *B. fruticulosa, B. insularis, B. juncea* cv. RH 7847, *B. villosa* and *B. spinescens* than on the susceptible control (Fig.3).

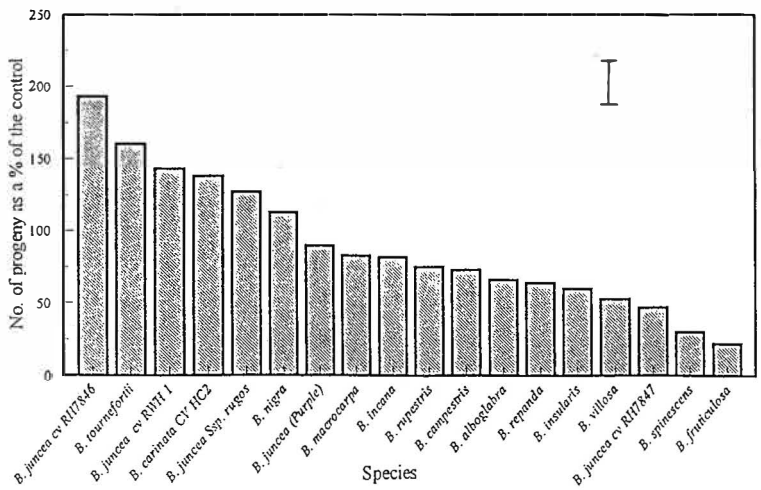

Fig. 3: Number of *Brevicoryne brassicae* progeny as a percentage of the control in the laboratory. Vertical bar=LSD (P=0.05).

In the field evidence of both antixenosis and antibiosis were demonstrated in *Brassica* species. Large numbers of aphids migrated into the plots in late June and showed a preference for the susceptible variety `Oliver'; 95% of this control variety was infested within 7 days of planting out while only 2% of *B. insularis* were infested. *B. fruticulosa, B. incana, B. macrocarpa, B. spinescens* and *B. villosa* var. *drepanensis* were also infested significantly less than the control indicating the presence of antixenosis resistance (Fig. 4).

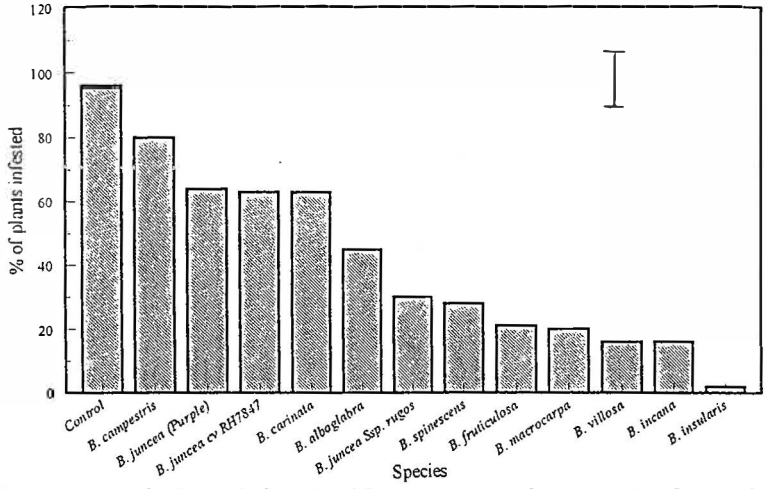

Fig. 4: Percentage of plants infested with *Brevicoryne brassicae* in the field in 1992. Vertical bar=LSD (P=0.05).

Observations were made later on in the season of development and survival of *B. brassicae* in the field plots, to determine levels of antibiosis. Four species were significantly more resistant than 'Oliver' (Fig. 5), three of which, *B. fruticulosa, B. insularis* and *B. spinescens*, had been shown to be colonised less at the time of aphid immigration at the beginning of the experiment. The fourth species, *B. juncea* cv. 7847, was preferred to the same extent as the control at the time of immigration but subsequent development and survival was much reduced.

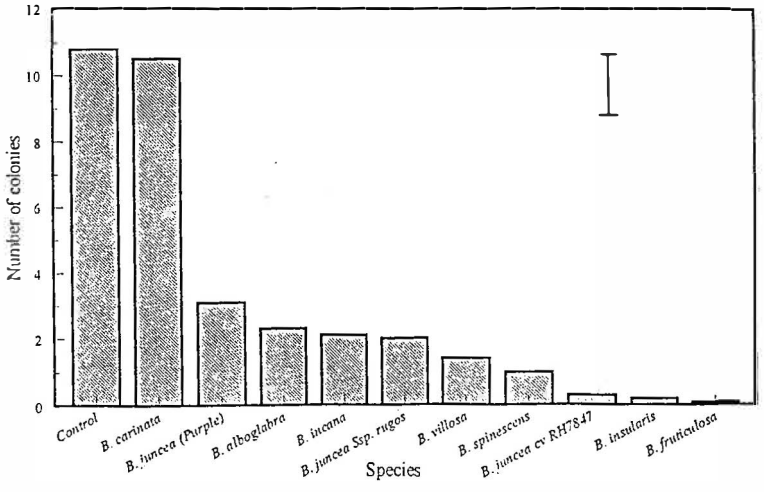

Fig. 5: Number of *Brevicoryne brassicae* colonies per plant in the field in 1992. Vertical bar=LSD (P=0.05).

Discussion

These experiments have demonstrated the existence of high levels of resistance in accessions of several *Brassica* species to the pests *A. proletella* and *B. brassicae.* The majority of earlier studies of host plant resistance to these pests has concentrated on the evaluation of cultivated *B. oleracea*, the horticultural brassicas. For example, Singh & Ellis (1993) reviewed the literature and noted that in the fifty years up to 1992, 950 genotypes of cruciferous plants had been tested against *B. brassicae* in 39 separate programmes. Very few of these studies had examined *Brassica* species. Two workers who tested wild *Brassica* species included *B. juncea* in their work and reported high levels of resistance (Hashmi, Shad & Chatha, 1985; Hussain, 1983). Our experiments confirmed high levels of resistance in this species.

Several of the species tested belong to the same cytodeme as *B. oleracea* and therefore share the same number of chromosomes (n=9). These include *B. cretica, B. montana, B. incana, B. insularis, B. rupestris* and *B. villosa* var. *drepanensis.* As these all cross with *B. oleracea* it should be relatively easy to produce F1 and F2 families and investigate the genetics of resistance. The highly resistant *B. fruticulosa* and *B. spinescens* have n=8 chromomes, and thus belong to a different cytodeme to *B. oleracea.* Transfering resistance from these wild species to horticultural brassicas is therefore likely to be more complicated.

It is important, but not essential, to understand the basis of resistance operating in these *Brassica* species. The laboratory and field experiments provided information on preferences for colonisation (antixenosis factors) and evidence of adverse effects on the subsequent development, survival and reproduction of insects (antibiosis factors). Certain species, for example *B. juncea,* were not significantly less preferred than the susceptible control variety `Oliver' but clearly possessed antibiosis resistance which prevented the development of large populations of aphids later in the season. In contrast, *B. incana* was shown to possess antixenosis early in the season and yet, later on, aphids colonies developed on the foliage and the overall level of resistance was partial not high. The accessions of other species and in particular *B. fruticulosa, B. insularis, B. repanda* and *B. spinescens* were shown to possess both antixenosis and antibiosis resistance and therefore show great promise as parents in breeding programmes designed to develop varieties of brassica crops resistant to *B. brassicae* and *A. proletella.*

Seed of certain accessions was in short supply and therefore only 20 of the accessions were tested against both pests. An examination of the results for these 20 accessions showed that there was good agreement in the various levels of resistance to both aphids and whiteflies. This is not unexpected as both insects feed in a similar manner and therefore probably have similar dietary requirements.

Résumé

Les espèces sauvages de *Brassica* en tant que sources de résistance à l'égard de *Brevicoryne brassicae* et d'*Aleyrodes proletella*

Trente espèces sauvages de *Brassica* ont été obtenues auprès de banques de gène et multipliées dans le but de produire suffisamment de semences pour en étudier la résistance à l'égard de *Brevicoryne brassicae* et *d'Aleyrodes proletella.*

Différents aspects d'antixénose, d'antibiose et de tolérance ont été étudiés en champ et en laboratoire durant 1992 et 1993.

Plusieurs espèces sont moins colonisées par les deux insectes, indiquant la présence d'antixénose. Certaines de ces espèces sont dotées d'une résistance de type antibiose, se manifestant par une survie et une descendance diminuée chez les ravageurs.

Les niveaux de résistance décelés chez les espèces de *Brassica* à l'égard de *Brevicoryne brassicae* et d'*Aleyrodes proletella* sont fortement corrélés entre eux.

Trois espèces, *Brassica fruticulosa*, *B. insularis* et *B. spinescens*, sont fortement résistantes aux deux espèces de ravageurs. Elles ont été choisies pour servir à développer des variétés résistantes de *Brassica* par un programme de sélection.

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Evaluation of cauliflower breeding lines resistant to cabbage root fly (*Delia radicum*)

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Summary

Successful selection for antixenosis resistance to cabbage root fly (*Delia radicum* L.) of individual cauliflowers based on egg counts around the stems occurred when the mean egg-laying was between 25 and 50 eggs per plant. Incorrect selection for low egg-laying could still not be excluded due to the random behaviour of the insect.

The performance of selected partially-resistant lines was assessed by:-

1. Counting the numbers of larvae and pupae in the soil around plants which had been exposed to variable, naturally-occurring pest populations. In cases, where the mean numbers of larvae and pupae per plant in the standard was below 10, a reduction of egg-laying of 42.1% - 66.7% was followed by a reduction in larvae and pupae of 25.3% - 68.4%. No definitive answer could be given as to whether resistance breaks at higher infestation levels. Selection for resistance to cabbage root fly also had an effect on bean seed fly, *D. platura* Meigen attack.
2. Offering diminishing choice situations to the cabbage root fly. The experiments showed that the partially-resistant line tested might retain its resistance in a no-choice situation.
3. Reducing the insecticide dose by 40% on the partially-resistant line (0.6n or 0.6g Dyfonate 5G (5% fonofos) per plant instead of 1g). In low pest population situations the reduction in numbers of larvae and pupae around the selected line sprayed with a reduced insecticide dose was comparable to the numbers around the standard sprayed with a normal dose.

Introduction

The investigations of antixenosis (non-preference) resistance in cauliflower (*Brassica oleracea* L. var. *botrytis*) to the cabbage root fly (*Delia radicum* L.) began in 1979 and from 1993-1995 focussed on the evaluation of partially-resistant breeding lines which had been bred following several cycles of selection.

Materials and Methods

The work has progressed through three more cycles of selection since 1992. Two odd-year cycles in 1993 and 1995 and one even-year cycle in 1994, were completed using the same methods as described by Freuler, Gagnebin & Strasser (1993).

The relative levels of antixenosis resistance of individual cauliflower plants exposed to a naturally-occuring fly population was assessed by counting eggs laid around the stem of a plant using the egg trap. Seed of selected plants was obtained during the second year of a selection cycle by an *in vitro* culture technique.

In 1993 the material tested included:-

- F5 breeding lines
- 3 F_3 lines received from M. Dickson, Cornell University, Geneva, N.Y., USA
- Goodman a commercial line
- F_1-F_4 generation plants of the selected line XIV/4/32/56/18 (from Imperator (XIV) and tested 1985-1991
- Snowball (susceptible standard)

In 1994 material tested included:-

- Breeding lines including some of which got to generation F6
- Commercial cauliflower varieties Talbot, Andes, Amazing, Goodman, Belot, Coleman, Fargot, Lateman and Silverado
- Kale cv. Fribor, which is highly resistant to turnip root fly, *Delia floralis* Fallen (Alborn *et al.*, 1985) and Imperator (standard)

In 1995 material tested included:-

- F6 generate breeding lines reached lines bred from crossing two partially resistant lines
- Commercial cauliflower varieties Belot, Asterix, Lateman, Goodman, Talbot, Amazing, Silverado, Fargo, Fremont, Lara, Nautilus, White Rock, Andes, Celesta, Coleman, Andes the Kale cv. Fribor.

New commercial cauliflower varieties were also screened for resistance to cabbage root fly by using a root damage index (RDI) recorded after harvest. The roots were graded in 4 categories:-

1. Clean (0% root cortex damaged)
2. Slight (25% root cortex damaged)
3. Moderate (50% root cortex damaged)
4. Complete (100% root cortex damaged).

The index was calculated as RDI=25b+50c+100d/a+b+c+d, where a, b, c and d were the numbers of plants in categories 1, 2, 3 and 4. To screen varieties a spring planting was carried out at the Les Fougères experimental station in the canton of Valais in 1993 and 1994 using a randomized block design with 3 replicates and sub-plots of 36 plants. In 1993 all plants and in 1994 half of the plants in a sub-plot were treated with 1g Dyfonate 5G (5% fonofos) per plant after planting.

The performance of the selected partially resistant lines with reduced egg laying was assessed by counting the numbers of larvae and pupae in the soil around the plants exposed to variable naturally-occuring pest populations in variable choice situations or treated with reduced doses of insecticide.

In all cases a standard layout with two 100 m^2 rectangular experimental plots was used, composed of approximately 280 plants and placed side by side. One plot was planted with the standard Imperator and the other with the experimental selected line. After a period which maximised cabbage root fly pupae formation but not later than 3 months after planting,

samples 20 x 20 x 20 cm were lifted from around each plant and the larvae and pupae (*D. radicum* and the bean seed fly, *D. platura* Meigen) washed out using a flotation technique. A block of 36 plants comprising 6 rows x 6 columns were sampled from each of the two experimental plots. The blocks were located mid-way along the common border of the two areas, the first column R1 (R=Reference Imperator) and S1 (S=selected line) of each block bordering on each other. Between 1992 and 1994 seven experiments were carried out at two sites exposed to a range of fly population densities.

The same experiment procedure was used to study the performance of selected lines under diminishing choice situations at two sites in 1993. At Les Fougères the mean numbers of larvae and pupae per plant were determined for each of the 6 columns of the standard or reference numbered R1-R6 and of the selected line numbered S1-S6. At Les Rives vegetable experimental farm near Changins in the canton of Vaud sampling was extended to columns R7-R13 and S7-S13.

In 1994 the influence of using a reduced dose of 0.6n or 0.6 g per plant of Dyfonate 5G (5% fonofos) on the number of larvae and pupae was studied at two sites. At Les Fougères two standard layouts were used, one for the sprayed and the other for the unsprayed plots. At Les Rives the experimental treatments were combined in one layout and each plot was split into two sub-plots. In this case 25 plants per sub-plot were sampled for extraction of larvae and pupae.

Results

The results of the selection cycles between 1993 and 1995 are shown in Tables 1-3. Varieties and lines are ranked according to the mean number of cabbage root fly eggs per plant.

In 1993 the egg-laying was low. The mean number of eggs per plant was 8.8 for Imperator and 9.9 for Snowball and varied from 5.3 for the least to 13.9 for the most infested line (or 2.6 - fold).

In 1994 the egg-laying was very low. The mean number of eggs per plant was 5.7 for Imperator and varyied from 1.5 for the least to 9.6 for the most attacked line (or 6.4 - fold). Fribor had a high level of resistance to cabbage root fly compared to the best breeding lines.

The 1995 experiment was subject to poor growing conditions. It is believed plant growth was hampered by a residual herbicide. Therefore only plants which grew to ≥1000 (diameter (cm) x height (cm)) were considered for statistical analysis. Although no significant differences were found between egg numbers in 1994 and 1995 the varieties Belot, Goodman and Talbot appeared in both years slightly better (not for Goodman in 1993 (Table 1)) and Silverado and Fargo slightly worser than the reference or standard variety. Asterix was ranked as the most resistant which is confirmed by the RDI screening (Table 4). The crossing B(♀) x C(♂) appeared to be promising but has to be tested again due to insufficient data.

Table 1. Egg counts in 1993 on cauliflower varieties and progenies selected for resistance (↙) or susceptibility (↗) to cabbage root fly during the series of odd years (Parents: X=Panda, VII=Coronado, XIV=Imperator).

No	Variety/line		Mean number of cabbage root fly eggs per plant	Significance (P=5%)
13	XIV/4/32/56/18 F4	↙	5.3	a
20	XIV/4/78/17/67/82 A	↙	6.1	a
1	VII/14/82/35/90/69	↙	7.1	ab
10	XIV/4 F1	↙	7.3	ab
6	XIV/4/32/56/18/35	↙	7.5	ab
9	XIV Reference 860554	-	8.8	abc
12	XIV/4/32/56 F3	↙	8.8	abc
21	XIV/4/78/17/67/82 B	↙	9.0	abc
4	X/11/92/71/97/64	↗	9.0	abc
2	X/11/56/82/7/6	↙	9.4	abc
17	7606-8 F3 (Dickson)	↙	9.6	abc
7	XIV/14/18/50/3/50	↗	9.8	abc
14	Snowball sensible reference	-	9.9	abc
5	XIV/4/32/30/18/92	↙	10.2	abc
19	Goodman	-	10.2	abc
3	X/11/92/71/97/58	↗	10.8	abc
11	XIV/4/32 F2	↙	11.0	abc
18	XIV/4/78/92/30/69	↙	11.9	bc
15	7606-2 F3 (Dickson)	↙	12.5	bc
16	7602-1 F3 (Dickson)	↙	13.4	c
8	XIV/14/18/50/3/92	↗	13.9	c

Table 2. Egg counts in 1994 on cauliflower varieties and progenies selected for resistance (↙) or susceptibility (↗) to cabbage root fly during the series of even years Parents: VII=Coronado, X=Panda, XIV=Imperator, XV=Elgon, XVI=Andes).

No	Variety/line		Mean number of cabbage root fly eggs per plant	Significance (P=5%)
13	X/20, 5/73/31/45/47/78	↙	1.5	a
12	X/20, 1/69/38/36/60/42	↙	1.5	a
3	Fribor 25350 2-2.25	-	1.7	a
20	XVI/47/59/63/40/52	↗	1.8	a
24	XVI/27/22/60/63/43	↙	1.9	a
A	VII/14/82/35/90/69	↙	2.1	a
B	X/11/92/71/97	↗	2.3	a
6	Talbot 25891	-	2.8	ab
22	XVI/47/59/63/40/76	↗	2.9	ab
D	XIV/4/78/17/67/82	↙	3.3	abc
21	XVI/93/39/12/4/4	↙	3.3	abc
5	Andes 3 920366	-	3.4	abc
C	XIV/14/18/50/3	↗	3.5	abc
9	Amazing 920369	-	4.3	abc
1	Goodman 852122	-	4.3	abc
17	XV/25/20/68/21/76	↙	4.4	abc
(4	Belot 27552	-	4.6)	
8	Coleman 920374	-	5.5	bc
15	XIV/66/14/4/58/99	↙	5.5	bc
25	XIV Reference 10.2022.01	-	5.7	bc
7	Fargo 30476	-	5.7	bc
2	Lateman 920367	-	6.0	c
(10	Silverado 20722	-	9.6)	

Table 3. Egg counts in 1995 on cauliflower varieties and progenies selected for resistance (↙) or susceptibility (↗) to cabbage root fly during the series of odd years (Parents:VII=Coronado, X=Panda, XIV=Imperator; B=VII/14/82/35/90, C=XIV/4/32/56/18). S=Significance (P=5%).

No	Variety/line		Mean no. of cabbage root fly eggs per plant	S	Mean plant development diam. (cm) x height (cm) ≥ 1000	No of plants development ≥ 1000
4	Belot 27620	-	13.1	a	1533	14
23	Asterix F1	-	13.8	a	1098	4
2	Lateman 35256	-	15.6	a	1210	5
3	Fribor 35850	-	16.4	a	1308	7
1	Goodman 35254	-	17.0	a	1340	9
6	Talbot 25891	-	19.8	a	1761	9
24	XIV Reference 10.2022.01	-	23.0	a	1332	3
9	Amazing 35486	-	24.2	a	1274	6
10	Silverado 20722	-	26.1	a	1827	15
7	Fargo 30476	-	27.4	a	1654	7
17	XIV/14/18/50/3/92/41	↗	28.3	a	1700	4
20	Fremont F1	-	31.4	a	1567	7
18	Lara	-	31.8	a	2080	15
19	Nautilus F1	-	40.8	a	1860	4
11	B(♀) x C(♂)	↙	0		1063	2
21	White Rock	-	7		1020	1
5	Andes-3 920366	-	7		1064	1
22	Celesta	-	54		1155	1
8	Coleman 35086	-	-		<1000	0
12	C(♀) x B(♂)	↙	-		<1000	0
13	B	↙	-		<1000	0
14	C	↙	-		<1000	0
15	X/11/56/82/7/6/39	↙	-		<1000	0
16	XIV/4/78/17/67/82B/39	↙	-		<1000	0

The results of the RDI screening are summarized in Table 4. Differences appeared between varieties in all experiments, but not within varieties between sprayed and unsprayed plants. The RDI screening method was easy but not very precise especially when the attack was rather low as in 1993 and 1994. It can be used as a tool to detect interesting varieties for selection purposes. In this respect the variety Asterix might be a worthwile candidate.

Table 4. Screening commercial cauliflower for resistance at Les Fougères in 1993 and 1994 using the root damage index (RDI). Dyfonate + : 1g Dyfonate 5G (5% fonofos) per plant after planting; Dyfonate - : no treatment; Significance of the RDI values transformed to arc sin.

Les Fougères 1993				
Variety	Dyfonate		RDI	Sign. (P=5%)
	+	-		
Asterix	x		6.1	a
Fargo	x		11.0	b
Goodman	x		13.7	bc
Lateman	x		14.4	bc
Fremont	x		14.5	bc
Pex-384	x		15.0	bc
Celesta	x		15.5	bc
Diplomat	x		15.5	bc
SG-4057	x		15.8	bc
Nautilus	x		17.4	c
Profil	x		18.3	c
(Check		x	29.7	-)

Les Fougères 1994				
Variety	Dyfonate		RDI	Sign. (P=5%)
	+	-		
Asterix	x		12.5	a
Lara	x		14.7	a
Asterix		x	15.0	a
Arizona	x		15.8	ab
Lara		x	15.8	ab
Pex-7000	x		17.5	ab
Fremont	x		17.5	ab
Fremont		x	20.0	ab
Pex-7000		x	20.0	ab
Tenere	x		20.8	ab
Aviso	x		20.8	ab
Aviso		x	21.7	ab
Amazing		x	22.5	ab
Tenere		x	22.5	ab
Arizona		x	22.5	ab
Celesta	x		23.3	ab
Amazing	x		23.3	ab
Celesta		x	28.3	b

Table 5 shows the selection effect expressed as the reduction in the number of larvae and pupae in partially resistant lines compared to the reference. Selection for resistance to *D. radicum* has also an effect on *D. platura*. For the selected line XIV/4/32/56/18 with 66.7% egg-laying reduction compared to the reference the reduction in the number of pupae was

over 40% for both Delia species when the number of larvae and pupae per plant in the reference was below 10. The selection effect seemed to disappear at 18 larvae and pupae per plant in the reference. More data are required to be able to determine whether resistance breaks and if it is the case at what critical pest population density.

For the selected line XIV/4/78/17/67/82 with a 42.1% egg-laying reduction the selection effect was also demonstrated for both *Delia* species. However, it varied quite a lot as the number of larvae and pupae per plant on the reference were very low in 1994.

Table 5. Selection effect expressed as a reduction in the number of *D. radicum* and *D. platura* larvae and pupae compared to the reference.

Year	Site	*Delia radicum*	*Delia platura*	Mean number of larvae and pupae per plant on the reference Imperator	Reduction of the number of larvae and pupae on the selected line %
Selected line: XIV/4/32/56/18 with 66.7% egg-laying reduction compared to the reference					
1992	Les Fougères	X		7	42.9
1993	Les Fougères	X		9.2	44.1
	Les Rives	X		18.2	0
	Les Rives		X	3.7	40.3
Selected line: XIV/4/78/17/67/82 with 42.1% egg-laying reduction compared to the reference					
1994	Les Rives 1	X		1.1	55.7
	Les Fougères	X		1.6	68.4
	Les Rives 2	X		2.5	25.3
	Les Rives 3	X		2.9	37.8
	Les Fougères		X	0.1	0
	Les Rives 2		X	0.1	21.4
	Les Rives 3		X	0.2	82.4
	Les Rives 1		X	0.3	89.3

In the diminishing choice experiments the choice was greatest between R1 and S1 plants and diminished gradually in the direction of R6 and S6. If a choice effect existed one would expect great larval and pupal numbers in R1 dropping gradually down in the direction of R6 and low numbers in S1 raising in the direction of S6. Considering the results at Les Fougères in 1993, shown in Table 6 and Figure 1 this does not seem to be the case. Hence the selected line might resist in a no-choice situation. Similar results were obtained at Les Rives in 1993 with additional sampling of columns R7-R13 and S7-S13 (Table 6) and presented in Figure 2 for *D. radicum* and in Figure 3 for *D. platura*.

Table 6. Mean number of cabbage root fly and bean seed fly larvae and pupae per plant under diminishing choice situations, Les Fougères, Les Rives 1993. R=Reference Imperator, S=selected line XIV/4/32/56/18.

Column	Mean number of larvae and pupae per plant		
	Delia radicum		*Delia platura*
	Les Fougères	Les Rives	
R13		11.7	3.0
R12		14.0	1.8
R11		31.0	6.2
R10		21.2	6.4
R9		18.6	2.0
R8		17.0	1.0
R7			
R6	8.2	9.3	0.7
R5	8.5	19.2	3.8
R4	11.2	14.0	2.6
R3	14.0	25.8	5.7
R2	7.8	20.3	7.5
R1	6.3	14.2	2.2
S1	5.3	15.8	2.2
S2	4.7	17.8	1.8
S3	5.3	21.2	2.2
S4	6.7	21.5	2.3
S5	2.4	14.4	1.6
S6	6.2	17.6	2.8
S7			
S8		18.8	3.8
S9		21.5	2.5
S10		21.4	1.4
S11		19.2	3.0
S12		16.2	1.5
S13		22.2	1.2

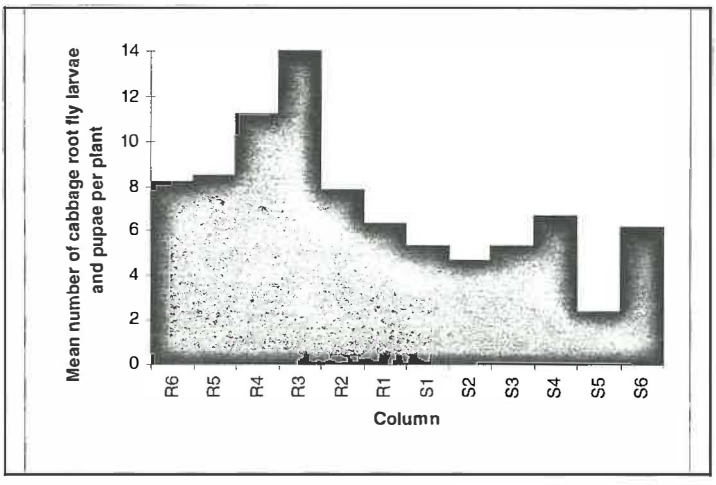

Fig. 1: Mean number of cabbage root fly larvae and pupae per plant under diminishing choice situations, Les Fougères 1993.R=Reference Imperator, S=selected line XIV/4/32/56/18.

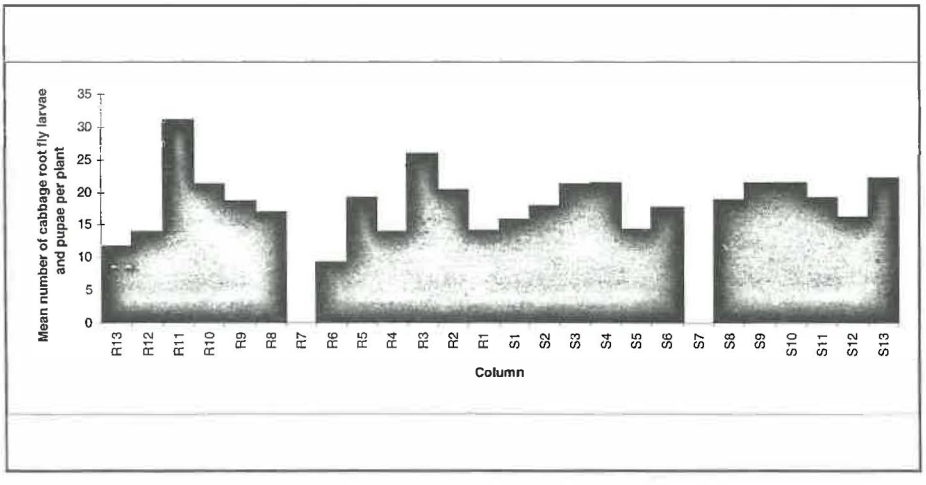

Fig. 2: Mean number of cabbage root fly larvae and pupae per plant under diminishing choice situations, Les Rives 1993. R=Reference Imperator, S=selected line XIV/4/32/56/18.

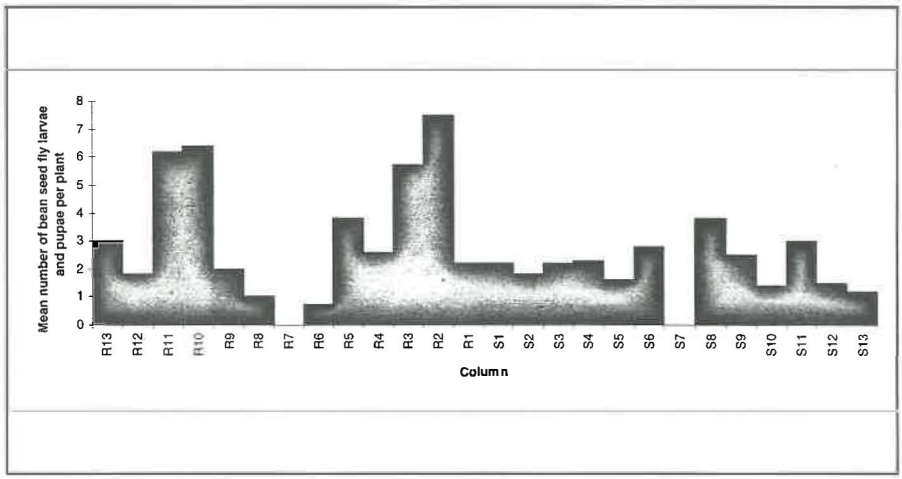

Fig. 3: Mean number of bean seed fly larvae and pupae per plant under diminishing choice situations, Les Rives 1993. R=Reference Imperator, S=selected line XIV/4/32/56/18.

The results of the reduced insecticide dose experiment are summarized in Tables 7 & 8. Although the numbers of larvae and pupae per plant were very low the results were consistent and as expected at both sites for *D. radicum*. The highest reduction of 96.2% was obtained when the unsprayed reference was compared with the selected line sprayed at a low dose. Looking at the reductions for the last two comparisons (68.4% for $R_{\text{no treatment}} \rightarrow S_{\text{no treatment}}$ and 64.7% for $R_{1n} \rightarrow S_{0.6\,n}$) further reduction of insecticide dose might be possible. For *D. platura* the number of larvae and pupae per plant were too low to permit interpretation of the results.

Table 7. Mean number of cabbage root fly and bean seed fly larvae and pupae per plant treated with a reduced insecticide dose, Les Fougères, Les Rives 1994. R=Reference Imperator, S=selected line XIV/4/78/17/67/82, 1n respecticvely 0.6n = 1g respectively 0.6g Dyfonate 5G (5% fonofos per plant).

Variety/line	Dose	Mean numbers of larvae and pupae per plant			
		Les Fougères		Les Rives	
		Delia radicum	*Delia platura*	*Delia radicum*	*Delia platura*
R	no treatment	1.58	0.11	1.28	0.52
R	1n	0.17	0.19	0.32	0.36
S	no treatment	0.50	0.17	0.44	0.44
S	0.6n	0.06	0.08	0.12	0.08

Table 8. Reduction in the mean numbers of cabbage root fly and bean seed fly larvae and pupae per plant treated with reduced insecticide dose, Les Fougères, Les Rives 1994. R=Reference Imperator, S=selected line XIV/4/78/17/67/82, 1n respectively 0.6n = 1g respectively 0.6g Dyfonate 5G (5% fonofos per plant).

Comparison	Reduction of the mean number of larvae and pupae per plant			
	Delia radicum		Delia platura	
	Les Fougères	Les Rives	Les Fougères	Les Rives
$R_{\text{no treatment}} \rightarrow S_{0.6n}$	96.2%	90.6%	27.3%	84.6%
$R_{\text{no treatment}} \rightarrow R_{1n}$	89.2%	75.0%	0%	30.8%
$S_{\text{no treatment}} \rightarrow S_{0.6n}$	88.0%	72.7%	52.9%	81.8%
$R_{\text{no treatment}} \rightarrow S_{\text{no treatment}}$	68.4%	64.6%	0%	15.4%
$R_{1n} \rightarrow S_{0.6n}$	64.7%	62.5%	57.9%	77.8%

Discussion

When a comparison is made of the levels of resistance of breeding lines between one cycle and the next, success and failure to increase the level of resistance were found to alternate. This is illustrated in Table 9 and Figure 4 for a breeding line between 1983 and 1993.

Table 9. A comparison of the relative resistance to egg-laying of a breeding line (P(1983)-F5(1993)) with Imperator (XIV) as parent compared to the reference variety.

Year	Variety/line	Mean number of cabbage root fly egg per plant	Relative egg laying compared to the reference %	Sign. (P=5%)
1983	XIV	11.0	100	-
1985	XIV	70.2		
	XIV/4 F1	53.7	76.5	n.s.
1987	XIV	42.2		
	XIV/4/32 F2	51.7	122.4	n.s.
1989	XIV	41.6		
	XIV/4/32/56 F3	39.5	94.8	n.s.
1991	XIV	8.4		
	XIV/4/32/56/18 F4	2.8	33.3	n.s.
1993	XIV	8.8		
	XIV/4/32/56/18/35 F5	7.5	85.2	n.s.

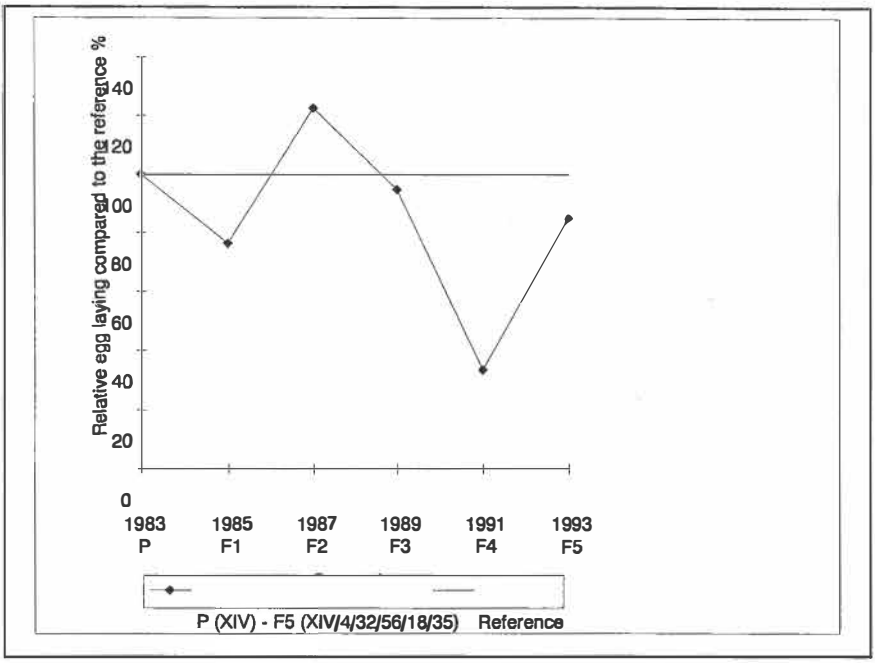

Fig. 4: Relative egg-laying of a breeding line (P(1983)-F5(1993)) with Imperator (XIV) as parent.

The experimental conditions for successful selection were not fullfilled every year. Looking at different parameters one may try to define these conditions more closely. One of the most important points is the level of the cabbage root fly population which influences the mean number of eggs laid per plant. Table 10 and Figure 5 show the egg-laying fluctuation around the reference variety from 1983-1994.

Table 10. Mean number of cabbage root fly eggs per plant around the reference variety from 1983-1994 (except 1986).

Year	1983	1984	1985	1986	1987	1988	1989	1990	1991	1992	1993	1994
Eggs in the reference	11.0	136.7	70.2	-	42.2	21.1	41.6	28.7	8.4	38.2	8.8	5.7

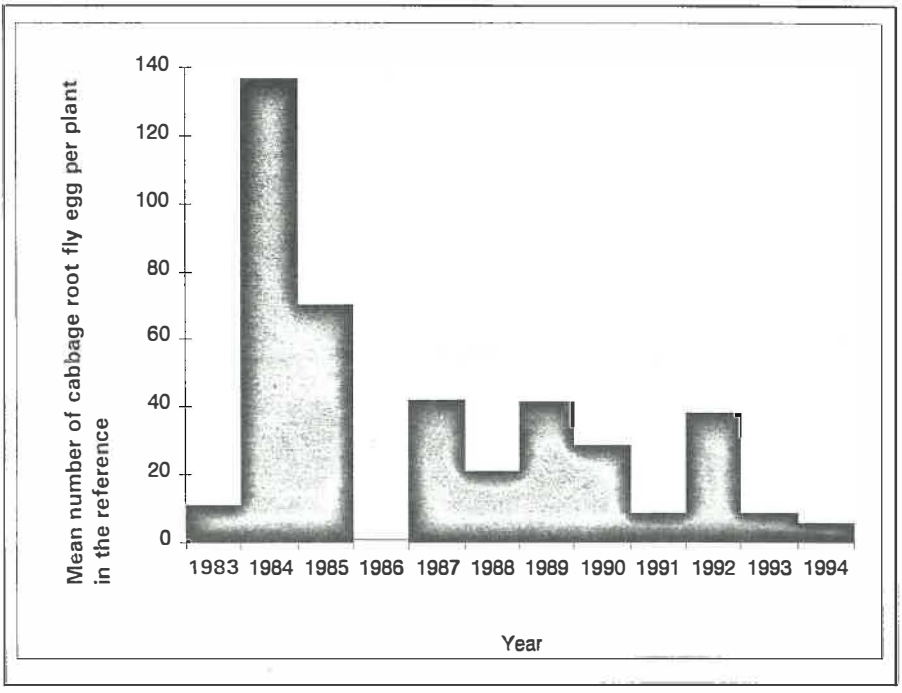

Fig. 5: Mean numbers of cabbage root fly eggs per plant on the reference variety 1983-1994 (except 1986).

During that period the numbers of eggs laid dropped greatly especially in 1991, 1993 and 1994. High temperatures in early spring and summer shifted the whole cycle of cabbage root fly and increased aestivation. In 1995 the planting date was therefore advanced in order to expose plants to the whole second generation of the fly.

In Table 11 the selection effect (for resistance (-) and susceptibility (+)) from one cycle to the next for some lines was compared with mean plant development for the whole experiment, mean numbers of eggs per plant on the reference variety, mean plant development and the egg-laying (mean number of eggs per plant, Min., Max., ratio Max./Min.) in the block where selection took place. A significant selection effect occurred when the mean egg-laying was between 25 and 50 eggs per plant. A high ratio between maximum and minimum egg-laying was not necessary. Low (<10 eggs per plant) and high egg numbers and little plant development were unfavourable for selection.

Unfortunately the behaviour of the fly itself cannot be influenced. As it lands on the plants randomly incorrect choice of a selected plant for low egg-laying cannot be excluded. In these cases a further cycle of selection is useless.

Table 11. Selection effect from one selection cycle to the next depending on egg-laying and plant growth.

Year	Mean plant dev. diam. (cm)	Ref. Mean no. eggs/ plant	Var./line	Gen.	Selected block					Selection effect	
					Mean plant dev.	Mean no. eggs/ plant	Min	Max	Ratio Max/ Min	Relative egg laying from one to the next gen.	Sign (P=5 %)
Selection for resistance, series of odd years											
1983		11.0	XIV	P		10.1	0	25	-	-24%	n.s.
1985		70.2	XIV/4	F1		58.0	25	119	4.8	+48%	n.s.
1987		42.2	XIV/4/32	F2		28.4	6	52	8.7	-28%	n.s.
1989		41.6	XIV/4/32/56	F3		42.5	12	73	6.1	-62%	n.s.
1991	883	8.4	XIV/4/43/56/18	F4	358	4.8	0	15	-	+15%	n.s.
Selection for resistance, series of even years											
1988		21.1	X/20, 1/69/38	F3		25/1	12	48	4.0	-103.6%	s..
1990	503	28.7	X/20,1/69/38/36	F4	425	1.4	0	4	-	+91.8%	n.s.
1992	1947	38.2	X/20,1/69/38/36/60	F5	2395	50.0	24	100	4.2	-70.4%	s.
Selection for susceptibility, series of odd years											
1983		11.0	XIV	P		16.3	1	45	45	-12%	n.s.
1985		70.2	XIV/14	F1		66.9	38	99	2.6	+57%	n.s.
1987		42.2	XIV/14/18	F2		55.8	27	128	4.7	-43%	n.s.
1989		41.6	XIV/14/18/50	F3		48.9	28	87	3.1	+194%	s.
1991	883	8.4	XIV/14/18/50/3	F4	1272	18.3	6	59	9.8	-134%	n.s.

In 1991 a descendant of Imperator (XIV) was shown to have a good level of resistance (33.3% relative egg laying compared to the reference after four selection cycles). To test the stability of the selected F4 line XIV/4/32/56/18, F1-F4 plants were grown together in 1993 and the results compared with the data obtained from 1985(F1)-1991(F4). The results are shown in Table 12 and Figure 6.

The two curves were very similar showing that the selection results were reproducible and independant of the annual conditions. It also means that the standard statistical test to discriminate between screened or selected plant material was too severe.

Table 12. Comparison of relative egg-laying of descendants (F1-F4) of Imperator (XIV) grown from 1985(F1)-1991(F4) and together in 1993.

Year	Variety/line	Mean no. of cabbage root fly egg per plant	Relative egg-laying compared to the reference %	Year	Variety/line	Mean no. of cabbage root fly eggs per plant	Relative egg-laying compared to the reference %
1985	XIV	70.2		1993	XIV	8.8	
	XIV/4 Fi	53.7	76.5		XiV/4 F	7.3	82.6
1987	XIV	42.2		1993	XIV	8.8	
	XIV/4/32 F2	51.7	122.4		XIV/4/32 F2	11.0	124.4
1989	XIV	41.6		1993	XIV	8.8	
	XIV/4/32/56 F3	39.5	94.8		XIV/4/32/56 F3	8.8	100
1991	XIV	8.4		1993	XIV	8.8	
	XIV/4/32/56/18 F4	2.8	33.3		XIV/4/32/56/18 F4	5.3	60.1

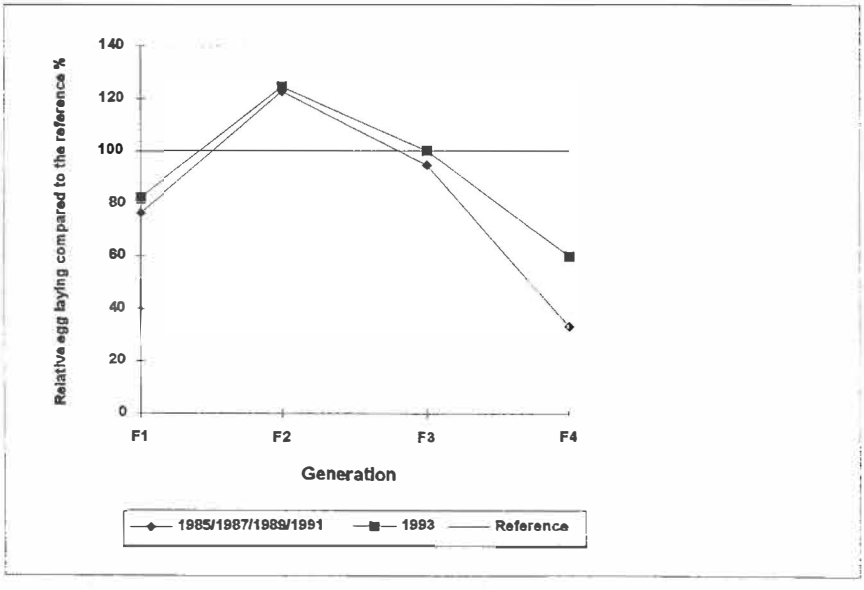

Fig. 6: Relative egg-laying of the selected F4 line XIV/4/32/56/18. Comparison of F1-F4 plants grown together in 1993 with the data obtained from 1985(F1)-1991(F4).

Résumé

Evaluation de lignées de chou-fleur sélectionnées pour la résistance à l'égard de la mouche du chou (*Delia radicum*)

La sélection de plantes individuelles de chou-fleur pour leur résistance du type antixénose à la mouche du chou (*Delia radicum* L.) est basée sur un comptage intensif des oeufs déposés au pied des plantes. Pourqu'elle puisse réussir, le niveau de ponte doit se situer entre 25 et 50 oeufs par plante en moyenne. Cependant, un mauvais choix d'une plante avec peu d'oeufs n'est pas exclu à cause du comportement de l'insecte qui se pose sur les plantes d'une manière aléatoire. Les lignées sélectionnées, partiellement résistantes, ont été évaluées sous différents aspects :

1. Dénombrement du nombre de larves et pupes dans le sol autour des plantes exposées à différents niveaux de populations du ravageur. Lorsque le nombre moyen de larves et pupes par plante était inférieur à 10 dans la variété de référence, une réduction de la ponte de 42.1% à 66.7% était suivie par une réduction du nombre de larves et pupes de 25,3% à 68.4%. On ignore encore si cette résistance est rompue lorsque les populations sont plus importantes. La sélection pour la résistance à l'égard de la mouche du chou a également un effet sur la mouche grise des semis *D. platura* Meigen.

2. hoix des plantes offertes aux ravageurs. Les résultats obtenus dans une expérimentation à choix dégressif montrent que la lignée partiellement résistante testée pourrait conserver ses qualités dans une situation de non choix.

3. Possibilité de diminuer la dose d'insecticide nécessaire dans le matériel partiellement résistant. Lorsque la réduction du pesticide est de 40% (0,6 n ou 0,6 g de Dyfonate 5G (5% fonofos) par plante au lieu de 1 g) et que la population est faible, le nombre de larves et de pupes dans la ligne sélectionnée est comparable à celui de la variété de référence traitée avec la dose normale.

Acknowledgement

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Multiple resistance in spring wheat to late-wheat shoot fly *Phorbia securis* (Dipt., Anthomyiidae) and frit flies *Oscinella* spp (Dipt., Chloropidae)

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Summary

The phenomenon of multiple resistance to frit flies and late-wheat shoot fly has been identified among common wheat cultivars and breeding lines originating from the South Eastern Institute for Agriculture (Saratov, Russia). In the majority of cases the cultivars, which had been found to be as resistant to one of the pests, were shown to be resistant to another insect as well. The mode of life of larvae of these two species in shoots is rather similar, so the same mechanism of resistance may be involved in their interactions with the host plant. Ancestral indigenous cultivars particularly cv. Poltavka seem to be the sources of this multiple resistance to the pests.

Introduction

Frit flies (FF) - *Oscinella frit* L. and *O. pusilla* Meig (Dipt.: Chloropidae) are important and common pests of cereals over the whole area of the cultivation of these crops in Russia. Late-wheat shoot fly (LWSF) *Phorbia securis* Tiensuu (Dipt.:Anthomyiidae) has been reported as an important pest of common wheat in the south of Russia and Ukraine. The resistance of cereals to FF has been investigated over many years. A number of plant characters (for the most part characteristics of plant anatomy and development which hamper larval penetration into shoots) has been shown to be associated with varietal resistance to FF (Shapiro, 1989; Sherbakov, 1984; Jonasson, 1988). The nature of wheat resistance to LWSF is not understood though some resistant and tolerant cultivars (mainly of winter wheat) have been reported (Nikolenko & Omeltchenko, 1977; Klechkovsky, 1985). Multiple resistance to several pest species is one of the most important aims in breeding for resistance to pests. In this connection the initial objective of the investigation described in this paper was to identify spring wheat cultivars possessing resistance to both FF and LWSF. Following the discovery of multiple host plant resistance to the pests the genealogy of resistant and susceptible wheat cultivars was compared in an attempt to reveal probable sources of resistance.

Materials and Methods

Spring wheat cultivars were tested for resistance to the pests under natural field conditions in Saratov (southern European part of Russia). The field trial was sown late sown late near St. Petersburg (North-Western region of the country) to coincide with a high population density of FF adults at the most vulnerable stage of plant development. LWSF

is the main dipterous stem borer in the first site mentioned, while FF dominates in the latter. The percentage of main tillers damaged by the pests was used as an index of resistance. The estimations were made at the tillering-boothing stages by conventional methods (Shapiro, 1978). Eighteen common spring wheat cultivars and breeding lines originating from the South-Eastern Institute for Agriculture (SEIA, Saratov) were used in the investigation.

Results and Discussion

The multiple resistance of spring wheat cultivars to LWSF and FF

The group of common wheat cultivars was shown to be resistant to LWSF during the three years of investigation. Certain cultivars, such as Lutescens 62, Saratovskaya 29 and Saratovskaya 55 had been shown to be resistant to FF as well (Shapiro, 1989; Sinelnikov, 1990). In this connection the levels of resistance to LWSF and FF were compared. As may be seen from Fig.1, in the majority of cases the cultivars which had been found resistant to one pest, were found to be resistant to another one as well. The same phenomenon was observed for the most susceptible cultivars.

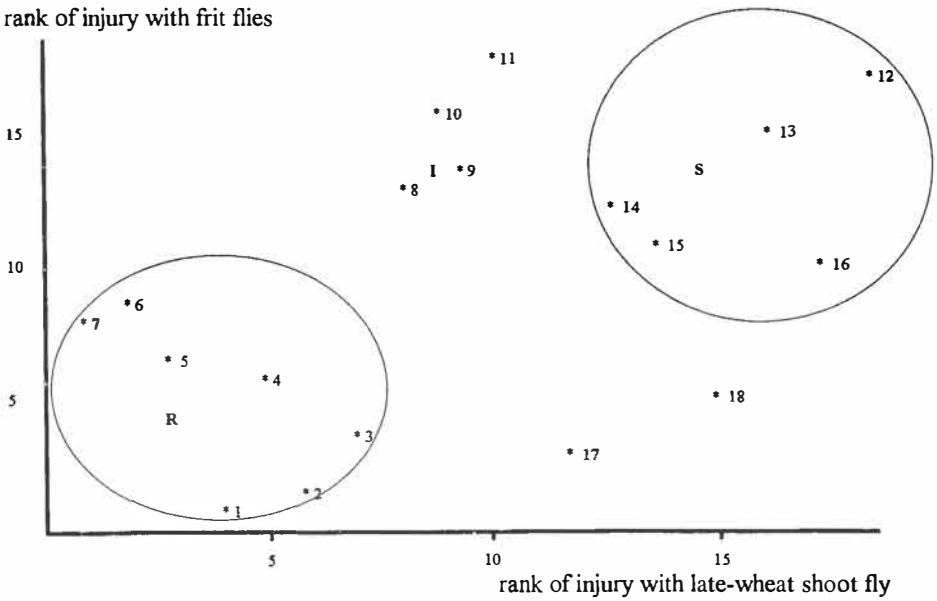

Fig. 1: The linkage between the resistance of spring common wheat cultivars to late-wheat shoot fly and frit flies in 1992. R - resistant; S - susceptible; I - intermediate. Cultivars and breeding lines: 1 - Albidum C-2011; 2 - Lutescens 62; 3 - Lutescens C-2033; 4 - Erithrospermum C - 2040, 5 - Saratovskaya 29; 6 - Saratovskaya 62; 7 - Saratovskaya 55; 8 - L 503; 9 - Lutescens C-1999; 10 - Saratovskaya 58; 11 - Saratovskaya 42; 12 -Saratovskaya 46; 13 - Albidum C-2015; 14 - Lutescens C2026; 15 - Erithrospermum C-2037; 16 - Erithrosperrnum C - 2039; 17 - Lutescens C - 2031, 18 - Saratovskaya 60. The Kendal index of rank correlation constitutes 0.42, $P<0.05$ (cv. NN 17 and 18 left out of consideration. The latter cultivar was found to be susceptible to FF in a separate experiment).

The mechanism of plant immunity to pests and other stresses has evolved as a non-specific reaction, so the basis of resistance to different phytophagan species may be of a similar nature (Shapiro,1985). The mode of life of larvae of these dipterous stem borers in the shoots is rather similar, so the same resistance mechanism may be involved in their interactions with the host plant. The same phenomenon of multiple host plant resistance to non-related pest species with similar feeding activity was also demonstrated in the case of cucumber interactions with spider mites and two thrips species (Mollema *et al.*, 1993, Razdoburdin, pers. commun.).

It is likely that this multiple resistance to the dipterous stem borers is based on antibiosis. Antixenotic resistance to FF is conditioned mainly by the lack of preferred oviposition sites on the host plant (Jonasson, 1980) whereas the different sizes of FF and LWSF adults dictates differences in their requirements for oviposition sites. Hence, it is unlikely that the multiple resistance to the dipterous stem borers is based on antixenosis.

Comparative studies of the genealogy of resistant and susceptible common wheat cultivars

The specific aim of releasing pest-resistant wheat cultivars was never an objective of the breeding programmes at the SEIA. Nevertheless a range of common wheat cultivars originating from this Institute has been found to be resistant to LWSF and FF. As resistance to pests is rare occurrence, it would be of interest to determine why so many resistant cultivars exist amongst the collection at the SEIA and to determine probable sources of this resistance. It can be concluded from the comparative studies of genealogy of the common wheat cultivars that the ancestral indigenous wheat forms are the source of this multiple resistance to FF and LWSF (Table 1).

Table 1. Origins of common wheat cultivars in the context of their multiple resistance to late wheat shoot fly and frit flies.

Origin of wheat cultivars	Number of cultivars	
	R	IS
From indigenous cultivars only	2	2
Indigenous cultivars + Kitchener (Canada); Nadadores 63 (Mexico)	2	00
Indigenous cultivars + Selkirk(Canada) 9.5 - 12.5%	0	03
Indigenous cultivars + Selkirk (Canada) 4.7 - 6.25%	2	20
Indigenous cultivars + Selkirk (10%) and other sources of foreign origin	0	0
Indigenous cultivars + Selkirk (6.25 - - 6.75%) and other sources of foreign origin	0	13

The hybridisation of indigenous cultivars with cv. Selkirk (Canada) resulted in the loss of resistance in the majority of cases. On the other hand, the use of cultivars Kitchener (Canada) and Nadadores 63 (Mexico) did not decrease the resistance to the flies. It is possible to trace the transmission of pest resistance in the sequence of cultivars (Fig.2). The indigenous cultivar Poltavka seems to be one of the most probable sources of resistance. Cv. Lutescens 62 which was bred from Poltavka through individual selection is known to be one of the most frit fly-resistant wheat cultivars (Shapiro, 1989).

In all likelihood the continual pest pressure resulted in the natural selection of pest-resistant forms within the Poltavka cultivar-population and other ancestral indigenous cultivars. This suggests, among other things, that the multiple resistance of common wheat cultivars to the two fly pests will be durable. The mechanisms of this resistance may prevent adaptation by these pest species.

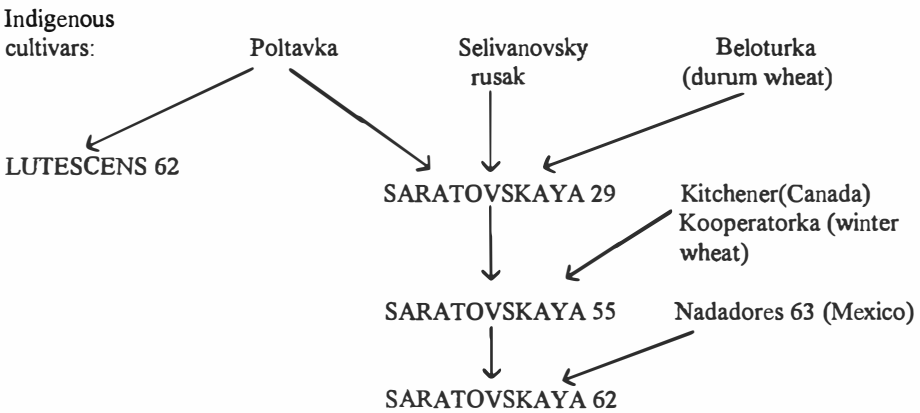

Fig. 2: Genealogy of some wheat cultivars resistant to late-wheat shoot fly and frit flies. Resistant cultivars are indicated in capital letters.

Conclusions

The phenomenon of multiple resistance to LWSF and FF has been identified among common spring wheat cultivars. The same resistance mechanism may be involved in interactions different pest species with the host plant. The indigenous wheat forms seem to be the sources of this multiple resistance of common wheat, and it is likely to be highly durable.

Résumé

Résistance multiple du blé de printemps à l'égard de *Phorbia securis* (Dipt., Anthomyiidae) et les oscinies *Oscinella* spp. (Dipt., Chloropidae)

Le phénomène de la résistance multiple à l'égard des oscinies et de *Phorbia securis* a été identifié parmi des cultivars communs de blé et des lignes sélectionnées provenant de l'institut d'agriculture de Saratov, dans le Sud-Est de la Russie. Dans la plupart des cas les cultivars ne sont pas uniquement résistants contre l'un des ravageurs cités. La biologique des asticots de ces espèces qui se trouvent dans les pousses, est assez semblable, de sorte que le même mécanisme de résistance pourrait être impliqué dans leurs interactions avec la plante-hôte. Les sources de cette résistance multiple semblent se trouver chez les anciens cultivars indigènes, en particulier le CV "Poltava".

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The morpho-physiological basis of resistance in cotton to sap-sucking insects

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Summary

Sap-sucking insects reduce the vigour of cotton plants enormously in the tropics and sub tropics. In India leafhoppers and thrips are of particular concern. With a view to finding out the physiological basis of resistance to these pests, five isogenic lines of a cotton variety H-777 carrying different morphological attributes viz. glandless (gl), red pigment (rd), fregobract (fg), okra leaf (ol), nectariless (nl) along with normal H-777 were evaluated for photosynthesis (ph), transpiration (tr) and stomatal conductance (sc). Isolines were grown using four treatments viz. irrigated + protected from insects, irrigated + unprotected, un-irrigated + protected from insects, un-irrigated + unprotected. Observations were recorded after 34, 65, 95 and 133 days after sowing. Various physiological processes were adversely affected at 65 days after sowing which synchronised with peak insect population pressure. Under un-irrigated and unprotected conditions, the leafhopper *Amrasca biguttula biguttula* caused a significant reduction in ph, stomatal conductance and transpiration on the leaves of red pigment, okra leaf and nectariless isolines. A decrease in ph and transpiration of glandless, fregobract and normal plants was marginal at the same pest population density. Heavy leafhopper populations on irrigated and unprotected plots resulted in a 30.3 to 75.0% decrease in ph and a 33.5 to 66.5% decrease in stomatal conductance without showing pronounced effect on transpiration. The glandless isoline was comparatively resistant to the adverse effect of leafhopper feeding. *Thrips tabaci* populations on red pigment, okra leaf glandless and gregobract lines in un-irrigated and unprotected plots caused a 26.2 to 46.1% decrease in ph without manifesting any marked effect on stomatal conductance and transpiration. In irrigated and unprotected plots, high thrips populations (67.3 - 111.2/leaf) caused a 36.6 to 48.9% reduction in ph without showing any pronounced effect on other characters except in okra leaf lines where stomatal conductance was reduced by 52.6%.

Introduction

Cotton leafhopper, *Amrasca biguttula biguttula* (Ishida) is the most serious sap-sucking pest of cotton in India and several other cotton growing countries (Singh & Agarwal, 1988). Thrips, *Thrips tabaci* Lindeman is occasionally serious on cotton worldwide (Bournier, 1994).

Leafhoppers introduce toxins to leaves during feeding that impair photosynthesis in proportion to the amount of feeding (Matthews, 1994). Subsequently leaves manifest downward curling, yellowing, reddening and finally drying out before shedding. Damage by large populations of thrips results in cotton fields developing a characteristic silvery colour. Necrotic areas later on become brown, leading to withering and falling of leaves.

Leafhopper-resistant varieties have been bred by introducing a pubescent character into commercial lines (Butter & Singh, 1993) but the adoption of pubescent cultivars has been limited by to the resurgence of other pests like spotted bollworm, whitefly and mites. Moreover, highly pubescent cultivars create problems for the cotton textile industry (Gannaway, 1994). However, there are other morphological attributes like red pigment, okra leaf, nectariless, glandless and fregobract which have been reported to confer resistance against several pests including pink bollworm, American bollworm, whitefly, bollweevil and cotton grey weevil (Kamboj, 1991; Butter & Singh, 1993). Information is lacking on the influence of these morphological traits on the incidence of leafhoppers and thrips, and their relationship with the physiological performance of leaves. The present studies were carried out to assess the role of morphological traits developed as isolines in the common genetic background of cultivar H-777 of *Gossypium hirsutum* L. in relation to the physiological performance of leaves as influenced by the incidence of leafhopper and thrips.

Materials and Methods

Seeds of five isogenic lines along with the commercial cultivar H-777 were obtained from the Department of Plant Breeding, Haryana Agricultural University. Seed was sown at the University Research Farm, by the dibbling method on 20 May 1991. The experiments were arranged in a randomised block design using five replicates of 45 sqm plots, spaced 60 x 30 cm between rows. The treatments were:-

T1. Irrigation on 30 July, 20 August and 17 September and foliar sprays of insecticides (CCSHAU, 1990).
T2. Irrigation as described in T1 but without insecticide.
T3. No irrigation but foliar sprays of insecticide.
T4. No irrigation, no insecticides.

The average annual rainfall at Hisar between June and October, is 357 mm but during the season under investigation only 150 mm rainfall occurred. Thus, irrigation was required to achieve maximum yield.

Records of insect populations

Weekly observations were made of the incidence of sap-sucking insects starting in the third week of July until crop maturity (2nd week of November). From each plot 10 plants were selected randomly to examine 30 leaves (3 leaves/plant) to record populations of leafhoppers and thrips. Insect infestations and damage do not overlap on the plant. Thrips are always confined to the upper 3 to 4 leaves of the plant whilst leafhoppers remain active in the middle and upper portion of the plant canopy. Hence, it is easy to separate and record populations and damage of both insects on the same plant. To record infestation levels, both the nymphs and adults of thrips were counted whereas only the nymphs of leafhoppers were counted on selected leaves.

Measurements of physiological parameters

On each sampling date, photosynthesis, stomatal conductance and transpiration rate were measured with a portable leaf chamber analyser (Model ADC, LCA-2). Measurements were made between 09.00 and 10.00 hours on days with full sunlight. In unprotected plots five fully-expanded leaves randomly selected in the top of the canopy on a plant infested with sap-

sucking insects were sampled. The corresponding leaves on plants in protected plots were also sampled. Insects were removed with a camel hair brush before recording physiological parameters. The first date of observation was 34 days after sowing, at the square initiation stage but before application of any irrigation and insecticidal sprays. At this stage the crop was free from thrips and harboured few leafhoppers (Table 1). The second observation was made 65 days after sowing which synchronised with peak populations of leafhoppers and thrips; the crop was at the active boll initiation stage. By this time the crop had been irrigated twice and had received two foliar sprays of insecticides. The third and fourth observations were made after 95 (boll opening initiation) and 133 (boll opening completion) days after sowing, respectively. Leafhopper and thrips populations were very low at these stages and the physiological functions of leaves in protected and unprotected plants also reduced insect activity. Hence, only the data for the first and second observations have been presented. To assess the cummulative effect of insect populations on the functioning of leaves, the data on leafhopper and thrips populations presented in Tables 1 to 7 represent the mean of the three observations recorded (15 days, 8 days and 1 day) and preceed to the day of sampling for physiological parameters.

Results and Discussion

The first observations of physiological parameters were made 34 days after sowing. Up to this period, the crop did not require any irrigation or protection from insects. Leafhopper populations early in the season ranged from 0.02 to 0.17 nymphs/leaf for the different isogenic lines (Table 1). At this stage, variations in physiological functions viz. transpiration (10.9 to 12.9 mmol $m^{-2}s^{-1}$), stomatal conductance (650 to 868 mmol $m^{-2}S^{-1}$) and photosynthesis (18.2 to 22.4 umol $m^{-2}s^{-1}$) of different isogenic lines indicated slight variability associated with morphological traits. The glandless, fregobract and H-777 lines appeared to be physiologically more efficient compared to other lines.

Table 1. Leaf transpiration, stomatal conductance and photosynthesis of cotton isolines of variety H-777 34 days after sowing (last week of July).

Isoline	Leaf hopper nymphs/leaf	Transpiration (m $molm^{-2}s^{-1}$)	Stomatal conductance (m mol $m^{-2}s^{-1}$	photosynthesis (u mol $m^{-2}s^{-1}$)
Red pigment	0.17	11.0	654	22.4
Okra leaf	0.12	10.9	650	18.2
Nectariless	0.05	11.9	775	19.2
Glandless	0.05	12.4	811	21.5
Fregobract	0.02	12.8	835	21.3
H-777	0.08	12.9	868	22.4
L.S.D. (P=0.05)	0.04	1.2	175	1.4

Effect of leafhopper on physiological parameters

Leafhopper populations in unprotected and irrigated plots (4.5 to 7.8 nymphs/leaf) were significantly higher than those in un-irrigated and unprotected plots (0.9 to 1.6 nymphs/leaf). Consequently the reduction in leaf photosynthesis (30.3 to 75.0%) due to leafhoppers (Table 2) in irrigated plots was also higher than in un-irrigated plots (0.7 to 69.9%). Photosynthesis in glandless, fregobract lines and the cultivar H-777 appeared to be less affected compared to the red pigment, okra leaf and nectariless lines under both irrigated and un-irrigated conditions. In these 3 lines reductions in transpiration rate under irrigated (6.8 to 22.3%) and un-irrigated (4.9 to 11.7%) conditions were also less compared to the other lines with corresponding 6.8 to 30.7 and 22.9 to 26.4% reductions (Table 3). Similar trends were observed for stomatal conductance records (Table 4). Reductions in leaf stomatal conductance of glandless (33.6%) and fregobract (33.5%) lines were significantly less than red pigment (43.3%), nectariless (60.3%) and okra leaf (66.5%) lines under irrigated conditions. Similar results were obtained in un-irrigated plots. Therefore, glandless and fregobract lines could be considered as potential sources of physiological resistance to leafhoppers. Reduction of photosynthesis and transpiration rates as a result of feeding by the leafhopper, *Empoasca fabae* in alfalfa (Flin *et al.*, 1990), aphids in potato (Veen, 1985) and broadbean (Alice & Thrower, 1981) have been reported earlier. However, the present study has revealed the possibility of evaluating resistance on the basis of the physiological performance of cotton lines against leafhoppers.

Table 2. Effect of leafhopper infestation on leaf photosynthesis of isolines of cotton variety H-777 65 days after sowing (last week of August).

Isoline	Leaf photosynthesis (u mol $m^{-2}s^{-1}$)					
	Irrigated			Un-irrigated		
	Protected	Unprotected	% reduction	Protected	Unprotected	% reduction
Red pigment	15.4 (0.2)	6.6 (6.9)	57.2	17.9 (0.3)	6.9 (1.3)	61.5
Okra leaf	15.2 (0.4)	6.0 (5.2)	60.5	15.9 (0.2)	7.2 (1.2)	54.7
Nectariless	20.7 (0.3)	5.2 (5.6)	75.0	17.6 (0.3)	5.3 (1.4)	69.9
Glandless	14.2 (0.4)	9.9 (4.5)	30.3	14.4 (0.3)	12.2 (1.6)	15.3
Fregobract	14.9 (0.2)	6.6 (7.8)	55.7	14.6 (0.4)	14.5 (1.3)	0.7
H-777	17.7 (0.2)	7.9 (4.4)	55.4	16.2 (0.2)	14.5 (0.9)	10.5
L.S.D. (P=0.05)	2.6	1.8		1.9	3.2	

Figures in parentheses are mean numbers of leafhopper nymphs per leaf.

Table 3. Effect of leafhopper infestation on leaf transpiration of isolines of cotton variety H-777 65 days after of sowing (last week of August).

Isoline	Leaf transpiration (m mol $m^{-2}s^{-1}$) Irrigated Protected	Irrigated Unprotected	% reduction	Unirrigated Protected	Unirrigated Unprotected	% reduction
Red pigment	11.8 (0.2)	11.0 (6.9)	6.8	12.5 (0.3)	9.2 (1.3)	26.4
Okra leaf	12.7 (0.4)	8.8 (5.2)	30.7	11.8 (0.2)	9.1 (1.2)	22.9
Nectariless	13.4 (0.3)	11.1 (5.6)	17.1	12.4 (0.3)	9.2 (1.4)	25.8
Glandless	12.6 (0.4)	11.2 (4.5)	11.1	11.9 (0.3)	11.2 (1.6)	5.9
Fregobract	11.7 (0.2)	10.9 (7.8)	6.8	12.8 (0.4)	11.3 (1.3)	11.7
H-777	13.0 (0.2)	10.1 (4.4)	22.3	12.2 (0.2)	11.6 (0.9)	4.9
L.S.D. (P=0.05)	1.5	1.2		N.S.	N.S.	

Figure in parentheses are mean numbers of leafhopper nymphs per leaf.

Table 4. Effect of leafhopper infestation on leaf stomatal conductance of isolines of cotton variety H-777 65 days after sowing (last week of August).

Isoline	Leaf stomatal conductance (m mol $m^{-2}s^{-1}$) Irrigated Protected	Irrigated Unprotected	% reduction	Unirrigated Protected	Unirrigated Unprotected	% reduction
Red pigment	760 (0.2)	431 (6.9)	43.3	993 (0.3)	451 (1.3)	54.6
Okra leaf	877 (0.4)	294 (5.2)	66.5	909 (0.2)	367 (1.2)	59.6
Nectariless	1112 (0.3)	441 (5.6)	60.3	1016 (0.3)	367 (1.4)	63.9
Glandless	788 (0.4)	523 (4.5)	33.6	916 (0.3)	777 (1.6)	15.2
Fregobract	683 (0.2)	454 (7.8)	33.5	1048 (0.4)	794 (1.3)	24.2
H-777	924 (0.2)	371 (4.4)	59.8	953 (0.2)	912 (0.9)	4.3
L.S.D. (P=0.05)	186	174		124	210	

Figure in parentheses are mean numbers of leafhopper nymphs per leaf.

Effect of thrips on physiological parameters

Thrips populations in unprotected and irrigated plots (67.3 to 111.2 nymphs and adults/leaf) were significantly higher than those in un-irrigated and unprotected plots (18.3 to 58.3/leaf). Reductions in leaf photosynthesis due to thrips attack varied between 36.6 and 48.9% in irrigated and 3.4 and 46.1% in un-irrigated plots for the different isogenic lines (Table 5). Under un-irrigated conditions nectariles and H-777 appeared to be less affected by thrips in terms of leaf photosynthesis. However, in the other treatments genotypic differences were inconsistent. Also leaf transpiration rate did not differ significantly among isolines and across treatments (Table 6). Changes in leaf stomatal conductance were also not proportional to the thrips population (Table 7) which indicates the unreliability of using the parameter for identification of sources of resistance. Buntin *et al.* (1988) also could not find any relationship between reduction in leaf photosynthesis and stomatal conductance and transpiration rates when peach leaves were injured by *Echinothrips americanus* feeding.

Table 5. Effect of thrips infestation on leaf photosynthesis of isolines of cotton variety H-777 65 days after sowing (last week of August).

Isoline	Leaf photosynthesis (u mol $m^{-2}s^{-1}$)					
	Irrigated			Unirrigated		
	Protected	Unprotected	% reduction	Protected	Unprotected	% reduction
Red pigment	17.4 (0.1)	10.2 (111.2)	41.4	18.3 (0.3)	13.5 (17.3)	26.2
Okra leaf	19.6 (0.2)	10.0 (79.6)	48.9	16.2 (0.2)	11.3 (18.3)	30.2
Nectariless	20.0 (0.8)	12.5 (83.6)	37.5	15.8 (0.1)	15.0 (26.2)	5.1
Glandless	23.2 (1.0)	14.7 (77.3)	36.6	18.8 (0.3)	10.6 (39.0)	43.6
Fregobract	15.9 (0.9)	9.2 (67.3)	42.1	19.1 (0.1)	10.3 (31.0)	46.1
H-777	19.2 (0.5)	10.6 (100.9)	44.8	14.7 (0.5)	14.2 (58.3)	3.4
L.S.D.(P=0.05)	2.4	2.1		1.9	2.3	

Figure in parentheses are mean numbers of thrips per leaf.

Table 6. Effect of thrips infestation on leaf transpiration of isolines of cotton variety H-777 65 days after sowing (last week of August).

Isoline	Leaf transpiration (m mol $m^{-2}s^{-1}$) Irrigated Protected	Irrigated Unprotected	% reduction	Unirrigated Protected	Unirrigated Unprotected	% reduction
Red pigment	12.5 (0.1)	11.9 (111.2)	4.8	12.7 (0.3)	11.8 (17.3)	7.1
Okra leaf	13.2 (0.2)	10.7 (79.6)	18.9	11.6 (0.2)	10.2 (18.3)	12.1
Nectariless	13.0 (0.8)	12.4 (83.6)	4.6	12.5 (0.1)	11.6 (26.2)	7.2
Glandless	12.9 (1.0)	12.4 (77.3)	3.9	12.4 (0.3)	11.4 (39.0)	8.1
Fregobract	12.5 (0.9)	12.4 (67.3)	0.8	12.8 (0.1)	11.3 (31.0)	11.7
H-777	13.9 (0.5)	12.8 (100.9)	7.9	11.6 (0.5)	11.5 (58.3)	0.9
L.S.D (P=0.05)	N.S.	N.S.		N.S.	N.S.	

Figure in parentheses are mean numbers of thrips per leaf.

Table 7. Effect of thrips infestation on leaf stomatal conductance of isolines of cotton variety H-777 65 days after sowing (last week of August).

Isoline	Leaf stomatal conductance (m mol $m^{-2}s^{-1}$) Irrigated Protected	Irrigated Unprotected	% change	Unirrigated Protected	Unirrigated Unprotected	% change
Red pigment	989 (0.1)	655 (111.2)	-33.8	1051 (0.3)	1107 (17.3)	+5.3
Okra leaf	1028 (0.20)	487 (79.6)	-52.6	929 (0.2)	515 (18.3)	-44.6
Nectariless	940 (0.8)	805 (83.6)	-14.4	1051 (0.1)	848 (26.2)	-19.3
Glandless	875 (1.5)	756 (77.3)	-13.6	962 (0.3)	818 (39.0)	-14.9
Fregobract	863 (0.9)	805 (67.3)	-6.7	1001 (0.1)	788 (31.0)	-21.3
H-777	1128 (0.5)	796 (100.9)	-29.4	777 (0.5)	860 (58.3)	+10.7
L.S.D. (P=0.05)	150	180		165	156	

Figure in parentheses are mean numbers of thrips per leaf.

Résumé

Bases morpho-physiologiques de la résistance du coton à l'égard d'insectes suceurs

Les insectes suceurs diminuent fortement la vigueur des plantes de coton dans les régions tropicales et subtropicales. En Inde, les cicadelles et les thrips sont à ce titre, d'intérêt particulier. Dans le but de trouver les bases physiologiques de la résistance à l'égard de ces ravageurs, cinq lignées isogéniques de la variété de coton H-777 présentant différents caractères morphologiques, à savoir : non glanduleux (gl) à pigmentation rouge (rd), à bractée réfléchie (fregobract) (fg), sans nectaires (nl) et la forme normale de H-777, ont été évaluées quant à leur photosynthèse (ph), transpiration (tr) et conductivité stomatale (sc). Les quatre traitements suivants ont été appliqués sur ces lignées : irrigation avec protection contre les insectes, irrigation sans protection, sans irrigation avec protection et sans irrigation ni protection. Les observations ont eu lieu 34, 65, 95 et 133 jours après le semis. Divers processus physiologiques ont été défavorablement touchés 65 jours après le semis, ce qui coïncidait avec la pression maximale de la population des ravageurs. La cicadelle *Amrasca biguttula biguttula* a causé dans la variante sans irrigation ni protection, une réduction significative du pH, de la conductivité stomatale et de la transpiration dans les feuilles des lignées isogéniques avec pigmentation rouge, feuilles d'okra et sans nectaires, alors qu'elle était insignifiante pour le pH et la transpiration pour celles dépourvues de glandes, à bractée réfléchie (fregobract) et pour la forme normale avec la même densité de population de la cicadelle. Lorsque celle-ci était très élevée, la variante avec irrigation sans protection a subi une diminution du pH de 30.3 à 75.0%, et de la conductivité stomatale de 33.5 à 66.5%, sans qu'il a ait un effet prononcé sur la transpiration. La lignée sans glandes a été relativement résistante à l'égard de la cicadelle. Des populations de *Thrips tabaci* ont causé une diminution du pH de 26.2 à 46.1% dans la variante sans irrigation ni protection sur les lignées avec pigmentation rouge, feuilles d'okra, dépourvues de glandes et à bractée réfléchie (fregobract), sans affecter notablement la conductivité stomatale ni la transpiration.

Des populations de thrips élevées (67.3 - 111.2 par feuille) ont provoqué dans la variante irrigation sans protection une chute du pH de 36.6 à 48.-9%. Il n'y avait pas d'effet prononcé sur les autres caractères sauf dans la lignée avec feuilles d'okra où la conductivité stomatale a chuté de 52.6%.

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Root flies and the root environment

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Summary

Secondary plant compounds are frequently altered by insect damage to the host plant. The concentrations of individual glucosinolates in brassica roots are radically altered by the damage of *Delia floralis* and *D. radicum* (Diptera: Anthomyiidae). *D. floralis* damage generally results in a rise in the concentration of aromatic glucosinolates and a relative fall in the concentration of aliphatic glucosinolates. *D. radicum* damage results in the concentrations of both aliphatic and aromatic glucosinolates rising. However, there is no clear evidence that glucosinolate profiles in roots are associated with different levels of antibiotic resistance to *D. radicum* or *D. floralis*. There are indications that the relationship between these pests and other plant components is less complicated. The percentages of plant fibre and lignin in the roots of brassicas increase following *D. floralis* damage. At the same time the concentrations of brassica root sugars are generally reduced by *D. radicum* and *D. floralis* damage. However, more importantly, root sugars appear to influence the larval development of both fly species. Changes in the root sugar content may be due to the physiological response of the plant to damage and to selective feeding by larvae. Evidence from field experiments indicates that the distribution of the feeding of *D. radicum* larvae differs on the roots of different plant genotypes. Increasingly detailed examination of the system may hold the key to the inter-relationship between the changing plant and insect feeding.

Introduction

Crucifer roots vary greatly in structure and chemical composition and consequently the material ingested by root fly larvae varies considerably. The major component of growing plant material is water which larvae ingest with the cellular contents and cell walls. Shaw *et al.* (1993) concluded that root dry matter was linked to antibiotic resistance to *Delia floralis* (Fall.) but not *Delia radicum* (L.) (Diptera: Anthomyiidae). The dry matter of plant material can be divided into cellular contents and cell walls, or fibre. The latter is composed of non-starch polysaccharides, cellulose, hemicelluloses and pectins, as well as the aromatic polymer lignin (Selvendran & Robertson 1990). Cellulose, a polymer of glucose, is a major constituent of fibre, but is indigestible by the majority of insects (Fonty & Gouet, 1989; Prins & Kreulen, 1991). Lignin increases the strength of plant tissue, allowing them to resist environmental stress and it protects plants against a large number of potential pathogens and pests (Wood,

1982).

Changes in the concentrations of cellular contents following insect damage show patterns which are reasonably consistent. *D. floralis* damage generally results in a rise in the concentration of aromatic glucosinolates and a relative fall in aliphatic glucosinolates (Birch *et al.*, 1990; 1992). However, both classes of glucosinolate have been shown to rise following *D. radicum* damage (Hopkins, 1994). No clear relationship has been found between root glucosinolates and larval performance. Sugars vary widely in the roots of mature swede plants (Bradshaw & Griffiths, 1990) but prior to the initiation of these studies little was known of the sugars in brassica roots prior to bulb development. Patterns in the relationships between root sugar content, insect larval performance and damage levels were investigated in these studies.

Progress

Turnip root fly attack and root chemistry

The total sugar content of undamaged swede (*Brassica napus* var. *napobrassica*) varied between 7% and 19% of the root dry matter and fell following *D. floralis* damage. Glucose and fructose content, which were very closely linked, entirely accounted for the fall in total sugar content. Pupal weight correlated strongly with concentrations of total and individual sugars (Hopkins *et al.*, 1993). Extension of this work to include kale (*B. oleracea* var. *acephala*) and rape (*B. napus* var. *napus*) demonstrated that concentrations of individual sugars (glucose, fructose and sucrose) varied between and within crop type and that sucrose concentrations fell following *D. floralis* damage to kales and rapes (Hopkins *et al.*, in press). *D. floralis* damage increased the fibre and lignin contents of the cultivars studied.

Cabbage root fly attack and root chemistry

D. radicum feeding on swede (good host), rape (intermediate host) and kale (poor host) caused a reduction in total root sugar content, as found for *D. floralis*. Changes in individual sugars were similar to those found for *D. floralis*. However, the sucrose content of some swede genotypes was reduced by *D. radicum* damage, whereas *D. floralis* damage did not affect its concentration. Relationships could be found between measures of *D. radicum* performance and root sugar content (Hopkins, 1994).

Turnip root fly and cabbage root fly feeding damage

Birch (1988) investigated the depth of larval penetration of *D. floralis* on different swede genotypes and found considerable differences in the ratio of external damage score to internal mines and the ratio of internal to external damage score. The pattern of larval feeding was simply different on the different genotypes tested.

Using swede and kale roots from a field experiment, *D. radicum* damage was scored according to existing indexes. It was also noted whether damage was on primary root or side root. Established root scoring techniques showed a fairly consistent variation of $x1.5$ between genotypes. However, scores showed cv. Doon Major was more likely to be damaged on the central bulb (P=0.002) than all other genotypes. The presence or absence of damage on the side roots was significantly (P<0.001) different between plant genotypes and ranged from a probability of 0.88 (kale cv. Fribor) to 0.33 (swede breeding line GRL aga) (Hopkins, 1994).

Discussion

Evidence for antibiosis resistance to root flies is not new, nor are the indications that it is important and requires detailed observation of damage. Swailes (1968) restricted *D.radicum* larvae to a small portion of the root and contrasted feeding on the intact skin with that on inner root tissues. It was concluded that skin characteristics were critical to the survival rates of *D.radicum* larvae. Finch & Coaker (1969) found the cortex of the root was not a barrier to larval penetration. Ryan & Behan (1973) identified cephalic sensory receptors on the larvae of *D. radicum* which were structurally adapted as chemoreceptors, odour receptors and mechanoreceptors. Behavioural studies of root feeding larvae are difficult and consequently uncommon. However, Koštál (1992) clearly demonstrated that *D. radicum* larvae utilise odour receptors to orientate to a range of volatile plant compounds. There are marked differences in the feeding strategies which have been adopted by *D. floralis* and *D.radicum*. Whilst *D. floralis* usually mines deeply within the root, *D.radicum* feeds in shallower mines which rarely penetrate the cortex. In addition, both species of fly have been shown to vary their feeding site on different plant genotypes (Birch, 1988; Hopkins, 1994). Although receptors have not been characterised or behavioural responses recorded it seems clear that root fly larvae must be responding to chemical and/or physical cues which influence feeding patterns on the roots of their host plants.

D. floralis and *D. radicum* performance are both linked to root sugar concentrations. However, there are difference in the changes in chemical composition with damage found for these root fly species. Considering the evidence presented above one possible explanation for this difference does become apparent. Due to the necessities of the chemical analysis, root samples are analysed and bulked from all the tissues of several roots. *D. floralis* mine deeply in the root and thus the whole root may well be representative of the tissue on which they feed. However, *D. radicum* are surface miners and do not penetrate deeply into the root tissue. In a sample of the whole root, the surface tissues make up a relatively minor component of the whole and these tissues probably have a different chemical composition.

Roots are not composed of uniform tissue and root fly damage is not evenly distributed over that tissue. To date, no data has been published on the distribution of sugars within the roots of developing brassicas and their influence on the intake of feeding root fly larvae. Consequently, even if the dietary requirements of *D. radicum* and *D. floralis* are identical, the effect of different plant genotypes will not necessarily be the same, owing to variation in feeding sites within the roots between the two species. Extensive work on these two species provide indications that many assessment of the damage or the plant's chemical composition may be inaccurate. Whilst it is important to be efficient when making selections in a large breeding programme it is also evident that detailed studies on mechanisms require more accurate assessment of the extent and the nature of the damage to the roots. Further work aims to improve knowledge of the distribution of compounds in brassica roots and the differences in the distribution of damage found on brassica roots.

Résumé

Mouches des racines et environnement racinaire

Les composés secondaires d'origine végétale sont souvent altérés par les dégâts d'insectes occasionnés aux plantes-hôtes. Chez *Brassica*, les concentrations racinaires des divers types de glucosinolates sont radicalement modifiées par les dégâts dus à *Delia floralis*

(Fall.) et *D. radicum* (L.) (*Diptera, Anthomyiidae*). *D. floralis* provoque généralement une augmentation de la concentration en glucosinolates aromatiques, et une baisse relative de la concentration en glucosinolates aliphatiques. *D. radicum* augmente les concentrations des deux sortes de glucosinolates. Cependant, il n'est pas démontré que les profils de glucosinolates des racines soient liés aux différents niveaux de résistance de type antibiose, à l'égard de *D. radicum* ou *D. floralis*. Certains indices tendent à montrer que la relation entre ces ravageurs et d'autres composants de la plante est moins complexe. Les taux de fibres et de lignine dans les racines de *Brassica* augmentent à la suite d'attaques par *D. floralis*. Parallèlement, les concentrations en sucres racinaires sont généralement réduites suite aux dégâts dus à *D. radicum* et *D. floralis*. De manière plus importante, les sucres racinaires semblent influencer le développement larvaire des deux ravageurs. Les modifications de teneurs en sucres pourraient provenir de la réaction physiologique de la plante à l'attaque, et à la nutrition sélective des larves. Des essais en champ indiquent clairement que les larves de *D. radicum* montrent des différences dans la localisation des choix trophiques sur les racines de divers génotypes de plantes-hôtes. Un examen plus détaillé du système pourrait fournir la clé régissant les relations entre les modifications physio-structurelles de la plante-hôte et la nutrition de l'insecte.

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Recent progress in understanding the role of leaf surface chemistry in susceptibility/resistance to cabbage and turnip root flies (*Delia radicum, D. floralis*)

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Summary

Collaborative studies continue to focus on host selection behaviour, contact chemoreception of oviposition stimuli and chemical analyses of leaf surface components, particularly glucosinolates and "CIF" (Cabbage Identification Factor). The main application of these studies is to target breeding for resistance to root flies. Our previous results showed that antixenosis was the main component of resistance operating in the brassicas studied (kales, swedes). The observed differences in preference for oviposition are largely due to quantitative differences in the methanol-soluble leaf surface compounds. Ovipositing females detect these chemicals by tarsal contact chemoreceptors. A procedure for the chemical fractionation of electrophysiologically-active compounds was developed. The resulting glucosinolate and CIF fractions were both found to be active in behavioural and electrophysiological assays. In behaviour assays, discrimination between resistant and susceptible swede genotypes was more distinct using the CIF than the glucosinolate fractions. With respect to the different levels of antixenotic resistance in the *Brassica* genotypes tested, the surface concentration of CIF seems to be most important, although certain glucosinolates also seem important as oviposition stimuli. Electrophysiological studies showed that in both *Delia* species chemoreceptors in some D-sensilla respond to glucosinolates. These sensilla are more sensitive to particular glucosinolates (e.g. glucobrassicin and glucobrassicanapin) and much less sensitive to others (e.g. glucoiberin or progoitrin). Some differences in sensitivity between the two species to particular glucosinolates were also found (e.g. glucotropaeolin). The C5-sensilla of *D. floralis* were less sensitive to CIF activity than those of *D. radicum*, indicating other differences between chemoreceptor systems of the two *Delia* species. A new LC-MS method was developed at SCRI to quantify CIF from small amounts of surface extracts. Initial results are promising, producing a strong correlation between CIF concentrations measured by LC-MS and those obtained indirectly from electrophysiological responses of C5-sensilla.

Introduction

Cabbage and turnip root flies (*Delia radicum, D. floralis*) are two major pests of horticultural crucifer crops. Insecticides frequently fail to control these pests because peak activity for egg-laying (up to 3 generations, over several months) varies locally and regionally. Therefore predicting when to apply insecticidal products is difficult. Also, in hot dry summers the active ingredients from spray or drench applications often fail to reach the roots where larval feeding usually occurs. Breeding for resistance to root flies has become a more achievable target because a) sources of strong and intermediate resistance have been identified (Alborn *et al.,* 1985; Birch, 1989) and b) the resistance mechanism(s) operating are now being fully characterised (Birch, 1988; Ruuth, 1988; Hopkins *et al.,* 1992; Baur *et al., in press*). Our major aim is to develop a rapid and reliable primary screening system for root fly resistance, based on chemical and/or molecular markers. A seedling screening system using selectable markers (chemical or molecular) could replace expensive and variable field trials in the early stages of the breeding programme, prior to field evaluation of more advanced breeding lines.

Recent progress

Behaviour and electrophysiology bioassays

Previous studies have clearly demonstrated that antixenosis (resulting in oviposition non-preference by females) is the major component of resistance operating in the *Brassicas* studied, although moderate levels of antibiosis and tolerance to root damage by larvae have also been detected (Ellis & Hardman, 1975; Alborn *et al.,* 1985; Birch, 1988; 1989). Detailed behaviour studies showed that female flies usually accept or reject a host plant as an oviposition site after landing and walking on the leaf surface and that host acceptance is mediated by non-volatile leaf surface chemicals (Hopkins *et al.,* 1992; Roessingh *et al.,* 1992a; b; Baur *et al.*, in press).

Based on UK-Swiss collaboration, our recent efforts have been to a) identify the main leaf surface chemicals involved in host recognition and stimulation of oviposition, b) assess the role of these compounds in host plant resistance, and, c) develop techniques to quantify these surface compounds from small amounts of extracts, as, for example, from individual leaves of small plants. Both behaviour and electrophysiological bioassays have been used as tools to guide the search for active compounds in complex leaf surface extracts and derived fractions. This approach has proved successful; the main oviposition stimuli (a new highly active compound referred to as "CIF" (Cabbage Identification Factor) and several known glucosinolates have now been identified for both root fly species (Roessingh *et al.,* 1992a; b; Simmonds *et al.*, 1994; Baur *et al.,* in press).

Furthermore, the tarsal chemoreceptors perceiving these compounds have been characterised (Roessingh *et al.,* 1992a; b; Isidoro *et al.,* 1994; Simmonds *et al.*, 1994). Ongoing structure-activity relationship (SAR) studies are comparing behavioural and electrophysiological responses to CIF and glucosinolates for cabbage and turnip root flies (Roessingh *et al.,* 1992a; Simmonds *et al.,* 1994; R. Baur & M.S.J. Simmonds, unpubl.). These studies show that glucosinolate receptors on the prothoracic tarsi of both species are especially sensitive to glucobrassicin (indole-based), glucobrassicanapin (aliphatic) and gluconasturtiin (aromatic).

Glucosinolate side chain modifications also modify activity. A significant correlation was found between the overall length of side-chain and stimulatory activity to *D. radicum* and

D. floralis. Slight modifications to the chemical structure also resulted in altered neural and behavioural activity. For example, the addition of a methoxy group resulted in a decrease in activity for neoglucobrassicin (compared with glucobrassicin). In contrast, sinalbin (hydroxylated glucotropaeolin) had the same electrophysiological activity as glucotropaeolin. Some glucosinolates (e.g. gluconasturtiin) possibly show dual behavioural activity, stimulating oviposition by *D. radicum* at low concentrations but depressing oviposition at increased concentrations. Thus female flies appear to use both qualitative and quantitative information from leaf surface glucosinolates when selecting hosts. Interesting inter-specific differences in behavioural and neural responses to certain glucosinolates (e.g. progoitrin, glucotropaeolin) were detected and are being further studied. The two *Delia* species also appear to differ in their detection of CIF on the leaf surface (Baur *et al.,* in press; R. Baur & M.S.J. Simmonds, unpubl.).

The non-glucosinolate compound CIF (Roessingh *et al.,* 1992b) appears to be a primary oviposition stimulus for both root fly species, being 2-3 orders of magnitude more active in stimulating the C5-sensilla of *D. radicum* than the most active glucosinolate tested, glucobrassicin. In oviposition choice assays the CIF fractions of leaf surface extracts from resistant and susceptible swedes received 1.8-4.6 times more eggs than the corresponding glucosinolate fractions, confirming the importance of CIF as a primary oviposition stimulus (Baur *et al., in press*). Based on an initial assessment of four *Brassicas* (1 susceptible, 2 partially resistant, 1 highly resistant in oviposition preference tests) the degree of susceptibility (i.e. preference by flies during oviposition) can be largely predicted from their profile of stimulatory leaf surface compounds (CIF and certain glucosinolates). In the two resistant kales tested we also obtained possible evidence for other, as yet unidentified, deterrent compound(s) determining the strong antixenosis exhibited in oviposition preference tests. Differences in wax chemistry between these plants may also be important in influencing the perception of more polar compounds (e.g. glucosinolates) to root flies during host recognition on the leaf surface (Shepherd *et al.,* 1995).

Leaf surface chemistry

Sensitive analyses of plant glucosinolates are now routine using HPLC and can be applied to leaf surface extracts and fractions (Hopkins *et al., in prep.*). The limited studies to date indicate that profiles of leaf surface glucosinolates differ between *Brassica* crops and cultivars/breeding lines. The ratios of individual glucosinolates found on the plant surface and internally also showed considerable differences (Roessingh *et al.*, 1992). Since adult female flies do not appear to rupture the leaf surface during the exploratory phase of host selection it is important to attempt to quantify compounds as they occur naturally on the plant surface. This necessitates very brief (5 second) dips of leaves into organic solvents, to minimise leaf surface damage and internal leakage of compounds from interior tissues. Using Scanning Electron Microscopy (SEM) we have checked that surface extraction of waxes and polar compounds using these brief dips does not damage cell structure on the leaf surface (Shepherd *et al.,* 1995).

A new Finnigan MAT LC-MS with electrospray interface (Liquid Chromatograph linked to a Mass Spectrometer) is being used at SCRI to develop a system for directly quantifying the leaf surface content of CIF, in collaboration with visiting Swiss chemists. The initial tests are promising. From a leaf surface extract it is now possible to measure directly CIF contents of *Brassicas*. Analyses of extracts from swede cv Doon Major (highly susceptible), SCRI swede breeding line GRL agaa (resistant) and kale cv Dwarf Green Curled gave results from LC-MS which correlated closely with indirect assessments of CIF using electrophysiological activity recorded from tarsal C5-sensilla of *D. radicum* (*in prep.*).

Further development and validation of the LC-MS screening method are currently in progress. Our knowledge of the plant chemicals involved in antixenotic resistance mechanisms to root flies and new analytical tools open up opportunities for developing a targeted screening programme for root fly resistance, based on chemical assessment of oviposition stimuli and deterrents on the leaf surface.

Résumé

Progrès récent dans la connaissance du rôle des composés chimiques de la surface foliaire dans la sensibilité/résistance à l'égard de la mouche du chou et de la mouche du radis *(Delia radicum, D. floralis)*

Les recherches poursuivies en collaboration se concentrent sur le comportement de choix de la plante hôte, la chimioréception de contact des stimuli de ponte et l'analyse chimique des composants de la surface foliaire, en particulier les glucosinolates et le "CIF" (Cabbage Identification Factor). Leur application principale vise la sélection variétale pour la résistance aux mouches des racines. Les résultats antérieurs ont montré que la résistance de type antixénose était la composante principale de résistance qui intervient chez les *Brassica* étudiés (choux, rutabagas). Les différences des préférences de ponte observées sont dues en grande partie aux variations quantitatives des substances de la surface foliaire solubles au méthanol. Celles-ci sont détectées par les chimiorécepteurs de contact situés sur les tarses des femelles prêtes à pondre. Une méthode de fractionnement chimique des composés électrophysiologiquement actifs a été développée.

Les fractions glucosinolates et CIF s'avèrent toutes deux actives dans les essais comportementaux et électrophysiologiques. Toutefois, les fractions CIF se sont montrées de plus importants facteurs de discrimination lors des essais comportementaux entre des génotypes de rutabaga résistants et sensibles. Eu égard aux différents niveaux de résistance antixénotique chez les génotypes de *Brassica* testés, la concentration en CIF de la surface foliaire semble primordiale, en terme de stimuli de ponte, bien que certains glucosinolates soient également importants. Des études électrophysiologiques ont montré que les chimiorécepteurs de quelques sensilla de type D répondent aux glucosinolates chez les deux espèces de *Delia.* Ces sensilla sont particulièrement sensibles à des glucosinolates donnés (p.e. glucobrassicine et glucobrassicanapine) et beaucoup moins à d'autres (p.e. glucoiberine ou progoitrine). Quelques différences de sensibilité entre les deux espèces existent également (p.e. pour la glucotropaeoline). Les sensilla du type C5 ont été moins activées chez *D. floralis* par le CIF que chez *R. radicum*, ce qui indiquent d'autres différences entre les systèmes de chimioréception des deux espèces de *Delia.* Une nouvelle méthode par LC-MS a été développée pour quantifier le CIF à partir de petites quantités d'extraits de surface foliaire. Les premiers résultats sont prometteurs, montrant une forte corrélation entre les concentrations de CIF mesurées par LC-MS et celles obtenues indirectement par les réponses électrophysiologiques des sensilla du type C5.

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Biologically active plant compounds in relation to plant resistance to insects: impact on pest metabolism

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Summary

Two types of insect physiological response to host plant antibiosis have been revealed. These types appear to be a characteristic feature of two groups of phytophagous insects which feed on either the reproductive or the vegetative organs of plants. In the first case the atreptic protective barrier (inferior nutrition) is of crucial importance in host plant resistance while in the latter case a physiological barrier (impact of secondary plant metabolites) plays a decisive role. Different types of alterations in insect metabolism in response to plant antibiosis have been observed.

Introduction

The use of host plant antibiosis makes it possible to suppress a pest species and decrease their harmful effect on crops. The effect of the host plant antibiosis is exhibited by diverse forms of insect response. It has been found that the antibiotic effect is achieved by a complex of both local and general morpho-physiological and biochemical alterations in the insect organism resulting in aggravation of their physiological state, lowering their biotic potential or death. A variety of plant properties has acquired the significance of protective barriers during the evolution of plant-insect interactions. The system of immunity of plants includes a number of barriers, such as atreptic, morphological, growth, physiological and ontogenetic ones (Shapiro & Vilkova, 1972). The antibiotic effect of the host plants on their consumers is determined mainly by the action of two barriers: atreptic (inferior nutrition) and physiological (influence of biologically active plant compounds) ones. The purpose of the investigation reported here was to study the physiological response of phytophagous insects in the context of location of their feeding sites on reproductive or vegetative plant organs. The atreptic barrier seems to be of major importance for the first mentioned group whereas a physiological barrier appears to be most important for the latter group of insects.

Material and Methods

Four test pest species, namely, the sun pest, *Eurygaster integriceps* Put. (Hem., Scutelleridae), cotton cutworm, *Helicoverpa armigera* Hbn., Lep.:Noctuidae), European corn borer, *Ostrinia nubilalis* Hbn. (Lep.:Pyralidae) and Colorado potato beetle, *Leptinotarsa decemlineata* Say.(Col.:Chrysomelidae) were used in the investigation.

Both natural and laboratory insect populations have been used in the investigation; the insects were reared on different genotypes of the host plants. The age of the insects was taken into account as well as their weight and physiological state. Hemolymph and a homogenate of the test insects were analysed by means of spectrophotometry and

chromatography. The following indices were determined as criteria of insect metabolism: activity of oxidation enzymes (succinatoxidase, ascorbinatoxidase, peroxidase, poliphenoloxidase) and acid phosphatase, content of lipids and proteins by Stroev (1986).

Results and Discussion

Insect feeding with reproductive organs: response to host plant antibiosis

A complex of the effects was observed when the sun pest specimens were reared on grain of resistant wheat cultivars, such as: disorientation of feeding behaviour, the increase in energy expenditure for searching suitable feeding sites and digestion and decline in survival along with the weight of the pest specimens and their fertility. The term "partial starvation" was proposed for the definition of this complex of effects. The retention of general dynamics of metabolic processes together with suppression of the metabolism to different extents (depending on level of starvation) are peculiar to this type of the insect response for the plant antibiosis. The character of the sun pest response to external impacts changes during its ontogenesis. The inferior nutrition at a period of post-embryonic development entailed physiological and functional depression of insect specimens.

Thus, a high energy cost of insect nutrition on resistant cultivars impairs the physiological state of the pest specimens resulting in depression of all the main vital functions (Fig.1).

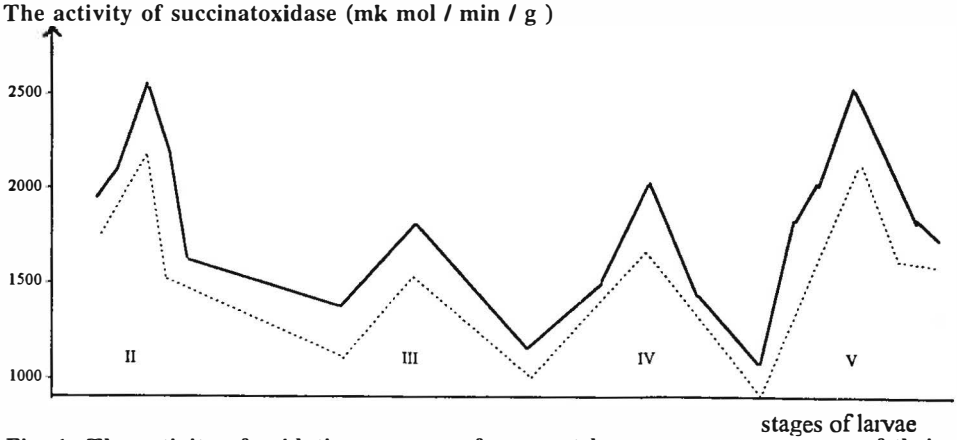

Fig. 1: The activity of oxidative enzymes of sun pest larvae as a consequence of their nutrition on wheat cultivars differing in their resistance to the pest.

——— - **susceptible**

····· - **resistant**

It has been shown that the level of vulnerability of endosperm bio-polymers to insect enzymes is of prime importance in resistance to the sun pest. This mechanism of the host plant resistance to insects was classified as an atreptic barrier. This atreptic barrier causes a peculiar kind of protection of plant biopolymers against hydrolitic decomposition by the sun pest enzymes, resulting in the suppression of metabolic processes especially the oxidative ones. The peculiarities of its impact on the insects allow to assign it to antibiotic type of resistance (Ivashchenko, 1971; 1976).

Insect feeding with vegetative organs: response to host plant antibiosis

The immunological functions of primary and secondary plant metabolites along with inhibitors of insect enzymes have been the focus of attention recently. Secondary plant metabolites are of major importance for the herbivorous insects which feed on vegetative organs of plants (e.g. Colorado potato beetle, cotton cutworm, European corn borer, etc.). Physiologically active plant compounds (secondary metabolites) influence the insect metabolism in a different way, resulting in another type of insect response (in comparison with their response to the influence of atreptic barrier of host plant resistance). A significant difference has been found in the level of metabolism of pests when fed on vegetative organs of crop genotypes differing in their pest resistance. Feeding with resistant crop cultivars entailed general depression of insect metabolism. The extent of varietal resistance to pests has been found to be directly related to the form and content of secondary metabolites. Fat and carbohydrate metabolism of pests on different cultivars has been analysed revealing specific alterations in utilization and energetic expenditure of plastic and energetic compounds. A greater expenditure and lesser accumulation of lipids by the insects was observed on resistant host plant cultivars. The increase in activity of acid phosphatase pointed to the onset of the pathological process well before the disruptions of insect metabolism were manifest. High activity of this enzyme testifies that the insect is mobilising reserve lipids or is unable to synthesize them (Table 1 & 2).

Table 1. The influence of gossypol on activity of oxidative enzymes in cotton cutworm larvae.

Oxidative enzyme in	Activity of enzyme in relation to nutrition to feeding substrate (Mean ± SEM)	
mkMol/min/gm	without gossypol	1.3-1.5% of gossypol
succinatoxidase	22.06 ± 0.29	12.80 ± 0.01
ascorbinateoxidase	19.10 ± 0.12	13.0 ± 0.4
peroxidase	13.22 ± 0.12	19.15 ± 0.46
poliphenoloxidase	15.66 ± 0.26	23.60 ± 0.29

Table 2. Content of lipids and activity of acid phosphotase in European corn borer larvae in the context of its feeding with maize genotypes differing in their resistance to the pest.

Hybrid, line	Index of resistance	Content of lipids mkg/g, mean ± SEM	Activity of acid phosphotase, mean ± SEM
WF - 9	S	285.0 ± 32.3	0.262 ± 0.037
KY 303	S	254.3 ± 64.5	0.217 ± 0.053
C 31 A	R	137.4 ± 29.7	0.682 ± 0.044
A 619	R	145.9 ± 31.2	0.543 ± 0.665

R - Resistant, S - susceptible

The total content and fraction constitution of proteins are other important indices of the physiological state of phytophagous insects. It has been shown that high concentrations of alkaloids and polyphenols in food entail an increase in total content of proteins and alterations of the albumin/globulin ratio. These effects give an indication of the protective response of the insect organism (Table 3).

Table 3. The content of proteins and their fractional composition in homogenates of Colorado potato beetle in the context of pest feeding with potato cultivars differing in their resistance to the pest.

Potato cultivars	Index of resistance	Content of proteins mkg Mean ± SEM	Protein fractions, %			
			albumin	globulin		
				α	β	γ
Nezabudka	S	110.0 ± 9.2	46.0	24.6	9.4	20.0
Lorch	S	120.0 ± 1.7	42.7	28.5	7.4	21.4
Rannia Roza	S	122.0 ± 1.7	44.6	26.7	11.7	17.0
Lvovsky bely	R	200.0 ± 6.7	58.0	2.0	12.0	28.0
Zarevo	R	175.0 ± 3.6	58.6	3.7	11.3	26.4
Temp	R	193.0 ± 4.2	57.0	3.9	13.7	25.4

The feeding of insects on crop genotypes characterised by high concentrations of alkaloids and polyphenols resulted in a significant decrease in the activity of a group of oxidases (succinatoxidase & ascorbinatoxidase). This effect suggests the disruption of insect energy and respiratory metabolism. At the same time the activity of another group of oxidase enzymes (peroxidase & poliphenoloxidase) has been found to be increased suggesting that the compersatory-adaptative response of insects was evolved.

Conclusions

Two types of response of phytophagous insects to host plant antibiosis have been revealed during the investigation. These two types of response determined by two different barriers for host plant protection: atreptic and physiological ones. The first type of response has been investigated using the sun pest, it seems to be peculiar to the insects which feed on the reproductive organs of the plants. A decrease in the total level of oxidative metabolism resulted from an inferior nutrition is characteristic of this type of response. The second type of response seems to be characteristic of insects which feed on plant vegetative organs. It manifests itself as disruption of the inter-relation between metabolic processes in an insect organism including pathological alterations of balance of lipid and protein metabolism and an increase in energy expenditure. Both types of insect physiological response to host plant antibiosis entail the decline in survival, fertility, etc., and eventually a change in the structure and levels of pest populations.

Résumé

Composés végétaux biologiquement actifs en rapport avec la résistance des plantes aux insectes : influence sur le métabolisme du ravageur

Deux types de réponse physiologique de l'insecte à l'antibiose de la plante-hôte ont étérévélés. Ils sont caractéristiques de deux groupes d'insectes phytophages, l'un vivant au dépens des organes reproducteurs, et l'autre, des parties végétatives des plantes. Dans le premier cas, la barrière atrophique protectrice (nutrition inférieure) est d'une importance décisive dans la résistance de la plante-hôte, alors que dans le deuxième cas une barrière physiologique (influence de métabolites secondaires des plantes) joue un rôle déterminant. Différents types de modifications du métabolisme de l'insecte en réponse à l'antibiose de la plante ont été observés.

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Induced chemical and physiological responses in brassicas to turnip and cabbage root fly (*Delia floralis, D. radicum*) attack

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Summary

Our studies are focussed on understanding the roles of glucosinolates and other brassica chemicals in determining host preference (adult oviposition) and host status (larval development) for *Delia floralis* and *D. radicum*. We have shown that root flies induce both local and systemic chemical and physiological changes (e.g. total sulphur, individual glucosinolates, sugars, lignin, compensatory plant growth) in their hosts during attack by larvae on roots. Collaborative experiments show that these interactions are complex, involving plant genotype, extent and nature of the damage, it's timing relative to plant growth stage and environmental stress factors. Artificial root damage does not fully mimic these effects. The ecological impact of these damage-induced plant responses as signals to the second (e.g. later attack by pests) and to the third (natural enemies) trophic levels are now being investigated. Initially, changes in key leaf surface (e.g. specific glucosinolates and novel compounds) and in volatile chemicals, induced by larval root damage, are being quantified and related to effects on adults' oviposition preferences.

Résumé

Réactions chimiques et physiologiques induites chez les *Brassica* par une attaque de la mouche du radis (*Delia floralis*) et de la mouche du chou (*D. radicum*)

Nos études se concentrent sur la compréhension des rôles des glucosinolates et d'autres substances chimiques des *Brassica*, en déterminant la préférence pour l'hôte (ponte des adultes) et le statut de ce dernier (développement larvaire) pour *Delia floralis* et *D. radicum*. Nous avons montré que ces ravageurs induisent à la fois des modifications chimiques locales et systémiques, ainsi que physiologiques (p.e. teneur totale en soufre, glucosinolates individuels, sucres, lignine, croissance compensatoire des plantes) dans leurs hôtes lors de l'attaque des racines par les asticots. Des essais en collaboration révèlent que ces interactions sont complexes, impliquant le génotype du végétal, l'étendue et la nature du dégât, sa coïncidence avec la phénologie de la plante et les facteurs environnementaux de stress. Ces effets ne peuvent être que partiellement imités par un dégât artificiel sur les racines. L'impact écologique de ces réactions induites chez les plantes par un dégât servant de signal au deuxième (p.e. attaques subséquentes de ravageurs) et au troisième (ennemis naturels) niveau trophique, sera étudié.

Dans un premier temps, les modifications des substances chimiques à la surface de la feuille (p.e. glucosinolates spécifiques et nouveaux composés) ainsi que celles des substances volatiles, induites par le dégât des larves aux racines, vont être quantifiées et mises en relation avec les effets sur les préférences de ponte des adultes.

Recent publications

Baur, R., Birch, A.N.E., Hopkins, R.J., Griffiths, D.W., Simmonds, M.S.J. & Stadler, E. (1995). Oviposition and chemosensory stimulation in the root flies *Delia radicum* and *D. floralis* in response to plants and extracts from four *Brassica genotypes*. *Entomologia exp. et appl.* (in press).

Shepherd, T., Robertson, G.W., Griffiths, D.W., Birch, A.N.E. & Duncan, G. (1995). Environmental effects on the composition of epicuticular wax from kale (*Brassica oleracea*) and swede (*B. napus*). *Phytochemistry* (in press).

Hopkins, R.J., Birch, A.N.E., Griffiths, D.W., Morrison, I.M. & McKinlay, R. (1995). Changes in dry matter, sugar, plant fibre and lignin contents of swede, rape and kale roots in response to turnip root fly damage. *Journal of Science of Food and Agriculture* (in press).

Comparison of feeding behaviour and development of two brassica pests *Brevicoryne brassicae* and *Myzus persicae* on wild and cultivated *Brassica* species and an analysis of the glucosinolate and amino acid profiles

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Summary

This paper describes experiments started in the summer of 1995 to investigate the importance of glucosinolates and nutrition to the feeding behaviour and development of the specialist, *Brevicoryne brassicae* (cabbage aphid) and the generalist, *Myzus persicae*, (peach potato aphid). Aphids were monitored electronically on *Brassica* species, *B. fruticulosa, B. spinescens, B. juncea, B. nigra, B. carinata. B. macrocarpa,* and *B. villosa* var. *depranensis* and cultivated brassica varieties. Aphids, monitored for 10 h on the underside of leaves, performed recognizable feeding behaviour on all species, aphids on susceptible plants showed differences in behaviour from aphids on resistant *Brassica* species. Analysis of individual glucosinolates in the wild *Brassica* species identified significant differences in the profiles and in the concentrations present in freeze dried leaves. Samples were also collected from phloem exudates, aphid honeydew, and aphid carcasses. The amino acid composition of these samples will also be determined. Leaf water potential of the *Brassica* species was measured using a pressure bomb.

Introduction

The specialist, *Brevicoryne brassicae* (L.) (cabbage aphid) (Homoptera. Aphididae) and the generalist, *Myzus persicae* (sulzer), (peach potato aphid) continue to increase in importance as pests of horticultural brassicas in most temperate regions. Currently control of aphids depends on insecticides but the production of resistant cultivars is under investigation.

Plants may be rejected due to physical factors such as hairiness, waxiness of cuticles or leaf colour or during probing from chemical cues, such as glucosinolates, present in the entire plant or translocated in the phloem. The nutrient status of the crop may also be important to some aphids species, in fact for many years current ideas have suggested that glucosinolates are important in stimulating feeding by the specialist *B. brassicae* while nutrients, represented by free amino acid concentrations, stimulate the generalist *M. persicae* (van Emden, 1990). The development of insect resistant varieties is a priority in brassica breeding programmes and the production of brassicas with altered glucosinolate profiles is now possible (Giamoustaris, *et al.*, 1994). It is important, however, in breeding crops resistant to the specialist that we do not make them more susceptible to generalist pests. The importance of glucosinolates/nutrients found in a range of wild brassica species and cultivated varieties to the development and fecundity of the two major aphid pests of brassica crops will be investigated by relating feeding behaviour to chemical analysis of glucosinolates/free

amino acids in freeze dried leaves of each plant species, from phloem exudates, aphid carcasses and aphid honeydew. This data will be used to determine the importance of glucosinolates/nutrients in mechanisms of resistance and which may be used by biological control agents such as predators and parasitoids in locating their prey (Weber, *et al.*, 1986).

Materials and Methods

Experimental design

A combination of 20 wild *Brassica* species and cultivated varieties which have previously shown differing resistance to *B. brassicae* were used in this experiment (Singh *et al.*, 1994). Plants for testing were randomised in six blocks (each block contained four plants of each accession) in a Tygan house. Limited temperature control in the house was achieved by raising or lowering the side flaps, but the temperature still fluctuated between 10° and 40 °C from July - September. Two replicates were sown at the same time and there were three sowings during the summer.

Aphid and plant material

Apterae of *B. brassicae* and *M. percicae* were mass reared from individual aphids on the susceptible standard cabbage, *brassica oleracea* var. *capitata* cv. 'Offenham Compacta' in a controlled environment chamber maintained at 18°C ± 2°C day and 15°C ± 2°C night respectively, with a photoperiod L16:D8.
Plants were raised in the Tygan house in 12 cm pots containing a well-drained coarse Levington compost and watered when the compost was dry.

Measurements of aphid performance

The methods used were those described by Pons & Tatchell (1995). Two alates (F_0) of each aphid species were caged, in 2.5 cm x 1cm clip-cages, on the fully-expanded fourth true leaves of each *Brassica* species. The alates were left until they had produced 5 young or for a maximum of 48 h. Nymphs were allowed to develop to adults within the clip-cages, the position of which was changed to minimize damage to leaves. The nymphs were observed daily except at weekends to estimate developmental time. Nymphal mortality was also recorded. When F_1 aphids were adults, two of each clone (when available) were caged individually on the next expanded leaves of the same plant or when neccessary leaves of the same species at a similar stage of development. These aphids were used to determine (a) the day their first offspring (F_2 generation) were born, (b) the pre-reproductive time, (c) the effective fecundity (Md) by allowing the aphids to reproduce during a period equivalent to the pre-reproductive time, (d) the reproductive rate, and (e) the intrinsic rate of increase $r_m = 0.738(\log_e Md)/d$ (Wyatt & White, 1977).

Signal recording of feeding behaviour

Feeding behaviour was recorded on plants between the four and six leaf stage. Feeding behaviour was recorded for 10 h using a modification (Cole *et al.*, 1993) of the equipment described by Tjallingii (1990). Adult apterous *B. brassicae* or *M. persicae* were tethered individually to the amplifier and placed on the abaxial surface of a fully-expanded leaf (Cole, 1994).

Waveform analysis. Recordings were made of the feeding behaviour of six aphids on individual plants of the wild *Brassica* species and crops varieties.

Terminology

1. **Non probing NP** - aphids on the leaf without stylet penetration or electrical stylet contact.
2. **Probe** - a period of stylet penetration.
3. **Pathway** - patterns A (initial penetration), B (salivation), and C (stylet pathway) were regarded as one pattern representing stylet pathway.
4. **Cell punctures** - short intracellular stylet penetrations during pathway.
5. $\mathbf{E_1}$ - sieve element salivation .
6. $\mathbf{E_2}$ - passive phloem ingestion.
7. **X** - xylem feeding.

Non-sequential parameters such as the duration of non-probing, stylet pathway, sieve element salivation E_1 and ingestion E_2, the time spent in xylem ingestion and the number of probes, were recorded. The summation of the behaviour patterns was expressed in a histogram to determine the % of time spent in each feeding activity for each plant tested.

Sampling of glucosinolates

Leaf samples were collected from plants grown in the Tygan house and leaves were immediately frozen in liquid nitrogen before freeze drying.

Phloem exudates were collected by a development of the method of King & Zeevaart (1974) from fully expanded leaves at the 4 - 6 true leaf stage. The petioles were cut with a sharp scalpel and the end of the petiole placed immediatedly into 200µl, 8mM EDTA solution, pH 6.8, contained in an eppendorf tube. The petiole was sealed into the eppendorf tube with strips of Parafilm and the whole placed into a small polythene bag with a dampened filter paper to reduce transpiration. Phloem exudate was collected for 24 h under normal daylight conditions at 20^0C.

Honeydew was collected in aluminium trays fitted into the clip-cages which were held in a horizontanl position by canes and plant ties for 72 h. Samples were stored at -20^0C for analysis.

Aphid carcasses were collected from five adult aphids which had remained for at least 72 h on the *Brassica* plants, these aphids were stored at -20^0 C before analysis.

Chemical analysis of glucosinolates

An approximately 1 g freeze dried leaf sample was extracted at 80°C (proportionally smaller samples were taken for phloem exudates, honeydew and aphid carcasses) , with 10 ml water and shaken for 15 minutes at this temperature in a water bath. After centrifugation 0.1 ml BaOAc/PbOAc was added to a 2ml sub-sample of extractant and re-centrifuged. Individual glucosinolates were analyzed after clean-up on DEAE Sephadex A25 and overnight de-sulphonation as the de-sulphoglucosinolates as described by Heaney, *et al.* (1985). Glucosinolate concentrations are expressed as mg/g dry weight.

Measurement of leaf water potential

This measurement was performed to evaluate any water stress in the leaves from which the aphids were feeding. The leaf was cut at the petiole and was placed in a humid plastic bag to prevent transpiration. The petiole was placed in a split bung which fitted into the lid of a pressure bomb, the bung formed a seal in the opening to the chamber and allowed the cut end of the petiole to protrude. When the petiole was cut the water contents of the xylem were drawn up into the petiole due to a negtive tension created during leaf transpiration. The pressure in the sealed chamber was slowly increased and the cut end of the petiole was watched carefully through a magnifying lens until visible quantities of water started to seep from the xylem vessels. At this point the rise in pressure was halted and the value taken as the water potential in the leaf (the pressure applied equals the tension of the water in the xylem when the leaf was intact). Typical values range from 7 to 17 bar.

Analysis of total free amino acids

A standard mixture of amino acids (Waters) containing 100 pmol/ul of each amino acid was derivatized and analysed by HPLC according to the methods in the Waters AccQ.Tag Chemistry package. Samples were chromatographed using a Kontron low pressure gradient former and Kontron high pressure pump to form a gradient of 0 - 33 % acetonitrile in aqueous buffer in 30 min. A wash step of 100 % acetonitrile was used to remove contaminents. Injections were made on a 46 mm i.d. x 150 mm Waters AccQ.Tag column at 37^{0}C and a flow rate of 1 ml/min. Derivatized amino acids were detected at 395 nm using a Kontron fluorescence detector.

Extracts of freed amino acids from freeze-dried leaves, phloem exudates, aphid honeydew and aphid carcasses, were derivatized and analyzed by HPLC as described above.

Results and Discussion

Preliminary results from electonically monitoring of feeding behaviour of *B. brassicae* and *M. persicae* are shown in Fig. 1 and 2. These figures indicate a significant reduction in passive phloem uptake in the resistant species *B. fruticulosa, B. spinescens* and *B. macrocarpa* for the brassica specialist *B. brassicae* which was closely related to the low fecundity of the aphids on these *Brassica* species. Reduced passive phloem uptake was also found to be highly correlated with the mean fecundity of *M. persicae* on *B. fruticulosa,* and *B. spinescens.* 'Offenham Compacta' which is susceptible to *B. brassicae* reduced the fecundity of *M. persicae* without reducing passive phloem uptake. The phloem in this case may have lower nutrient status as measured by the amino acid composition or the glucosinolate composition of the phloem may be the cause of reduced development, while not deterring feeding. The biggest difference in the feeding behaviour between the two aphid species was the significantly increased time spent in xylem feeding by *M. persicae.* An unusual occurence for *B. brassicae* except on resistant species such as *B. fruticulosa* and *B. spinescens.* Xylem feeding has also been recorded for *Nilaparvata lugens* (Stal), the brown planthopper, on resistant rice varieties (Hopkins, 1991).

The glucosinolate profiles of the *Brassica* species is shown in Fig. 3. The indolyl glucosinolate was only found in significant amounts in the cabbage, cauliflower and Brussels sprouts varieties but may be responsible for the reduced development of *M. persicae* on these crops. Previous reports have suggested that *B. brassicae* performs better than *M. persicae* on diets containing glucosinolates while *M. persicae* performs better when the supply of nutrients is greater as in senescent leaves (van Emden, 1990). Quantitatively, the present results do

not agree with this hypothesis but may indicate that the the presence of individual glucosinolates, such as the indolyl glucosinolates, may be important to the feeding strategies of the two pests.

Honeydew collected from *M. persicae* appeared much more watery than that collected from *B. brassicae* which may be related to the time spent in xylem feeding observed during monitoring of feeding behaviour. In addition, significantly higher quantities of glucosinolates were detected in the honeydew of *M. persicae* than *B. brassicae.* The unexpected xylem feeding may represent a mechanism of removing toxic secondary plant compounds from the generalist aphid system. The concentration of glucosinolates found in the carcasses of *M. persicae* was found to be significantly lower than in *B. brasssicae.* The specialist aphid may sequester the glucosinolates as protective compounds against predators or parasitoids. The differences in glucosinolate composition of specialist and generalist honeydew and carcasses may provide interesting information in further studies designed to integrate resistant varieties and biological control of aphid pests by predators and parasitoids.

Although we already have a number of interesting preliminary results it will only be possible to draw any final conclusions when the last samples have been analysed. But this experiment may allow closer and more detailed investigations into the interactions between aphids, their host-plants and other biological control strategies. The results of which will enable plant-breeders to know more accurately which factors are important in breeding for resistance to *Brassica* pests.

Résumé

Comparaison du comportement trophique et du développement de deux ravageurs des *Brassica, Brevicoryne brassicae* et *Myzus persicae* sur des espèces de *Brassica* sauvages et cultivées, et analyse des profils des glucosinolates et des acides aminés

Ce travail décrit les essais entrepris dès l'été 1995 pour étudier l'importance des glucosinolates et de la nutrition dans le comportement trophique et le développement d'un spécialiste, *Brevicoryne brassicae* (L.) (puceron cendré du chou) et d'un généraliste, *Myzus persicae* (Sulzer) (puceron vert du pêcher). Les pucerons ont été observés électroniquement sur les espèces *Brassica fruticulosa, B. spinescens, B. juncea, B. nigra, B. carinata, B. macrocarpa* et *B. villosa* var. *depranensis* et des variétés de *Brassica* cultivées.

Les insectes ont montré un comportement alimentaire reconnaissable sur toutes les espèces pendant le suivi, d'une durée de 10 heures, à la surface inférieure de la feuille; cependant les pucerons ont un comportement différent sur les plantes sensibles et sur les espèces de *Brassica* résistantes. L'analyse de glucosinolates individuels dans les espèces sauvages de *Brassica* a révélé des différences significatives dans les profils et les concentrations de feuilles lyophilisées. Des échantillons provenaient également d'exsudats de phloème, de miellat de pucerons et de cadavres de pucerons. Leur composition en acides aminés sera également déterminée. Le potentiel hydrique foliaire des espèces de *Brassica* a été mesuré au moyen d'une enceinte pressurisée.

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Isolation of allomones from phloem sap of aphid-resistant lettuce by bioassay guided fractionation

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Summary

The monogenic, dominant and absolute resistance factor of lettuce (*Lactuca sativa*) to the aphid *Nasonovia ribisnigri* (Homoptera, Aphididae) is located in the phloem sap of the plant. In a dual choice bioassay aphids showed a preference for "susceptible phloem" which was collected by EDTA chelation and dissolved in artificial diet. Attempts are being made to isolate the allomones through bioassay guided fractionation. The first fractionation steps show that a fairly polar compound is involved.

Introduction

The absolute, monogenic resistance of lettuce (*Lactuca sativa* L.) to the aphid *Nasonovia ribisnigri* (Nr-gene, Eenink *et al.*, 1982; Reinink & Dieleman, 1989; van Helden *et al.*, 1993) is based on the interruption of sap uptake after the aphid stylets have started ingestion from a phloem sieve element (van Helden & Tjallingii, 1993). Chemical comparison of phloem sap samples for sugars, amino acids, proteins and UV absorbing secondary plant compounds showed no consistent differences between near-isogenic resistant and susceptible lettuce lines (van Helden *et al.*, 1994a; b).

A dual choice bioassay using EDTA-collected phloem sap dissolved in artificial diet (van Helden *et al.*, 1995) showed that *N. ribisnigri* is able to distinguish between resistant and susceptible phloem sap. His bioassay is now used for a "classical" bioassay-guided fractionation of the allomones involved in the resistance. he first results, together with some of the problems encountered during this process are described.

Materials and Methods

Plants and aphids

Plants were grown and aphids (*N. ribisnigri* biotype Wn1) were reared as described earlier (van Helden *et al.*, 1993; 1994a). Larvae were used when 4 to 5 days old (L3 stage). Two sets of near isogenic lettuce lines were used: SUS(susceptible, genotype nrnr) and RES(resistant, NrNr) (SET1), SUS2 (nrnr) and RES2 (NrNr) (SET2).

Phloem extraction procedure

Phloem sap exudate was collected by placing excised leaves from 4-6 week old plants in an aqueous solution with 8 mMol EDTA and 5 mMol phosphate buffer at pH 6 for 20 h. The "crude EDTA extract" was stored at -20 °C.

Bioassays

All dual choice tests were prepared in a laminar flow cabinet. Materials were cleaned with 70% EtOH in water prior to use. ll test solutions were filtered through a 0.45 µm micropore filter. Phloem extracts (or fractions) were dissolved at 20 mg/ml (or equivalent after fractionation) in a complete artificial diet (Harrewijn & Noordink, 1971). A dual choice test was constructed using Fuji Sealon Film^{R}, with two adjacent test solutions as described by van Helden *et al.* (1995) (see Fig. 1), always comparing the twin fractions from resistant and susceptible plants of the same isogenic set. 15-20 larvae of *N. ribisnigri* were introduced and the tests were placed in a 22°C incubator with weak HF-fluorescent illumination from the top. Transparent yellow plastic foil was placed under the light source to stimulate settling on the diet. Aphid distribution was determined after 24 and 48h.

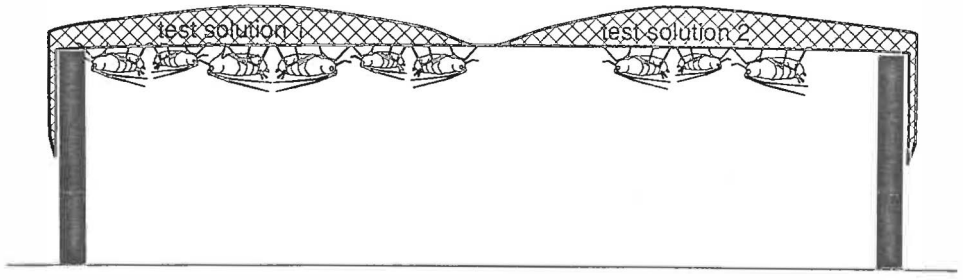

Fig. 1: Cross section of a dual choice bioassay. 15-20 larvae of *Nasonovia ribisnigri* can freely select a feeding site on a test solution.

Sample preparation and fractionation

The method used to remove the EDTA was described by van Helden et al. (1995), but another cation exchange column (CHELEX 100) was used. This EDTA free extract will be referred to as Ed-extract.

Solvent partitioning

Different partitioning steps were tried using different organic solvents. All solvents (p.a. quality) were purified by an extra distillation step prior to use.

Solvent partitioning was tried using the combinations shown in Table 1. Because biological activity was found in the aqueous fraction after BuOH partitioning, an attempt was made to extract all biological activity from the (lyophilized) aqueous fraction by adding BuOH. After ultrasonic mixing and centrifugation the supernatant was collected, evaporated to dryness and tested.

Direct solvent partitioning of the crude EDTA extract (so without EDTA removal) against BuOH was also tried. The activity of the water fraction was not tested in this case.

C-18 fractionation

The organic fraction from solvent partitioning of the crude phloem exudates against BuOH was divided in three fractions using solid phase extraction with a C18 column. Eluents were H_2O/MeOH mixtures of 98:2 (fraction 1), 33:67 (fraction 2) and 0:100 (fraction 3). Fractions were evaporated to dryness, dissolved in H_2O, lyophilized and tested.

Table 1.

A. Summary of the different solvent partitioning experiments of Ed-extracts and crude EDTA extracts.

Water Fraction	Org. Fraction	Biological activity	
		Water	Organic
Ed-extract *	BuOH	+	++
Ed-extract	Butanone	+	++
Ed-extr.+HCOOH,pH 2.3	BuOH	?	++
Ed-extr.+10% NH_4HCO_3	BuOH/iPrOH 1:1	?	?
Crude EDTA extract	BuOH	NT	++

B. Ultrasonic extraction of a lyophilized aqueous phase (which resulted from the solvent partitioning of Ed-extract with BuOH, see *) with BuOH.

Residual fraction	BuOH soluble	?	+/?

++ = strong preference for susceptible extract in nearly all replicates and all tests, + = preference for susceptible extract in most replicates of most tests. ? = test results not clear. NT = not tested

Statistics

Bioassay results were compared by Wilcoxon's signed rank tests at 5% significance level.

Results and Discussion

Results of the different tests are presented in Tables 1 and 2.

A change was made from a Varian CBA cation exchange column (a silica lattice with carboxymethyl as the active group) to Chelex 100 (styrene lattice with iminodiacetic acid group) since this material after loading with Lead (II)ions gave better EDTA removal. However, some coloured (brown/yellow) components were still retained on the column, so the lead-loaded column was not 100% EDTA specific.

As the first objective was to remove the relatively large amounts of sugars and amino-acids present in the Ed-extracts solvent partitioning was used against (water saturated) butanol. The first attempts showed poor results because of impurities in the

solvent, therefore only distilled butanol was used in all subsequent experiments. This resulted in relatively low amounts of the dry matter (DM) (around 5% of the DM of the crude EDTA extract) in the organic phase. This butanol fraction showed clear activity in the bioassays. However, the aqueous phase also contained a clear activity in the bioassay (Table 1, Fig. 2 top half). An attempt was made to increase the amount of DM dissolving in the organic phase by adding a volatile salt (NH_4HCO_3) to the aqueous phase and at the same time increasing the polarity of the organic phase by adding iso-propanol (50% v/v), or adding acid (HCOOH) to the aqueous phase. Although in both cases an increase of DM in the organic phase was obtained, it was not possible to show that all biological activity had transferred to the organic phase (see Table 1). The bioassays were probably hampered by impurities in the volatile salts and the acids. It is also possible that the extracts changed due to these additions (e.g. hydrolysis). A low number of aphids settling on the diets was observed and no preference for susceptible over resistant material. The organic phase showed a good response in most cases, the (desired) absence of a response in the polar phase could not be confirmed beyond doubt.

Table 2. Results of C18 fractionation experiments after 24 and 48 Hr, 10 replicates per test. * = significant difference in preference at $\alpha < 0.05$.

Fraction		Fraction 1		Fraction 2		Fraction 3	
Eluens H_2O/MeOH		98:2		33:67		0:100	
Plant lines	Time(h)	SUS	RES	SUS	RES	SUS	RES
Set1	24	121 *	44	77	71	78	45
	48	95 *	61	56	49	99 *	30
Set2	24	102 *	46	42	59	51	63
	48	128 *	26	32	41	39	73

Subsequent extraction of the lyophilized aqueous fraction with BuOH yielded a very small amount of DM in the BuOH. Biological activity in this fraction, and absence in the residual (water soluble) fraction could not be confirmed beyond doubt (Fig. 2 left middle).

It was decided to continue with a fraction produced by solvent partitioning of the raw EDTA extract (so with the EDTA) against BuOH. This fraction showed good biological activity in all experiments. This was then divided into three fractions on a small C18 column using H_2O/MeOH mixtures (Fig. 2 right and bottom). Only the first fraction (eluent H_2O/MeOH 98:2) always showed very clear biological activity. This suggests that the active compound is a fairly polar compound, possibly a glycoside, with moderate solubility in butanol and little affinity for the C18 chromatography resin.

During the fractionation steps it was observed that the amount of aphids settling on the diet increased. Possibly the loss of deterring compounds which are present in both resistant and susceptible phloem extracts (van Helden *et al.*, 1995), increased the acceptability of the remaining fractions. The tests which showed the clearest discrimination also had the largest amount of aphids settling on the diets. The two sets of

isogenic lines showed similar results for all experiments.

The bioassay, though perfectly suitable for showing differences between extracts, is not very suitable for showing gradual differences. Large differences between replicates within the same test did occur, and results of diferent (replicate) tests were not always identical. Therefore, results of different test cannot be compared, apparently the quality of the extracts or the aphids used was not constant.

On several occasions a complete inversion of the reaction was recorded. This supports the hypothesis that the resistance is based on a (normally stimulating) compound which acts as a feeding deterrent due to an increase in concentration in the resistant phloem. This phenomenon could also cause problems in the present bioassay due to concentration effects. This hypothesis can be investigated by comparing different concentrations of the compound in one test, and testing the diet with or without this compound, but only when the allomones are separated from all possible interfering compounds.

Since the aqueous fraction also exhibited some activity we cannot be sure that the compound(s) which are active in the last fractions represent all active substances. If an allomone is identified a more specific extraction procedure might clarify this.

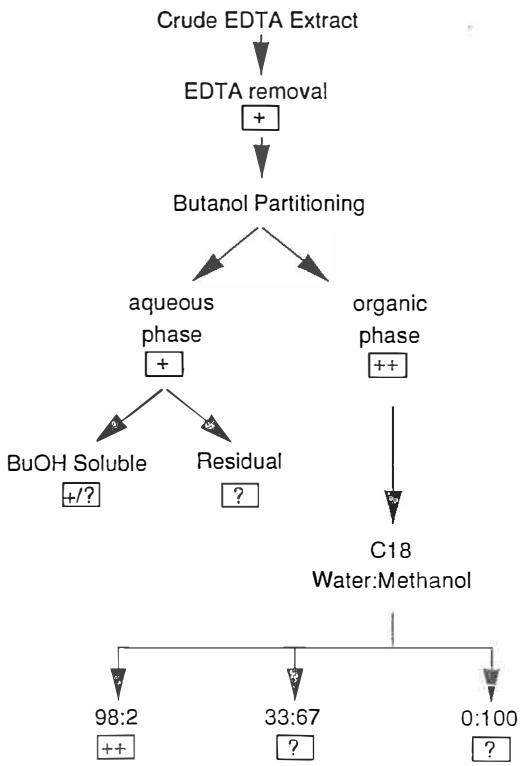

Fig. 2: Fractionation tree showing the different fractionation steps. Results of bioassays comparing twin fractions of resistant and susceptible extracts are given as ++ (very clear preference for susceptible extract) + (preference for susceptible extract) or ? (no preference) in the rectangles at each fractionation step.

Résumé

Isolation d'allomones dans le phloème de laitue résistante au puceron de la laitue à l'aide de fractionnement piloté par biotest

La résistance monogénique, dominante et absolue de la laitue (*Lactuca sativa*) au puceron de la laitue, *Nasonovia ribisnigri* (Homoptera, Aphidiae) est localisée dans le phloème de la plante. Les pucerons montrent, dans un biotest à double choix, une préférence pour le "phloème sensible" qui a été recueilli par chélation à l'EDTA et dissous dans un milieu nutritif artificiel. Nous tentons actuellement d'isoler les allomones à l'aide de fractionnement piloté par biotest. Les premières étapes de fractionnement montrent qu'une substance nettement polaire est impliquée.

Acknowledgements

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Development of a technique for assessing antibiosis resistance in brassica species to the cabbage aphid, *Brevicoryne brassicae*

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Summary

The cabbage aphid, *Brevicoryne brassicae* (L.), is a serious pest of brassica crops in many parts of the world. Several different methods involving field, glasshouse and laboratory facilities have been used to identify sources of resistance to this pest. Field studies were found to be valuable for locating antixenosis resistance when the plants were exposed to large immigrant populations of *B. brassicae*. However, more precise experimental conditions have been found necessary to locate antibiosis resistance. Several methods described in the past for evaluation of antibiosis resistance to cabbage aphid had drawbacks which made them difficult, unreliable and time-consuming. With these difficulties in mind improved techniques have been devised involving the raising of test plants, rearing of insects, caging of aphids on plants and assessing resistance using different parameters. These techniques are discussed in this paper.

Introduction

The cabbage aphid, *Brevicoryne brassicae* (L.), is an important pest of brassica crops worldwide. There is a growing concern over the widespread dependence of growers on pesticides to control pests and there is an urgent need for the development of environmentally safe methods of aphid control. The cultivation of resistant varieties of crops offers a desirable method of reducing chemical inputs. In the last two decades 25 studies were done in different countries to locate sources of resistance to cabbage aphid (Singh & Ellis, 1993) which is an indication of the urgent need to develop resistant varieties. Despite concerted efforts, little success has been achieved in releasing commercial varieties resistant to cabbage aphid. One of the reasons for delayed success seems to be the lack of appropriate screening methods to identify sources of resistance. Most of the studies reported in the literature were done exclusively under field conditions where it is essential to establish high insect populations to provide intense pressure for a number of seasons. It is also essential to synchronise the plant development with aphid immigration. These ideal conditions rarely exist in the field. Fluctuating field conditions often lead to inconsistencies in the results obtained and, on occasions, total failure of the experiment. Hence, there is a need to supplement field studies with laboratory experiments which are reproducible, reliable and minimise risks of selecting susceptible plants. Laboratory experiments also provide the opportunity of screening resistant plant material against pest biotypes which is extremely difficult under field conditions at a single site.

We have attempted to develop a standardised screening technique for identifying antibiosis resistance to *B. brassicae* using techniques devised by other workers as a basis (Van Emden, 1966; Dunn & Kempton, 1972; Lara *et al.*, 1979; Ronquist & Ahman, 1990). This technique is easy to follow, reliable, less time consuming and has produced consistent results. The technique involves the following steps:

1. Seed germination

In resistance studies, it is essential to use healthy and disease-free plants of the same physiological stage. This requires knowledge of seedling viability and speed of development. Direct seeding in growth media in plastic pots (Root & Olson, 1969; Miles *et al.*, 1982) or wooden boxes (Lammerink, 1968) may lead to variability in plant growth or even death of weak and diseased seedlings. Therefore to select healthy seedlings, germination should be carried out under controlled conditions. Seeds of most *Brassica* species germinate well at 19°C and, depending on species, take 5 to 8 days to produce seedlings ready for pricking out into pots. Germination is achieved by placing seeds on water-soaked filter paper (9 cm diameter Whatman Filter Paper 91, wet strengthened qualitative grade), placed inside a polystyrene petri-dish of the same diameter. The petri-dish is then sealed with parafilm (American National Can TM/Greenwich CT.06836) to avoid dehydration, labelled top and bottom before placing inside an incubator maintained at 19°C.

2. Plant raising

The potting medium used for test plants must ensure uniform and healthy growth. Standard potting soils (Miles *et al.*, 1982) or mixtures of loam, sand, peat and fertilizer (Root & Olson, 1969) have been found adequate for the purpose. We used Fisons Professional Levington M2 compost which has a medium structure, medium nutrients and standard pH in plastic pots (7 x 7 x 8 cm) which were satisfactory for growth of *Brassica* plants for the entire experimental period.

Procedure: Pots (generally 12 pots/genotype) were filled with M2 compost to within 1 cm of the pot rim. Two seedlings of uniform growth were pricked out into each pot from the petri-dishes. After five days one seedling was removed if both were still alive. Plastic white labels (10 x 1 cm) were used to identify the pots of each genotype. Pots were arranged on mobile benches in the glasshouse equipped to maintain adequate irrigation, temperature and light conditions for growth of the plants. Depending on the *Brassica* species, plants at the four to five leaf stage were ready in 3 to 5 weeks to transfer to controlled environment rooms (maintained at 20°C, RH 70%, Day : Night cycle, 16 : 8 h) in the insect rearing unit for antibiosis studies. These conditions were similar to those used by others in similar experiments; for example, 20°C, RH 60%, light regime 20 L : 4 D (Root & Olson, 1969), 18°C, RH 70%, Day : Night cycle 16 : 8 h (Dunn & Kempton, 1972), 20°C, RH 70%, light regime 20 L : 4 D (Ronquist & Ahman, 1990).

3. *B. brassicae* cultures

Four- to five-week old plants were used to maintain stock cultures of *B. brassicae* in the same controlled environment rooms as mentioned above. The plants were grown in pots in plastic trays (36 x 26 x 10 cm) which were placed inside perspex acrylic cages (39 x 39 x 39 cm). These cages had a wooden base, perspex top and sides and a detachable nylon

mesh front. The back was fitted with fine nylon netting (mesh size 102μ) to provide adequate ventilation and light conditions inside the cage but to exclude other insects and mites. The aphid culture originated from the field at HRI Wellesbourne and was started with the progeny of a single apterous aphid as recommended by van Emden (1966) and Wearing (1972). Overcrowding of the culture was avoided by maintaining insects on stock plants in several cages. The rearing cages were always kept on benches beneath fluorescent lights suitable for the growth of plants. The stock culture could be maintained on various brassica crops. To avoid adaptation to a single host the culture was rotated among three brassica species.

4. Caging of aphids on plants

Securing clip cages on plants: Clip cages were made according to the design of van Emden (1972). Ten plants of each genotype were arranged in a plastic tray (36 x 26 x 10 cm) and clip cages were fitted to the first and second expanded leaves from the top of each plant. Clip cages were supported by empty plastic pots to reduce the stress resulting from the weight on soft and tender leaves of some *Brassica* genotypes

Release of aphids in clip cages: Three healthy apterous *B. brassicae* from the stock culture were released into each clip cage on the lower side of a leaf and left for 16 to 18 h overnight. On the next day (day 0), in the morning only the first born nymph in each clip cage was retained while the adults and remaining nymphs were carefully removed using a camel hair brush so as to avoid disturbance of the nymph and any injury to the leaf. The trays containing plants with aphids in clip cages were kept inside perspex acrylic cages as described above for the stock cultures. The cages were kept in the same controlled environment room as described above. The position of the trays in the cages was changed frequently to avoid any light or position effect on plant growth. Releases of one or two adult aphids/clip cage were also recommended by other workers (Van Emden, 1966; Root & Olson, 1969; Dunn & Kempton, 1969; Lara *et al.*, 1979; Miles *et al.*, 1982; Hussain, 1983; Ronquist & Ahman, 1990) but by caging 3 adults timely production of nymphs was assured. The nymphs retained in the clip cages completed their entire post-natal development on the experimental plants where they were permitted to feed on leaves of only one age category.

5. Recording of biological parameters for antibiosis

The first born nymph retained in a clip cage was observed on every other day for 6 days and then daily for another 5 days to record the date on which the first offspring was produced. From these records it was possible to determine the pre-reproductive period. For another 6 days the numbers of offspring produced were counted and carefully removed from the cages on every other day making sure not to disturb the mother aphid. In the remaining period, daily observations determined the day on which the female stopped reproducing and the day of death. Thus the number of progeny and reproductive period were calculated for adults which had completed their entire development on the experimental plants. At each inspection, clip cages were moved together with the insects to the upper leaves so as to maintain the same leaf position throughout the insects' life. To determine survival rate, records were made of the nymphs surviving after 4 and 8 days following caging. The observations of various biological parameters *viz.* pre-reproductive period, fecundity, reproductive period and survival rates were useful in identifying antibiosis resistance in brassica crops to *B. brassicae*. Of these, fecundity and survival are the most frequently used criteria.

Résumé

Développement d'une technique d'évaluation de la résistance de type antibiose des espèces de *Brassica* à l'égard du puceron cendré du chou, *Brevicoryne brassicae*

Le puceron cendré du chou, *Brevicoryne brassicae* (L.) est un ravageur important dans les cultures de *Brassica* de nombreuses régions du monde. Plusieurs méthodes différentes en champ, serre et laboratoire, ont été utilisées pour identifier des sources de résistance à l'égard dudit puceron. Les études en champ se sont avérées valables pour localiser une antixénose chez les plantes soumises à de fortes populations d'immigrants de *B. brassicae*. Cependant la localisation de l'antibiose nécessite des conditions expérimentales plus précises. Plusieurs méthodes y relatives décrites antérieurement ont souffert d'inconvénients les rendant difficiles à mettre en oeuvre, inexactes et demandant beaucoup de temps. Ceci a conduit à l'élaboration de techniques améliorées comprenant l'élevage de plantes test et d'insectes, le confinement des pucerons sur les plantes et l'évaluation de la résistance à l'aide de différents paramètres. Ces techniques sont discutées dans ce travail.

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Plant characteristics influence biological control agents: implications for breeding for host plant resistance

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Summary

Environmentally benign pest control may employ biological control in combination with host plant resistance. Natural enemies of herbivores that are used in biological control inhabit or visit the crop plants and feed on the herbivores. Therefore the natural enemies may be influenced by a range of plant characteristics, either independently of the herbivore or mediated through herbivore activities. Among these are plant volatiles, secondary plant chemicals, plant morphology, plant tissues and products that are used as a source of nutrition, and visual and vibrational cues. The interaction between host plant resistance and biological control can range from antagonistic, through neutral to synergistic, depending on the kind of interactions among plants and natural enemies occurring in a certain crop system. In this paper the possibilities of successfully integrating biological control and host plant resistance are discussed.

Introduction

Plants have two general ways of defence against herbivorous arthropods. They may combat their enemies directly through characteristics that negatively affect herbivore, or they may do so indirectly by enhancing the effectiveness of natural enemies of herbivores. These two ways of plant defence have been exploited by man to develop two methods of environmentally-benign pest control: biological control and host plant resistance (Figure 1).

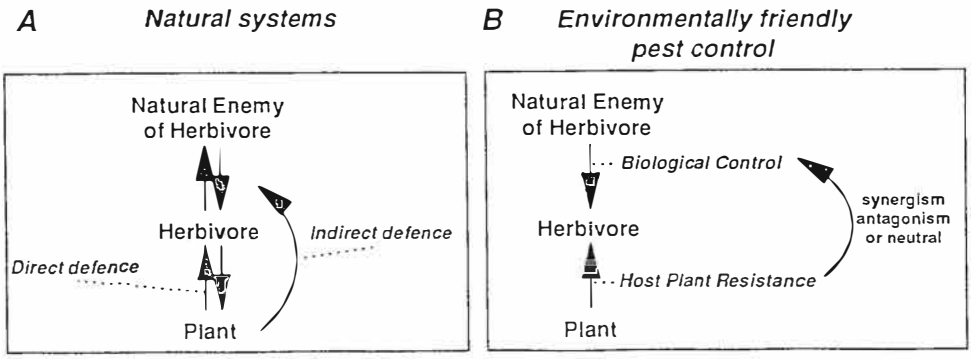

Fig. 1: (A) Direct and indirect defence of plants and (B) their counterparts in pest control.

The objective of biological control is to keep a pest organism under the damage threshold through the use of natural enemies of the herbivores, such as parasitoids and predators. The objective of breeding for resistance is to select plant cultivars that prevent pest organisms from developing populations above the damage threshold. Breeding for resistance is often targeted at absolute resistance, which implies that the crop cultivars do not sustain herbivores at all. However, just as pest species can become resistant to synthetic pesticides, so too can they develop resistance to plant cultivars. Basically, the higher the level of resistance of the host plant to the herbivore, the stronger the selection pressure on the pest to develop resistance. Therefore, plant breeders should develop partially-resistant cultivars. In the past, pest control strategies have often combined resistant cultivars with pesticide applications. However, environmentally-benign pest control should integrate resistant cultivars with biological control. Although it was often considered that the two control strategies are synergistic, this is not *a priori* obvious. Plant characteristics not only affect herbivores, but they may also affect the plant-inhabiting or visiting natural enemies of herbivores. This latter aspect has been well documented in the past 15 years (Price *et al.*, 1980; Boethel & Eikenbary, 1986; Dicke & Sabelis, 1988; van Lenteren & De Ponti, 1990). Therefore, changes in plant characteristics through breeding for resistance to herbivores are likely to result in changes in the way plants affect the natural enemies. Strikingly, in an analysis of all published studies on interaction among host plant resistance and biological control, Hare (1992) found that out of 16 cases only two showed synergism, while in six cases antagonism among the two control strategies was found.

Current political pressures to reduce pesticide application will lead to more and more situations where host plant resistance and biological control could be integrated. For such an integration to be successful, studies are needed on the overall effect of the integration. Otherwise plant breeding programmes may result in a pest control programme that is less rather than more effective. Obviously, antagonism is not only undesirable in individual cases but it could also suppress new developments in environmentally-benign pest control.

Plant characteristics that affect natural enemies of herbivores

Plants can affect natural enemies in various ways, either independently of the herbivore or mediated by the herbivore. The best-known plant characteristics in this respect are briefly described below.

Plant toxins and digestibility reducers

There are many plant secondary chemicals that affect herbivore performance and herbivores are believed to have evolved ways of overcoming their effects. For example, herbivores may sequester toxic plant compounds with the consequence that natural enemies are affected once they attack these herbivores. There are many examples of plant secondary chemicals interfering with predators, parasitoids and pathogens of herbivores (e.g. Barbosa, 1988; Krischik, 1991; Rowell-Rahier & Pasteels, 1992).

On the other hand, digestibility reducers lead to decelerated development, which may prolong exposure to natural enemies in certain vulnerable stages and thus enhance the effectiveness of natural enemies (e.g. Price *et al.*, 1980; Loader & Damman, 1991).

Plant morphology

Cuticle thickness, glandular and non-glandular trichomes and plant architecture are examples of plant characters that can affect herbivores. These characteristics have often been used in plant breeding programmes to select for cultivars that are less susceptible to herbivores (e.g. Obrycki, 1986). Although such characteristics may impede herbivore

movement and the accessibility of plant contents to small herbivores they may also have direct effects on the behaviour of natural enemies that are of a similar size. Moreover, natural enemies usually move over plant surfaces much more than herbivores. Plant morphology can either impair or enhance natural enemy effectiveness. Reduced effectiveness may result from impairment of natural enemy movements by non-glandular trichomes, entrapment of enemies by glandular trichomes and reduced enemy adhesion on smooth plant surfaces (Eigenbrode & Espelie, 1995). Knowledge of plant morphology hampering natural enemy movement has been employed to improve control of greenhouse whitefly (*Trialeurodes vaporariorum*) with the parasitoid *Encarsia formosa* on cucumber (van Lenteren & De Ponti, 1990). On commercial cultivars trichomes strongly impeded movement of the parasitoid. In a breeding programme, van Lenteren & de Ponti (1990) used a hairless cucumber variety and obtained a half-hairy cucumber hybrid by crossing this with commercial, hairy varieties. Hairiness was determined by one dominant gene with intermediary inheritance. On the hairless variety the parasitoids moved so fast that they ran over the whitefly larvae without noticing them, but on the half-hairy variety they had a walking speed twice that on the hairy variety, which resulted in higher encounter rates with host larvae. In greenhouse experiments the performance of the parasitoids was better on half-hairy hybrids than on a commercial, hairy variety, yielding a satisfactory level of control. The seed of the half-hairy hybrid is now available for commercial development by breeding companies.

Enhanced effectiveness can result from plant structures that provide shelter to carnivores, such as domatia that are used by ants or mites (O'Dowd & Willson, 1989).

Nutritional value of plants to natural enemies

Natural enemies can use various plant tissues and plant products, such as floral and extra-floral nectar, plant sap, and pollen, as sources of food (Hagen, 1986). This may result in prolonged presence or long-term arrestment of natural enemies on the plant during periods of herbivore absence. Often this is additional to provision of shelter to natural enemies.

Visual and vibrational cues

Herbivore presence and activities on plants result in visual and vibrational cues that may be used by parasites and predators during foraging. Plant characteristics can decisively affect the intensity of these stimuli. For instance, leaf thickness can determine whether caterpillars produce holes or only windows, which result in different visual cues to their natural enemies; plant morphology affects the transmission of vibrational cues throughout the plant and thus may impair the ability of natural enemies to perceive these cues related to their victim (Dicke, 1995).

Another plant characteristic that can affect natural enemies is the emission of chemical cues. These cues provide a major source of information to foraging arthropod natural enemies. They will be considered separately in the next paragraph.

Chemical plant cues that mediate searching behaviour of beneficial organisms

Foraging behaviour of arthropod parasites and predators is influenced to a large extent by chemical cues, both from their herbivore victims and from the food of the herbivores. This phenomenon has been extensively investigated and many reviews stress that plant chemicals play an essential role in host searching behaviour by arthropods (e.g. Vinson, 1976; Nordlund *et al.*, 1988; Dicke *et al.*, 1990b; Vet & Dicke, 1992; Turlings *et al.*, 1993b). That natural enemies respond to constitutively produced plant volatiles has been known for a long time (Vinson, 1976; Nordlund *et al.*, 1988). However, constitutive plant odours seem to have

a limited information content and may be of particular importance to specialist natural enemies that attack specialist herbivores (Vet & Dicke, 1992). An exciting discovery was made in the past decade: the production of plant volatiles that attract parasites and predators can be induced by herbivore damage (Dicke & Sabelis, 1988; Dicke *et al.,* 1990a; Turlings *et al.*, 1990). In other words, plants have a chemical burglar alarm. Herbivore-induced plant volatiles appear to play an important role in natural enemy foraging behaviour (reviewed by Dicke *et al.*, 1990b; Vet & Dicke, 1992; Turlings *et al.*, 1993b; Dicke, 1994; Takabayashi *et al.*, 1994) and are likely to play a role in many plant-herbivore-natural enemy interactions (Dicke, 1994).

Constitutive natural enemy attractants and plant breeding

In a study of cotton, Elzen *et al.*, (1985) reported more than a 100-fold difference between different cultivars in the emission of volatile terpenes that attract the parasitoid *Campoletis sonorensis*. That differences in the amount of plant-produced parasitoid attractants can decisively affect pest control was demonstrated in a field study of cabbage-aphid-parasitoid interactions by van Emden (1986). More aphids (*Brevicoryne brassicae*) were found on a 'resistant' cultivar than on a 'susceptible' cultivar, when parasitoids (*Diaeretiella rapae*) were present. He showed that the susceptible cultivar, which produced significantly more (2.4 times) of the parasitoid attractant allyl isothiocyanate, was significantly preferred by the parasitoids and had significantly more mummified aphids than the resistant cultivar. This is one of the few examples where the effect of plant volatiles has been investigated in the field and the laboratory. It shows that the terms 'susceptible' and 'resistant' are context-specific and that plant breeding practices may be counter-productive if the effect of natural enemies is not taken into consideration.

Herbivore-induced natural enemy attractants and plant breeding

It has been known for a long time that natural enemies can discriminate from a distance between odours from un-infested plants and odours from plants that are infested by herbivores (Vet & Dicke, 1992). However, it has been known for only 10 years that the volatiles involved are produced by the plant in response to herbivory (Dicke & Sabelis 1988; Dicke *et al.*, 1990a; b; Turlings *et al.*, 1990). This has been demonstrated for several plant-herbivore-natural enemy systems (for reviews see Dicke, 1994; Vet & Dicke, 1992; Turlings *et al.*, 1993b). The induced plant volatiles are released after herbivory and natural enemies discriminate between herbivore-damaged and mechanically-damaged plants. Moreover, herbivore-induced plant volatiles can even be specific for the herbivore that inflicts the damage: natural enemies can sometimes discriminate between plants damaged by different herbivore species (Sabelis & Van de Baan, 1983; Dicke *et al.*, 1990b; Vet & Dicke, 1992; Turlings *et al.*, 1993b). The role of herbivore-induced plant volatiles in extermination of herbivore populations seems to be essential (Sabelis & van der Meer, 1986; Dicke *et al.*, 1990b). Plant cultivars may differ in their attractiveness to natural enemies through herbivore-induced plant volatiles. For instance, the degree of infestation of bean plants by the two-spotted spider mite, *Tetranychus urticae*, that was needed for attraction of predators of the herbivores differed between cultivars (Dicke *et al.*, 1990b) and chemical analyses showed differences in the composition of the volatile blend emitted by different apple cultivars after infestation by *T. urticae* (Takabayashi *et al.,* 1991).

Herbivore-induced natural enemy attractants and plant breeding

So far, more than 15 plant species, more than 10 herbivore species and more than 10 natural enemy species have been used in different combinations in studies on herbivore-

induced natural enemy attractants (Dicke & Sabelis, 1988; Dicke *et al.*, 1990b; Vet & Dicke, 1992; Turlings *et al.*, 1993b; Takabayashi *et al.*, 1994; Dicke 1994). Almost all research conducted on herbivore-induced natural enemy attractants has been done with agricultural plant species. In all cases natural enemies discriminate between herbivore-infested plants and mechanically-damaged plants. The cultivars used in these studies were not selected for their ability to produce herbivore-induced natural enemy attractants. It may be expected that plant cultivars that are currently used have lost the ability to attract natural enemies through induced volatiles partly or completely, because of the absence of selection for this trait in breeding programmes. Therefore, it is of great significance that the characteristic has been found in all plant species studied so far. It will be interesting to investigate wild relatives of crop plants with respect to the quantity and quality of their herbivore-induced volatiles. If wild relatives produce higher amounts of herbivore-induced volatiles, crossing with wild ancestors may yield cultivars with a higher degree of natural enemy attraction.

The use of behaviour-modifying chemicals (info-chemicals or semio-chemicals) in biological control practices with natural enemy arthropods has received much speculation. The spatial distribution of info-chemicals relative to that of the pest individuals appears to be important in order to improve rather than to impair natural enemy foraging behaviour (see Dicke *et al.*, 1990b for discussion). Herbivore-induced plant volatiles have several advantages in this respect: their spatial distribution is closely linked to that of the herbivores and they are produced in much larger amounts than herbivore volatiles. In addition, the application of these info-chemicals does not need any action by man. Basically, it can be seen as the recruitment of biological control agents by the plant after being attacked by herbivores. An important additional aspect for pest control is that these plant-produced volatiles do not need registration under pesticide laws.

Thus, in analogy to the study on constitutive plant volatiles by Van Emden (1986), the effect of herbivore-induced plant volatiles deserves to be incorporated in plant breeding practices. To do so, one should compare different cultivars for the the production of natural enemy attractants in response to herbivore infestation. Recent progress in the basic knowledge of the signal-transduction process may facilitate this comparison of cultivars. It is laborious to compare the induction in response to herbivore infestation for all cultivars. Herbivory can be simulated by application of herbivore oral secretions (Turlings *et al.*, 1990; 1993a; Mattiacci *et al.*, 1995). Several attempts have been made to identify the active components in these herbivore secretions (Turlings *et al.*, 1993a; Mattiacci *et al.*, 1995) and recently an elicitor in the oral secretion of caterpillars of the large cabbage white, *Pieris brassicae*, was identified as ß-glucosidase. This enzyme induces the production of volatiles by cabbage that attract the parasitoid *Cotesia glomerata* (Mattiacci *et al.*, 1995). Elicitor identification may facilitate the comparison of cultivars for their ability to produce natural enemy attractants.

Implications for future selection programmes

A wide variety of plant attributes appears to influence the effect of natural enemies on herbivore populations. From an applied perspective this may seem to represent a rather confusing situation: for successful integration to occur between breeding host plants for resistance and selecting natural enemies for biological control one needs to screen all possible interactions between plants and natural enemies. Although it is valuable to have all this knowledge, it is evidently impractical to obtain all this information before integration of plant breeding and biological control can be started. Thus, priorities must be set. The major lesson to be learned is that although host plant resistance and biological control can be synergistic it is not self-evident that this is the case (Boethel & Eikenbary, 1986). Thus, both plant breeders and entomologists should take into consideration the constraints of each other's

practices where integration of host-plant resistance and biological control is to be achieved, either intentionally or implicitly.

In order to make a sound prediction, collaborative studies should be carried out, that take into consideration the most important pests in a certain crop and the most important plant characteristics affecting natural enemies that have potential for biological control of these pests. In the case of selecting cultivars that are best suited for biological control the following aspects should be taken into account: (a) the effect of different cultivars on the pest organism, (b) the effect of different cultivars on the beneficial organism and (c) the effect of different cultivars on the interaction of the beneficial with the pest organism. In the case of selecting natural enemies that are compatible with a certain plant cultivar this concerns: (a) which species of natural enemies are compatible with the most important plant trait affecting the natural enemies, (b) is it possible to select within a natural enemy species for genotypes that are better suited on the cultivar or crop plant under investigation and (c) is the performance of such selected natural enemy species or genotypes in the interaction with the pest organism satisfactory? Only through an integrated selection procedure an optimal integration of host plant resistance and biological control can be reached.

The scenario depicted above still involves a large amount of research and several pitfalls may be encountered when taking this path. For instance, during breeding for resistance against one herbivore species, increased susceptibility to another species may arise (Beck & Maxwell, 1976). Analogously, there is a risk that selection for a characteristic that favours one species of beneficial organism interferes with the effectiveness of another beneficial species. Yet, these are the risks that are inherent to plant breeding. When investing in a programme to integrate partial host plant resistance with biological control, this will lead to prolonged use of a resulting cultivar because of the slower rate of herbivore adaptation (Gould *et al.*, 1991). Because of potential interference with other herbivore-natural enemy interactions it is most profitable to start with projects that deal with crop plants that have relatively few pest species and where much knowledge exists on the biological control agents that are used to control these pests. This would favour starting with greenhouse crops, because biological control in greenhouses is well-established, much knowledge exists on the biological control agents and the number of pests is relatively low compared to outdoor crops or perennial systems such as fruit orchards. The first example that has yielded success actually relates to a greenhouse system: cucumber-greenhouse whitefly-*Encarsia formosa* (Van Lenteren & De Ponti, 1990).

Finally, an approach that results in application in the shortest period of time is to use the empirical method. If a characteristic is known that improves the effectiveness of a biological control agent, this should be tested under agricultural conditions. If successful for the beneficial organism under consideration while no negative effects are observed for other beneficial species, the characteristic should be incorporated into management programmes. Subsequent steps will be to intensify research along the lines described above, in order to increase understanding of how, why and when this aspect is important in the multitrophic system under consideration. In doing so, future programmes may be developed more efficiently.

Conclusions

In conclusion, host plant resistance and biological control are highly valuable components of environment-friendly pest control. However, these two components are not *a priori* compatible. The biological control agents are active on the plant and are thus

affected by a wide range of plant traits that can influence their effectiveness either directly or through interactions with the pest organism. In order to develop an integrated pest management programme that incorporates both methods of pest control, we should consider how the crop affects the beneficial organisms. Ideally, we should exploit the impact of plants on beneficial organisms both in selection procedures that select for the best plant cultivar for agriculture and in selection procedures that select for beneficial organisms that are most suitable for biological control. It is important to keep in mind that in doing so, we are likely to be forced to set priorities. Not all combinations of traits, either in the plant or in the beneficial organism may be biologically realistic. For instance, some plant species may have invested considerably in direct defense rather than in indirect defense, i.e. via natural enemies of herbivores (Dicke, 1994). In crop plants originating from such plants, there may be more possibilities of modifying plant traits that directly affect the herbivore rather than traits that indirectly affect the herbivore through its natural enemies. Although the notion that plant traits may decisively influence the effectiveness of beneficial organisms is a recent one, a few studies have already shown that it is easily possible to exploit this knowledge and to expand the employment of environmentally-benign pest control. These studies show that cooperation of plant breeders and entomologists can lead to innovative new developments in environmentally-sound pest control.

Résumé

Les caractéristiques des plantes influencent les auxiliaires utilisés pour la lutte biologique : implications sur la sélection de plantes-hôtes résistantes

Une protection des plantes respectueuse de l'environnement peut combiner la lutte biologique avec la résistance des plantes-hôtes. Les auxiliaires utilisés en lutte biologique habitent ou visitent les plantes cultivées et se nourrissent des herbivores. En conséquence, ils peuvent être influencés par une série de caractéristiques des végétaux, soit indépendamment, soit par l'intermédiaire des activités de l'herbivore. Ainsi, on notera les substances volatiles et les composés secondaires des plantes, leur morphologie, les tissus et les produits des végétaux servant de source de nutrition, et enfin leurs propriétés visuelles et vibratoires. L'interaction entre la résistance d'une plante-hôte et la lutte biologique peut s'étendre de l'antagonisme à la synergie en passant par un état neutre. Elle dépend de la nature des interactions entre la plante et les ennemis naturels présents dans un système de culture donné. Ce travail discute les possibilités d'intégration de la résistance des plantes-hôtes et de la lutte biologique.

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Suitability of the ornamental crop *Gerbera jamesonii* for spider mites and the attraction of predators in response to spider mite damage

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Summary

The intrinsic rate of increase (r_m) of the spider mite, *Tetranychus urticae*, has been determined on nine commercial cultivars of *Gerbera jamesonii*. Significant differences between the cultivars in their suitability for spider mites were found. The r_m ranged from 0.090 on the cultivar Bianca to 0.242 on the cultivar Sirtaki.

The ability of gerbera plants to produce volatiles attractive to predators in response to spider mite damage has been investigated. When tested in a Y-tube olfactometer the predatory mite *Phytoseiulus persimilis*, a natural enemy of spider mites, was attracted to gerbera leaves with spider mite infestation. When a choice was given between undamaged leaves and artificially-damaged leaves, the predatory mites prefer undamaged leaves. This indicates that the attraction of predators to spider mite infested leaves was not a response to volatiles that are passively released by gerbera after wounding. This supports the hypothesis that spider mite damage induces gerbera to produce certain volatiles that attract predatory mites. Possible implications for biological control of spider mites on gerbera are discussed.

Introduction

Plants have several ways of defending themselves against herbivores. Certain plant species affect herbivores directly by the production of toxic substances. This forms the basis of breeding for host plant resistance. Besides this direct defense, many ways exist to affect herbivores indirectly. Certain plant characteristics can have a positive effect on natural enemies of herbivores. These plant characteristics can be extremely important in biological control of herbivores (Dicke, 1995). Since the use of host plant resistance and biological control as pest control strategies is increasing, it is important to investigate direct and indirect defense systems of plants and to analyse possible interactions.

A large number of cultivars of the ornamental crop, *Gerbera jamesonii* (Asteraceae) are grown commercially. These cultivars not only differ in the shape and colour of the flower, but also in leaf characteristics such as size, colour, toughness, hair structure and hair density (Sütterlin et al., 1992; Krips, unpublished data). As well as these differences that have not been selected for, differences between cultivars in their suitability for herbivore species may exist.

The spider mite, *Tetranychus urticae* Koch, is an important pest of Gerbera. In Dutch greenhouse vegetable crops, spider mites are successfully controlled by the predatory mite *Phytoseiulus persimilis* Athias-Henriot (Helle & Sabelis, 1985). In ornamentals, however,

the use of biological control is not very well developed, because sales requirements for ornamentals generally do not permit any damage by herbivores Gerbera appears to be a promising crop for biological control of spider mites as only the flowers are sold while spider mite damage is mainly found on the leaves.

In the interaction between spider mites and predatory mites the production of spider mite-induced volatiles by the host plant plays an important role. Many plant species have been shown to respond to spider mite damage by producing a blend of volatiles that attract predatory mites (see Dicke & Sabelis, 1988 for a review). The ability of a plant to interact with predatory mites through the production of volatiles is considered to be essential in local extermination of the spider mites by their predators (Sabelis & Van der Meer, 1986).

This paper addresses the questions as to whether gerbera cultivars differ in suitability for spider mites and whether gerbera plants are able to respond to spider mite damage by producing a volatile blend that attracts predatory mites.

Material and Methods

Plants, spider mites and predators

Gerbera plants were obtained from commercial growers and were subsequently grown in a greenhouse in our department (20-30°C, 50-70% RH and a photoperiod of at least 16 h.). Plants used for experiments were six to nine months old. Spider mites were collected from a commercial gerbera greenhouse and were subsequently reared in our laboratory on the gerbera cultivar Sirtaki. Predatory mites were originally obtained from commercial mass rearings for biological control. In our laboratory they were reared on spider mites (*Tetranychus urticae*) on Lima bean leaves.

Suitability of gerbera cultivars for spider mites

Leaf disks, 2 cm in diameter were cut from specific gerbera cultivars and placed upside down on wet cotton wool. Eggs differing upto 8 hours in age were collected from spider mites that had fed for at least three days on the gerbera cultivar that was used for the experiment. On each leaf disk one spider mite egg was placed after which the development from egg to adult was followed. For each cultivar the age-specific reproduction and the age-specific mortality of the spider mites were determined by counting the number of eggs per adult female and the number of surviving mites. This was done daily during the start and at the end of the experiment and at least twice a day around the age of reproducing the first young. For each cultivar the intrinsic rate of population increase (r_m)of the spider mites was calculated by using the equation given by Birch (1948): $\Sigma \exp(-r_m x) l_x m_x s_x = 1$, where l_x is the age specific survival, m_x is the age specific reproduction and s_x is proportion of females in the offspring of a female at age x. It was assumed that the proportion of females remained constant with the age of the mother and did not differ between cultivars. To obtain a value for s_x, data for six cultivars were pooled and the proportion of females was determined, which was 0.77.

The experiment was performed at 25 ± 0.5°C, ≥65% RH and a photoperiod of L:D=16:8. One week after the start of the experiment most of the eggs had hatched after which the mites were transferred to fresh leaf disks. Thereafter the mites were transferred to fresh leaf disks at least once every three days. After 21 days the experiment was terminated. Many of the mites dispersed from the leaf disks into the surrounding wet cotton wool. These mites were re-placed on leaf disks once. If they moved into the wet cotton wool for a second time, they were discarded. Only data gathered from these mites before

they walked into the wet cotton wool for the second time were used in the analysis and these mites were not used for determining the age-specific mortality. The results for the mites that dispersed into the wet cotton wool are given separately as the loss of spider mites by dispersal. An initial number of 45 eggs per cultivar was used and the experiments were repeated until a minimum number of 15 adult females per cultivar was reached.

Because eggs could easily be damaged during transfer to the leaf disks, egg-mortality, which represents the age-specific mortality between day 0 and day 1, was measured in a separate experiment. Hereto approximately 60 adult female mites that had fed for at least 3 days on the cultivar used for the experiment were placed on leaf disks that were positioned upside down on wet cotton wool. After 8 hours the adult females were removed and the eggs present on the leaf disks remained untouched and were kept at 25 ± 0.5°C, ≥65% RH and a photoperiod of L:D=16:8. The fraction of eggs that hatched was determined. Eggs that did not hatch within 10 days were assumed to be dead.

Response of predatory mites to spider mite damage and artificial damage

To obtain leaves with spider mite damage at least seven detached gerbera leaves (cv. Sirtaki) were incubated with at least 75 adult female spider mites each, and were kept individually in glass vials containing tap water for seven days at 23 ± 2°C, 70 ± 10% RH and continuous light. An identical number of control leaves was incubated without spider mites. These leaves were also kept for seven days under identical conditions. To obtain leaves with artificial damage, the upper surface of seven detached gerbera leaves (cv. Sirtaki) was severely rubbed with a wet cotton pad covered with carborundum and afterwards rinsed with tap water. Seven other leaves were only rinsed with tap water. These leaves were used immediately afterwards for determining attractiveness of predatory mites.

Attractiveness to predatory mites was tested in two-choice situations using the Y-tube olfactometer method described by Takabayashi and Dicke (1992). Predatory mites were starved for 3 hours before the start of the experiment. Twenty mites per set of leaves were tested and each experiment was repeated 3 times.

Results

Suitability of gerbera cultivars for spider mites

Table 1 shows the r_m values of spider mites for nine gerbera cultivars. Gerbera cultivars differed greatly in their suitability for spider mites. The intrinsic rate of increase varied from 0.090 on Bianca to 0.242 on Sirtaki.

The loss of spider mites by dispersal showed substantial variance between the cultivars varying from 26% on Donga to 61% on Sirtaki. However, there was no correlation between a cultivar's resistance to spider mites and the dispersal of spider mites on that specific cultivar.

Response of predatory mites to spider mite damage and artificial damage

When tested in a two-choice situation, two-thirds of the predatory mites chose gerbera leaves with spider mite damage. However, predatory mites significantly preferred undamaged leaves when the alternative was leaves with artificial damage.

Table 1. Intrinsic population growth-rate (r_m) of spider mites on nine different gerbera cultivars, percentage loss of spider mites by dispersal and the number of females on which r_m values are based.

Cultivar	r_m	% loss in 21 days	number of females
Sirtaki	0.242	61	43
Donga	0.215	26	27
Estelle	0.149	57	26
Fame	0.171	29	27
Rondena	0.206	33	46
Sardana	0.193	32	40
Fireball	0.127	56	21
Bianca	0.090	49	20
Bourgogne	0.145	49	32

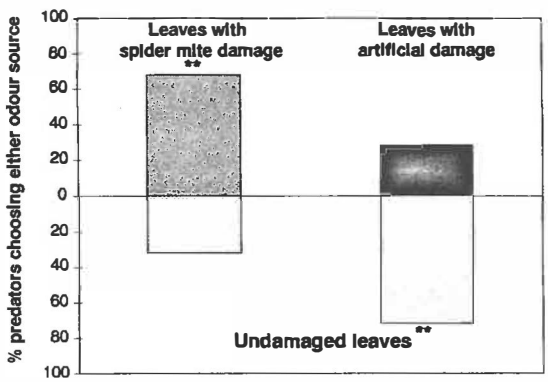

Fig. 1: Response of predatory mites in a Y-tube olfactometer when a choice was given between undamaged gerbera leaves (cv. Sirtaki) and either leaves with spider mite-damage (n=59) or artificially damaged leaves (n=60). Predators significantly preferred leaves with spider mite damage above undamaged leaves (X^2-test, P=0.004) and significantly preferred undamaged leaves above artificially damaged leaves (X^2-test, P=0.001). In the first experiment the percentage of predators that did not make a choice at all was 0% while this was 12% in the second experiment.

Discussion

Gerbera cultivars differ largely in their suitability for spider mites but this phenomenon has never been selected for. This offers great potential for breeding for spider mite resistance in gerbera. However, as this resistance is only partial, control of spider mites will still have to rely on either pesticide application or biological control. Because the use of biological control by gerbera growers is increasing, an investigation should be made of whether spider mite resistance in gerbera can be combined with biological control of spider mites in an integrated pest management system. Several examples are known of

compatibility of host plant resistance and biological control, however examples of incompatibility have been reported as well (Boethel & Eikenbary, 1986; Van Lenteren & De Ponti, 1990; Gould *et al.*, 1991). Therefore, to optimise integrated control of spider mites, the way spider mite resistance influences the effectiveness of predatory mites should be investigated.

In the interaction between spider mites and their predators the production of volatiles attractive to predators plays an important role (Dicke & Sabelis, 1988). For bean it has been demonstrated that the volatiles responsible for the attraction of predatory mites are of plant origin (Dicke *et al.* 1990b). Spider mites themselves are not attractive to predatory mites (Sabelis & Van de Baan, 1983; Sabelis *et al.*, 1984). This shows that after infestation by spider mites the plant is induced to produce volatiles that attract the natural enemies of spider mites. The ability of a plant to produce these volatiles and thereby arrest predatory mites in a prey patch seems to be essential in local extermination of spider mites by their predators (Sabelis & Van der Meer, 1986).

As well as many other plant species the host plant gerbera becomes attractive to predatory mites after spider mite infestation. Predatory mites prefer undamaged leaves when the alternative is artificially-damaged leaves. This shows that the attraction of predators to spider mite infested leaves is not a response to volatiles that are passively released after the plant is wounded. This indicates that spider mite infestation induces gerbera to produce predator-attracting volatiles.

Since it has been shown that commercial gerbera cultivars differ largely in resistance to spider mites it may well be that large differences also exist in the ability to produce predator-attracting volatiles. Large quantitative differences between two apple cultivars have been found in the production of amongst others 4,8-dimethyl-1,3(E),7-nonatriene, (E)-β-ocimene and methyl salicylate (Takabayashi *et al.*, 1991; 1994), which are known to attract predatory mites (Dicke *et al.*, 1990b). Dicke *et al.* (1990a) showed differences between two bean cultivars in the level of infestation by spider mites necessary for attraction of predatory mites. It should be stressed that if spider mite resistance is incorporated in an integrated system to control spider mites in gerbera, the ability of gerbera to attract the natural enemies of the spider mites should not be overlooked. This will be investigated by our group in the near future.

Résumé

La convenance de la plante d'ornement *Gerbera jamesonii* pour les acariens et l'attraction des prédateurs en réponse aux dégâts de l'acarien jaune commun

Le taux intrinsèque d'accroissement (r_m) de l'acarien jaune commun *Tetranychus urticae* a été déterminé sur neuf cultivars commerciaux de *Gerbera jamesonii*. Des différences énormes ont été mises en évidence. Le r_m s'étend de 0.090 pour le cultivar "Bianca", à 0.242 pour "Sirtaki".

La faculté des plantes de gerbera de produire des substances volatiles capables d'attirer les prédateurs, en réponse à une attaque d'acariens, a été étudiée. L'ennemi naturel des tétranyques, l'acarien prédateur *Phytoseiulus persimilis*, placé dans un olfactomètre à tube en Y, est attiré par les feuilles de gerbera infestées d'acariens. Lorsque le choix porte sur des feuilles indemnes et artificiellement endommagées, les acariens préfèrent les premières. Ceci indique que l'attraction des prédateurs par les feuilles infestées d'acariens n'est pas une réponse à des volatiles qui seraient diffusées passivement par les gerberas après blessure.

Ceci soutient l'hypothèse que les dégâts dus aux acariens induisent la production de certains volatiles par les gerberas, capables d'attirer les acariens prédateurs. Les conséquences possibles sur la lutte biologique contre les acariens sur gerbera sont discutées.

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Stable resistance to the cereal aphid *Sitobion avenae* F. in the ancient diploid wheat *Triticum monococcum* L.

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Summary

A clone of the cereal aphid *Sitobion avenae* was maintained for two years on either susceptible wheat genotypes (*Triticum aestivum* cv. 'Arminda'; *Triticum monococcum* line Tm47) or highly resistant wheat genotypes (*T. monococcum* lines Tm44 and Tm46). The intrinsic rate of natural increase (r_m) of the four aphid colonies was evaluated on their host plants at the beginning of the experiment, 9 months, 12 months and 24 months later. The feeding behaviour of the aphid colonies maintained respectively on 'Arminda' and Tm44 was monitored on both plants at t=0 and two years later using an electronic method (DC-EPG).

The levels of resistance/susceptibility of the four wheat genotypes tested did not change within 24 months, whether the particular aphid colony had been reared on resistant or susceptible plants. The feeding behaviour of aphids was slightly modified after two years of continuous rearing on Tm44 but remained highly inhibited on resistant wheat when compared with the susceptible wheat genotype 'Arminda.'

Thus, in spite of rearing *S. avenae* continuously for two years on resistant plants and despite the strong selection pressure exerted by these plants on the aphid colonies, no resistance-breaking biotypes were detected.

Introduction

The cereal aphid *Sitobion avenae* F. is one of the main aphid pests of winter wheat as a result of its direct damage in Western Europe (Schepers, 1989). A high level of resistance to this aphid was found in some lines of the ancient diploid wheat *Triticum monococcum* L. (Di Pietro *et al.*, 1993; Caillaud *et al.*, 1994). A survey of 60 clones of *S. avenae* collected in a locality in Brittany (France) showed that the ability to develop and reproduce on the resistant wheat Tm44 was characterized by being highly and continuously variable; 90% of the new born nymphs of some clones survived until adulthood whereas practically no nymphs survived until the end of the experiment in the case of certain other clones (Caillaud *et al.*, 1995a). A study of the genetics of resistance showed that this trait was primarily determined by autosomal loci, partially-dominant and encoded by many genes with small additive effects (Caillaud et al, in prep.). The clonal feature of the variation observed in *S. avenae* agressivity on *T. monococcum* lines and the main characteristic of the genetic control of this variability indicates the potential for evolutionary shifts in resource use and thus for overcoming *T. monococcum* resistance.

In an attempt to determine whether more agressive clones could emerge in *S. avenae* populations, the present study investigated the effect of the selection pressure exerted by resistant *T. monococcum* lines on *S. avenae*. A clone of the cereal aphid was maintained on four wheat genotypes, either resistant or susceptible, for two years. The possible changes in the aphid performances on every genotype in the course of time were evaluated by measuring their intrinsic rate of natural increase (r_m) on four different dates during this two-year period. As feeding behaviour plays a key role in host acceptance, small behavioural changes may precede and/or explain a subsequent change in the aphid fitness on a host. Thus, the possible changes in the feeding behaviour of aphids maintained on resistant plants during the two years was also studied by monitoring their stylet penetration activities at the beginning of the experiment and two years later.

Material and Methods

Aphid and plant material

A holocyclic clone of *S. avenae*, Sar2 was used, which was collected from wheat in the Rennes basin in 1987 and reared continuously on the susceptible *Triticum aestivum* L. cv. 'Arminda.' The performance and feeding behaviour of Sar2 was shown to be consistently affected by resistant *Triticum monococcum* L. lines (Caillaud *et al.*, 1994; Caillaud *et al.*, 1995b). The four wheat genotypes used were chosen on the basis of their susceptible/resistant status assessed in previous experiments (Di Pietro *et al.*, 1993; Caillaud *et al.*, 1994) : 'Arminda' is a very susceptible cultivar of wheat (susceptible; S1); Tm47 is the only susceptible line of *T. monococcum* that has been described (S2); Tm46 and Tm44 were two *T. monococcum* lines in which the resistance mechanisms were different (resistant; respectively R1 and R2) (Caillaud *et al.*, 1995c).

Four aphid colonies were established by releasing fifteen larvae of Sar2 either on 'Arminda', Tm47, Tm46 or Tm44 (two-leaf growth stage). These four aphid colonies are referred to as SaAr, Sa47, Sa46 and Sa44 respectively. They were maintained on these plants for two years, plants being replaced every 15 days. Aphids of every colony were transferred to the susceptible plant 'Arminda' for one generation before an experiment in order to start off with aphids for which weight and general physiological status were uniform.

Aphid and plant cultures, as well as experiments, were performed in growth chambers at 20°C+1°C, and L:D 16:8 (5.86 W/m^2).

Measurement of aphid performance

The intrinsic rate of natural increase r_m (Birch, 1948) is known to be a good measure of aphid population fitness. The r_m of every aphid colony on its rearing plant was evaluated as described elsewhere (Caillaud *et al.*, 1995a) at the beginning of the experiment (first aphid generation, G1), after 9 months (G25), 12 months (G35) and 24 months (G70) (Fig. 1).

For each generation, the r_m values recorded on every plant genotype were compared using a one-way analysis of variance (Anova) followed by the multiple mean comparison test of Student-Newman-Keuls.

Electroning recording of stylet penetration activity

The feeding behaviour of SaAr and Sa44 was monitored on resistant Tm44 and susceptible 'Arminda' after 70 generations of continuous rearing on both plants (G70) withthe DC-EPG method (Tjallingii, 1978; 1988) as described elsewhere (Caillaud *et al.*, 1995b).

The forty parameters evaluated on each EPG recording (see Caillaud *et al.*, 1995b) were subjected to a two-way analysis of variance (factor 1=aphid colony; factor 2=plant genotype) followed by a Student-Newman-Keuls test.

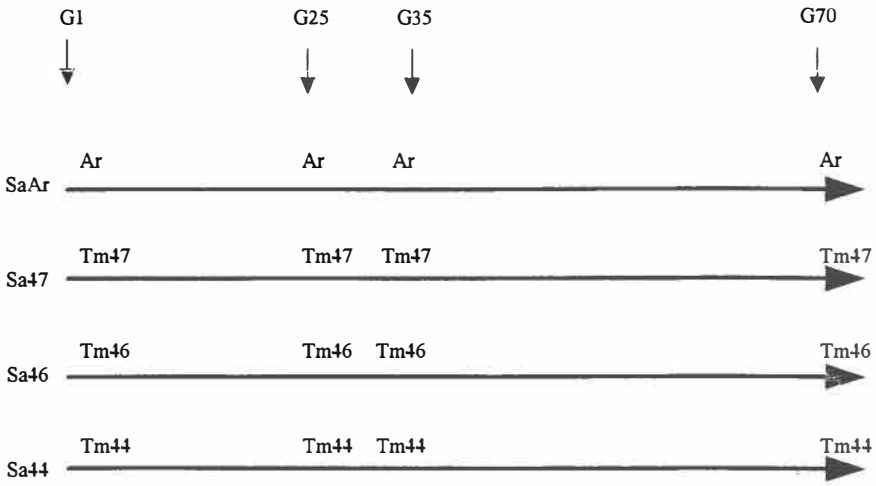

Fig. 1: Experiments performed on 4 aphid colonies maintained respectively on Arminda (SaAr), TM47 (Sa47), Tm46 (Sa47) and TM44 (Sa44) for two years and then tested regularly in the course of time on their particular host plant.

Results

At the beginning of the experiment (G1), the four wheat genotypes 'Arminda', Tm47, Tm46 and Tm44 could be clearly distinguished from each other; as described previously (Di Pietro *et al.*, 1993), 'Arminda' and Tm47 appeared as susceptible plants while Tm44 and Tm46 were shown to be highly resistant lines. Nine months later (G25), 12 months later (G35) and 24 months later (G70), 'Arminda' and Tm47 were still behaving as susceptible genotypes for *S. avenae* while Tm44 and Tm46 were shown to severely affect aphid performance (Table 1). Even after two years of continuous rearing on Tm44 and Tm46, 30 to 75% of the larvae settled on these resistant genotypes died before becoming adult and adult survival remained low (5 days on average).

Table 1. Intrinsic rate of natural increase (r_m) of *S. avenae* colonies maintained for two years on 'Arminda', Tm47, Tm46 or Tm44 and then regularly studied (aphid generations n°1 to 70) on their particular host plant.

	'Arminda' (S1)	Tm47 (S2)	TM46 (R1)	Tm44 (R2)	Plant effect
G1	**0.26 a**	**0.2b**	**0.15c**	**0.1d**	**F=74.37, p=0,*****
G25	(0.25/0.27) **0.23a**	(0.16/0.23) **0.18b**	(0.10/0.20) **0.05c**	(0.02/0.17) **0.04c**	**F=73.66, p=0,*****
G35	(0.20/0.26) **0.23a**	(0.14/0.21) **0.16b**	(-0.12/0.22) **0.1c**	(-0.1/0.19) **0.11c**	**F=95.03, p=,*****
G70	(0.21/0.25) **0.25a** (0.21/0.28)	(0.10/0.22) **0.2b** (0.16.0.25)	(00/0.20) **0.06d** (-0.11/0.23)	(00/0.22) **0.1c** (0.003.0.20)	**F=5.45, p=0,*****

The feeding behaviour of the aphid colony maintained on Tm44 for two years was the same at the beginning of the experiment and two years later (Fig. 2). The time elapsed between first access to the leaf and first sap ingestion (ts-E2) on Tm44 appeared to be as long for Sa44 as it did for the aphid colony maintained continuously on 'Arminda', SaAr. The total time spent by Sa44 ingesting sieve sap (d-E2) was not significantly different from that of SaAr. Nevertheless, other characteristics of aphid feeding behaviour were affected by continuous rearing of *S. avenae* on a resistant genotype (15 to 44 variables measured on each EPG recording). The time needed to reach the phloem vessels (ts-E1) on susceptible plants appeared to be significantly higher for Sa44 than for SaAr (212 against 95 min. on average). Conversely, the total time spent by Sa44 in EPG patterns E1 (d-E1) and G (d-G) were twice as few as those of SaAr on susceptible plants as well as on resistant ones (d-E1 : F=9.2; p<0.01; 1ddl; d-G :F=11.3; p<0.01; 1ddl). Finally, the total time spent in exhibiting non-penetration patterns (d-np) on Tm44 appeared to be significantly higher for Sa44 when compared with SaAr (F=12.9; p<0.01; 1ddl).

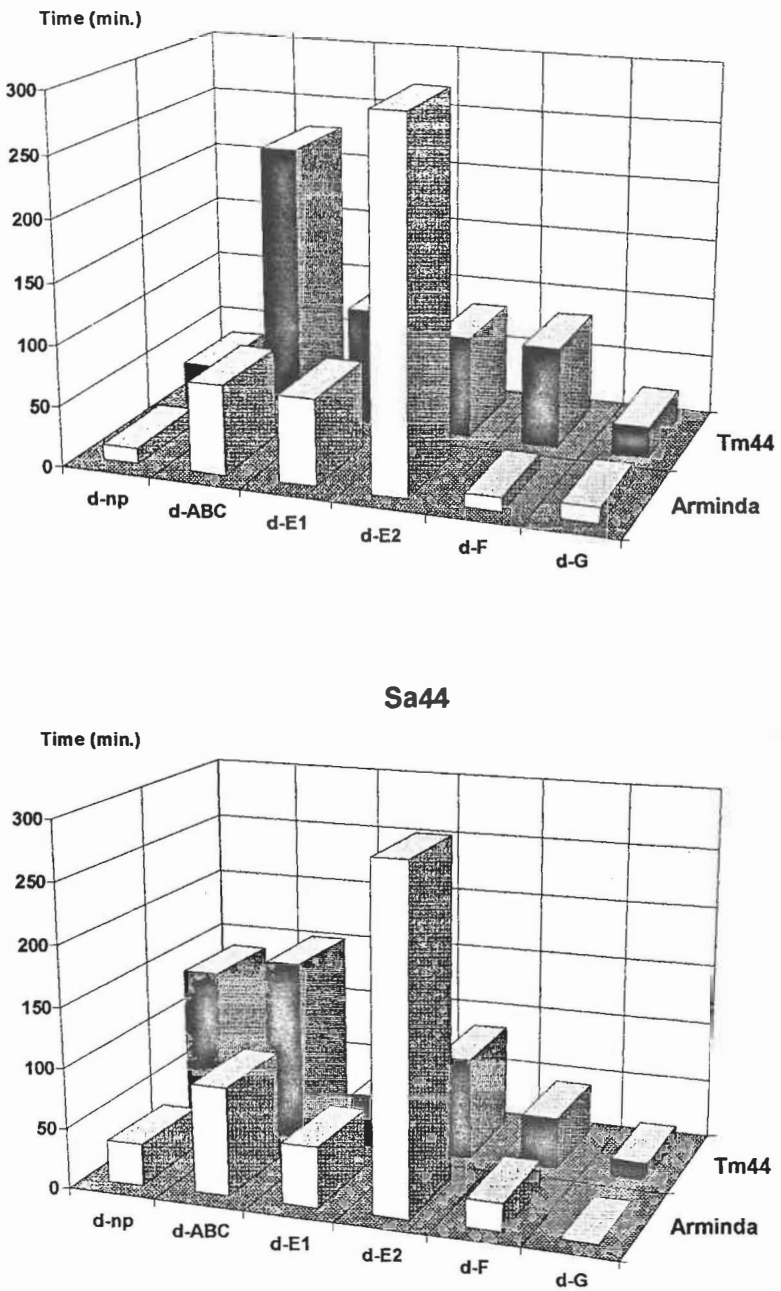

Fig. 2: Total duration of each EPG pattern (np to G) of SaAr (aphid colony continuously maintained on 'Arminda' before the experiment) and Sa44 (aphid colony maintained on Tm44 for two years before the experiment) on 'Arminda' (susceptible wheat) and Tm44 (resistant wheat).

Discussion

Continuous rearing for 70 generations on resistant plants did not result in a revert-selection for more agressivity of *S. avenae* thus indicating that both *S. avenae* non-agressivity and *T. monococcum* resistance were stable characters, at least at the phenotypic level. Nevertheless, certain changes that did not affect the resistance status of *T. monococcum* Tm44 to Sar2 were detected in the feeding behaviour of this clone after being reared on this resistant genotype for two years. The amount of time spent in non-penetration activities (the aphid is walking or motionless) and the time needed to reach the phloem vessels might have been higher for aphids maintained on poor hosts(Tm44) during the two years. This is probably because these aphids were weaker than aphids that have been well fed on 'Arminda' during the same period. It is not clear why the amount of time spent in prepenetrating of the phloem vessels for sap ingestion (E1) and iningesting mainly water from the xylem vessels (G) was far less important for aphids maintained on poor hosts during the two years.

The stability of *T. monococcum* resistance to Sar2 shown in this experiment is of interest to plant breeders. Nevertheless, two characteristics of this experiment suggest that it is premature to believe *T. monococcum* resistance could not be overcome in the future. Firstly, this experiment was performed using a simple clone of the aphid and *S. avenae* populations are known to contain many different clones, even on a very local scale (Weber, 1985; Lowe, 1981). As some clones were shown to be more agressive on resistant *T. monococcum* than others (Caillaud *et al.*, 1995a), selection pressure exerted by a resistant plant in these more realistic conditions could favour the most agressive clones. Moreover, the experiment presented here did not consider the effect of sexual reproduction on the emergence of new recombinants which, combined with a resistance selection pressure, could speed up the emergence of agressive clones in aphid populations. Secondly, this experiment examined phenotypic traits and more information about the possible changes that selection pressure can induce may be obtained when considering traits of higher resolution. Although enzymatic markers were recently proved to provide a useful tool for studying the genetic structure of *S. avenae* populations (Dedryver *et al.*, 1995), the use of genetic markers such as the RAPD's, successfully developed recently for another cereal aphid, *Rhopalosiphum padi* L. (Simon *et al.*, in prep.), may be of greatest interest. Thus, selection experiments involving a mixture of clones, two different modes of reproduction (parthenogenesis versus sexual reproduction) and higher resolution traits are now needed in order to get an accurate prediction of the durability of *T. monococcum* resistance to *S. avenae*.

Résumé

Stabilité de la résistance au puceron vert de l'avoine *Sitobion avenae* F. chez l'ingrain diploïde *Triticum monococcum* L.

Un clone du puceron vert de l'avoine *Sitobion avenae* a été maintenu pendant deux ans sur des génotypes de blé sensibles (*Triticum aestivum* cv. "Arminda"; lignée Tm 47 de *Triticum monococcum*) ou fortement résistants (lignées Tm 44 et Tm 46 de *T. monococcum*).

Le taux naturel d'accroissement intrinsèque (r_m) des quatre colonies de pucerons a été évalué sur leur plante d'élevage au début de l'expérience puis après 9, 12 et 24 mois. Le comportement trophique des colonies de pucerons maintenues sur "Arminda" et Tm 44 a été suivi au temps t = 0 et deux ans plus tard, au moyen d'une méthode électronique (DC-EPG).

Le niveau de résistance et sensibilité des quatre génotypes de blé n'a pas changé durant ces 24 mois, quelles que soient les caractéristiques de sensibilité ou de résistance des plantes sur lesquelles la colonie de pucerons a été élevée. Le comportement trophique des pucerons s'est légèrement modifié après un élevage continu de deux ans sur Tm 44. L'inhibition est néanmoins restée élevée sur le blé résistant en comparaison du génotype sensible "Arminda".

Ainsi, malgré un élevage permanent de *S. avenae* pendant deux ans sur un végétal résistant et la forte pression de sélection exercée par ces plantes sur les colonies, aucun biotype capable de rompre la résistance n'a été détecté.

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Biotypic variation in aggressiveness of western flower thrips to susceptible and partially resistant cucumbers

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Summary

Reproduction of eight European strains of western flower thrips, *Frankliniella occidentalis* and one strain from New Zealand was determined on leaf discs from one susceptible and two partially resistant cucumber (*Cucumis sativus* L.) genotypes. Significant differences between strains were observed. However, none of the strains had a higher reproduction than the control strain from The Netherlands. The strain from New Zealand had a lower level of reproduction on the susceptible and on one of the resistant cucumber genotypes. A cross was made between the control strain from The Netherlands and the strain from New Zealand. The strains do hybridize and the reproduction of the F_1 females from the reciprocal crosses was determined. The reproduction of the F_1 was comparable to that of the strain from The Netherlands, suggesting that a higher reproduction rate is dominantly inherited.

Introduction

Western flower thrips, *Frankliniella occidentalis* (Pergande) is a major pest world wide. In The Netherlands it is one of the most important pests in many ornamental and vegetable crops in greenhouses (Mantel & van de Vrie, 1988). Introduction of host plant resistance could help to manage this pest. At CPRO-DLO cucumber genotypes have been selected with partial resistance to western flower thrips (Mollema *et al.*, 1993) using a thrips strain that was collected in The Netherlands. The existence or development of more aggressive biotypes poses a major threat to host plant resistance to insects. Therefore biotypic variation and inheritance of virulence in this insect were studied.

Materials and Methods

Plant material and insects

A susceptible cucumber inbred line "G6" and two partially resistant accessions 9104 and 9140 (CPRO-DLO numbers) were used (Mollema *et al.*, 1993). Thrips strain NL1 was collected in 1988 on cucumber in Wageningen, The Netherlands, and maintained on flowering cucumber plants, cv. 'Autumn Green' (Mollema *et al.*, 1990). Strain NL2 was collected from chrysanthemum in a greenhouse in The Netherlands in 1991 and reared on flowering chrysanthemums (van Dijken *et al.*, 1994). In 1993-1994, strains of *F. occidentalis* were collected from different countries and reared on bean pods and bee pollen

in 0.5 l glass jars in a climate chamber (L:D=16:8 h., T=25°C, RH=60-70%). The origin of the strains is summarized in Table 1. Samples (200 individuals) of strains NL1 and NL2 were used to initiate cultures on bean pods in the same way. These bean-reared strains were used in the experiments.

Table 1. Origin of strains of *F. occidentalis* used in experiments.

Code	Country	Host plant source
NL1	Netherlands	Cucumber
NL2	Netherlands	Chrysanthemum
GE	Germany	Gerbera
FR	France	Bean
IT	Italy	Bean
SP	Spain	Cotton
HU	Hungary	Bean
SWI	Switzerland	Bean
SWE	Sweden	Brassica
NZ	New-Zealand	Egg-plant

Reproduction of randomly collected females

Reproduction of randomly-collected females was determined on leaf discs. Leaf discs (ϕ=8 cm) were taken from basal leaves, and 10 females placed on a disc, 4 discs per cucumber accession. After a two-day pre-adaptation period thrips were transferred to fresh leaf discs where they were allowed to oviposit for 24 h. After four days the numbers of hatched larvae were recorded.

Reproduction of synchronized females

To obtain the same aged females, thrips were synchronized on leaf discs from susceptible cucumber G6. Thrips were allowed to oviposit for one day on leaf discs after which they were carefully removed. After four days the hatched larvae were transferred to fresh leaf discs. Thrips were transferred to fresh leaf discs every four days until emergence. Reproduction was determined on day 4, 5 and 6 after emergence on leaf discs (ϕ=1.2 cm, 1 female/disc) in wells of tissue culture plates according to Soria & Mollema (1995).

Cross between strains NL1 and NZ

Virgin females and males of strains NL1 and NZ were put on leaf discs for one day in combinations NL1*NL1, NZ*NZ, NL1*NZ and NZ*NL1 (n=24). Thrips mated readily. Females were allowed to lay eggs and the offspring reared until adult. The sex-ratio of the F_1 was determined and F_1 females were used in a reproduction experiment (same as with synchronized females) on G6 and 9140.

Results and Discussion

Reproduction of randomly collected females

Reproduction of the control strain NL1 on susceptible G6 was 4.1 larvae/female/day. On the resistant genotypes 9104 and 9140 reproduction was 0.9 and 2.7. Strains Ge, FR, SP SWE and NZ reproduced less on G6 than strain NL1. On 9104 none of the strains differed significantly in reproduction from NL1 and on 9140 only the strain from New Zealand had a significant lower reproduction (1.6 larvae/female/day) compared to strain NL1. In conclusion: there are significant differences in reproduction rates between strains. None of the strains tested had a higher reproduction than the reference strain NL1. The strain from New Zealand had a lower reproduction than strain NL1 on two of the three cucumber accessions.

Reproduction of synchronized females

Reproduction of synchronized females of strain NZ was significantly lower than that of strain NL1 on both G6 and 9140 (Fig. 1). It can be concluded that with synchronized females similar results were obtained as in experiments with randomly-collected females. Again there is a significantly lower level of reproduction of strain NZ compared to strain NL1 on both G6 and 9140.

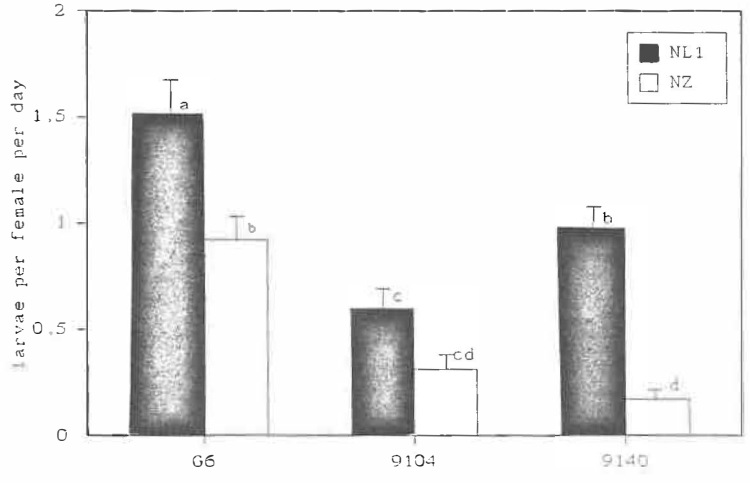

Fig 1: Mean reproduction+SEM (larvae/female/day) of *F. occidentalis* from strains NL1 and NZ on susceptible cucumber G6 and partially resistant 9104 and 9140.

Cross between strains NL1 and NZ

More than 50% of the F_1 from the crosses between strain NL1 and NZ was female (Table 2).

Table 2. Sex-ratio (proportion females) ±SEM of the F_1 of crosses between strain NL1 and NZ.

NL1♀xNL1♂	NL1♀xNZ♂	NZ♀xNZ♂	NZ♀xNL1♂
0.59 ± 0.06	0.78 ± 0.03	0.69 ± 0.06	0.51 ± 0.05

Western flower thrips is haplo-diploid; unmated females produce only male offspring. Therefore it can be concluded that in all crosses thrips mated successfully. Reproduction of F_1 females from strain NZ was lower than reproduction of F_1 females from strain NL1. This was the case both on the susceptible G6 and on the resistant 9140 (Fig. 2).

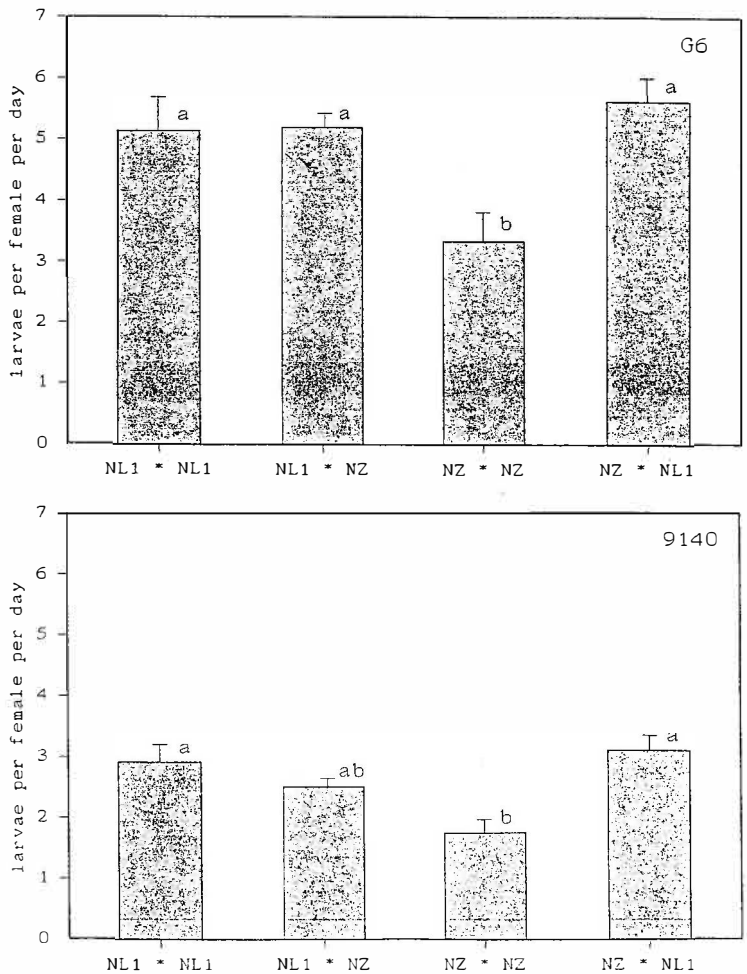

Fig. 2: Reproduction of F_1 females from crosses between strains NL1 and NZ on susceptible cucumber G6 (above) and partially-resistant cucumber 9140 (below).

Reproduction of F_1 females from the reciprocal crosses between the two strains was comparable to the higher reproduction of the F_1 females from strain NL1 (Fig 2). This suggests that a higher reproduction rate is dominantly inherited.

Résumé

Variation biotypique de l'agressivité du thrips de Californie vis-à-vis de concombres sensibles ou partiellement résistants

La reproduction de huit lignées européennes et d'une lignée néo-zélandaise du thrips de Californie, *Frankliniella occidentalis* (Pergande) a été déterminée sur disques foliaires d'un génotype sensible et de deux génotypes partiellement résistants de concombre (*Cucumis sativus* L.). Des différences significatives entre les lignées ont été observées. Néanmoins aucune d'entre elles n'a présenté de reproduction supérieure à celle de la lignée hollandaise de référence. La lignée néo-zélandaise montre une reproduction plus faible sur le génotype sensible et sur un des génotypes résistants de concombre.

Après croisement des souches hollandaise et néo-zélandaise, la reproduction des femelles hybrides F_1 issues des croisements réciproques a été déterminée.
Elle est comparable à celle de la référence hollandaise, ce qui suggère que le taux supérieur de reproduction est un caractère héréditaire dominant.

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Interaction between cereal phenolics and grain aphid (*Sitobion avenae* Fabr.)

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Summary

Moderately resistant winter wheat cultivars (rich in phenolics) had a significant effect on colonisation, feeding behaviour and physiology of the grain aphid, *Sitobion avenae* (Fabr.). When tested *in vitro*, naturally-occurring phenolic compounds in cereals prolonged grain aphid development time and reduced its fecundity and intrinsic rate of natural increase (r_m). They also increased the number of aphid probes and shortened the duration of total probing as well as phloem salivation and phloem ingestion. In addition, the cereal phenolics modulated activity of aphid butyrylcholinesterase, an important enzyme in insect nervous impulse transmission. The importance of the phenolic compounds in the resistance of cereals to the grain aphid is discussed.

Introduction

Numerous studies have reported the occurrence of antixenotic and antibiotic resistance in cereal cultivars to the grain aphid, a serious pest of cereals in western and eastern Europe (Sotherton & van Emden, 1982; Leszczynski, 1987; Hinz, 1989). Generally, varietal resistance to herbivorous insects is conditioned by various plant features acting as resistance factors. These include secondary metabolites, which are important plant allelochemicals against insect pests (Frankel 1969, Whitaker & Feeny 1971). Within cereal allelochemicals, phenolic compounds, hydroxamic acids and indole alkaloids are believed to play an important role in cereal resistance to aphids (Zinger *et al.*, 1985; Leszczynski *et al.*, 1989; Leszczynski & Dixon, 1990; Escobar & Niemeyer, 1993).

The role of phenolics in this process has been extensively studied and a negative correlation between the content of phenolic compounds in cereal tissues and the level of aphid infestation has been found (Todd *et al.*, 1971; Dreyer & Jones, 1981; Leszczynski *et al.*, 1985). There is also evidence that aphid infestation results in elevation of phenol content and induction of activity of the enzymes involved in phenol metabolism (Leszczynski, 1985; Havlickova & Cvikrova, 1995). However, there is still no detailed information on the interaction between phenolics and cereal aphids.

In the present paper we report on the influence of cereal phenolics on grain aphid colonisation, feeding behaviour and physiology. In addition, the effect of these allelochemicals on activity of the aphid target enzyme, butyrylcholinesterase is discussed.

Materials and Methods

The mixed population of grain aphid, *S. avenae,* used in the experiments came from a stock culture kept at the Agricultural & Pedagogic University (Siedlce, PL). The aphids were reared in the controlled environment room on two week-old seedlings of barley.

Four wheat cultivars were used in the experiments: two moderately resistant, Saga and Grana and two susceptible - Emika and Liwilla (Leszczynski, 1987a). When seedlings were tested, seeds were germinated in the controlled environment room maintained at 20-22°C and at 16 h light: 8 h dark photo period. The seedlings were grown in a medium nutrient compost in 15 cm diameter plastic pots, 10 seedlings per pot. The plants were regularly watered and no extra fertiiiser added.

Colonisation and growth and development parameters of the grain aphid performed on wheat were measured according to Leszczynski (1987a). The EPG (electrical penetration graphs) recordings of the grain aphid feeding behaviour were performed according to Tjallingii (1978).

The total phenol content in the flag leaf was estimated as described earlier (Leszczynski *et al.*, 1989). GC-MS analysis of the phenolic compounds was done using the technique described by Bakowski (1995).

The influence of the selected phenolics on grain aphid growth and development and feeding behaviour was tested as described earlier (Leszczynski, 1987b) and Leszczynski *et al.* (1995), respectively. The effect of the phenolic compounds on activity of the aphid butyrylcholinesterase was determined after Brestkin *et al.* (1985) and Zhu & Brindley (1992). Analysis of variance followed by Duncan's multiple range test were used to analyse the data.

Results and Discussion

The experiments showed that the moderate resistance of wheat varieties was associated with cereal phenolics. This was generally true when content of total phenols was correlated with grain aphid performance on wheat cultivars in the field and laboratory.

In particular, colonisation of wheat cultivars by alates during migration was negatively correlated with the content of total phenols in the flag leaves (Table 1). The grain aphid feeding behaviour was clearly different on moderately resistant wheats (rich in phenols) than on susceptible ones (poor in phenols). Probing of peripheral tissues and phloem sap ingestion were much shorter on resistant wheats while phloem salivation was prolonged (Table 1).

Moreover, aphid growth and reproduction on these cultivars was limited. The development time was prolonged while fecundity and the intrinsic rate of natural increase (r_m) were reduced (Table 1).

Table 1. Comparison of the total phenol content (mg/g d.w.) extracted with EtOH and grain aphid colonisation, growth and development and probing behaviour on resistant and susceptible wheats.

Parameters studied	Resistant cultivars		Susceptible cultivars	
	Saga	Grana	Emika	Liwilla
Total phenols	4.82 a	4.70 a	4.18 ab	2.81 b
Number migrants per 100 stems	2.00 a	7.00 a	10.00 a	14.00 a
Pre-reproductive period (in days)	12.10 a	11.45 ab	11.25 ab	10.95 b
Daily fecundity	2.24 c	2.34 c	2.78 b	3.08 c
r_m	0.1799	0.1984	0.2080	0.2144
Peripheral tissue probing (%)	25.00	21.20	30.10	28.90
Phloem salivation (%)	23.70	11.10	9.20	8.00
Phloem ingestion (%)	32.70	44.60	41.10	52.50

Values not followed by the same letter are significantly different at the 0.05% level (Duncan's test).

When tested *in vitro*, selected phenolic compounds had a similar affect on the grain aphid feeding behaviour and physiology. There was an increase in the number of probes and pathway time and a significant reduction in total probing time and duration of phloem phase in comparison to untreated seedlings (Table 2).

Table 2. Effect of the phenolic compounds (2.5 mM) on grain aphid feeding behaviour (after Leszczynski *et al.*, 1995).

Aphid activity	Control	Ferulic acid	Sinapic acid
Number of probs	2.3 a	3.0 a	9.5 b
Total probing (s)	26930 a	3351 b	19109 b
Total pathway (s)	2347 a	22389 ab	6061 c
Phloem salivation (s)	1513 a	669 a	1110 a
Phloem ingestion (s)	21764 a	18127 ab	11465 b

Values not followed by the same letter are significantly different at the 0.05% level (Duncan's test).

The phenolics tested also prolonged grain aphid pre-reproductive period and decreased daily fecundity and values of the intrinsic rate of natural increase (Table 3).

Table 3. Effect of the phenolic compounds (500 ppm) on the grain aphid growth and development.

Phenolics	Prereproductive period (days)	Daily fecundity	r_m
Control	10.2 c	3.00 a	0.2519
Coumarin	11.1 b	2.34 b	0.2250
Quercetin	11.9 a	2.37 b	0.2053
Ferulic acid	12.4 a	2.06 c	0.1921
p-Coumaric acid	12.5 a	1.88 c	0.1761

Values not followed by the same letter are significantly different at 0.01 % (Duncan's test).

In addition, phenolic compounds modulated activity of grain aphid cholinesterases. The modulation was particularly significant for the activity of butyrylcholinesterase (Table 4) - a unique cholinesterase so far found only in cereal aphid tissues (Brestkin *et al.*, 1985; Leszczynski *et al.*, 1995).

Table 4. Effect of phenolic compounds on activity of grain aphid butyrylcholinesterase as a percentage of the control (= 100%).

Phenolics	Concentration (mM)		
	3×10^{-2}	3×10^{-3}	3×10^{-4}
Coumarin	101.8	96.0	79.2
p-Coumaric acid	104.3	103.4	72.2
Ferulic acid	77.3	77.2	76.3
Quercetin	92.1	52.1	50.0

The results presented here give a more detail description of the phenolic compounds action against grain aphid. Since the phenolics are distributed on the plant surface e.g. furanocoumarins, in the peripheral tissues and in the phloem sap it is not surprising that they have a significant affect on grain aphid colonisation, feeding behaviour and physiology. As a result this group of cereal allelochemicals probably play a role in both resistance mechanisms: antixenosis and antibiosis.

The resistance mechanisms are based on the action of the cereal allelochemicals against the aphid at the molecular level. Our earlier studies showed that within cereal phenolics there are important substrates and/or modulators of grain aphid detoxifying enzymes, secreted with the aphid saliva and into the aphid gut (Leszczynski *et al.*, 1993; Urbanska *et al.*, 1995). The data presented here indicate that they can also modulate activity of the aphid target enzymes.

This is extremely important since the cholinesterases play a key role in transmission of nerve impulses across insect synapses and any modulation of the enzymes activity stimulates a quick response in insect behaviour (Brattsten, 1988). Allelochemicals that inhibit or inactivate acetylcholinesterase cause acetylcholine to accumulate in the cholinergic nerve fibres throughout the central and peripherial nervous systems of insects; this can result in paralysis or even death. This is usually not observed when the grain aphid feeds on resistant wheats, rich in the phenolics, however, on the modulatory resistant cultivars the aphids are often reluctant to settle and feed.

Therefore we believe that phenolic compounds are important in cereal resistance to the grain aphid and they should be considered during breeding of new cereal varieties resistant to aphids. Further studies are in progress to determine the biochemical mechanisms of cereal resistance to aphids.

Résumé

Interaction entre les composés phénoliques des céréales et le puceron vert de l'avoine (*Sitobion avenae* Fabr.)

Des cultivars de blé d'automne (riches en composés phénoliques) modérément résistants ont fortement affectés la colonisation, le comportement alimentaire et la physiologie du puceron vert de l'avoine, *Sitonion avenae* (Fabr.). *In vitro*, les composés phénoliques naturellement présents dans les céréales ont prolongé la durée de développement et réduit la fécondité et le taux intrinsèque d'accroissement naturel (r_m) du puceron. Ils sont aussi à l'origine de l'augmentation du nombre de piqûres de test effectués par le puceron et du raccourcissement de la durée de l'ensemble de ces tests, ainsi que de la salivation et de l'ingestion du phloème. En outre, les composés phénoliques des céréales ont modulé l'activité de la butyrylcholinesterase, une importante enzyme impliquée dans la transmission des impulsions nerveuses chez les insectes. L'importance des composants phénoliques dans la résistance des céréales à l'égard du puceron vert de l'avoine est discutée.

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Changes in the pattern of phenolic acids induced by aphid infestation in two winter wheat cultivars

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Summary

Significant changes were found in the activity of phenylalanine ammonia-lyase (PAL) and the content of free phenolic acids in the leaves and ears of two winter wheat cultivars, Regina (partially resistant) and Zdar (susceptible) after infestation by *Metopolophium dirhodum* (Md), *Rhopalosiphum padi* (Rp) and *Sitobion avenae* (Sa). The infestation led to the increase in PAL activity in leaves and ears of both cultivars. The difference in response to aphid infestation between cultivars was more significant in leaves. In spite of higher PAL activity, the level of total free phenolic acids in infested leaves of susceptible Zdar did not change significantly. In leaves of resistant Regina the content of phenolics even decreased by about 25% and 45% when infested with Rp and Md, respectively. The decrease was caused mainly by the decline of p-coumaric acid content. The level of dominant phenolic acid in ear extracts, p-coumaric, increased after 5a infestation approximatelly twice and there were no significant differences between Regina and Zdar. The possible role of phenolic acids in cereal resistance to aphids is discussed.

Introduction

Phenolic compounds with their antimicrobial and allelochemical properties play an important role in plant resistance and they are also involved in the process of host-plant selection by cereal aphids. A correlation between the levels of phenolic compounds and hydroxamic acid in wheat plants and their resistance to aphids has been found (Leszczynski, 1985; Leszczynski *et al.*, 1989). Niemeyer *et al.* (1989) which give rise to differences between wheat cultivars in their ability to accumulate these compounds as a result of aphid feeding. The study of the metabolism of phenols in the leaves of susceptible and relatively resistant winter wheat varieties after artificial infestation by *Rhopalosiphum padi* L. revealed significant changes in the content of total phenolics, phenylalanine and tyrosine ammonia-lyases and peroxidase activities (Leszczynski, 1985).

In this paper we describe changes in the activity of phenylalanine amonia-lyase and the content of free phenolic acids in the leaves and ears of two winter wheat cultivars differing in resistance and sensitivity to the grain aphid, *Sitobion avenae* (F.), (Havlíčková, 1993; Havlíčková & Sýkorová, 1994) partially resistant Regina and susceptible Zdar after infestation by *Metopolophium dirhodum* (Walker), *R. padi* and *S. avenae*.

Materials and Methods

Two winter wheat cultivars of Czechoslovak origin, Regina and Zdar, were used to study changes in phenolic metabolism caused by the cereal aphids, *Rhopalosiphum padi* (L.), *Metopolophium dirhodum* (Walker) and *Sitobion avenae* (F.).

Plant material and aphid infestation

Test plants were cultivated in Mitcherlich pots in a ventilated greenhouse under natural daylight. At the end of booting (49 GS) the plants in individual pots were infested with either *M. dirhodum* or *R. padi*. Infestation of ears by *S. avenae* was carried out at the beginning of anthesis. Uninfested plants (ears) of similar habit as infested ones served as the control. Both infested and uninfested plants (ears) were covered by nylon bags. The aphids were removed 14 days later. The leaves and ears of infested and uninfested plants were cut off, weighed and frozen in liquid nitrogen before PAL and phenolic acid determination.

Phenylalanine ammonia-lyase assay

Phenylalanine ammonia-lyase (PAL, EC 4.3.1.5) was extracted and its specific activity determined by the method described by Jangaard (1974). The absorbance of cinnamic acid produced in the assay mixture was determined at 275 nm using a VSU 2-P spectrophotometer (Zeiss, Germany). The amount of the enzyme catalysing the formation of 1 µmol cinnamic acid in 1 min at 40°C was defined as one enzyme unit.

Determination of phenolic acids

Free phenolic acids were obtained from a methanol extract of tissue ground in liquid nitrogen as described earlier (Cvikrová *et al.*, 1988) and analysed by means of HPLC using a Pye Unicam PU 4002-Video Liquid Chromatograph with a Spherisorb 5 ODS column (250 x 4.6 mm). Two solvents were used: A - 5 mM citric acid + 5 mM sodium dihydrogen orthophosphate + 0.3 mM caprylic acid (adjusted to pH 2.0 by addition of phosphoric acid), and B - 80% (v/v) methanol. Elution conditions were as follows: flow rate 0.5 ml min^{-1}, linear gradient from 10 to 35% B for 60 min, then from 35 to 50% B for 15 min, from 50 to 100% B for 5 min and finally 100% B for 5 min and 5 min from 100 to 10% B. The column eluate was monitored at 260 and 300 nm using a Multichannel detector PU 4021. Authentic compounds (Serva, Germany) were used as references for quantitative analyses.

Results

The infestation caused a large increase in PAL activity in leaves and ears of both cultivars. The difference in response to aphid infestation between cultivars was more significant in the leaves. The infestation with Md and Rp of the partially-resistant Regina evoked comparable induction of PAL activity (approximately three times higher than the activity determined in control plants), while in the susceptible Zdar the enzyme activity was significantly increased only after Md infestation (by about 325%). The infestation of ears of both cultivars with Sa resulted in a slight increase in PAL activity only (Table 1).

Table 1. Changes in the activity of PAL in the leaves and ears of two wheat cultivars infested by *Metopolophium dirhodum* (Md), *Rhopalosiphum padi* (Rp) and *Sitobion avenae* (Sa) in comparison to uninfested plants (control - C). Values represent the means ± SE of 4-6 replicates.

	PAL activity (mU g^{-1} DW)			
	C	Md	Rp	Sa
leaves Regina	27.3 ± 4.6	75.5 ± 10.5	82.5 ± 12.3	-
leaves Zdar	83.7 ± 9.1	86.9 ± 9.6	271.7 ± 32.5	-
ears Regina	5457.1 ± 490.5	-	-	6145.7 ± 528.1
ears Zdar	4928.5 ± 394.2	-	-	5751.4 ± 456.1

The increase in PAL activity coincided with the elevation of the content of free phenolic acids only in ears of both cultivars (by about 70% and 60% in resistant and susceptible cultivars, respectively; Table 2). The content of dominant phenolic acid, p-coumaric acid, increased after Sa infestation approximately twice and there were no significant differences between its content in Regina and Zdar.

Table 2. Content of free phenolic acids in uninfested ears (C) in plants infested with *S. avenae* (Sa). Results are the means of 2-4 determinations with deviations not exceeding 10 to 15%. Regina - resistant cultivar; Zdar - susceptible cultivar. CA = cinnamic acid, pCA = p-coumaric acid, FA = ferulic acid, pHBA = p-hydroxybenzoic acid, VA = vanillic acid,SA = sinapic acid.

Cultivar	Infestation	Phenolic acids (µg g^{-1} DW)					
		pHBA	VA	pCA	FA	SA	CA
Regina	C	0.37	0.46	2.97	0.43	0.74	trs
	Sa	0.57	0.68	5.63	0.66	0.94	0.06
Zdar	C	0.46	0.57	2.46	0.34	-	0.14
	Sa	0.51	0.60	5.06	0.34	trs	trs

In contrast, infestation of leaves of both cultivars with Rp and Md did not induce the increase in the pool of free phenolic acids. In spite of a high activity of PAL the level of total free phenolic acids in Rp infested leaves of susceptible Zdar did not change significantly. In the leaves of resistant Regina the content was decreased by about 25% and 45% upon Rp and Md infestation, respectively (Table 3). The decrease was caused mainly by the decline of p-coumaric acid.

Table 3. Content of free phenolic acids in the leaves of two wheat cultivars from uninfested plants (C) and infested by *M. dirhodum* (Md) and *R. padi* (Rp). Results are the means of 2-4 determinations with deviations not exceeding 10 to 15%. Abbreviations see Table 2.

Cultivar	Infestation	Phenolic acids ($\mu g\ g^{-1}$ DW)				
		pHBA	VA	pCA	FA	CA
Regina	C	0.30	0.50	1.87	0.36	0.10
	Md	0.36	0.51	0.46	0.15	0.20
	Rp	0.35	0.74	0.39	0.26	0.22
Zdar	C	0.41	0.81	0.68	0.32	0.23
	Md	0.68	0.73	0.68	0.21	0.21
	Rp	0.35	0.65	0.56	0.35	0.17

Discussion

The degree of aphid resistance in wheat was found to be positively correlated with the content of free phenols (Ciepiela, 1989a). Phenylalanine ammonia-lyase is generally considered to be the key enzyme in phenylpropanoid biosynthesis catalysing deamination of L-phenylalanine to trans-cinnamic acid. The induction of PAL activity observed in our experiment in both leaves and ears of susceptible and partially-resistant cultivars is in agreement with results reported earlier (Leszcynski, 1985; Ciepiela, 1989 b). Increase in the enzyme activity of the infested tissues may be caused either by the injury of plant cells (typical plant cell response to stress) or may be induced by the enzymes contained in aphid saliva. The relatively high level of p-coumaric acid determined in leaves and ears of the control and infested Regina and Zdar cultivars suggests the possibility of tyrosine ammonia-lyase (TAL) involvement in the biosynthesis of phenolics in tissues of test plants. Induction of PAL and TAL activity was observed in ears of a relatively resistant winter wheat cultivar after infestation by *Sitobion avenae* (Ciepiela, 1989 b) and in the leaves of both susceptible and relatively resistant cultivars infested by *Rhopalosiphum padi* (Leszczynski, 1985).

The increase in PAL activity coincided with the elevation of the level of free phenolic acids only in ears of both cultivars. The content of free phenolic acids in infested leaves was even lower than that in the control and our resuts were unexpected regarding the stimulation of PAL activity in infested leaves. The decrease was caused mainly by the decline in p-coumaric acid content. The p-coumaric acid serves as a precursor for the biosyntheses of chalcons and iso-flavonoids and so its decline in infested leaves may be connected with the syntheses of further phenylpropanoid defense compounds. This hypothesis is supported by the discovery of three un-identified substances of phenolic character in relatively high concentrations in infested leaves (two in the susceptible cultivar and one in the partially-resistant cultivar).

In order to obtain further information about the role of phenolic substances in aphid resistance further research will be focused on both the identification of newly-synthesised phenolic substances in infested leaves and on the rate of phenolic acid incorporation into the cell walls of cultivars.

Résumé

Modifications de la composition en acides phénoliques induites par l'infestation de deux cultivars de blé d'automne par les pucerons

L'activité de la phenylalanine ammonia-lyase (PAL) et les teneurs en acides phénoliques libres ont changé d'une manière significative dans les feuilles et les épis de deux cultivars de blé d'automne, Regina (partiellement résistant) et Zdar (sensible) après une infestation de *Metopolophium dirhodum* (Md), *Rhopalosiphum padi* (Rp) et *Sitobion avenae* (Sa). Une augmentation d'activité de PAL dans les feuilles et les épis des deux cultivars a été notée. La différence de réponse des cultivars à l'infestation de pucerons a été significativement plus appuyée dans les feuilles. Malgré une activité de PAL élevée, le niveau des acides phénoliques libres ne change pas d'une manière significative dans les feuilles infestées de Zdar, qui est sensible. Dans celles de Regina, qui est résistant, la teneur en composés phénoliques a diminué de 25% sous l'effet de Rp et de 45% sous l'action de Md. La diminution est due principalement à une baisse de teneur en acide p-coumarique. Dans les épis, le niveau de ce dernier, a approximativement doublé suite à une infestation par Sa, sans différences significatives entre Regina et Zdar. La contribution éventuelle des acides phénoliques à la résistance des céréales à l'égard des pucerons est discutée.

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Mechanism of resistance to *Trialeurodes vaporariorum* in *Cucumis* spp.

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Summary

The greenhouse whitefly *Trialeurodes vaporariorum* (Westwood) is the vector of the virus causing a yellowing disease in melon crops cultivated under greenhouse conditions in southern Europe. One-hundred and fifty-eight accessions of *C. melo* and several wild species of the genus *Cucumis* had been previously evaluated for resistance to the yellowing virus. One accession of each of *C. metuliferus*, *C. meeusii*, and *C. dipsaceus* species was selected for the experiments because of its resistance to the yellowing virus. No accession of *C. melo* was completely resistant, although an accession of *C. melo* var. *agrestis* showed mild and delayed symptoms and was also selected. Several components of *T. vaporariorum* life history were estimated on these selected accessions and on *C. melo* cv. 'Bola de Oro' (susceptible control). *C. metuliferus* and *C. dipsaceus* accessions proved to be susceptible to the whitefly. Antixenosis and antibiosis mechanisms of resistance against *T. vaporariorum* appeared to exist in the *C. melo* var. *agrestis* accession. In the *C. meeusii* accession, antixenosis resistance mechanisms were detected. Since the cross-compatibility barriers with the cultivated species which are present in the wild species studied do not exist in *C. melo* var. *agrestis*, the accession of this variety could be used in a breeding programme to introduce yellowing disease resistance in to cultivars with agronomic value.

Introduction

One of the yellowing-diseases that affect melon crops cultivated under plastic greenhouses in south Europe is caused by a closterovirus (Jordá-Gutierrez *et al.*, 1993) transmitted by the greenhouse whitefly *Trialeurodes vaporariorum* (Westwood) (Soria *et al.*, 1991). The control of this disease is difficult because of the wide virus host-range (Cura *et al.*, 1990) and the polyphagous nature of the vector *T. vaporariorum* (Mound & Halsey, 1978). To overcome virus diseases, the use of varieties with genetic resistance to the virus and/or to the vector is the most effective, and in some cases the only, control method (Fraser, 1990; Gray & Moyer, 1993). These resistant varieties can be used together with other control methods (physical, chemical, or biological) in an integrated disease management programme. One-hundred and fifty-eight accessions of *Cucumis melo* L. and several wild species of the genus *Cucumis* had previously been evaluated for resistance to the yellowing virus under natural and controlled infection conditions. One accession of each of *C. metuliferus*, *C. meeusii*, and *C. dipsaceus* species was resistant to the yellowing virus. No accession of *C. melo* was completely resistant, but an accession of *C. melo* var. *agrestis* showed mild and delayed symptoms (Soria *et al.*, 1989; Nuez *et al.*, 1991). Under natural infection conditions,

yellowing-affected genotypes supported higher *T. vaporariorum* populations than those accessions little or not affected (Soria *et al.*, 1989). Mechanisms of resistance to *T. vaporariorum* may be present in the above mentioned accessions.

Materials and Methods

Free-choice test

An experiment with three replicates was conducted in a glasshouse. Each replicate consisted of four plants of each *C. melo* var. *agrestis*, *C. meeusii* and *C. metuliferus*, and *C. melo* cv. 'Bola de Oro' (susceptible control) at the two-true-leaf stage which were placed randomly beneath a structure 1x1x0.8 m covered with an insect-proof muslin net (30 threads per cm). Eight-hundred whiteflies were released inside the structure. The whiteflies had been randomly collected, without separating the sexes, from a culture of the insect. Seventy-two hours later, enough time for *T. vaporariorum* to recognise the host-plant (van Lenteren & Noldus, 1990), the insects were removed using a small oral suction-tube, and the plants were kept in an isolated chamber. Thirty-five days later, the number of empty pupal cases per leaf and per plant were counted as this parameter is directly related to the fertility (number of viable eggs). At the same time, the number of leaves per plant was counted as a measure of the vegetative growth of genotypes. The mean daily minimum and maximum temperatures during the period were 18.8±2°C and 25.6±2°C, and relative humidities were 61.4±9% and 80.0±3%.

Non-choice test

Twenty plants of each accession of *C. melo* var. *agrestis*, *C. metuliferus*, *C. dipsaceus* and *C. melo* cv. 'Bola de Oro' (susceptible control), were used to feed one recently-emerged female of *T. vaporariorum* which was placed in a clip-on cage on the first leaf of each plant in a glasshouse. The single whitefly was transferred every 48 h to the next higher leaf on the same plant. The number of empty pupal cases was recorded on each leaf where the whiteflies had fed. Additionally, the number of eggs laid (fecundity) by eight-day-old females of *T. vaporariorum*, the percentage of pre-adult mortality (by counting the number of eggs laid which did not reach the adult stage), and the adult longevity of the offspring were estimated on *C. melo* var. *agrestis*, since this appeared to be the most promising genotype for future breeding, and on the susceptible control *C. melo* cv. 'Bola de Oro'. Eight-day-old females were chosen since greenhouse whiteflies show a higher reproduction rate around this age (Romanow *et al.*, 1991). The mean daily minimum and maximum temperatures were 17.3±1.9°C and 25.2±1.5°C, and the relative humidities were 53.1±10.5% y 83.5±10.5%.

The results of both experiments were subjected to analysis of variance and treatment means ere compared using the Student-Newman-Keuls test (Sokal & Rohlf, 1969).

Results and Discussion

Free-choice test

Under free-choice conditions, significant differences were found among the accessions ($p<0.001$) for the number of empty pupal cases per plant. *C. meeusii* was the most resistant accession as it showed the lowest number of empty pupal cases, followed by *C. melo* var. *agrestis*, but not significantly different (Table 1). The susceptible control *C. melo* cv. 'Bola

de Oro' supported a higher number of empty pupal cases per plant. According to den Nijs & Custers (1990), a high level of resistance to *T. vaporariorum* was to be expected in *C. metuliferus*. However, the accession of *C. metuliferus* used in this experiment supported the highest number of empty pupal cases (Table 1).

Considering the number of empty pupal case per leaf, significant differences ($p<0.001$) were observed among accessions. The accessions maintained the same order as in the above experiment. Again, the lowest number of empty pupal cases occurred on *C. meeusii* and *C. melo* var. *agrestis*, and the highest number on *C. metuliferus* (Table 1).

Table 1. Mean number of *T. vaporariorum* empty pupal cases per leaf and per plant under free-choice conditions, and mean number of leaves per plant.

Genotype	No. of empty pupal cases/plant	No. of empty pupal cases/leaf	No. of leaves
C. meeusii	101.3 c *	22.3 b	5.2±1.1
C. melo var. *agrestis*	237.4 c	54.0 b	4.4±1.0
C. melo cv. 'Bola de Oro'	537.1 b	124.3 a	4.5±1.1
C. metuliferus	1026.7 a	148.0 a	6.9±1.0

* Means with the same letter are not significantly different, $p<0.05$, Student-Newman-Keuls test.

Although at the beginning of the experiment all the plants were at the two true-leaf stage, *C. metuliferus* had more leaves than the other accessions at the time whiteflies were removed one week later (Table 1). *T. vaporariorum* appeared to benefit from this faster vegetative growth of *C. metuliferus* as it provided good conditions for feeding and colonisation. This observation supported that of Noldus *et al.* (1986) who reported that *T. vaporariorum* preferred younger, more tender tissues.

Antixenosis mechanisms of resistance to *T. vaporariorum* appeared to exist in accessions of *C. melo* var. *agrestis* and *C. meeusii*. The antixenosis would explain why, under field conditions (natural infection), the insect avoids *C. melo* var. *agrestis* and chooses attractive genotypes for feeding or egg-laying such as the susceptible *C. melo*. Whitefly colonies could only establish on *C. melo* var. *agrestis* when the insect population was very high. Symptoms of yellowing-disease would then appear late in the growing season and not in all plants, as previously observed by Soria *et al.* (1989).

Non-choice test

The analysis of variance revealed that the accessions had a signifficant effect ($p<0.01$) on the number of empty pupal cases obtained from a female *T. vaporariorum*. The number of empty pupal cases per plant was not significantly different among *C. dipsaceus*, *C. metuliferus*, and the susceptible control *C. melo* cv. 'Bola de Oro' (Table 2). The number of empty pupal cases on *C. melo* var. *agrestis* plants was considerably lower (Table 2). Because there were no significant differences between the number of eggs laid by single females on *C. melo* var. *agrestis* and the susceptible control *C. melo* cv. 'Bola de Oro' (Table 2), the decrease in the number of empty pupal case on *C. melo* var. *agrestis* appeared to be due to the high pre-adult mortality (Table 2). The pre-adult mortality could be a

consequence of the presence of one or more toxic chemical compounds in the plant tissues or the lack of some essential food material (Painter, 1968). *Trialeurodes vaporariorum* adult longevity was significantly reduced on *C. melo* var. *agrestis* than on the susceptible control (Table 2). These results appeared to indicate the presence of antibiosis resistance to *T. vaporariorum* in *C. melo* var. *agrestis.*

Table 2. Mean number of empty pupal case per individual of *T. vaporariorum*, and mean number of eggs laid per eight-day-old female, pre-adult mortality (%), and adult longevity of the offspring (days) on different genotypes.

Genotype	No. of empty pupal cases	No. of eggs	Pre-adult mortality	Adult longevity
C. melo var. *agrestis*	15.7 b*	14.3 a	92.9 a	9.3 a
C. melo cv. Bola de Oro	69.8 a	20.6 a	46.9 b	17.3 b
C. metuliferus	69.9 a	--	--	--
C. dipsaceus	72.7 a	--	--	--

* Means with the same letter are not significantly different, $p<0.05$, Student-Newman-Keuls test.

The yellowing virus is not transmitted either mechanically or by seed, but it is efficiently transmitted by its vector (Soria et al., 1991). To keep melon crops free from yellowing-disease, *T. vaporariorum* would have to be eliminated from the entire crop. Nevertheless, research to obtain commercial cultivars that incorporate the resistance to *T. vaporariorum*, combined with either chemical or biological insect control, would decrease the insect population sufficiently to delay the disease outbreak. Since the cross-compatibility barriers with the cultivated species which are present in the wild species studied (Esquinas-Alcazar & Gulick, 1983; Beharav & Cohen, 1994) do not exist in *C. melo* var. *agrestis*, this particular accession of this variety could be used in a breeding programme to introduce yellowing disease resistance in cultivars with agronomic value.

Résumé

Mécanisme de résistance chez *Cucumis* spp. à l'égard de *Trialeurodes vaporariorum*

La mouche blanche des serres *Trialeurodes vaporariorum* (Westwood) est le vecteur du virus responsable d'un jaunissement dans les cultures de melon en serre du sud de l'Europe. Cent cinquante huit variétés de *C. melo* et plusieurs espèces sauvages du genre *Cucumis* ont été évaluées antérieurement pour leur résistance à ce virus. Une obtention de chacune des espèces *C. metuliferus, C. meeusii,* et *C. dispacens* a été choisie pour les essais en raison de leur résistance à l'égard du jaunissement. Aucune obtention de *C. melo* ne s'est montrée complètement résistante, bien qu'une obtention de *C. melo* var. *agrestis* ait extériorisé des symptômes atténués et différés. Elle a également été incluse dans l'essai. Plusieurs composants du cycle de développement de *T. vaporariorum* ont été estimés sur ces obtentions

sélectionnées et sur *C. melo* cv. "Bola de Oro" (témoin sensible). Les obtentions de *C. metuliferus* et *C. dipsacens* se sont avérées sensibles à l'égard de la mouche blanche. Des mécanismes de résistance à *T. vaporariorum* du type antixénose et antibiose semblent semanifester chez l'obtention de *C. melo* var. *agrestis*; ils ont également été détectés pour l'antixénose, chez l'obtention de *C. meensii*. Puisque *C. melo* var. *agrestis* ne présente pas de barrières de compatibilité de croisement avec les espèces cultivées, à la différence des espèces sauvages étudiées, cette obtention peut être utilisée dans un programme de sélection pour introduire la résistance au jaunissement dans les cultivars qui présentent une valeur agronomique.

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The role of flower volatiles in host attraction and recognition by the raspberry beetle, *Byturus tomentosus*

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Summary

Fundamental and applied studies have been initiated in order to understand the role(s) of flower volatiles in host finding and recognition by adults of the raspberry beetle, *Byturus tomentosus*. Behavioural assays using a linear track olfactometer have shown that female beetles clearly prefer host (raspberry; *Rubus idaeus*, hawthorn; *Craetaegus monogyna*) to non - host (oilseed rape; *Brassica napus*) flower volatiles. They also prefer the flower volatiles of susceptible red raspberry cv Glen Prosen to those emitted from flowers of the highly resistant related species, *Rubus phoenicolasius*. The phenological stage of the developing raspberry flower also influences the characteristic volatile profile and appears to convey additional information to the female beetles when searching for feeding and oviposition sites. Chemical investigations of entrained flower volatiles or ether extracts indicate that > 100 chemical compounds are emitted, with profiles characteristic of the plant species and its phenological stage. Electrophysiological techniques using electroantennogram and single cell recordings (with IACR, Rothamsted) are being applied to "shortlist" active chemicals from these complex profiles for behaviour testing. Recent results using olfactometers and a wind tunnel indicate that raspberry beetles use several flower compounds, in specific ratios, to identify suitable hosts for feeding and ovipostion. We have identified several of the main flower volatile components involved in host recognition by raspberry beetles and are using this information to target breeding for the development of resistant *Rubus* genotypes and to design more effective field traps for monitoring beetle activity.

Introduction

The raspberry beetle, *Byturus tomentosus* (Degeer), is a major pest of cultivated and wild *Rubus* species (raspberries and blackberries, and hybrid berries) in Europe, and a closely related species, *B. unicolor*, (raspberry fruitworm) causes similar damage in N. America. *B. tomentosus* larvae normally inflict more damage than adults, by tunnelling into the developing fruit, allowing entry of pathogens or directly contaminating harvested fruit. Adult beetles can also damage unopened buds, unfolding leaves of first year canes and opened flowers during feeding. Adults usually start to emerge from the soil in late April to mid-May, and in warm weather migrate to flowers of other Roseaceous plants, including hawthorn, until raspberries begin to flower. They then return to early flowering raspberry cultivars, where they feed, mate

and females lay eggs mainly on the stamens. Larvae emerge from eggs after a few days where they initiate fruit damage.

At present insecticides are routinely applied to the ripening fruit ("green/pink" stage) close to harvest and targeted against eggs and recently emerged larvae, before they have caused significant fruit damage. As part of a general strategy at SCRI to develop more environmentally-benign control methods for pests of soft fruit (Brennan *et al.,* 1992; Gordon, 1992; Gordon *et al.*, 1993) we are investigating the fundamental biology and behaviour of raspberry beetles. Our main emphasis is on the role of host plant volatiles in attraction to and recognition of host plants by soft fruit pests. Sources of resistance to raspberry beetle in wild *Rubus* species including *R. coreanus, R. craetigifolius, R. occidentalis* and *R. phoenicolasius* have been reported in the past and have been used in breeding programmes (Briggs *et al.,* 1982) but little is known about the resistance mechanisms involved. One of the long term aims at SCRI is to breed pest - resistant raspberry cultivars, based on a detailed knowledge of susceptibility/resistance mechanisms operating. Our approach should enable us to develop selectable markers (chemical and/or molecular) for pest resistance and also identify plant - derived attractants, repellents and deterrents which complement host plant resistance in sustainable IPM programmes.

Recent progress

Behaviour studies

a) Tests on hosts and non - hosts: A linear track olfactometer, modified from the original design (Sakuma & Fukami, 1985), is being used to quantify behavioural responses of adult male and female raspberry beetles to flower volatiles and to identified chemical components. These olfactometer tests have provided clear evidence that flower volatiles from raspberry or from other Roseaceous hosts are highly attractive in a choice with a control (moist air). In similar choice tests between a non - host (oilseed rape flower volatiles) and the moist air control no clear preference was observed (Woodford *et al.,* 1992 ; papers *in prep.*). Further olfactometer choice tests between flower volatiles from the host (raspberry) and a non - host (oilseed rape) again showed a clear preference for the raspberry flower volatiles. Interestingly, raspberry beetles that emerged early in the 1991 season, before raspberry flowered, showed a distinct preference for hawthorn volatiles (early season "temporary host" for adult aggregation and feeding) over raspberry volatiles (normal host for mating, oviposition and larval feeding). However, this preference for hawthorn flower volatiles was not seen in later tests over three subsequent seasons. These and other studies indicate that the phenological stage of the host flower may alter the chemical composition of volatiles and hence their relative degree of attraction to raspberry beetles during host finding.

b) Tests on beetle - resistant *Rubus* species: *R. phoenicolasius* (Japanese wineberry) was selected as a "model" beetle - resistant wild *Rubus* species for behavioural and chemical studies. This species was reported by Briggs *et al.,* (1982) as being the most resistant in comparative "sleeve" (muslin bags or nylon stockings tied over fruiting laterals) inoculation tests under field conditions. Under their experimental conditions raspberry beetles laid very few eggs on flowers on *R. phoenicolasius* and the fruit were virtually free from larval infestation, indicating antixenosis as a main component of resistance. Recent olfactometer tests, giving female beetles the choice of flower volatiles from *R. phoenicolasius* and the red raspberry cv. Glen Prosen, clearly showed that beetles have a strong preference for red raspberry. *R. phoenicolasius* is covered in glandular hairs which emit a distinctive aromatic odour.

In more recent "sleeve" inoculation tests at SCRI (S.C. Gordon *et al., unpublished*) high levels of beetle resistance were also noted in a purple raspberry breeding line, indicating that the source of resistance may be from a *R. occidentalis* (black raspberry) parent. The *R. occidentalis* cv Munger has previously been reported to be highly resistant to raspberry beetle (Jennings *et al.,* 1977). The mechanism of this resistance in purple raspberry selections to raspberry beetle is currently being investigated.

Flower volatile chemistry

Flower volatiles from four raspberry cultivars and from wild hawthorn were entrained on the porous polymers Haysep Q and Tenax TA. These were analysed on an automated thermal desorption (ATD) - gas chromatography (GC) - mass spectrometry (MS) system at SCRI and by GC-MS analyses of ether extracts in a collaborative study with IACR, Rothamsted (Robertson *et al.,* 1993). The major classes of compounds (> 100 detected in each entrainment) included aliphatic and aromatic hydrocarbons, aldehydes, ketones, alcohols and esters, monoterpenes and sesquiterpenes and a number of unusual nitrogen compounds. The volatile profiles of the four raspberry cultivars at full flowering were complex but exhibited only minor differences between them. Volatiles of hawthorn flowers, although similar to raspberry, contained elevated levels of several compounds not found in raspberry cultivars. The volatile profile of the resistant wild species *R. phoenicolasius* contained elevated levels of terpenoid and other compounds, in addition to the typical volatile profile of red raspberry (G. Robertson *et al., in prep.*). The chemical identities of possible repellent compounds in this and other wild *Rubus* species are currently being investigated at SCRI using ATD - GC - MS (see below).

Field observations from a joint AFRC (now BBSRC) Link programme with St Andrews University (Wilmer *et al.*, in press) and confirmatory olfactometer tests at SCRI indicate that the preference of the female for oviposition sites is strongly influenced by the phenological stage of the raspberry flower. In a second series of entrainment experiments we investigated changes in volatile profiles during flower development of raspberry cv. Glen Prosen. ATD-GC-MS analyses of volatiles from green buds, flowers, green fruit, pink fruit and ripe red fruit showed major changes in the composition of the odour profile. As the flowers matured, levels of "green leaf" volatiles declined whilst several monoterpenes increased. During fruit ripening several additional compounds appeared, which are highly characteristic of raspberries, followed by the production of higher levels of several types of acetates (Robertson *et al.,* 1995). Further experiments are now in progress to identify the key behaviourally - active compounds which convey information on the development state of the flower and fruit to raspberry beetles.

Electro-antennogram studies to target chemical identification

In collaborative studies between SCRI and IACR Rothamsted, components of raspberry and hawthorn flower extracts separated by GC were passed over a raspberry beetle antenna. In the initial experiments, electrophysiological recordings were made from the whole antenna (an electroantennogram = EAG) and from individual olfactory receptors (single cell recording) of raspberry beetles at IACR. Several peaks which showed electrophysiological activity were identified by GC-MS and are currently undergoing further EAG and behaviour bioassays. Our most recent results using olfactometers, a wind tunnel and EAG dose - response studies indicate that both qualitative (number of chemical components in the odour blend) and quantitative (ratios of components) characteristics of the flower profile are important to elicit a normal behavioural response (equivalent to flowers) by beetles.

Discussion

We have shown that flower volatiles are important in host finding (attraction) and recognition (potential feeding and/or oviposition sites). Furthermore, raspberry beetles discriminate between hosts, non-hosts/resistant *Rubus* species using olfactory information from flower volatiles. Raspberry beetles also appear to obtain extra information on the phenological stage and physiological condition of the developing fruit from characteristic volatile profiles emitted from buds, opened flowers, green and fully ripened fruits. This olfactory information modifies beetle behaviour in host selection for feeding and/or oviposition, but is probably only one of several types of cues used. Ongoing field trials and scanning electron microscope studies indicate that visual and contact stimuli are also important.

Our chemical studies have revealed the complexity and dynamic nature of volatile profiles emitted from host and non-host flowers. In most analyses > 100 compounds are detected, many of which are minor components more characteristic of the host than some of the major components (e.g. more ubiquitous "green leaf" volatiles). A further complexity in obtaining "biologically relevant" chemical data for plant - insect studies is that each extraction or entrainment method and GC-MS system used will produce different sets of chemical data, both in terms of relative proportions of components detected and in the classes of compounds trapped. It is an impossible task to isolate or purchase all the compounds identified and perform behavioural assays. Because of this we, like many others, have adopted a strategy of using electrophysiology to identify compounds which the insect's antennae can detect. This then provides a "short-list" of candidate volatiles which must be characterised in terms of their behavioural effects (e.g. attraction, repulsion) over a range of naturally - occurring concentrations. Research at SCRI and in collaboration with IACR, Rothamsted has led to the identification of a number of candidate volatiles for further studies. Although this list of electrophysiologically-active compounds is not yet complete it has enabled us to show that both qualitative (the number of chemical components) and quantitative (ratios of components in the odour blend) appear to be important in order to elicit a strong behavioural response. Similar observations, indicating the importance of the "optimal blend" of volatile components for attracting insects to baited traps have been reported for the sunflower seed weevil (Roseland *et al.,* 1992). This information is now being used to design improved traps for monitoring raspberry beetle activity in the field and to target plant breeding for raspberry beetle resistance.

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Résumé

Rôle des substances volatiles florales dans l'attraction et la reconnaissance de l'hôte par le ver des framboises, *Byturus tomentosus*

Des recherches fondamentales et appliquées ont été engagées pour déterminer le rôle des substances volatiles des fleurs dans la recherche et la reconnaissance de l'hôte par l'adulte du ver des framboises, *Byturus tomentosus*. Les essais menés dans un olfactomètre à flux linéaire ont montré que les femelles du coléoptère préfèrent clairement les substances volatiles des fleurs de plantes-hôtes (framboisier, *Rubus idaeus* ; aubépine, *Crataegus monogyna*) à celles de non-hôte (colza , *Brassica napus*). Elles préfèrent également les substances volatiles des fleurs de framboisier cv "Glen Prosen" à celles émises par l'espèce parente hautement résistante *Rubus phoenicolasius*. Le stade phénologique de la fleur du framboisier en formation influence aussi le profil des substances volatiles et fournit d'autres informations à la femelle du ravageur lorsqu'elle cherche des sites de nutrition et de ponte. L'analyse chimique par entraînement des substances volatiles des fleurs ou par extraction à l'éther indique que plus de 100 substances sont émises, avec des chimio-profils caractéristiques de l'espèce végétale et de son stade phénologique. Grâce aux techniques électrophysiologiques comportant des électroantennogrammes et des enregistrements sur cellules individuelles (avec IACR, Rothamsted), une liste de substances actives est en élaboration à partir de ces profils complexes, pour des tests de comportement. Les résultats récents obtenus en olfactomère et en tunnel font penser que l'adulte du ver des framboises utilise plusieurs composants floraux, dans des proportions spécifiques, pour identifier les hôtes qui conviennent à sa nutrition et à sa ponte.

Nous avons identifié plusieurs des composants volatiles majeurs des fleurs qui sont impliqués dans la reconnaissance de l'hôte par le ravageur, et utilisons cette information pour cibler la sélection en vue de développer des génotypes résistants de *Rubus* et des pièges plus efficaces pour suivre l'activité de l'insecte en plein champ.

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