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

Coventry, United Kingdom
13-16 September 1992

Edited by P.R. Ellis & J. Freuler

INTRODUCTION

Sixth Triennial Meeting

The sixth triennial meeting of the Working Group was organised by Bob Ellis from Horticulture Research International and held from 13 to 16 September 1992 at the University of Warwick, Coventry, Great Britain. The meeting was opened by Professor Chris Payne, Chief Executive of Horticulture Research International, Wellesbourne. Professor Payne welcomed the participants to the heart of England 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 excursions to Horticulture Research International, at Wellesbourne, Warwick and to the National Centre for Organic Gardening at Ryton, Coventry. On the second evening of the Conference the participants visited the Royal Shakespeare Company's theatre, 'The Other Place' to see the play 'King Richard 111'.

The meeting was largely sponsored by the IOBC with contributions from Eucarpia (the European Association for Research on Plant Breeding) and the Vegetable Research Trust (a Horticulture Research International charity). Grants were made to seven participants who were facing financial difficulties in attending the conference.

A total of 45 participants from 11 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-three papers were presented during the conference and three additional reports received for inclusion in this Bulletin, the proceedings of the conference.

Future 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 1995, the proposed date for the next meeting.

2.   Venue for the Next Meeting

Three locations for the next meeting of the Working Group in 1995 were proposed:- The Netherlands, Scotland and Sweden. After debate about ease of transport, costs of accommodation and rotation of hosts it was agreed that the meeting in 1995 would be held in Wageningen, The Netherlands and that the local organiser would be Chris Mollema.

3.   Convenor of the Working Group

Bob Ellis, the present Convenor believed it would be wise to elect his successor. Nick Birch, from the Scottish Crop Research Institute was willing to stand and was unanimously elected. His appointment will need to be ratified by the IOBC Council. He will take up office 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.

Examples of successful Project Groups were mentioned. For example, the Carrot Fly Group, formed in 1976 under the leadership of Bob Ellis and involving participants from five European countries, carried out collaborative experiments at 12 sites in five countries over two seasons. Meetings of the Project Group were held in Denmark, England and The Netherlands. It was agreed that this had been a very successful example of a Project Group. More recently the Lettuce Aphid Project Group had been led by Kees Reinink and Frans Dieleman from The Netherlands. This Group organised collaborative experiments in 1990 and 1991 at twelve sites in six countries; the results of this collaboration are published in this Bulletin. Once more it was considered to have been a most successful Project Group.

At the meeting in Marcelin, Switzerland in 1989 three other Project Groups were formed. The Group concerned with the western flower thrips, organised by Chris Mollema, had involved mainly Dutch seed companies but Chris was keen to expand the activities and include research scientists from other countries. Erich Stadler's Project Group on Plant Surface Chemistry/Root flies had completed collaborative work which is published in this Bulletin. Once more Erich encouraged other interested scientists to contact him about future collaboration. A Project Group led by Maarten Van Helden on EPG and Stylet Cutting work had been active in The Netherlands. One of the collaborators, Freddie Tjallingii, organised workshops and

training sessions on EPG techniques which had been attended by several Working Group members. It was decided to strengthen the Groups activities and to disseminate information through the Aphid Resistance Newsletter which is organised by Jens Weibull.

The feasibility of forming two new Project Groups was discussed: Jens Weibull will coordinate a Group on Cereal Aphids and Rosemary Cole will investigate the possibility of setting up a Group on Aphid Biotypes.

5.   Publications

a)   Aphid Resistance Newsletter. Jens Weibull 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. Bob Ellis urged participants to send their final versions of papers to him as soon as possible. It is planned to submit the whole Bulletin to the publishers early in 1993.

6.   Other Points

a)   Aad Van Der Arend expressed an Industry Viewpoint on the Working Group and its activities. He spoke on behalf of all the representatives of the seed industry and fully supported the Working Group in its objectives. There was excellent collaboration between members of the Group and the industry and it was important for everyone to maintain contact and discuss problems in pest control. He felt sure industry would continue with its support and attend future meetings in strength.

b)   Chris Mollema stressed the importance of members of the Working Group attending the meetings of similar societies in other countries. For example, several members of this Working Group had attended meetings of the Plant Resistance to Insects Workshop in the USA and found these meetings profitable.

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

P R Ellis   February 1993

SIXTH MEETING OF THE IOBC/EUCARPIA WORKING GROUP ON BREEDING FOR RESISTANCE TO INSECTS AND MITES

University of Warwick, Coventry, Great Britain

13-16 September 1992

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MECHANISMS OF RESISTANCE TO THE CABBAGE AND TURNIP ROOT FLIES: COLLABORATIVE FIELD, BEHAVIOURAL AND ELECTROPHYSIOLOGICAL STUDIES

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Summary

Collaborative studies are targeted at understanding the mechanisms of resistance and susceptibility to *Delia radicum* and *D. floralis* in brassicas, so that rapid and sensitive laboratory-based screens can be designed for use in plant breeding programmes. Previous field and field cage studies identified antixenosis as the major component of root fly resistance, resulting in reduced oviposition by adults of both species. Four *Brassica* genotypes representing a range of antixenotic resistance were selected for detailed experiments. Behavioural analysis revealed that adult flies of both species predominantly rejected resistant plants after walking on the leaf surface. Tests of leaf surface extracts sprayed on to artificial leaves demonstrated that the oviposition elicited by the methanol-soluble fractions of the four brassicas closely reflected the level of oviposition preferences of *D. floralis* on the young plants. Electro physiological responses of *D. radicum* and *D. floralis* indicated that specific tarsal contact chemoreceptors were stimulated by these leaf surface chemicals. These methanol-soluble fractions were found to include not only several known glucosinolates but also at least one other very active compound, currently being identified. The combined use of behavioural bioassays with contact electrophysiology has proved invaluable in isolating the key oviposition stimulants on the leaf surface and could provide a powerful tool in screening for pest resistance in the future.

Introduction

Recent field trials using swedes which are partially resistant to turnip root fly (TRF, *Delia floralis* (Fallen)) and cabbage root fly (CRF, *D. radicum* (L.)) in combination with half the normal rate of insecticide have demonstrated the potential for integrated control of these two important brassica pests (Ruuth & Hellqvist, 1989; Taksdal, 1992; McKinlay & Birch, 1992). In order to assist plant breeders to screen for greater levels of root fly resistance we have continued to research into ways of improving the conventional screening methodology. Initially field trials were used to select sources of combined resistance to both pests and also to determine that antixenosis (reduced egg laying) was the main component of resistance (Alborn, Karlsson, Lundgren & Stenhagen, 1985; Birch 1988). Field cages were then used to screen cultivars and breeding lines for greater levels of antixenosis (Birch, 1989).

At this point a more detailed collaborative study of the resistance mechanism(s) was undertaken on four brassicas, selected to exhibit a wide range of antixenotic resistance to one or both *Delia* species. We chose swede cv. Doon Major (susceptible to TRF and CRF), swede breeding line GRF aga (partially resistant to TRF and CRF), kale cv. Dwarf Green Curled (moderately resistant to TRF and CRF) and kale cv. Fribor (strongly resistant to TRF but not tested against CRF by Alborn *et al.*, 1985). This project currently involves behavioural, chemical and electrophysiological studies at three research centres.

Progress

Insect behaviour

Detailed observations of the oviposition behaviour of TRF and CRF on young plants of the four brassicas indicated that both root fly species use leaf surface chemicals to discriminate between resistant and susceptible plants. Leaf surface extracts from the swedes and kales were sprayed on to artificial leaves (Roessingh & Städler, 1990) and the egg laying activities elicited by the surface extracts were assessed. Results from the methanol-soluble fractions of the extracts tested in Wädenswil (CRF) and Dundee (TRF) correlated with results from oviposition on the whole plants (Hopkins, Birch, Griffiths & McKinlay, 1992). Further behavioural tests on CRF using a range of purified glucosinolates sprayed on to artificial leaves showed that some of these compounds were more active than others (Roessingh, Städler, Fenwick, Lewis, Nielsen, Hurter & Ramp, 1992). For example, in a multiple choice bioassay CRF females differentiated between artificial leaves treated with different glucosinolates. Seven leaves were treated with one of seven glucosinolates and one leaf was untreated and acted as a control. Compared with the control, at concentrations as low as $10^{-7}M$, females laid more eggs on leaves treated with gluconasturtiin, gluconapin, glucobrassicin and glucobrassicanapin. This latter glucosinolates also stimulated oviposition of the TRF (Simmonds *et al.*, in prep.).

Electrophysiological activity of leaf surface extracts and fractions on tarsal receptors

Root flies have several types of receptors (chemosensilla) on their tarsi and labellum. These receptors are used by the adult female to detect compounds on the plant surface and assess the plant's suitability as a site for egg laying. The responsiveness of the main tarsal receptors to leaf surface chemicals of the four brassicas have been studied at Wädenswil (CRF) and London (TRF). Progress was greatly assisted by chemical fractionation techniques recently developed at Wädenswil, which led to the discovery of a potent oviposition stimulant, provisionally called "CIF" (cabbage identification factor) until fully identified (Roessingh, Städler, Hurter & Ramp, 1992; Roessingh *et al.*, 1992). This compound, apparently not a glucosinolate, stimulates receptors in a pair of tarsal sensilla (type C_5 sensilla). It is several orders of magnitude more active as an oviposition stimulant than any of the glucosinolates tested. Interestingly, this newly discovered compound does not stimulate receptors in the type D tarsal sensilla, which are responsive to glucosinolates.

These electrophysiological screening methods were used to bioassay leaf surface extracts and fractions from the four selected brassicas. Ion-exchange columns were used to prepare fractions from the surface extracts and screened for activity against the "CIF"-sensitive C_5 sensilla of TRF and CRF. Initial results indicate that one or more as yet unidentified compounds (including one major stimulant with "CIF"-type activity) are involved in oviposition preference, in addition to the glucosinolates. We are continuing to study electrophysiologically the activity of more purified fractions in an attempt to isolate the fractions that contain the stimulatory compound(s), which will then be purified and their chemical structure(s) determined.

Discussion

Collaborative studies involving entomologists, plant breeders and chemists have enabled us to make progress towards understanding the mechanisms of susceptibility and resistance to root flies in brassicas. We have further evidence, supporting earlier work by Alborn *et al.* (1985) that leaf surface chemicals play a major role in determining the oviposition preferences of root flies. From our studies comparing the behaviour and receptor sensitivity of the two *Delia* species, it seems that in general *D. floralis* and *D. radicum* respond almost identically to our four selected brassicas and also to their extracted leaf surface chemical profiles. Overall, electrophysiological activity correlated well with bioassay results of the leaf surface fractions on artificial leaves and also with oviposition behaviour on whole plants.

Initial chemical studies of the surface extracts of the four selected brassicas suggest that although some glucosinolates are involved in stimulating ovipositions, the major stimulatory compound(s) are contained in "non-glucosinolate" ion-exchange fractions. For example, the relative electrophysiological responses to "non-glucosinolate" fractions from surface extracts of the most resistant kale Fribor are lower than the responses to the most susceptible swede Doon Major. This suggests that the observed differences in levels of antixenotic resistance of the four brassicas may be due to quantitative variation of one or more key oviposition stimulant(s) contained in this "non-glucosinolate" fraction and maybe to a lesser degree, also in

other fractions. Once the full chemical structure of "CIF" and some not yet identified compounds have been determined we should be able to confirm its presence in the "non-glucosinolate" fraction and to measure its concentration in the surface extracts of the four brassicas. Since the necessary chemical fractionation procedures are relatively simple prior to electrophysiological recording, these techniques have the potential to be developed as a powerful tool in developing chemical screening methods of pest resistance in future plant breeding programmes.

Acknowledgements

Résumé

Mécanisme de résistance aux mouches du chou et du radis: collaboration pour les recherches au champ ainsi que pour les études comportementales et électrophysiologiques

Des efforts de collaboration sont destinés à la compréhension des mécanismes de résistance et de sensibilité des *Brassica* à l'égard de *Delia radicum* et *D. floralis*, dans le but de déterminer une méthode de laboratoire utilisable dans les programmes de sélection, à la fois rapide et sensible pour l'évaluation du matériel végétal. Des études antérieures en champ et dans des cages extérieures ont révélé l'antixénose comme étant le facteur le plus important de la résistance aux mouches, résultant en une diminution des pontes par les adultes des deux espèces. Quatre génotypes de *Brassica* ayant montré une palette de résistance antixénotique ont été choisis pour des expériences plus fines. Les analyses de comportement ont révélé que les diptères adultes des deux espèces rejettent d'une manière prédominante les plantes résistantes après avoir parcouru la surface foliaire.

Si les extraits des surfaces foliaires sont pulvérisés sur des feuilles artificielles, on peut démontrer que l'oviposition obtenue avec les fractions solubles au méthanol des quatre *Brassica* reflète de près les préférences d'oviposition de *D. floralis* sur de jeunes plantes.

Les réactions électrophysiologiques de *D. radicum* et de *D. floralis* indiquent que des chimiorécepteurs tarsaux de contact sont stimulés par ces substances de la surface foliaire. Ces fractions solubles au méthanol contiennent plusieurs glucosinolates connus et au moins un autre composé très actif dont les travaux d'identification sont en cours.

La combinaison d'essais comportementaux et d'électrophysiologie de contact s'est avérée très efficace à l'isolation des stimulants-clés de la surface foliaire et pourrait constituer à l'avenir un puissant outil dans la sélection pour la résistance aux insectes.

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BREEDING FOR CABBAGE ROOT FLY (Delia radicum) RESISTANCE IN CAULIFLOWER

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Summary

Progress in breeding for resistance to cabbage root fly in cauliflower is reported. Selection for parental lines was continued on a biennial cycle. This resulted in the production of the F_4 generation for the odd-year cycle lines in 1991 and the F_4 for the even-year cycle lines in 1992. The greatest difference between lines was achieved in 1990 when the least preferred line had 1.3 and the most preferred line had 28.7 eggs/plant; this represented a 22-fold difference in egg-laying. It is difficult to achieve steady progress in improving levels of resistance between one generation and the next. Segregation for high and low egg numbers of descendants from one parent was observed for the first time in the F_4 in 1991. Certain of the 1991 breeding lines possessing promising levels of resistance were tested in the field in the presence of a high cabbage root fly population. The root damage indices for these lines was correlated with egg-laying records.

Introduction

Integrated pest management (IPM) programmes utilise partial resistance to pests in crop plants because it permits a reduction in chemical treatments by either spraying less frequently or by reducing the dose of insecticide. Reduced chemical inputs may also have positive effects on parasites and predators of the target pest.

Materials and Methods

Since 1979, investigations have been made of antixenosis (non-preference) resistance in cauliflower (*Brassica oleracea* L. var. *botrytis* L.) to the cabbage root fly (*Delia radicum* L.). Cauliflower is regionally an important crop and although it is very attractive to cabbage root fly the tolerance threshold of this stem crucifer for cabbage root fly is much higher than that of root crucifers.

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The relative level of resistance was determined by counting eggs laid around the stem of the plant. Egg counts on a great number of plants has become feasible through the use of the egg trap. The cultivars were grown in the field in experimental plots and exposed to a naturally occurring fly population.

The experimental site was located at Lullier near Geneva. It has proved to be suitable for this work as the fly population is sufficiently high to reveal genetic variation among the plants without compromising their survival.

The experimental lay out used was a randomised block design with 5 replicates. Each subplot comprised 20 plants in a double row, and 8 plants in a subplot were sampled for eggs.

In order to synchronise plant growth with maximum egg laying which takes place during July and August, the sowing time chosen was the beginning of May followed by planting out before mid-June.

Egg counts were taken 4-5 times at 10-day intervals between the beginning of July and the end of August. This ensured that 68%-92% of the eggs laid were trapped (Freuler *et al.*, 1984), which is believed to be sufficient to minimise an inevitable effect on cauliflower which was described by Ellis, Hardman, Crisp & Johnson (1979) for radish where a modification of the attractiveness of the plant intervenes according to its physiological age.

In order to avoid the effects of plant size on egg laying the development of plants in subplots was recorded. Between the end of July and the beginning of August the average height and diameter of the largest and smallest plants per subplot was recorded. Subplots which represented the extreme of development were discarded for selection and statistical analysis.

The selection scheme which involved setting up breeding lines from non-attractive individual plants of proven commercial value was determined by the fact that differences in egg counts, although significantly different for cultivars, were even more different for individual plants within a cultivar (Freuler & Gagnebin, 1984). Selection took place at the end of the egg recording period in September and took into account leaf and curd quality as well as antixenosis.

In these trials the growing period chosen resulted in plants maturing at low temperatures and in short days, creating poor seeding conditions. Thus, in 1983, the *in vitro* culture technique was introduced. Plants selected in the autumn were multiplied during the winter. Regenerated plantlets taken from the same curd were vernalised and flowered the following year. These were used for cross- and self-pollination in cages in a glasshouse. Thus two biennial selection cycles could be started, one occurring in odd years commencing 1983 and one occurring in even years starting in 1984.

In order to detect undesirable effects of *in vitro* culture, checks were made of plants raised from the cultures. The characters examined included leaf, stem and inflorescence development as well as development of reproductive parts including

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flowers, flower stems, flower number, self-pollination capacity, fruit and seed (Gagnebin & Freuler, 1988). In addition, after several tube transfers selected lines were grown in the field to check curd development.

Progress in the selection programme was estimated by comparison with standard cultivars mainly Imperator (XIV) beginning in 1981 but also cv. 'Panda' (X) starting in 1989. Comparisons were made of egg numbers on progenies selected for resistance (✓) or susceptibility (↗). After egg counts have been adjusted to these standards comparisons can be made between parents and progenies.

Results

Since the last meeting of the working group in Marcelin in 1989 (Freuler et al., 1990), three more selection cycles have run: two even-year cycles in 1990 and 1992 and one odd-year cycle in 1991.

In 1990, when most lines had reached the third generation (Table 1) egg laying was generally low. The mean number of eggs per plant varied from 1.3 to 28.7 (or 22-fold). Most of the eggs were found on parent reference line No. 21. Lines 9, 10 and 11 were all derived from this parent but had not responded to selection to the same extent (differences were not significant). We observed no response for selection for susceptibility.

Table 1: Egg counts in 1990 on cauliflower varieties and progenies selected for resistance (✓) or suceptibility (↗) to cabbage root fly during the series of even years

Nr.	Variety/line			Mean number of cabbage root fly eggs per plant	Significance
18	XVII/64/56	✓		1.3	a
5	X/20,1/69/38/36	✓		1.4	a
10	XIV/66/14/4	✓		1.5	a
17	XVII/30/21	✓		2.5	ab
1	III/44/34/17	✓		4.8	ab
6	X/20,5/9/21/45	✓		6.0	abc
11	XIV/66/14/42	✓		6.4	abc
7	X/20,5/73/31/45	✓		7.4	abc
13	XV/25/20/68	✓		7.8	abc
14	XVI/27/22/60	✓		7.8	abc
12	XV/25/5/87	✓		8.8	abc
15	XVI/47/59/63		↗	9.5	abc
16	XVI/93/39/12	✓		10.1	abc
4	X/20,1/31/26/38	✓		11.0	abc
3	III/44/54/27		↗	11.5	abc
9	XIV/35/38/44	✓		12.1	abc
19	XX/4	✓		13.9	bc
2	III/44/34/8	✓		17.4	cd
8	XIV/35/38/23	✓		26.1	de
21	XIV	-		28.7	e

In 1992, most lines had reached the fourth generation (Table 2) and egg laying was generally high. The mean number of eggs per plant varied much less and ranged from 19.8 to 49.4 (or 2.5-fold). The parent reference line was again amongst the lines with most eggs. No response to selection for susceptibility was observed.

Table 2: Egg counts in 1992 on cauliflower varieties and progenies selected for resistance (✓) or suceptibility (✗) to cabbage root fly during the series of even years

Nr.	Variety/line			Mean number of cabbage root fly eggs per plant	Significance
17	XVII/30/21/48	✓		19.8	a
18	XX/4/25	✓		22.7	ab
16	XVII/30/21/28	✓		25.2	abc
19	XX/4/29	✓		28.4	abcd
4	X20,1/31/26/38/23	✓		31.5	bcde
3	III/44/54/27/81		✗	32.7	cdef
2	III/44/34/17/97	✓		32.9	cdef
6	X/20,5/73/31/45/47	✓		33.1	cdef
9	XIV/35/38/44/80	✓		33.2	cdef
1	III/44/34/8/66	✓		33.9	cdef
8	XIV/35/38/23/63	✓		34.0	cdef
15	XVI/27/22/60/63	✓		35.4	def
12	XV/25/20/68/21	✓		35.7	def
5	X/20,1/69/38/36/60	✓		37.0	def
13	XVI/93/39/12/4	✓		37.7	def
14	XVI/47/59/63/40		✗	38.1	def
20	XIV 860554	-		38.2	def
21	XIV 1992	-		39.9	efg
10	XIV/66/14/4/58	✓		41.3	efg
7	X/20,5/73/31/45/78	✓		41.9	efg
22	Kathmandu	-		42.7	fg
23	Snowball	-		43.0	fg
11	XV/25/5/87/97	✓		49.4	g

In 1991, most lines had reached the fourth generation (Table 3) and egg laying was generally low. The mean number of eggs per plant varied from 2.8 to 28.9 (or 10-fold). This time, the reference line was ranked near the top of the list. We observed a good response for selection for susceptibility as all lines selected for high egg numbers were at the bottom of the list. The most interesting case occurred within descendants of the reference line where we found for the first time good segregation between selected lines for resistance (No. 11) and susceptibility (No. 16). The result of the four generations appear in Fig. 1.

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Table 3:   Egg counts in 1991 on cauliflower varieties and progenies selected for resistance (✓) or suceptibility (↗) to cabbage root fly during the series of odd years

Nr.	Variety/line		Mean number of cabbage root fly eggs per plant	Significance
11	XIV/4/32/56/18	✓	2.8	a
2	VII/14/82/35/90	✓	6.8	ab
20	XIV	-	8.4	abc
12	XIV/4/32/30/18	✓	9.6	abc
18	XIX/4/39	✓	11.5	bc
14	XIV/4/78/17/31	✓	12.3	bc
10	XIII/15/27/33/90	✓	12.6	bc
9	XIII/15/27/33/81	✓	12.8	bc
15	XIV/4/78/17/67	✓	13.0	bc
13	XIV/4/78/92/30	✓	13.2	bc
17	XV/1/81/32/18	✓	13.2	bc
19	X	-	13.7	bc
8	XIII/15/4/48/48	✓	14.8	bcd
1	III/16/98/54/46	✓	14.8	bcd
7	XIII/15/4/48/83	✓	15.3	cd
6	XIII/8/32/18/42	↗	22.1	de
5	X/11/92/83/53	↗	22.7	e
16	XIV/14/18/50/3	↗	24.5	e
4	X/11/92/71/97	↗	28.9	e

Discussion

An examination of all the results indicates certain anomalies. It would be expected that in a programme of repeated selection the parent reference line would be ranked further down the list alongside lines with high numbers of eggs. In the case of the even-year cycle this is the case but it is not evident in the odd-year cycle where the reference line moves up and down the ranked list.

It was suspected that the reference line itself was not stable and so a comparison was made of plants raised from old and new seed (Table 2, Nos. 20 & 21) grown alongside two susceptible cultivars (Table 2, Nos. 22 & 23). The result show that the reference line is consistently susceptible. It is therefore concluded that resistance was lost between generations (for example Table 1, No. 10 (1990) → Table 2, No. 10 (1992)). This may have resulted from selection of the wrong plant or due to problems in the *in vitro* culture. It is possible that selection needs to continue for further generations - segregation occurred only in the F_4 with other lines - or we have reached a stage where the level of resistance cannot be enhanced.

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In the meantime we have tested selected lines in a cauliflower-growing area where there is a high population of cabbage root fly. This was done in 1992 in the Valais region testing some of the 1991 selections. The roots were scored using a root damage index (Winfield & Wardlow, 1966; Thompson, Percivall & Edmonds, 1990) (Table 4). These damage index results confirmed the egg laying records.

Table 4: Cabbage root fly attack on some selected 1991 material exposed to a high fly population in the Valais in 1992

Nr.	Variety/line		RDI	Significance
2-11	XIV/4/32/56/18	✓	20.5	a
4-3	X/11/56/82/7	✓	21.5	a
3-2	VII/14/82/35/90	✓	25.9	ab
6-4	X/11/92/71/97	✗	26.5	ab
1-20	XIV	-	27.3	ab
5-16	XIV/14/18/50/3	✗	29.5	b

mean number
of eggs
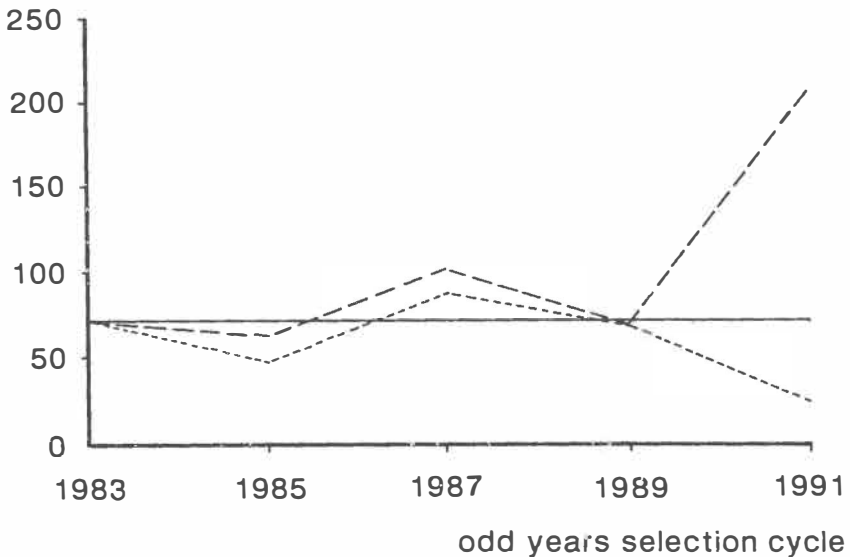per plant

odd years selection cycle

Fig. 1: Segregation in F_4 between selected lines for resistance (---------- XIV/4/32/56/18) and susceptibility (- - - - XIV/14/18/50/3) when compared with their common parent, the reference (——— XIV)

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Résumé

La sélection du chou-fleur résistant à la mouche du chou (*Delia radicum*)

La sélection parentale a été poursuivie selon un cycle bisannuel pour atteindre la F_4 en 1991 pour le cycle des années impaires et en 1992 pour celui des années paires. La plus grande différence entre les lignées est obtenue en 1990 où l'on trouve 1.3 oeufs/plante pour la lignée la moins appréciée et 28.7 oeufs/plante chez la plus appréciée, ce qui signifie une différence d'oviposition d'un facteur 22. On observe une certaine difficulté à augmenter le niveau de résistance d'une génération à l'autre. Une ségrégation entre beaucoup et peu d'oeufs chez des descendants du même parent s'est manifestée pour la première fois en F_4 en 1991. Quelques lignées de 1991 montrant un niveau de résistance prometteur, ont été exposées en champ à une forte pression du ravageur. Les indices de dégâts sont en corrélation avec les dénombrements d'oeufs.

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RESISTANCE IN SWEDES TO THE TURNIP ROOT FLY AND ITS RELATION TO INTEGRATED PEST MANAGEMENT

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Summary

The swede cultivars Melfort, Angus, Vige, Gry and Ruta were grown with either no chemical treatment or chlorfenvinphos at half and normal (2.5 kg a.i./ha) dose in field experiments. The relative resistance of the cultivars to *Delia floralis* changed both with increasing levels of attack and with the chemical treatments. Chemical treatments generally reduced the differences between the cultivars. The average effect of the half dose was about 84% of that of the full dose. Half dose gave equal or better control with the most resistant cultivars than full dose with the susceptible cultivars. Spatial separation of swede crops over different distances and by natural barriers from a site with a high number of root fly pupae reduced the oviposition pressure. The reduction in indices of damage and increase in percentage of marketable roots were more pronounced in the resistant cv. S7.7.90, than in the less resistant cv. Vige and the susceptible cv. Ruta. Breeding, based on resistance in swede cultivars from northern Norway has produced plant material with highly improved resistance to *D. floralis* compared to Vige. Further selection does not seem to have increased resistance, but has improved the commercial quality of the material. The relationship of the results to integrated pest management is discussed.

Introduction

Variation in local ecologies, as well as certain biological conditions may constrain the use of some methods to control brassica root flies. Two such examples are: (1) co-existence of the turnip root fly (*Delia floralis* (Fallen)) and the cabbage root fly (*D. radicum* (L.)) (Finch & Collier, 1988), and (2) variation between local populations of the turnip root fly in emergence dates (Taksdal, 1992b). Both conditions may preclude forecasting attack based on accumulated day-degrees.

Plant resistance, if it is effective against both root fly species, seems to avoid such problems. This paper presents attempts to include partial resistance in swedes (*Brassica napus* L.) in methods of integrated pest management which do not depend on detailed information about the biology of local populations of the brassica root flies.

The two brassica root fly species co-exist at Særheim Research Station. However, in the research discussed in this paper, *D. floralis* was dominant.

Plant resistance and doses of chlorfenvinphos

Materials and Methods

Field experiments were carried out in 1987, 1988 and 1989 using five swede cultivars: The partially resistant cvs Angus, Melfort and Vige and the susceptible cvs Gry and Ruta. The cultivars were combined in a split-plot design with no chemical treatment or chlorfenvinphos (as Birlane granulated, Shell) applied at 1.25 kg a.i./ha (half normal dose) and at 2.5 kg a.i./ha. The levels of damage increased from 1987 to 1989, thus giving an opportunity to study interactions between plant resistance and chemical treatments at different levels of damage (Taksdal, 1992a).
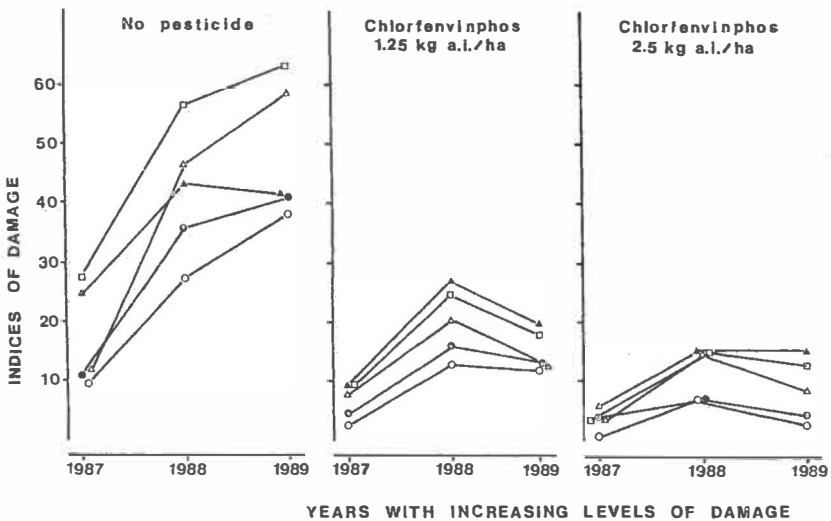
Fig. 1: Indices of damage at different levels of pesticide use during three years with increasing levels of damage. Five swede cvs included: Melfort; Angus; Vige; Gry; Ruta.

Results

There was an overlying trend for indices of damage in all three years to indicate a decreasing degree of resistance to *D. floralis* in the following order: Melfort, Angus, Vige, Gry, Ruta. However, the interactions showed that the relative resistance changed both with levels of damage, and with the dose of chlorfenvinphos.

Melfort and Angus were the two most consistently resistant cultivars, regardless of levels of damage, or use of pesticide (Fig. 1). In the absence of pesticide the resistance of Vige seemed to break down at high levels of damage, while the relative resistance of Gry improved (Taksdal, 1992a). Thus these two cultivars changed places in the ranking of resistant and susceptible cvs from 1987 to 1989, while the situation in 1988 was intermediate.

In untreated plots Ruta was the most susceptible cultivar. However, Gry appeared to respond less to chemical treatments than other cultivars, and appeared the most susceptible at both doses of chlorfenvinphos (Fig. 1).

Chemical treatment reduced the differences between cultivars. This is most obvious when the mean values in percent grade 1 of total yields of the two most resistant cultivars are compared with those of the two most susceptible cultivars (Fig. 2). When resistance alone did not give satisfactory control, the effect of half dose as a percentage of full dose effect varied from 77 to 99. With high levels of damage in 1988 and 1989, half dose of chlorfenvinphos gave better results with the resistant cvs than full dose with the susceptible.
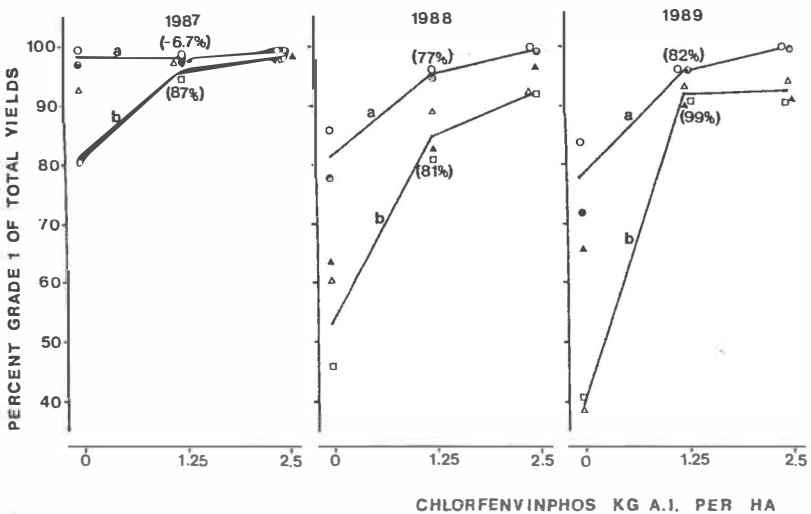
Fig. 2: Percent grade 1 yield at different levels of pesticide use during three years with increasing levels of damage. Curves give the mean values of the two (a) most resistant and (b) most susceptible cvs each year based on levels in the absence of pesticide. Effects of half dose in percent of full dose given in (). Swede cvs: Melfort; Angus; Vige; Gry; Ruta.

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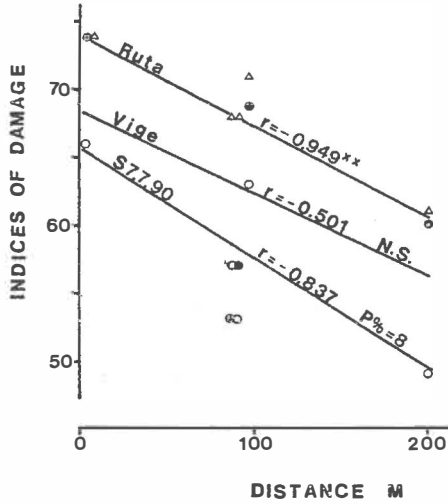
Fig. 3: The correlations between indices of damage and separation by five distances from a previously infested site in three swede cvs with decreasing level of resistance: S7.7.90; Vige; Ruta.
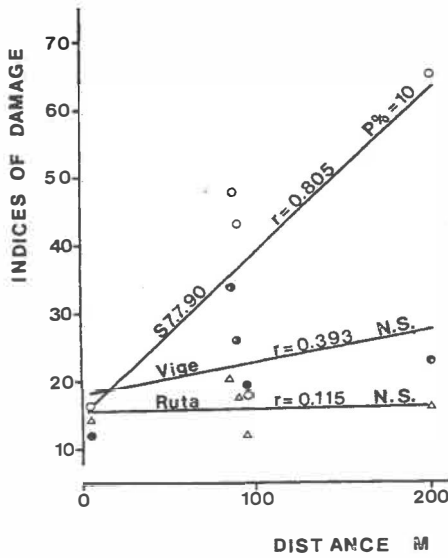
Fig. 4: The correlations between percent grade 1 of total yield and separation by five distances from a previously infested site in three swede cvs with decreasing levels of resistance: S7.7.90; Vige; Ruta.

Crop separation and root fly attack

Materials and methods

In 1991 eight field experiments were sited between 3 m and 550 m away from a 0.2 ha site infested with high numbers of root fly pupae from a 1990 swede crop. In each experiment the three cvs S-7.7.90 (most resistant), Vige (less resistant) and Ruta (susceptible) were planted in three randomized blocks. Cauliflowers were also planted for sampling root fly eggs by the flotation method (Taksdal, 1992b). At the longest distances of separation from the infested site, eggs were laid by flies migrating from a neighbour's field. This paper, therefore, includes the distances 3 m, 87 m, 90 m, 97 m and 200 m. The two last distances contained windbreak barriers.
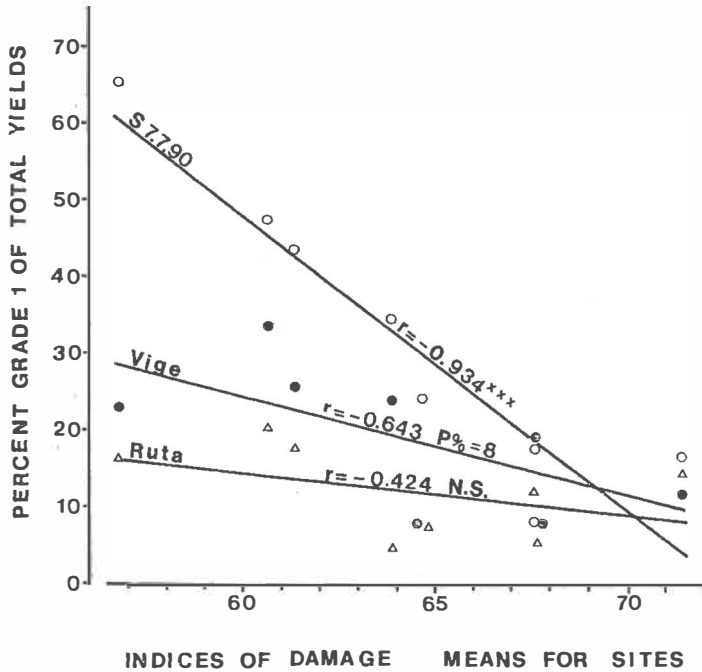
Fig. 5: The correlations between percent grade 1 and eight sites with different levels of root fly damage in three swede cvs with decreasing levels of resistance: S7.7.90; Vige; Ruta.

Results

Windbreaks were important in reducing oviposition. The relationship between egg numbers and root fly damage was not significant. In more detailed analyses of the cultivars, however, the most resistant cv. S7.7.90 was significantly less damaged with increasing separation than Vige and Ruta (Taksdal, 1992b) (Fig. 3). The most striking difference was the increase in percent grade 1 of total yields (Fig. 4).

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Analyses were carried out to relate the percentages of grade 1 for the cvs to the general level of damage at all experimental sites without including distance as a covariate. Cultivar S7.7.90 showed a larger increase in percent grade 1 yields with reduction in damage levels than did Vige and Ruta (Fig.5).

Breeding for new resistant swede cultivars

Materials and methods

A breeding programme for improving swede vegetable cultivars was started in 1986 based on open crosses between Vige and other cvs expected to possess resistance to root flies (cvs Stenhaug and Brandhaug). Selection for resistance to *D. floralis* has been carried out since 1988 in field experiments at Saerheim.

Results

The differences in indices of damage indicate that the selections represent a significant improvement in root fly resistance over cvs Vige and Ruta (Table 1). There was no clear improvement in degree of resistance after 1989. However, the uniformity and commercial value of the selections improved.

Table 1: Indices of damage and yield in grade 1 of the four most promising selections in 1989, 1990 and 1991 compared with Vige and Ruta.

1989 (18 selections)[1]			1990 (28 selections)[1]			1991 (18 selections)[1]		
Seln.	Yield	Indices	Seln.	Yield	Indices	Seln.	Yield	Indices
	grade 1 of			grade 1 of			grade 1 of	
cvs	t/ha	damage	cvs	t/ha	damage	cvs	t/ha	damage
Vige	37	54	Vige	17	66	Vige	33	51
Kv. 9,5	51	31	S19.7	32	45	S19.7.1	65	35
Kv. 1,5	49	36	S7.10	31	47	S19.8.4	55	36
Kv. 9.7	47	39	S19.6	28	47	S7.10.3	57	38
Kv. 9.8	57	40	S19.8	30	47	S7.7.2	59	40
Ruta	44	51	Ruta	7	74	Ruta	32	59
L.S.D. P=0.05	15	13		11	10		N.S.[2]	13

[1] Total numbers of selections each year

[2] N.S. = not significant

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Discussion

The major application of plant resistance is its contribution to integrated programmes of pest control (Ellis, 1988). The development of resistant cultivars may be time-consuming and expensive, but their application in the field is simple and does not require detailed biological observations of the pests involved. To maintain this advantage the resistant cultivars should be combined with other biological or chemical control methods in an integrated pest management programme. The use of reduced doses of pesticides, an extended crop rotation which includes spatial separation and restricting root fly movement by introducing windbreaks or other barriers are examples of such methods.

The impact of different levels of attack on the expression of resistance (Fig. 1) focuses the need for combined pest control strategies. The effect of half dose chlorfenvinphos on resistant cultivars compared with full dose on susceptible cultivars appears to indicate that half dose would generally give satisfactory control. However, growers may find it profitable to apply the full dose on resistant cultivars to obtain a higher yield of marketable roots. Forecasting levels of attack could give a rationale for the use of reduced doses in the field. The use of the lowest satisfactory dose in each situation would probably also maximise possible potentiation ("synergism") between plant resistance and insecticides (van Emden, 1991). This approach would, however, re-introduce the cost of biological observations for development and application.

The crop movement experiment indicated some benefit from spatial separation, and that this was most pronounced for the most resistant cultivar (Figs 3 and 4). The most significant results were found when the general level of damage at each site, regardless of distance of separation, was compared with the percent grade 1 of total yields for each cultivar (Fig. 5). Thus potentiation of resistant cultivars compared with susceptible cultivars may occur with any factor reducing the general level of damage in the field.

In the breeding programme the differences between Vige and the best selections (Table 1) equal the differences between Vige and Melfort in Fig. 1 (no pesticide). The value of this level of resistance is thus illustrated by the investigation on plant resistance and doses of chlorfenvinphos. The cultivar S7.7.90 used in the crop separation experiment is also from this breeding programme. The level of resistance has apparently not increased in recent years. The programme will therefore aim to improve the uniformity and commercial value of the selections.

Acknowledgement

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Résumé

**La résistance du rutabaga à la mouche du radis et ses liens avec la
production intégrée**

Les cultivars de rutabaga Melfort, Angus, Vige, Gry et Ruta ont été cultivés
dans des essais en plein champ selon trois variantes: témoin sans traitement, 1/2 dose
et dose normale de Chlorfenvinphos (2,5 kg m.a./ha). La résistance relative des cvs.
à *Delia floralis* change en fonction du niveau d'attaque et de la dose de
Chlorfenviphos employée. Les traitements chimiques réduisent généralement les
différences entre les cvs. L'effet moyen de la 1/2 dose correspond à environ 84% de
celui de la dose normale. La 1/2 dose combat le ravageur aussi bien ou mieux sur le
cv. le plus résistant que la dose normale sur le cv. le plus sensible. Des cultures
séparées spatialement (par éloignement ou par des barrières naturelles) de sites à
fortes populations de mouches, bénéficient d'une baisse de la pression de ponte. La
réduction de l'index des dégâts et l'augmentation du taux de racines
commercialisables sont plus prononcées chez le cv. résistant S7.7.90, que chez le cv.
moins résistant Vige et chez le cv. sensible Ruta. Des sélections, basées sur la
résistance des cultivars de rutabaga provenant du nord de la Norvège, ont produit un
matériel végétal ayant une résistance à *D. floralis* nettement renforcée par rapport au
cv. Vige. Une sélection ultérieure ne semble pas avoir augmenté la résistance, mais
elle a amélioré la qualité commerciale du matériel. Les liens avec la production
intégrée sont discutés à la lumière de ces résultats.

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SOURCES, MECHANISMS AND BASES OF RESISTANCE IN CRUCIFERAE TO THE CABBAGE APHID, Brevicoryne brassicae

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Summary

Progress is reported on the identification of sources, mechanisms and bases of resistance in Cruciferae to cabbage aphid, *Brevicoryne brassicae*. In 39 field and glasshouse/laboratory trials about 950 genotypes have been evaluated during the last 50 years. Of these, 93 were reported to possess moderate to high levels of resistance. The red and glossy nature of plants are major factors conferring antixenosis resistance while the factors governing antibiosis have not been investigated in detail. The difficulties associated with studies of resistance in *Brassica* crops to the cabbage aphid and future areas for investigation are discussed in this review.

Introduction

The cabbage aphid, *Brevicoryne brassicae* ((L.), is one of the most severe pests of cruciferous crops in many different parts of the world. It is a particularly serious pest of horticultural brassicas including broccoli, Brussels sprouts, cabbage, cauliflower, collard, kale, kohl-rabi, mustard, rape, swede and turnips. Large colonies of aphids may stunt or kill plants in the early stages of growth and later on, their contamination reduces the market value of the crop. High populations distort actively-growing leaves, causing them to curl. The pockets and folds created by this distortion offer shelter to the aphids, thus enabling them to escape pesticide treatments. Repeated applications are therefore frequently needed to keep the crop free of aphids. In addition, cabbage aphid is capable of transmitting viruses to the crops. To overcome these problems, environmentally-safer methods such as the development of cultivars resistant to this insect are receiving attention. The literature on cabbage aphid contains numerous reports on the bionomics (Markkula, 1953; Baran, 1970), population dynamics (Otake, 1966; Raworth, 1984), biological control (Wilson, 1960; Paetzold & Vater, 1967) and taxonomy (Blackman & Eastop, 1984). However, the information on integrated control (Theunissen, 1989) and host plant resistance (Dunn, 1978; Laska, 1987; Auclair, 1989) has been only partly covered. Since host plant resistance is emerging as an important component of integrated pest management, it was considered desirable to survey the literature and review the studies of resistance to the cabbage aphid.

As early as 1944, Breakey and Carlson observed less damage by this pest on flat-head cabbage cultivars as compared to round-head cultivars. Since then, about 39 studies (Table 1) have been carried out to identify resistant sources in cruciferous plants to this pest. More than 950 genotypes were evaluated in the last 50-year period and about 93 of these were reported to show some level of resistance. The information concerning sources of resistance in major brassica crops is summarised below:

1.   Cabbage, *Brassica oleracea* L. var. *capitata* (L.) Alep.

In eight field experiments (Sr. Nos 1-8, Table 1), 34 genotypes were found to be resistant to cabbage aphid expressed either as antixenosis, antibiosis or tolerance or a combination of these factors. Green (Verma, Bhagchandani, Singh & Lal, 1981), red (Radcliffe & Chapman, 1965; Ellis & Hardman, 1988) and glossy (Ellis and Hardman, 1988; Edelson & Dickson, 1988) leaves were attributed to be the basis of resistance. In addition, thick parenchyma in the mesophyll (Bogdanov, Asyakin & Shapiro, 1988; Asyakin & Ivanova, 1989) and low wax content in red lines (Karl & Eisbein, 1987) were considered to induce resistance. Flat-head cultivars were also reported to be less damaged than other cultivars.

2.   Brussels sprouts, *Brassica oleracea* L. var. *gemmifera* Zenk.

In five studies (Sr. Nos 9-13, Table 1), six cultivars and two clones were resistant to cabbage aphid. Of these, a glossy strain showed antixenosis (Way & Murdie, 1965) because of its low wax content. The remaining cultivars listed as resistant expressed either tolerance or antibiosis.

3.   Swede or rutabaga, *Brassica napus* L. ssp. *rapifera* (Metz.)

Thirteen genotypes showed field resistance to cabbage aphid in three trials conducted in New Zealand (Sr. Nos 14-16, Table 1) on the basis of yield and visual damage. In these trials the basis and mechanisms of resistance were not established.

4.   Rape, *Brassica napus* L.

In six trials (Sr. Nos 17-22, Table 1) only 4 genotypes were reported as resistant. Among these, the cultivar Aphis Resistant Rape was found to be resistant in New Zealand, Australia and England and it was considered to be due to both antibiosis and antixenosis mechanisms.

5.   Kale, *Brassica oleracea* L. var. *acephala* DC.

In two trials on kales (Sr. Nos 23-24, Table 1), six cultivars were identified as resistant in Brazil. M.R.P. 2620 and M. 1811 showed antixenosis and tolerance but

the remaining four possessed antibiosis resistance. Details of the mechanisms of resistance were not established.

6.   Cauliflower, *Brassica oleracea* L. var. *botrytis* L.

There were only two trials exclusively carried out on cauliflower (Sr. Nos 25-26, Table 1) but no material resistant to cabbage aphid was identified. However, de Ponti (1984) reported that light green cultivars were less damaged than others.

7.   Comparatives studies on members of Cruciferae

Thirteen trials (Sr. Nos 27-39, Table 1) included various *Brassica* crops and wild species. Among these genotypes of swede (Doon Spartan; Calder), turnip(York Globe; Green Globe), kale (marrow stem kale; Rawara), kale X rape *hybrid (Line 3)*, *Sinapis alba* L. (white mustard), rape (825; 827), *Crambe abyssinica* (Prophet; 304399; Indy), *C. juncea* (314075; 325274), *B. carinata* (Carinata), *B. trilocularis* (Y.S.L. 5), *B. juncea* (RL-10), radish (Scarlet Globe), broccoli (Broc 3; Broc 4; Broc 5), cauliflower (Caul1; PI 234599; Glossy Andes), collard (Robson-Georgia; Green Glaze; South Carolina Glaze; White Green Glaze), kale (KCR 4; Glazed Vates) were found resistant. Antibiosis and antixenosis were identified as the principal mechanisms of resistance. The basis of resistance was not investigated although it was reported that resistant genotypes of broccoli, cauliflower, kale and collards were glossy in nature. Plants of Line 3 had glaucous leaf surfaces.

Problems associated with resistance to cabbage aphid

Seasonal shifts in resistance and undesirable resistance factors

Red foliage is a well-documented antixenosis factor for incoming alates of the cabbage aphid but it loses its significance as the season progresses. Thus, red cultivars are heavily infested by cabbage aphid as well as *Pieris rapae* (L.) (Radcliffe & Chapman, 1965; 1966; Ellis & Hardman, 1988). Aphis Resistant Rape was found to express resistance to cabbage aphid in multilocation trials and over several seasons but loses its resistance during the flowering phase. Early Half Tall which is a non-glossy, green leaved cultivar also exhibited resistance to cabbage aphid in the early part of the season but later on supported high population of the aphid (Dodd & van Emden, 1979). Glossiness of leaves is considered to be another important factor conferring resistance to cabbage aphid but such cultivars are severely damaged by the flea beetles, *Phyllotreta cruciferae* (Goeze) and *P. striolata* (F.) (Stoner, 1990; 1992). Furthermore, the level of resistance offered by glossiness is variable under different environmental conditions (Dickson & Eckenrode, 1975). Hence, glossiness may not be a desirable factor to breed into new crop cultivars.

Table 1: The sources, mechanisms and basis of resistance in Brassicas to the cabbage aphid

Sr. No.	Crop (No. of genotypes)	Resistant genotypes	Mechanism of resistance	Basis of resistance	Field/ Glasshouse/ Lab.	Location	Reference
1.	Cabbage (8)	Flat Dutch Red Acre Red Hollander	- Antixenosis Antixenosis	- Red colour Red colour	Field	Madison, USA	Radcliffe & Chapman (1965)
2.	Cabbage (15)	Flat Dutch Racine Market	- -	- -	Field	Madison, USA	Radcliffe & Chapman (1966)
3.	Cabbage (47)	All Season EC 99825 Round Sure Head	Tolerance Tolerance Tolerance	Green leaf Green leaf Green leaf	Field	Katrain, India	Verma et al. (1981)
4.	Cabbage (55)	G 10127 G 10128 Red Drumhead Yates Giant Red	- - - -	Glossy leaf Glossy leaf Red colour Red colour	Field	Wellesbourne, England	Ellis & Hardman (1988)
5.	Cabbage (11)	St-R1	Antixenosis + Antibiosis	Red colour + Low wax	Field & Lab.	Aschersleben, Germany	Karl & Eisbein (1987)
6.	Cabbage (13)	NY 1381 NY 10118	- -	Glossy leaf Intermediate-glossy	Field	Weslaco, USA	Edelson & Dickson (1988)

Sr. No.	Crop (No. of genotypes)	Resistant genotypes	Mechanism of resistance	Basis of resistance	Field/ Glasshouse/ Lab.	Location	Reference
7.	Cabbage (26)	Stakhanovka 1513	-	Thick parenchyma	Field	European Russia, USSR	Asyakin & Ivanova (1989)
		Taininskaya 11	-	Thick parenchyma			
		Uzbekistanekav	-	Thick parenchyma			
		Iyunskaya	-	-			
		Nomer Pervi polarnyk	-	-			
		206	-	-			
		Skorasepelya	-	-			
		Nomer Pervi	-	-			
		griboraski 147	-	-			
		Zolotoi Gertav 1432	-	-			
		Salava 1305	-	-			
		Rusinovka	-	-			
		Turkis	-	-			
		Bewama	-	-			
		Danish Ballhead	-	-			
		Amager St.	-	-			
		Amager Rossebos	-	-			
		Late St.	-	-			
		Kharkovaskaya					
		Zimovka 1474					
8.	Cabbage	-	-	Flat head	Field	Puyallup, USA	Breakey & Carlson (1944)
9.	Brussels sprouts (3)	Glossy strain	Antixenosis	Less Wax	Field	Ascot, England	Way & Murdie (1965)
10.	Brussels sprouts (31)	Darkmar 21	Tolerance	-	Field	Wellesbourne, England	Dunn & Kempton (1971)
		Vremo Inter	Tolerance	-	Field		
		Seven Hills	Tolerance	-	Field		
		ICI	Antibiosis	-	Lab.		
11.	Brussels sprouts (8)	Early Half Tall	Tolerance	-	Field	Wellesbourne, England	Dunn & Kempton (1972a)
		Gronalto	Tolerance	-	Field		
12.	Brussels sprouts (9)	Clone BG 10	Antibiosis	-	Field & Lab.	Wellesbourne, England	Dunn & Kempton (1972b)

Sr. No.	Crop (No. of genotypes)	Resistant genotypes	Mechanism of resistance	Basis of resistance	Field/ Glasshouse/ Lab.	Location	Reference
13.	Brussels sprouts (7)	Early Half Tall	Antibiosis	Less amino-acids	Field & Glasshouse	Reading, England	Dodd & Van Emden (1979)
14.	Swede (45)	New Zealand Yellow-fleshed Sensation Wilhelmsburger Otofte Bangholm Wilby Otofte Granhovsdet Hunsvalle Balder Vilmorin's White-flesh green-top Vilmorin's White-flesh purple-top Calder	- - - - - - - - -	- - - - - - - - -	Field	Christchurch, New Zealand	Palmer (1953)
15.	Swede (5)	NZ Calder NZ Sensation NZ Resistant	- - -	- - -	Field	Auckland & Canterbury, New Zealand	Lamb (1953)
16.	Swede (7)	Tina	-	-	Field	Christchurch, New Zealand	Lammerink & Hart (1985)
17.	Rape (2)	Aphis Resistant-Rape	Antibiosis	-	Field	Lincoln, New Zealand	Palmer (1960)
18.	Rape (3)	Aphis Resistant-Rape	- -	- -	Field	Tasmania, Australia	Lamp (1965)
19.	Rape (4)	Aphis Resistant-Rape Clone 6G	Antixenosis + Antibiosis Antixenosis + Antibiosis	- -	Field & Lab. Field & Lab.	Wellesbourne, England	Dunn & Kempton (1969)
20.	Rape (2)	Resistant (825)	-	-	Field	Canterbury, New Zealand	Lowe (1973)
21.	Rape (7)	Stamm 1	Antibiosis	-	Lab.	Gottingen-Weende, Germany	Weber et al. (1986)

Sr. No.	Crop (No. of genotypes)	Resistant genotypes	Mechanism of resistance	Basis of resistance	Field/ Glasshouse/ Lab.	Location	Reference
22.	Rape (11)	Nil	-	-	Glasshouse	Rostock, Germany	Hinz & Daebeler (1981)
23.	Kale (13)	MRP 2620 M 1811	Antixenosis + Tolerance Antixenosis + Tolerance	- -	Field & Lab. Field & Lab.	Jaboticabal, Brazil	Lara et al. (1978)
24.	Kale (12)	MRP 2446 Roxa M Jundiai M Mococa	Antibiosis Antibiosis Antibiosis Antibiosis	- - - -	Field & Lab. Field & Lab. Field & Lab. Field & Lab.	Jaboticabal, Brazil	Lara et al. (1979)
25.	Cauliflower (-)	-	-	Light-green leaf	Field	Wageningen, Netherlands	Ponti (1984)
26.	Cauliflower (4)	-	-	-	Field	Ganeshkind, India	Khaire et al. (1986)
Miscellaneous Brassica Crops							
27.	Swede (4) Turnip (2) Rape (1) Kale (1)	Doon Spartan Calder York Globe Green Globe - -	- - - -	- - - -	Field	Auckland, New Zealand	Lamb (1960)
28.	Collard (1) Kale (1) Cabbage (1) Brussels sprouts (1) Broccoli (1)	Robson-Georgia - - -	- -	- -	Field	Ithaca, USA	Pimentel (1961)
29.	Kale (1) White mustard (1)	Marrow stem kale White mustard	Antixenosis Antixenosis	Non waxy leaves Wax quality	Field	Cambridge, England	Thompson (1963)
30.	Swede (2) Rape (3)	Calder Rape (825) Rape (827)	Antixenosis Antixenosis Antixenosis	- - -	Field	Canterbury, New Zealand	Lamb & Lowe (1967)

Sr. No.	Crop (No. of genotypes)	Resistant genotypes	Mechanism of resistance	Basis of resistance	Field/ Glasshouse/ Lab.	Location	Reference
31.	Yellow Rocket (1) Broccoli (1) Chinese cabbage (1) Collard (1)	Not given - - -	Antibiosis	-	Lab.	Ithaca, USA	Root & Olson (1969)
32.	Crambe abyssinica (31) C. juncea (2) Other Cruciferous entries (480)	Prophet 304399 Indy 314075 325274 -	Antibiosis Antibiosis Antibiosis Antibiosis + Antixenosis Antibiosis + Antixenosis	- - - - -	Glasshouse	Ames, USA	Jarvis (1982)
33.	Brassica carinata (1) B. trilocularis (1) B. juncea (1) B. napus (1) B. campestris (1) B. nigra (1)	carinata YSL-5 RL-16 - - -	Antixenosis + Antibiosis Antizenosis + Antibiosis Tolerance	- - -	Field	Tandojam, Pakistan	Hussain (1983)
34.	Radish (1) Cabbage (1) Cauliflower (1)	Scarlet Globe - -	Antixenosis	-	Lab.	Amman, Jordan	Mustafa (1986)
35.	Brassica spp. (4)	-			Field	Faisalabad, Pakistan	Hasmi et al. (1985)
36.	Giant Kale (1) Rape (2) Rape x Kale hybrids (4)	Rawara - Line 3	- -	- -	Glasshouse Glasshouse & Field	Lincoln, New Zealand	Quazi (1988)
37.	Brassica oil seed (6)	-			Lab.	Uppsala, Sweden	Ronquist & Ahman (1990)

Sr. No.	Crop (No. of genotypes)	Resistant genotypes	Mechanism of resistance	Basis of resistance	Field/ Glasshouse/ Lab.	Location	Reference
38.	Broccoli (4)	Broc 3	-	Glossy leaf	Field	New Haven, USA	Stoner (1990)
		Broc 4	-	Glossy leaf			
	Cauliflower (7)	Cauli	-	Glossy leaf			
	Kale (4)	KCR 4	-	Glossy leaf			
	Collard (4)	Glazed Vates	-	Glossy leaf			
		Green Glaze	-	Glossy leaf			
		South Carolina Glaze	-	Glossy leaf			
		White Green Glaze	-	Glossy leaf			
	Brussels sprouts (5)	-	-				
39.	Broccoli (6)	Broc 3	-	Glossy leaf	Field	New Haven, USA	Stoner (1992)
		Broc 5	-	Glossy leaf			
	Cauliflower (5)	PI 234599	-	Glossy leaf			
		Glossy Andes	-	Glossy leaf			
	Collard (4)	Green Glaze	-	Glossy leaf			
		South Carolina Glaze	-	Glossy leaf			
		White Green Glaze	-	Glossy leaf			
	Kale (4)	-					

Emergence of biotypes

Two cultivars of rape *viz.* Aphis Resistant Rape and Rangi account for a high proportion of the acreage of fodder rape in New Zealand because of their resistance to cabbage aphid. In 1968, a population of cabbage aphid was discovered which readily infested these resistant cultivars while a population from another source could not attack these cultivars (Lammerink, 1968). The former was designated as biotype NZ-2, the latter being NZ-1. A swede cultivar 'Tina' showed resistance against dry rot, clubroot, cauliflower mosaic virus, turnip mosaic virus and the cabbage aphid biotype NZ-1 but was found to be susceptible to biotype NZ-2 (Lammerink & Hart, 1985). Dunn and Kempton (1972b) also discovered a similar situation in Brussels sprouts. The clones derived from resistant lines failed to express resistance against populations of cabbage aphids collected from different locations in England and failure was attributed to different biotypes.

Lack of knowledge on the biochemical basis of resistance

Attempts have been made to correlate variability in levels of amino acids (van Emden, 1972; Weibull & Melin, 1990), wax quality (Thompson, 1963; Karl & Eisbein, 1987), mustard oil glycosides (Dodd & van Emden, 1979; Weber, Oswald & Zollner, 1986) and other chemical components (Miles, Aspinall & Rosenberg, 1982) with performance of cabbage aphid but no significant relationships have been identified in relation to resistance. There are reports which indicate that aphid alates are attracted to cruciferous plant volatiles (Pettersson, 1973; Nottingham, Hardie, Dawson, Hick, Pickett, Wadhams & Woodcock, 1991) but this aspect of biology has not been investigated in relation to resistance. It is possible that poor quality of carbohydrates (Evans, 1938) and sugar : nitrogen ratio of more than 1.4 as well as osmotic pressures in excess of 13 atm. (Weismann & Baran, 1969) could induce resistance to cabbage aphid. However, this needs further testing.

Performance of cabbage aphid-resistant plants against other aphid pests

Lipaphis erysimi pseudobrassicae (Davis) and *Myzus persicae* (Sulzer) are also important aphid pests on *Brassica* crops. Their response to changes in plant/leaf physiology and nutritional components are different from those of the cabbage aphid (van Emden, 1966; Daiber, 1970; Wearing, 1972; van Emden & Bashford, 1971; 1976; Ronquist & Ahman, 1990). This may explain why cultivars found resistant to cabbage aphid did not perform well against other aphids (Palmer, 1956; 1960; Margetts, 1963; Way & Murdie, 1965; Lamp, 1965; Kennedy & Abou-Ghadir, 1979).

Prospects for the future

It is evident from the literature that there are potential sources of resistance to cabbage aphid. However, there is a need to accelerate the process of screening by including a large number of accessions so as to select resistant lines with better horticultural qualities. There is considerable information on the mechanisms of

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resistance operating in aphid resistance lines but there is little information on the factors (particularly biochemical) governing resistance. In the past there have been difficulties in assessing the biochemical components of phloem sap but modern techniques (Weibull & Melin, 1990) may be employed to carry out these studies. Very little effort has been made to compare aphid resistance under both field and controlled conditions. This type of study is essential to establish mechanisms and bases of resistance and also the detection of biotypes. In certain *Brassica* crops such as swede and rape, a concerted effort has been made to breed resistant cultivars (Lammerink & Hart, 1985) against cabbage aphid but little effort has been made to investigate the inheritance of resistance. Quazi (1988) provided the evidence of single dominant gene resistance to cabbage aphid in kale and kale X rape hybrids, although it was believed to be controlled polygenically (Russell, 1978). More recently the genetics of the glossy character in different cole crops has been established (Stoner, 1990; 1992). There is a need to identify additional sources of resistance so that opportunities can be created for selection of commercially- acceptable, high quality brassica crops.

Résumé

Sources, mécanismes et bases de la résistance des crucifères à *Brevicoryne brassicae*

Un compte rendu sur les progrès dans l'identification des sources, des mécanismes et des bases de la résistance des *Cruciferae* au puceron cendré du chou, *Brevicoryne brassicae* (L.) est présenté. Pendant les dernières 50 années, 950 génotypes ont été passés en revue dans 39 essais en champ ou en serre/laboratoire. Parmi eux, 93 montrent des niveaux de résistance moyens à élevés. La couleur rouge et le lustre des plantes sont les facteurs principaux de la résistance antixénotique, mais ceux régissant l'antibiose n'ont pas été étudiés en détail. Les difficultés liées aux études de la résistance des cultures de *Brassica* au puceron cendré du chou et les futurs champs d'investigations sont discutés.

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A SEARCH FOR RESISTANCE TO INSECTS
IN SPRING OILSEED RAPE

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Summary

Out of approximately 90 populations of spring oilseed rape (*Brassica napus*) tested for resistance to insects in a field trial in 1990, 11 populations were considered to be of interest for a more thorough study in 1991, along with two populations from somatic hybrids between *Eruca sativa* and rape. Comparisons between an insecticide-treated (TRE) and an untreated (UNT) set of the populations showed a dramatic effect of insects on the crop. In general, untreated plants were shorter, had more side shoots and podless stalks but fewer pods. Pod production was shifted from main to side shoots but the significantly lower yields in the UNT-set, showed that plants were unable to compensate for insect attack. All of these changes in plant architecture are typical responses to pollen beetle (*Meligethes* spp.) infestation. In the UNT-set symptoms related to pollen beetle attack were correlated with seed yield whereas symptoms related to attack by flea beetles (*Phyllotreta* spp.), brassica pod midge (*Dasineura brassicae*) and turnip seed weevil (*Ceutorhynchus assimilis*) were not. From a plant breeding point of view it was encouraging that certain populations yielded more than the mean of the standard varieties Puma and Legend in the UNT-set. However, the yield of the best-yielding population in the UNT-set was only 36% of that in the TRE-set. Further, correlations between symptoms of insect attack during the two years of study were poor. Infestation rate by *D. brassicae* was the only exception. Thus no suitable plant source was found to start a breeding programme for insect resistance in oilseed rape.

Introduction

The present study was aimed at finding plant genotypes which might serve as resistance sources in a breeding programme for insect-resistance in spring-sown *Brassica* oilseeds. Insect pests cause considerable damage to such crops in Sweden. Pollen beetles, *Meligethes aeneus* Fab. in particular, are the most severe pests (Nilsson, 1987). Their feeding on buds and flowers causes abscission of buds, stunting of the plants, loss of apical dominance, retarded plant development and a shift from main shoot to side shoots for pod production (Tatchell, 1983; Nilsson, 1988). Sometimes the apical part of the stem is also fed upon (Nilsson, 1987). Other insects like flea beetles of the genus *Phyllotreta* feeding on the cotyledon, the aphid

Brevicoryne brassicae L., and the pod-infesting gall midge *Dasineura brassicae* Winn. and the weevil *Ceutorhynchus assimilis* Payk. may also cause damage to the crop (Nilsson, 1981). In Sweden, the economy of growing spring-sown *Brassica* oilseeds often depends on the application of insecticides (Nilsson, 1987). Breeding for resistance is one possible mean of reducing this dependence.

Materials and methods

The initial search for resistance was made in 1990 among 92 populations of rape (*Brassica napus* L.). Most of the populations were crosses between Asian and Swedish rapes, and between rape re-synthesized from various *Brassica oleracea* L. and *Brassica campestris* L. forms and Swedish rapes. The populations were grown in two sets of single-plot experiments. One set (TRE) was sprayed 10 times with pyrethroids and methoxichlor from cotyledon stage until end of flowering and another set (UNT) was untreated. These experiments were placed in a large field with other spring oilseed trials. The plots were 5 m long and consisted of 5 rows of plants. Plant density was approximately 300 plants per m^2 and fertilization rate was 125, 15 and 30 kg/ha respectively of N, P and K.

Based on the ratings in the 1990 experiments 11 populations (Table 1) were selected for a new trial in 1991 with three repeats of each population in an UNT- and a TRE-set. In addition, two populations resulting from somatic hybridisations between *Eruca sativa* Mill. and rape were included along with the same two rape varieties used as standards in 1990 and one Indian *E. sativa* variety. The plot size and the management of the experiments were the same as in 1990. The TRE-set was sprayed with insecticides 5 times. The plots were laid out in a lattice-design and the TRE- and the UNT-set were placed adjacent to one another only separated by a guard row of 5 m long rape plots surrounding each set. The plot results were used as inputs in an ANOVA performed by the software NOBIS from Nordisk Statistik & Datakonsult HB.

Most of the recordings were the same in 1990 and 1991. Since the results in 1990 were only based on one plot per population those recordings were only used to select populations of interest for the 1991 trial and to make correlations between results from the two years.

Growth stage was recorded at weekly intervals in one plot each of UNT and TRE from bud formation until start of maturation. When the crop was in the cotyledon stage, plant samples were collected from the UNT-experiment to count number of feeding holes made by *Phyllotreta* flea beetles, predominantly the species *Phyllotreta undulata* Kutch. Twenty plants were collected from each plot by taking every 10th plant in the mid-row. The plants were placed in plastic bags and kept frozen at about -4°C until the number of feeding holes on the cotyledons were recorded under a microscope.

Table 1:   Populations of brassicaceous oilseeds tested in 1991. In 1990 all but
            pop. 12-14 were included. x = sexual hybridizations, + = somatic
            hybridizations.

Population No.	Origin
1	Asian rape x Swedish rape cv. Puma
2	Asian rape x Swedish rape Sv 02372
3	Puma x (Swedish turnip rape cv. Emma + cauliflower)
4	Puma x (Emma + cauliflower)
5	Asian rape x Puma
6	Mediterranean x Swedish rape Sv 02347
7	(Chinese cabbage x Savoyan cabbage) x Sv 02372
8	Asian rape x Swedish rape cv. Korall
9	(Chinese cabbage x Savoyan cabbage) x Puma
10	Puma x Rapid cycling population of rape
11	Asian rape x Sv 02372
12	Swedish rape cv. Hanna + *E. sativa*
13	Hanna + *E. sativa*
14	*E. sativa* cv. RTM-2
15	Puma (standard)
16	Legend (standard)

When most of the insecticide-treated plots had ceased to produce flowers
another plant sample was taken, again by picking every 10th plant in the mid-row.
Ten plants per plot were collected from both the UNT- and the TRE-experiment.
The plants were preserved at about -4°C until examination. Height of the main
raceme was then recorded along with number of primary side shoots, total number of
pods, number of pods on the main shoot, number of podless stalks, number of buds,
number of pods that were yellowish, split open or missing due to infestation by larvae
of the gall midge *D. brassicae* and number of pods with exit holes made by the larvae
of the weevil *C. assimilis*. Plants that were violet and malformed due to attack by the
aphid *B. brassicae* were counted in 1990. No such plant occurred in the 1991 samples.
In 1991, but not in 1990, the crop was harvested, dried, cleaned and weighted plot-
wise. Contents of crude fat and chlorophyll were analyzed population-wise for UNT
and TRE separately by standard methods (Troëng, 1955; Johansson & Appelqvist,
1984).

Results in 1991

Within-TRE-comparisons

Even though the insecticide-treatment did not fully protect the plants in the
TRE-set, the insect attack was considerably lower in the TRE- than in the untreated

UNT-set, judged by infestations rate of *D. brassicae* and *C. assimilis* (Table 3). Thus differences between the entries in the TRE-set in plant height, number of side shoots, total number of pods, number and proportion of pods on the main shoot, number of potential pods (i.e. number of pods + number of podless stalks), proportion of podless stalks and proportion of buds (i.e. number of buds/(number of potential pods + number of buds) Table 2) should mainly be due to phenotypic differences between the plant populations unrelated to the effects of insect herbivores. None of the populations yielded significantly more than the mean of the two standards when protected by insecticide but nos. 4, 9 and 14 yielded significantly less (Table 4).

Between TRE- and UNT-comparisons

There were significant differences between the TRE- and the UNT-set in all the variables compared by Two-way ANOVA (Tables 2, 3 & 4). In most of the populations, plants in untreated plots were shorter than plants in insecticide-treated plots. All the populations had more side shoots but fewer pods in the UNT- than in the TRE-set. Number of pods as well as proportion of pods on the main shoot was much reduced when the plants were exposed to insects and proportion of stalks without pods was normally much higher when no insecticide had been applied (Table 2). Number of potential pods were also affected by the treatment, being higher in the UNT-set just as proportions of buds normally was. All of these changes in plant architecture may be attributed to pollen beetle attack. Pods were attacked by *D. brassicae* and *C. assimilis* in insecticide-treated plots but generally in lower frequency than in untreated plots (Table 3). The yield was considerably lower in the UNT-set than in the TRE-set (Table 4).

In four out of the eight variables describing plant architecture there were significant interactions between population and treatment effects in the ANOVA (Table 2). Thus the various plant populations were differentially affected by exposure to insects in number of side shoots produced, number but not proportion of pods on the main shoot, number of potential pods and proportion of podless stalks. All of these plant characteristics are influenced by pollen beetle attack. Further there were interactions between population and treatment effects in two other variables, infestation by *D. brassicae* and yield (Tables 3 & 4).

Correlations between yield and symptoms of insect attack

In the UNT-set yield level was positively correlated with number of pods on the main shoot (r = 0.69), proportion of pods on the main shoot (r = 0.77) and negatively correlated with number of potential pods (r = -0.77), proportion of podless stalks (r = -0.61) and proportion of buds (r = -0.53). None of the other variables in Tables 2 and 3 reached the 5% probability level of significance for correlation with yield (df = 13, r = ±0.51; *E. sativa* was excluded from the analysis). Thus only symptoms which could be attributed to pollen beetle attack were correlated with yield. However also in the TRE-set there were significant negative correlations between the number of potential pods (r = -0.53) and proportion of buds (r = -0.58) with yield indicating that those may be inherent characteristics correlated with yield.

Table 2: Plant characteristics and symptoms of insect attack on spring oilseed populations in 1991. Means from three plots per population and set (TRE = treated with insecticide; UNT = untreated). Standards: No. 15 = Puma; No. 16 = Legend. LSD-test: +, - and *, = indicate which populations that differ significantly from the mean of the two standards, and in which direction, at the probability levels 5 and 1% respectively.

Population No.	Set	Plant height (cm)	No. of side shoots	Total no. of pods	No. of pods on main shoot	% pods on main shoot	No. of potential pods	% podless stalks	% buds
1	TRE	89+	3.3	39	16	43	105	59	0.0
	UNT	83*	4.3	18	5	37	214	88*	1.1
2	TRE	90+	3.3	44	17	45	105	56	0.0
	UNT	75	5.3*	22	4=	22	208	89*	1.4
3	TRE	87	3.2	50	20	47	120	57	0.0
	UNT	80*	4.7	23	6	28	211	84+	1.6
4	TRE	94*	3.6	47	18	42	138+	65+	0.1
	UNT	85*	4.7	19	3=	17-	329*	93*	2.3
5	TRE	88+	2.5=	35	13	51	72	52	0.0
	UNT	81*	3.3=	18	5-	34	169	84+	0.1
6	TRE	90+	3.3	29	18	67*	72	59	0.0
	UNT	83*	4.3	17	9*	50+	98	79	0.5
7	TRE	102*	2.7=	61+	22*	51	153*	57	0.0
	UNT	92*	4.7	35	6	22	251*	84	2.8
8	TRE	83	2.6=	28	17	67*	69	58	0.0
	UNT	78+	4.3	18	6	41	148	85+	1.7
9	TRE	97*	2.8-	39	19	64+	141+	70*	2.0
	UNT	97*	4.3	26	6	26	281*	88*	1.7

Table 2 continued:

Population No.	Set	Plant height (cm)	No. of side shoots	Total no. of pods	No. of pods on main shoot	% pods on main shoot	No. of potential pods	% podless stalks	% buds
10	TRE	76	2.8=	27	13	62+	58–	54	0.0
	UNT	70	4.0	19	6	39	105	77	0.7
11	TRE	91*	3.0–	44	18	54	95	52	0.0
	UNT	78+	5.0+	24	6	32	161	84+	2.3
12	TRE	80	3.1	36	17	68*	78	57	0.0
	UNT	83*	5.3*	31	7	30	162	79	1.6
13	TRE	96*	3.7	53	18	39	140+	58	0.0
	UNT	82*	5.3*	27	5	25	209	89*	1.8
14	TRE	54=	4.6+	22–	7=	36	52=	60	1.0
	UNT	53=	5.0+	17	3=	27	38=	66=	0.0
15	TRE	76	3.7	27	17	56	87	56	0.0
	UNT	70	4.7	27	6	35	163	81	1.4
16	TRE	79	3.8	48	17	36	113	56	0.0
	UNT	65	4.0	30	7	33	137	76	1.7
ANOVA population	F=	17.2	5.9	4.3	6.2	4.5	11.4	6.3	0.8
	P=	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	ns
set	F=	32.9	198.2	85.1	686.3	105.1	116.7	794.4	24.8
	P=	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
pop. x set	F=	1.0	2.5	1.2	3.4	1.5	2.6	3.7	1.1
	P=	ns	1.1%	ns	0.1%	ns	0.8%	0.1%	ns

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Table 3: Plant characteristics and symptoms of insect attack on spring oilseed populations in 1991. Means from three plots per population and set (TRE = treated with insecticide; UNT = untreated). Standards: No. 15 = Puma; No. 16 = Legend. LSD-test: +, - and *, = indicate which populations that differ significantly from the mean of the two standards, and in which direction, at the probability levels 5 and 1% respectively.

Pop. No.	Set	No. of *Phyllotreta* punctures	% pods infested by:	
			D. brassicae	*C. assimilis*
1	TRE		7.4	1.3
	UNT	1.3	46.6+	5.4
2	TRE		8.5+	1.7
	UNT	1.7	48.3*	1.6
3	TRE		5.0	0.1
	UNT	1.0	37.0	0.6
4	TRE		4.3	0.5
	UNT	1.7	40.1	1.5
5	TRE		10.6*	1.7
	UNT	1.9	41.9	2.2
6	TRE		7.1	1.1
	UNT	1.6	38.4	3.3
7	TRE		3.1	1.7
	UNT	1.3	27.0	0.9
8	TRE		8.6+	1.0
	UNT	0.8	34.1	2.2
9	TRE		6.2	0.7
	UNT	1.9	24.1	0.9
10	TRE		8.3+	1.6
	UNT	1.1	38.9	3.3
11	TRE		10.5*	0.8
	UNT	1.4	47.7+	2.0
12	TRE		5.8	1.3
	UNT	1.2	32.7	2.3
13	TRE		7.0	0.9
	UNT	1.0	25.1	2.2
14	TRE		0.0-	0.0
	UNT	1.5	2.6=	0.0
15	TRE		4.3	2.3
	UNT	1.5	27.5	2.0
16	TRE		4.5	1.0
	UNT	1.2	40.2	3.6
ANOVA population	F=	1.4	9.6	2.3
	P=	ns	0.0%	1.9%
set	F=		651.9	14.3
	P=		0.0%	0.1%
pop. x set	F=		4.8	1.3
	P=		0.0%	ns

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Table 4: Plant characteristics and symptoms of insect attack on spring oilseed populations in 1991. Means from three plots per population and set (TRE = treated with insecticide; UNT = untreated). Standards: No. 15 = Puma; No. 16 = Legend. LSD-test: +, - and *, = indicate which populations that differ significantly from the mean of the two standards, and in which direction, at the probability levels 5 and 1% respectively. Oil and chlorophyll content analysed population-wise.

Pop. No.	Set	Seed yield (kg/plot at 15% H_2O)	% Oil content	Chlorophyll content (ppm)
1	TRE	1.70	42.2	52
	UNT	0.15	36.6	154
2	TRE	1.61	42.9	32
	UNT	0.16=	36.1	200
3	TRE	1.58	40.9	68
	UNT	0.25	36.6	135
4	TRE	1.36-	42.6	56
	UNT	0.14=	36.0	276
5	TRE	1.48	42.0	50
	UNT	0.21	36.0	132
6	TRE	1.88	44.2	36
	UNT	0.67*	38.4	109
7	TRE	1.50	41.0	67
	UNT	0.11=	35.0	328
8	TRE	1.78	43.1	35
	UNT	0.31	39.3	129
9	TRE	1.21=	41.2	89
	UNT	0.09=	36.1	254
10	TRE	1.51	44.6	35
	UNT	0.49*	38.9	108
11	TRE	1.43	42.0	35
	UNT	0.22	36.0	145
12	TRE	1.76	43.7	42
	UNT	0.39*	39.1	175
13	TRE	1.48	43.2	53
	UNT	0.26	38.1	160
14	TRE	0.28=	38.3	73
	UNT	0.08=	35.5	117
15	TRE	1.60	44.9	35
	UNT	0.26	38.4	200
16	TRE	1.78	43.5	38
	UNT	0.23	37.0	184

ANOVA

population	F=	15.2
	P=	0.0%

set	F=	1709.8
	P=	0.0%

pop. x set	F=	6.9
	P=	0.0%

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Another possibility is that even though insecticides were applied repeatedly there was enough influence of pollen beetles to affect bud production and yield.

Within UNT-comparisons

Populations 6, 10 and 12 yielded significantly more than the mean of the standards when no insecticide protected the crop (Table 4). Population 6 also differed significantly from the standards in being taller and by having a higher number and proportion of pods on the main shoot (Table 2). Population 10 did not differ from the mean of the standards in any of the variables studied, apart from the mean yield. Population 12 was taller and had a higher number of side shoots than the standards (Table 2).

There were only small differences between the populations in plant phenology and the highest yielding population number 6 for example was intermediate to the early standard Legend and the late standard Puma.

Yield and quality aspects

Seed yield was significantly positively correlated with oil content in the TRE (r = 0.73) but not in the UNT-set (Table 4) and seed yield was significantly, negatively correlated with chlorophyll content in both of the sets (r = -0.65 and r = -0.62 respectively; df = 13, p < 5%; r = ±0.51, *E. sativa* was excluded from the analysis).

Correlations between 1990 and 1991 in symptoms of insect attack

In the TRE-sets, there was a significant correlation between 1990 and 1991 in the number of potential pods (r = 0.75, p < 1%, df = 11) but not in the number of developed pods and proportion of podless stalks.

In the UNT-sets, there was a significant, <u>negative</u> correlation between the two years in number of pods (r = -0.58, p < 5%). There was no correlation between number of potential pods, proportion of podless stalks, number of *Phyllotreta* feeding punctures or proportion of pods with exit holes by *C. assimilis* larvae. There was, however, a positive correlation between 1990 och 1991 in proportion of pods with *D. brassicae* symptoms (r = 0.68, p < 5%).

Discussion

The present study shows how severe effects of insects may be to spring oilseed rape in Sweden. The architecture of the plants was changed due to pollen beetle attack and the population yields were considerably lower. Other studies designed to estimate damage to spring-sown *Brassica* oilseeds by insects have found effects ranging from a somewhat increased yield to a 70% reduction in yield (Gould, 1975;

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Free & Williams, 1978; Tatchell, 1983; Tulisalo & Wuori, 1986; Nilsson, 1987; Axelsen & Nielsen, 1990).

From a plant breeding point of view it was encouraging to find that some of the test populations gave higher yields than the standard varieties when no insecticide was applied. However the highest yielding population gave only 36% of the yield it produced when insecticides were applied. Another factor discouraging the initiation of a breeding programme with any of these populations was the discrepancy between ratings made during the two years. Only infestation rate by the gall midge *D. brassicae* was consistent among the populations, but the infestation rate was not correlated with yield. Thus even though the plant collection tested was more variable than normal breeding material no suitable plant source was found to start breeding for resistance to insects in oilseed rape. Efforts have been made in India to breed for resistance to the aphid *Lipaphis erysimi* Kalt. (Sekhon & Åhman, 1992) and in Canada for resistance to *Phyllotreta* spp. (Lamb, 1989) but no insect resistant oilseed rape variety has been marketed yet.

Acknowledgements

Résumé

Recherche de la résistance aux insectes chez le colza

Parmi environ 90 populations de colza de printemps (*Brassica napus*) étudiées pour leur résistance aux insectes dans un essai en champ en 1990, 11 ont été considérées comme dignes d'intérêt pour une étude approfondie en 1991, en plus de 2 populations d'hybrides somatiques entre *Eruca sativa* et le colza.

Des comparaisons entre une série de populations traitées (TRE) et non traitées (UNT) aux insecticides montrent un effet impressionnant des insectes sur la culture. D'une manière générale, les plantes non traitées sont plus courtes, ont plus des pousses latérales et plus de tiges fructifères, mais moins de siliques. La production de siliques se déplace de la tige principale aux tiges secondaires, mais le rendement significativement plus bas dans la série UNT montre que les plantes ne sont pas en mesure de compenser l'attaque des insectes. Toutes ces modifications à l'architecture de la plante sont des réactions typiques à l'infestation par le méligèthe (*Meligethes spp.*).

Dans les variantes UNT, les symptômes d'attaque provoqués par le méligèthe sont corrélés avec le rendement des graines, alors que cela n'est pas le cas pour les altises (*Phyllotreta spp.*), ni pour la cécidomyie des siliques (*Dasyneura brassicae*), ni pour le charançon des siliques (*Ceutorhynchus assimilis*).

Du point de vue de la sélection, il est encourageant de noter que certaines populations ont un meilleur rendement que la moyenne des variétés standard Pluma et Legend dans la série UNT. Cependant, le rendement de la meilleure population dans la série UNT n'atteint que 36% de celui de la population correspondante dans la série TRE. De plus, les corrélations entre les symptômes d'attaque provoqués par les insectes ont été faibles pendant les deux années d'expérience. Seul le taux d'infestation par *D. brassicae* fait exception. De ce fait, aucune source convenable de résistance n'a pu être trouvée pour débuter un programme de sélection du colza pour la résistance aux insectes.

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DETERMINATION OF ALLOZYME VARIATION WITHIN AND BETWEEN POPULATIONS OF Brevicoryne brassicae USING CELLULOSE ACETATE ELECTROPHORESIS

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Summary

Allozymes from individual aphids were used as markers to examine the structure of cabbage aphid, *Brevicoryne brassicae* colonies originating in the UK, France and USA during the summers of 1991 and 1992. Aphids were collected from four field experiments at Horticulture Research International, Wellesbourne and from locations within the UK, also from INRA Centre de Recherches de Rennes, France; and New York State Agricultural Experiment Station, Geneva, USA. Eighteen enzyme systems were examined using cellulose acetate electrophoresis but only 6-phosphogluconate dehydrogenase showed polymorphism (six alleles) in the three populations. The population samples from each country had genotype frequencies which conformed to Hardy-Weinberg (H-W) expectations but, as expected, the geographic isolation of the populations produced significant differences in the genotype frequencies.

Introduction

The cabbage aphid, *Brevicoryne brassicae* (L.), is an important pest of brassicas in most temperate regions. Reproduction is parthenogenetic, although a sexual phase may occur naturally during winter conditions. Currently, cabbage aphid control depends on insecticides. However, there is an urgent need to reduce pesticide use and therefore the production of resistant cultivars are under investigation. The occurrence of insect populations that differed in their ability to damage crop cultivars was first recognized by Painter (1930); these different populations were later called biotypes. The evolution of biotypes has been related to single gene resistance mechanisms (vertical resistance) which is easy to transfer by breeding rather than polygenic (horizontal resistance) which is difficult to breed but does not produce cultivar-resistant biotypes. Plant breeders efforts would greatly benefit from an understanding of the genetic variability of aphid populations against which they are developing resistance. Geographic differences or narrow genetic variability in pest populations could give misleading results for plant screening trials. Dunn & Kempton (1972) recorded differences in reproductive capabilities on Brussels sprouts lines cloned for resistance to cabbage aphid, when grown in different regions of the UK. This paper studies the genotypic variation, using allozyme variation, in *B. brassicae*

populations in four field experiments at Horticulture Research International, Wellesbourne; INRA Centre de Recherches de Rennes, France; and New York State Agricultural Experiment Station, USA, during the summers 1991 and 1992.

Cellulose acetate electrophoresis has become established as a technique for determining population genetics since 1985 (Richardson, Baverstock & Adams, 1986; Easteal & Boussy, 1987). Preliminary experiments showed the enzyme 6-phosphogluconate exhibited allelic variation (6 alleles) which could be used as useful markers to examine the structure of the three populations.

Materials and Methods

Stability of allozyme variants

To be useful markers to study aphid populations the allozyme variants produced parthenogenetically from individual female aptera must be stable over time. Clones were isolated from field-collected populations and reared on brassica plants for six generations. The original aptera and three individuals from the successive generations were typed using cellulose acetate electrophoresis of 6-phosphogluconate dehydrogenase.

Field collections

B. brassicae were collected from untreated guard rows from four field experiments at Horticulture Research International, Wellesbourne during the summers of 1991 and 1992. Samples were taken from infested leaves and frozen intact in 1.5 ml microcentrifuge tubes in liquid nitrogen. The four field experiments were located as follows:- Little Cherry, Deep Slade, Long Meadow and Townsend fields during 1991 and Cottage Field, Pump Ground West, Gravel Pits and Big Ground during 1992. Live samples of aphids were also received from INRA, Centre de Recherches de Rennes, France and freeze dried samples were sent from New York State Agricultural Experiment Station, Geneva, USA (aphids were frozen intact in liquid nitrogen then freeze dried).

Electrophoresis

Titan III cellulose acetate plates (76 mm x 76 mm) were obtained from Helena Laboratories with the Super Z-12 applicator kit. Individual aphids were placed in sample wells with 5 ul Tris/HCl, pH 8.0 for grinding with a spatula. Before use the cellulose acetate plates were soaked for 30 min in running buffer then blotted dry. Using the applicator 10 extracts were applied to each plate which was then placed acetate side down on the wicks in the electrophoresis tank (three plates were run concurrently). Extracts were resolved at 200 V/20 mA for 45 min in 0.1 M Tris-EDTA-Maleate-MgCl2 pH 7.4 as running buffer. Plates were stained for 6-phosphogluconate dehydrogenase activity according to the method of Richardson *et al.* (1986).

Data analysis

Gene frequencies among the three geographic regions sampled (UK, France and USA) were analyzed using Biosys-1 (Swofford & Selander, 1989) to calculate allele frequencies and two genetic distance measures: Nei's unbiased measure (Nei, 1978) and Rogers modified measure (Wright, 1978) were used.

Results

B. brassicae showed a low level of polymorphism, only 6-GPDH showed a significant level of variation in 18 enzyme systems investigated but the allozyme variants produced parthenogenetically from individual female aptera were stable in time. Using cellulose acetate electrophoresis there appears to be one locus for 6-Phosphogluconate dehydrogenase (6-PGDH) which is polymorphic with six allelomorphs recorded. Bands A, C and B were recorded in the descending order of frequency and differed in their mobility by about 10% (Fig. 1), while bands F, E, and D were much less frequent but had much greater mobility. Heterozygotes AB, AC, AB, BC, AF, AE, AD, DE, were recorded with reducing order of frequency. The AA genotype was always present in the populations sampled, both at HRI, Wellesbourne UK, New York State Experiment Station, Geneva, USA and INRA, Rennes, France with allele frequencies > 0.5.

A comparison of allele frequencies for four Wellesbourne plots (Deep Slade, Long Meadow, Little Cherry and Townsend) sampled during 1991 (Fig. 2) showed that for individual plots genotype frequencies deviated from Hardy Weinsberg (H-W) expectations. However, when the data were bulked from each plot there was better agreement with H-W predicted frequencies. Sampling from four similar plots (Big Ground, Gravel Pits, Pump Ground West and Cottage Field) during early 1992 (Fig. 3) again indicated differences between plots and a comparison of allele frequencies from the early populations of *B. brassica* during 1991 and 1992 showed some temporal variation (Fig. 4).
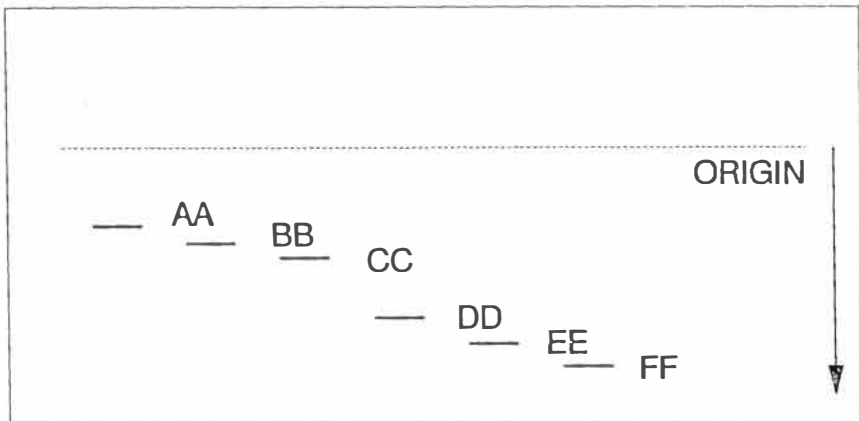

Fig. 1: Cellulose acetate electrophoresis 6-PGDH from *Brevicoryne brassicae*

DEEP SLADE

LONG MEADOW

LITTLE CHERRY

TOWNSEND

■ AA ◨ AC ⊞ CC ◨ BB □ BC ◪ AB

Fig. 2:   Comparison of allele frequencies, four Wellesbourne plots, both
            populations 1991

BIG GROUND

GRAVEL PITS

PUMP GROUND

COTTAGE FIELD

■ AA ◨ AC ⊞ CC □ BB □ BC □ AB ◪ AF

Fig. 3:   Comparison of allele frequencies, four Wellesbourne plots, early
            population 1992

50

1991   1992

■ AA ⊠ AC ▦ CC ⧄ BB ☐ BC ◩ AB ⊠ AF
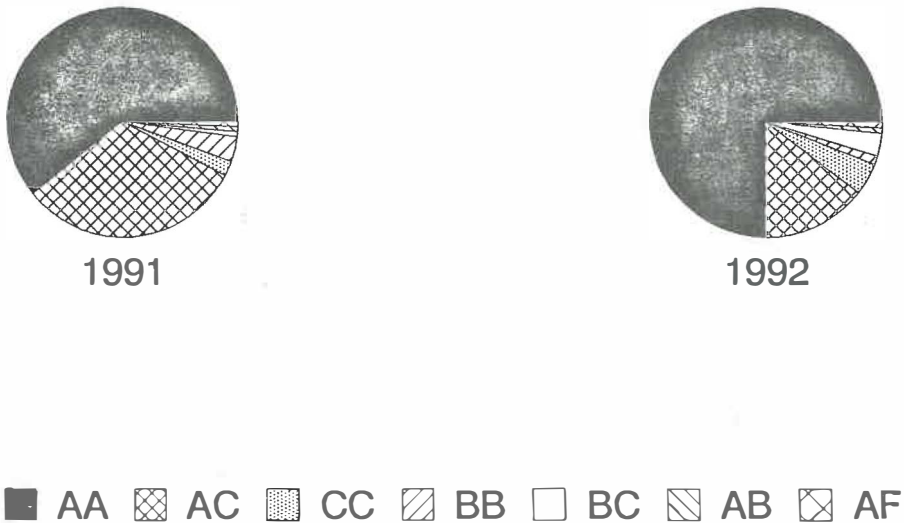
Fig. 4:   Comparison of allele frequencies, Wellesbourne, early populations
               1991/92

When the aphid sample received from Geneva, USA was analysed two allelomorphs DD and EE not recorded in the UK were observed with a reduced frequency of CC and BB alleles. The most common allelomorph in each was population was still found to be the homozygote AA but the two populations could easily be distinguished (Fig. 5). A small French population also sampled during September/October 1991 showed very little variation the most common allelomorph being the homozygote AA; however, a heterozygote AE was also recorded (Fig. 6).

There are two populations of *B. brassicae* each year and allele frequencies showed a significant difference between the two populations in 1991. During the early population (early July/August) the frequency of genotypes other than AA was > 0.4 while this was reduced to 0.15 in the late population (September/October) (Fig. 7).
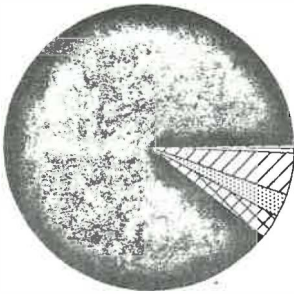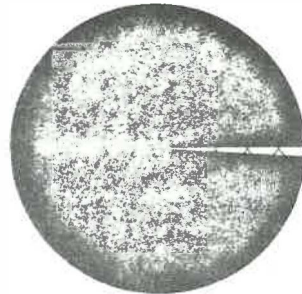
WELLESBOURNE   USA

■ AA ⊠ AC ☑ DD ▦ EE ◣ DE ⧄ BB ▩ CC
■ AD ▒ AE ◇ AF ◨ AB □ BC ▨ FF
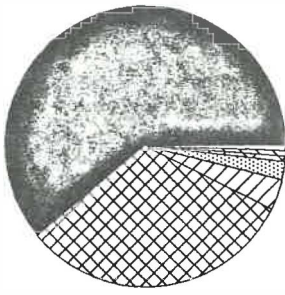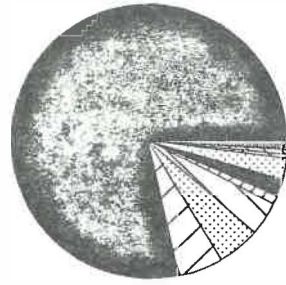
Fig. 5:   Comparison of allele frequencies, Wellesbourne/USA, 1991

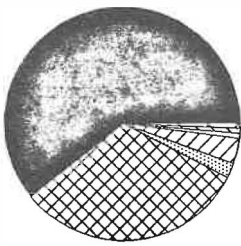WELLESBOURNE   FRANCE
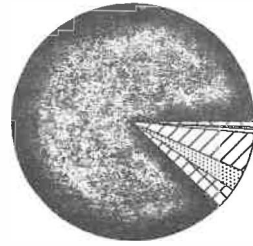
■ AA ⊠ AC ▦ CC ⧄ BB □ BC ◨ AB ◇ AE

Fig. 6:   Comparison of allele frequencies, Wellesbourne/France, 1991

EARLY
JULY/AUG

LATE
SEPTEMBER/OCT

■ AA ⊠ AC ▦ CC ▨ BB □ BC ◩ AB ◌ AF

Fig. 7:   Comparison of allele frequencies, Wellesbourne, early and late
            population 1991

Discussion

   B. brassicae showed a low but stable level of polymorphism, only 6-GPDH
showed a significant level of variation in 18 enzyme systems investigated. This level
of polymorphism is much less than that found for *Sitobion avenae* and *S. fragariae* by
Loxdale & Brookes (1990) or for *Acyrthosiphon pisum* (Simon, Parent & Auclair,
1982). However, in nineteen taxonomically diverse aphid species the level of
polymorphism was < 12% (Tomiuk & Wohrmann, 1983). Thirteen 6-GPDH
genotypes were recorded so at least this number of clones exist during the asexual
phase of reproduction. Figs. 2 and 3 indicate the number of aphids samples required
to investigate the genetic variation present in a population at a location. As this
sampling indicates smaller sample sizes can produced biased results which will not be
in agreement with Hardy-Weinsberg equilibrium.

   This preliminary investigation revealed interesting variations in *B. brassicae*
populations in different locations and in the two populations observed within each
year. Further experiments are required to clone aphid genotypes and investigate their
stability, fecundity, parasitism and responses to resistant cultivars. Cloning of aphid
genotypes will also determine contamination by other organisms such as
hymenopterous endoparasitic wasps and entomophagous fungi. Parasitism of cabbage
aphids by *Diaeretiella rapae* was quite common and resulted in the loss of the 6-
GPDH band. Also of interest is the variation in aphid genotypes, as indicated by 6-

GPDH allele frequencies observed between the early and late populations of *B. brassica* during both 1991 and 1992. The variation in allele frequencies from the most common allelomorph AA is reduced from 0.4 to 0.15 from the early to the late aphid population. This suggests that only the parthenogenetically reproducing common allelomorph AA remains to infest crops later in the year and that other allelomorphs form sexuals which migrate.

Much more sampling over a number of years is required to determine how temporally-stable populations are in terms of genetic structure and the proportion of gene-flow which face the introduction of new aphid-resistant cultivars.

Résumé

La détermination de la variation des allozymes au sein et entre trois populations de *Brevicoryne brassicae* à l'aide de l'électrophorèse sur cellulose d'acétate

Des allozymes de pucerons individuels ont servi de marqueurs pour examiner la structure des colonies de *Brevicoryne brassicae* collectées en GB (HRI, Wellesbourne et autres provenances), en France (INRA, Rennes) et aux U.S.A. (New York State Agricultural Experiment Station) durant les étés 1991 et 1992.
Parmi les 18 systèmes enzymatiques étudiés à l'aide d'électrophorèse sur cellulose d'acétate seul la 6-phosphogluconate déhydrogénase a montré un polymorphisme (6 allèles) dans les trois populations. Les fréquences des génotypes de tous les échantillons sont conformes à la loi de Hardy-Weinberg (H-W). Mais comme on pouvait s'y attendre, l'isolation géographique des populations s'est traduite par des différences significatives des fréquences de génotypes.

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SURVEY OF APHID SPECIES ON LETTUCE

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Summary

At the fifth meeting of the IOBC/EUCARPIA working group on Breeding for Resistance to Insects and Mites at Marcelin, Switzerland in 1989 a cooperative project was initiated. The goals of this study were: 1) to determine which aphid species are most important on field-grown lettuce in Europe; 2) to test accessions with different levels of resistance in several countries against the local aphid populations. A total of 59 experiments were carried out in 1990 and 1991 at eleven sites in six countries; Czechoslovakia (1), England (1), France (2), Germany (1), The Netherlands (4) and Switzerland (2). In both years *Nasonovia ribisnigri* was the most common aphid species. *N. ribisnigri* was about twice as numerous as *Macrosiphum euphorbiae*, which was the second most common species. Third in frequency was the root aphid (*Pemphigus bursarius*), which was, however, absent in most of the experiments. *Uroleucon sonchi* was only present in high numbers in a few experiments in the Netherlands. *Myzus persicae* was present in high numbers in only one experiment in Germany. All other leaf aphid species were absent or of only minor importance. Thus, breeding of lettuce for resistance to aphids should be focussed on *N. ribisnigri*, *M. euphorbiae* and *P. bursarius*. Accessions with monogenic resistance to *N. ribisnigri* and *P. bursarius* showed absolute resistance in all experiments where the corresponding aphid species occurred. Quantitative differences in numbers of aphids per accession were observed, but these did not always correspond with results from previous antibiosis tests.

Introduction

Lettuce (*Lactuca sativa*) is a suitable host for many aphid species. Blackman and Eastop (1984) list the following species as pests of lettuce: *Pemphigus* species, *Neotrama caudata*, *Trama troglodytes*, *Aphis gossypii*, *Aphis fabae*, *Aphis citricola*, *Uroleucon formosanus*, *Uroleucon cichorii*, *Uroleucon ambrosiae/pseudoambrosiae*, *Nasonovia ribisnigri*, *Aulacorthum (Neomyzus) circumflexum*, *Aulacorthum solani*,

Acyrthosiphon lactucae and *Macrosiphum euphorbiae*. Aphids are a serious problem in lettuce growing, both under glass and outdoors. Because the presence of aphids in the head renders the crop unmarketable, lettuce growers frequently apply insecticides in periods favourable to the development of aphids. Furthermore, aphids spread virus diseases and can cause reduced or abnormal growth.

Host resistance is an attractive alternative for chemical control of aphids in lettuce. Numerous investigations have been carried out on lettuce to find resistance to several aphid species and to study the inheritance of resistance. Resistance to the following aphid species has been studied: *N. ribisnigri* (Dunn & Kempton, 1980b; Eenink, Groenwold & Dieleman, 1982a; Eenink, Dieleman & Groenwold, 1982b; Eenink & Dieleman, 1983), *Myzus persicae* (Eenink & Dieleman, 1977; 1980; Reinink, Dieleman & Groenwold, 1988; Reinink, Dieleman, Jansen & Montenari, 1989), *M. euphorbiae* (Dunn & Kempton, 1980b; Reinink & Dieleman, 1990a; 1990b), *Uroleucon sonchi* (Reinink & Dieleman, 1990a; 1990b) and *Pemphigus bursarius* (Dunn, 1960; 1974; Dunn & Kempton, 1980a). An almost absolute level of major gene resistance is available for two aphid species, *N. ribisnigri* and *P. bursarius*, while for *M. persicae, M. euphorbiae* and *U. sonchi* high levels of quantitatively inherited partial resistance (antibiosis) were found. Because there are so many different aphid species and because most sources of resistance are specific to one species, breeding of lettuce lines with a high level of resistance to all aphid species is an enormous task. Therefore, choices have to be made which aphid species receives priority in a breeding programme. To make a rational decision, information is needed on the relative importance of the aphid species in areas of lettuce production. However, such information is almost completely lacking. In general, growers do not identify their aphid species and reports in the literature on this topic are out dated and mostly based on few observations.

According to Ainsworth & Ogilvie (1939) *M. euphorbiae* was the most common aphid on lettuce in the west of England, with *Uroleucon (Dactynotus) sonchi* and *N. ribisnigri* occurring only occasionally. They never found *M. persicae* on lettuce. Broadbent, Tinsley, Budden & Roberts (1951) listed three species as common on lettuce on two farms in Buckinghamshire (UK): *N. ribisnigri* (most numerous), *M. euphorbiae* (second in numbers) and *M. persicae* (occurring occasionally). Giustina (1972) studied the aphid species occurring in glasshouses in the Paris region and listed *M. persicae* as the most numerous species, and *M. euphorbiae, N. ribisnigri* and *A. solani* as less numerous. Hinsch, Vail, Tebbets & Hoffmann (1991) reported that *M. persicae* and *M. euphorbiae* were most prevalent on iceberg lettuce grown in the coastal area of California, while *A. gossypii* was the most common species in the San Joaquin Valley, California.

In 1989, at the fifth meeting of the IOBC/EUCARPIA Working Group on Breeding for Resistance to Insects and Mites at Marcelin, Switzerland, a cooperative project was initiated to produce new and more comprehensive data on the relative importance of aphid species on field-grown lettuce in several European countries. A second objective of this cooperation was to test accessions which had shown differences in resistance in previous research in several countries against the local aphid populations. The result of this initiative was called the "Cooperative Lettuce Aphids Project" and the results are presented here.

Materials and Methods

Scientists and breeders participated at 11 locations in the Cooperative Lettuce Aphids Project (Table 1, Fig. 1). At each location three experiments (spring, summer and autumn) were planned both for 1990 and 1991.

Table 1:   Participants in the Cooperative Lettuce Aphid Project

Code	Participant	Company	Location	Country
CH1	J. Freuler & S. Fisher	St. Féd. Rech. Agron. de Changins	Nyon	Switzerland
CH2	B. Hurni	Eidg. Forsch. Anstalt	Wädenswil	Switzerland
CS	P. Láska & L. Bocák	Res. Inst. Vegetable Growing and Breeding	Olomouc	Czechoslovakia
D	C.P.W. Zebitz	University Hannover	Hannover	Germany
F1	P.Clausel & A. Amiraux	Rijk Zwaan France	Aramon	France
F2	J.P. Moreau & B. Maisonneuve	INRA	Versailles	France
GB	P.R. Ellis	HRI	Wellesbourne	Great Britain
NL1	Th. Jacobs	Royal Sluis BV	Enkhuizen	Netherlands
NL2	A. van der Arend	Leen de Mos BV	's Gravenzande	Netherlands
NL3	J. Velema & A. Bazelmans	Rijk Zwaan BV	De Lier	Netherlands
NL4	F.Dieleman & R. Groenwold	WAU, Dept. Entomol. CPRO-DLO	Wageningen	Netherlands

At all sites the same lettuce accessions were tested. In 1990 seven accessions were included in the experiments, of which two were discontinued in 1991 (Table 2).

Cultural methods were applied according to local practice, with the exception of pesticides, which were not used. The experiments utilised a randomized block design with three replicates. In 1990 each plot consisted of two rows of 10 plants, bordered by two rows of the butterhead cultivar Soraya. In 1991 each plot consisted of 10 rows of 10 plants, and the whole experiment was surrounded by a border of

Soraya. It was recommended that the experiment should be located in the neighbourhood of other lettuce crops. Lettuce seeds, randomization schemes and observation sheets were provided by Reinink. An Illustrated Guide to Lettuce Aphids, containing photographs and drawings with identification keys of *N. ribisnigri*, *M. persicae*, *M. euphorbiae*, *U. sonchi*, *A. solani*, *A. circumflexum*, an *Aphis* species and *P. bursarius* was produced by Dieleman and Reinink and sent to all participants.

Fig. 1: Locations of experiments

Table 2:   Lettuce accessions with their level of resistance to *Nasonovia ribisnigri* (Nr), *Macrosiphum euphorbiae* (Me), *Myzus persicae* (Mp), *Uroleucon sonchi* (Us) and *Pemphigus bursarius* (Pb). Resistance levels taken from Dunn & Kempton (1980a; 1980b) and from Reinink & Dieleman (1990a)

Accession	Resistance[1]					Type
	Nr	Me	Mp	Us	Pb	
PIVT313	+	-	+ +	+	+	cos
Reskia	+	-	+	-	+ +	butterhead
PIVT364 (PI169497)	+	-	-	-	+	loose leaved
Avoncrisp	+	+ +	?	+ +	--	crisp
Liba	+	-	-	-	+	butterhead
Batavia Chou de Naples [2]	+	+ +	?	+ +	?	batavia/crisp
PIVT280 (*Lactuca virosa*) [2]	--	-	-	-	?	wild species

[1] + +: very susceptible; +: susceptible; - : partially resistant; -- : completely resistant;
    ? : not tested, probably susceptible.
[2] Only included in the 1990 experiments.

When the plants were mature and ready for harvest one final and destructive evaluation was done. Twenty plants of each accession were lifted and the roots were evaluated for symptoms of root aphid (*P. bursarius*). Then, the numbers of adult leaf aphids (both winged and apterous) on each plant were scored for each accession. In the first experiment in 1990 20 plants were scored per plot, using a 0-3 scale (0: no adults; 1: less than 10 adults; 2: 10-100 adults and 3: more than 100 adults). However, this method was too time consuming and after the spring experiment of 1990 the observation method for leaf aphids was changed. The number of plants per plot evaluated for leaf aphids was reduced to a minimum of four. Instead of scoring, all adult leaf aphids on these plants were counted by peeling off the leaves. Evaluated plants were taken from the centre of the plot.

Results

In 1990 a total of 29 experiments were carried out (Table 3). In the following sections experiments are classified as heavily infested when at least 70% of the plants had aphids. Plants were classified as having an intermediate level of infestation when between 40 and 70% of the plants were attacked.

In the spring experiments *N. ribisnigri* and *M. euphorbiae* were most frequent. In two spring experiments (NL2, F1) a high infestation of *P. bursarius* was recorded. In only one spring experiment (D) was a high infestation of *M. persicae* found. According to the participant, the observation date of this experiment coincided with the peak of migration and inflight of *M. persicae*. More than 90% of the *M. persicae* aphids that were scored were winged virginoparae. *Aphis* species, *U. sonchi* and *A. solani* were found in low frequencies in only a few spring experiments.

Most summer experiments in 1990 had low infestation levels. In five experiments (GB, CH1, CH2, F1, F2) a medium to high level of infestation with *P. bursarius* was found. Only in the west of the Netherlands (NL1, NL2, NL3) medium to high levels of *N. ribisnigri* and *M. euphorbiae* were maintained. The following species occurred in low frequencies: *Aphis* species, *U. sonchi*, *M. persicae*, *Neomyzus* sp. (NL4), *A. solani* and *Acyrthosiphon scariolae* (CH1).

Also in the third series of experiments, conducted in late summer to early autumn of 1990, most experiments had low infestation levels. Only at two locations, again in the west of the Netherlands (NL2, NL3), were high frequencies of *N. ribisnigri*, *M. euphorbiae* and *U. sonchi* recorded. The following species were detected only in low frequencies: *P. bursarius*, *M. persicae*, *A. solani* and *A. scariolae* (CH1).

In 1991 a total of 30 experiment were carried out (Table 4). Again, in the spring experiments *N. ribisnigri* and *M. euphorbiae* were dominant. Eight of the 11 spring experiments had a medium to high level of infestation of *N. ribisnigri*, while six experiments had a medium to high infestation of *M. euphorbiae*. One of the spring experiments (NL4) had a medium level of infestation of *U. sonchi*. Two experiments (CH1, F1) had a medium level of infestation of *P. bursarius*.

Table 3: Percentage of plants infested with aphids of the species *Nasonovia ribisnigri* (Nr), *Macrosiphum euphorbiae* (Me), *Myzus persicae* (Mp), *Uroleucon sonchi* (Us), *Aulacorthum solani*, *Aphis* spp. (A) and *Pemphigus bursarius* (Pb) in the spring, summer and autumn experiments of 1990.

Code	Observation date	Nr	Me	Mp	Us	As	A	Pb
Spring								
GB	27-6	17	82	23	-	-	-	-
D	15-6	89	82	75	-	-	6	-
NL1	14-6	79	86	3	15	-	-	-
NL2	20-7	85	88	-	14	-	-	24
NL3	28-6	83	88	43	26	-	24	78
NL4	13-6	99	100	6	25	40	-	-
CS	?-6	69	69	-	-	36	-	-
CH1	11-6	11	60	4	-	-	-	-
CH2	8-6	68	43	30	-	-	-	-
F1	16-6	-	-	-	-	-	-	67
F2	9-6	76	71	-	-	-	-	7
Summer								
GB	21-8	-	-	-	-	-	-	52
D	31-7	6	30	-	-	-	6	-
NL1	23-7	65	35	10	18	-	-	-
NL2	6-8	87	65	-	25	-	-	18
NL3	1-8	58	89	10	45	-	13	20
NL4	23-7	40	44	34	7	-	-	-
CS	30-7	19	7	-	1	6	6	-
CH1	30-8	-	35	-	-	-	19	47
CH2	17-7	36	4	-	-	-	-	68
F1	26-6	-	-	-	-	-	-	72
F2	29-6	24	33	-	-	-	-	73
Autumn								
D	11-10	-	32	-	-	-	-	-
NL2	15-9	87	86	-	77	-	-	2
NL3	?-9	67	90	36	86	5	-	-
NL4	11-9	-	13	-	15	-	-	-
CS	9-10	17	22	5	-	22	-	23
CH1	30-11	48	39	6	21	-	-	-
F2	7-9	14	26	5	7	-	-	33

Table 4: Percentage of plants infested with aphids of the species *Nasonovia ribisnigri* (Nr), *Macrosiphum euphorbiae* (Me), *Myzus persicae* (Mp), *Uroleucon sonchi* (Us), *Aulacorthum solani*, *Aphis* spp. (A) and *Pemphigus bursarius* (Pb) in the spring, summer and autumn experiments of 1991.

Code	Observation date	Nr	Me	Mp	Us	As	A	Pb
Spring								
GB	27·6	15	85	7	-	-	-	-
D	10·7	42	38	-	-	-	3	-
NL1	1·7	100	78	17	10	-	-	-
NL2	22·6	10	18	-	7	-	-	3
NL3	24·6	99	99	4	3	1	3	-
NL4	9·7	100	90	-	60	-	-	-
CS	25·6	98	34	3	2	1	3	-
CH1	25·6	84	71	8	7	38	11	48
CH2	13·6	92	20	-	-	-	-	-
F1	11·7	38	17	18	-	-	-	44
F2	26·6	94	51	8	-	-	-	17
Summer								
GB	26·7	97	98	3	-	-	35	2
D	11·9	17	7	-	-	-	3	34
NL1	25·8	52	12	-	10	-	-	-
NL2	30·8	87	67	-	25	-	-	38
NL3	13·8	13	8	-	18	-	-	*
NL4	5·8	-	8	-	12	-	-	-
CH1	9·8	-	-	-	-	-	-	59
CH2	16·7	30	10	-	-	-	-	74
F1	11·9	-	8	-	-	-	-	-
F2	5·7	73	37	8	8	-	-	1
Autumn								
GB	4·9	23	17	-	-	-	-	-
D	8·10	23	11	-	-	-	-	35
NL2	4·10	22	63	-	95	-	-	15
NL3	16·9	83	79	-	72	-	-	*
NL4	8·9	3	15	-	30	-	-	2
CH1	13·11	23	41	30	-	2	-	-
CH2	6·9	23	43	-	-	-	-	13
F1	29·11	13	22	5	-	-	-	-
F2	23·9	18	6	-	-	-	-	14

In the summer experiments of 1991 the infestation levels again were low in most experiments. There were four experiments (GB, NL1, NL2, F2) with medium to

high levels of infestation of *N. ribisnigri* and two experiments (GB, NL2) with medium to high levels of *M. euphorbiae*. *P. bursarius* was present in medium to high levels in four experiments (D, NL2, CH1, CH2).

Also in most of the late summer-early autumn experiments of 1991 low aphid infestation levels were found. Only experiment NL3 had a high infestation with *N. ribisnigri*, while four experiments (NL2, NL3, CH1, CH2) had a medium to high infestation with *M. euphorbiae*. *U. sonchi* was present in high frequency in two experiments (NL2, NL3). Only one experiment (D) had a medium level infestation of *P. bursarius*. *A. solani* and *Aphis* spp. were absent or only present in low frequencies in the 1991 experiments.

The results from the 59 experiments carried out in 1990 and 1991 are summarized in Table 5. This table shows clearly that *N. ribisnigri* and *M. euphorbiae* were the most common aphid species, followed by *P. bursarius*. Judged by the percentages of plants infested, the two dominant species were about equally frequent. However, at the same percentage of plants infested, the absolute numbers of *N. ribisnigri* were much higher than those of *M. euphorbiae*. This is illustrated in Fig. 2, that shows the relationship between the logarithm of the absolute number of aphids counted and the percentage of plants infested with *N. ribisnigri* and *M. euphorbiae* for the 1991 experiments. The regression line for *N. ribisnigri* lies above the line for *M. euphorbiae*. Averaged over all 43 experiments in which the adult aphids per plant were counted, the mean number per plant was 7.8 for *N. ribisnigri* and 3.2 for *M. euphorbiae*. Thus, *N. ribisnigri* was the dominant aphid species in these experiments. *U. sonchi* seems to be a Dutch problem: the six experiments in which this aphid species was found in medium to high frequencies were all located in the Netherlands. *A. solani* and *Aphis* species did not occur frequently.

Table 5: Number of experiments with a high, intermediate or low level of infestation for each aphid species. Summarized results of Tables 3 and 4.

Species	Level of infestation			
	High >70%	Intermediate 40-70%	Low <40%	Absent
N. ribisnigri	19	8	23	9
M. euphorbiae	17	10	28	4
M. persicae	1	1	23	34
U. sonchi	4	2	22	31
A. solani	0	0	9	50
Aphis spp.	0	0	12	47
P. bursarius[1]	8	7	14	28

[1]Infestation on susceptible cultivars

Table 6 presents the accession means for the percentage of plants with symptoms caused by root aphid in experiments with a medium to high infestation level.

Table 6:   Mean percentage of plants with symptoms of root aphid (*P. bursarius*) in experiments with at least 40% of the susceptible plants infested.

Accession	1990	1990-91
PIVT313	87	74
Reskia	91	75
PIVT364	80	62
Avoncrisp	2	1
Liba	79	58
Batavia C.N.	88	
PIVT280	1	
# of experiments	5	13

Table 7:   Mean number of adults leaf aphids per accession in experiments with at least 40% of the plants infested

Accession	*N. ribisnigri*		*M. euphorbiae*		*U. sonchi*	
	1990	1990-91	1990	1990-91	1990	1990-91
PIVT313	14^b	10^a	8.3^b	6.7^a	12.3^c	12.5^c
Reskia	57^c	40^b	4.6^a	7.4^a	0.2^a	2.1^a
PIVT364	11^b	12^a	3.3^a	6.5^a	1.9^{ab}	4.1^{ab}
Avoncrisp	23^b	14^a	6.5^{ab}	7.1^a	5.3^{bc}	6.3^{bc}
Liba	5^b	8^a	4.7^{ab}	6.6^a	2.0^{ab}	1.7^a
Batavia C.N.	27^{bc}		7.9^b		5.5^{bc}	
PIVT280	0^a		11.4^b		2.0^{ab}	
# of experiments	7	19	7	18	1	4

The monogenic resistance of the cultivar Avoncrisp to *P. bursarius* provided almost complete resistance in all experiments with infestation with root aphid. *Lactuca virosa* (PIVT280) also showed nearly absolute resistance to *P. bursarius*. On average

the accessions Liba and PIVT364 had a percentage of damaged plants about 10% lower than the other susceptible accessions.

To evaluate whether the lettuce accessions showed different levels of attack by the leaf aphid species, those experiments were selected in which the number of adult aphids per species was counted (thus excluding the earliest experiments, in which scores were used) and for each aphid species those experiments were selected in which that species had at least an intermediate level of infestation. Significant differences between accessions were calculated from an analysis of variance on transformed data. The transformation used was the logarithm of the counted number of aphids plus 1. Least significant differences were calculated by taking the accession × experiment interaction mean square as error term. Table 7 presents the mean number of aphids per accession for *N. ribisnigri*, *M. euphorbiae* and *U. sonchi*. For *M. persicae*, *A. solani* and *Aphis* spp. the data did not allow a comparison of the accessions.

Percentage of plants infested
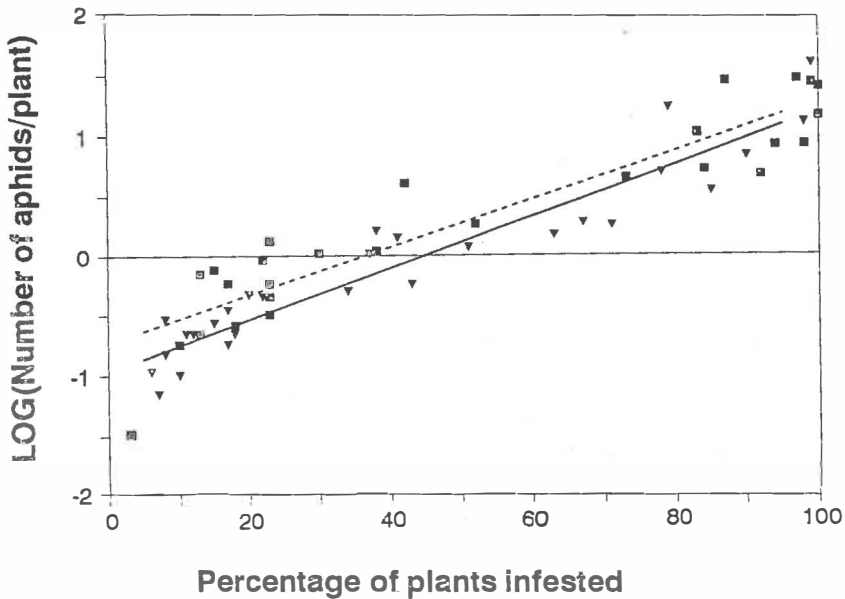
Fig. 2:   Relationship between the logarithm of the absolute number of aphids counted and the percentage of plants infested with *N. ribisnigri* (squares, dotted regression line; r = 0.93) and *M. euphorbiae* (triangles, drawn regression line, r = 0.95) for the 1991 experiments.

The *L. virosa* accession used in the 1990 experiments has been used in breeding programmes as the donor of the Nr-gene, which provides absolute resistance to *N. ribisnigri* (Eenink *et al.*, 1982a). In the experiments performed in 1990 the *L. virosa* accession remained free of *N. ribisnigri* (Table 7). The butterhead cultivar Reskia had significantly higher numbers of *N. ribisnigri* than the other accessions.

For *M. euphorbiae* the experiments with seven accessions carried out in 1990 showed some significant differences between accessions, with PIVT280 (*L. virosa*) having the highest numbers and PIVT364 the lowest (Table 7). In contrast, the results calculated for five accessions in 18 experiments did not show any significant differences between the accessions.

In 1990 only one experiment had sufficient numbers of *U. sonchi* to compare the accessions. In this experiment Reskia, PIVT364, Liba and PIVT280 had low numbers of *U. sonchi*, while PIVT313 had high numbers of this species (Table 7). The combined analysis of the 1990 and 1991 experiments for five accessions again showed PIVT313 to be more attacked than the other species. Again Reskia, PIVT364 and Liba had the lowest numbers of *U. sonchi*, while Avoncrisp had intermediate numbers.

Discussion

The field experiments conducted in 1990 and 1991 within the framework of the Cooperative Lettuce Aphid Project provided good information on the relative importance of the various aphid species on lettuce in several European countries. In both years *N. ribisnigri* was the most common aphid species. *N. ribisnigri* was about twice as numerous as *M. euphorbiae*, which was second in numbers. *U. sonchi* seems to be a local Dutch problem, as it was only found in high numbers in a few experiments in the Netherlands. *M. persicae* was present in high numbers in only one experiment, where the observation date coincided with the peak of migration and inflight of alate virginoparae. All other leaf aphid species were absent or of only minor importance. Third in numbers was the root aphid (*P. bursarius*), which was, however, absent in the majority of experiments.

Although the experiments were only carried out in two different years, we feel that the conclusions on the relative importance of the aphid species on lettuce can be extrapolated from these two years. Firstly, because both years gave similar results, and secondly, because the results are in line with our previous experiences. For plant breeders these results indicate that breeding of lettuce for resistance to aphids should be focussed on *N. ribisnigri*, *M. euphorbiae* and *P. bursarius*. Monogenic absolute resistances are available for *N. ribisnigri* and *P. bursarius*. The experiments provided no indications that local populations of *N. ribisnigri* or *P. bursarius* were insensitive to these resistances. Also quantitative differences in numbers of aphids per accession were observed for *N. ribisnigri*: the butterhead cultivar Reskia had a significantly higher number of *N. ribisnigri* than the other accessions.

For *M. euphorbiae*, Reinink and Dieleman (1990a) detected high levels of antibiosis in various lettuce accessions. However, the resistance of lettuce accessions to *M. euphorbiae* as measured in antibiosis tests (Table 2) did not show a relationship with the numbers of *M. euphorbiae* observed per accession in these experiments (Table 7). Unexpectedly, the large differences in antibiosis, which were very manifest under glasshouse test conditions, did not produce large effects in the field. Preference could play an important role in the field. For instance, the very dark green colour of *L. virosa* and PIVT313 could attract *M. euphorbiae*, notwithstanding

the relatively high level of antibiosis of these genotypes. Because the accessions used in these experiments had a very different growth habit, plant size could also be important in explaining the differences between results from the field and from standardized tests. Genotype × environment interactions could also be important. Poehling, Tenhumberg, Yakti and Prüter (1990) demonstrated that the partial resistance of faba bean to *Aphis fabae* was strongly influenced by temperature. There could also be biotypes of *M. euphorbiae* with a lower sensitivity to the partial resistance in the accessions used. The results indicate that more research is needed to clarify the relationship between antibiosis tests, performed under standardized conditions in the laboratory or glasshouse, and the performance of lettuce accessions in the field.

Differences between accessions were also found for *U. sonchi* (Table 7). These differences corresponded better with the results from antibiosis tests (Table 2) than the results for *M. euphorbiae*.

Thus, the Cooperative Lettuce Aphids Project has in an efficient way provided useful information on the relative frequencies of aphid species in field-grown lettuce in Europe. The results of the project have, however, raised new questions about the relationship between measurements of partial resistance under standardized conditions and the number of aphids found on field-grown plants.

Résumé

Etude des pucerons de la laitue

Lors de la dernière réunion du groupe de travail, la décision a été prise de conduire une étude en commun sur les pucerons de la laitue avec les buts suivants:
1. déterminer la fréquence relative des différentes espèces de pucerons sur la laitue de plein champ;
2. vérifier la concordance des différences de sensibilité à plusieurs espèces de puceron des cultivars étudiés dans plusieurs pays européens.

Les expériences étaient conduites en 1990 et en 1991. Chaque année, trois essais ont eu lieu dans 12 localités de 6 pays: Tchécoslovaquie (1x), Angleterre (2x), France (2x), Allemagne (1x), Pays Bas (4x) et Suisse (2x). Les essais furent évalués à la maturité des plantes, en comptant ou estimant le nombre de pucerons de chaque espèce. Dans chaque site, les mêmes lignées de laitue ont été testées, soit sept en 1990 et cinq en 1991. Au total, 29 essais ont été menés en 1990 et 30 en 1991. Les résultats de ces 59 expériences sont présentés.

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THE CHEMICAL BASIS OF RESISTANCE TO Nasonovia ribisnigri (APHIDIDAE) IN LETTUCE

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Summary

The monogenic absolute resistance of lettuce to the aphid *Nasonovia ribisnigri* was studied using Electrical Penetration Graphs (EPG), behavioral studies and chemical analysis. We tried to find differences between isogenic resistant and susceptible lettuce lines. The only clear difference in behaviour showed up after a phloem sieve element was reached and feeding started (E2 pattern). The difference may be caused by chemical differences in the phloem sap composition or mechanical blocking of the sieve element or aphid stylets during feeding. Chemical analysis of the phloem was hampered by difficulties during the collection of phloem by stylet amputation. EDTA-phloem exudation and honeydew were used as substitutes. Honeydew was collected using *Myzus persicae*. Samples of several sources were analyzed for sugars, amino acids, secondary plant compounds and proteins. So far no chemical differences have been found. A good bioassay is necessary to test the performance of aphids on phloem extracts from susceptible and resistant plants.

Introduction

Absolute resistance to *Nasonovia ribisnigri* (Mosley) was transferred from *Lactuca virosa* to *L. sativa*. The resistance is based on a single dominant gene (Nr-gene, Eenink, Groenwold & Dieleman, 1982a; b). Direct Current (DC) Electrical Penetration Graphs (DC-EPG's) from *N. ribisnigri* on resistant and susceptible lettuce (*L. sativa*) (Helden & Tjallingii, 1990; 1992) showed that the behaviour differed only after the aphid had reached the phloem. Possible resistance mechanisms include mechanical blocking of the stylets or the sieve element after puncturing or the chemical composition of the phloem sap (Helden & Tjallingii, 1992). This article describes the preliminary results of the analyses of different "phloem sap" samples for amino-acids, sugars, proteins and secondary plant substances. Phloem sap samples were collected by EDTA chelation (King & Zeevaart, 1974), honeydew (Banks & Macaulay, 1964; Sasaki, Aoki, Hayashi & Ishikawa, 1990) and stylectomy (Unwin, 1978; Weibull, Ronquist & Brishammar, 1990).

Materials and methods

Aphids and plants

Mass rearing of *N. ribisnigri* and *M. persicae* and culture of plants were performed as described by Helden & Tjallingii (1990). The lettuce lines used were: Taiwan lettuce (susceptible) and two (near) isogenic lettuce lines which differed only in the Nr-gene (Helden & Tjallingii, 1990). Plants were about 4 weeks old (6-8 fully expanded leaves).

Stylectomy

Stylectomy was performed in the laboratory at $22 \pm 1°C$ under artificial illumination (HF fluorescent tubes ca. 6000 Lux). *M. persicae* adults were wired for EPG in the afternoon and left overnight on the test plant. The next morning they were checked, those which showed pattern E2 were used for stylectomy with a microcautery unit (Syntech, modified after Unwin, 1978).

Honeydew collection

This was performed in a greenhouse at $22 \pm 1°C$ and continuous light by SONT high pressure sodium lamps. 5-10 *M. persicae* adults were placed in a honeydew collection cage as described by Eenink, Dieleman, Groenwold, Arts & Clerkx (1984). *N. ribisnigri* adults were placed on the other side of the leaf. After 1 day the petri dish bottom of the cage was replaced by a new one with 2 ml of N-hexadecane. The original idea of honeydew collection in oil was described by Banks & Macaulay (1964) who used mineral-oil. This was changed to using n-Hexadecane which is much lighter than water (density 0.77 g/ml). Honeydew droplets falling into the oil sank to the bottom and were collected with a glass capillary and frozen at $-20°C$.

EDTA extracts

Extracts were made by placing excised leaves in a reaction vial with 0.7 ml of water containing 8 mMol EDTA, 5 mMol $Na_2S_2O_5$ (anti-oxidant) and 5 mMol sodiumphosphate-buffer (pH 6). Leaves and vials were placed in a transparent plastic container (100% R.H.) in the greenhouse under continuous light. After 22 ± 2 h the leaves were removed and the solution pooled and frozen at $-20°C$.

Analyses

Sugars were analyzed using a sensitive sugar analyzer (Gruppen, Hoffmann, Kormelink, Voragen, Kamerling & Vliegenthart, 1992), concentrating on mono- and disaccharides. Total sugar content was determined by anthrone reagent (Handel, 1967). Amino acids were analyzed using an ion exchange amino acid analyzer. All amino acids could be analyzed in this way with the exception of proline. Proteins

were analyzed by SDS-PAGE on a 12% mini slab gels after precipitation by acetone (Joosten, 1991). Bands were silver stained. Secondary plant substances were separated on a RP-HPLC system with a 23 cm C_{18} column (Macherey-Nagel) and a Diode Array detector at 200-400 nm using a water/acetonitrile Gradient.

Results

Yield

Honeydew collection yielded samples of 5 - 20 μl per cage. EDTA extracts were about 0.5 ml per leaf. The sugar contents of the EDTA samples was 0.1 to 0.5%, depending on leaf size and age. Stylectomy samples could only be collected from Taiwan lettuce in very small ($< 1 \mu$l) samples. Stylectomy was successful but phloem sap flow stopped after 5-10 seconds on the other lines, yielding only a few nanoliters. Sample size was too small to do analyses. In order to compare samples all concentrations are given as percentage of the total amount.

Sugars

The sugar analyses showed mainly sucrose and some glucose and fructose in comparable amounts (Fig. 1a). Honeydew samples also contained traces of starch oligomers. No difference was observed for resistant versus susceptible lines. The Taiwan stylectomy sample contained only sucrose whereas the EDTA sample also showed fructose and glucose (Fig. 1b).
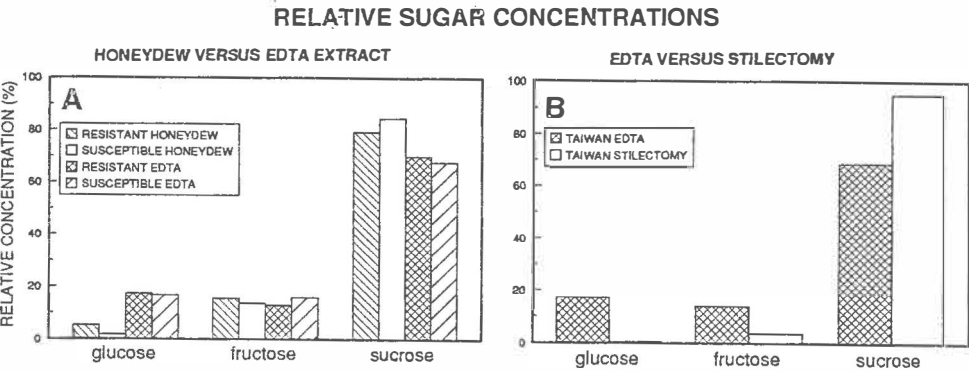
Fig. 1:   Relative sugar concentrations in samples. a. comparison of honeydew and EDTA samples. b. comparison of EDTA and stylectomy samples.

Amino Acids

Amino-acid analyses of stylectomy-collected phloem sap was not performed. Analyses of other samples showed glutamine as the most prevalent compound. No clear difference between resistant and susceptible lines was found for honeydew or EDTA samples (Fig. 2c,d). There were however clear differences between the relative amounts of aminoacids in the EDTA and honeydew samples (Fig. 2a,b). EDTA samples where higher in glutamine and alanine and lower in serine, they contained asparagine while honeydew did not and yielded no glycine, cysteine and Gamma isobuteric acid while honeydew did contain these compounds.

Proteins

No differences were found in protein composition between resistant and susceptible lines. Honeydew contained little protein and less different proteins than EDTA samples.

Secondary-plant compounds

The analyses of the samples by HPLC showed many peaks in the chromatogram. Several peaks differed considerably in size and ratio with different samples. However no consistent differences between lines or between sample collection methods were observed.

Discussion

Sugars

The results of the sugar analyses showed that in the phloem sap sucrose is the only important sugar as has been reported for most other plants. We think that the glucose and fructose found were sucrose breakdown products. No other sugars were detected in the honeydew of *M. persicae*.

Amino acids

Several authors have compared the amino acids in EDTA samples and honeydew with stylectomy samples (Rahbé, Delobel, Calatyud & Febvay, 1991; Weibull *et al.*, 1990). Rahbé *et al.* (1991) reported a closer resemblance between honeydew and stilectomy collected phloem sap than EDTA extracts and stylectomy samples. We found clear differences between EDTA and honeydew samples. Stylectomy samples were not analyzed. Amino acid concentrations in honeydew can differ because of the aphids "processing" the phloem sap (Sasaki *et al.*, 1990).

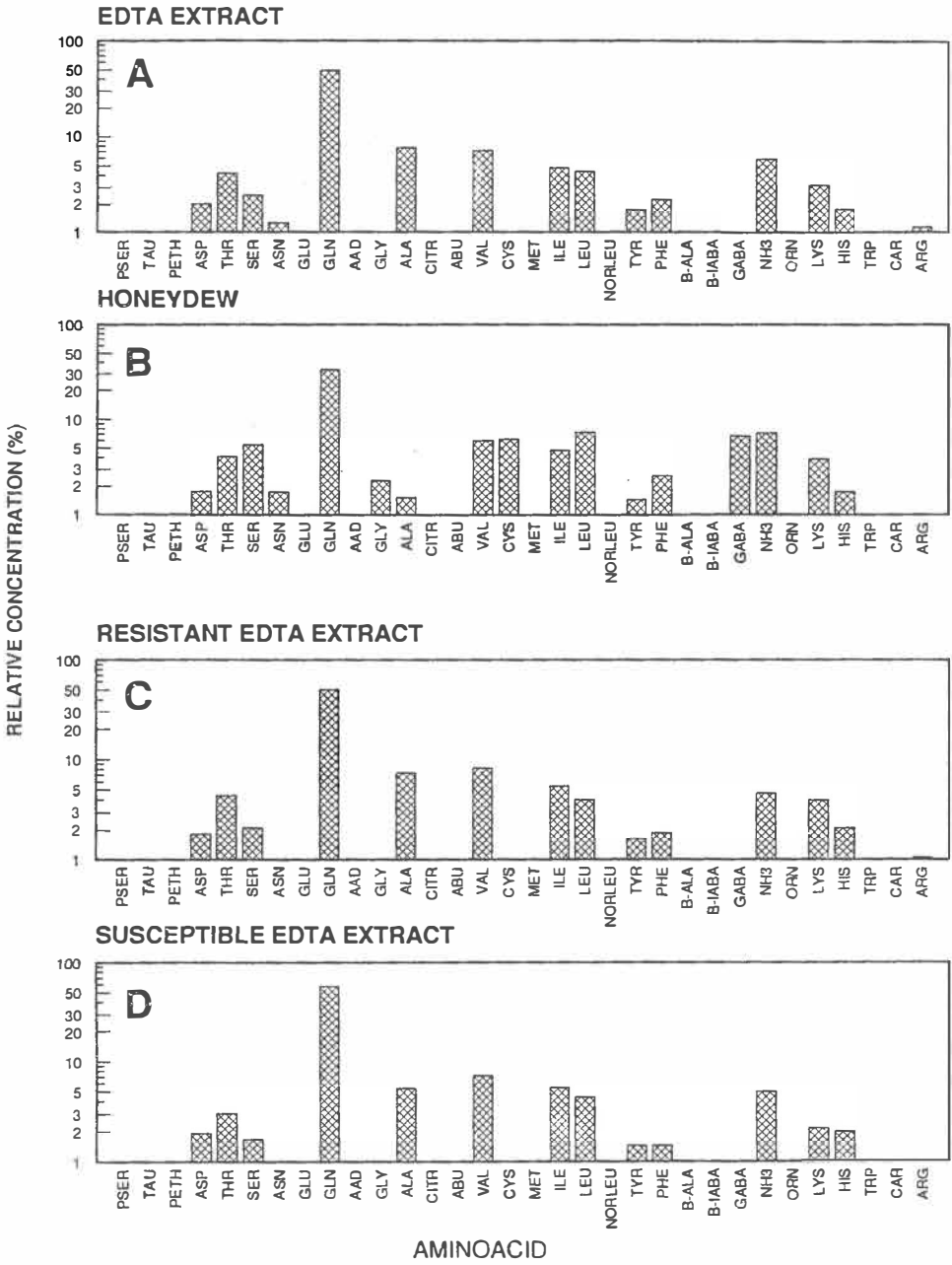# RELATIVE CONCENTRATIONS OF AMINOACIDS

Fig. 2:   Relative concentrations of aminoacids in samples. a. EDTA extract, b. Honeydew extract, c. EDTA extract from resistant line, d. EDTA extract from susceptible line.

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It is not clear why some amino acids (cysteine and glycine) and other compounds (GABA) occur in the honeydew and not in EDTA samples. It is possible that these amino acids are produced by the aphid or its symbionts. It is also possible that their concentration in the samples is too low to be detected in the EDTA samples. Some scarce amino acids like arginine can be used completely.

Proteins

No proteinase activity has been reported for aphids. Therefore honeydew was expected to contain the proteins taken up by the aphid with the phloem sap. There were several clear bands in the protein gel. However the EDTA extracts showed many more bands. We therefore suspect that the EDTA extracts also contain many non-phloem proteins.

Secondary Plant Substances

The detection method used is not able to detect all secondary plant substances. The chromatograms showed only the substances which are well separated with this method, show UV absorption and are present in amounts above the detection limit. The negative results of the analyses can not exclude the role of secondary plant substances.

Possibility of induced resistance

So far we have only analyzed honeydew from *M. persicae*, even though *N. ribisnigri* was also present on the other side of the leaf to cover the possibility of induced resistance. It is still possible that an induced defence reaction of the plant acts only very locally, so only against *N. ribisnigri* in one sieve element cell. Comparison of honeydew from different aphid species on the same lettuce line might give an indication. For the EDTA samples no effort was made to activate a possible induced resistance.

Reliability of different samples

Clear differences were detected between the different methods of sample collection. Unfortunately there was not enough material to analyze the phloem sap collected by stylectomy for all the components. The reliability of the EDTA samples is questionable. The effects of the EDTA on the formation of callose is clearly not the only effect. The leaf areas in the EDTA solution deteriorate during the collection and the leaves lose turgor possibly leading to contamination. Also the honeydew is not a good representation of the phloem sap since it has to pass through the aphid first. The reliability of each technique can be different for each collection method. For major components like amino-acids and sugars possible contaminations or changes are probably less important than for other minor components.

The resistance mechanism

The results of the analyses did not show any clear differences between resistant and susceptible lines. However this is no proof that the chemical composition of the phloem sap is not important. Analytical methods were limited and incomplete. It is unlikely that the absolute resistance against *Nasonovia ribisnigri* is based on minor differences in sugars or amino acids; secondary plant substances are better candidates. However an exhaustive study of the composition of the phloem sap will be very time consuming. Therefore a bioassay where phloem sap samples can be offered to the aphids is necessary to be able to continue this work in a sensible way. The incorporation of the samples into artificial diets has several problems like toxicity of compounds (EDTA), concentration of samples and sample size. With a working bioassay it will be easier to approach the origin of resistance. Stepwise separation and purification of the samples is then possible. A possible resistance mechanism based on mechanical blocking of sieve plate pores or aphid stylets can be excluded when differences in aphid behaviour are found using a bioassay based on extracts in an artificial diet.

Résumé

La base chimique de la résistance de la laitue à *Nasonovia ribisnigri* (*Aphididae*)

Des recherches sur la résistance monogénique totale de la laitue à *Nasonovia ribisnigri* ont été conduites, à l'aide d'enregistrements électriques de pénétration (EPG = Electrical Penetration Graph), d'études de comportement et d'analyses chimiques.

Nous avons tenté de trouver des différences entre des lignées de laitue isogéniques résistantes et sensibles. La seule différence de comportement marquée apparaît lorsqu'une cellule criblée du phloème est atteinte et que la nutrition commence (schéma E2). La différence peut être due aux variations de la composition chimique de la sève du phloème, ou au blocage mécanique de la cellule criblée ou des stylets du puceron durant la nutrition. L'analyse chimique du phloème a été entravée par des difficultés survenues lors des prélèvements par amputation du stylet. En remplacement, des exsudats de phloème et du miellat ont été utilisés, ce dernier étant prélevé chez *Myzus persicae*. Des échantillons de plusieurs provenances ont été analysés quant à leur teneur en sucre, en acides aminés, en substances secondaires des plantes et en protéines. Aucune différence chimique n'a encore été décelée. Une bonne méthode biologique sera nécessaire pour tester la performance des pucerons élevés sur des extraits de phloème provenant de plantes sensibles ou résistantes.

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EVALUATING THE RESISTANCE TO Frankliniella occidentalis IN CUCUMBER: METHODS, GENOTYPIC VARIATION AND EFFECTS UPON THRIPS BIOLOGY

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Summary

A new method for mass-rearing and production of synchronized stages of the western flower thrips (*Frankliniella occidentalis*) has been developed. In addition, an automatic, quick and accurate technique for the quantification of thrips damage has been devised. These two techniques were used for the inoculation and damage-rating of cucumber plants. The search for sources of resistance to thrips in cucumber was started with a screen of several hundred accessions. The accessions that met the criterion "less than 50 % damage in relation to the susceptible control" were re-evaluated. This test revealed accessions with partial levels of resistance (relative damage level 0.3 - 0.5 in relation to the susceptible control). Finally, the most resistant accessions were tested at a mature plant stage. This search revealed significant genotypic variation for resistance to thrips in cucumber. The levels of resistance of mature plants grown under conditions of normal practice in which the thrips were exposed for several generations, were greater than in the screening tests, which used young plants. In a separate investigation the effect of the resistance upon the life-history components of the thrips was studied. In this study it was shown that survival and reproduction was particularly affected.

Introduction

Since the early 1980s the western flower thrips (*Frankliniella occidentalis* (Pergande)) has been a major pest worldwide of many crops (Brødsgaard, 1989). The damage caused by this insect is direct (feeding punctures and scarring) and indirect (fruit malformation and transmission of Tomato Spotted Wilt Virus). The control of this polyphagous thrips species is difficult because it is very small and hides easily and because it has developed resistance to many pesticides. The problem could be solved by the introduction of resistant crop varieties. Therefore, CPRO-DLO started a project to develop a method of selecting thrips-resistant genotypes in cucumber.

First of all, the elementary biology of the thrips was studied on a susceptible cultivar (Mollema, Steenhuis & van Rijn, 1990). This investigation and contacts with other researchers led to knowledge about handling, mass-rearing, synchronization, ways of inoculation and evaluation of thrips damage. In addition, a method was developed of measuring life-history parameters of thrips. This method permits the analysis of effects of resistance upon individual thrips (e.g. survival, reproduction and development). The techniques for inoculation and evaluation of damage were tested by comparing cucumber genotypes differing in susceptibility to spider mites (Mollema, van der Hoeven, Steenhuis & Groot, 1989). In this experiment it was shown that cucumber lines selected for resistance to spider mites (De Ponti, 1979) are also resistant to western flower thrips. Since thrips is an insect with great adaptive potential, more knowledge about the genotypic variation of resistance to this insect in cucumber was desired. Therefore, a research project was aimed at (1) the detection of other sources of resistance; (2) whether such accessions retain resistance at the mature plant stage; (3) the relationship between thrips and spider mite resistance and (4) the effects of resistance upon thrips biology.

Methods

Mass-rearing and synchronization of thrips was done in glass jars containing small cucumber fruits and a few grains of pollen purchased commercially. The thrips thrive on the skin of the fruits. To obtain large numbers of young first instar larvae, the thrips were allowed to lay their eggs in new fruits for one day. After this period the fruits were brushed free of thrips and stored at 25°C for three days. On the third day after egg laying equal numbers of young larvae were deposited on the first true leaf of a cucumber seedling. Two weeks after inoculation the damage of the same leaf was evaluated.

Thrips damage was quantified automatically using an Image-Analysis device. This system (i.e. a video-camera, a personal computer and a software program) determined the number of spots, the total area and the percentage of damage per leaf fast and accurately. The level of resistance was expressed in relation to the susceptible control cultivar "Corona".

Survival and reproduction of individual thrips was determined on leaf discs deposited inside the wells of a tissue culture plate (Mollema et al., 1990). The leaf discs had a diameter of 1.5 cm and were stored at 25°C. In these experiments, one susceptible (CPRO-DLO inbred line "G6") and six resistant genotypes were used.

Spider mites were reared in a greenhouse on plants of the susceptible cultivar "Corona". Three weeks after sowing, six plants per genotype were inoculated with ten female spider mites each. Evaluation of damage by spider mites was done as described by De Ponti (1978).

<center>Results</center>

Sources of resistance

A selected group of cucumber accessions from the CPRO-DLO genebank was tested together with genotypes with known resistance to other insect pests. In total 350 accessions were screened using four replicates. The 60 accessions that met the criterion "less than 50% damage in comparison to the susceptible control cultivar "Corona" were evaluated a second time in 11 replicates. These experiments showed significant differences for resistance among the accessions.

The 12 accessions with the highest level of resistance (about 60 - 70% less damage than the susceptible control cultivar) were also tested at the mature plant stage in the greenhouse. This test (allowing the thrips to develop several generations before the final evaluation) showed even larger genotypic differences (Fig. 1).

Effects upon the biology of thrips

It was shown that survival and reproduction are negatively affected by the resistant cucumber accessions. On the resistant accessions, survival was less than 20 % and reproduction less than 50% of the level of the susceptible control (Table 1).

Table 1:   Reproduction of young western flower thrips females on seven cucumber genotypes

Cucumber genotype	Number of larvae per female in first four days	Percentage survival of larvae until emergence to adults
G6	6.77	56
9104	3.05	4
9127	2.84	4
9130	2.29	3
9140	3.19	3
9143	2.37	17
9153	3.12	13

Relation with resistance to spider mites

The accessions which showed the highest level of resistance in the screening for resistance to thrips were evaluated separately for resistance to spider mites. This experiment showed that the thrips-resistant accessions were also resistant to spider mites (Table 2).

<center>79</center>
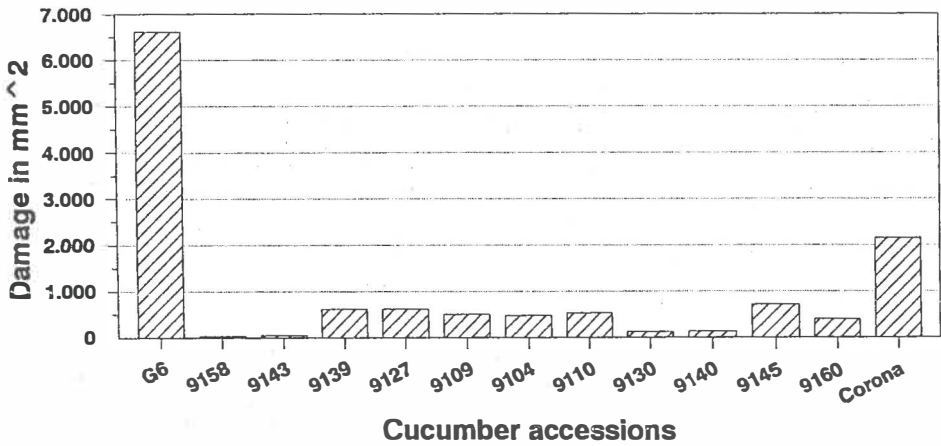
Fig. 1: Damage in mm^2 six weeks after inoculation. (Average of two plants per accession and four leaves per plant).

Table 2. Relation between genotypic resistance to western flower thrips and resistance to spider mite. Both evaluations were done under comparable greenhouse conditions. Average damage levels to four leaves per plant.

Cucumber genotype	Average damage by thrips six weeks after inoculation (in mm2 x 1000)	Average damage by spider mite four weeks after inoculation (0-5 scale)
G6	6.6	1.5
Corona	2.2	2.2
9104	0.6	0.0
9109	0.6	0.3
9110	0.6	0.2
9127	0.7	0.0
9130	0.1	0.1
9139	0.7	0.5
9140	0.1	0.3
9143	0.1	0.3
9145	0.8	0.7
9158	0.0	0.1
9160	0.4	1.1

Conclusions

The fairly high levels of resistance found in several cucumber accessions provide good prospects for future breeding programmes. What is particularly encouraging is that the resistance affects not only the survival, but also the reproduction of the thrips. An interesting phenomenon is the combined resistance to thrips and spider mites. A comparable situation of combined resistance to thrips and spider mite was found previously in cotton (Trichilo & Leigh, 1988). Considering the similar way of feeding of these two organisms, the same resistance mechanism may be effective to both of them.

Résumé

Evaluation de la résistance du concombre à *Frankliniella occidentalis*: méthodes, variation génotypique et effets sur le thrips

Une nouvelle méthode d'élevage de masse et de production synchronisée des stades du thrips de Californie (*Frankliniella occidentalis*) a été développée. En plus, une technique automatique, rapide et exacte, a été mise au point pour quantifier le dégât provoqué par les thrips. Ces deux procédés sont utilisés pour infester les plantes de concombre et pour évaluer les dégâts successifs. Pour déceler des sources de résistance au thrips chez le concombre, plusieurs centaines d'obtentions ont été passées en revue. Pour ce faire, quatre plantes par obtention ont été évaluées. Ultérieurement, le critère d'évaluation a été fixé à un niveau de dégâts observés d'au moins 50% plus faible que celui du témoin sensible. Cette évaluation, à 11 répétitions, a fait apparaître des résistances partielles ayant un niveau de dégâts de 0.4 à 0.7, alors qu'il était de 1.4 pour le témoin sensible. Les obtentions les plus résistantes ont finalement été examinées au stade phénologique de maturité. Cette recherche a révélé des variations génétiques significatives en ce qui concerne la résistance du concombre au thrips. Les niveaux de résistance sont même plus élevés chez les plantes matures en conditions de serre et qui ont supporté des thrips pendant plusieurs générations.

Lors d'autres expérimentations, l'effet de la résistance sur les composantes du cycle biologique du thrips a été étudié. Il en ressort que la survie et la reproduction sont particulièrement affectées.

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BREEDING TOMATOES FOR WHITEFLY-VECTOR RESISTANCE

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Summary

The tobacco or sweet potato whitefly, *Bemisia tabaci*, is the limiting factor in tomato production in sub-tropical and tropical countries mainly because of its ability to transmit the Tomato Yellow Leaf Curl Virus (TYLCV). Approximately 4 h of inoculation feeding are required to infect a tomato plant. Whitefly-resistant or so called vector-resistant cultivars reduce virus transmission significantly. In laboratory experiments a range of tomato breeding lines was tested by confining whiteflies in clip-cages on the underside of the leaves. Honeydew excreted by whiteflies was collected on a microscope slide, washed off, Anthrone was added and then the optical density of the liquid determined using a spectrophotometer. The quantity of total sugars in this honeydew was determined by reference to a calibration curve. In field experiments on a range of tomato breeding lines the rate of TYLCV infection of plants was used as a criterion to determine levels of vector resistance. A comparison was made between laboratory and field experiments to relate honeydew excretion to virus infection levels in the field. A good correlation was obtained between the two assessments. Using these laboratory and field techniques it was possible to identify resistance in *Lycopersicon pennellii*. Selections were made within a BC_1 family derived from a cross between a whitefly-susceptible *L. esculentum* and the resistant *L. pennellii*. An F_2 population has been bred from this material which will be used in further breeding work.

Introduction

The tobacco (sweetpotato) whitefly, *Bemisia tabaci* (Gennadius), is the limiting factor in tomato, *Lycopersicon esculentum* L., production in warm countries mainly because the pest transmits Tomato Yellow Leaf Curl Virus (TYLCV) (Anon., 1983; Duffus, 1987). This whitefly attacks outdoor as well as protected crops. In temperate regions it is a pest in glasshouses. About 4 h of feeding are sufficient to inoculate a healthy tomato plant with virus (Cohen & Nitzany, 1966). Since the virus cannot be

eliminated from infected plants, the only ways to prevent virus infections are by controlling the vector or by growing virus-resistant cultivars (Pilowsky & Cohen, 1974). However, virus-resistant cultivars are very rare. Whitefly-resistant, or so called vector-resistant cultivars, are capable of reducing virus transmission considerably even in virus-infectible crops if the time taken to affect the vector is shorter than the time needed for a viruliferous whitefly to inoculate a healthy plant. This hypothesis has been confirmed for whiteflies in cassava (Bellotti & Kawano, 1980), cotton (Jones, 1987), in *Lycopersicon pennellii* accessions, as well as in the F_2 hybrids of tomato x *L. pennellii* (Berlinger & Dahan, 1987) and for vectors other than *B. tabaci* in various crops (Cohen, 1982a; 1982b; Jones, 1987; Pathak, 1970; Thresh, 1983).

In this report we describe a rapid and reliable screening method for detecting vector resistance to *B. tabaci* in tomatoes. It is based on a high correlation ($r = 0.95$) between the amount of excreted honeydew and the incidence of TYLCV in inoculated plants (Berlinger *et al.*, 1990). It was also found that the threshold levels of honeydew varied with *Lycopersicon* species.

Materials and Methods

Screening methods

Laboratory experiments were conducted under controlled conditions. The amount of excreted honeydew was directly related to feeding activity and thus the probability of virus transmission; it was quantified by confining whitefly adults on the underside of a tomato leaf by means of a clip-on-cage (a modified Munger-cell). The plants were tested at the 4-5 true leaf stage. Total soluble sugars were determined by collecting the honeydew on a microscope glass cover slide, then washing it off using 1 ml of distilled water. 2 ml of Anthrone (0.2%) was then added. The optical density of this solution was recorded by a spectrophotometer at 620 nm. The absolute amount of the total sugars in the honeydew was then derived from a pre-prepared calibration curve (Berlinger *et al.*, 1990).

The rate of TYLCV infection of the plants was used as a criterion to determine levels of vector resistance. The plants were tested in the field during July-August, at the peak of whitefly occurrence and virus transmission. The proportion of infected plants were recorded weekly.

Results and Discussion

The first experiment was designed to verify the relationship between honeydew excretion in the laboratory and virus infection in the field. In this experiment F_2 hybrids of a tomato cv. x *L. pennellii* were tested, and compared with either of the two parents. Those hybrids in the laboratory on which the amount of excreted honeydew on the seedlings before planting was below the previously set threshold, did not become virus infected in the field. The resistant *L. pennellii* parent did not become infected either. However, on some of the hybrids and on the susceptible

tomato parent the amount of honeydew collected was above the threshold. All these plants became infected in the field. In a second experiment in which F_3 hybrids of the same parents as mentioned above were tested, the same pattern of result was found.

The method described above provided a means of selecting a whitefly resistant *L. pennellii* and thus justification for starting a breeding programme. Recently an F_2 population derived from a cross between a whitefly susceptible *Lycopersicon esculentum* and the whitefly resistant *L. pennellii* was screened in a field test for vector resistance. Most of the plants became severely infected by TYLCV. However, two plants were found to be highly resistant to the whitefly and were not infected by the virus. These two plants were selected and crossed with a susceptible tomato to produce the BC_2 generation for further selections.

Résumé

Sélection de la tomate pour sa résistance à l'aleurode du tabac, vecteur de virus

L'aleurode du tabac, *Bemisia tabaci*, est un facteur limitant dans les pays subtropicaux et tropicaux, principalement en raison de sa faculté de transmettre le virus de l'enroulement jaune de la tomate (TYLCV - Tomato Yellow Leaf Curl Virus). Environ 4 heures de nutrition d'inoculation sont nécessaires pour l'infection d'une plante de tomate. La résistance des cultivars à la mouche blanche (résistance au vecteur) réduit la transmission du virus d'une manière significative. Une série de lignées de tomate ont été examinées en laboratoire en maintenant des mouches blanches dans des cagettes fixées à la face inférieure des feuilles. Le miellat secrété par les mouches blanches a été capté sur un porte-objet, lavé et additionné d'anthrone. La densité optique a ensuite été déterminée par spectrophotométrie. La quantité de sucre total dans le miellat a été déduite en se référant à une courbe d'étalonnage. Le taux d'infection d'une série de lignées de tomate par le TYLCV a servi de critère d'évaluation du niveau de résistance au vecteur lors d'essais au champ. Une bonne corrélation entre l'évaluation en laboratoire, par l'analyse du miellat, et l'évaluation au champ par la détermination du niveau d'infection virale a été constatée. Par ces techniques, il a été possible d'identifier la résistance chez *Lycopersicon pennellii*. Des sélections ont été opérées dans une famille BC_1 dérivée d'un croisement entre *L. esculentum*, sensible, et *L. pennelli* résistant à la mouche blanche.

A partir de ce matériel, une population F_2 a été obtenue qui va être utilisée pour un travail ultérieur de sélection.

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INTERACTIONS BETWEEN RESISTANCE GENES IN RASPBERRY AND APHID BIOTYPES

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Summary

Plant breeders have been successfully using major genes for resistance to the virus vector aphid *Amphorophora idaei* for more than 40 years. Currently most of the UK raspberry hectarage is planted with cultivars possessing one or more of these genes, which is imposing a strong selection pressure for virulent aphid biotypes. In order to assess the risk of the breakdown of genetic resistance caused by aphid virulence a rapid diagnostic test for *A. idaei* biotypes is urgently needed. Progress is reported on the development of DNA probes which are being used to detect differences in RFLP patterns of aphid populations and clones of biotypes. Results from a two year survey of raspberry aphids from UK plantations indicate that virulent biotypes able to break the most widely deployed resistance gene (A_1) are now much more widespread than indicated by Briggs' original survey in 1965. Ongoing investigations into the mechanisms of aphid resistance are also discussed, in relation to leaf surface chemistry of plants containing resistance gene A_{10}, currently effective against all known *A. idaei* biotypes and now being used in the breeding programme to replace gene A_1.

Introduction

The large raspberry aphid, *Amphorophora idaei* (Börner), is currently the only natural vector of importance for four viruses commonly found infecting raspberry in Europe. More than 12 major genes for resistance to *A. idaei* are reported in *Rubus*, differing in their effectiveness against the four known biotypes of *A. idaei*. Because the major resistance gene A_1 has been widely used by plant breeders and growers for more than 40 years it is likely that there has been strong selection pressure on aphid populations for virulent *A. idaei* biotype 2, which is able to overcome this gene. Plant breeders have now introduced a stronger type of resistance, conferred by gene A_{10}, which is effective against all known biotypes of *A. idaei*.

To optimise the usefulness of the A_{10} resistance gene in aphid control strategies a deeper understanding of the mechanism is needed. In addition, breeders

and growers need to know which *A. idaei* biotypes are abundant, so that they can assess the risk of the breakdown of genetic resistance. Existing tests to identify *A. idaei* biotypes, based on differential colonisation of a range of resistant raspberry cvs, are too slow and laborious for monitoring large numbers of aphid samples from field surveys. A rapid diagnostic test for biotypes is therefore urgently needed.

Progress

Mechanisms of resistance - aphid settling behaviour

Work has continued to investigate the precise mechanisms of resistance, using the major gene for *A. idaei* resistance A_{10} in cv. Autumn Bliss. Previous experiments have shown that leaf surface chemicals are involved in the initial settling response of *A. idaei* on susceptible and resistant raspberry cultivars (Birch & Jones, 1988).

Further investigations of initial settling behaviour (20 minutes after placing on upper leaf surface) confirmed that apterous aphids on cv. A. Bliss (R) spent more time walking (43%) between upper and lower leaf surfaces (six surface changes) than on susceptible cv. Malling Jewel (15% walking, one surface change). Antennal ablation did not alter after settling behaviour on cv. M. Jewel (S) apart from increasing time to the first five minute probe, but markedly changed behaviour on cv. A. Bliss (R), so that aphids spent less time walking and settled sooner on the under surface of leaves. These results support those of previous olfactometer studies, suggesting that leaf volatiles are involved in short range host recognition and initial acceptance by *A. idaei* (Birch, Jones, Woodford & Jones, 1991).

The floating leaf bioassay was used to test the response of *A. idaei* to leaf surface wax components. When leaflets of cv. A. Bliss (R) were wiped with hexane to remove surface chemicals, bioassays showed that resistance to *A. idaei* was decreased by more than 40% compared to untreated leaves. When leaflets of cv. A. Bliss (R) were painted on one half with cellulose acetate dissolved in acetone and the resulting dry film peeled off, most aphids moved to the treated half of the leaflet (i.e. surface wax removed) within two hours (Jones, Birch, Griffiths, Robertson, McNichol & Hall, 1991).

Mechanisms of resistance - leaf surface chemistry

Progeny seedlings of an A. Bliss (R) x M. Jewel (S) cross, each characterised for resistance to biotypes 1 and 2 of *A. idaei*, were used to study leaf surface chemistry in more detail. Detached leaflets were briefly dipped in dichloromethane and the extracts concentrated by rotary evaporation. Following GC-MS analysis, 13 major peaks were identified. Discriminant analysis of the peak areas indicated that the resistance category of the 28 progeny seedlings could be estimated with 96% success (Robertson, Griffiths, Birch, Jones, McNicol & Hall, 1991). Behavioural bioassays on selected compounds from the three classes of dominant compounds (esters, tocopherols, amyrins) are currently in progress to see if they are directly involved in the resistance mechanism.

We are also currently using thermal desorption GC-MS of entrained leaf odours to identify plant volatiles from the resistant and susceptible progeny seedlings produced from the A. Bliss x M. Jewel cross. Preliminary results indicate that there are differences in the complex chemical profiles of leaf volatiles from the two sets of progeny plants, but the behaviourally-active components have not yet been identified.

Molecular probes of A. idaei *biotypes*

Currently in the UK more than 80% of the raspberry hectarage is planted with cultivars containing *A. idaei* resistance genes. This has inevitably led to selection of virulent biotypes of *A. idaei* in some locations, particularly against the most commercially used resistance gene A_1.

A survey to assess the changing abundance of *A. idaei* biotypes in relation to localised selection pressures have not been carried out in the UK for at least 20 years. At that time the three virulent biotypes of *A. idaei* were not abundant among UK populations (Briggs, 1965). We are now developing a rapid molecular diagnostic test using RFLP (Restriction Fragment Length Polymorphism) analysis, to distinguish biotypes of *A. idaei*. Initial attempts using an M13-derived DNA probe on digested aphid DNA samples revealed differences between clonal laboratory populations belonging to three different biotypes. Recent analysis of RFLPs using a ribosomal DNA probe showed that within each *A. idaei* clone all aphids were the same, but field populations sampled from raspberry cvs with different resistance genes tended to have distinct patterns. Our two year survey of UK *A. idaei* populations indicates that localised *A. idaei* populations often contain a mixture of biotypes (as identified by RFLP patterns) and that virulent biotype 2 is now widespread, due to adaptation to resistance gene A_1 (Birch, Fargette, Harrower, Malloch, Jones, Phillips & Catley, 1992). The apparent abundance of *A. idaei* populations with virulence to A_1 means that raspberry growers will become increasingly reliant on cvs containing gene A_{10}. It is hoped that durability of the A_{10} gene can be maximised by careful management of its use in future IPM strategies.

Résumé

Interactions entre des gènes de résistance chez le framboisier et les biotypes de pucerons

Les sélectionneurs ont du succès depuis plus de 40 ans en utilisant les principaux gènes de résistance au puceron *Amphorophora idaei*, vecteur de virus. A l'heure actuelle, la plus grande partie de la surface occupée par le framboisier en Grande Bretagne est plantée de cultivars contenant un ou plusieurs de ces gènes, ce qui exerce une forte pression de sélection pour des biotypes virulents de puceron. Afin d'évaluer le risque d'un échec de la résistance génétique dû à la virulence du puceron, une méthode de test rapide servant au diagnostic des biotypes d'*A. idaei* est d'une nécessité urgente.

Des sondes de DNA ont été développées et sont utilisées pour détecter les différences dans le schéma du RFLP des populations de puceron et des clones de

biotypes. Les résultats d'une évaluation des populations du puceron du framboisier en Grande Bretagne, durant deux ans, montrent que des biotypes virulents, capables de surmonter le gène de résistance le plus largement introduit (A_1), sont maintenant beaucoup plus répandus qu'en 1965, année d'un premier relevé effectué par Briggs. Les recherches actuelles sur les mécanismes de résistance contre le puceron sont également discutées. Elles sont en relation avec la chimie de la surface des feuilles des plantes pourvues du gène de résistance A_{10}, efficace contre tous les biotypes d'*A. idaei* connus, et qui est utilisé dans les programmes de sélection pour remplacer le gène A_1.

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DEVELOPMENT OF ROSY APHID <u>Dysaphis plantaginea</u> ON A TOLERANT APPLE CULTIVAR 'FLORINA'

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Summary

A study of the biology of rosy apple aphid, *Dysaphis plantaginea*, on the tolerant cultivar 'Florina' was carried out under controlled conditions and compared with 'Golden', a susceptible cultivar. Survival of larvae and instar duration, as well location of aphids on apex, leaves or stems of grafted potted plants were recorded. Survival and fecundity of apterous virginoparous females was tested for aphids reared on 'Golden' or 'Florina', and then transferred to the same or to the other cultivar. The results showed that there was a nutritional effect of 'Florina' on aphids which resulted in increased instar duration, lower survival rate and lower fecundity. Insect location on the plant was also affected, many aphids moving from the leaves to the stems of plants.

Introduction

The rosy apple aphid, *Dysaphis plantaginea* Pass., is a major pest in European orchards, causing severe leaf-rolls and long term crop losses on susceptible cultivars.

The biology of *D. plantaginea* has been described on different cider apples cultivars (Bonnemaison, 1959).

The fresh fruit cultivar 'Florina-Querina', from the INRA breeding programme, is resistant to scab and tolerant to mildew and fire blight (Lespinasse, Olivier, Lespinasse & Le Lezec, 1985). This cultivar is resistant to rosy aphid, as shown in studies carried out in orchards and in the glasshouse from 1984 to 1989 (Vénard, 1987; Marboutie, 1989; Rat-Morris, 1990). Both antibiosis and tolerance seem to be involved in this resistance, in contrast to the hypersensitivity of *Malus robusta* Mal 59 (Briggs, 1967; Alston & Briggs, 1970; Massonié, Maison, Meymérit & Lespinasse, 1981). Experiments on 'Florina' when compared with 'Golden' and hypersensitive cultivars have shown that virginoparous adults females depart from the plant 22 to 37 h after infestation, suggesting host acceptance problems (Bouey, 1990).

In order to maximise progress in this subject, we have made detailed studies of the biology of rosy applied aphid on 'Florina' under controlled conditions.

Materials and Methods

Plant material

'Golden' was chosen for aphid mass-rearing and as a susceptible control cultivar. 'Golden' and 'Florina' were grafted on 'Golden' seedlings and kept in pots under controlled conditions (photophase 16 h, 20 ± 1°C, 7000 to 8000 lux; scotophase 8 h, 18 ± 1°C). Only plants 50 - 80 cm high were used, that is with 8 to 20 fully-developed leaves. Leaves were quoted from apex to base: leaf 1 is the first leaf beneath the apex.

Excised leaves were used for the fecundity study. Leaves numbered 3 or 4 were collected. The petiole was inserted in a hemolyse tube filled with water, through a cotton mesh top. Each leaf in its tube was placed in a clear polystyrene box (15 x 10 x 2 cm) with its underside down. Tubes were positioned on a modelling clay support. Boxes were kept in the same conditions as grafted plants and leaves changed every 8-10 days.

Aphids

Dysaphis plantaginea was reared in the same conditions as described above, on grafted 'Golden', and raised from one fundatrix collected in the orchard that year. Every 10 days, young virginoparous adult females were allowed to lay for 24 h on new 'Golden' plants (mass-reared) or on 'Florina' for the different studies. As alate *D. plantaginea* all emigrate to plantain (Bonnemaison, 1959), migration of larvae and apterous adults from one test plant to another was prevented by standing the pots in water on a tray.

Results and Discussion

Survival of pre-adult aphids and instar duration

Five first instar aphids (L1) from the culture, aged less than 24 h, were placed on the upper side of leaf 1 of 10 'Florina' and 10 'Golden' plants, giving 50 in total per cultivar. Their development was observed every day for 10 days.

Survival (Fig. 1) was practically the same on the two cultivars, with most mortality during the first three days. As the first instar was about 0.7 mm, it was not possible to find every aphid on a plant, especially on 'Florina' which is more hairy than 'Golden'. So, for the settling period, it was hard to prove that mortality was higher on 'Florina'. There was no evidence of host acceptance problems, as larvae may move about on the plant.

Instar duration (Table 1) was longer on 'Florina' for each instar. Development time from L1 to adult was the same as found on the other susceptible cultivars (Bonnemaison, 1959): 10-11 days at 20°C. On 'Florina' after 11 days, only 30% of surviving aphids were adults, compared with 80% on 'Golden'. In this experiment,

the influence of 'Florina' on aphid development had more effect on instar duration and development time than on mortality.

Table 1: Development of *D. plantaginea* on 'Golden' and on 'Florina' over 11 days at 20°C in the laboratory in terms of the proportion of surviving aphids (5 L1 per plant, 10 plants per cultivar)

TIME (days)	0	1	2	3	4	5	6	7	8	9	10	11
% 'Golden'												
L1	100	43	11	10	0	0	0	0	0	0	0	0
L2	0	57	89	90	19	13	4	4	19	0	0	0
L3	0	0	0	0	81	52	48	28	11	7	0	0
A L4	0	0	0	0	0	35	48	64	70	56	32	13
W L4	0	0	0	0	0	0	0	4	0	7	4	0
A Ad.	0	0	0	0	0	0	0	0	0	30	60	79
W Ad.	0	0	0	0	0	0	0	0	0	0	4	8
% 'Florina'												
L1	100	72	44	32	4	0	0	0	0	0	0	0
L2	0	28	56	68	83	42	29	18	8	4	0	0
L3	0	0	0	0	13	58	67	55	50	42	35	19
A L4	0	0	0	0	0	0	4	27	38	46	39	38
W L4	0	0	0	0	0	0	0	0	4	8	9	10
A Ad.	0	0	0	0	0	0	0	0	0	0	17	33
W Ad.	0	0	0	0	0	0	0	0	0	0	0	0

(L = larvae, A = apterous, W = winged, Ad = adult virginoparous female)

Survival and location on the plant when transferred from 'Golden' to 'Florina' and vice versa

Transfer from 'Golden' to 'Florina', and 'Golden' to 'Golden' for control. The same techniques were used as described above. Survival of aphids was observed for 10 days, recording their location on apex, leaves or stems (Fig. 2). In this experiment, survival was greater on the control (Fig. 3) than in the previous assay, while it was similar for aphids transferred to 'Florina'. A higher proportion of aphids moved from leaves to stems on 'Florina' than they did on 'Golden'.

Transfer from 'Florina' to 'Golden', and 'Florina' to 'Florina'. Eleven first instar larvae were released on each plant, in order to provide sufficient aphids for a 10 day experiment. Survival and location were observed from 7 to 20 days, because of the extention of the development time (Figs 4 & 5). Aphid instars were recorded on the 13th, 17th and 20th days (Table 2).

The results showed that when the first generation feeds on 'Florina', the offspring have a high mortality rate, even when they are transferred to 'Golden'. The

results also showed that *D. plantaginea* was able to feed on 'Florina' for two generations (we have reared it in the laboratory for more than four generations).

On 'Golden', aphids were located only on the apex and leaves, while aphids stayed mainly on the stems on 'Florina'. On susceptible cultivars, *D. plantaginea* preferred to settle on the leaves (Forrest, 1987). Further studies are in progress to understand this behaviour. It is planned to investigate whether stylet penetration is easier or whether there are repellent compounds present in the leaves or attractants present in stems?

When aphids were transferred from 'Florina' to 'Golden', the larvae were still third instar after 10 days instead of adult as they were when transferred from 'Golden' to 'Golden' (Table 2). In transfers from 'Florina' to 'Florina', instar durations were longer than those spending the first generation on 'Florina'. The number of winged adults aphid was higher in the second generation on 'Florina', than when the aphids were transferred to 'Golden'. It is hard to compare these results with those obtained for the first generation on 'Florina' because the proportion of winged adults is not the same for all generations in the aphid culture.

Table 2:   Development of *D. plantaginea* when transferred from 'Florina' to 'Golden' or to 'Florina' over 20 days, at 20°C in the laboratory in terms of the proportion of surviving aphids (11 L1 per plant, 10 plant per cultivar)

TIME (days)	10	13	17	20
% Florina/Golden				
L1	0	0	0	0
L2	0	5	0	0
L3	100	40	6.5	0
A. L4	0	45	20	0
W. L4	0	10	6.5	0
A. Ad.	0	0	67	86.5
W. Ad.	0	0	0	13.5
Total	19	20	17	15
% Florina/Florina				
L1	37.5	0	0	0
L2	50	28.5	0	0
L3	12.5	21.5	0	0
A. L4	0	7	18	9
W. L4	0	43	27	0
A. Ad.	0	0	0	9
W. Ad.	0	0	55	82
Total	16	14	11	11

(L = larvae, A = apterous, W = winged, Ad = adult virginoparous female)

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Aphids (number)
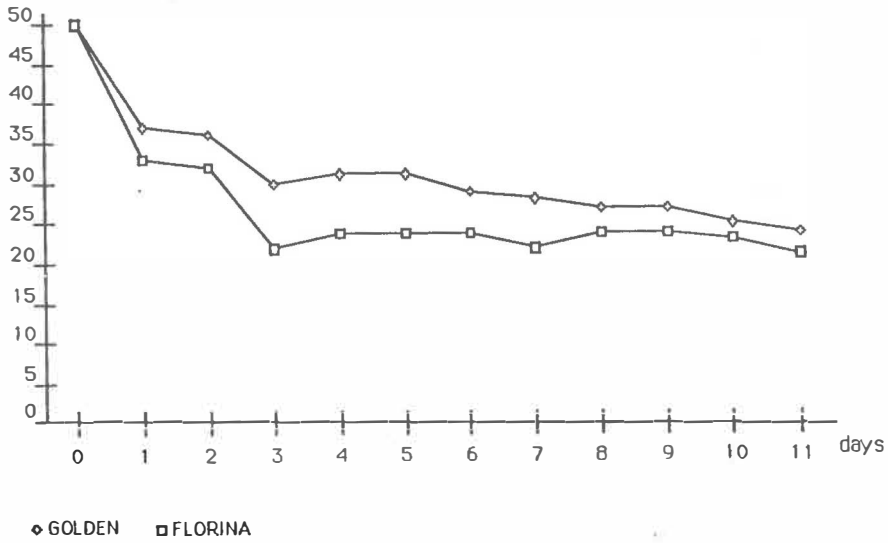
◇ GOLDEN   □ FLORINA

Fig. 1:   Numbers of *D. plantaginea* surviving on 'Golden' and on 'Florina', in relation to time at 20°C in laboratory (5 L1 per plant, 10 plant per cultivar).

Virginoparous female survival and fecundity

Excised leaves were used in these experiments to be certain of finding all offspring. Four combinations were tested, using young females released less than 24 h after the last moult (Table 3).

Table 3:   Total offspring of *D. plantaginea* when reared on 'Golden' or 'Florina', and then transferred to the same cultivar or to the other.

Pre-adult development	Adult life	leaves (nb)	Total offspring (mean)
Golden	Golden	30	50.67 ± 5.27
Golden	Florina	30	36.14 ± 3.71
Florina	Golden	20*	30.60 ± 4.63
Florina	Florina	30	28.34 ± 3.99

*   leaves came from trees with only 6 leaves, and therefore not as mature as in other treatments.

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New-born offspring were removed every day (Fig. 6). Survival was recorded for as long as possible (Fig. 7). Because of the poor quality of the leaves, survival of 'Florina'/'Golden' females decreased too much for the derivation of meaningful fecundity results to be obtained for this combination. Nevertheless, for the other combinations, there was a gradient in fecundity, as expected and in agreement with the hypothesis of an antibiosis effect of 'Florina' even when the complete pre-adult development took place on 'Golden'. When the females fed on 'Florina' during their adult life too, fecundity decreased still further. Results for the 'Florina'/'Golden' combination suggest that the effect of 'Florina' lasted when the females were put back on their susceptible host. Survival of adult virginoparous females on 'Florina' was higher than survival of larvae.

Conclusions

The biology of *D. plantaginea* on the 'Florina' apple cultivar is different from its biology on hypersensitive cultivars. Increased development time and instar duration, high mortality after the first generation on 'Florina' and decreased fecundity, all contribute to lower populations on this tolerant host. Studies of the biology under controlled conditions confirmed earlier observations in orchards and the glasshouse: both antibiosis and tolerance were identified as the basis of resistance of 'Florina' to rosy aphid. It is important to note that in orchards, apterous females are not likely to move from infested trees e.g. transfer from 'Golden' to 'Florina', and *D. plantaginea* populations cannot easily increase by means of exogenous aphids as they can for *Aphis pomi* de Geer. Behaviour under laboratory conditions, for example, movement from leaves to stems, must always be compared with behaviour in the field. This programme is to be continued and will investigate histological and biochemicals factors conferring resistance.

Résumé

Le développement du puceron cendré du pommier, *Dysaphis plantaginea*, sur la variété de pomme tolérante "Florina"

La biologie du puceron cendré du pommier, *Dysaphis plantaginea* Pass., a été étudiée sur la variété tolérante "Florina", en conditions contrôlées, et comparée à celle sur la variété sensible "Golden". La survie des larves et la durée des différents stades, ainsi que leur emplacement sur les feuilles apicales ou sur la tige des plantes greffées en pot sont rapportés. Sont examinées la survie et la fécondité des virginipares aptères obtenus à partir de pucerons, élevés sur "Golden" ou "Florina" et transférés sur la même variété ou sur l'autre.
Les résultats montrent qu'il y a un effet trophique sur "Florina" se traduisant par une durée plus longue des différents stades, ainsi qu'une baisse de la survie et de la fécondité. Quant à l'emplacement, on constate une migration des pucerons des feuilles vers la tige des plantes.

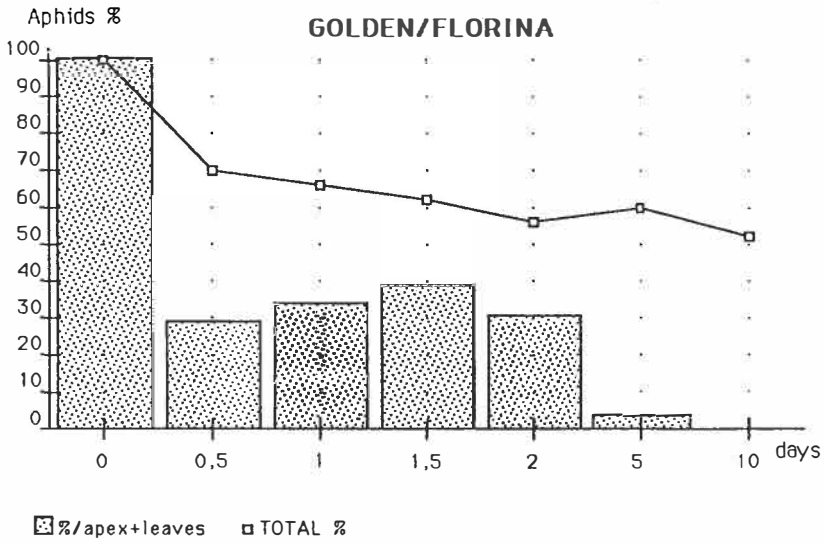Aphids %   GOLDEN/FLORINA

⊡ %/apex+leaves   □ TOTAL %

Fig. 2:   Location and surviving of *D. plantaginea* when transferred from a susceptible host 'Golden' to a tolerant host 'Florina' (5 L1 per plant, 10 plants per cultivar).

Aphids %   GOLDEN/GOLDEN
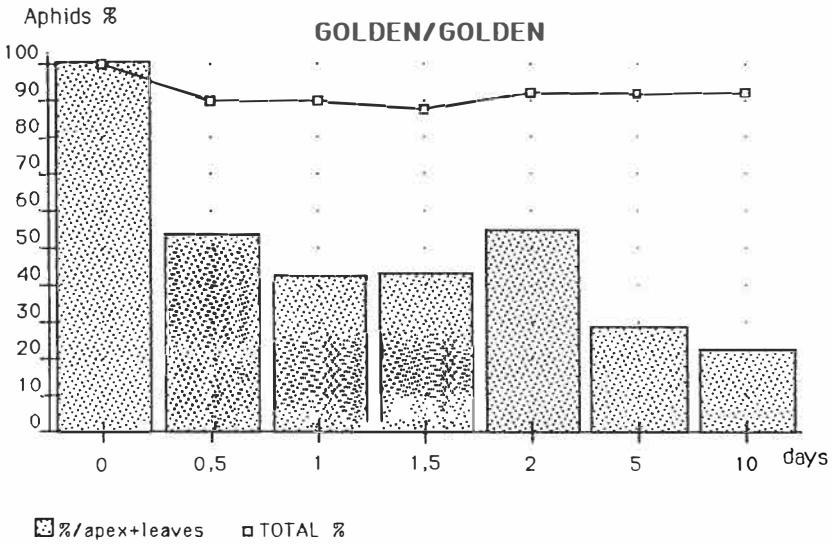
⊡ %/apex+leaves   □ TOTAL %

Fig. 3:   Location and surviving of *D. plantaginea* when transferred from 'Golden' to 'Golden' (5 L1 per plant, 10 plants per cultivar).
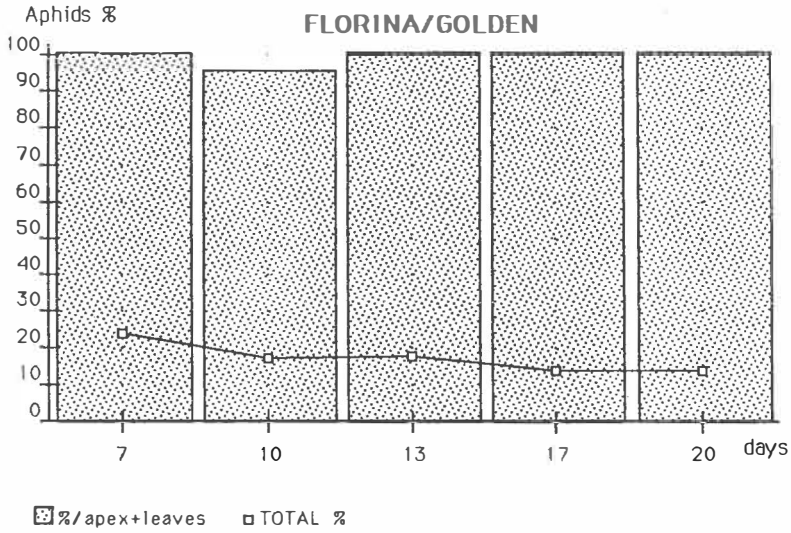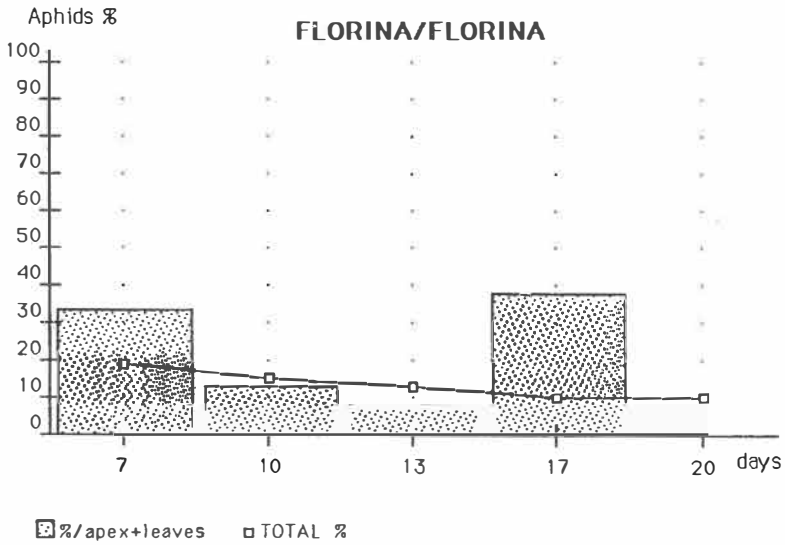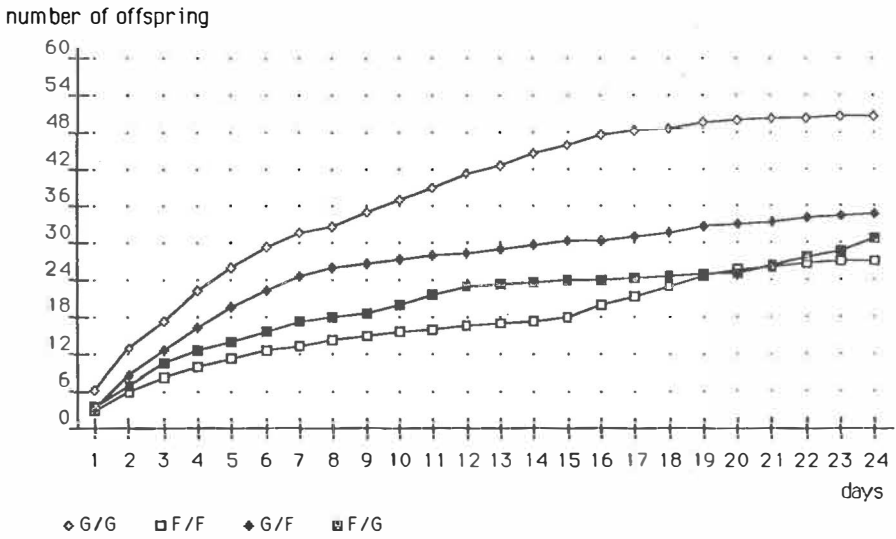
Fig. 4:   Location and surviving of *D. plantaginea* when transferred from 'Florina' to 'Golden' (11 L1 per plant, 10 plants per cultivar).

Fig. 5:   Location and surviving of *D. plantaginea* when transferred from 'Florina' to 'Florina' (11 L1 per plant, 10 plants per cultivar).

number of offspring

◇ G/G   □ F/F   ◆ G/F   ■ F/G

Fig. 6:   Fecundity of *D. plantaginea* when transferred from 'Golden' (G) to
           'Golden or 'Florina' (F) and vice versa, in relation to time, on excised
           leaves at 20°C (mean per day).

surviving aphids %

◇ G/G   □ F/F   ◆ G/F   ■ F/G
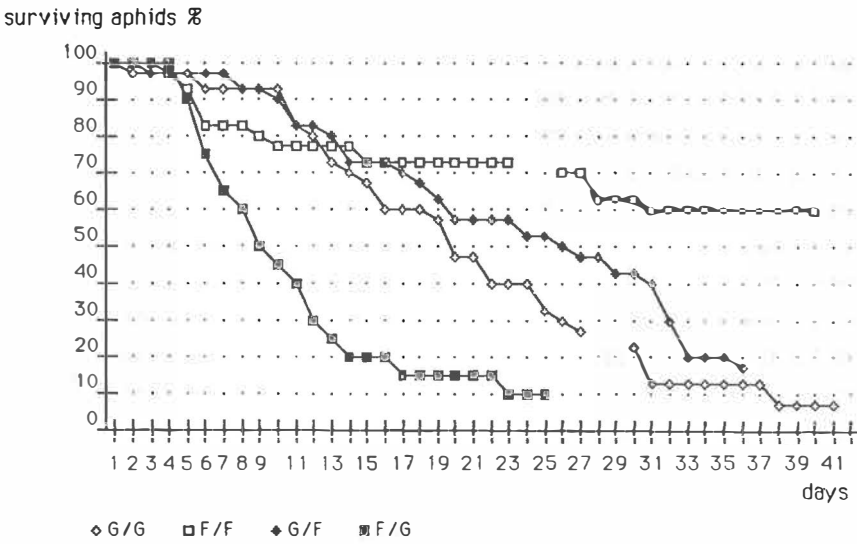
Fig. 7:   Surviving of *D. plantaginea* (apterous virginoparous females) when
           transferred from 'Golden' (G) to 'Golden' or 'Florina' (F) and vice
           versa, in relation to time, on excised leaves at 20°C.

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THE INTERACTION BETWEEN Liriomyza trifolii AND DIFFERENT CHRYSANTHEMUM CULTIVARS

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Summary

A detailed analysis of the impact of resistance to *Liriomyza trifolii* in chrysanthemum was made. Three different experiments were used to seperate non-preference effects in choice situations from oviposition and antibiosis to larval development. Non preference was found, as well as reduced oviposition. When comparing survival in the different larval stages, only the first and second larval stage were influenced by resistance. Since these different effects of resistant plant genotypes occur in different cultivars, resistance can be improved by combining factors which reduce oviposition with those which reduce larval survival.

Introduction

As a first step in breeding for resistance it is necessary to know whether resistance to the pest insect involved is a heritable character present within the genetic variation of the crop. This aspect of resistance has been studied extensively by several workers for chrysanthemum (*Dendranthema grandiflora* (Tzvelev.) and the serpentine leafminer fly *Liriomyza trifolii* (Burgess) (Webb & Smith, 1969; Alverson & Gorsuch, 1982; De Jong & van de Vrie, 1987). These studies focused on differences in overall measures of resistance such as the number of large (third instar) mines or pupae per plant in a choice situation. In this kind of experimental design it is difficult to tell apart the separate contributions of host plant effects on oviposition choices, adult mortality and larval survival. Of course the characters chosen were useful in the assessment of the potential for breeding leafminer-resistant chrysanthemums. There turned out great variation was discovered in existing commercial cultivars, and therefore the potential was clearly present.

To set up breeding programmes for insect resistance, however, needs more information than just the knowledge that genetic variation for the trait is present. It is important to know if different mechanisms exist and whether they can be combined to yield a stronger resistance. Therefore a programme was started to analyse these mechanisms. As a first step a detailed analysis of the interaction between the leafminer and different chrysanthemum cultivars was needed. In this paper the results of this first step will be discussed. Further steps in this programme will involve:

* the analysis of morphological and chemical plant properties influencing resistance,
* the assessment of the importance of environmental factors in the expression of these mechanisms, and
* an analysis of the genetical make up of these plant properties.

Resistance can be complete (no damage) or partial (some damage, but less than on a susceptible plant). It can be antixenotic (e.g. causing avoidance behaviour in the herbivore: this is also called non-preference) or antibiotic (causing death of adult or larval stages of the herbivore). Resistance can influence the adult stage of the herbivore, or the larval stage, or both.

In order to determine which types of resistance occur in this particular plant-insect interaction, three different experiments were set up. The first one was designed to measure non-preference, the second one to measure direct effects of plant quality on oviposition, and the third one to measure antibiosis to larval development.

Experiments

Non preference

Non-preference was measured by offering a mixed population of plant genotypes, with 30 genotypes each replicated ten times (for details see Van Dijk, De Jong, Van der Knaap & Van der Meijden, in prep.). The total number of mines was counted on each plant as a measure of the number of eggs laid. This is possible, because no significant differences in egg hatching exist between genotypes (as will be shown later). As is clear from the range in total numbers of mines in Fig. 1, the distribution of total number of mines over genotypes was far from random. Some genotypes were clearly avoided, while others were preferred. Interestingly, no significant relation existed between preference by the ovipositing female and subsequent larval survival (Fig. 1). The female does not always select the genotypes that are best for her progeny. Whether the non preference traits shown here are of any use to the breeder depends on the customers demands. It could be of use when a large variety of cultivars is grown in an intermixed system (which is the case in Great-Britain) but would be less profitable in systems growing one cultivar (as is quite common in Dutch glasshouses).

Oviposition in a no-choice situation

The direct effect of six genotypes on oviposition was measured in a no-choice experiment, using ten plants per genotype in two series. Four pairs of one day old flies were left together with a single plant for two days. Later, plants were harvested and eggs counted.
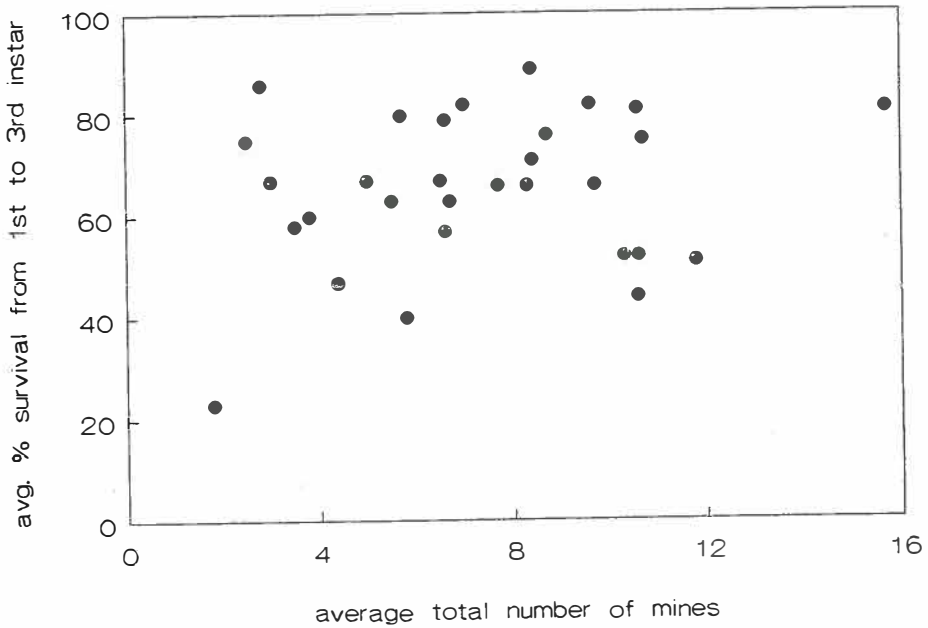
Fig. 1:   Relationship between total number of *L. trifolii* mines (preference) in a choice experiment with 30 chrysanthemum genotypes and larval survival from first to third instar. (r = 0.19, n.s.). (Pooled standard error of total number of mines is 1.7; F-ratio (ANOVA) = 3.588, significance level < 0.0001).

The results are given in Fig. 2. In the first series the differences in number of egss seem quite clear. Cultivar A is least suitable for oviposition while cultivar F is the most suitable one. However, in the second series the ranking order of cultivars changes. Therefore differences in oviposition show little consistency. The absolute difference between the two series is caused mainly by a difference in fly quality; in the first series 17% of the females were still alive after two days, while in the second series 51% were still alive. The second series in particular showed very little variation in fly survival between plant genotypes. The difference in oviposition between A and F found here can thus not be explained in terms of an antibiotic effect on the adult female. It must therefore be attributed to other factors, for instance, repellence or inferior food quality hindering egg development in the female fly. We can conclude that differences are far less clear in no-choice situations compared with choice situations. As will be shown in the next experiment, larval survival has a much more dramatic effect, and since this experiment shows little consistency, suitability for oviposition in no-choice situations seems a difficult and un-important trait for a breeder to work with.
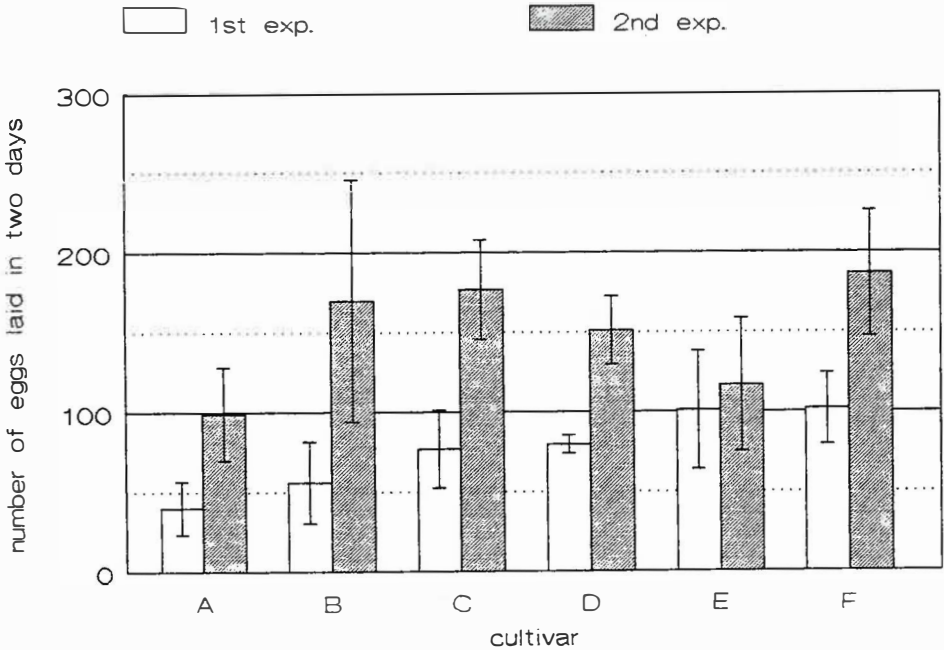
oviposition in a no choice situation

Fig. 2: Oviposition in a no-choice experiment with six genotypes. Averages and confidence limits. Two series each with five plants per genotype, tested one week apart. Adult female survival was on average 17% in the first and 51% in the second series.

Resistance and larval survival: antibiosis effects

Antibiosis to the larvae was measured using six other plant genotypes in a similar no-choice experiment, this time releasing two pairs of flies for a single day to avoid over-crowding and to synchronize development. First instar mines were allowed to develop on five plants per genotype. They were then harvested and examined for first instar larvae and unhatched eggs. Larvae were allowed to develop into third instar in another ten plants per genotype, after which plants were put in plastic bags to collect pupae. Numbers of larvae from each instar on each leaf were recorded daily.

As is shown by Fig. 3, large differences existed between the plant genotypes tested with respect to the proportion of eggs surviving to the pupa stage. As is shown by Figs. 4 to 6, these differences were not caused by differences in egg hatching, but mainly as a result of differences in survival in the first larval instar, with additional differences in survival in the second larval instar. Third instar larvae in all cultivars all survived to the pupa stage. In addition to this, as is shown in Fig. 7, the number of days required to reach the third larval instar was clearly increased in the genotypes showing reduced larval survival.

oviposition and pupa yield on six cvs.
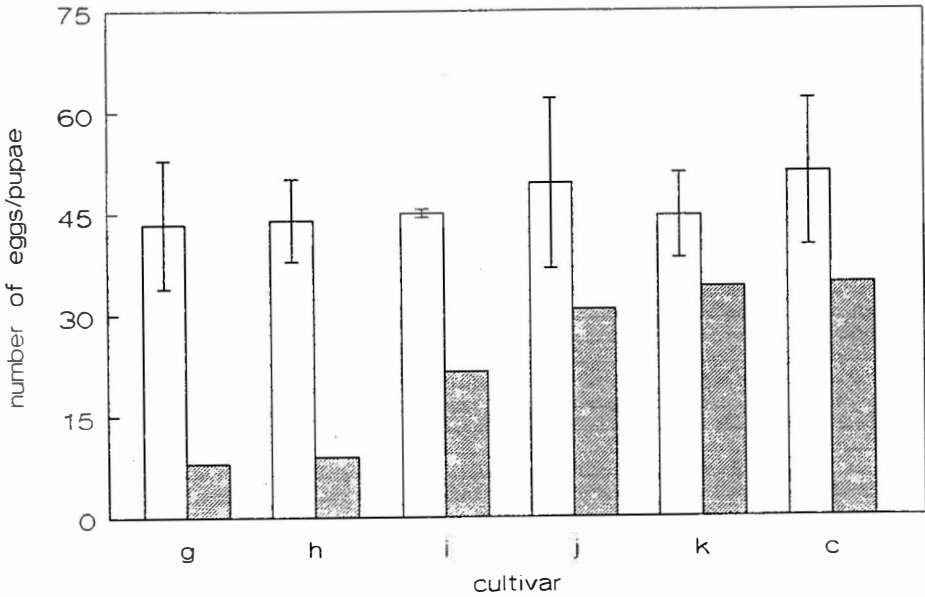
Fig. 3: Number of eggs laid (with s.e.) and number of pupae produced on six genotypes in a no-choice experiment.
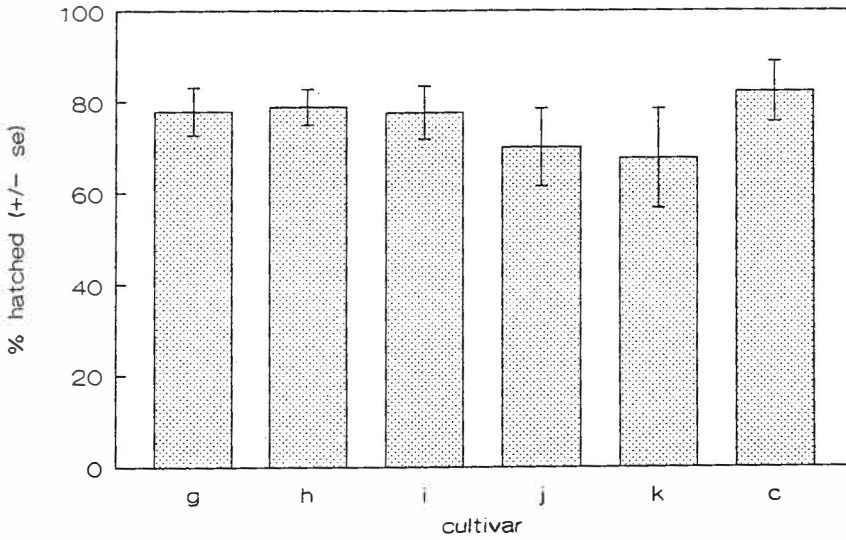
Fig. 4:   Survival in the egg hatching stage of *Liriomyza trifolii* on six
            chrysanthemum genotypes. Averages and standard errors.

survival in first instar on six cvs.
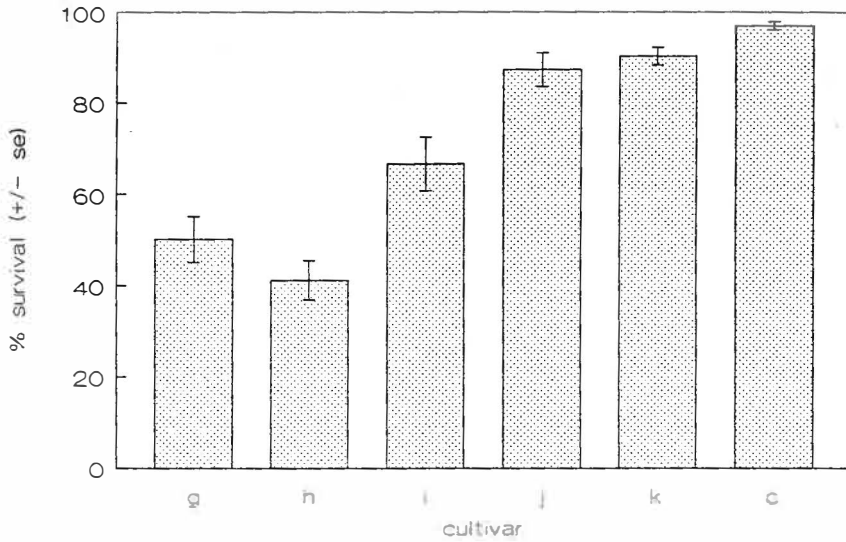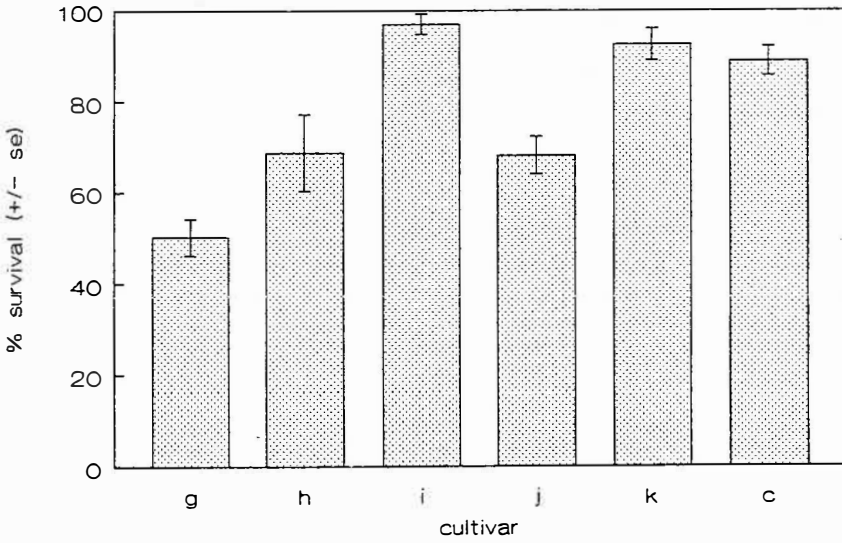

Fig. 5:   Survival in the first larval instar of *Liriomyza trifolii* on six
            chrysanthemum genotypes. Averages and standard errors.

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Fig. 6:   Survival in the second larval instar of *Liriomyza trifolii* on six
chrysanthemum genotypes. Averages and standard errors.

developmental time on six cultivars
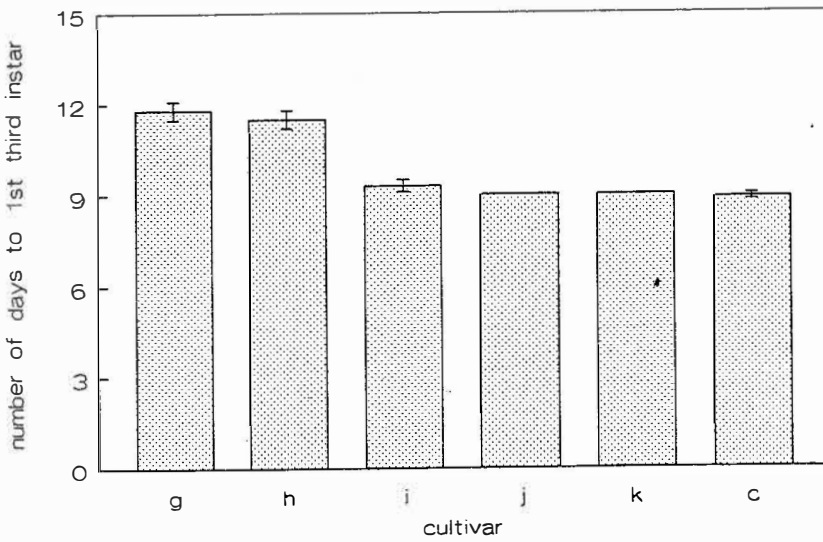
Fig. 7:   Average number (and s.e.) of days needed to reach the third instar for
larvae of *L. trifolii* on six chrysanthemum genotypes.

Conclusion

It is clear from these results that resistance operates at different developmental stages of the fly. The resistance affects fly preference, it affects the number of eggs laid in a no-choice experiment to some extent, and it drastically influences survival of first and second instar larvae and developmental time. As is already shown in Fig. 1, these effects are not always combined in one genotype. While some chrysanthemum cultivars are unattractive for oviposition, others reduce larval survival. Therefore it seems useful to try and combine the different plant properties causing these effects, in order to produce higher levels of resistance to *Liriomyza trifolii* in chrysanthemum. Since very little leafminer damage is acceptable in chrysanthemum, a type of resistance which greatly reduces oviposition and produces high first instar mortality would be the best combination to aim for in the future.

Résumé

La résistance du chrysanthème (*Dendranthema grandiflora*) à la mouche mineuse (*Liriomyza trifolii*)

Un programme de recherche de 5 ans a été mené sur l'interaction entre le chrysanthème et la mouche mineuse, les propriétés de la plante responsables de la résistance (partielle), l'interaction entre ces propriétés et les facteurs environnementaux, enfin leurs bases génétiques.
Les résultats rapportés ici se concentrent sur l'étude de l'interaction entre la mouche mineuse et divers cultivars de chrysanthème. On constate des variations dans la ponte, dans la survie des stades larvaires et dans le temps de développement.
Une variation génétique des chrysanthèmes est démontrée pour plusieurs aspects des performances de l'insecte. Les génotypes réduisant la ponte ne sont pas forcément les mêmes que ceux aboutissant à une faible survie larvaire. Des croisements devront être pratiqués pour combiner les qualités de résistance des divers cultivars de chrysanthème.

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THRIPS (Frankliniella occidentalis (PERGANDE)) RESISTANCE IN CHRYSANTHEMUM; THE IMPORTANCE OF POLLEN AS NUTRITION

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Summary

The resistance of five chrysanthemum cultivars to western flower thrips, *Frankliniella occidentales* was investigated in the laboratory. Both cultivars with and without flowers differed significantly in their resistance to thrips. Twenty to 60 times as many thrips were found on plants with flowers compared to plants without flowers. Five additional experiments were done to determine the importance of chrysanthemum pollen as food for thrips. In two experiments the thrips were confined to a small area on chrysanthemum petals. A significant, positive effect of pollen on fecundity and larval growth was found. The addition of chrysanthemum pollen to petals improved the nutritive value for *F. occidentalis*. However, when whole flowers were used in three different experiments no pollen-effect was found. It is suggested that in whole flowers thrips feeds preferentially on another substance with a high nutritive value such as young female reproductive tissue or nectar. In addition, differences in resistance to thrips between flowering chrysanthemum cultivars was not caused by variation in pollen production.

Introduction

The use of pesticides for crop protection is a major threat to the environment. One possible way of reducing pesticide use is to breed pest-resistant cultivars. Chrysanthemum is an important ornamental crop in the dutch economy but its production is threatened by the thrips species *Frankliniella occidentalis* (Pergande) which is a major pest on this and other crops. A project has been initiated at Leiden to develop a biotechnological test to scan chrysanthemum (*Dendranthema grandiflora* (Tzvelev.) seedlings for resistance to pests such as *F. occidentalis* (Pergande) and *Liriomyza trifolii* (Burgess). In order to develop a resistance test it is necessary for the mechanisms of resistance to be studied in detail.

In this paper we describe experiments designed to investigate the resistance to thrips of different chrysanthemum cultivars with and without flowers, the importance of pollen in thrips nutrition and the effect of pollen production on thrips resistance.

Materials and methods

Plant and insect source

Six chrysanthemum cultivars (FR17, CR15, FR05, FR07, CR14, V01) which were thought to vary in resistance were selected. These cultivars are referred to as cv 1 to cv 6. Cuttings of these cultivars were grown in a growth room which was programmed for 20°C, 70% RH, and a 8L:16D photoperiod.

A thrips strain that had been reared for several months on the flowers of the chrysanthemum cultivar FR16 was used for all experiments. The thrips rearing conditions were 20°C day and night temperature, 70% RH and a 12L:12D photoperiod. The experimental conditions were similar to the thrips rearing conditions.

Thrips resistance of chrysanthemum cultivars with and without flowers

Five chrysanthemum cultivars were used to measure thrips resistance (cv 1 to cv 5) which was recorded in terms of population growth. Ten equally sized plants with and without flowers of each cultivar were put in a whole-plant cage into which twenty adult female thrips were released. After four weeks the thrips were counted. Data were analysed by ANOVA and the means separated by the Duncan's multiple range test.

Pollen in thrips nutrition and the role of pollen in thrips resistance

Five experiments were conducted to study the importance of pollen as food for thrips and the role of pollen in thrips resistance. Data were analysed with a paired t-test.

In the first and second experiment the value of pollen as food for thrips was investigated. Pollen obtained from cultivar 4 was added to the petals of 6 chrysanthemum cultivars (cv 1 to cv 6). Two petals were used, 1 with and 1 without pollen from each of 18 plants (exp. 1) or 24 plants (exp. 2) per cultivar. In the first experiment one first instar larva was confined to a petal with or without pollen (Fig. 1). The growth of the larvae was determined by measuring their length on the first and fourth day. In the second experiment one adult female was confined to a petal with or without pollen (Fig. 1). Fecundity was measured by counting the number of eggs the females laid within 24 hours.

In the third, fourth and fifth experiments the role of pollen in thrips resistance was determined. In these experiments thrips resistance of whole, chrysanthemum flowers treated in different ways was measured by putting the flowers with seven adult female thrips in pots. After 6 days the numbers of larvae were counted. In this case fecundity and egg survival were taken as measure of resistance.
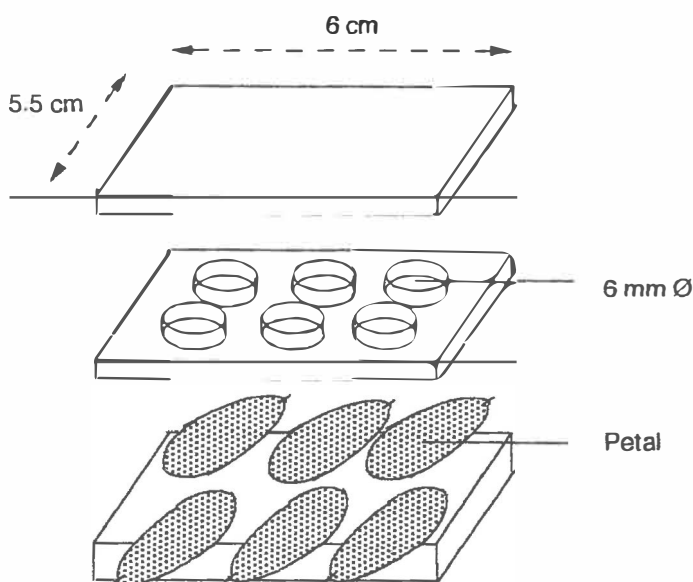
Fig. 1:   Thrips are confined to chrysanthemum petals with or without pollen. The plexiglas plates are held together with binder clips.

In experiment 3, pollen obtained from cultivar 4 was added to whole chrysanthemum flowers of four different cultivars (cv 1 to cv 3 and cv 6). Two flowers were tested for resistance, 1 with and 1 without extra pollen from each of 20 plants per cultivar.

In experiment 4, 13 plants of cultivar 2 were used. The anthers were cut away from 2 flowers on each plant. The anthers were then removed from one flower and the anthers left on the other flower. The flowers were then tested for thrips resistance.

In experiment 5 the pollen production per flower was determined for all cultivars (cv 1 to cv 6) using twenty plants per cultivar. The pollen production of a single flower on a plant was compared with the thrips resistance of another flower of the same plant. A Spearman's rank correlation test was used to determine the correlation between pollen production and thrips resistance.

Results

Thrips resistance of chrysanthemum cultivars with and without flowers

The five chrysanthemum cultivars, both with and without flowers differed significantly in their resistance to thrips. In addition, 20 to 60 times as many thrips were found on plants with flowers compared to plants without flowers (Fig. 2).

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When thrips were confined to chrysanthemum petals, a significant positive effect of pollen was found on larval growth (Fig. 3a) and fecundity (Fig. 3b). The level of the pollen effect was different for each chrysanthemum cultivar.
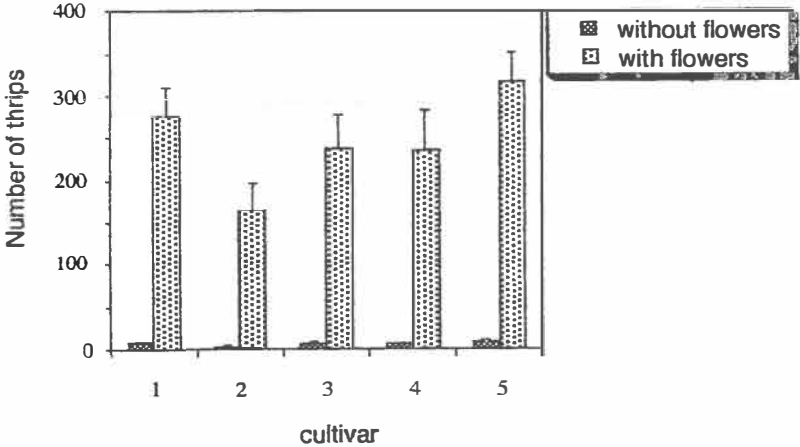

Fig. 2:　　　Thrips resistance in chrysanthemum cultivars with and without flowers.

No effect of the addition or removal of pollen was found in the experiment with whole flowers (Fig. 4a and 4b). In addition no correlation was found between pollen production and thrips resistance for any cultivar (r_s = -0.486, p = 0.28) (Fig. 4c).

The high numbers of larvae found in whole flowers of cultivar 6 (exp. 3 and exp. 5) were not explained by the numbers of eggs which females laid in petals (exp. 2).

Discussion

Differences in thrips resistance between chrysanthemum cultivars were found for both plants with and without flowers. Plants without flowers proved to be far more resistant than plants with flowers. Therefore it is important to know which factor determines thrips resistance in plants with flowers.

In the literature it is suggested that the higher numbers of thrips in chrysanthemum flowers are a result of pollen being present (Oetting, 1991; Teulon & Penman, 1991; Trichilo & Leigh, 1988). Therefore it could be possible that the differences in thrips resistance between the flowering cultivars were caused by

variation in pollen production. In the first and second pollen experiments pollen proved to be a good food for thrips. However, there was no correlation found between the pollen production per flower and the thrips resistance. A possible explanation could be that the quality of the pollen also played a role. However, when pollen of good quality was added to whole chrysanthemum flowers in experiment 3, no effect was found. There was no effect of pollen removal either. Therefore we suggest that in whole chrysanthemum flowers thrips feeds preferentially on another substance with a high nutritive value, such as young female reproductive tissue or nectar.

In order to prevent thrips damage in chrysanthemum growing, breeders may resort to isolating plants with flowers and using resistant cultivars. These resistant cultivars will not necessarily be the ones with low pollen production.
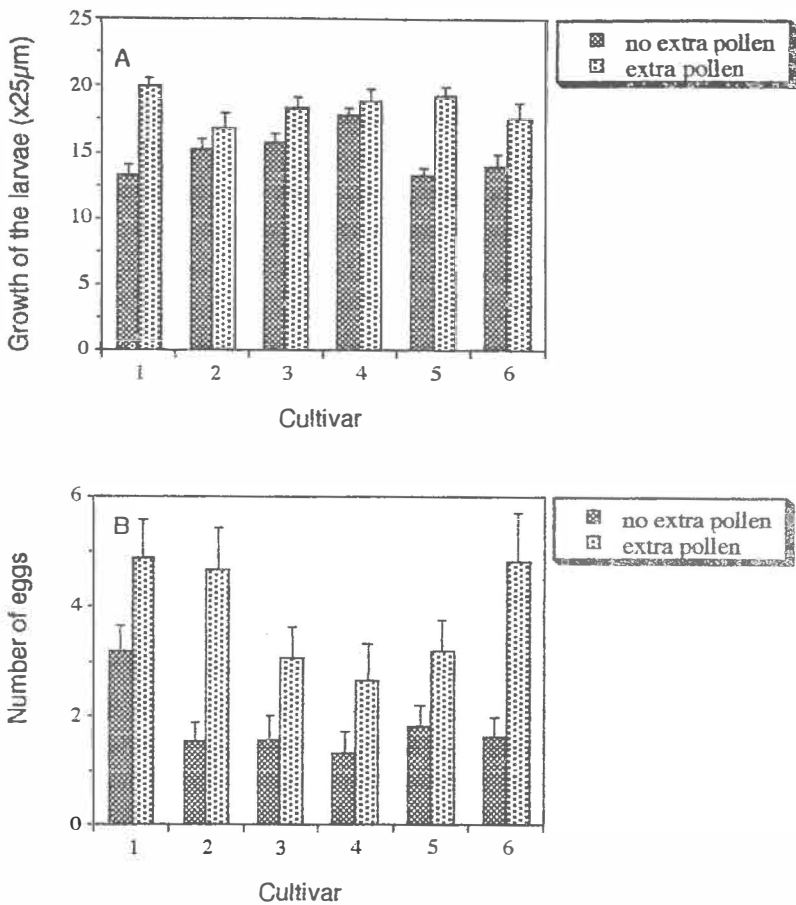

Fig. 3: The influence of pollen addition to chrysanthemum petals on larval growth (A) and fecundity (B).
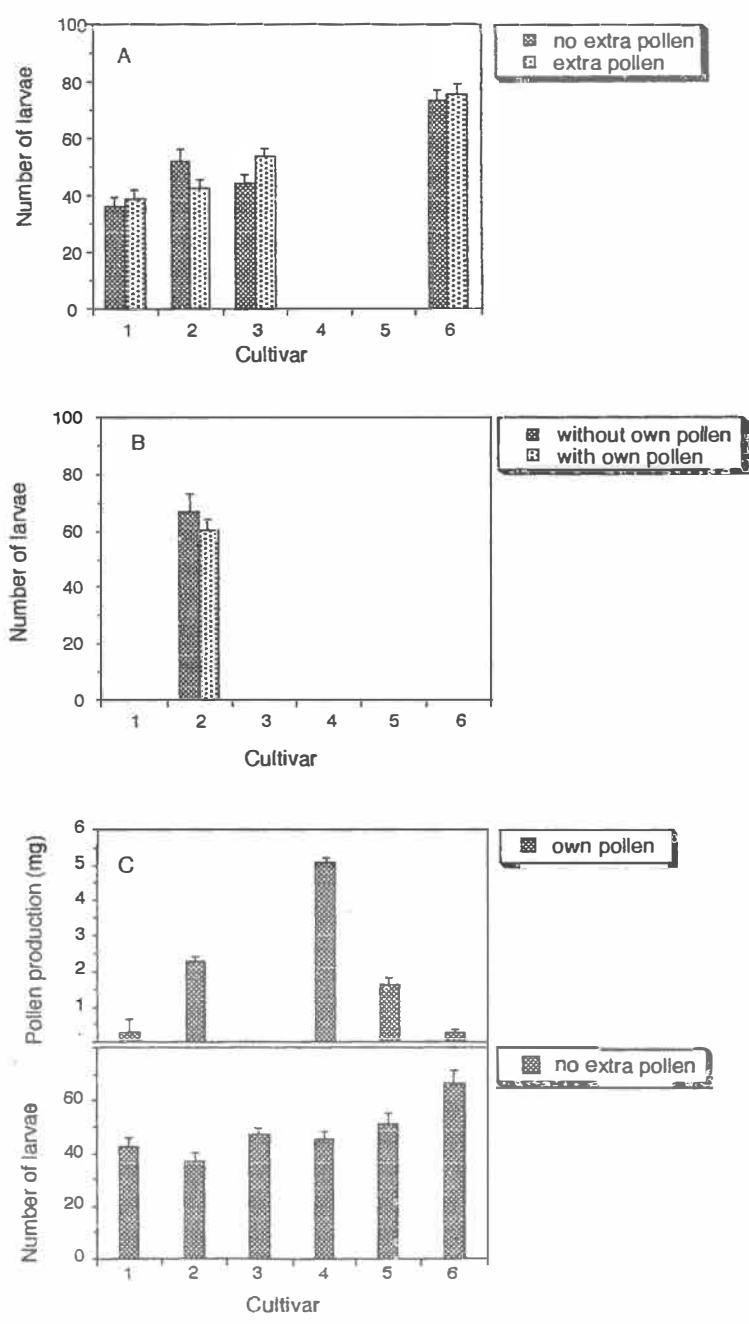
Fig. 4: Number of larvae in whole chrysanthemum flowers with and without extra pollen (A), in flowers with and without anthers (B) and compared to own pollen production per flower (C).

Résumé

La résistance du chrysanthème au thrips (*Frankliniella occidentalis*); l'importance du pollen servant de nutrition

L'utilisation de pesticides pour la protection des plantes est une des menaces les plus importantes pour l'environnement. Une possibilité de réduire leur emploi passe par la sélection de variétés résistantes aux ravageurs. Un projet a débuté, destiné à développer une méthode biotechnologique permettant l'examen de la résistance des plantes de chrysanthème à *Frankliniella occidentalis* et *Liriomyza trifolii*. Pour ce faire, les mécanismes de résistance ont été étudiés en détail.
Lors d'une expérience préliminaire, la résistance au thrips de cinq cultivars de chrysanthème, avec et sans fleurs, a été évaluée. Les plantes avec fleurs hébergent 20 à 60 fois plus de thrips que celles sans fleurs. Selon la littérature, ceci serait dû à la présence de pollen. En conséquence, il est possible que les différences de production de pollen soient à l'origine des différents niveaux de résistance chez ces cvs.
Cinq essais ont ensuite été menés pour connaître l'importance du pollen dans la nutrition des thrips. A cet effet, les thrips ont été confinés sur des portions de ligules de chrysanthèmes. L'adjonction de pollen de chrysanthème a montré un effet significatif sur la fécondité et la croissance larvaire en améliorant la valeur nutritive des pétales pour les thrips. Néanmoins, lorsque des fleurs entières sont utilisées, aucun effet dû au pollen n'est constaté. Dans ce cas, les thrips se nourrissent de préférence d'autres parties hautement nutritives, telles que les jeunes tissus reproducteurs femelles ou le nectar. De plus, les différences de résistance au thrips chez les cvs. de chrysanthème en fleurs ne sont pas corrélées à la variation de la production de pollen.

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THE RESISTANCE OF DIFFERENT LINES OF <u>Triticum</u> SPECIES TO THE APHID <u>Sitobion avenae</u>

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Summary

Screening experiments have been undertaken under controlled conditions to find new sources of antibiosis resistance to *Sitobion avenae*, within diploid *Triticum* material. Values of the intrinsic rate of increase (rm) of the aphid on each cultivar were used as indices of resistance. All 88 lines tested revealed a relative resistance compared to a susceptible hexaploid line but only 17 showed a high level of resistance. The stability of different levels of resistance found in four *T. monococcum* genotypes has been tested over a period of one year after continuous rearing of the aphid on each genotype : no erosion of resistance was observed. The genetic variation of *S. avenae*, represented by four different clones of the aphid has been confronted with the same *Triticum* resistant lines. An inter-clonal variability in the aphid response to resistant wheats has been revealed.

Introduction

Sources of antibiosis to the English grain aphid, *Sitobion avenae* F., have been found in hexaploid wheat genotypes (Lowe, 1984; Lee, 1984; Di Pietro & Dedryver, 1986), but these were not high levels of resistance. Nevertheless, partial resistance can be helpful in reducing the frequency of aphid outbreaks and consequently the number of insecticide applications in programmes of cereal protection (Acreman, 1984).

High resistance levels are, of course, more valuable for incorporation in cereal breeding programmes. Such high levels have been observed by Sotherton & Van Emden (1982) and Spiller & Llewellyn (1986) for the three most important cereal aphids in ancient wheat varieties, especially in *T. monococcum* (L.). Our own preliminary experiments have concentrated on resistance to *S. avenae* in diploid *Triticum* material. Three lines of *T. monococcum* have been selected for their high level of antibiosis against this aphid and one for intermediate level, for comparison with a susceptible hexaploid cultivar Arminda in further investigations.

This paper presents the assessments of antibiosis resistance to *S. avenae* found after screening 88 genotypes belonging to three diploid species : *T. monococcum* (L.), *T. baroticum* (Boiss.), *T. urartu* (Tum.).

The other aim of this work was to test the stability of the resistance discovered. Experiments were carried out on the four *T. monococcum* lines first selected, to evaluate the durability of resistance against stocks of a *S. avenae* standard clone maintained on each line.

The resistance of these lines was tested against aphid genetic variability using three other *S. avenae* clones collected in the Rennes basin.

Materials and methods

Seeds of the 88 available lines of diploid *Triticum* species and of the susceptible hexaploid culitvar Arminda were obtained from the INRA breeding station at Le Rheu.

Unvernalised seedling plants (2-leaf stage) were grown and all experiments performed at 20 ± 1°C and 16 h photoperiod. Experimental aphids were taken from stocks reared under the same conditions on Arminda except for the stability experiments where the aphids were taken from a stock maintained on each *Triticum* line tested.

The intrinsic rate of natural increase (rm) (Birch, 1948) was used as a measure of the level of resistance of each line tested. For its evalutation, five newly-born aphids were caged on plants : nymph survival and pre-reproductive development time were recorded. Subsequent fecundity values for the apterous adults which were obtained from and replaced on new seedling plants, were recorded for eight days.

Some cultivars display a chlorotic reaction after aphid feeding. The presence of these chlorotic spots was noted.
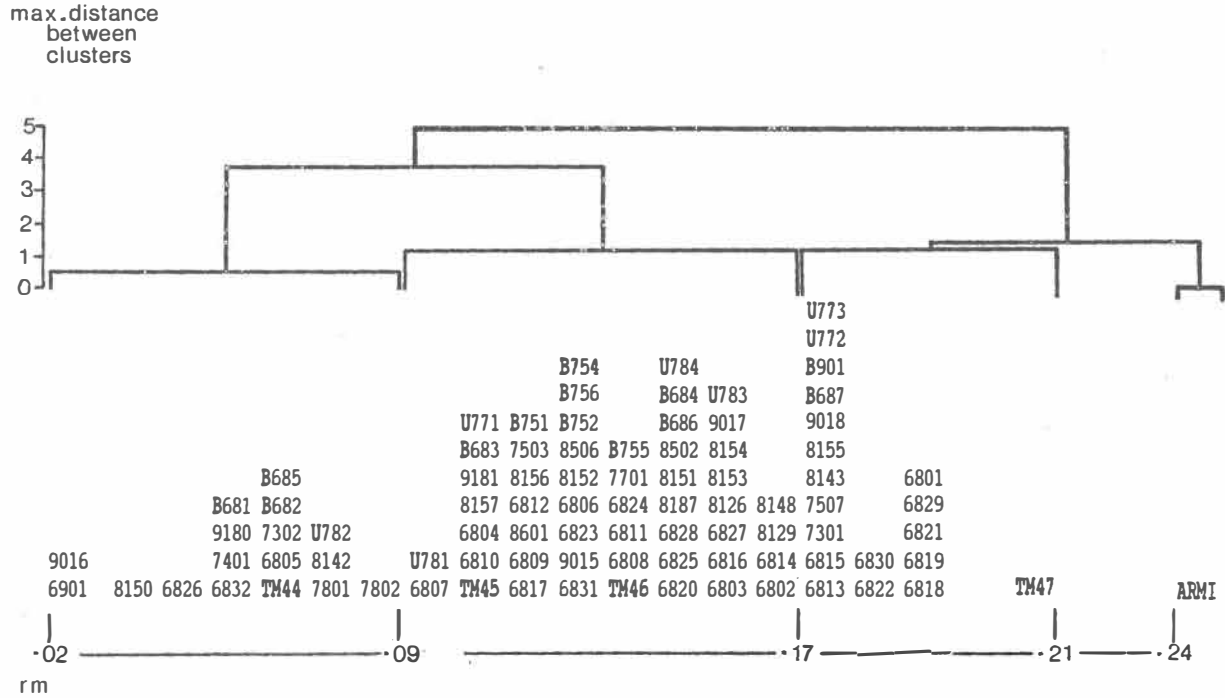
Results

Screening for new resistant lines in diploid Triticum *species*

Values for rm, expressed for the aphid on the different diploid lines were compared with those obtained on the susceptible Arminda wheat (rm = 0.24 ± 0.03) and on the Tm44 resistant line (rm = 0.06 ± 0.12). Values for the 87 diploid lines ranged from 0.02 to 0.21. The lowest values of rm indicated the most resistant cultivars and vice versa. A complete Linkage Cluster Analysis (SAS Institute, 1985) distinguished four groups of rm values (Fig. 1).

The hexaploid line, Arminda, was more susceptible than any of the diploid lines. A group of 19 diploid lines (including Tm 47) was relatively susceptible (0.21 < rm < 0.17). Compared with Arminda, aphid performance on these lines was not affected in terms of nymph or adult survival. However the pre-reproductive period was longer and the adult fecundity reduced. A group of 52 lines was classed as intermediate (0.09 < rm < 0.16). In this group, all the aphid performance parameters were slightly affected by an antibiosis effect. Lastly, a group of 16 lines is

Fig. 1: Cluster Analysis on rm values of *S. avenae* on 88 diploid *Triticum* lines and one hexaploid cv. Arminda.

max.distance
between
clusters

5—
4—
3—
2—
1—
0—

```
                                                    U773
                                                    U772
                                        B754   U784      B901
                                        B756   B684 U783  B687
                              U771 B751 B752   B686 9017  9018
                              B683 7503 8506 B755 8502 8154  8155
                       B685   9181 8156 8152 7701 8151 8153   8143   6801
                 B681 B682    8157 6812 6806 6824 8187 8126 8148 7507   6829
                 9180 7302 U782   6804 8601 6823 6811 6828 6827 8129 7301   6821
     9016        7401 6805 8142   U781 6810 6809 9015 6808 6825 6816 6814 6815 6830 6819
     6901   8150 6826 6832 TM44 7801 7802 6807 TM45 6817 6831 TM46 6820 6803 6802 6813 6822 6818      TM47        ARMI
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·02 ———————————— ·09 ———————————————————————— ·17 ——— ———— ·21 ——— ·24

rm

comparable to Tm44 (rm < 0.09). This group was characterized by a significant effect on aphid development : nymph survival was lower than 70 % and the average adult longevity less than 8 days. In the same time, the prereproductive period is longer and fecundity dramatically reduced.

There was no apparent relationship between the level of resistance and the species of diploid wheat : *Triticum* genotypes of each of the three species could be found in any resistance group. In addition, genotypes which developed isolated chlorotic spots after aphid feeding could be found in any group regardless of its level of resistance.

Durability of T. monococcum *resistance to a standard* S. avenae *clone*

The standard *S. avenae* clone was reared for 1 year separately on each of the five selected *T. monococcum* (Tm44, Tm45, Tm46 and Tm47) and on cv. Arminda. The rate of increase of the aphid was evaluated at the beginning of the experiment, then after 9 and 12 months (Table 1).

Table 1: The intrinsic rate of increase (and confidence interval) of *S. avenae* after 0, 9, 12 months of continuous rearing on 5 wheat genotypes (15 to 50 replicates)

	S T 0	S T 9	S T 12
ARMI (S)	0.25 .22 / .28	0.23 .20 / .26	0.23 .21 / .25
Tm 44 (R)	0.08 -.04 / .20	0.04 -.11 / .19	0.11 .00 / .22
Tm 45 (R)	0.10 .02 / .19	0.03 -.13 / .19	0.05 -.01 / .19
Tm 46 (R)	0.14 .05 / .22	0.05 -.11 / .21	0.10 .00 / .20
Tm 47 (I)	0.21 .18 / .25	0.18 .14 / .21	0.16 .10 / .22

Differences between the three experiments were apparent : the rm decreased after the first date for most of the genotypes tested. This can be explained by variation in experimental conditions rather than by a weakening of the aphid after continuous rearing. Apart from this, the levels of resistance presented by the three lines Tm44, Tm45, Tm46 were maintained on the three dates, compared to the susceptible Arminda. Chlorotic spots appeared at all times on Tm44 and Tm45 only.

Stability of the T. monococcum *resistance against variability in* S. avenae *populations*

The three *S. avenae* clones collected in the Rennes basin differed from the standard clone mainly because of their colour, caryotype and feeding effect on plants : presence of chlorotic spots (*) or not (-)

      - Clone 1   :   light green   2n = 18   (-)
      - Clone 39   :   light orange   2n = 19   (-)
      - Clone 48   :   brown   2n = 19   (*)
      - STD Clone :   dark green   2n = 18   (*)

Aphid performance of the four clones was evaluated against the same five lines (Table 2).

Table 2:   The comparative rate of increase (and confidence interval) of 4 *S. avenae* clones feeding on 5 wheat genotypes (10 replicates).

	ARMI	Tm 44	Tm 45	Tm 46	Tm 47
CLONE 1	0.26 .24 / .28	0.15 .10 / .21	0.18 .15 / .20	0.20 .16 / .24	0.21 .19 / .23
Cl. 39	0.27 .24 / .31	0.16 .14 / .19	0.18 .14 / .21	0.20 .15 / .24	0.22 .17 / .27
Cl. 48	0.26 .23 / .29	0.11 .03 / .19	0.12 .07 / .17	0.15 .11 / .20	0.20 .19 / .21
STD CL.	0.24 .21 / .27	0.06 -.15 / .26	0.10 .03 / .17	0.15 .09 / .20	0.22 .19 / .25

For any clone tested, the rm of *S. avenae* was higher on Arminda than on the three resistant lines Tm44, Tm45, Tm46. Tm47 was generally intermediate between the two groups. Nevertheless two groups of clones can be distinguished : with clones 1 and 39, resistance levels of Tm44, Tm45 and Tm46 were lower than with clone 48 and the standard clone. In addition, the chlorotic spots which appeared after the two last clones fed on Tm44 and Tm45 lines, were not apparent with clones 1 and 39.

Discussion-Conclusion

Evidence has been provided of the existence of high levels of resistance to *Sitobion avenae* within diploid *Triticum* genotypes. The intrinsic rate of natural increase of the aphid proved to be a vailable index of antibiotic resistance which depended not only on development time and fecundity (Birch & Wratten, 1984) but also on nymph survival.

This biological parameter was as greatly affected on seedlings of the most resistant lines like Tm44 as on the diploid lines evaluated by Sotherton & Van Emden (1982).

The screening experiments on diploid species have revealed at least three new resistant *T. monococcum* genotypes which could prove to be more interesting for breeders than the Tm44 line. This is because of their high level of resistance combined with a lack of chlorotic reaction to aphid feeding.

The resistance appeared to be stable after one year and maintained its level when confronted with the genetic variability of *S. avenae* clones in our experiments even though the levels of resistances were slightly modified. This confirms the observations of Lowe (1981) on resistance in *T. aestivum* cultivars to colour forms of this aphid.

All these results justify the start of an investigation of the genetics of the resistance factors revealed, after verification of the maintenance of these levels of antibiosis at tillering, stem elongation and/or at least ear emergence for the most interesting diploid genotypes.

Résumé

La résistance de différentes lignées de *Triticum monococcum* à *Sitobion avenae*

3 cultivars de *T. monococcum* ont prouvé leur résistance à *S. avenae* en comparaison à une lignée intermédiaire et un cultivar sensible de *T. aestivum*. Le taux de développement intrinsèque du puceron élevé sur les génotypes résistants est fortement réduit à cause de la forte mortalité larvaire et le faible taux de reproduction.

La stabilité de la résistance a été examinée sur une période d'une année en élevant un clone de *S. avenae* sans interruption sur des cvs. sensibles et résistants. Aucune érosion de la résistance n'est intervenue.

L'effet de la résistance a été étudié sur plusieurs clones de *S. avenae* provenant du bassin de Rennes (Bretagne, France). Une grande variabilité interclonale dans la réaction du puceron face aux cvs. résistants a été relevée. Cependant tous les clones présentent un taux inférieur de survie ou/et de reproduction.

Parmi 86 génotypes de blé diploïde passés en revue pour découvrir d'autres sources de résistance, un petit nombre de génotypes a révélé des qualités comparables aux sources de résistance connues actuellement.

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THE EFFECT OF RESISTANT WHEAT (Triticum monococcum LINES) ON DEVELOPMENT AND REPRODUCTION OF Sitobion avenae

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Summary

To investigate the influence of plant resistance stress on the reproductive performance of the English grain aphid (*Sitobion avenae*, Fabr.), a study was made of the longevity, fecundity, adult weight and gonad status of aphids reared from birth, either on resistant or susceptible plants, and transferred as apterae to both species. Results were compared with those obtained for aphids starved 10 or 14 hours daily during larval or adult life. Plant resistance experienced by adults resulted in larger differences in each parameter measured than the same plant experience in the course of larval life. Aphid growth and reproduction on resistant hosts were very similar to that for aphids subjected to semistarvation.

Introduction

The English grain aphid, *Sitobion avenae* F., occurs sporadically causing yield losses of up to 10% in Northern Europe, and is one of the important vectors of the Barley Yellow Dwarf Virus (BYDV). Research on the performance of *S. avenae* on different wheat genotypes revealed low levels of resistance amongst cultivars of *Triticum aestivum* L. (Lowe, 1984; DiPietro & Dedryver, 1986) and a more promising source of resistance may be an ancient diploid wheat, *Triticum monococcum* L. (Sotherton & Van Emden, 1982; Spiller & Llewellyn, 1986; DiPietro *et al.*, in prep.). To date, little attention has been paid to elucidating the physiological basis of this resistance.

Among the possible mechanisms of varietal resistance to aphid attack, the nutritional status of the insect on its host plant has often been reported (Painter, 1951; Maltais & Auclair, 1957). In a previous study, plant penetration activities of *S. avenae* on resistant *T. monococcum* lines was monitored using an electronic method (EPG technique, Tjallingii, 1978). Recordings over 8 hours showed that phloem ingestion was greatly reduced on resistant wheat, when compared with a susceptible cultivar (Caillaud *et al.*, 1992), suggesting that plant resistance could affect the aphid feeding behaviour.

The purpose of this study was to investigate the effect of plant resistance on the reproductive performance of *S. avenae*. Studies were also conducted in order to compare plant resistance and semi-starvation effects on aphid growth and reproduction.

Materials and Methods

First instar larvae were obtained from virginoparae alatae of *S. avenae* (clone SAR2, collected in the Rennes basin, France) cultured on wheat seedlings (*T. aestivum* L. cv. ARMINDA). The following genotypes were used for the experiments: a commercial susceptible *T. aestivum* cultivar (ARMINDA-S), a highly resistant *T. monococcum* line (TM44-R), and a moderate resistant *T. monococcum* line (TM47-R). Experiments were performed on unvernalised seedling plants (2-leaf stage). Plants and aphids were grown and maintained at 20°C ± 1°C and a photoperiod of 16 h.

In a first experiment, aphids were subjected during their development and adult life to one of the 4 treatments shown in Fig. 1. Adults obtained from nymphs reared on the susceptible wheat ARMINDA, were either (i) left on ARMINDA (SS group = AR-AR group), (ii) or transferred to resistant plants (SR group = AR-TM44 or AR-TM47 groups). Adults obtained from nymphs reared on the resistant TM44 or on the semi-resistant TM47 were either (i) left on TM44 or TM47 (RR group = TM44-TM44 or TM47-TM47 groups), (ii) or transferred to ARMINDA (RS group = TM44-AR or TM47-AR groups). Adult apterae were allowed to reproduce for 40 days. Longevity and the daily number of offspring produced during this period were recorded. In a separate study, adults were weighed and dissected when they had just moulted (E0), after seven or ten days of reproduction (respectively E7 and E10). Five embryo classes were identified according to differences in eye structure.
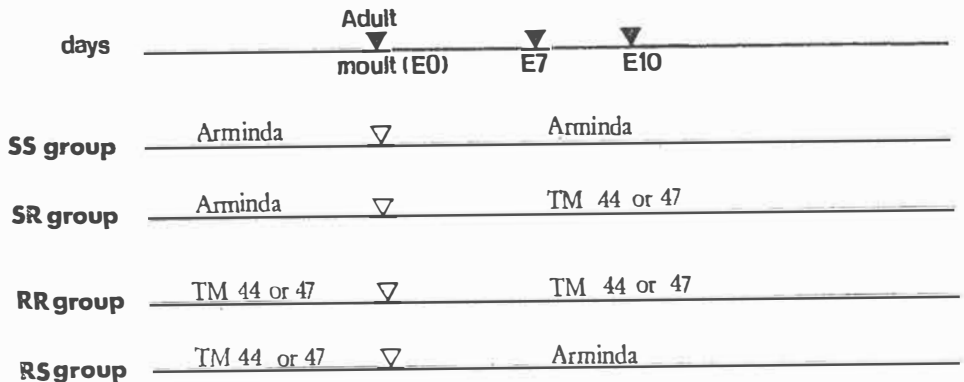

Fig. 1: Aphid-plant combinations. Quality of host plant (resistant or susceptible), timing over 40 days and starvation. E0, E7 and E10: when aphids were weighed and dissected.

In a second experiment, aphids were subjected to semi-starvation by keeping them off plants during 10 hours or 14 hours daily in plastic Petri dishes containing moistened filter paper. They were starved (i) during nymphal life and then transferred to the susceptible cultivar ARMINDA when they had developed into apterae, (ii) during adult life. For each group, the weight and gonad status were studied at the time of adult moult; longevity and fecundity were recorded for a period of 40 days.

An analysis of variance followed by the parametric SCHEFFE test was conducted for both experiments with the SAS general linear models procedure.

Results

Effect of plant resistance on aphid growth and reproduction

Weight and gonad status of newly-moulted adults

On moulting to the adult stage, aphids reared on resistant plants were significantly lighter than they were when maintained throughout larval life on susceptible plants (69% lighter for TM44, 30% for TM47). The number of ovarioles was found constant in all cases, but each of the ten ovarioles contained fewer embryos when aphids were reared on TM44 (3 to 5, giving a total number of embryos of 39.3), than when they were kept on ARMINDA (5 to 7 embryos, giving a total number of 59.8).

Changes in weight and gonad status during adult life:

When aphids were transferred from TM44 to ARMINDA, their weight increased by 41% during the first week of adult life and doubled after 10 days. Their number of mature embryos remained constant during the first 10 days whereas it decreased in the group AR-AR. After 10 days, they had significantly more embryos than at adult moult (44.1 versus 39.3) and this increase was mainly due to changes in the number of end-of-tract embryos (5th class of embryos).

Adult weight and total number of embryos was greatly affected when aphids were transferred from ARMINDA to TM44 (745 versus 386 microgrammes after 7 days on resistant plants). On the 10th day of adult life, they contained twice as few well-developped embryos than adults continuously maintained on ARMINDA. Their number of end-of-tract embryos was reduced by 26% from E0 to E10, whereas it was reduced by 12% in the AR-AR group.

Adult longevity, fecundity and daily offspring:

Prolonged rearing on resistant plants resulted in poor adult survival and reduced aphid fecundity (5.5 larvae/adult for TM44 when 56.3 larvae/adult were produced on ARMINDA; Table 1).

Table 1:   Adult survival and overall fecundity on different host plants
            (S = ARMINDA, susceptible; R = TM44 or TMN47, resistant).
            Figures with the same letter are not significantly different.

TREATMENT	LONGEVITY (Days)	NUMBER OF OFFSPRING
SS	34.8 (a)	56.3 (a)
SR(44)	7.6 (b)	13.6 (ed)
SR(47)	28.5 (a)	36.7 (bc)
RR(44)	6.2 (b)	5.5 (e)
RR(47)	23.3 (a)	28.3 (cd)
R(44)S	30.7 (a)	41.8 (abc)
R(47)S	31.1 (a)	48.9 (ab)

Aphid longevity and overall fecundity was not significantly affected by resistance experience in larval life if they were transferred to susceptible plants at adult moult (compare SS and RS groups, Table 1) but their daily offspring production differed. In the AR-AR group, 41% of the nymphs were produced within the first week (maximum production on the third day of adult life). In the TM44-TM44 group, only 27% of the nymphs were produced during the first seven days and the maximum nymph production was observed on the 11th day.

Aphids that were transferred at the beginning of adult life from susceptible to resistant plants (SR group) had a longevity comparable to that of aphids kept on resistant plants throughout their life (RR group). Similarly, overall fecundity was the same irrespective of whether the larvae were reared on resistant or susceptible plants (compare RR and SR groups, Table 1). In AR-TM44 and TM44-TM44 groups, daily offspring production displayed a rapid decline and oviposition ceased about two days before death of adults.

Effect of semi-starvation on aphid growth and reproduction

Starvation during larval life

Nymphs starved for 10 or 14 hours per day suffered a high mortality (32.3% and 65% respectively) and reached maturity after 13.1 and 15.05 days respectively.

On moulting to the adult stage, 14 hour-starved aphids were 60% lighter than they were if its not significant its not different reared on susceptible plants (Table 2, (A)). Similarly, the total number of embryos was reduced by semi-starvation, and did not differ whether aphids were reared on TM44 or starved 14 hours daily. The number of ovarioles in the ovaries remained constant and equal to ten.

Table 2:   Comparative effect of starvation and plant resistance on (A) newly moulted adult weight and number of embryos (starvation during larval life), (B) longevity and overall fecundity (starvation during adult life).

(A) Starvation during larval life

	Starvation 10h	Starvation 14h	TM44	TM47
Weight (ug)	368 (b)	295 (ab)	246 (a)	540 (c)
N embryos	46 (b)	42 (ab)	39 (a)	52 (c)

(B) Starvation during adult life

	AR-Starvation 10h	AR-Starvation 14h	AR-TM44	AR-TM47
Longevity (days)	22 (b)	13 (a)	14 (a)	35 (c)
Fecundity (N nymphs)	14 (b)	8 (a)	7 (a)	25 (c)

Starvation during adult life

Longevity and overall fecundity of aphids starved 14 hours daily were comparable to that of aphids kept on TM44 after adult moult (Table 2, (B)). Aphids subjected to 10h-starvation had a significantly lower longevity and fecundity than aphids kept on resistant lines ARMINDA or TM47 when developed into apterae.

Conclusion and Discussion

The ovariole number remained constant for all sizes of individuals, irrespective of starvation or plant resistance experience and seems therefore to be determined by intrinsic factors early in development, as reported by Leather & Wellings (1981). In contrast, the number of embryos per ovariole was shown to be variable in this experiment, and appeared to be a consequence of the entire developmental history. Nevertheless it is difficult to state whether this variability was determined by an actual effect of extrinsic factors such as host quality or starvation on ovaries development, or if there was a differential nymphal mortality of individuals in different weight classes in response to nutritional quality.

Larvae of *S. avenae* were able to compensate for poor nymphal growth on resistant plants if transferred to high quality plants, i.e. the susceptible cultivar. Aphids responded to the improved conditions by delayed embryo maturation as well as by an increase in the length of adult life and in the number of embryos contained in the ovaries (there were slightly more offspring produced than the number of

embryos present at adult moult). This latest result suggested that the development of a small number of new embryos may have occured after the adult moult. Although aphid reproductive performance was shown to have become very comparable to that of aphids kept continuously on high quality plants, they never achieved the same longevity and overall fecundity: compensation was, therefore, not complete. Such re-establishment has been reported in the case of aphids subjected to a nutrient stress: Grüber and Dixon (1988) showed that nymphs of *Metopolophium dirrhodum* reared on barley seedlings cultured on sand (poor nutritional quality) could compensate partly for poor larval growth if transferred to barley seedlings cultured on compost (high nutritional quality).

Development on a suitable host during larval life did not favour aphids when reared on resistant hosts after their adult moult: adult experience seemed to be more important than larval experience in determining aphid reproductive performance. The embryo maturation rate was reduced in response to poor host-plant quality.

Fasting periods and rearing periods on resistant plants apppeared to produce similar effects on aphid growth and reproduction. The resistant wheat line TM44 had the same effect as a 14h daily starvation.

These results strongly suggest that *T. monococcum* resistance could act in the form of a nutritional stress. Aphid feeding rate may be reduced on resistant varieties and/or there are qualitative differences between resistant and susceptible plants (Auclair, 1959; Auclair & Cartier, 1960) although convergent data indicate that the balance of main macro in the sap could not be regarded as the principal and only factor conferring resistance (Febvay *et al.*, 1988).

Résumé

L'effet du blé résistant (*Triticum monococcum*) sur le développement et la reproduction de *Sitobion avenae*

L'influence de la qualité de l'hôte sur la performance reproductive du puceron vert de l'avoine a été examinée en étudiant la longévité, la fécondité globale, la production journalière de descendants et le développement des gonades chez les virginipares aptères soumises à l'un des quatre traitements suivants: des adultes obtenus à partir de nymphes élevées sur des plantes sensibles (*Triticum aestivum* L. cv. Arminda) ont été laissés sur Arminda (groupe SS), soit transférés sur des plantes résistantes (4 lignées de *T. monococcum* L. (groupe SR); des adultes obtenus à partir de nymphes élevées sur plantes résistantes ont été soit laissés sur *T. monococcum* (groupe RR) soit maintenus sur Arminda (groupe RS).

Les pucerons élevés sur plantes résistantes pendant leur vie larvaire et adulte sont soumis à une mortalité élevée et un faible taux de reproduction (RR). En revanche, ceux qui sont transférés sur des plantes sensibles, dès qu'ils sont devenus adultes, sont capables de compenser le faible développement larvaire (RS) (il n'y a pas de différences significatives de longévité et de fécondité des adultes entre les variantes RS et SS). La condition SR a pour conséquence une performance reproductive si réduite que cette influence défavorable de la qualité de l'hôte sur

l'adulte semble masquer l'influence favorable reçue durant la vie larvaire (il n'y a pas de différences significatives entre les groupes SR et RR).

Nos résultats suggèrent que l'effet de la résistance des lignées de *T. monococcum* sur *S. avenae* est comparable à celui d'un stress de nutrition. Pour cette raison, nous étudions le comportement nutritionnel du puceron sur des plantes résistantes et sensibles à l'aide d'enregistrements électriques de pénétration (EPG = Electrical Penetration Graph) et de méthodes histologiques.

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HYDROXAMIC ACIDS IN WHEAT: ANTIBIOSIS, ANTIXENOSIS AND EFFECTS UPON APHID SUSCEPTIBILITY TO AN INSECTICIDE

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Summary

A screen of 47 worldwide cultivars of *Triticum* (mainly *T. aestivum*) was conducted in which the concentration of the hydroxamic acid DIMBOA ranged between 1 and 8 mmol/kg fresh wt. When alatae of the aphid *Sitobion avenae* were released among replicated test seedlings, there were highly significant correlations between aphid antixenosis and DIMBOA levels in the seedlings.

The effects of DIMBOA levels in cultivars of wheat on the antibiosis and tolerance of the aphid *Sitobion avenae* to the insecticide, deltamethrin were investigated. Over 48 hours the mean relative growth rate was found to differ significantly between nymphs of *S. avenae* reared on wheat cultivars containing different levels of DIMBOA. Nymphs exposed to higher levels of DIMBOA suffered the greatest reduction in growth rate and showed a significantly greater susceptibility to deltamethrin, particularly at lower doses. The LD_{50}, adjusted for weight, was reduced by 73% for nymphs reared on high DIMBOA seedlings. The value of these results in work leading to the production of aphid-resistant cultivars is discussed.

Introduction

Hydroxamic acids (Hx) are secondary plant chemicals present in Gramineae (Niemeyer, 1991; Copaja, Barría & Niemeyer, 1991; Barría, Copaja & Niemeyer, 1991; Copaja, Niemeyer & Wratten, 1991) showing significant deleterious effects on organisms such as fungi, bacteria and insects on cereals (Niemeyer,1988a; Xie, Arnason, Philogène & Lambert, 1990). Information from experiments in which aphids are reared on artificial diets containing DIMBOA (2,4-dihydroxy-7-methoxy-1, 4-benzoxazin-3-one), the main Hx in wheat (Niemeyer, Pesel, Franke & Francke, 1989; Niemeyer, Pesel, Copaja, Bravo, Franke & Francke, 1988), and from electronic monitoring of aphid feeding (Argandoña, Corcuera, Niemeyer & Campbell, 1983;

Givovich & Niemeyer, 1991), suggests that DIMBOA exerts both toxic and antifeedant effects, with complete inhibition of feeding by the aphid *Schizaphis graminum* (Rondani) at a concentration of 12 mM DIMBOA (Argandoña *et al.*, 1983).

The maximum recorded levels of Hx in cultivated wheat range from 1.4 to 10.9 mmol/kg fresh weight (Niemeyer, 1988b; Thackray, Wratten, Edwards & Niemeyer, 1990; Copaja *et al.*, 1991). Higher levels are found in some wild Triticeae (Copaja *et al.*, 1991a; Barría *et al.*, 1991; Niemeyer, Copaja & Barría, 1991), with wild *Secale* having a maximum concentration of nearly 40mmol/kg fresh wt (Barría *et al.*, 1991). There is therefore germplasm potentially availiable for breeding programmes aimed at producing cultivars with higher Hx levels.

The use of host-plant resistance to pests is of increasing importance as conventional chemical control has been shown to produce undesirable environmental side effects (Greig-Smith, Frampton, & Hardy, 1992); chemical control may also decline in efficiency through increased pesticide resistance displayed by some pests (Metcalf, 1983). New pesticides are becoming more expensive to develop with a less attractive monetary return for agrochemical research and development companies than some prospective host-plant resistant breeding programmes (Wratten, Martin, Rhind & Niemeyer, 1991).

Previous research has shown the possibility of reducing pesticide inputs when these compounds are used in conjunction with host-plant resistance. The concentration of the insecticide parathion required to kill 50% of *Myzus persicae* (Sulz.) on a partially resistant chrysanthemum cultivar was less than half that needed on a susceptible cultivar (Selander, Makkula & Tiittanen, 1972). Tolerance of an organophosphate-resistant strain of the same aphid species to malathion was significantly decreased when aphids were reared on a moderately resistant Brussels sprouts cultivar (Mohamad & van Emden, 1989). Raman (1977) compared the susceptibility to dimethoate of the leafhopper, *Empoasa dolichi* (Germar.) by spraying susceptible and resistant cowpea cultivars. The same concentration causing 75% mortality on the susceptible cultivar gave 94% mortality on the resistant one. Similar increases in mortality were found with the cereal aphid, *Metopolophium dirhodum* (Walker) on resistant wheat cv. Emmer, compared with the susceptible cv. Maris Kinsman (Attah & Van Emden, 1992).

Due to differences between some wild Gramineae and modern wheats, in agronomical traits such as grain quality, yield, growth habit and adaptation to different environments, it may be advantageous to demonstrate usefully high levels of Hx in modern hexaploid wheat cultivars. To investigate this potential, a screen of 47 worldwide cultivars of *Triticum* (mainly *T. aestivum*) was conducted. Ten of these cultivars were selected and assessed in antibiosis and antixensis bioassays for their suitability for using the aphid pest species *Sitobion avenae* (Nicol, Copaja, Wratten & Niemeyer, 1992). The effects of DIMBOA levels on the tolerance of *S. avenae* to the insecticide, deltamethrin were then investigated using two cultivars of high and low DIMBOA content, respectively. The value of these results in work leading to the production of aphid-resistant cultivars is discussed in relation to enhanced natural predation levels arising from reduced pesticide input.

Materials and Methods

Quantification of DIMBOA content in wheat seedlings

The method of extraction and quantification closely followed that of Copaja, Niemeyer & Wratten (1991). The shoot of a seedling, cut at its junction with the seed, was macerated progressively with three batches of 0.33 ml distilled water using a pestle and mortar. After 15 minutes, 1-2 drops of 0.1N H_3PO_4 were added to the extract to bring it to pH 3. The sample was then centrifuged at 13,000 rpm for 15 minutes and the supernatant filtered (0.45μm). Aliquots of 50 or 100 μl were then injected into a Gilson 712 HPLC using a Lichrospher 100RP-18 (5 μm) column (125 x 4 mm). The gradient profile of solvent A (MeOH) and solvent B (0.5 ml H_3PO_4 in 1 l H_2O) was 0 - 9 min: 30% A to 50% A; 9 - 9.5 min: 50% A to 30% A; 9.5 - 10 min: constant at 30% A. Flow rate was 1.5 ml/min and the detection was carried out at 263 nm. The retention time of DIMBOA at 263 nm was 3.5 ± 0.2 mins. The reference compound DIMBOA was obtained from ethereal extracts of *Zea mays* (L.), as described by Queirolo, Andreo, Niemeyer & Corcuera (1983).

Screening of cultivars for DIMBOA content

A screen of 47 worldwide cultivars of *Triticum* (mainly *T. aestivum*) was conducted. For each cultivar approximately 15 seeds were planted in each of ten 6-cm-diameter plastic pots containing vermiculite and allowed to germinate in a plant growth room. The temperature was 20°C with a range of 2°C; relative humidity ranged from 45 - 65%; photoperiod was 12 h and light intensity was 440 μE m^2s^1. Four, five, six and seven days after planting, one healthy seedling of representative size from each pot was cut at the junction with the seed and its length was measured, The sample was weighed and frozen at -20°C ready for subsequent Hx analysis.

Aphid antixenosis experiments

Ten wheat cultivars previously analysed for DIMBOA (Nicol *et al.*, 1992) were selected to include DIMBOA concentrations representing the full range found during screening. Within this range, cultivars were selected which were at the same height at four days; also as wide a range of countries of origin as possible was included.

For each selected cultivar four seeds were planted in each of 27 plastic spittle pots of 4 cm diameter and 4 cm depth containing vermiculite. The pots were placed in 27 randomised blocks, each block containing a full replicate of the ten cultivars, in the growth room described above. On the morning of the fourth day after planting, seedlings were thinned to one seedling per pot, providing plants of similar size in each of ten pots per cultivar. These pots were re-arranged in a randomised block design of ten distinct blocks, each block containing a full replicate of the ten cultivars, within a metal arena measuring 0.5 x 0.5 x 0.15 m. The arena was then filled to just above pot level with vermiculite; PTFE suspension had previously been painted on the inside edge of the arena to prevent the escape of crawling aphids. Four hundred alate *S. avenae* collected from a non-clonal culture 24 hours previously and stored

without food in polyestyrene specimen tubes were then dispersed from the tubes evenly over 25 equally spaced sites in areas between the plants. Three counts were made at two, four and six hours after infestation, respectively, of the number of alatae settled on each seedling. Immediately after the last count, aphids were removed gently with a fine brush and the seedlings cut, measured, weighed and frozen ready for Hx analysis, as described above.

Calculation of mean relative growth rate (MRGR) of aphids

To establish that the cultivars chosen because of their different Hx concentrations, did differ in their levels of resistance under the conditions of the experiment, seedlings of the *Triticum* cvs Altar and Dollarbird were planted in 6 cm-diameter plastic pots containing vermiculite, in separate culture boxes under the same conditions as the aphid culture. Fifty nymphs of *S. avenae* were individually weighed using a torsion balance with a sensitivity of $\pm$ 0.005mg, and placed singly in separate clip cages. Twenty-five cages were subsequently clipped onto 4-day-old seedlings of each of the two cultivars, respectively. After 48 h the nymphs that had settled were re-weighed individually and their MRGR calculated using the following formula (van Emden, 1969):

$$\frac{log_e \text{ final weight (mg)} - log_e \text{ initial weight (mg)}}{2}$$

Effect of pesticide on aphids reared on different cultivars

Approximately 25 seeds of *Triticum aestivum* (L.) cv. Dollarbird and *Triticum durum* (L.) cv. Altar were sown in each of three pots, respectively. The pots were placed in separate boxes under the same conditions as those for aphid culturing. After four days 150 adult apterous *S. avenae* from the stock culture were transferred to the test seedlings in each of the two respective culture boxes. Three days later, 120 first instar nymphs were removed from each culture with a fine paintbrush and weighed in batches of five individuals. Ten of these aphids were then transferred back into each of 12 small polyestyrene tubes per cultivar ready for subsequent pesticide testing on the same day. Twenty four hours prior to this latter analysis, 1 ml of pesticide of known concentration had been pipetted onto filter paper in each of two petri dishes per dose for each cultivar. The concentrations used were 0.1, 0.03125, 0.0156, 0.01 and 0.0067 field concentration. For the analysis, ten nymphs cultured on either of the two cultivars were placed each of the petri dishes and the number of aphids alive, moribund or dead were recorded at the same time intervals as in the initial dose range test. The aphids were subsequently transferred to clean petri dishes containing only barley leaves and the number that were live, moribund or dead were recorded after a further 24 h.

The equation below was used to correct the LC50 values for the difference in the mean weight of the aphids reared on the two different cultivars, respectively; the units of LC50 wt are mg/g aphid fresh wt.

$$\text{LC50wt} = \frac{\text{LC50 (mg/l)}}{\text{fresh wt of 10 aphids (mg)}}$$

Results

The DIMBOA concentrations of the cultivars studied by Nicol *et al.*, 1992 ranged from 0.99 to 8.07 mmol/kg fr. wt. The majority of seedlings had maximal levels of DIMBOA four days after planting. In every cultivar the amount of DIMBOA decreased rapidly two days after the maximal level and it was assumed that whole plant concentrations would not rise to this level again (Argandoña, Niemeyer & Corcuera, 1981).

The relationship between the mean number of alate aphids per cultivar and mean DIMBOA concentration per cultivar is shown in Fig. 1. There was a significant negative relationship between the mean number of alate *S. avenae* present on each cultivar after each of the assessment periods 2, 4 and 6 h and the mean DIMBOA level per cultivar. However, the relationship did not differ significantly (intercept or slope) for each period so the data for the three periods was pooled. The relationship for the pooled data was: (log y = 0.84 - 0.22x, r = -0.81, $P < 0.001$). There was no significant relationship between the mean number of alate *S. avenae* present on each cultivar and the mean seedling height per cultivar (pooled assessment periods: log y = -0.46 + 0.22x, r = 0.31, $P > 0.05$). The ranking of the DIMBOA content of the ten cultivars selected for the bioassay, analysed under non - bioassay and bioassay conditions was strongly positively correlated (Spearman's rank correlation; $r_s = 0.77$, $P < 0.02$), (Nicol *et al.*, 1992).

There was a significant difference ($\underline{t} = 3.279$; $P < 0.005$, d.f. = 40) between the mean values of MRGR over 48 h for nymphs of *S. avenae* cultured on two different *Triticum* cultivars. The mean MRGR of nymphs cultured on cv. Altar was 0.150 mg/mg/day, 46.4% lower than that for cv. Dollarbird, which was 0.280 mg/mg/day. The initial weight of nymphs did not differ significantly between the two cultivars ($\underline{t} = 1.943$; $P > 0.05$, d.f. = 40).

The regression lines in Fig. 2 represent probit mortality after 45 mins at different doses of deltamethrin for nymphs reared on cv. Altar ($\chi^2 = 5.689$; $P > 0.05$, d.f. = 8) and cv. Dollarbird ($\chi^2 = 9.099$; $P > 0.05$, d.f. = 6), respectively. The χ^2 values indicate that the relationships did not differ significantly from the (pre-transformation) sigmoid dose-response model. The LC50 of nymphs cultured on cv. Altar was 0.119 mg a.i./l, significantly lower than that of nymphs from the cultivar containing lower DIMBOA levels, which was 0.528 mg a.i./l ($\underline{t} = 8.954$; $P < 0.001$, d.f. = 16). The aphids cultured on cv. Altar were significantly lighter ($\underline{t} = 3.304$; $P < 0.005$, d.f. = 46) than those on cv. Dollarbird. When the values were adjusted for

weight the LC50 wt (0.074 mg a.i./g aphid fresh wt) for nymphs on Altar was 73% lower than the LC50 wt (0.324 mg a.i./g aphid fresh wt) for nymphs on cv. Dollarbird (t = 8.874; P < 0.001, d.f. = 16).
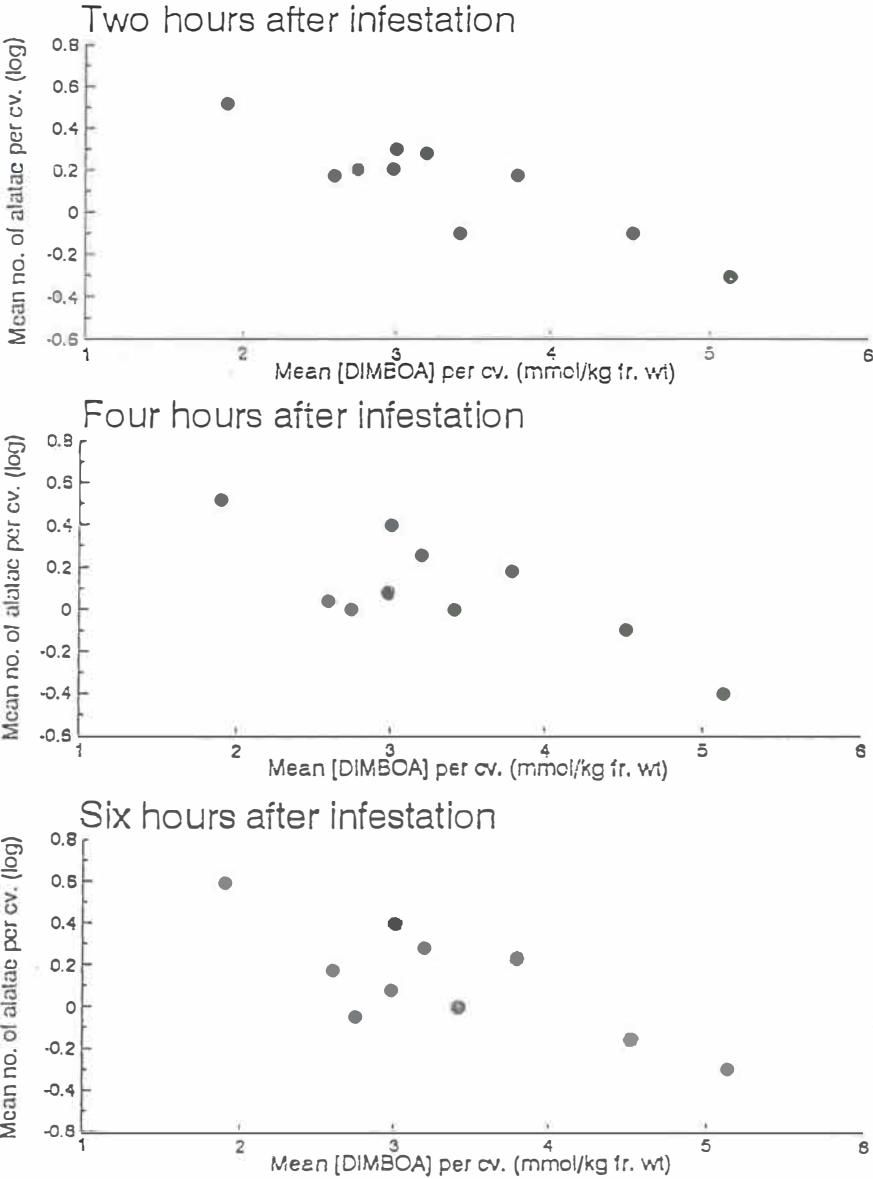

Fig. 1: The relationship between the mean number of alate *Sitobion avenae* settling per cultivar and the mean DIMBOA concentration at 2, 4 and 6 hours after initial infestation.
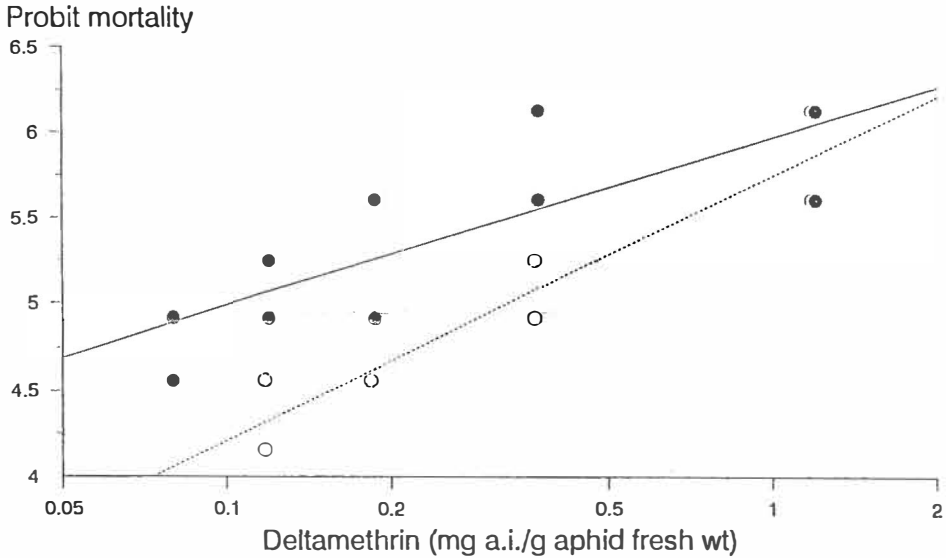
Probit mortality

Fig. 2.   The susceptibility to deltamethrin (adjusted for aphid weight) for nymphs of *Sitobion avenae* reared on *Triticum*, cv. Altar (—•—) and cv. Dollarbird (---o---), containing hight and low levels of DIMBOA, respectively.

Discussion

Nicol *et al.*, 1992, demonstrated a wide range of DIMBOA concentrations in the 47 cultivars screened. The range was very similar to that demonstrated by Copaja, Niemeyer & Wratten (1991) for a screen of cultivars from within one country (Chile), implying that the DIMBOA levels in currently-grown wheat seedlings may not extend far beyond those in the earlier Chilean screen. However, the levels of DIMBOA displayed confirm the potential of Hx as possible aphid resistance factors in modern cultivars.

The fact that the range of DIMBOA concentration of the ten selected cultivars was strongly positively correlated between non-bioassay and bioassay conditions, suggests that despite some individual variation, the relative difference in DIMBOA content between the cultivars remained similar under both sets of conditions. Any individual variation recorded may be due to the effects of induction from aphid feeding or the different conditions experienced by the wheat seedling, such as the lower density of planting under bioassay conditions. Most published work on Hx levels and aphid resistance has concerned antibiosis, in which aphids have usually been confined to a plant of a particular cultivar (Argandoña, Luza, Niemeyer & Corcuera, 1980; Bohidar, Wratten & Niemeyer, 1986; Thackray *et al.*, 1990), or dual choice tests between two cultivars. This work is the most complete antixenosis bioassay to date concerning this aphid/biochemical interaction.

Aphids reared on the *Triticum* cv. Altar, containing a higher level of DIMBOA than cv. Dollarbird, had a significantly lower mean relative growth rate over 48 h, demonstrating an antibiosis effect upon this pest species *S. avenae*.

Analysis of probit mortality showed that the effect of the pesticide was significantly different for nymphs reared on the two cultivars. The nymphs that experienced higher levels of DIMBOA were less tolerant to the insecticide. Some effect may be expected due to their slightly smaller size, but when LD50 values were adjusted for weight the effect was still significant.

This interaction between host-plant resistance and pesticide effects, if shown in the field, could allow reduction of pesticide dosage. In addition, the lower rate of pesticide application may subsequently have less detrimental effect upon beneficial insects (van Emden, 1987; van Emden & Wratten, 1990).

Résumé

Les acides hydroxamiques du blé: antibiose, antixénose et effets sur la sensibilité du puceron *S. avenae* à un insecticide.

Dans un test impliquant 47 cultivars de *Triticum* (principalement *T. aestivum*) en provenance du monde entier, la concentration en acide hydroxamique DIMBOA a varié de 1 à 8 mmol/kg de poids frais. Des essais d'introduction du puceron *Sitobion avenae* parmi de jeunes plantules ont montré qu'il y a une corrélation hautement significative entre leur antixénose à l'égard du ravageur et leur teneur en DIMBOA. En outre, les effets de la teneur en DIMBOA des cultivars de blé sur les rapports entre l'antixénose et la tolérance de *S. avenae* à la deltamethrin ont été étudiés. Après 48 heures, le taux de croissance relative moyenne des nymphes de *S. avenae* différait significativement selon la teneur des cultivars en DIMBOA. Les nymphes exposées à des niveaux de DIMBOA élevés ont montré la plus importante diminution de croissance, ainsi qu'une sensibilité significativement plus grande à la deltaméthrine, surtout aux faibles doses. La DL 50, ajustée au poids, a été réduite de 73% chez les nymphes élevées sur des plantules à haute teneur en DIMBOA. Ces

résultats sont discutés dans le cadre des possibilités de production de cultivars résistants au puceron.

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USE OF Hordeum vulgare SUBSP. spontaneum WHEN BREEDING APHID RESISTANT BARLEY - PROGRESS AND PROBLEMS

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Summary

Since 1990 efforts have been made to transfer resistance to the bird-cherry aphid, *Rhopalosiphum padi*, from *Hordeum vulgare* subsp. *spontaneum* to cultivated barley. Selection was made among segregating F_2 populations, whereby the best plants in the best families were chosen. F_3 families from retained plants will subsequently be evaluated. Although a complete genetic analysis has not been made, resistance appears to be quantitative and governed by several "minor" genes. In this paper problems related to the selection of resistant plants are discussed.

Background

Host plant resistance to the bird-cherry aphid, *Rhopalosiphum padi* (L.), in wild barley species was demonstrated by Weibull (1987). Unfortunately most of these wild relatives are incompatible with cultivated barley and, until the present gene transfer methods are improved, this resistance will remain inaccessible. However, *Hordum vulgare* subsp. *spontaneum* (C. Koch.) Thell. has been found to contain a wealth of new and interesting resistance genes (Lehmann, 1991). Therefore, during a great *R. padi* outbreak in 1988 approximately 500 *H. vulgare* subsp. *spontaneum* lines were screened in the field near Uppsala, Sweden. Of these 500 lines, 30 were selected and re-tested in the laboratory.

Methods

The 30 selected lines were re-tested by infesting them with individual aphid larvae (one/plant) and measuring their weight increase after a six-day period. The results showed that the majority of the lines were in fact less suitable as host plants than a barley control cultivar included in the same tests (cv. Tellus; $X^2 = 4.172$, $P < 0.05$). Eventually crosses were made with two of the best lines on which individual aphid growth was reduced by 50%. The cultivar 'Golf' was used as the crossing parent. The F_1 kernels produced were grown on to the F_2 generation before selection

was made. Twenty-five seeds of each F_2 population were sown. However, as some seedlings did not emerge the final number of plants was slightly reduced. Each plant was evaluated as above by measuring aphid weight increase over a six-day period. Selection was made firstly by comparing population means and secondly by retaining the best plants in the best population. The crossing parents were also tested along with the populations. Selected plants were grown on to the F_3 generation and are now in the process (June 1992) of being evaluated for *R. padi* resistance. Similarly a first backcross generation (BC_1F_2) has been developed which will be tested later in 1992. Crosses with a barley yellow dwarf virus (BYDV) resistant source (cv. Coracle) failed to produce viable seeds.

Results and Discussion

F_2 segregation patterns in both crosses showed continuous distributions. The best population means were either on the same level as the resistant parent or even slightly lower. Mean aphid weight was 30-40% less compared to the susceptible parent cv. 'Golf'. Phenotypically the plants were intermediate with many of the wild type characters, such as weak stem, thin stature and traits associated with ear development (weak rachilla etc.). The BC_1F_1 plants were more of the normal type. The continuous distribution among segregates indicates the influence of several minor genes having additive effects. This is the situation for BYDV resistance in oats (Qualset, Lorens, Ullman & McGuire, 1990) where resistance is inherited in a quantitative manner. This type of resistance can be enhanced by intercrossing genotypes showing resistance and selecting for good transgressive segregates in early generations, i.e. a form of recurrent selection.

Problems

A.   *Selecting for aphid weight*

Using aphid weight or aphid mean relative growth rate as indicators of host plant suitability has several drawbacks. Although all efforts were made to standardize the aphid material it is likely that nymphs showed individual variation when it came to growth. In general this variation was less than that caused by the host plant on which the aphid was feeding. However, if the degree of host plant resistance is low the risk of getting spurious correlations increases rapidly.

B.   *Other selection criteria*

Ideally, plant selection should be based on a particular plant character, either physical (e.g. hairiness) or chemical (eg. antifeedants). Gramine is an indole alkaloid in barley which has been suggested to have detrimental effects on aphid growth and feeding behaviour (Zuñiga, Varanda & Corinera, 1988). Research is in progress in Sweden to evaluate a larger collection of barley for this particular relationship. Similarly, concentrations of the hydroxamic acid DIBOA showed high correlations with *R. padi* performance on wild *Hordeum* species (Barría, Copaja & Niemeyer,

1992). Such traits would be much easier to screen for and follow in segregating material. Characters like these are most likely to be simply genetically inherited and should be easy to transfer into modern cultivars. However, their true effects on aphid performance still has to be determined.

C.   When to select?

As indicated above it is probable that the resistance found in the *H. vulgare* subsp. *spontaneum* lines is quantitatively inherited. Therefore resistant plants could have been selected in the F_1 and F_2 generations, followed by hybridization to produce good transgressions. However, F_1 seeds are often very few and weak, and they may have a long dormancy. This may result in unacceptable variation which is not due to true resistance. On the other hand testing F_2 populations would mean screening a larger quantity of plant material, which is immensely tedious if aphid growth is used as a resistance trait. However, comparing F_2 population means appears to be the least risky approach for selection purposes.

D.   Plant selection

In the above work the 'best' plants in the 'best' F_2 population were selected. How confident can a breeder be that the selected plants really are resistant and were not selected because that particular aphid "had a bad week"? Aphids vary in quality despite efforts to produce homogenous material. In this study aphid numbers were intentionally kept at one/plant so as not to create crowding effects. But with a single aphid/plant there is a risk of getting missing values simply because aphids disappear! The only apparent solution is therefore to test F_3 plants and again compare them with the parents.

Conclusions

Host plant resistance to *R. padi* does exist in *H. vulgare* subsp. *spontaneum* lines and is probably quantitatively inherited. At present the selection methods are too imprecise. Perhaps plant selection in the future should be based upon the analysis of certain plant chemicals which have detrimental effects on aphid behaviour and growth.

Résumé

L'utilisation de *Hordeum vulgare* subsp. *spontaneum* dans la sélection de l'orge pour la résistance au puceron du merisier à grappes *Rhopalosiphum padi* L. - progrès et difficultés

Depuis 1990, un travail de transfert et de résistance à *Rhopalosiphum padi* L. de *Hordeum vulgare* subsq. *spontaneum* à l'orge cultivée est poursuivi. La sélection a été opérée parmi les populations F_2 en ségrétation, en choisissant les meilleures

plantes dans les meilleures familles. Les familles F_3, issues de ces plantes, seront retestées. Même si une analyse génétique complète n'a pas été effectuée, il apparaît que la résistance est quantitative et régie par plusieurs gènes mineurs. Les difficultés de la sélection pour la résistance des plantes sont discutées.

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HOST PLANT RESISTANCE IN SUGARCANE (Saccharum officinarum) TO THE LOPHOPID PLANT HOPPER Pyrilla perpusilla (HOM; LOPHOPIDAE)

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Summary

Antibiosis tests were carried out using first and third instar nymphs of *Pyrilla perpusilla* (Homoptera : Lophopidae) on 23 cultivars of sugarcane, namely SL 7103, SL 8302, SL 8306, SL 8406, SL 8606, SL 8618, Co 62175, Co 775, M 550-60, M 34-45, F -148, MANA 77-58, LF 72-2246, LF 74-3152, LF 75-507, LF 76-2694, LF 76-5958, LF 82-9999, RB 70-194, H 38-2915, VOMO, RAGNAR and VATU obtained from the germplasm collection of the Sugarcane Research Institute of Sri Lanka. Parallel experiments were conducted to determine the level of hydroxamic acid content in the leaves and the structure of the leaf surface. Five cultivars selected on the basis of the results of the antibiosis tests were subjected to the antixenosis experiments. The results showed that the mean relative growth rate (M.R.G.R.) differed significantly between cultivars (from 0.03191 to 0.05487 $\mu g/\mu g$/day for first instar nymphs and from 0.3251 to 0.04557 $\mu g/\mu g$/day for the third instar nymphs) and did not change signficantly over a narrow range of plant growth stage. Results of the experiments on morphological characters of the cultivars revealed that leaves of two cultivars which had low M.R.G.R.s for the first instar nymphs, bear erect and sharp spines which may be the basis of resistance to the insects. Two hydroxamic acids, DIBOA and DIMBOA were found in different concentrations in sugarcane plants.

Introduction

The sugarcane plant hopper *Pyrilla perpusilla* Walker (Homoptera:Lophopidae) is a serious pest of sugarcane in the Oriental region. It has also been recorded as a pest on other crops such as maize (*Zea mays* L.), wheat (*Triticum sativum* Lamk.), sorghum (*Sorghum halpense* Wall.), millet (*Pennisetum typhodideum* L.), oats (*Avena sativa* L.) and barley (*Hordeum vulgare* L.). All types of wild grasses growing in and around sugarcane fields are also recorded as alternate hosts for *P. perpusilla* (Avasthy, 1973; Chaudhary, Kaushik, Singh & Mrig, 1987).

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Both nymphs and adults of the insect suck sap from the pholem through the main veins of leaves. In the case of outbreaks, every part of the leaf is attacked and as a result of continuous draining of sap, the leaves become pale and wilted. The growth of the plant is also arrested. Apart from the direct injury to the plant, the honey dew excreted by the insect serves as an ideal substrate for the development of sooty moulds (*Capnodium* sp.) which affects first photosynthesis and then crop yield (Butani, 1964; Bindra, Singh & Chaudhary, 1979; Asre, Gupta & Pawar, 1983). Poor germination of seed materials and problems for milling with affected cane have also been recorded as after effects of *Pyrilla* infestation (Saxena, 1969; Kalra, 1973).

The control of this pest has become a necessesity as it causes losses of up to 34% in the sucrose content, up to 26% in the purity of sugar, up to 50% in sugar recovery and up to 28% in crop yield (Rahman & Nath, 1940; Gupta & Avasthy, 1954; Agarwal, 1969; Avasthy, 1973; Varma, 1986).

The feasibility of controlling the pest using different methods has been studied since the begining of the century but control at present involves biological and chemical methods. Eighteen parasitoids, nineteen predators and six pathogens have been recorded as biological control agents for *P. perpusilla*. Up to now only, the egg parasitoid *Tetrastichus pyrillae* Crawford (Hymenoptera : Eulophidae) and the nymphal and adult parasitoid *Epiricania melanoleuca* Fletcher (Lepidoptera : Epipyropidae) have been used in biological control programmes with varying degrees of success (Singh & Dayal, 1975; Hamid, Mohyuddin & Mohammad, 1986). Many different methods such as dusting, fogging and spraying (aerial and ground) of chemicals have been tried but present recommendations include aerial or ground applications of organophosphorus insecticides such as malthion (Bindra *et al.*, 1970; Dang & Tyagi, 1975).

The observations of preference by *P. perpusilla* for some sugarcane cultivars in the natural environment has been recorded since 1929 (Narayanan, 1953; Rajak, Pawar, Misra, Prasad, Varma & Singh, 1987). Most researchers observed that the soft, broad, succulent characteristic of the leaves attracted the pest. Khanna, Sharma & Hussain (1950) discovered that the cultivar Bo 3 had a phloem which is protected with a shield formed by the fusion of the vascular sheath and sclerenchymatous rib below it; this shield interferes with feeding.

This paper reports the results of antibiosis and antixenosis tests for *P. perpusilla* on 23 sugarcane cultivars and a preliminary investigation of the chemical aspects of the resistance mechanism of sugarcane. This part of the programme involves the study of 112 cultivars from the germplasm collection of the Sugarcane Research Institute of Sri Lanka, the aim being to reduce pest outbreaks in plantations and to establish a foundation for host plant resistance research in sugarcane for *P. perpusilla*.

Materials and Methods

Sugarcane plants

Seed sets of the following sugarcane cultivars were received from the Sugarcane Research Institute of Sri Lanka. They were planted in separate pots (dia. 30 cm) in sterilized compost using four replicates in a glasshouse maintained at 25-27°C and 70% RH.

SL 7103	MANA 77-58	F - 148
SL 8302	LF 76-2694	M 550-60
SL 8306	LF 82-9999	M 34-45
SL 8406	LF 74-3152	RB 70-194
SL 8606	LF 76-5958	H 38-2915
SL 8618	LF 75- 507	VOMO
Co 62175	LF 72-2246	RAGNAR
Co 775		VATU

Insects

Eggs of *P. perpusilla* were received from the International Institute of Biological Control, Pakistan. The nymphs that hatched out from these eggs were reared on four month-old sugarcane plants in culture boxes maintained at 26-27°C and 70% RH.

Preliminary studies

Preliminary experiments were conducted in a culture room maintained at 26-27°C and 70% RH to determine the duration of the nymphal period, the minimum number of weighings necessary and the effect of the age of plants in the antibiosis experiments.

An experiment was carried out to determine the range of the nymphal period of *P. perpusilla* for the antibiosis tests. The weight and size of different nymphal stages of *Pyrilla* were considered in order to select appropriate nymphs for experiments. It was decided to conduct the experiments using the first three instars of nymphs due to difficulties in identifying the other two instars in laboratory cultures. During the experiment, 10 nymphs of *P. perpusilla* were enclosed separately in clip cages (diam. 4.5 cm) on the second leaf of the plant (five months-old) and weighed once in two days, during the period from first to third moult. The mean relative growth rate (M.R.G.R.) and coefficient of variation were calculated for 2, 4, 6, 8 and 10 days intervals respectively. The nymphal duration which represents the maximum weight difference with lowest coefficient of variation was selected for the antibiosis experiments.

An experiment was designed to determine the minimum number of weighings in order to save time and to minimize disturbances of the insects. Ten newly-moulted third instar nymphs were enclosed in clip cages separately on the second leaf of two

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plants five months-old. These ten insects divided into groups of five. One batch of insects was weighed once in two days and the other was weighed two times, once at the beginning and then at the end of an eight day period. The mean relative growth rate for the eight day period as well as that for each of the two day periods were compared to determine whether there were any significant differences. The appropriate time gap between the two weighings was then decided.

The influence of plant age on the mean relative growth rate was investigated using three groups each consisting of four, newly-moulted third instar nymphs which were enclosed separately in clip cages on the second leaf of 6, 10, 13 and 16 week-old plants of the cultivar Co 775. The insects were weighed after a period of eight days and M.R.G.R. for the insect for the respective age of the plant was calculated. These results were compared to see whether there was an effect of the age of the plant on the growth of the insect.

Antibiosis experiments

Antibiosis experiments were carried out on first instar nymphs to investigate the effect of spines on the leaf surface on the growth rate of the insect, an effect which may not influence larger instars.

First instar nymphs were caged (two insects in two separate clip cages [dia. 4.5 cm] on the ventral surface of the second leaf of the plant) on four replicates of the 22 different cultivars. The insects were weighed and recorded at the beginning and the end of the seven day period (duration of the first instar period). Four first instar nymphs were caged on the second leaf of the reference cultivar Co 775, in order to compare the results obtained with other cultivars. The mean of the two replicates on a single plant was used to calculate overall plant means and four replicates of the cultivars were used to calculate the M.R.G.R. for the various cultivars.

An antibiosis test for third third instar nymphs was carried out using newly-moulted third instar nymphs which were weighed and transferred to clip cages (diam. 4.5 cm) on the ventral surface of the second leaf of 22 different cultivars (2 insects per plant for four replicates of each cultivar). The insects were weighed after a period of eight days. The same procedure was carried out with four insects caged on two plants of the reference cultivar Co 775 (two insects per plant). The mean of the two cages in a replicate was used to calculate the overall plant mean and the mean of the four replicates was used to calculate the M.R.G.R. for each cultivar.

Antixenosis experiments

Antixenosis experiments were carried out to test the preference of *Pyrilla* for four selected and the reference cultivar which gave different M.R.G.R. for the third instar nymphs (RAGNAR, 0.03251; SL 7103, 0.03737; F-148, 0.04186 VATU, 0.04557 and Co 775, 0.05018). The second leaf of two of the five cultivars was offered to 20 third instar nymphs which were kept inside a closed perspex box (27.5 x 15 x 10 cm) (the top of the box was covered with a mosquito net). The number of insects on

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leaves of the different cultivars were recorded after a period of 12 h. The experiment was repeated four times for each pair of cultivars.

Preliminary studies on biochemical compounds in leaves

Samples were collected from the tip and the base of the second leaf of each cultivar and weighed separately. The individual samples were macerated progressively with three batches of 0.33 ml H_2O using a pestle and mortar. After 15 min, 1-2 drops of 0.1N H_3PO_4 were added to the extract to bring it to pH 3. The samples were then centrifuged at 13,000 rpm for 15 min. and the supernatant filtered (0.45μm). Aliquots of 50 or 100 μl were then injected into a Gilson 712 HPLC using a Lichrospher 100RP-18 (5 μm) column (125 x 4 mm). The gradient profile of solvent A (0.5 ml H_3PO_4 in 11 H_2O) and solvent B (MeOH) was 0-7 min: 26% B to 30% B; 7-7.05 min: 30% B to 100% B; 7.05-9 min: constant at 100% B; 9-9.05 min: 100% B to 30% B: 9.05-12 min: constant at 30% B. Flow rate was 1.5 ml/min and the detection was carried out at 263 nm. The retention time at 263 nm for DIBOA was 3.50 ± 0.15 mins, DIMBOA Glucocide was 3.75 ± 0.15 mins and DIMBOA was 4.70 ± 0.15 mins. The reference compounds were obtained from etheral extracts of wheat seedlings, as described by Queirolo, Andero, Niemeyer & Coreuera (1983).

Morphological characteristics of leaves

Electron microscope photographs of the ventral surface of the second leaf of each cultivar were taken and compared with the M.R.G.R. obtained for the first instar nymphs which were reared on the different cultivars.

Results

Preliminary studies

The results indicated that the most suitable range for the antibiosis test was the third nymphal period which had the maximum range of weight difference of 0.00124 g (Table 1). The minimum coefficient of variation (9.84%) was observed for the period of eight days duration (Table 2). Therefore antibiosis tests were carried out on third instar nymphs for a period of eight days just after the second moult.

Table 1.   The duration and the weight differences of the second and third instar nymphs of *P. perpusilla* at 27°C and 70% RH

Second instar		Third instar	
Duration	Weight difference	Duration	Weight difference
8 ± 1.5 days	56 ± 1 x 10^{-5}g	10 ± 2.2 days	124 ± 3 x 10^{-5}g

Table 2.   Mean Relative Growth Rate of third instar nymphs of *P. perpusilla* for 2, 4, 6, 7 and 10 day intervals at 27°C and 70% RH

Duration (days)	Mean M.R.G.R. (μg/μg/day)	Standard deviation	Coefficient of variation (%)
2	0.03706	0.00920	24.82
4	0.03883	0.00670	17.49
6	0.03635	0.00579	15.94
8	0.03627	0.00357	9.84
10	0.03707	0.00369	9.96

The results of the experiments to determine the minimum number of weighings for the antibiosis tests showed that there were no significant differences between the M.R.G.R.s of the third instar nymphs of disturbed (weighing was done once in two days) and undisturbed (weighing was done only beginning and the end of the eight day period) insects (Table 3). Therefore, it was decided to weigh the third instar nymphs only at the beginning and at the end of the eight day duration for the antibiosis test.

Table 3.   Mean relative growth of *P. perpusilla* at the two days and eight days durations at 27°C and 70% RH

| Duration of weighings (days)	
Average M.R.G.R. for once in two day period	M.R.G.R. (μg/μg/day) for eight day period
0.03706	
0.03891	
0.03891	0.03702
0.03707	

In the experiments designed to determine the effect of plant age on the growth of the insect. There were no significant differences between the M.R.G.R.s of the insects reared on plants aged 6, 10, 13, and 16 weeks (Table 4).

Table 4.   Mean relative growth rate of the third instar nymphs of *P. perpusilla* on 6, 10, 13 and 16 weeks old plants at 27°C and 70% RH

| Age of plant (weeks)	M.R.G.R. (μg/μg/day)
6	0.04817
10	0.04855
13	0.04871
16	0.04837

The results of tests for the first instar nymphs showed that the M.R.G.R.s of the insects varied from 0.03191 $\mu g/\mu g/day$ (M 550-60) to 0.05487 $\mu g/\mu g/day$ (LF 74-3152) within the 23 cultivars tested (Fig. 1). The mean M.R.G.R. observed for the reference cultivar (Co 775) was 0.05018. The electron microscope photographs showed that the two cultivars M 550-60 and SL 8606 have sharper and more erect spines than the other cultivars and the leaves of the cultivar RB 70-194 bear the lowest density of spines.
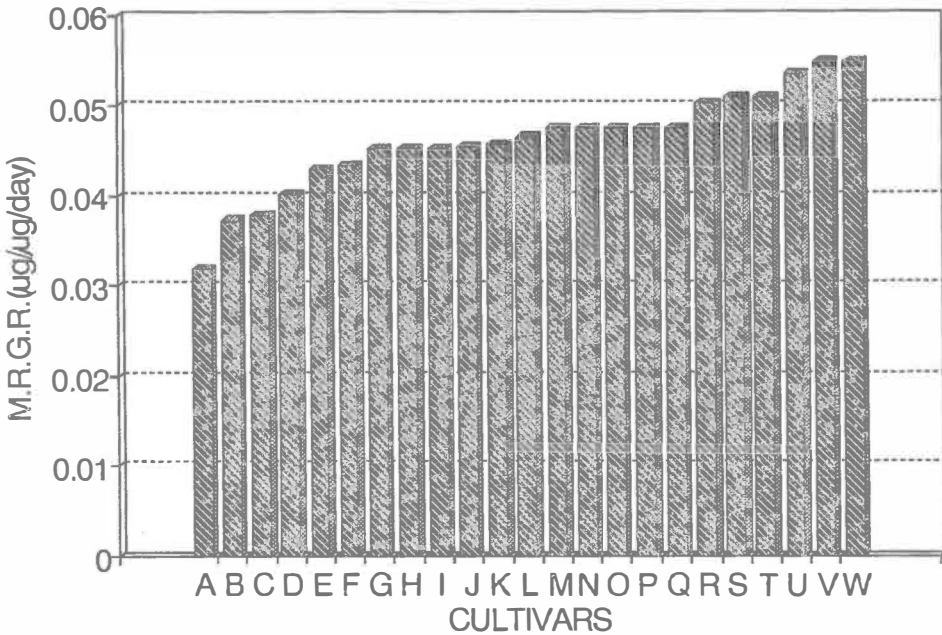
A - M 550-60; B - SL 8606; C - RAGNAR; D - VOMO; E - LF 76-2694;
F - LF 76-5958; G - RB 70-194; H - MANA 77-58; I - SL 8618; J - SL 8302;
K - SL 8306; L - H 38-2915; M - LF 82-9999; N - LF 72-2246; O - VATU;
P - SL 7103; Q - M 34-45; R - Co 775; S - LF 75-507; T - Co 62175; U - SL 8406;
V - F - 148; W - LF 74-3152

Fig. 1.   M.R.G.R. of first instar nymphs of *P. perpusilla* on 23 cultivars

The results of the experiment on the third instar nymphs showed that the M.R.G.R.s of the insects did not vary very much within the 23 cultivars tested (from 0.03251 μg/μg/day [RAGNAR] to 0.04612 μg/μg/day [VATU]). The M.R.G.R.s of the insects for all the experimental cultivars were below that of the reference cultivar Co 775. The M.R.G.R.s for the cultivars M 550-60 and SL 8606 were lower as well as for the first instars, compared to the other cultivars (Fig. 2).
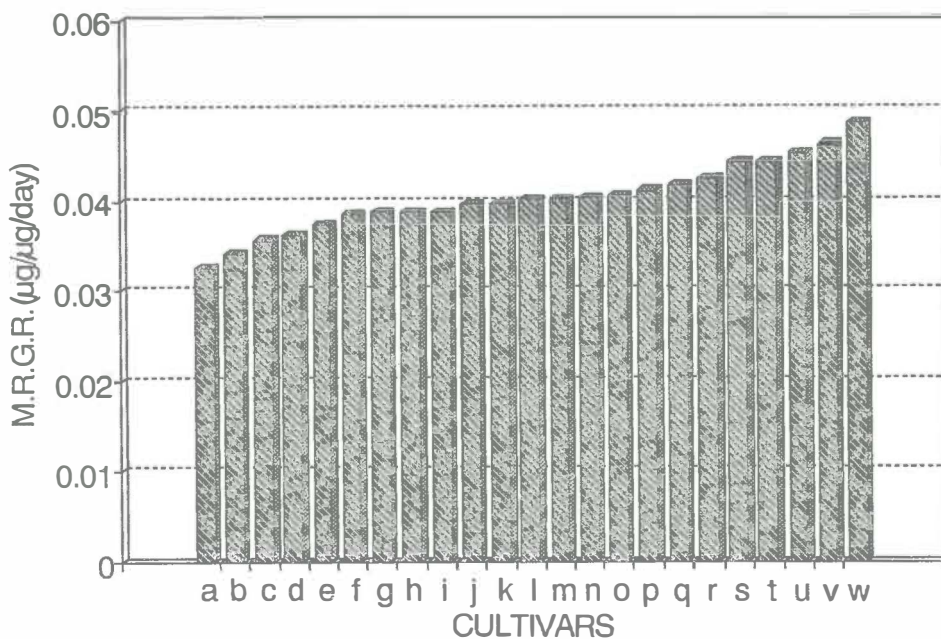
a - RAGNAR; b - Co 62175; c - M 550-60; d - SL 8606; e - SL 7103; f - VOMO;
g - SL 8302; h - LF 76-2694; i - RB 70-194; j - H 38-2915; k - SL 8618; l - SL 8306;
m - LF 76-5958; n - LF 75-507; o - LF 82-9999; p - M 34-45; q - F - 148;
r - LF 72-2246; s - SL 8406; t - LF 74-3152; u - MANA 77-58; v - VATU; w - Co 775

Fig. 2.   M.R.G.R. of third instar nymphs of *P. perpusilla* on 23 cultivars

Antixenosis experiments

The results of the experiments were first analysed to determine whether there was agreement between the four replicates of each combination of cultivars. The results of the analysis showed that there was no significant differences between the four replicates. Subsequent analysis using a χ^2 test showed that there were no significant differences in the preferences of the insect for the different combinations of cultivars offered except for the cross involving the two cultivars VATU and RAGNAR. *P. perpusilla* prefered the cultivar VATU to RAGNAR (Table 5).

Table 5.   Preference for different cultivars of sugarcane by the plant hopper *P. perpusilla*

Varieties tested	χ^2 values
RAGNAR x SL 7103	3.62
RAGNAR x F - 148	1.52
SL 7103 x F - 148	0.32
RAGNAR x VATU	4.52*
SL 7103 x VATU	1.02
F - 148 x VATU	1.05
RAGNAR x Co 755	2.82
SL 7103 x Co 775	0.62
F - 148 x Co 775	0.12
VATU x Co 775	1.02

* significant at $P = 0.05$

Preliminary studies on biochemical compounds in leaves

The results of this experiment showed that hydroxamic acids (DIBOA and DIMBOA) were present in different concentration in the leaves of the sugarcane (DIBOA 0.10 - 0.55 MMol/Kg and DIMBOA 0 - 0.08 MMol/Kg per fresh weight). It was also found that the level of DIBOA and DIMBOA in the tip of the leaves was lower than in the base of the leaves.

Discussion

From the results of the preliminary studies, it was decided that an eight day period with two weighings at the begining and the end of the period would give satisfactory results for the antibiosis test. There was no effect of plant age on the mean relative growth rate of the insect during this period.

The difference in the M.R.G.R.s from 0.03612 (M 550-60) to 0.05487 (LF 74-3152) of newly-emerged first instar nymphs among the 23 cultivars may possibly be due to their difficulty in living on the leaf surface of different cultivars. The electron microscope photographs suggest that the characteristic of sharp and erect spines on the leaf surface of the two cultivars M 550-60 and SL 8606, affest the growth of the first instar nymphs as the insects at this stage of growth have a smaller body with thinner cuticle. The small differences in the M.R.G.R.s for the third instar nymphs showed that there is probably no significant effect of the different cultivars on this stage of the insect.

The results of the antixenosis test suggested that *P. perpusilla* only showed a significant preference for the cultivar VATU over RAGNAR. Selection of cultivars for antixenosis experiments was based on the results of the antibiosis test. The

research will be continued further and concentrate on hydroxamic acids in sugarcane.

Acknowledgement

Résumé

La résistance de la canne à sucre (*Saccharum officinarum* L.) à la cicadelle *Pyrilla perpusilla* (*Hom*; *Lophopidae*)

L'effet de l'antibiose sur le premier et le troisième stade larvaire de *Pyrilla perpusilla* a été examiné sur 23 variétés de canne à sucre. Celles-ci ont été obtenues auprès de la banque de gènes de l'Institut de recherche sur la canne à sucre du Sri Lanka. Voici la liste des cultivars: SL 7103, SL 8302, SL 8306, SL 8406, SL 8606, SL 8618, Co 62175, Co 775, M 550-60, M 34-45, F-148, MANA 77-58, LF 72-2246, LF 74-3152, LF 75-507, LF 76-2694, LF 76-5958, LF 82-9999, RB 70-194, H 38-2915, VOMO, RAGNAR et VATU.

Des essais parallèles, destinés à déterminer la concentration d'acides hydroxamiques dans les feuilles, ainsi que la structure de la surface foliaire ont été conduits. Les cinq variétés retenues sur la base des résultats de l'examen de l'antibiose ont été soumises aux expérimentations sur l'antixénose.

Les résultats montrent que la vitesse relative moyenne de développement M.R.G.R. (= mean relative growth rate) varie entre les variétés (de 0.03191 à 0.05487 $\mu g/\mu g$/jour pour le premier stade larvaire et de 0.3251 à 0.4557 $\mu g/\mu g$/jour pour le troisième stade larvaire) et ne change pas drastiquement à l'intérieur d'une plage étroite des stades de développement de la plante.

Les résultats concernant les essais sur les caractères morphologiques des variétés montrent que les feuilles des deux variétés ayant un M.R.G.R. faible chez le premier stade larvaire portent des épines érigées et acérées pouvant être à l'origine d'une résistance aux insectes. Deux acides hydroxamiques, DIBOA et DIMBOA, ont été trouvés à différentes concentrations dans les plantes de canne à sucre.

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BIOCHEMICAL RESISTANCE IN WILD SPECIES OF GROUNDNUT (Arachis) TO Spodoptera litura (LEPIDOPTERA:NOCTUIDAE)

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Summary

This paper reports on recent results in the identification of the mechanisms of resistance in wild species of *Arachis* to *Spodoptera litura*. Two wild species, *Arachis paraguariensis* and *A. chacoensis*, and the hybrid of *A. chacoensis* x *A. hypogaea* ICGS11 were highly resistant to larvae of *S. litura*, whereas the cultivated groundnut, *A. hypogaea* ICGS11 was highly susceptible. The observed resistance was associated with the presence of several foliar quercetin diglycosides and caffeoylquinic acids which were isolated and determined during this study. The analogous compounds rutin and chlorogenic acid were toxic to the larvae of *S. litura* and the toxicity was dose dependent. The concentration of these compounds in the foliage of the resistant plants was greater than the optimal concentrations of both rutin and chlorogenic acid. The potential use of the identified compounds in groundnut resistance breeding programmes against *S. litura* and other lepidopteran pests of groundnut is discussed.

Introduction

The tobacco armyworm, *Spodoptera litura* (Fab.), is a polyphagous foliage feeding insect which is distributed throughout South and East Asia and Western Australia (Feakin, 1973). In India *S. litura* has been reported as an increasingly important pest of groundnut (Amin & Mohammad, 1980). Wightman & Amin (1988) have attributed this increase in pest status to an increase in post-rainy season cropping resulting in the continuous exposure of the crop to the pest.

The development of cultivars of groundnuts with resistance to *S. litura* has been proposed as an essential aspect of integrated pest management programmes of groundnut in India (Wightman & Amin, 1988). A few cultivars of the cultivated groundnut, *Arachis hypogaea* L., have been shown to tolerate defoliation by *S. litura* (Wightman, Dick, Ranga Rao, Shanower & Gold, 1990) but there are currently no known resistant cultivars. In contrast, all of the wild species of *Arachis* investigated so far have a detrimental effect on the feeding behaviour and development of larvae of *S. litura* (Stevenson, 1992; Stevenson, Blaney, Simmonds & Wightman, in press). Physical characteristics of the leaves have been implicated as factors which are

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partially responsible for the observed resistance, although foliar phytochemicals in aqueous methanol extracts are considered to be the most important contributors to the resistance in wild species of *Arachis* (Stevenson, Padgham, Wightman & Moss, 1992; Stevenson *et al.*, in press).

This paper describes the importance of polar phenolic compounds from the leaves of *Arachis chacoensis* and *A. paraguariensis* in the resistance of these species to *S. litura*, and discusses their potential use in breeding cultivars of *A. hypogaea* resistant to *S. litura*.

Methods

Plant material

Wild and cultivated species of groundnut foliage were provided by the ICRISAT Centre, Hyderabad, India and were selected for insect bioassays and chemical analysis as described previously (Stevenson, 1992; Stevenson *et al.*, in press). The species examined were the two wild species, *Arachis paraguariensis* (Chod. et Hassl.) and *A. chacoensis* (nom. nud.), the susceptible control cultivar *A. hypogaea* L. ICGS11 and the F_1 hybrid of a cross between *A. chacoensis* and *A. hypogaea* ICGS11.

Bioassays

Only larvae of known age and feeding history were used in bioassays. Pre-feeding neonate larvae were selected when less than 6h old. Third stadium larvae were selected 6 to 12h after ecdysis and were deprived of food for 2h before being exposed to the groundnut leaves.

Previous studies have shown that larval growth and behavioural data using excised groundnut leaves provides information which accurately reflects the effect of the unexcised leaves in the field (Stevenson *et al.*, in press). Using excised leaves allows experiments to be conducted under controlled conditions to minimise sample variation, is reproducible and convenient requiring relatively few larvae. One excised leaf of one of the plants was placed in a capped plastic cup with the exposed end of the petiole touching a circle of wet filter paper. One weighed third stadium larva was placed in the cup. Larvae were incubated at 26°C ± 2°C and larvae were re-weighed after 24 and 48 h. Larval weight change was evaluated as a percentage of the original weight. Experiments were replicated 10 times for each species/cultivar.

Rutin and chlorogenic acid are very similar to the compounds isolated from the foliage of the wild species of groundnut and were thus used as representative compounds in insect bioassays. Solutions of known concentration of rutin (Quercetin-3-rhamnosylglucoside) in methanol were poured onto the dry components of a Gram flour/sorghum leaf-based artificial diet (Taneja & Leuschner, 1985) and were left for 24 h to allow the methanol to evaporate. Chlorogenic acid [5-caffeoylquinic acid (IUPAC, 1976)] was added to the artificial diet as a solution

in water. Control diets were set up for both rutin and chlorogenic acid diets to ensure that any solvent effects of evaporating methanol could be evaluated. The treated and control diets were allowed to set in 25 ml plastic cups after which 5 neonate larvae were placed onto the diets. Larvae were incubated at 26°C ± 2°C and after 10 days larvae were weighed and larval development was evaluated as actual larval weight. Each treatment was replicated 10 times.

Chemical analysis

Freeze dried, powdered groundnut foliage (10 g) of each species analysed was extracted in 250 ml of hot methanol for 4 h. The filtered extract was made up to 750 ml with 250 ml of water and 250 ml of ether: ethylacetate (2:1) and the apolar fraction was discarded. The polar fraction was evaporated to give a concentrated extract equivalent to 1 g/ml of plant material in water.

Thin layer and two-dimensional paper chromatography had shown there to be considerable differences between groundnuts in their profiles of flavonoids (Stevenson, 1992). Further analysis and isolation of compounds for determination was carried out using HPLC (Merck-Hitachi pump 6200 with Waters 991 diodearray detection). An isocratic solvent delivery of 16% acetonitrile in 5% acetic acid was used with an end-capped, Lichrosorb RP-8 column (25 cm x 4.6 i.d.).   The concentration of flavonoid glycosides was determined by comparison of peak areas with a 1 mg/ml rutin standard. Compounds were isolated by collecting them manually from the diode array outlet and their structures were determined by standard techniques of flavonoid identification (Markham, 1982).

The analysis of caffeoylquinic acids was conducted using the same apparatus as described above but with the following solvent delivery of methanol (A) and 5% acetic acid (B): 0-10 min, 5%A at 1.5 ml/min to 16%A at 2.0 ml/min; 10-13 min, 16%A at 2.0 ml/min to 25%A at 2 ml/min; 13-16 min, 25%A at 2.0 ml/min to 5%A at 1.5 ml/min. The concentrations of the caffeoylquinic acids in *A. paraguariensis* were determined by comparison with a standard solution of 1 mg/ml chlorogenic acid.

Results

After 24 and 48 h, larvae feeding on the leaves of *A. hypogaea* ICGS11 gained significantly more weight (> 7 times after 48 h) than larvae feeding on the leaves of the *A.chacoensis* x *A. hypogaea* hybrid. Larvae feeding on the hybrid gained significantly more weight than larvae on *A. chacoensis* (Mann-Whitney; $P < 0.05$). Larvae lost significantly more weight (Mann-Whitney; $P < 0.05$) on leaves of *A. paraguariensis* than on *A. chacoensis* (Table 1).

Table 1: The weight change (% ± sem) of 3rd stadium larvae on the leaves of wild and cultivated species of *Arachis* and the F_1 hybrid (*A. hypogaea* ICGS11 x *A.chacoensis*)

Species	Weight change 24 hours		(% ± sem) 48 hours	
A. hypogaea ICGS11	34.4	(9.03)	136.7	(18.33)
A. chacoensis	- 6.4	(1.99)*	- 2.1	(2.90)*
F_1 Hybrid	11.8	(6.01)*	18.8	(7.10)*
A. paraguariensis	-14.6	(4.39)*	no data	

* indicates that the weight change after 24 and 48 h was significantly different to that of *A. hypogaea* ICGS11 (Mann-Whitney; $P < 0.05$)

The distribution and foliar concentration of the major flavonoid diglycosides in the wild and cultivated species are shown in Table 2. There were variations in the flavonoid profiles and concentrations between the foliar extracts of the groundnuts (Table 2). No quercetin diglycosides were detected in *A. hypogaea* ICGS11 which means that they were absent or at extremely low concentrations. The concentration of quercetin diglycosides in *A. paraguariensis* was 1.80 mM and in *A. chacoensis* was 4.60 mM.

Quercetin-3-arabinosylgalactoside and quercetin-3-digalactoside were both absent from the foliage of *A. hypogaea* ICGS11 but were both present in the foliage of *A. chacoensis* and the F_1 hybrid, suggesting that their presence in the hybrid was due to the parent *A. chacoensis*.

Table 2: The distribution and concentration (mM) of major flavonoid glycosides in the foliage of two wild species and one cultivar of *Arachis* and the F_1 hybrid of a cross between *A. chacoensis* and *A. hypogaea* ICGS11

Compounds	*A. hypogaea* ICGS11	*A. chacoensis*	Hybrid	*A. paraguariensis*
Quercetin-3-arabinosylgalactoside	-	3.10	1.35	-
Quercetin-3-digalactoside	-	1.50	0.65	-
Quercetin-3-rhamnosylgalactoside	-	-	-	1.80
Kaempferol-3-rhamnosylgalactoside	-	-	-	1.56
Caffeoylquinic acids	-	-	-	4.00
TOTAL conc Quercetin diglycosides (MmM)	0	4.60	2.00	1.80

- = absent or not detected

Two caffeic acid esters which were identified in *A. paraguariensis* but were absent or not detected in *A. chacoensis* and *A. hypogaea* are also included in Table 2. These compounds have yet to be fully characterised although preliminary structural analysis does indicate that they are caffeoylquinic acids (Stevenson, 1992).

Figure 1 shows the weight of larvae after 10 days feeding on diets containing increasing concentrations of either rutin or chlorogenic acid. Larval growth was significantly lower on all diets treated with rutin and chlorogenic acid than on the control diets. The inhibition of larval growth increased with increasing concentration of rutin or chlorogenic acid in the diet. At a concentration of 3 mM or more there appeared to be little increase in the inhibitory effect of the compounds on the larvae suggesting that this concentration was close to optimal.
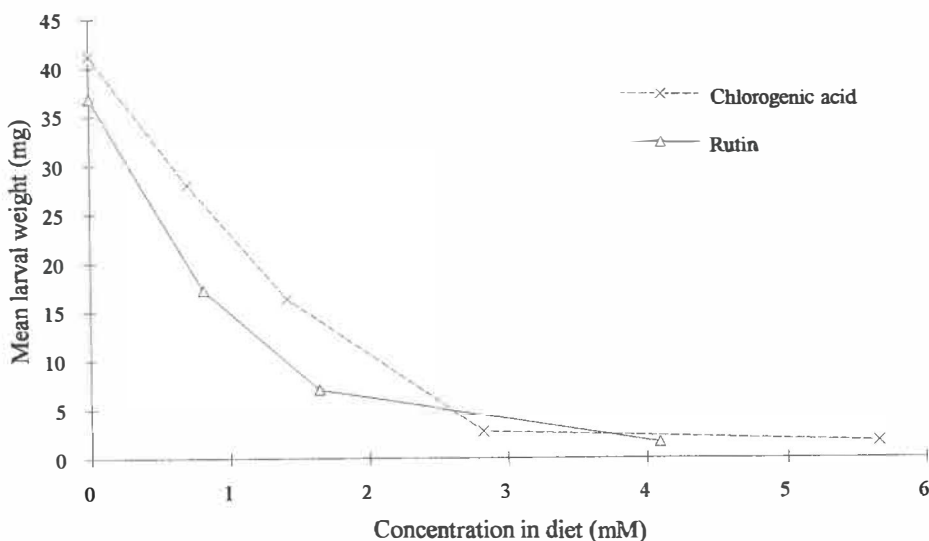
Fig. 1.   Larval weight after 10 days on diets incorporating chlorogenic acid and rutin: a dose response.

Discussion

The potential importance of quercetin-3-diglycosides and caffeoylquinic acids in the resistance of crop plants to attack by Lepidoptera is well established (Shaver & Lukefahr, 1969; Elliger, Wong, Chan & Waiss, 1981; Isman & Duffey, 1982; Duffey & Bloem, 1986; Lindroth & Petersen, 1988; Duffey & Felton, 1991 and references therein). In this study both rutin and chlorogenic acid severely inhibited larval development when presented to larvae at the same concentrations as the equivalent compounds were found in the foliage of the respective plants. This suggests that these compounds are at least partially responsible for the resistance of groundnuts to the larvae of *S. litura*.

This study has also shown that resistance identified in the wild species, *A. chacoensis*, is heritable in that the *A. hypogaea* cultivar ICGS11 was highly susceptible to the larvae of *S. litura* but the foliage of the F_1 hybrid was resistant. Quercetin-3-arabinosylgalactoside and quercetin-3-digalactoside were present in both the F_1 hybrid and *A. chacoensis* which supports the proposed role of these flavonoids in the resistance of the groundnut foliage to the larvae. However, larvae feeding on the foliage of the F_1 hybrid gained significantly more weight than the larvae feeding on the foliage of the resistant wild species *A. chacoensis*. This difference in effect may be accounted for by the fact that the concentration of the flavonoids in the foliage of the F_1 hybrid was lower than in *A. chacoensis*.

The concentration of the quercetin diglycosides in *A. paraguariensis* was marginally lower than that in the F_1 hybrid. However, the foliage of *A. paraguariensis* was more resistant than that of *A. chacoensis*. This difference could be accounted for by the fact that the total foliar concentration of "toxic" compounds in the foliage of *A. paraguariensis*, including both caffeoylquinic acids and quercetin diglycosides, was almost 6 mM; a higher concentration than the total concentration of "toxic" compounds in the foliage of *A. chacoensis*.

The compounds proposed as being responsible for the resistance in wild species of *Arachis* can be identified by relatively simple chromatography. It is conceivable, therefore, that they might have a role as genetic markers of resistance and be used in the selection of resistant progeny from breeding programmes. The genus *Arachis* is diverse and the species within the genus are subdivided into sections based on anatomical characteristics and cross-compatability (Resslar, 1980). Thus hybridisation between species within the same section is possible, e.g. *A. chacoensis* x *A. hypogaea* both of which are grouped into section *Arachis*. However, *A. paraguariensis* is grouped in section *Erectoides* and cannot be hybridised with *A. hypogaea* by conventional breeding methods (Stalker & Moss, 1987). The transfer of characters from some wild species of *Arachis* will thus depend on artificial methods of gene manipulation. The availability of resistance markers e.g. phytochemicals, would provide an appropriate technique for the selection of transformed plant material.

Isman & Duffey (1982) and Duffey & Bloem (1986) reported that the response of the larvae of *Heliothis zea* and *Spodoptera exigua* to rutin and chlorogenic acid was related to the dose in much the same way as the dose response reported in this paper for larvae of *S. litura*. Wiseman, Gueldner, Lynch & Severson (1990) reported that caffeoylquinic acids were responsible for the resistance of centipedegrass to *Spodoptera frugiperda* and Lindroth & Peterson (1988) reported that ingested rutin reduced development and increased mortality of the larvae of *Spodoptera eridania*. *S. exigua*, *S. frugiperda* and *H. zea* are all important pests of groundnuts in addition to *S. litura* (Feakin, 1973). Thus it is likely that the compounds so far identified in extracts of groundnut foliage may have a potential role in the resistance of the crop to a wide spectrum of foliage-feeding insects in different agro-ecological zones.

Résumé

La résistance biochimique d'espèces sauvages de l'arachide (*Arachis spp.*) à *Spodoptera litura (Lepidoptera : Noctuidae)*

Spodoptera litura est devenu, ces dix dernières années, un ravageur de plus en plus important de l'arachide (*Arachis hypogaea* L.) en Inde. Ceci s'explique par les nouvelles techniques permettant la culture de post-mousson, qui, cumulées à la culture traditionnelle en saison des pluies, assurent au ravageur la disponibilité permanente de cette plante-hôte. La résistance inhérente à la plante offre un grand intérêt pour contenir le ravageur et protéger la culture. Même si quelques cultivars d'*A. hypogaea* présentent une certaine tolérance à la défoliation, aucun n'est résistant au ravageur selon les connaissances actuelles. Par des essais de nutrition et de comportement, en champ et au laboratoire, plusieurs espèces sauvages d'*Arachis* ayant un niveau élevé de résistance aux larves de *S. litura* ont néanmoins pu être décelées. Deux espèces, *A. chacoensis* et *A. paraguariensis*, ont été particulièrement étudiées.

Des extraits de feuilles (à 50% méthanol) de ces deux espèces sauvages ont été fournis à des larves nourries sur milieu artificiel; il en est résulté une inhibition significative de leur développement, en comparaison de larves ayant absorbé un extrait de feuilles d'arachide cultivée (*A. hypogaea* TMV2). Les analyses chromatographiques et chimiques des extraits actifs des deux espèces sauvages ont permis d'identifier plusieurs diglycosides de quercetine, ainsi que des acides caffeoylquiniques, qui s'avèrent être soit absents, soit présents à très faibles concentrations dans les extraits analogues des arachides cultivées non résistantes.

A des concentrations identiques à celles constatées dans les feuilles des espèces sauvages, la rutine (diglycoside de quercetine) et l'acide chlorogénique (acide caffeoylquinique) inhibent sévèrement le développement larvaire. Par contre, à des concentrations équivalentes à celles observées dans le feuillage des arachides cultivées, aucune différence n'a été constatée par rapport à la nutrition sur milieu témoin.

Il en est conclu que les composants identifiés pourraient représenter une source de résistance utilisable dans les programmes de sélection de l'arachide, à condition que leur impact environnemental sur les autres méthodes de lutte antiparasitaire et les conditions écologiques locales soient admissibles. L'usage de ces composants comme marqueurs phénotypiques est également discuté.

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RESISTANCE MECHANISMS TO APHIDS IN GROUNDNUTS

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Groundnut production in the semi-arid tropics can be severely limited by diseases and pests. In West Africa, Groundnut Rosette Disease (GRD) is the major virus disease transmitted by the aphid vector, *Aphis craccivora*. Resistance to the groundnut rosette virus (GRV) complex has been identified in a cultivated genotype but breeding from it has proved difficult. An alternative approach which may prove valuable is resistance to the vector. During field trials, with high aphid infestation levels, the incidence of GRD on one cultivated genotype, EC36892 (= ICG 5240) was significantly lower than others.   The genotype developed rosette symptoms after inoculation in laboratory conditions and this suggested that the field resistance of EC 36892 was based on resistance to the aphid vector.   Subsequent observations of behaviour and measurements of aphid development in screenhouse conditions confirmed that EC 36892 offered resistance to the insect particularly in adverse conditions, i.e. heavy rainfall, when higher aphid mortality was recorded on EC 36892 compared with susceptible genotypes.   This paper describes the methodology used to investigate resistance ie. electronic recording, bioassays etc, and evidence that the mechanism of aphid resistance in EC 36892 is linked to high concentrations of a condensed tannin, procyanidin.

Résumé

Les mécanismes de résistance de l'arachide aux pucerons

La production de l'arachide dans des conditions tropicales semi-arides peut être fortement entravée par des maladies et des ravageurs. En Afrique de l'Ouest, la maladie de la rosette de l'arachide (GRD = Groundnut Rosette Disease) est la virose principale transmise par le puceron vecteur, *Aphis craccivora*. La résistance au complexe du virus de la rosette de l'arachide (GRV = Groundnut Rosette Virus) a été identifiée chez un génotype cultivé, mais sa sélection s'est avérée difficile. Une approche plus prometteuse pourrait être la recherche d'une résistance au vecteur. Lors d'essais en champ, avec de fortes infestations de pucerons, un génotype cultivé, EC36892 (=ICG 5240) a été moins marqué par la GRD que les autres.   Après inoculation en laboratoire, ce génotype a développé des symptômes de la rosette, ce qui suggère que la résistance au champ de EC36982 est basée sur la résistance au vecteur.

Les observations ultérieures du comportement et les mesures du développement du puceron en serre ont confirmé la résistance de EC36982 à l'insecte et en particulier lorsque les conditions atmosphériques sont mauvaises, p. ex. fortes pluies, ce qui se traduit par une plus grande mortalité chez les pucerons sur EC36892

que sur les génotypes sensibles.

Les méthodes d'étude de la résistance, p. ex. enregistrements électroniques, essais biologiques, etc., sont décrites.

Le mécanisme de la résistance d'EC36892 au puceron est lié aux fortes concentrations d'un tannin condensé, la procyanidine.

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DETOXIFYING ENZYMES OF THE GRAIN APHID

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Summary

Peroxidase, polyphenol oxidase, N-demethylase, glutathione S-transferase and UDP-glucose transferase activity was found in the grain aphid, *Sitobion avenae*. The first two enzymes in the saliva and the other three in homogenates of whole aphids. With the exception of glutathione S-transferase the activity of these enzymes was lower in aphids fed on moderately resistant wheat cultivars, which contained high concentrations of the cereal allelochemicals: phenolic compounds and hydroxamic acids, than on susceptible cultivars. The main hydroxamic acid - DIMBOA acted as a strong inhibitor of the aphid's detoxifying enzymes at concentrations as low as one hundredth of those that affect the survival and reproduction of the aphid. Some of the enzymes exist as isoenzymes, which may enable them to react with various substrates. The significance of these findings is discussed.

Introduction

For the breeding of cereal cultivars that are resistant to phytophagous insects it is necessary to understand the chemical interaction between these two groups of organisms. It is well known that phytophagous insects are adversely affected by host-plant allelochemicals and show biochemical counter-adaptations. One such biochemical adaptation is enzymatic detoxification (Ahmad, 1986; Ahmad, Brattsten, Mullin & Yu, 1986).

Numerous studies indicate that three groups of allelochemicals: hydroxamic acids (Beck, Dunn, Routley & Bowman, 1983; Bohidar, Wratten & Niemeyer, 1986; Leszczyński & Dixon, 1990), phenolic compounds (Todd, Getahun & Cress, 1971; Dreyer & Jones, 1981; Leszczyński, 1985; Leszczyński, Wright & Bądowski, 1989) and indole alkaloids (Zuniga & Corcuera, 1986; Kanehisa, Tsumuki, Kawada & Rustmani, 1990) are involved in the resistance of cereals to aphids. There are also three groups of detoxifying enzymes in sucking-piercing insects: 1) egzoenzymes, which are secreted in saliva into host-plant tissues, 2) first phase detoxifying enzymes that convert highly toxic lipophilic compounds into water-soluble and less toxic metabolites, 3) second phase detoxifying enzymes that generate water-soluble conjugates, which are easily excreted (Brattsten, 1988; Miles & Peng, 1989).

This paper reports on the activity of two egzoenzymes: peroxidase and polyphenol oxidase, one I phase detoxification enzyme: N-demethylase and two II phase detoxification enzymes: glutathione S-transferase and UDP-glucose transferase in the grain aphid. Changes in the activity of these enzymes in aphids fed on wheat cultivars with different levels of allelochemicals were recorded and the structure and molecular weights of some of these enzymes was determined.

Materials and methods

The grain aphid, *Sitobion avenae* (F.), used in the experiments came from stock cultures kept at the University of East Anglia in Norwich and the Agricultural and Pedagogic University of Siedlce.

Four Polish winter wheat cultivars: two moderately aphid resistant - Grana and Saga and two susceptible - Emika and Liwilla (Leszczyński, 1987) were used in this study.

The allelochemical content of the wheats was determined as described earlier: phenolic compounds (Leszczyński et al., 1989) and hydroxamic acids (Leszczyński, & Dixon, 1990).

Detection of the peroxidase and polyphenol oxidase activity in the saliva of the grain aphid was determined using diets containing pyrogallol and 3,4-dihydroxyphenylalanine as the respective substrates. Activity of the aphid peroxidase and polyphenol oxidase was measured according to Fehrman & Dimond (1967) and Miles (1964), respectively.

Homogenates of whole grain aphids were used to determine the activity of the detoxifying enzymes. Glutathione S-transferase activity was estimated as described earlier (Leszczyński & Dixon, 1992). Activity of the UDP-glucose transferase was determined according to Morello & Repetto (1979). The activity of O-demethylase and N-demethylase, using p-nitroanisole and p-chloro-N-methylaniline as substrates, respectively, was estimated by means of a slightly modified version of the method described by Hansen & Hodgson (1971).

Structure and molecular weights of the glutathione and UDP-glucose transferases were determined after separation of the aphid homogenates by gel filtration followed by SDS-polyacrylamide gel electrophoresis, as described previously (Leszczyński, Matok & Dixon, 1992).

The results given in the Tables were subjected to an analysis of variance followed by Duncan's test.

Results

The moderately resistant cultivars had much higher concentrations of total phenols and hydroxamic acids than the susceptible wheats (Table 1).

Table 1: Content of total phenols (mg/g dry weight) and hydroxamic acids (μM/g fresh weight) in moderately resistant and susceptible cultivars of wheat

Cultivar of wheat	Total phenols (flag leaf)	Hydroxamic acids (seedlings)
Resistant		
Saga	23.85 a	2.68 b
Grana	20.89 b	3.35 a
Susceptible		
Emika	8.73 c	1.89 c
Liwilla	7.06 c	2.12 c

Values not followed by the same letter are significantly different ($P < 0.05$; Duncan's test)

The grain aphid secreted both peroxidase and polyphenol oxidase in it's saliva. The highest activity of salivary peroxidase was found in alates and of polyphenol oxidase in adult apterae (Table 2). Peroxidase and polyphenol oxidase activity was also detected in homogenates of whole aphids. The activity of both enzymes was much lower in aphids fed on the flag leaves of moderately resistant wheat cultivars than on the susceptible cultivar Liwilla (Table 3).

Table 2: The activity of peroxidase (A430/30 min/mg protein) and polyphenol oxidase (A 460-30 min/mg protein) in the saliva of the grain aphid

Developmental stage	Peroxidase	Polyphenol oxidase
Larva	0.050 c	0.034 c
Apterage	0.090 b	0.105 a
Alate	0.115 a	0.070 b

Values not followed by the same letter are significantly different ($P < 0.01$; Duncan's test).

Table 3.   The activity of peroxidase (A 430/30 min/mg protein) and polyphenol oxidase (A 460/30 min/mg protein) in homogenates of grain aphids fed on the flag leaves of moderately resistant and susceptible cultivars of wheat

Cultivar of wheat	Peroxidase	Polyphenol oxidase
Resistant		
Saga	0.21 b	0.10 b
Grana	0.11 c	0.12 b
Susceptible		
Liwilla	0.33 a	0.17 a
Emika	-	-

Values not followed by the same letter are significantly different ($P < 0.05$; Duncan's test)

Activity of the aphid's first phase detoxifying enzyme: N-demethylase, with the exception of the results for aphids fed on Emika, was also higher in aphids fed on susceptible than on moderately resistant cultivars. Using p-nitroanisole as a substrate revealed no significant O-demethylase activity in the grain aphid homogenates.

The activity of the aphid's second phase detoxifying enzymes: glutathione S-transferase and UDP-glucose transferase showed an opposite trend. The activity of glutathione S-transferase, which is in the cytosolic fraction of an aphid homogenate, was much higher in aphids fed on the moderately resistant wheats, and the activity of the UDP-glucose transferase, which is located in the microsomal fraction, was higher in the aphids fed on the susceptible cultivars (Table 4).

Preliminary studies of the structure and molecular weights of the grain aphid's glutathione S-transferase and UDP-glucose transferase, indicate that these enzymes are composed of subunits. The glutathione S-transferase consisted of three subunits, with molecular weights of 28,500, 27,500 and 26,000. Gel filtration and SDS-PAGE revealed that the microsomal fraction contained several forms of the UDP-glucose transferase. The molecular weights of the three most active fractions were estimated to be 68,000; 66,000; and 35,000 (Fig. 1).

Table 4: Activity of the detoxifying enzymes: N-demethylase (pmoles p-chloroaniline/min/mg protein), glutathione S-transferase (nmoles CDNB/min/mg protein) and UDP-glucose transferase (μmoles p-nitrophenol/30 min/mg protein) in homogenates of grain aphid fed on seedlings of moderately resistant and susceptible cultivars of wheat

Wheat cultivar		Enzyme		
		N-demethylase	Glutathione S-transferase	UDP-glucose transferase
Resistant				
	Saga	263.8 ab	153.8 a	0.14 c
	Grana	345.7 a	140.3 b	0.50 b
Susceptible				
	Liwilla	463.0 a	125.9 c	0.61 a
	Emika	70.8 b	111.9 d	0.67 a

Values not followed by the same letter are significantly different ($P < 0.01$; Duncan's test)
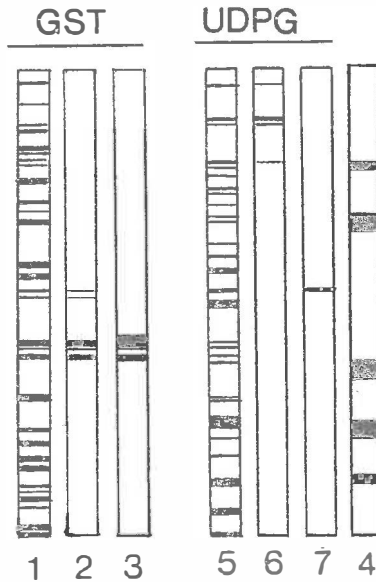
1 - total cytosolic fractions; 2 - GST active fractions; 3 - GST bovine standard;
4 - Sigma Dalton Mark VI Proteins; 5 - total microsomal fractions;
6.7 - UDPGT active fractions

Fig. 1: SDS-PAGE separation of fplc active fractions of the grain aphid gluthathione S-transferase (GST) and UDP-glucose transferase (UDPGT).

Discussion

These results indicate that the grain aphid has a well developed detoxifying enzyme system, which enables it to partially detoxify cereal allelochemicals. The presence in saliva of peroxidase and polyphenol oxidase, which oxidise and polymerize a wide range of allelochemicals enable the aphid to neutralise allelochemicals in host-plant tissues. Thus the first step in the detoxification of allelochemicals occurs to some extent before the food is ingested.

The activity of the other enzymes help aphids further detoxify the allelochemicals contained in the ingested food. As many cereal allelochemicals are water soluble, the enzymes of the second phase of detoxification may be important in their metabolism (Ahmad, 1986). Our studies showed a high activity of II Phase detoxifying enzymes: glutathione S-transferase and UDP-glucose transferase, in grain aphid tissues. With the exception of glutathione S-transferase the enzymes showed a much lower level of activity in aphids fed on resistant than on susceptible cultivars. This suggests that the higher concentrations of the allelochemicals present in moderately resistant cultivars of wheat inhibit the activity of the detoxifying enzymes. The nature of the inhibition is still not clear but it has been shown in vitro that DIMBOA markedly inhibits the activity of glutathione S-transferase and UDP-glucose transferase (Leszczyński & Dixon, 1992; Leszczyński et al., 1992). It is of considerable interest that the strong inhibitory action of DIMBOA on aphid enzymes was observed at concentrations one hundredth of that which has a deterrent and/or antibiotic effect on the aphid.

The possible absence of O-demethylase in the grain aphid is particularly interesting because the cereal allelochemicals that have -OMe groups, e.g. DIMBOA, tricin and ferulic acid, are more toxic for cereal aphids than their non methoxyl derivatives (Dreyer & Jones, 1981; Zuniga, Argandona, Niemeyer & Corcuera, 1983; Leszczyński, Warchol & Niraz, 1985). This implies that the grain aphid might not be well endowed to deal with such allelochemicals.

That the grain aphid's detoxifying enzymes exist as isoenzymes, possibly with different substrates specificities, may enable it to detoxify a wider range of cereal allelochemicals. Further research is needed to determine the chemical interaction between cereals and aphids at the molecular level.

Résumé

Les enzymes de détoxication chez le puceron vert de l'avoine

Pour sélectionner et cultiver des variétés de céréales résistantes aux ravageurs et/ou maladies, quelques connaissances des interactions chimiques entre les céréales et ces groupes d'organismes sont nécessaires. Il est bien connu que les insectes phytophages développent des adaptations biochimiques aux substances alléliques de leur plante hôte, dont la détoxification enzymatique.

De nombreux travaux font état des substances allélochimiques de plantes (p. ex. acides hydroxamiques, composés phénoliques et alcaloïdes indoliques) impliquées

dans la résistance des céréales aux pucerons. Les trois groupes les plus importants des enzymes de détoxification chez les insectes piqueurs-suceurs sont: 1) des egzoenzymes sécrétés par la salive dans les tissus de la plante hôte, 2) des enzymes de détoxification de la première phase (soit des monoxygénases à plusieurs substrats dépendant du cytochrome P-450) et 3) des enzymes de détoxification de la deuxième phase, qui s'attaquent aux molécules relativement plus solubles à l'eau.

Ici est rapportée l'activité des péroxydase, polyphénol oxydase, O-demethylase, N-demethylase, glutathion S-transferase et UPD-glucose transferase chez le puceron vert de l'avoine. Les modifications de l'activité de ces enzymes chez les pucerons se nourrissant de variétés de blé qui contiennent différentes concentrations de substances allélochimiques sont décrites. Les structures et les poids moléculaires de certains de ces enzymes ont été déterminés.

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HOST PLANT ACCEPTANCE BY APHIDS: AN EPG ANALYSIS

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Summary

Aphids spend a considerable time probing and walking on a plant before accepting it as a host; acceptance here is defined as reaching a phase of sustained ingestion from a sieve element. An average period of 2-5 h spent in this activity is not exceptional. However, during stylet penetration known as a probe, the sieve elements can be reached within 10-20 min or even earlier. The questions arise as to why aphids need all this time, and why are they so hesitant after reaching a sieve element of an acceptable host plant. Many different principles have been suggested as the basis of host plant selection and the principles relating to chewing insects have often been used as a model. The comparability of aphids with chewing insects in respect of food plant selection will be discussed in the light of recent results obtained by transmission electron microscopy (TEM) and from using the electrical penetration graph (EPG) technique.

Introduction

Aphid probing or stylet penetration behaviour is believed to be needed: (1) in order to acquire appropriate chemical information relating to internal gustation (Wensler, 1977) and (2) to reach the sieve elements in the vascular bundle for actual feeding. Transmission electron microscopy (TEM) has shown that *Aphis fabae* (Scop.) punctures many, if not all, the plant cells along the stylet track in its host *Vicia faba* (L.) (Hogen Esch & Tjallingii, 1990; Tjallingii, 1990b). The stylet path, however, remained completely extracellular and cells generally survived these punctures. It was interesting that 11 sieve elements were reached and punctured before sap feeding started from the last one. This sieve element had evidently been punctured earlier, as could be reconstructed on the basis of salivary sheath tracks. These data suggest that once a sieve element is reached its acceptability is not necessarily a foregone conclusion. It remains unclear from these studies whether the sieve element suitability was due to plant reactions to aphid activities, to motivational factors, or to intrinsic behavioral properties.

Evidence of sap ingestion could not be inferred from histological studies but the electrical penetration graph (EPG), recorded from this aphid previous to TEM

processing, indicated it unequivocally. It has been shown that EPG's can record aphids activities during plant penetration (Tjallingii, 1988). Thus, mechanical work, brief punctures of plant cells (potential drops (pd)), saliva secretion, active and passive ingestion and other activities can all be derived from EPGs. Wave-form E2, indicative of sap ingestion from a sieve element, has been shown to last for more than 1.5 h before stylectomy. Before reaching the ultimate E2, several shorter E wave-form periods were identified, each containing a good deal of E1 (Tjallingii, 1990a). This E1 wave-form has not been related to any aphid activity as yet. As E2 is restricted to sieve elements, any E1 preceding an E2 without interruption of the intracellular potential level should also have come from the same element. In fact E2 is always preceded by E1 and typically for 30-40 s.

Hogen Esch & Tjallingii (1992) showed that also when E1 lasted longer than the typical 30-40 s and was not followed by E2, the stylets were inside a sieve element. However, from the same study it became clear that sieve elements can be punctured in the same brief manner as is shown in all other types of cells. Therefore, three different EPG phenomena can reflect sieve element punctures: a pd (usually 5 s), an E1 wave-form (usually longer than 5 s) and an E1-E2 sequence during which E1 typically lasts for 30-40 s and E2 which may last for minutes, hours, or days.

The aim of the present study was to investigate how representative the situation described above is for this and other aphid-plant combinations. Using EPG recording this was studied without the very time consuming TEM, so that a reasonable number of replicates could be tested.

Materials and methods

A. fabae was reared on broad beans (cv. Drie Maal Wit) with a photoperiod of 16 h and at 20°C. Eight apterous adults were wired and connected simultaneously to the EPG amplifier. During an 8 h period EPG's of about 15 aphids per treatment, on bean stems and leaves were stored on a computer hard disk. For this acquisition, successive EPG analysis the STYLET 2.0 computer program in ASYST^{RM} was developed and used. Starting time and duration of wave-form patterns were scored. Only separate penetrations (PEN) and sieve element puncture (E) data are presented here. Within the sieve element punctures distinction was made between the first (1st E) and later punctures. And within these, the first longer than 10 min (E > 10 min) and any longer than one hour (E > 1 h) were distinguished. Also, the number and duration of separate penetrations were scored.

Results

Penetration behaviour appeared to be composed of a series of successive penetrations (PEN) of increasing duration separated by decreasing non-penetration periods (Table 1). As time progresses, duration of sieve element puncture within penetrations increased (E/PEN) whereas the number of new punctures (new E) increases upto the fourth hour and then decreases gradually. This implies that the duration of each puncture often lasted even more hours.

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Table 1:   Mean numbers (#), durations (h) or relative time (%) spent by *A. fabae* on bean leaves in each of the 8 successive hours.

hour		1	2	3	4	5	6	7	8
PEN	(#)	4.6	2.3	2.4	1.2	1.5	0.6	0.3	0.5
PEN	(h)	0.75	0.87	0.87	0.91	0.93	0.95	0.97	0.96
E/PEN	(%)	0.5	1.9	4.5	18.2	43.5	65.1	64.6	78.5
new E	(#)	0.1	0.3	0.2	0.8	0.5	0.5	0.5	0.5

There is no reason to consider the stem as a less (1st E) or more (E > 10 min) suitable feeding site. On leaves it takes somewhat longer to reach the sieve elements (Fig. 1) than on stems but this is not statistically significant. Most, but not all, E > 10 min lasted more than one hour.
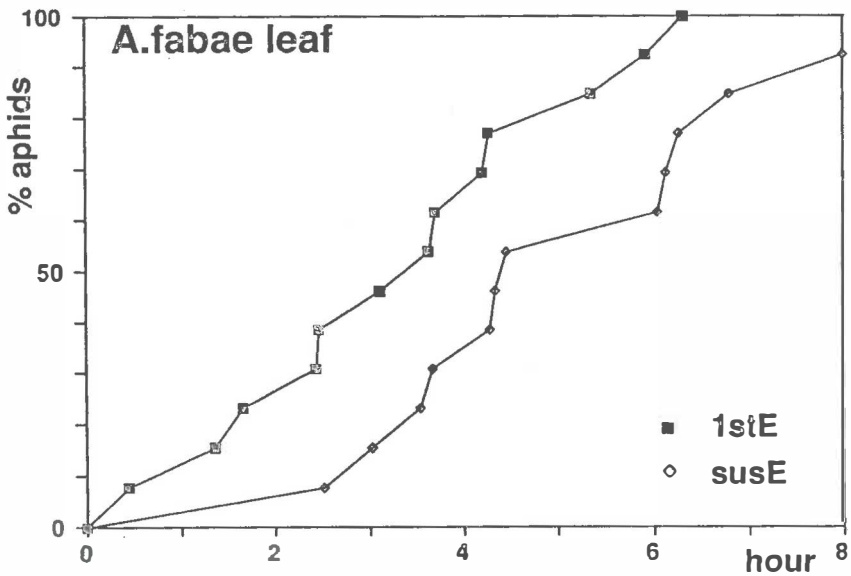

Fig. 1:   Percentage of aphids that reached a sieve element (1stE) and started sustained phloem ingestion (susE) on leaves. Though the 1stE is not the real first sieve element puncture, it clearly demonstrates the considerable delay.

Discussion and Conclusions

It appears that the time needed for aphids to accept a plant for feeding takes many hours. Other aphid species showed the same or even significantly longer periods (Tjallingii & Mayoral, 1992). It is unlikely that wire effects or other artefacts have biassed these results as they have been confirmed by control experiments recording honeydew secretion by free aphids. Many probes were needed and within these probes a number of sieve element punctures generally preceded sustained phloem sap feeding; this may be used as an indication of plant acceptance as discussed earlier (Tjallingii, 1990b), especially, since most E > 10 min lasted longer than 1 h.

Using the 1st E here as a criterion for reaching a sieve element may overestimate this parameter as has been indicated earlier (Hogen Esch & Tjallingii, 1992). As a consequence, the delay between reaching a sieve element and sustained ingestion is underestimated. The conclusion, that the delay between reaching and acceptance of the sieve elements is considerable, remains unaltered.

The earlier morphological evidence for such a delay (Tjallingii, 1990b) is supported by this study. Why these early E1 or short E1-E2 punctures do not lead to sap feeding remains unclear. Evidence that the same sieve element, rejected during an early puncture, was apparently accepted later, seems to support the hypothesis that a possible plant reaction may provide important cues for the real acceptance of the phloem sap. This is an attractive explanation but, at present, it remains mere speculation.

Résumé

L'acceptation de la plante hôte chez les pucerons

Les pucerons usent un temps considérable à tester et à se déplacer sur leur propre plante hôte avant de l'accepter (une période moyenne de 2 à 5 h n'est pas exceptionnelle). On parle d'acceptation quand il y a ingestion soutenue à partir d'une cellule criblée du phloème. Néanmoins, pendant un sondage, c'est-à-dire une pénétration du stylet, les cellules criblées sont parfois atteintes en 10-20 min ou même plus rapidement. Pourquoi alors les pucerons ont-ils besoin d'autant de temps et pourquoi sont-ils si "hésitants", même après avoir touché une cellule criblée appartenant à leur propre plante hôte?

Un grand nombre de principes différents ont été avancés pour déterminer la base de cette sélection de la plante hôte. Souvent ils sont fondés sur ceux utilisés comme modèles pour les insectes broyeurs. La comparaison des modalités que présentent ces deux groupes d'insectes en choisissant leur plante hôte, à la lumière des résultats récents obtenus par la microscopie électronique par transmission (TEM = Transmission Electron Microscopy) et par la technique des enregistrements électriques de pénétration (EPG = Electrical Penetration Graph) est discutée.

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THE RELATIONSHIP BETWEEN THE CHEMICAL COMPOSITION OF SUGAR BEET LEAVES AND THE DEVELOPMENT OF BLACK BEAN APHID Aphis fabae

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Summary

In the years 1986 to 1988 the development of black bean aphid *Aphis fabae*, was investigated on eight sugar beet cultivars in a field cage of the insectary type. During the same period the chemical composition of beet leaves (reducing sugars, sucrose, total and protein nitrogen, and total phenols) was compared. It was found that two measures of aphid development, i.e. total aphids index and larvae index for individual cultivars differed significantly in every season. The highest indices of aphid development were recorded for diploid cultivars (A.Janasz-AJ 3 and A.Janasz-AJ 4) and polyploids (AJ-Poly 1 and AJ-Polycama). The leaves of these cultivars always contained more sucrose and nitrogen, especially in the form of protein but less phenols than the leaves of mono-germ cultivars (PN-Mono 1,3) which supported the least aphid development. The cultivars were consistently different in their content of the various compounds analysed. There were significantly positive correlations between both the total aphids index and larvae index and the sucrose content in leaves, the % of sucrose in total sugars, and the % of protein in the form of nitrogen. These correlations confirmed the fact that the chief stimuli for aphid feeding on sugar beet are sucrose and protein substances.

Introduction

It has been shown in previous studies (Luczak & Gabrys, 1990) that sugar beet (*Beta vulgaris* L.) cultivars differ in their susceptibility to the invasion and direct feeding of black bean aphid (*Aphis fabae* Scop.). These investigations also indicated that under field conditions the dynamics of aphid development on beet cultivars was different.

These earlier studies suggested the existence of differences in the chemical composition of the leaves of sugar beet cultivars. To investigate this possibility both *Aphis fabae* development and the chemical composition of leaves were analysed in experiments carried out in an insectary over three years. The content of reducing sugars, sucrose, nitrogen and phenol compounds were determined.

Sugars and nitrogen are the main stimuli in the feeding of aphids (Maltais & Auclair, 1962; Beck, 1965; Srivastava & Auclair, 1971). Moreover they play an important role in the processes of growth and development of many aphid species (Harrewijn, 1970; Mittler, Dadd & Daniels, 1970; van Emden & Bashford, 1971; Malik, 1989; Ciepiela, 1991). Apart from the constituents mentioned above which largely determine the nutritional value of the plants for aphids other chemical substances of a repellent nature are also present in plants (Erlich & Raven, 1967; Mc Key, 1974; Seigler & Price, 1976). These are secondary plant substances which have a negative effect on aphid development. Amongst these, phenols are particularly toxic (Todd, Getahun & Cress, 1971; Bernays & Chapman, 1977; Leszczynski, Warchol & Niraz, 1985).

Experiments were undertaken to answer the question whether variation in *Aphis fabae* development was related to differences in the biochemical composition of sugar beet cultivars.

Materials and methods

The life history of black bean aphid was investigated on eight sugar beet cultivars AJ-Poly 1, AJ-Polycama, A.Janasz-AJ 3, A.Janasz-AJ 4, PN-Mono 1, PN-Mono 3, PN-Mono 4, Annomono 1 in the years 1986-1988. The observations were carried out in a field cage of the insectary type at the Mydlniki Agricultural Experimental Station. Single sugar beet plants were grown in plastic pots in the cage. After the appearance of the first *Aphis fabae* colonies on beets grown in an open field the pot plants were isolated with bolting cloth. One wingless parthenogenetic individual was then placed on each plant in the cage. These wingless aphids were collected in the open field and transferred to beet leaves. Ten plants infested with insects and 10 control plants (without aphids) of each cultivar were used for chemical analysis. Observations on *Aphis fabae* development were carried out at 5 - 6 days intervals until they completed their development. On each occasion the numbers of adult insects and larvae produced were counted. Pest development was determined using the so-called aphids index, i.e. mean total number of aphids per plant and the number of larvae produced per plant.

In the period of maximum infestation of insects the leaves of the test cultivars were sampled. Immediately after sampling the material for chemical analysis was dried at 105°C. It was then ground and stored in glass containers. The concentrations of reducing sugars (glucose, fructose), non-reducing sugars (sucrose) as well as total and protein nitrogen were assessed in these samples: reducing sugars were estimated following the colorimetric ferricyanide procedure according to Ting (1956) and Furnholmen, Windefordner, Knapp & Dennison (1964). The sucrose content was determined using the same method after inversion with 25% hydrochloric acid. Total nitrogen was established by the Kjeldahl method and protein nitrogen according to the same procedure with the exception that, before ashing a sample, all soluble nitrogen fractions were removed with boiling acetic acid solution. Phenol content was examined in fresh leaves. Immediately after harvest, phenols were extracted from samples with hot re-distilled water. 100 ml of the extract was collected from 10 g of fresh leaves. The sum of total phenols was determined using

the Folin-Ciocalteu reagent according to the procedure described by Johnson &
Schaal (1957) and modified by Seevers & Daly (1970).

All the chemical analyses were performed using three replicates. Significant
differences between cultivars were determined by the Duncan's test. The
relationships between *Aphis fabae* development and the chemical composition of
leaves were described using correlation co-efficients "r".

Results and discussion

The pattern of black bean aphid development varied on the different cultivars.
In each year of the investigations the greatest increase in aphid population was
observed on two diploid cultivars A.Janasz-AJ 3 and A.Janasz-AJ 4. This was
confirmed by the large indices, i.e. total aphids index and larvae index (Table 1). The
aphid also bred successfully on the polyploids AJ-Poly 1 and AJ-Polycama.

Table 1: Development of black bean aphid on sugar beet cultivars in field cages
in 1986 - 1988

Cultivar	Total aphids index			Larvae index		
	1986	1987	1988	1986	1987	1988
AJ - Poly 1	110.5	260.1	123.2	87.6	181.8	95.4
AJ - Polycama	157.2	159.5	114.6	134.8	78.6	83.2
A.Janasz - AJ 3	384.0	163.2	216.6	313.7	105.8	170.2
A.Janasz - AJ 4	196.5	360.9	188.6	160.3	189.2	148.2
PN - Mono 1	117.2	6.0	54.4	86.6	2.0	40.4
PN - Mono 3	51.5	6.0	49.8	32.5	2.0	36.4
PN - Mono 4	159.7	139.4	105.4	87.9	66.0	83.0
Annomono 1	126.0	140.6	119.6	101.7	88.8	91.0
L.S.D. ($P = 0.05$)	143.2	225.6	142.8	97.8	86.5	98.2

The poorest *Aphis fabae* development was found on the mono-germ cultivars PN-
Mono 1 and PN-Mono 3. The smallest numerical indices of aphid development were
recorded on the latter in the three years. In 1987, particularly, low values for the
total aphids index and larvae index were observed. These results suggest clearly that
the various cultivars affect larval growth and the physiology of black bean aphid in
different ways. It appears that mono-germ cultivars of sugar beet have the most
negative affect on larval development of this pest.

Chemical analyses indicated the presence of significantly different concentrations of sugars, nitrogen compounds and total phenols in the cultivars tested (Table 2). Differences were particularly evident in the content of sucrose, nitrogen (especially in the form of protein) and phenolic compounds when a comparison was made between the poly-germ cultivars (polyploids: AJ-Poly 1, AJ-Polycama and diploids: A.Janasz-AJ 3, A.Janasz-AJ 4) and the mono-germ cultivars (PN-Mono 1, 3, 4 and Annomono 1).

Table 2: Content of sugars, nitrogen and phenol compounds in leaves of sugar beet cultivars during the development of black bean aphid

Cultivar	Year	Sugars (% dry weight)		Nitrogen (% dry weight)		Phenols (μg/g, fresh weight)
		reducing	sucrose	total	protein	
AJ-Poly 1	1986	4.74	3.56	3.75	3.06	380.1
	1987	7.02	2.84	3.95	3.02	315.5
	1988	7.14	3.24	4.33	3.71	390.0
AJ-Polycama	1986	8.74	2.34	4.34	3.48	420.2
	1987	7.68	2.03	4.02	2.95	336.5
	1988	8.86	1.98	4.18	2.98	440.3
A.Janasz-AJ 3	1986	5.07	2.13	3.62	2.83	280.2
	1987	7.21	2.64	3.90	2.95	380.6
	1988	7.84	2.37	4.54	3.82	360.5
A.Janasz-AJ 4	1986	4.73	3.22	4.05	3.33	370.2
	1987	5.86	3.24	4.27	3.69	290.0
	1988	6.22	2.73	4.13	3.19	330.5
PN-Mono 1	1986	7.11	1.65	4.05	2.75	345.5
	1987	6.36	1.40	3.68	2.69	540.0
	1988	9.14	1.15	4.21	3.31	445.3
PN-Mono 3	1986	7.00	1.77	4.13	3.23	346.7
	1987	6.66	1.53	3.87	2.85	565.0
	1988	7.12	1.36	4.06	2.71	455.5
PN-Mono 4	1986	4.47	2.44	3.87	2.86	470.0
	1987	5.33	2.36	4.05	2.62	385.6
	1988	6.85	1.92	4.11	3.15	420.2
Annomono 1	1986	7.19	1.34	3.63	2.86	450.5
	1987	7.98	1.14	3.87	2.86	370.3
	1988	8.84	1.24	4.08	3.45	410.0
L.S.D.($P = 0.05$)		0.78	0.45	0.15	0.11	17.6

In practically every year the poly-germ cultivars (characterised by greater *Aphis fabae* population increase) contained more sucrose and nitrogen compounds but less phenols than the mono-germ cultivars. The above data suggests that the successfull development of black bean aphid on sugar beet depends on a high content of sucrose and nitrogen compounds in leaves; these appear to be the main stimuli for insect feeding. The important role of these two constituents is supported by the highly significant correlations between the indices of aphid development and the content of sucrose and its percentage as total sugars as well as the concentration of protein nitrogen as % of total nitrogen (Table 3). The role of sucrose, the most important stimulus for aphid feeding, was determined in previous studies (Beck, 1965; Srivastava & Auclair, 1971). Furthermore Kieckhefer & Derr (1967); Mittler *et al.*, (1970) and

Table 3: Relations between the development of black bean aphid and the content of sugars, nitrogen and phenol compounds in sugar beet leaves (means for cultivars and years)

Component / Aphid index	Correlation co-efficient $r_{exp.}^{x}$
Sucrose / Total aphids	0.507
Sucrose / Larvae	0.447
Sucrose as % total sugars / Total aphids	0.508
Sucrose as % total sugars / Larvae	0.439
Protein nitrogen as % total nitrogen / Total aphids	0.396
Protein nitrogen as % total nitrogen / Larvae	0.427
Protein: Sugars / Total aphids	0.397
Total phenols / Total aphids	- 0.390

[x] - correlation statistically significant
$r_{theor.}$ $(P = 0.05) = 0.389$

Srivastava & Auclair (1971) proved that high concentrations of sucrose in artificial solutions increase fecundity and survival of aphids. Nitrogen compounds appear to be important for aphid growth as indicated by: Harrewijn, 1970; van Emden & Bashford, 1971; Malik, 1989 and Ciepiela, 1991.

Quantitive differences between cultivars in their content of total phenols and statistically significant negative correlations between the level of these compounds and the total aphids index ($r = - 0.390$) confirm the toxic effects of these constituents on *Aphis fabae*. The harmful effects of phenolic compounds were demonstrated in earlier investigations concerning cereal aphids (Todd *et al.*, 1971; Leszczynski *et al.*, 1985).

Résumé

La relation entre la composition chimique des feuilles de betterave sucrière et le développement du puceron noir de la fève *Aphis fabae*

De 1986 à 1988, le développement du puceron noir de la fève a été étudié sur 8 cultivars de betterave sucrière dans une enceinte extérieure (type insectarium). Parallèlement la composition chimique des feuilles de betterave (sucres réducteurs, sucrose, azote total et azote protéinique et phénols totaux) a été comparée. Il a été observé que deux mesures pour le développement des pucerons, soit l'index de l'ensemble des pucerons et l'index des larves diffèrent chaque saison de manière significative pour chaque cultivar pris individuellement. Les indices de l'ensemble des pucerons les plus élevés ont été observés chez les cultivars diploïdes (A.Janasz-AJ 3 et A.Janasz-AJ 4) et polyploïdes (AJ-Poly 1 et AJ-Polycama). Les feuilles de ces cultivars contenaient toujours plus de sucrose et d'azote, spécialement sous forme de protéine, mais moins de phénols que les feuilles des cultivars monogermes (PN-Mono 1,3) qui offraient les conditions les plus mauvaises au développement des pucerons. Les analyses des teneurs en divers composés chez les cultivars ont fourni des différences cohérentes. Il existe une corrélation positive entre les deux indices de développement des pucerons et la teneur en sucrose des feuilles, le % de sucrose dans le sucre total et le % de protéine sous forme d'azote. Ceci confirme que le sucrose et les substances protéiques sont les stimuli-clés de la nutrition des pucerons sur betterave sucrière.

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THE ROLE OF CHEMICAL FACTORS IN SUGAR BEET RESISTANCE TO INVASION BY BLACK BEAN APHID Aphis fabae

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Summary

The differences in the content of green (chloropyll a + b) and yellow-orange (carotenoids) pigments in leaves of 8 sugar beet cultivars and their effect on *Aphis fabae* invasion were investigated in 1989 and 1990. The largest numbers of winged and wingless aphids as well as attacked plants were observed on two diploid cultivars A.Janasz-AJ 3 and A.Janasz-AJ 4. The greatest amounts of yellow-orange and large quantities of green pigments were found in the leaves of these cultivars. The severe aphid infestation was also observed on two polyploid cultivars AJ-Poly 1 and AJ-Polycama. These cultivars were characterised by a high content of yellow-orange pigments in leaves and the high ratio of these pigments to green ones. The fewest winged aphids and the smallest degree of plant infestation were found on the mono-germ cultivars PN-Mono 1 and PN-Mono 3. During the two-year investigation these cultivars were characterised by the lowest content of green and yellow-orange pigments in leaves. The influential role of yellow-orange pigments in the *Aphis fabae* invasion was confirmed by the statistically significant and positive correlation co-efficients between the level of carotenoids in leaves and the percentage of infested plants as well as the number of insects per plant.

Introduction

Black bean aphid (*Aphis fabae* Scop.) is one of the most damaging pests of sugar beet, *Beta vulgaris* L. Aphid feeding causes considerable reduction in the assimilation area available in sugar beet plants. In the case of aphid invasion of young plants this reduction may be as high as 92% (Hurej, 1991). The diminution of assimilating tissues eventually results in a decrease of crop fresh weight and root sugar content. The sugar decrease amounts to 4 - 25% depending on the cultivar (Luczak & Gabrys, 1990). A reduction in *Aphis fabae* damage could be achieved by growing more resistant cultivars, i.e. those which are less infested by winged migrants or those which restrict reproduction and development of this insect.

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The invasion of host plants by winged migrants is largely associated with the stimulation of visual receptors (Kennedy, Booth & Kershaw, 1961). One of the main stimuli affecting this process is plant colour. The results of many investigations indicated the positive phototaxis of aphids towards the green-yellow part of the spectrum (Kennedy *et al.*, 1961; Kring, 1967; Coon & Pepper, 1968). This means that concentrations of particular groups of pigments: green, yellow, orange may play an important role in the process of aphid invasion of plants (Leszczynski, Warchol & Niraz, 1985).

The aim of this study was to determine the effect of the content of green (chlorophyll a + b) and yellow-orange pigments as well as their interrelation with black bean aphid invasion of various sugar beet cultivars.

Materials and methods

In the years 1989 and 1990 *Aphis fabae* infestation of sugar beets was investigated in field experiments. Eight sugar beet cultivar were tested: AJ-Poly 1, AJ-Polycama, A.Janasz-AJ 3, A.Janasz-AJ 4, PN-Mono 1, PN-Mono 3, PN-Mono 4, Annomono 1 which were of various degrees of polyploidy as well as genetically mono- and poly-germ. The investigations were carried out in plots at the Agricultural Experimental Station, Agricultural University, Mydlniki near Cracow. The experiments were carried out using a randomized block design and four replicates. The area of a single plot was 24 m^2.

Aphis fabae invasion was determined by recording the numbers of aphids and the percentages of infested plants. The numbers of insects were estimated on one hundred plants chosen at random from each plot. The selected plants were marked at the time of the appearance of the first migrants on beets. Observations were made once a week until aphids had completely disappeared. On each occasion all leaves on the marked plants were inspected and the total numbers of aphids, winged migrants as well as infested plants were recorded.

The infestation of cultivars was described by deriving means (obtained from seven observations in 1989 and from six in 1990) for four parameters: the percentage of attacked plants by all aphids, by winged migrants, the total number of insects per plant, and the number of winged migrants per plant. Significant differences were determined using the Student's "t" test.

In parallel with the entomological observations, the estimation of green (chlorophyll a + b) and yellow-orange (carotenoids) pigments in leaves was carried out. The chemical analyses were performed in the first ten days of July, i.e. after the maximum appearance of *Aphis fabae* on beets. Pigment contents were determined using fresh leaves according to the method of Lichtenthaler & Wellburn (1983). The leaf samples were taken from 20 plants of each cultivar (5 x 4 replicates). The absorption of scetone solutions was measured at the following wavelengths: chlorophyll a - 663, chlorophyll b - 646, carotenoids - 470 nm. The relationships between the *Aphis fabae* infestation and the content of pigments in leaves were assessed using linear correlation co-efficients "r".

Results

The numbers of *Aphis fabae* and the percentage of attacked plants were higher in 1989 than in 1990. In 1989 the aphid invasion of beets started on 9 June and lasted until 20 July. The maximum numbers of insects and the largest percentages of infested plants were observed on 27 June. Values for the various cultivars ranged from 92.6 - 422.1 individuals/plant and 58.2 - 92.0% of invaded plants. During this period and during the whole period of observation the least infested plants by all aphids and winged migrants as well as the smallest number of insects per plant were found on the mono-germ cultivars PN-Mono 3, PN-Mono 1 and PN-Mono 4 (Table 1). The lowest percentage of attacked plants but the highest number of aphids per plant were observed on the cultivar Annomono 1.

Table 1:   Infestation of sugar beet cultivars by black bean aphid, at Mydlniki in 1989 (means of seven observations)

Cultivar	Plant infestation (%) by aphids		Number of aphids per plant	
	total	winged	total	winged
AJ - Poly 1	45.7	17.7	394.1	56.3
AJ - Polycama	42.3	15.1	441.7	63.1
A.Janasz - AJ 3	48.9	19.2	933.1	133.3
A.Janasz - AJ 4	54.2	20.7	434.7	62.1
PN - Mono 1	36.0	9.0	315.0	45.0
PN - Mono 3	28.5	8.0	268.8	38.4
PN - Mono 4	40.1	13.4	359.1	51.3
Annomono 1	38.4	9.4	1033.2	147.6
L.S.D.(P = 0.05)	8.1	3.8	238.4	33.2

The two diploid cultivars A.Janasz-AJ 3 and A.Janasz-AJ 4 as well as polyploids AJ-Poly 1 and AJ-Polycama were the most preferred by black bean aphid.

In 1990 a slightly later and less numerous *Aphis fabae* invasion of beets was observed. It began in the third week of June and lasted until the end of July. In this year both the maximum (recorded on 11 July) and the average (from six observations) records of infestation were slightly lower but differed significantly for the various cultivars (Table 2). The relative degrees of infestation of the cultivars was similar to that noted in 1989.

In both years the content of leaf pigments differed significantly between cultivars (Table 3). In 1989 the lowest levels of green pigments but highest levels of yellow-orange pigments were recorded. In both years the highest amounts of carotenoids and chlorophylls were observed in the leaves of the diploid cultivars A.Janasz-AJ 4 and A.Janasz-AJ 3.

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Table 2: Infestation of sugar beet cultivars by black bean aphid, at Mydlniki in 1990 (means of six observations)

Cultivar	Plant infestation (%) by aphids		Number of aphids per plant	
	total	winged	total	winged
AJ - Poly 1	41.2	13.1	259.8	16.2
AJ - Polycama	41.9	13.0	142.8	15.6
A.Janasz - AJ 3	40.3	18.0	282.6	17.4
A.Janasz - AJ 4	39.0	20.1	298.2	12.0
PN - Mono 1	21.4	4.9	229.2	10.2
PN - Mono 3	24.5	4.2	134.4	8.4
PN - Mono 4	28.5	8.9	142.8	9.0
Annomono 1	22.5	3.7	312.6	25.2
L.S.D.(P = 0.05)	6.7	5.2	57.4	5.7

Table 3: Content of leaf pigments (mg/g of fresh weight) in sugar beet cultivars in 1989-1990

Cultivar	Chlorophylls a + b		Carotenoids		Carotenoids / Chlorophylls a + b	
	1989	1990	1989	1990	1989	1990
AJ-Poly 1	1.195	1.196	0.272	0.241	0.228	0.202
AJ-Polycama	1.205	1.229	0.269	0.233	0.223	0.189
A.Janasz-AJ 3	1.295	1.451	0.300	0.240	0.231	0.165
A.Janasz-AJ 4	1.570	1.650	0.333	0.262	0.212	0.159
PN-Mono 1	1.050	1.104	0.231	0.222	0.220	0.201
PN-Mono 3	1.080	1.154	0.233	0.214	0.215	0.185
PN-Mono 4	1.355	1.411	0.268	0.222	0.198	0.157
Annomono 1	1.110	1.379	0.249	0.241	0.224	0.175
L.S.D(P=0.05)	0.084	0.078	0.026	0.024	0.022	0.020

Polyploids AJ-Poly 1 and AJ-Polycama were also characterised by a high content of yellow-orange pigments and the high ratio of carotenoids to chlorophylls. The lowest level of green and yellow-orange pigments was found in the mono-germ cultivars PN-Mono 1 and PN-Mono 3.

A comparison of the correlation co-efficients "t" showed that yellow-orange pigments played the most important role in *Aphis fabae* invasion of sugar beet

(Table 4). This was confirmed by the high positive and statistically significant relationships between the four parameters of aphid invasion of beets and the carotenoid content in leaves.

Table 4:   Relations between the infestation of sugar beet by black bean aphid and the content of leaf pigments (means for cultivars and years)

Pigment / Index of infestation	Correlation co-efficient $r_{exp.}$ [x]
Chlorophylls a+b/Plant infestation (%) by winged	0.578
Carotenoids / Plant infestation (%) by aphids	0.816
Carotenoids / Plant infestation (%) by winged	0.763
Carotenoids / Total aphids per plant	0.492
Carotenoids / Winged aphids per plant	0.650
$\dfrac{\text{Carotenoids}}{\text{Chlorophylls a + b}}$ / Total aphids per plant	0.547

[x] - correlation statistically significant

$r_{theor.}$ (P = 0.05) = 0.468

Discussion and conclusions

The two-year field observations on *Aphis fabae* confirmed the previous reports on differences in aphid invasion of sugar beet cultivars differing in degree of polyploidy as well as those which were mono- or poly-germ (Luczak & Gabrys, 1990). Differences in the content of green and yellow-orange leaf pigments were determined in order to explain this phenomenon. Winged migrants of *Aphis fabae* appeared more frequently and invaded most heavily two diploid cultivars A.Janasz-AJ 3 and A.Janasz-AJ 4. The aphid population on these cultivars was very high, too. In the two-year investigation the leaves of these cultivars showed the highest levels of yellow-orange pigments and the largest amount of chlorophylls. The highest numbers of winged migrants and the most successful development of *Aphis fabae* were also recorded on the polyploids AJ-Poly 1 and AJ-Polycama. These cultivars were also characterised by a high content of yellow-orange pigments and a high ratio of those pigments to chlorophylls.

The smallest numbers of winged individuals as well as the lighest invasion of plants were found on the mono-germ cultivars (PN-Mono 1, PN-Mono 3) containing the least leaf pigments.

The above data and results of detailed observations on the behaviour of summer winged forms of *Aphis fabae* (David & Hardie, 1988; Hardie, Poppy &

David, 1989; Nottingham & Hardie, 1989) indicate that the preferences of migrants of this aphid for host plants depends on leaf colour. It appears that a higher content of yellow-orange pigments in leaves actively stimulates the attraction of winged individuals. This is confirmed by high positive and statistically-significant co-efficients of correlation between the level of carotenoids in leaves and the four parameters of aphid invasion of beet. The positive relationships between infestations of this aphid and the amount of carotenoids were also found in the case of red beets (Luczak & Gaweda, 1991).

Résumé

Le rôle des facteurs chimiques dans la résistance de la betterave sucrière à la colonisation par le puceron noir de la fève (*Aphis fabae*)

Les différences de teneur en pigments verts (chlorophylle a + b) et jaune-orange (caroténoïdes) dans les feuilles de 8 cultivars de betterave sucrière et leur effet sur la colonisation par *Aphis fabae* ont été étudiés en 1989 et 1990. Le plus grand nombre de pucerons ailés et aptères et de plantes attaquées ont été observés sur les deux cultivars diploïdes A.Janasz-AJ 3 et A.Janasz-AJ 4. La teneur la plus élevée en pigments jaune-orange et une grande quantité de pigments verts ont été déterminées sur ces c.v.s.

Cette forte infestation de pucerons a aussi été remarquée sur les deux cultivars polyploïdes AJ-Poly 1 et AJ-Polycama. Leurs feuilles se caractérisent par une teneur élevée de pigments jaune-orange par rapport aux pigments verts. Le plus petit nombre de pucerons ailés et le niveau d'infestation des plantes le plus bas ont été observés chez les cultivars monogermes PN-Mono 1 et PN-Mono 3, dont les feuilles se sont caractérisées, durant la période expérimentale, par les teneurs les plus basses en pigments verts et jaune-orange. Le rôle des pigments jaune-orange lors de la colonisation par *Aphis fabae* est confirmé par la mise en évidence d'une corrélation entre la teneur en caroténoïdes dans les feuilles et le taux de plantes infestées, ainsi que le nombre d'insectes par plante.

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A REVIEW OF THE HOST PLANTS OF THE CABBAGE APHID, Brevicoryne brassicae (HOMOPTERA, APHIDIDAE)

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Summary

The literature concerning host plants of the cabbage aphid, *Brevicoryne brassicae*, has been surveyed as part of an investigation of resistance to this pest in cruciferous plants. In order to determine the validity of records of non-cruciferous species being reported as hosts, tests were carried out at Wellesbourne in which plants were infested with cabbage aphid. In addition, eight new records of *Brassica* species have been added to the list as a result of a series of field and laboratory experiments. Plant species have been classified as: 1) true host plants, those supporting reproduction over one or more generations; 2) members of the cruciferae that are potential hosts; 3) plants mistakenly regarded as hosts; and 4) members of the family Cruciferae that are non-hosts. It is concluded that cabbage aphid is an oligophagous insect with hosts largely confined to the family Cruciferae and with a few hosts in the Resedaceae.

Introduction

Though European in origin, the cabbage aphid, *Brevicoryne brassicae* (L.) is widely distributed in temperate and sub-tropical regions of the world. Field crops including broccoli, Brussels sprouts, cabbage, cauliflower, collard, kale, kohl-rabi, mustard, radish, rape, swede and turnips are damaged severely by this pest and regularly warrant the application of insecticide to control the insect. In addition, cabbage aphid is the vector of about 20 plant viruses. These include cabbage black ring spot, cabbage ring necrosis, cauliflower mosaic and radish mosaic virus (Blackman & Eastop, 1984).

In recent years the cabbage aphid has increased in importance as a pest for several reasons including the intensification of brassica crop production, the ever-increasing demand for blemish-free produce, a reduced range of insecticides and severe problems in achieving complete control of the pest on many crops. At Wellesbourne a search is being made for sources of resistance to cabbage aphid that can be used in breeding programmes to develop new cultivars of brassica crops. These will help growers reduce their dependence on insecticides for controlling this pest.

The literature on the cabbage aphid contains numerous reports about bionomics (Markkula, 1953; Baran, 1970), population dynamics (Otake, 1966; Gilbert & Hughes, 1971; Raworth, 1984), biological control (Wilson, 1960; Paetzold & Vater, 1967), host plant resistance (Dunn & Kempton, 1972; van Emden, 1978; Auclair, 1989) and factors affecting formation of winged forms (alates) (Broadbent, 1949). In 1953, Markkula published an extensive account of host plants of this pest. During the last four decades, however, more studies have been carried out on the incidence of cabbage aphid in field crops. Hence there is a need to update the earlier information. There is some confusion over the true nature of host plants of this insect. This is due in part to the migratory habit of the pest, as winged forms are frequently caught in traps placed in non-host crops and often land on non-host plants. These observations could result in reports of cabbage aphid "establishing" on non-host crops.

In this report, we classify plants in four categories *viz.* true host plants (Table 1), members of the cruciferae that are potential hosts (Table 2), plants mistakenly regarded as hosts (Table 3) and members of the Cruciferae that are non-hosts (Table 4).

Table 1:   True host plants of *Brevicoryne brassicae* (L.)

A.   *Family Cruciferae*

1.	*Alliaria officinalis* Andrz.
2.	*Alyssoides utriculatum* (L.) Med.
3.	*Arabidopsis thaliana* (L.) Heyneh.
4.	*Arabis allioni* DC.
5.	*A. alpina* L.
6.	*A. bellidifolia* Jacq.
7.	*A. coerulea* Haenke
8.	*A. hirsuta* (L.) Scop.
9.	*A. procurrens* Waldst. & Kit.
10.	*A. suecica* Fr.
11.	*Armoracia rusticana* (Lam.) G.M. Sch.
12.	*Barbaraea stricta* Andrz.
13.	*B. verna* (Miller) Asch.
14.	*B. vulgaris* L.
15.	*Berteroa incana* (L.) DC.
16.	*Brassica adpressa* Boiss.
*17.	*B. alboglabra* L.H. Bailey
18.	*B. campestris* L., Hn

var. *silvestris* (Lam.) Hiit.
f. *genuina* Lund & Kj.
var. *oleifera* auct. p.p.
f. *annua* (Rchb.) Hiit.
f. *biennis* (Rchb.) Hiit.
var. *rapa* (L.) Hn

19.	B. chinensis L.
20.	B. elongata Ehrh.
21.	B. fruticulosa Cyrillo
*22.	B. incana Ten.
*23.	B. insularis Moris
24.	B. juncea (L.) Coss., Czern.
	*subsp. rugosa
*25.	B. macrocarpa Guss.
26.	B. napus L., em. Metzg.
	var. oleifera DC. p.p.
	f. annua (Schuebl. & Mart.) Thell.
	f. biennis (Schuebl. & Mart.) Thell.
	var. napobrassica (L.) Peterm.
27.	B. nigra (L.) Koch
28.	B. oleracea L. em. DC.
	var. acephala DC.
	var. botrytis L.
	var. capitata L.
	f. alba (Lam.) DC.
	f. rubra L.
	var. gemmifera DC.
	var. sabauda L.
	var. gongyloides L.
29.	B. oxyrrhina Coss.
30.	B. pekinensis Rupr.
*31.	B. repanda (Willd.) DC.
	subsp. maritima (Rouy) Heywood
*32.	B. rupestris Rafin.
33.	B. sabularia Willk. ex Willk. & Lange
34.	B. sinapistrum Boiss.
*35.	B. spinescens Pomel
36.	B. tournefortii Gouan
*37.	B. villosa
	subsp. drepanensis (Caruel) Damanti
38.	Bunias erucago L.
39.	Cakile maritima Scop.
40.	Camelina sativa (L.) Crantz
41.	Capsella bursa-pastoris (L.) Med.
42.	C. grandiflora Boiss.
43.	Cardamine hirsuta L.
44.	C. pratensis L.
45.	Cardaminopsis halleri (L.) Hayek
46.	Carrichtera annua Prantl
47.	Chorispora tenella (Pall.) DC.
48.	Clypeola ionthlaspi L.
49.	Cochlearia arctica Schlecht
50.	C. danica L.

51. *C. glastifolia* L.
52. *Crambe maritima* L.
53. *Descurainia sophia* (L.) Webb & Berth.
54. *Diplotaxis muralis* (L.) DC.
55. *D. tenuifolia* (L.) DC.
56. *Draba incana* L.
57. *D. oxycarpa* Boiss. & Heldr.
58. *D. rupestris* R.Br., Lindbl.
59. *Eruca vesicaria*
     subsp. *sativa* (Mill.) Thell.
60. *Erucastrum abyssinicum* (A. Rich.) O.E. Schulz
61. *E.nasturtiifolium* (Poir.) O.E. Schulz
62. *E. pollichii* Sch. & Sp.
63. *Erysimum canescens* Roth
64. *E. cheiranthoides* L.
65. *Hesperis lutea* Maxim.
66. *Isatis aleppica* Moris, nec Scop.
67. *I. tinctoria* L.
68. *Lepidium amplexicaule*
69. *L. armoracia* Fisch.
70. *L. draba* L.
71. *L. graminifolium* L.
72. *L. heterophyllum* (DC.) Benth.
73. *L. latifolium* L.
74. *L. ruderale* L.
75. *L. sativum* L.
76. *L. virginicum* L.
77. *Lunaria annua* L.
78. *Myagrum perfoliatum* L.
79. *Peltaria turkmena* Lipsky
80. *Raphanus caudatus* L.
81. *R. maritimus* Sm.
82. *R. raphanistrum* L.
83. *R. sativus* L.
     var. *radicula* Pers.
     var. *niger* (Mill.) Pers.
84. *Rapistrum perenne* (L.) All.
85. *R. rugosum* (L.) All.
86. *Roripa silvestris* (L.) Bess.
87. *Schivereckia doerfleri* Bornm.
88. *Sinapis alba* L.
89. *S. arvensis* L.
90. *S. pubescens* L.
91. *Sisymbrium altissimum* L.
92. *S. austriacum* Jacq.
93. *S. officinale* (L.) Scop.

94. *Thlaspi alpestre* L.
95. *T. alpinum* Crantz
96. *T. arvense* L.
97. *T. goesingense* Halac.
98. *T. praecox* Wulf.
99. *Turritis glabra* L.

B. *Family Resedaceae*

1. *Reseda alba* L.
2. *R. luteola* L.

The list includes the plant species given by Markkula (1953) except those shown with an asterisk.

Table 2: Cruciferous plants that are potential hosts of *Brevicoryne brassicae* (L.)

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1. *Biscutella auriculata* L. - Markkula (1953)
2. *B. cichoriifolia* Lois. - Markkula (1953)
3. *B. laevigata* (L.) - Studzinski & Malachowska (1972)
4. *Bunias orientalis* (L.) - Studzinski & Malachowska (1972)
5. *Cardaria draba* (L.) Desv. - Studzinski & Malachowska (1972)
6. *Cheiranthus cheiri* L. - Markkula (1953)
7. *Conringia orientalis* (L.) Andrz. - Studzinski & Malachowska (1972)
8. *Erucastrum gallicum* (Willd.) O.E. Schulz - Studzinski & Malachowska (1972)
9. *E. obtusangulum* Reichenb. - Borner (1921)
10. *Hesperis matronalis* L. - Studzinski & Malachowska (1972)
11. *Hutchinsia alpina* (L.) R.Br. - Studzinski & Malachowska (1972)
12. *Iberis amara* L. - Studzinski & Malachowska (1972)
13. *Lobularia maritima* L. - Studzinski & Malachowska (1972)
14. *Malcolmia maritima* (L.) R.Br. - Markkula (1953)
15. *Matthiola incana*
              var. *annua* Voss (M.a.(L.) Sweet). - Markkula (1953)
16. *M. incana* (L.) R.Br. - Potgieter & DuRR (1961)
17. *Matthiola sp.* - Bodenheimer & Swirski (1957)
18. *Moricandia arvensis* DC. - Markkula (1953)
19. *Nasturtium officinale* R.Br. - Studzinski & Malachowska (1972)
20. *Neslia paniculata* (L.) Desv. - Studzinski & Malachowska (1972)
21. *Peltaria alliacea* Jacq. - Markkula (1953)
22. *Ricotia lunaria* DC. - Markkula (1953)
23. *Sisymbrium loeselii* L. - Studzinski & Malachowska (1972)
24. *Vogelia paniculata* (L.) Horn. - Markkula (1953)

Table 3: Plants mistakenly regarded as hosts of *Brevicoryne brassicae* (L.)

1. Artichoke - Anonymous (1920)
2. Beans - Zaumeyer (1933)
3. Beets - Bogdanova-Katokova (1918)
4. *Beta vulgaris* cv *macrorhiza* - Potgieter & DuRR (1961)
5. Celery - Hill (1987)
6. Cotton - Plotnikov (1915)
7. *Dahlia* sp. - Bodenheimer & Swirski (1957)
8. Lettuce - Apablaza (1984)
9. *Linum* sp. - Potgeiter & DuRR (1961)
10. Lucerne - Averin (1913)
11. Pears - Uvarov (1914)
12. Potato - Bodenheimer & Swirski (1957); Dyakonov & Milchenko (1982)
13. Spinach - Blanchard (1925)
14. Tobacco - Pinto Da Fonseca (1934); Takaoka (1976)
15. *Tropaeolum* - Hille Ris Lambers (1950)
16. Vetch - Ewing (1914)
17. *Vicia faba* - Chowfla & Thakur (1987)

Table 4: Cruciferous plants which are not hosts of *Brevicoryne brassicae* (L.)
(Markkula, 1953)

1. *Alyssum altaicum* C.A. Mey.
2. *A. argenteum* Vilm.
3. *A. corymbosum* Boiss.
4. *A. gemonense* L.
5. *A. marshallianum* Andrz. ex DC.
6. *A. montanum* L.
7. *A. repens* Baumg.
8. *A. reptans* Baumg.
9. *Bunias orientalis* L.
10. *Cheiranthus menziesii* Benth. & Hook.
11. *Coronopus didymus* (L.) Sm.
12. *Erysimum asperum* DC.
13. *E. aurantiacum* Leyd.
14. *E. hieraciifolium* L.
15. *E. pulchellum* J. Gay
16. *E. wittmannii* Zaw.
17. *Fibigia eriocarpa* Boiss.
18. *Iberis amara* L.
19. *I. sempervirens* L.
20. *I. umbellata* L.
21. *Roripa palustris* (Leyss.) Bess.

Classification of host plants

True host plants

These plants are suitable for survival and reproduction of the aphids over one or more generations and have been evaluated for their suitability under both natural and controlled conditions. Of 101 plant species listed as true hosts, 99 belong to the Cruciferae and 2 to the Resedaceae (Table 1). Among these, 93 plant species were identified as true hosts by Markkula (1953). Another eight *Brassica* species were tested by Singh, Ellis & Pink (in prep.), which are marked in the list with an asterisk. Reports of cabbage aphid host plants during the last 4 decades have not been referred to separately, unless the reports either contain additional host plants or delete names in the list presented by Markkula (1953).

Members of the cruciferae that are potential hosts

These carry extremely low populations (nymphs and adults) of aphids in the field but have not been evaluated for their ability to support aphid populations over one or more generations under controlled conditions. However, low aphid numbers on such plants suggest that they are unsuitable for the build-up of large aphid populations or that the plants were observed at a stage in growth which was unfavourable for aphid development. We have categorised these plants as potential hosts. Twenty four cruciferous plants have been placed in this group (Table 2).

Plants mistakenly regarded as hosts

Several plant species have been reported as hosts of the cabbage aphid on the basis of: 1) the presence of alates on crop plants; 2) the capture of alates in field traps in non-host crops; 3) the potential of the cabbage aphid to transmit viral diseases; 4) incorrect quotation of the original work by the abstracting services (e.g. beans and lettuce (Table 3)); 5) the mistaken identity of the insect. We have compiled a list of 17 plant species (Table 3) misquoted as hosts. These have been mentioned by the names, common or latin, given by the original authors. From this list, we tested tobacco, cotton, spinach, lettuce, lucerne, potato, celery and *Vicia faba* as hosts of cabbage aphid under controlled conditions. They were unsuitable for survival or reproduction of the cabbage aphid and therefore should no longer be considered as hosts.

Markkula (1953) also tested spinach, *Phaseolus vulgaris* L. (beans), *Beta vulgaris* L., *Tropaeolum majus* L., *T. canariense* Hert. and tobacco and concluded that these also were not host-plants of the cabbage aphid.

Members of the Cruciferae that are non-hosts

Markkula (1953) presented a list of 21 cruciferous plants that he considered were not host plants (Table 4) of cabbage aphid on the basis of his own studies and

information gleaned from the literature. Among these, *Iberis amara* L. and *Bunias orientalis* L. were found by Studzinski and Malachowska (1972) to support nymph and adult populations of aphid in the field. We have included these two species as potential host plants. In view of this discrepancy, there is a need to carry out further work on these two host plants.

Conclusions

All the available evidence suggests that the cabbage aphid, *Brevicoryne brassicae* (L.) should be considered as an oligophagous pest of the family Cruciferae. However, there has been a reliable, solitary occasion when it was reported to survive on members of the Resedaceae, a plant family closely related to the Cruciferae. In this respect the cabbage aphid resembles closely the cabbage root fly, *Delia radicum* L. (Finch & Ackley, 1977). In addition, certain workers have included species from other plant families in the host range of the cabbage aphid, without evaluating their suitability for aphid survival and reproduction. We showed that many of these plants do not support cabbage aphid. This confirms the view that the mere presence of an insect on a plant species should not justify the plant being classed as a host.

Résumé

Un recensement des plantes hôtes du puceron cendré du chou, *Brevicoryne brassicae* (*Homoptera, Aphididae*)

La littérature concernant les plantes hôtes du puceron cendré du chou, *Brevicoryne brassicae*, a été passée en revue lors d'une investigation de la résistance des crucifères à ce ravageur. La validité des mentions d'espèces-hôtes n'appartenant pas à la famille des crucifères a été déterminée par infestation artificielle de plantes à Wellesbourne. De plus, 8 nouvelles espèces de *Brassica*, non connues comme hôtes, ont été ajoutées à cette liste d'essai, suite à une série d'expérimentations en champ et en laboratoire. Les espèces végétales ont été classées comme suit: 1) plantes hôtes vraies permettant une reproduction pendant une ou plusieurs générations; 2) plantes hôtes transitoires offrant nourriture et habitat durant de courtes périodes; 3) plantes considérées comme hôte par erreur et 4) représentants de la famille des crucifères, mais non hôtes. La conclusion est que le puceron cendré du chou est un insecte oligophage trouvant la plupart de ses hôtes dans la famille des crucifères et quelques-uns parmi les *Resedaceae*.

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