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# WORKING GROUP "BREEDING FOR RESISTANCE TO INSECTS AND MITES

# GROUPE DE TRAVAIL "AMELIORATION DES PLANTES POUR LA RESISTANCE CONTRE LES INSECTES ET LES ACARIENS"

"In collaboration with EUCARPIA"

MARCELIN (SWITZERLAND),

4-6 SEPTEMBER 1989

EDITED BY
EDITE PAR *P.A. ELLIS & J. FREULER*

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WORKING GROUP

# "BREEDING FOR RESISTANCE TO INSECTS AND MITES"

PROCEEDINGS OF THE FIFTH WORKSHOP

MARCELIN (SWITZERLAND), 4-6 SEPTEMBER 1989

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GROUPE DE TRAVAIL

# "AMELIORATION DES PLANTES POUR LA RESISTANCE CONTRE LES INSECTES ET LES ACARIENS"

COMPTES - RENDU DE LA CINQIEME REUNION A

MARCELIN (SUISSE), 4-6 SEPTEMBRE 1989

EDITED BY
EDITE PAR *P.R. ELLIS & J. FREULER*

IOBC / WPRS BULLETIN
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# INTRODUCTION

## Fifth Triennial Meeting

The fifth triennial meeting of the Working Group was organised by Jost Freuler and held from 4 to 6 September 1989 in Marcelin, Switzerland. It was followed by an excursion to the Federal Agricultural Research Station at Changins, an arboretum and several other centres. This meeting, like the previous four, was supported by EUCARPIA, the European Association for Research on Plant Breeding which provided a grant to assist certain participants with travel and subsistence costs. Thirty four participants from research institutes, universities and seed companies in 9 countries attended the meeting. Four workshops were held over two days at which 27 papers were presented. Time was allowed for discussion of individual papers and for more general topics. Several seed companies were represented at the meeting, reflecting the close links that the members of this Working Group have with industry.

The subjects receiving most attention were:

1. Techniques for the evaluation of resistance in crops to pest attack.

2. Morphological and biochemical basis of resistance.

3. Genetics of resistance in crop plants to pests.

4. Identification of biotypes of aphids and other pests.

5. Resistance to virus vectors.

6. The exploitation of partial resistance in integrated programmes of pest control.

At the meeting a special vote of thanks was extended to Orlando de Ponti, the first Convenor of the Working Group and who lead the Group enthusiastically and wisely through the first 10 years of its existence.

The proceedings of this meeting are included in this Bulletin.

## Future Activities

At this meeting in Marcelin one session was assigned to a discussion of the future activities of the Working Group. The membership has been maintained since the Group's foundation in 1976 which indicates the continuing interest in this field of research and crop protection. This work can only grow in importance as demands for more pest-resistant varieties increase thereby reducing chemical use and environmental pollution. Closer ties have been formed between research workers and seed companies to promote the development of resistant crop varieties and to ensure that the resistance is used in agriculture.

It was decided to continue holding the highly successful triennial meetings and to establish Project Groups which would increase active collaboration between scientists of different nations. No changes to the Working Group's organisation or activities were considered necessary.

Members belong to other Working Groups and participate in their activities. For example, during the period 1985-1987 several members participated in collaborative experiments organised by a member of the Working Group on Integrated Control in Field Vegetable Crops; the results of these experiments have been published - see IOBC Bulletin 1988/XI/1.

The Aphid Resistance Newsletter will continue to keep interested workers in close contact and well-informed on progress in research programmes on these pests. At present the Newsletter is edited by J. Weibull, Sweden, and it has 13 contributors from 7 countries.

Two new Project Groups are planned:

a) Lettuce Aphid Group: Leader: K. Reinink, The Netherlands. This Group will involve members from at least 6 countries and will aim to survey the incidence of aphid species on lettuce in different parts of the Western Palearctic region. The information gathered will be used to ensure that resistant plant material can be deployed wisely and future requirements for resistant lettuce varieties planned.

b) Western Flower Thrips Group: Leader: C. Mollema, The Netherlands. This Group will collaborate in the investigation of insect/plant relationships concentrating on the development of techniques for the identification of resistance to western flower thrips in plants.

The next meeting of the Working Group will be held in September 1992 at the AFRC Institute of Horticultural Research, Wellesbourne, Warwick, CV35 9EF, Great Britain.

The creation of additional Project Groups is also being investigated, for example a Group to explore the use of EPG (electrical penetration graph) techniques in recording aphid stylet activity on resistant and susceptible host plants.

**IOBC/WPRS WORKING GROUP BREEDING FOR RESISTANCE TO INSECTS AND MITES**
**Details of triennial meetings of the Working Group**

| Date | Venue | Participants | Countries | Papers |
|---|---|---|---|---|
| Dec.1976 | Wageningen, The Netherlands | 61 | 16 | 26 |
| Apr.1980 | Canterbury, Great Britain | 43 | 10 | 29 |
| Apr.1983 | Capbreton, France | 42 | 11 | 32 |
| Sept.1986 | Hundestedt, Denmark | 28 | 8 | 32 |
| Sept.1989 | Marcelin, Switzerland | 34 | 8 | 27 |

P R Ellis
Convenor

## LIST OF PARTICIPANTS

| Name | Address |
|---|---|
| J J Berg | Rijk Zwaan Zaadteelt en Zaadhandel, BV., Burgermeaster Crezeelaan 40, postbus 40, 2678 ZG De Lier, THE NETHERLANDS |
| M J Berlinger | Entomology Laboratory, Gilat Regional Experiment Station, Mobile Post Negev 85-820, ISRAEL |
| N C Berlinger | Entomology Laboratory, Gilat Regional Experiment Station, Mobile Post Negev 85-820, ISRAEL |
| N Birch | Zoology Department, Scottish Crop Research Institute, Invergowrie, Dundee, DD2 5DA, Scotland, GREAT BRITAIN |
| G D van Blokland | Royal Sluis, Postbox 22, 1600 AA Enkhuizen, THE NETHERLANDS |
| D den Hollander | Rijadijk 61, NL-3161 HM Rhoon, THE NETHERLANDS |
| F L Dieleman | Department of Entomology, Agricultural University, P O Box 8031, 6700 EH Wageningen, THE NETHERLANDS |
| S D Eigenbrode | Department of Entomology, New York State Agricultural Experiment Station, Cornell University, Geneva, NY 14456-0462, UNITED STATES OF AMERICA |
| P R Ellis | Entomology Section, AFRC Institute of Horticultural Research, Wellesbourne, Warwickshire, CV35 9EF, GREAT BRITAIN |
| J Freuler | Station Fédérale de Recherches Agronomiques de Changins, CH-1260 Nyon, SWITZERLAND |
| J E Frey | Eidg. Forschungsanstalt, CH-8820 Wädenswil, SWITZERLAND |
| F Gagnebin | Laboratoire Bioénergétique, Université de Genèva, CH-1254 Lullier, SWITZERLAND |
| J A Hardman | Entomology Section, AFRC Institute of Horticultural Research, Wellesbourne, Warwickshire, CV35 9EF, GREAT BRITAIN |
| R Herr | Vorderer Alter Berg 7, D-7507 Pfinztal, FEDERAL REPUBLIC OF GERMANY |
| S Hofstede | Sluis & Groot Research, Zaadunie BV, P O Box 26, NL-1600 AA Enkhuizen, THE NETHERLANDS |

| | |
|---|---|
| B Hurni | Eidg. Forschungsanstalt, CH-8820 Wädenswil, SWITZERLAND |
| H Legutowska | Department of Applied Entomology, Warsaw Agricultural University, 02-766 Warszawa, ul. Nowoursynowska, POLAND |
| C Mollema | Centrum voor Plantenveredelingsonderzoek CPO, Postbus 16, 6700 AA Wageningen, THE NETHERLANDS |
| S Morse | Biology Department, Building 44, The University, Southampton, SO9 5NH, GREAT BRITAIN |
| M Morse | 151 Blackmoor Gate, Furzton, Milton Keynes, Bucks, MK4 1DW, GREAT BRITAIN |
| J K Nielsen | Chemistry Department, Royal Veterinary and Agricultural University, 40 Thorvaldsensej, DK-1871 Frederiksberg C, DENMARK |
| S M Niraz | Biochemistry Department, Agricultural and Teachers University, (WSRP), Institute of Applied Biology, ul. Prusa 12, 08-110 Siedlce, POLAND |
| J E Peterson | US Vegetable Laboratory, 2875 Savannah Highway, Charleston, South Carolina, SC 29414, UNITED STATES OF AMERICA |
| H M Poehling | Institut für Pflanzenkrankheiten und Pflanzenschutz, Universität Hannover, Herrenhäuserstrasse 2, D-3000 Hannover 21, FEDERAL REPUBLIC OF GERMANY |
| C Prüter | Institut für Phytomedizin, Universität Hohenheim, Otto-Sander Strasse 5, D-7000 Stuttgart 70, FEDERAL REPUBLIC OF GERMANY |
| K Reinink | Centrum voor Plantenveredelingsonderzoek CPO, Postbus 16, 6700 AA Wageningen, THE NETHERLANDS |
| J M Schalk | US Vegetable Laboratory, 2875 Savannah Highway, Charleston, South Carolina, SC 29414, UNITED STATES OF AMERICA |
| M G Solomon | Entomology Section, AFRC Institute of Horticultural Research, East Malling, Maidstone, Kent, ME19 6BJ, GREAT BRITAIN |
| E Stadler | Eidg. Forschungsanstalt, CH-8820 Wädenswil, SWITZERLAND |
| R Strasser | Laboratoire Bioénergétique, Université de Genèva, CH-1254 Lullier, SWITZERLAND |

| | |
|---|---|
| C Terrettaz | Service Phytosanitaire Cantonal, Ecole Cantonale d'Agriculture, CH-1950 Châteauneuf/Sion, SWITZERLAND |
| M van Helden | Department of Entomology, Agricultural University, P O Box 8031, 6700 EH Wageningen, L-6709 Wageningen, THE NETHERLANDS |
| A A van Herp | Nickerson-Zwaan, Postbus 4, 1747 ZG, Tuitjenhorn, THE NETHERLANDS |
| A von Eggermond | De Ruiter Zonen BV, Veredelings-en Produktiebedrijven van Hybride Zaden, Postbus 4, BL-2665 ZG Bleiswijk, THE NETHERLANDS |
| C P W Zebitz | Institut für Pflanzenkrankheiten und Pflanzenschutz, Universität Hannover, Herrenhäuserstrasse 2, D-3000 Hannover 21, FEDERAL REPUBLIC OF GERMANY |

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Workshop 1

# PROTECTED and OUTDOOR VEGETABLE CROPS: BRASSICAS and CARROTS

# RESISTANCE AND SUSCEPTIBILITY TO TURNIP ROOT FLY (DELIA FLORALIS) IN BRASSICAS

A.N.E. BIRCH
Zoology Department, Scottish Crop Research Institute,
Invergowrie, Dundee DD2 5DA, Scotland

Summary
Further progress has been made on swede resistance, studying attack of resistant genotypes under 'choice' and 'no choice' field conditions. Field cages have been used to identify swedes with antixenosis and antibiosis type resistance. Oviposition preference has also been examined using the IHRW design turntable system to study effects of leaf extracts sprayed on to plastic leaves. Egg inoculation experiments have been used to examine levels of antibiosis and tolerance to larval attack in fodder and oilseed rapes. The significance of metabolic changes induced in roots after larval attack are also being investigated.

## 1. Introduction

Initial studies at SCRI on resistance mechanisms to turnip root fly (TRF) in swede and rape have shown that antixenosis (reduced attraction for egg laying), antibiosis (reduced larval development) and tolerance (ability to withstand root damage) are important components. Screening brassicas against TRF has initially involved field evaluations but has now mainly been replaced by field cage, laboratory and glasshouse testing. Currently the mechanisms of each resistance component and their genetic control are being studied.

## 2. Progress

### Field evaluations, swede

Field experiments in 1985 and 1986 (Birch, 1988) demonstrated that TRF egg laying on susceptible cvs Doon Major and Sator Øtofte was 4-8 times higher than on resistant swede cv. Angus. A field experiment comparing Angus grown in one large block ('no choice') with Angus grown in an adjacent 4 cultivar ('choice') experiment showed that the level of antixenosis in Angus was only slightly reduced when oviposition preferences effects were operating (Birch, 1988).

Indications that Angus was also partially resistant to cabbage root fly (CRF, D. radicum) under field conditions were confirmed in field trials at ESCA (Scotland) and IHR Wellesbourne (England). The degree of antixenosis effective against CRF was variable between sites and years, but under heavy CRF attack 34 times more eggs were laid on susceptible control cultivars than on Angus.

Field experiments also indicated that root antibiosis was a second but less

important resistance component, especially against TRF (also see glasshouse assessments). In two year trials, external and internal root damage (using a 4 scale RDI) was significantly less on Angus and Melfort (RDI 1.2-1.8) than susceptible control cultivars (RDI 2.2-2.8). Larval penetration into the root was greatly reduced on resistant swedes. The inability to penetrate resistant roots was not correlated with tissue hardness, but may be associated with some component of higher dry matter (Birch, 1988).

Field cage assessment, swede

In 1988 a field cage method was evaluated as a possible alternative to field trials for identifying sources of TRF resistance (Birch, 1989). Fifteen swede cultivars and breeding lines were selected, to include two susceptible controls (Doon Major, Sator Øtofte), four resistant controls (Angus, Melfort, Vige, Mustiala) and nine other genotypes. The test involved the controlled release of adult TRF and the use of egg traps on potted plants. Significant levels of antixenosis and antibiosis type resistance were identified, and there was a reasonable agreement for control cultivars between field cage results and those from previous field trials. The test method was particularly useful for assessing antixenosis levels relatively quickly (about 40% less time than soil sampling for eggs in the field).

Laboratory tests, swede and rape

A three tier mini turntable (IHR Wellesbourne design) has been used to investigate oviposition preference by TRF on young (4-8 wk old) plants. Three times as many eggs were laid on susceptible swede cv. Doon Major than on Angus, reflecting results from field and field cage experiments. Tests on rapes indicated a strong preference for certain genotypes. Up to four times as many eggs were laid on forage rape breeding line 84411 compared with the least preferred cultivar, Samo (Birch & MacFarlance Smith, 1988). The greatest difference in oviposition preference was found between kale cv. Fribor (73 eggs/plant) and chinese cabbage cv. China King (3 eggs/plant). Leaf extracts from resistant and susceptible brassicas sprayed on to artificial leaves are currently being tested in an oviposition bioassay. Oviposition stimuli are being isolated for chemical identification.

A laboratory bioassay was devised to investigate the response of newly hatched TRF larvae to root chemicals. Time-lapse studies showed that larvae were attracted to root exudates and root cores from swedes. However, there was no obvious preference for diffusable root chemicals from susceptible compared with those from resistant swede genotypes (Birch, 1989).

Glasshouse assessments, swede and rape

TRF egg inoculation tests confirmed that swede cvs Angus and Melfort were significantly less damaged than susceptible cultivars (Birch, 1988). The maximum depth of larval penetration was also significantly reduced on resistant roots. The proportion of pupae developing after standardised inoculation levels was lower on Angus and Melfort (25-32%) compared with susceptible control cultivars (44-49%), indicating a low level of antibiosis resistance.

Inoculation tests on forage and oilseed rapes indicated considerable variation in tolerance to root damage (Birch & MacFarlane Smith, 1988). Forage rape cvs Bonar and Lair supported relatively high numbers of TRF larvae (21-

27/root) without severe effects on root and shoot growth. In comparison, forage rape cv. Samo supported relatively low numbers of larvae (5/root) but suffered severe wilting and root reduction.

Recent studies have demonstrated changes in rape root glucosinolate metabolism, induced by TRF attack (Birch et al., 1989). For example, after 6 weeks larval feeding on cv. Samo roots, the proportion of aliphatic glucosinolates was reduced and aromatic types increased, compared with control plants. This shift was largely due to increased (88%, relative to controls) levels of indole-based glucosinolates. The biological significance of these changes are currently being investigated, since these indole compounds are structurally related to recently discovered brassica phytoalexins.

## 3. Résumé

L'étude de la résistance du rutabaga a été poursuivie en champ en observant l'attaque de génotypes résistants en condition de "choix" et de "non choix". A l'aide de cages au champ on a identifié des rutabagas ayant des résistances de type antixenose et antibiose. La table rotative développée à l'Institut de recherches horticoles à Wellesbourne, a servi à l'étude de l'effet d'extraits de feuille pulvérisés sur des attrapes en plastique, sur la préférence de ponte. L'inoculation de colzas utilisés soit pout l'affouragement soit pout la production des graines avec des oeufs du ravageur a été utilise afin de préciser le degré d'antibiose et de tolérance des plantes aux attaques larvaires. L'importance des changements métaboliques induits dans les racines par les attaques est également étudiée.

## 4. References

BIRCH, A.N.E. 1988. Field and glasshouse studies on components of resistance to root fly attack in swedes. Ann. appl. Biol. 113: 89-100.

BIRCH, A.N.E. & MACFARLANE SMITH, W.H. 1988. Laboratory tests on turnip root fly attack of forage and oilseed rapes. Tests of Agrochemicals and Cultivars 9. Ann. appl. Biol. 112 (supplement), 78-79.

BIRCH, A.N.E. 1989. A field cage method for assessing resistance to turnip root fly in brassicas. Ann. appl. Biol. 115: 321-325.

BIRCH, A.N.E., GRIFFITHS, D.W. & MACFARLANE SMITH, W.H. 1989. Changes in forage and oilseed rape root glucosinolates in response to attack by turnip root fly. J. Sci. Food Agric.: (in press).

BIRCH, A.N.E. 1989. Interactions between root flies and brassica roots - clues to alternative control strategies. Aspects of Applied Biology 22: 289-295.

# Current studies on resistance to cabbage root fly (Delia radicum) in cauliflower

J. FREULER[1], F. GAGNEBIN[2] & R. STRASSER[2]

[1] Station fédérale de recherches agronomiques de Changins, CH-1260 Nyon, Switzerland
[2] Université de Genève, Laboratoire bioénergétique, CH-1254 Lullier, Switzerland

Abstract

The work which started in 1979 progresses in a biennial cycle in spite of the annual character of the selected species. To obtain seeds it is necessary to use in vitro culture as crop establishment has to coïncide with the activity of the pest.

F2 or F3 stages occur in even years, whereas F3 plants occur in odd years and are checked in this year. An examination of the mean number of eggs laid per plant shows that in 1988 for the first time significant difference between an F3 line and the control variety were obtained. Some lines have evolved significantly towards relative resistance without being different from the control.

The observed individual variability should permit further improvement in the level of resistance.

Selection for resistance to cabbage root fly (Delia radicum) in cauliflower began in 1979. This crop is of economic importance in Switzerland. It is also a stem cruciferous crop with a higher tolerance threshold for the fly than that of root cruciferous crops. Partial resistance could therefore offer a way of reducing chemical treatments for this pest (GAGNEBIN & FREULER, 1988).

The experimental site was located near Geneva, where a natural pest population proved to be sufficiently consistent and high to permit inbreeding selection without compromising the survival of host plants.

As non-preference resistance is being investigated, the number of eggs laid on individual plants is the most important criterion for selection. Egg counts on a great number of plants has become feasible with the egg trap (FREULER & FISCHER, 1982).

The experimental lay out used was a randomized block design with 5 replicates. Each subplot comprised 20 plants in a double row, and 8 plants were sampled for eggs. In order to synchronise plant growth and maximum egg laying which takes place during July and August, the sowing time chosen was the beginning of Mai followed by planting out before mid-June. Eggs were collected 4-5 times every 10 days between the beginning of July and the end of August. This ensured that 68%-92% of the laid eggs were trapped. Selection followed soon afterwards and ended in September. The quality of the leaves and the curd was considered as well as the number of eggs.

The growing period of the crop chosen in these trials meant that plants matured when daylength and temperature had decreased, creating poor conditions for seed production. Therefore, the in vitro culture technique was introduced in 1983 (LÊ, 1984). Plants selected in the fall were multiplied during the winter and regenerated plantlets obtained from the same curd flowered the following year together in cages for allogamous and autogamous pollination. Hence two biannual selection cycles could be started, one occurring in odd years from 1983 onwards

and the other occurring in years starting in 1984.

In order to detect undesirable effects of in vitro culture, after several tube transfers selected lines were periodically cropped in the field to observe normal curd production. Agronomic characteristics of the progenies were checked in the experimental plot and yields were recorded in a plot grown under normal agricultural practices.

Progress in selection was estimated by using standard types (nr. XIV since 1981 and X since 1989) for a comparison of egg numbers on progenies selected for resistance (↙) or susceptibility (↗).

Tab. 1 lists details of the plant material tested.

From 1979 to 1981 parents were tested. Tab. 2 provides the mean number of eggs per plant as a measure of the level of resistance. Significant differences between the varieties were present in 1979 and 1980, but there was more variation within varieties. Nr. X and XIV appear to be the most interesting lines on an examination of egg numbers.

Tab. 3 shows the egg counts on cauliflower varieties and selected progenies during the series of odd years. In 1983 selection was difficult because of low egg numbers and there were no significant differences between varieties. This resulted in only slight differences in 1985.

In 1987, significant differences increased, but there was no difference between standard and resistant progenies. Progenies of nr. XV were consistent in selection for susceptibility.

Selection is continuing during 1989. Progeny of nr. XIX had significantly fewer eggs than the standard. Note the deviation in that the most resistant progeny has been selected for susceptibility.

Similar observations can be made for the series of selections in even years (tab. 4). Nr. X showed interesting variability as some progenies proved to be statistically different.

When egg counts are adjusted in relation to the standard, comparisons between parents and its progenies can be made. Various situations have been observed up to now. For example: no effect of selection for resistance resp. susceptibility is shown in tab. 5 resp. tab. 6.

Tab. 7 shows a temporary effect of selection for resistance. No effect of selection for resistance with temporary deviation for susceptibility appears in tab. 8.

Tab. 9 shows the reverse effect of selection for susceptibility and finally expected effect of selection for susceptibility resp. resistance appear in tab. 10 resp. tab. 11.

Further cycles of selection will be required with the most promising plant material to improve the level of resistance to cabbage root fly.

Résumé

Etudes en cours sur la résistance à la mouche du chou (Delia radicum) chez le chou-fleur

Le travail, commencé en 1979, se poursuit à un rythme bisannuel malgré le caractère annuel de l'espèce sélectionnée. L'obtention de semences nécessite de passer par la culture in vitro étant donné que la mise en culture doit tenir compte du vol du ravageur.

La série des années paires est au stade F2 ou F3, alors que nous contrôlons cette année les F3 de la série des années impaires.

En considérant la moyenne des oeufs pondus au pied des plantes, on constate pour la première fois, en 1988, chez F3, une différence significative avec la variété considérée comme témoin. Certaines lignées ont sensiblement évolué dans le sens d'une résistance relative sans pour autant se démarquer du témoin.

Les différences individuelles doivent permettre de renforcer encore le phénomène de résistance.

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REFERENCES

FREULER, J. & FISCHER, S. 1982. Description d'un piège à oeufs pour la mouche du chou, Delia brassicae Wiedemann. Mitteilungen Schweizerische Entomologische Gesellschaft 55: 77-85.

GAGNEBIN, F. & FREULER, J. 1988. Progress in the studies of resistance to cabbage root fly (Delia radicum) in cauliflower. IOBC-WPRS Bulletin XI/3: 67-69.

LÊ, C.L., 1984. Rapport d'activité 1982-1983 de la Station fédérale de recherches agronomiques de Changins, CH-1260 Nyon.

Tab. 1 : Summary of details of plant material tested for resistance to cabbage root fly.

| Cauliflower variety | | | Year of creation | Variety or progeny tested | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| No | Name | Obtaining or origine | | 1979 | 1980 | 1981 | 1983 | 1984 | 1985 | 1986 | 1987 | 1988 | 1989 |
| I | SAXA | Besson-Decroux | 1945 | X | X | X | X | | X | | X | | |
| II | SELANDIA | " | 1955 | X | X | X | X | | X | | X | | |
| III | SUCCES | " | 1948 | X | X | X | X | X | X | X | X | X | X |
| IV | WESLANDIA | " | 1955 | X | X | | | | | | | | |
| V | IDOL | " | 1962 | X | X | X | X | | X | | X | | X |
| VI | GRANDESSE | " | 1960 | | X | | | | | | | | |
| VII | CORONADO F1 | " | 1976 | X | X | X | X | | X | | X | | X |
| VIII | PERFECTION | " | 1964? | X | X | | | | | | | | |
| IX | OPAAL | Blanck | 1976 | X | | | | | | | | | |
| X | PANDA | " | 1976 | X | X | X | X | X | X | X | X | X | X |
| XI | FORTADOS | " | | X | X | | | | | | | | |
| XII | NEVADA | " | 1974 | X | X | | | | | | | | |
| XIII | BRENDO | " | 1976 | X | X | X | X | | X | | X | | X |
| XIV | IMPERATOR NOUVEAU | " | 1950 | X | X | X | X | X | X | X | X | X | X |
| XV | ELGON | " | 1982 | | | | X | X | X | X | X | X | X |
| XVI | ANDES | " | 1982 | | | | | X | | X | | X | |
| XVII | Nyi R 7802-2 | Dickson | | | | | | | | X | | X | |
| XVIII | Roi des Géants | Vatter | 1945 | | | | | | | X | | X | |
| XIX | Ny 9120 | Dickson | | | | | | | | | X | | X |
| XX | Early Green Glazed (Australia) | " | | | | | | | | | | X | |
| XXI | Local cauliflower (Nepal) | Fischer | | | | | | | | | | X | |

Tab. 2 : Level of resistance of cauliflower varieties to cabbage root fly from 1979 to 1981

| | Variety | Mean nb. eggs/plant | S.D. (P=0.05) | Total nb. eggs / plant Min. | Max. |
|---|---|---|---|---|---|
| **1979** | | | | | |
| **1st plantation** | | | | | |
| | III | 13 | a | 3 | 25 |
| | II | 22 | ab | 8 | 64 |
| | V | 22 | ab | 5 | 49 |
| | IV | 25 | b | 3 | 98 |
| | I | 27 | b | 2 | 68 |
| | VIII | 29 | b | 7 | 44 |
| | VII | 32 | b | 4 | 67 |
| **2nd plantation** | | | | | |
| | XIII | 25 | a | 3 | 50 |
| | XI | 33 | a | 5 | 67 |
| | X | 36 | ab | 14 | 60 |
| | XIV | 36 | ab | 8 | 73 |
| | IX | 44 | ab | 9 | 124 |
| | XII | 49 | b | 7 | 104 |
| **1980** | X | 28 | a | 7 | 113 |
| | XIV | 36 | a | 13 | 65 |
| | II | 37 | a | 12 | 64 |
| | III | 38 | a | 9 | 78 |
| | XII | 38 | a | 15 | 50 |
| | XIII | 40 | a | 10 | 75 |
| | V | 41 | a | 12 | 59 |
| | XI | 41 | a | 22 | 64 |
| | VI | 55 | ab | 35 | 78 |
| | VII | 57 | ab | 2 | 107 |
| | VIII | 70 | b | 18 | 144 |
| | IV | 75 | bc | 28 | 105 |
| | I | 102 | c | 29 | 221 |
| **1981** | X | 65,5 | a | | |
| | XIV | 71,2 | a | | |
| | II | 76,7 | a | | |
| | V | 85,8 | a | | |
| | XIII | 85,9 | a | | |
| | I | 96,2 | a | | |
| | III | 106,0 | a | | |
| | VII | 107,9 | a | | |

Tabl. 3 : Egg counts on cauliflower varieties and selected progenies for resistance (↙) or susceptibility (↗) to cabbage root fly during the series of even years from 1983 to 1989.

| 1983 | | | 1985 | | | 1987 | | | 1989 | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Variety or progeny | Mean nb. eggs/plant | S.D. (P 0.05) | Variety or progeny | Mean nb. eggs/plant | S.D. (P 0.05) | Variety or progeny | Mean nb. eggs/plant | S.D. (P 0.05) | Variety or progeny | Mean nb. eggs/plant | S.D. (P 0.05) |
| XV | 8.1 | a | VII/14 ↙ | 49.7 | a | (V/11/14 ↙ | 25.8 | -) | XIII/8/32/18 ↗ | 28.4 | a |
| II | 8.3 | a | X/3 ↗ | 51.2 | a | XIX - | 31.1 | a | XIX/4 ↙ | 29.3 | ab |
| III | 8.4 | a | XIV/4 ↙ | 53.7 | ab | VII/14/82 ↙ | 38.0 | ab | XIV/4/32/30 ↙ | 32.8 | abc |
| XIII | 8.7 | a | V/11 ↙ | 54.2 | ab | VII/14/47 ↙ | 40.9 | ab | XIV/4/78/92 ↙ | 33.3 | abc |
| V | 9.8 | a | I/15 ↙ | 55.2 | ab | III/16/98 ↙ | 41.6 | ab | XIII/15/27/33 ↙ | 34.0 | abcd |
| I | 10.7 | a | XIII/15 ↙ | 55.4 | ab | XIV reference | 42.2 | ab | III/16/98/54 ↙ | 35.2 | abcd |
| X reference | 10.9 | a | II/7 ↙ | 57.7 | ab | XIII/8/32 ↗ | 42.2 | ab | X reference | 35.8 | abcd |
| XIV reference | 11.0 | a | III/2 ↗ | 57.8 | ab | (VII/14/60 ↙ | 46.3 | -) | XIV/4/78/17 ↙ | 36.4 | abcd |
| VII | 11.3 | a | XIV/14 ↗ | 59.0 | ab | X/11/92 ↗ | 46.7 | abc | XIII/15/4/48 ↙ | 36.9 | abcd |
| | | | I/16 ↗ | 60.1 | ab | V/11/69 ↙ | 47.2 | abc | X/11/92/83 ↗ | 37.0 | abcd |
| | | | II/18 ↗ | 60.5 | ab | XIII/15/4 ↙ | 48.4 | abc | VII/14/82/35 ↙ | 37.5 | abcd |
| | | | XV/1 ↙ | 63.0 | ab | X/11/56 ↙ | 48.9 | abc | XIV/4/32/56 ↙ | 39.5 | abcd |
| | | | XIII/8 ↗ | 63.5 | ab | XIV/4/78 ↙ | 49.2 | abc | VII/14/47/75 ↙ | 40.3 | bcd |
| | | | III/16 ↙ | 64.5 | ab | XIV/4/32 ↙ | 51.7 | abc | XIV/14/18/50 ↗ | 40.6 | bcd |
| | | | X/11 ↙ | 64.8 | ab | (I/15/92 ↙ | 53.7 | -) | VII/14/47/56 ↙ | 41.2 | cd |
| | | | V/11 ↗ | 65.5 | ab | XV/1/81 ↙ | 54.6 | bc | XIV reference - | 41.6 | cd |
| | | | XIV reference | 70.2 | ab | XIII/25/27 ↙ | 55.7 | bc | X/11/56/82 ↙ | 44.2 | cd |
| | | | XV/9 ↗ | 76.1 | b | XIV/14/18 ↗ | 59.5 | bc | XV/1/81/32 ↙ | 44.6 | cd |
| | | | | | | XV/9/43 ↗ | 66.4 | c | X/11/92/71 ↗ | 45.6 | d |
| | | | | | | (II/7/96 ↙ | 68.0 | -) | (V/11/69/24 ↙ | 53.6 | -) |
| | | | | | | (II/7/96 ↙ | 69.2 | -) | | | |

Tabl. 4. Egg counts on cauliflower varieties and progenies selected for resistance (↙) or susceptibility (↗) to cabbage root fly during the series of even years from 1984 to 1988.

| 1984 | | | 1986 | | | | 1988 | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Variety or progeny | Mean nb. eggs/plant | S.D. (P 0.05) | Variety or progeny | | Mean nb. eggs/plant | S.D. (P 0.05) | Variety or progeny | | Mean nb. eggs/plant | S.D. (P 0.05) |
| X/20, 2 | 102.3 | a | XVII | - | 19.7 | a | XVII/30 | ↙ | 9.9 | a |
| X reference | 120.0 | ab | X/20,5/9 | ↙ | 22.2 | ab | (XX | - | 10.7 | -) |
| XIV reference | 136.7 | abc | XV/44 | ↗ | 23.8 | abc | XVII/64 | ↙ | 11.3 | ab |
| X/20,1 | 151.9 | abc | XVI/93 | ↙ | 25.7 | abc | X/20,5/9/21 | ↙ | 14.4 | abc |
| X/20,5 | 153.3 | abc | III/44 | ↗ | 26.0 | abc | III/44/34 | ↙ | 16.7 | bcd |
| XVI | 158.2 | bc | X/20,5/64 | ↗ | 26.5 | abc | XIV/66/14 | ↙ | 17.4 | bcde |
| X/20,3b | 158.4 | bc | X/20,1/12 | ↙ | 26.5 | abc | XIV/35/38 | ↙ | 17.5 | bcde |
| III | 189.2 | c | X/20,3b/86 | ↙ | 26.9 | abc | XVI/93/39 | ↙ | 18.2 | cdef |
| XV | 191.8 | c | XVI/27 | ↙ | 27.6 | abc | III/44/54 | ↗ | 20.9 | cdef |
| | | | X/22 | ↙ | 28.6 | abc | XIV reference | | 21.1 | def |
| | | | XIV/35 | ↙ | 29.3 | abc | X/22/32 | ↙ | 21.1 | def |
| | | | XVI/47 | ↗ | 30.4 | abcd | XV/25/20 | ↙ | 21.5 | def |
| | | | X/20,5/73 | ↙ | 31.0 | abcd | XVI/27/22 | ↙ | 21.9 | def |
| | | | XIV/66 | ↙ | 32.1 | abcd | XV/25/5 | ↙ | 22.6 | def |
| | | | X/20,1/31 | ↙ | 33.3 | abcd | X/20,1/69/38 | ↙ | 22.9 | def |
| | | | X/20,5/29 | ↙ | 35.5 | bcd | X/20,1/31/26 | ↙ | 23.0 | def |
| | | | XV/25 | ↙ | 35.7 | bcd | XVIII/34 | ↙ | 23.8 | ef |
| | | | X/20,1/69 | ↙ | 36.2 | cd | XVI/47/59 | ↗ | 24.3 | ef |
| | | | XVIII | - | 42.8 | d | X/20,5/73/31 | ↙ | 24.9 | f |
| | | | | | | | XXI | - | 34.7 | g |

**Tab. 5 No effect of selection for resistance**

| Year | Variety or progeny | | Corrected number of eggs | S.D. (P=0.05) |
|---|---|---|---|---|
| 1981 | X | - | 65,5 | a |
| 1984 | X/20,1 | ↙ | 79,1 | a |
| 1986 | X/20,1/31 | ↙ | - | - |
| 1988 | X/20,1/31/26 | ↙ | 77,6 | a |

**Tab. 6. No effect of selection for susceptibility**

| Year | Variety or progeny | | Corrected number of eggs | S.D. (P=0.05) |
|---|---|---|---|---|
| 1984 | XVI | - | 82,4 | a |
| 1986 | XVI/47 | ↗ | - | - |
| 1988 | XVI/47/59 | ↗ | 82,0 | a |

**Tab. 7. Temporary effect of selection for resisance**

| Year | Variety or progeny | | Corrected number of eggs | S.D. (P=0.05) |
|---|---|---|---|---|
| 1983 | VII | - | 73,1 | b |
| 1985 | VII/14 | ↙ | 41,9 | a |
| 1987 | VII/14/47 | ↙ | 69,0 | b |
| 1989 | VII/14/47/56 | ↙ | 70,5 | b |

**Tab. 8. No effect of selection for resistance with temporary deviation for susceptibility**

| Year | Variety or progeny | | Corrected number of eggs | S.D. (P=0.05) |
|---|---|---|---|---|
| 1983 | XIII | - | 56,3 | a |
| 1985 | XIII/15 | ↙ | 54,3 | a |
| 1987 | XIII/15/27 | ↙ | 94,0 | b |
| 1989 | XIII/15/27/33 | ↙ | 58,2 | a |

**Tab. 9. Reverse effect of selection for susceptibility**

| Year | Variety or progeny | | Corrected number of eggs | S.D. (P=0.05) |
|---|---|---|---|---|
| 1984 | III | - | 98,6 | a |
| 1986 | III/44 | ↗ | - | |
| 1988 | III/44/54 | ↗ | 70,5 | b |

**Tab. 10. Expected effect of selection for susceptibility**

| Year | Variety or progeny | | Corrected number of eggs | S.D. (P=0.05) |
|---|---|---|---|---|
| 1983 | XV | - | 52,4 | a |
| 1985 | XV/9 | ↗ | 74,3 | a |
| 1987 | XV/9/43 | ↗ | 112,1 | b |

**Tab. 11. Expected effect of selection for resistance**

| Year | Variety or progeny | | Corrected number of eggs | S.D. (P=0.05) |
|---|---|---|---|---|
| 1984 | XV | - | 99,9 | a |
| 1986 | XV/25 | ↙ | - | |
| 1988 | XV/25/20 | ↙ | 72,6 | b |

# BEHAVIORAL BASIS OF RESISTANCE TO DIAMONDBACK MOTH LARVAE IN GLOSSY LEAFED *BRASSICA*

S. D. EIGENBRODE and A. M. SHELTON.
Department of Entomology, Cornell University, New York State Agricultural Experiment Station, Geneva, NY 14456, USA.

## SUMMARY

First instar diamondback moth larvae move faster, establish fewer mines, and have higher mortality on glossy leafed, highly resistant *Brassica* genotypes descended from the cauliflower PI234599 than on nonglossy controls. A correlation between larval mortality and larval movement rates on several genotypes indicates that the larval movement behavior is directly related to resistance. Increased movement rates and reduced feeding could increase larval exposure to biotic and abiotic mortality factors. Morphology, quantity, and chemical composition of epicuticular leaf waxes affect movement and feeding behaviors of the larvae and are implicated in producing the resistance in glossy brassicas.

## INTRODUCTION

Glossy leafed *Brassica oleracea* breeding lines descended from cauliflower PI234599, are highly resistant to the diamondback moth. This resistance is due either to the glossy leaf itself or to unknown linked characters (1). The resistance results from high mortality of first instar larvae (2, 3). Studies are in progress to better understand this mechanism and to determine if the glossy trait is required for expression of resistance or can be separated from characters linked to the glossy trait. In this paper we report the results of studies of the behavior of first instar diamondback larvae on resistant glossy and susceptible nonglossy brassicas, and on leaf waxes from these plants.

## EXPERIMENTS

### Observations of Larvae on Whole Plants

Approximately 500 neonate ~~larvae~~ were placed on a middle age leaf of resistant and susceptible cabbages. Location and condition of the larvae and number of feeding mines established was determined at 3 hours and 24 hours after the inoculation. Additionally, the dispersal of larvae from the inoculated leaf was quantified using an index, calculated as

$\sum_{i=1}^{k} pi(Li)$, where pi is the proportion of larvae found on leaf i, Li is the leaf position relative to the inoculated leaf, and k is the total number of leaves on the plant. A large index indicates greater dispersal.

Larvae disperse more rapidly from the inoculated leaf, establish fewer mines, and are more likely to be found moribund on resistant plants (Table 1). We hypothesize that glossy resistant plants are rejected by neonate larvae, resulting in increased mortality due in part to starvation, and also, that increased movement and failure to establish protective mines results in increased exposure to predation and environmental stresses.

Table 1: Comparison of diamondback moth larval survival and feeding and dispersal on susceptible 'Round-Up' (R-Up) and glossy resistant 2518 cabbage in the field (SE).

| Hours after inoc. | proportion moribund larvae | | mines/larva | | dispersal index | |
|---|---|---|---|---|---|---|
| | R-Up | 2518 | R-Up | 2518 | R-Up | 2518 |
| 3h | 0.021 (0.01) | 0.07 (0.02) | 0.71 (0.08) | 0.06 (0.11) | 1.18 (0.15) | 3.01 (0.29) |
| P | 0.05 | | ns | | 0.012 | |
| 24h | 0.08 (0.02) | 0.30 (0.04) | 3.96 (0.46) | 0.46 (0.43) | 2.43 (0.46) | 5.81 (0.55) |
| P | 0.001 | | 0.001 | | 0.003 | |

Movement Rates of Individual Larvae

Movement rates of individual larvae were measured for five minutes on intact leaves of resistant and susceptible brassicas. Test lines were NY2535, a nonglossy cabbage showing partial resistance to diamondback moth, PI261597, a collard with a glossy gene nonallelic with the PI234599 glossy gene, NY8329 and NY3891, cabbages descended from the PI234599, and susceptible 'Round-Up' cabbage. Plants of the these lines were also inoculated with large numbers of larvae and larval survival determined at pupation. Movement rates were highest on the most resistant glossy cabbages, lower on the less resistant glossy collard, and lowest on resistant and susceptible nonglossy types (Table 2). Among these five lines, resistance (mortality) was correlated with movement rate ($R = 0.833$; $R^2 = 0.781$; $P < 0.02$; $df = 4$) supporting the hypothesis that movement rate is directly related to resistance.

Table 2: Percent survival of diamondback moth to pupation and movement rates of neonate diamondback moth larvae (cm/min) on five Brassica genotypes

| Genotypes | Percent survival | No. of Plants Inoculated | cm/min | No. of Larvae |
|---|---|---|---|---|
| Normal Bloom | | | | |
| 'Round-Up' | 30.83±2.18a | 12 | 0.12±0.01c | 39 |
| NY2535 | 14.17±1.56b | 12 | 0.15±0.02c | 40 |
| Glossy | | | | |
| PI 261597 | 9.22±1.08c | 12 | 0.37±0.04b | 21 |
| NY3891 | 2.22±0.41d | 12 | 0.41±0.05b | 38 |
| NY8329 | 0.78±0.16d | 12 | 0.53±0.05a | 36 |

Values (± SE) with same letter in a column are not significantly different (P = 0.05)

Role of Leaf Waxes

Removal of waxes with dichloromethane reduces larval movement rates on glossy 8329 and increases movement rates on nonglossy susceptible 'Round-Up', eliminating the behavioral difference on the two genotypes, and implicating the leaf surface waxes in producing the behavioral response by the larvae (Table 3). Mechanical polishing of these two genotypes results in a greater increase in larval movement on 'Round-Up', eliminating the difference between the two genotypes, and implicating morphology of the leaf surface waxes in producing the behavioral response by the larvae (Table 3).

Table 3: Movement rates of neonate diamondback moth on leaves (cm/min) on susceptible 'Round-Up' and resistant glossy 8329: A. effect of removal of leaf epicuticular waxes; B. effect of polishing leaf epicuticular waxes

A. Removal of Leaf Waxes

| | 'Round-Up' | 8329 |
|---|---|---|
| Waxed | 0.13±0.01c (35) | 0.51±0.05a (42) |
| Dewaxed | 0.26±0.02b (35) | 0.27±0.05b (43) |

B. Polishing Leaf Waxes

| | 'Round-Up' | 8329 |
|---|---|---|
| Untreated | 0.29±0.03b (35) | 0.52±0.06a (42) |
| Polished | 0.49±0.05a (35) | 0.58±0.07a (43) |

Values with the same letter in each experiment are not significantly different (P = 0.05).

The waxes removed with dichloromethane and hexanes from glossy resistant 8329 and susceptible 'Round-Up' plants were redeposited on glass slides. The morphology of the waxes from the two genotypes did not differ substantially when deposited on glass. Neonate larval behaviors were quantified on these substrates, for 5 minutes per observation, using a computer-assisted monitoring system. Larvae spend the same amount of time biting and spinning on waxes deposited at 10 µg wax/$cm^2$ from the two genotypes; at 65 µg wax/$cm^2$, larvae spend more time in these two behaviors on waxes from susceptible plants (Table 4). In addition, there are significant behavioral differences in response to amount of wax, regardless of genotype (10µg vs 65µg: searching P = 0.004; biting P = 0.002; length of bite P = 0.008). This suggests that the reduced wax on glossy plants (59.7 µg/$cm^2$ on 'Round-Up' vs. 10.7 µg/$cm^2$ on glossy 8329) also contributes to the resistance. These results indicate that wax chemistry, as well as structure, influences the behavior of diamondback moth larvae and may contribute to resistance in the glossy plants.

Table 4: Mean time diamondback moth larvae spend biting and spinning during five minutes on leaf waxes from susceptible 'Round-Up' and resistant glossy 8329 cabbage

| Solvent | Wax Source | Wax/ $cm^2$ | Biting | Spinning |
|---|---|---|---|---|
| Dichloro-methane | 'Round-Up' | 10µg | 14.0±3.0ecd | 15.0±3.7abc |
| | | 65µg | 35.2±6.8ab | 27.1±6.8ab |
| | 8329 | 10µg | 16.9±3.1ecd | 16.2±4.9abc |
| | | 65µg | 28.0±5.2bc | 15.4±3.9abc |
| Hexane | 'Round-Up' | 10µg | 21.7±5.2bcd | 19.9±5.8abc |
| | | 65µg | 48.0±8.8a | 29.6±6.7a |
| | 8329 | 10µg | 18.1±4.4ecd | 25.4±6.8ab |
| | | 65µg | 18.7±3.4ecd | 15.8±4.3abc |
| | Paraffin | 10µg | 13.6±6.2ecd | 15.9±8.0abc |
| | | 65µg | 2.08±0.5ed | 10.6±4.1bc |
| - | - | 0.0g(glass) | 2.3±0.8e | 4.8±2.5c |

Means (± SE) with same letter in a column are not significantly different (P = 0.05)

## CONCLUSIONS

Resistance in glossy brassicas to the diamondback moth is associated with increased movement rates and reduced feeding of first instars. We hypothesize that this association is causal. The direct involvement of the leaf waxes in producing behavioral

differences related to resistance indicates that the resistance is due to, rather than linked to, the glossy trait. Additional experiments are needed to confirm the hypothesis that increased movement leads to exposure related high mortality on the glossy plants.


## RÉSUMÉ

Les larves du premier stade de la teigne des crucifères se déplacent plus vite, produisent moins de mines et ont une mortalité plus élevée sur les feuilles sans cuticule cireuse des génotypes de *Brassica* à forte résistance descendants du chou-fleur PI 234599. Une corrélation entre la mortalité des larves et l'intensité des mouvements de celles-ci sur plusieurs génotypes indique que le comportement de locomotion des larves peut être directement mis en relation avec la résistance. Il se pourrait que les larves se déplaçant plus et se nourrissant moins soient plus exposées à des facteurs de mortalité biotiques et abiotiques. La structure, l'épaisseur et la composition chimique de la couche cireuse épicuticulaire de la feuille influencent la locomotion et le comportement de nutrition des larves, et sont impliqués dans la résistance chez les *Brassica* non pruineux.


## REFERENCES


1. LIN, J., ECKENRODE, C. J. & DICKSON, M. H., 1983. Variation in Brassica oleracea resistance to diamondback moth (Lepidoptera: Plutellidae). J. Econ. Entomol. 76: 1423-1427.
2. LIN, J., DICKSON, M. H. & ECKENRODE, C. J., 1984. Resistance of Brassica lines to the diamondback moth (Lepidoptera: Yponomeutidae) in the field, and inheritance of resistance. J. Econ. Entomol. 77: 1293-1296.
3. EIGENBRODE, S. D., SHELTON, A. M. & DICKSON, M. H., 1990. Two types of resistance to the diamondback moth in cabbage. Environ. Entomol. In press.

# EXTRACTS OF NORMAL BLOOM RESISTANT CABBAGE REDUCE SURVIVORSHIP OF DIAMONDBACK MOTH LARVAE

S. D. EIGENBRODE, A. M. SHELTON and M. H. DICKSON.
First author's address: Department of Entomology, Cornell University, New York State Agricultural Experiment Station, Geneva, NY 14456, USA.

## SUMMARY

Normal bloom cabbage genotypes with partial resistance to the diamondback moth have been developed at the New York State Agricultural Experiment Station at Geneva. Polar (water soluble) fractions of ethanol extracts of several of these genotypes, when added to meridic diets, reduce the survival of diamondback larvae, as compared with extracts from susceptible controls. This reduction in survival is correlated with levels of resistance of the intact plants in the field. Hexane fractions of the same extracts are not active, nor are extracts from a highly resistant glossy genotype descended from PI234599. Polar toxins or antifeedants are thus at least partially responsible for the resistance in the normal bloom lines but do not account for resistance in the glossy lines. Efforts are underway to characterize the active materials further.

## INTRODUCTION

Cabbage breeding lines, developed at the New York State Agricultural Experiment Station at Geneva, have resistance to the diamondback moth, Plutella xylostella (L.). These lines are being investigated to determine the mechanisms conferring resistance to this insect. Resistance is due to reduced survival of first instar larvae (1, 2). In this paper we report the results of efforts to extract active compounds from several of these resistant breeding lines.

## METHODS

Three normal bloom resistant lines, NY2503, NY2506, NY2535, a glossy line NY2518, and one susceptible hybrid, 'Round-Up', were used in these tests. In previous damage screening trials these lines ranked, from most to least damaged: 'Round-Up' > NY2506 > NY2503 > NY2535 > NY2518.

Plants were transplanted into field plots eight weeks after germination. Identical plantings were made on 11 June and 8 July, 1987, at the Vegetable Research Farm near Geneva, New York. All tests were performed using plants which had been in the field for at least six weeks, but before heading occurred.

### Larval Survival on the Genotypes

Nine plants per line, in each planting, were inoculated with 200 diamondback moth eggs each from our laboratory culture.

Plants were inoculated by pinning aluminum foil with eggs to an inner frame leaf (3). When larvae reached early fourth instar, the plants were destructively sampled and all larvae counted. Actual larval survival was estimated using a mean egg hatch rate of 51.8±4.3%, obtained from a sample of 30 similar aluminum foil egg sheets placed in the field.

In a second experiment, leaf clip cages, 4 cm in diameter, were attached to inner frame leaves of all five lines and inoculated with ten diamondback moth eggs. Survival, determined when insects reached third instar, was calculated as above. This experiment was initiated on 21 July (22 cages per line) and 28 July (12 cages per line).

Larval Survival on Diet with Extracts of the Genotypes

One hundred grams of leaf tissue were removed from inner frame leaves of four plants from each cabbage genotype. The samples were immediately plunged into 70° C ethanol, to minimize enzymic activity, and extracted for 15 minutes. In the laboratory, the crude extracts were homogenized, filtered, and concentrated under reduced pressure. The residue was re-extracted with hexane, followed by water, to produce nonpolar and polar fractions. Filtered polar fractions were again concentrated and added to a standard artificial diet (4) at a rate of four grams leaf equivalent per gram (g LE/g). Nonpolar fractions were mixed with cellulose granules and evaporated, with agitation, to dryness. The coated cellulose was added to diet at the 4 g LE/g diet rate. Untreated controls were prepared for each test by adding water or uncoated cellulose to diet at the same rates used to produce treated diets. Cups (30 ml) with diet were inoculated with 10 diamondback moth eggs and survival was determined at fourth instar. Mean percent survival was calculated as:

$$\text{Adjusted \% survival} = \left(1 - \left(\frac{\text{\%survival control-\% survival treatment}}{\text{\% survival control}}\right)\right) \times 100$$

This test was repeated five times using nonpolar extracts and seven times using polar extracts for a total of 276 and 388 observations respectively.

## RESULTS

Larval Survival on the Genotypes

In the two inoculations, NY2535 reduced larval survival by 50% and 60% respectively, compared with 'Round-Up'. The glossy NY2518 reduced survival by >99% relative to 'Round-Up'. Survival on NY2506 and NY2503 was not significantly different than that on 'Round-Up'. Similar patterns occurred in leaf cages but survival on NY2503 was also significantly less than on 'Round-Up' (Table 1).

Larval Survival on Diet with Extracts of the Genotypes

Significant reduction in larval survival occurred on artificial diet supplemented with polar extracts of NY2503 and

NY2535, relative to diet treated with 'Round-Up' extracts (Table 2). Survival on diet treated with extract from NY2506 and glossy NY2518 was not different from that on 'Round-Up'-extract treated diet. No significant differences occurred among diets supplemented with nonpolar extracts and survival on the nonpolar diets was enhanced relative to pure diet controls (adjusted survival > 100%).

Table 1: Percent survival to pupation of diamondback moth larvae placed on five cabbage genotypes (whole plants or in leaf cages)

| | | Check | Test Lines | | | |
|---|---|---|---|---|---|---|
| Type | Date | 'Round-Up' | 2506 | 2503 | 2535 | 2518 (glossy) |
| plant | 24 July | 60.30±11.60a | 61.62±3.56a | 42.88±6.14ab | 30.22±3.56b | 0.22±0.15c |
| plant | 17 Aug. | 16.00±2.04a | 14.50±1.92a | 14.82±2.12a | 6.32±0.68b | 0.16±0.18c |
| cage | Aug. 3 | 35.0±7.8a | 36.6±9.9a | 13.3±4.5b | 16.6±6.4b | 0.80±0.91c |
| cage | Aug. 7 | 56.7±5.4a | 39.2±6.6ab | 32.5±5.8b | 37.5±9.5b | 0.0±0.0c |

Means (± SE) in each row with the same letter are not statistically different

Table 2: Percent survival of diamondback moth on artificial diet treated with polar and nonpolar extracts of five cabbage genotypes

| | Control | Test Lines | | | |
|---|---|---|---|---|---|
| Extract | 'Round-Up' | 2506 | 2503 | 2535 | 2518 (glossy) |
| polar | 94.2±4.2a | 84.6±5.6abc | 76.3±4.0c | 80.2±4.4bc | 93.1±4.1ab |
| nonpolar | 106.6±4.5a | 100.6±5.1a | 117.3±9.2a | 101.5±5.7a | 106.3±4.5a |

Means (± SE) in the same row with the same letter are not significantly different

## DISCUSSION

With the exception of the glossy NY 2518, survival on whole plants was reflected in larval survival on diets supplemented with polar extracts from the plants. This suggests that resistance in the Geneva nonglossy lines depends, in part, on plant compounds present in the polar fraction. Work on the further isolation of chemical resistance factors has proven difficult. Activity is lost with further fractionation which may indicate that several compounds must be present together to adversly affect the insects. Additionally, because the activity in the extracts does not fully reflect the resistance levels of the source plants, and because resistance in the nonglossy lines is quantitatively inherited (unpublished data), it appears likely that resistance in these

lines is due to the cumulative effect of several minor characters including the compounds present in the polar extracts.

Resistance in the glossy line NY2518 does not depend on compounds extractable in ethanol but is due to other mechanisms (Eigenbrode and Shelton, this volume).

## RÉSUMÉ

Des génotypes de choux typiques à cuticule cireuse montrant une résistance partielle à la teigne des crucifères, ont été développées à New York State Agricultural Experiment Station de Geneva. Si on rajoute des fractions polaires (solubles à l'eau) d'extraits à l'éthanol de plusieurs de ces génotypes au milieu alimentaire artificiel, on constate que la survie des larves de ce lépidoptère est réduite comparée aux extraits de témoins sensibles. Cette diminution de survie est corrélée avec les niveaux de résistance des plantes indemnes au champ. Les fractions à l'héxane du même extrait, ainsi que les extraits d'un génotype non cireux descendant du PI 234599, et à forte réeistance, n'ont pas montré d'activité. Des toxines polaires ou des antiappétants sont donc, partiellement au moins, responsables de la résistance des lignées à cuticule cireuse, mais cela n'est pas le cas pour les lignées non pruineuses. De nouvelles recherches sont entreprises pour caractériser les principes actifs.

## REFERENCES

1. LIN, J., ECKENRODE, C. J. & DICKSON, M. H., 1983. Variation in Brassica oleracea resistance to diamondback moth (Lepidoptera: Plutellidae). J. Econ. Entomol. 76: 1423-1427.

2. EIGENBRODE, S. D., SHELTON, A. M. & DICKSON, M. H., 1990. Two types of resistance to the diamondback moth in cabbage. Environ. Entomol. In press.

3. ANDALORO, J. T., ROSE, K. B., SHELTON, A. M., HOY, C. W. & BECKER, R. F., 1983. Cabbage growth stages. New York's Food and Life Sciences Bulletin No. 101.

4. BIEVER, K. D. & BOLDT, P. E., 1971. Continuous laboratory rearing of the diamondback moth and related biological data. Annals of Entomol. Soc. of Amer. 64: 651-655.

# EFFECTS OF PLANT COMPOUNDS ON HOST PLANT ACCEPTANCE IN SOME CRUCIFER-FEEDING INSECTS: HOW CAN THIS INFORMATION FACILITATE BREEDING FOR RESISTANCE.

JENS KVIST NIELSEN

Chemistry Department, Royal Veterinary and Agricultural University, Thorvaldsensvej 40, DK-1871 Frederiksberg C.

Summary

Host plant recognition in crucifer specialist insects is mediated by 1) glucosinolates and their hydrolysis products, 2) other stimulatory compounds present in the host plants, and 3) inhibitory compounds. A short review of the role of these factors in different insects is given. Resistance in cultivated crucifers is often based on antixenosis (non-preference), but the actual mechanisms and the chemicals involved are largely unknown. It is important to know the type of resistance factor present in different breeding lines in order that appropriate selection and breeding procedures can be adopted.

## 1. Introduction

A number of insect species have specialised in feeding on members of the plant family Cruciferae. All members of this plant family appear to contain a characteristic group of compounds, the glucosinolates. The glucosinolates always occur together with specific enzymes, myrosinases, which hydrolyse the glucosinolates to simpler products, e.g. isothiocyanates, thiocyanates, and nitriles. The kind of hydrolysis products formed depend amongst other things on the nature of the parent glucosinolate, the plant species, and the presence of other compounds in the hydrolysate (Fenwick et al., 1983).

The interaction between the glucosinolate complex and cruciferous insects is well established, but recent results have demonstrated that the presence of particular glucosinolates or hydrolysis products is not the only factor influencing host plant acceptability for these insects. It is the intention of the present paper to give a short review of the chemical factors utilized by cruciferous insects to discriminate between different species or cultivars of crucifers. The paper deals specifically with effects on behaviour. It is suggested that at least three different mechanisms could influence resistance which is based on antixenosis (non-preference), and that a better knowledge of these factors might be helpful in designing appropriate breeding programmes.

## 2. Glucosinolates and their hydrolysis products

A stimulatory effect of glucosinolates on feeding in cruciferous insects has been demonstrated frequently (Larsen et al.,1985; Lerin, 1980; Ma, 1972; Nayar & Thorsteinson, 1963; Nielsen, 1988 and references therein). A similar effect on oviposition has been demonstrated in certain Lepidoptera and Diptera (Ma & Schoon-

hoven, 1973; Nair & McEwen, 1976; Sömme & Rygg, 1972; Zohren, 1968). The hydrolysis products are usually less efficient than their parent glucosides in stimulating feeding and oviposition (Nair & McEwen, 1976; Nayar & Thorsteinson, 1963; Tanton, 1977; Terofal, 1965). However, the volatile isothiocyanates are known to attract insects from some distance, and many cruciferous insects can be caught in traps emitting these substances (Finch & Skinner, 1982; Görnitz, 1956).

The presence of glucosinolates and their hydrolysis products may allow insects to discriminate between crucifers and non-crucifers which do not contain these compounds. However, there is little evidence that insects discriminate between species and cultivars of crucifers by means of specific reactions to particular glucosinolates present in the most acceptable plants. Some observations from our laboratory demonstrate this limited role of glucosinolates: 1) The glucosinolates from non-host plants were as stimulatory and sometimes even more stimulatory than glucosinolates from host plants to two flea beetle species (Nielsen, 1978b; Nielsen et al., 1979a). 2) Five plant species with identical glucosinolate patterns were treated differently by several beetle species. Two of these plants, horseradish (*Armoracia rusticana*) and black mustard (*Brassica nigra*), were acceptable to the monophagous horseradish flea beetle (*Phyllotreta armoraciae*) in laboratory experiments, while three other plant species, pennycress (*Thlaspi arvense*), garlic mustard (*Alliaria petiolata*), and a candytuft species (*Iberis umbellata*) were unacceptable (Nielsen et al., 1979a). Black mustard was highly acceptable for the oligophagous *Phyllotreta nemorum,* while horseradish, pennycress, and garlic mustard were of intermediate acceptability, and *Iberis umbellata* was unacceptable (Nielsen, 1978a,b). These five plant species were also treated differently by adults of *Ceutorhynchus constrictus* (Nielsen et al., 1989) and by 5th instar larvae of *Pieris brassicae* and *P. napi* (Terofal, 1965). 3) Increasing the concentration of glucosinolates on leaf discs of *Cheiranthus, Erysimum*, and *Iberis* species and cotyledons of oilseed rape (*Brassica napus* var. *oleifera*) had no effect on the acceptability for several flea beetle species (Larsen et al., 1985; Nielsen, 1978a).

Glucosinolates may vary in their ability to stimulate feeding and oviposition (Larsen et al., 1985; Nair & McEwen, 1976; Nayar & Thorsteinson, 1963; Nielsen, 1978a; Nielsen et al., 1979a; Schoonhoven, 1972), but insects may be unable to discriminate between plants having high concentrations of less stimulatory glucosinolates and lower concentrations of more stimulatory compounds. At least in butterfly larvae, this limited discriminatory ability may be related to the low number of sensory cells responding to these compounds (Schoonhoven, 1972).

Some glucosinolates are known to yield different hydrolysis products in different plant species, e.g. sinigrin (allylglucosinolate) is hydrolysed to allyl thiocyanate in pennycress, while in most other species it is hydrolysed to allyl isothiocyanate. This difference may affect colonization rates in the field (Feeny, 1983), but the laboratory experiments described above suggest that other factors are more important for the ability of beetle species to discriminate between pennycress and other sinigrin containing plants. There is no evidence that specificity in host plant recognition in any species of Coleoptera or Lepidoptera is determined by particular hydrolysis products released from glucosinolates.

Differences in acceptability of radish (*Raphanus sativus*) of

different ages to the cabbage root fly (*Delia radicum*) are correlated with the amounts of volatile hydrolysis products released during autolysis for one hour at 40°C. (Ellis et al., 1980). Since volatile isothiocyanates and other plant odours are generally unable to stimulate oviposition in the cabbage root fly (Nair & McEwen, 1976; Zohren, 1968) as well as the related turnip root fly (*D. floralis*) (Alborn et al., 1985; Sömme & Rygg, 1972), this effect may be caused mainly by the parent glucosinolates. Plant odours are attractants for the root flies (Finch & Skinner, 1982) and they also seem to increase general activity (Wallbank & Wheatley, 1979). Plants lacking volatile isothiocyanates may be less readily found by ovipositing females (Finch, 1978), but the role of volatiles in mediating differences in acceptability of cruciferous plants is still uncertain.

In conclusion, there appear to be no reported good examples, where the acceptability of particular crucifer species to an insect is determined by a particular pattern of glucosinolates and their hydrolysis products. The compounds are important stimulants of behaviour leading to host plant selection, but the ability to discriminate between different crucifers seems to depend on other factors.

### 3. Other stimulatory compounds

Some common nutrients like sugars, amino acids, and ascorbic acid have been found to influence feeding on artificial substrates and/or to stimulate particular sensory cells in larvae of certain Coleoptera and Lepidoptera (Ma, 1972; Mitchell, 1978; Nayar & Thorsteinson, 1963; Schoonhoven, 1972; Van Loon, 1988). It is not known whether differences in the content of particular nutrient compounds in plants have any influence on the acceptability of the plants to insects.

Only a few secondary compounds have been identified which are behavioural stimulants for cruciferous insects, but not related biosynthetically to the glucosinolates. However, there is evidence for the presence in *Brassica* varieties of such compounds which stimulate oviposition in the cabbage root fly (Schöni et al., 1987), the turnip root fly (Alborn et al., 1985), and in *Pieris rapae* (Renwick & Radke, 1988). Other compounds from the host plants stimulate feeding in species of *Ceutorhynchus* (Nielsen, 1989b; Nielsen et al., 1989) and *Phyllotreta* (Nielsen et al., 1979b). Two flavonol glycosides have been identified from horseradish leaves. They stimulate feeding in the monophagous horseradish flea beetle, *P. armoraciae*, but not in the related oligophagous species, *P. nemorum* (Nielsen, 1978b; Nielsen et al., 1979b). Mixtures of sinigrin and the flavonol glycosides are more stimulatory than any of the pure compounds (Nielsen et al., 1979b). The horseradish flea beetle is able to distinguish between the flavonol glycosides from horseradish and similar glycosides found in several other crucifers. Therefore, the responses to the flavonol glycosides seem to be important for the ability to discriminate between host and non-host plants. However, the interactions are complex since the responses of the horseradish flea beetle to plants and to the flavonol glycosides vary with age and previous experience of the beetles (Nielsen, unpubl.). Sinapic acid and an anthocyanin (cyanin) stimulate feeding on artificial diets in *Pieris brassicae* larvae (Van Loon, 1988).

## 4. Inhibitory compounds

The role of feeding deterrents in host plant selection of several flea beetle species has been evaluated. The acceptability of leaf discs from a number of glucosinolate containing plant species was measured. Extracts were prepared from the same plants, and the contents of feeding deterrents in the extracts were measured and related to the acceptability of the leaf discs. In the oligophagous *P. nemorum* there was a good negative correlation between the acceptability and the content of feeding deterrents in the investigated plants (Nielsen, 1978b). Feeding deterrents also influenced the acceptability of plants for the monophagous horseradish flea beetle, but this species rejected some plants which did not contain deterrents (Nielsen, 1978b).

Flea beetle species differred somewhat in their sensitivity to different classes of feeding deterrents. *P. nemorum* was very sensitive to cucurbitacins and did not feed on *Iberis* species containing these compounds. On the other hand it was relatively insensitive to cardenolides, and it fed on *Cheiranthus* and *Erysimum* species containing cardenolides (Nielsen, 1978a,b, 1989a). Other flea beetle species were very sensitive to cardenolides and did not feed on plants containing cardenolides (Nielsen, 1978a,b). These results suggest that inhibitory compounds are important in host plant selection of flea beetles. On the other hand, it was not possible to detect any deterrent or toxic effects of cucurbitacins and cardenolides on *Pieris rapae* larvae when these compounds were presented in an artificial diet. The reasons why *P. rapae* larvae reject *Iberis* and *Erysimum* species is therefore still unknown (Usher & Feeny, 1983). Recently, cardenolides have been found to be oviposition deterrents for *Pieris brassicae* (Rothschild et al., 1988) and *P. rapae* (Renwick et al., 1989).

A number of compounds, especially alkaloids, inhibit feeding and stimulate a so called deterrent receptor on the mouthparts of *Pieris brassicae* larvae (Schoonhoven, 1972). However, none of these compounds are present in crucifers, and the role of deterrents in host plant recognition in this insect is still unknown.

The cabbage root fly is sensitive to inhibitory compounds, since oviposition on acceptable plants can be reduced by extracts from frass of the garden pebble moth *Evergestis forficalis* as well as by extracts from garlic mustard leaves (Jones & Finch, 1987). Sinapic acid is the active component in frass of the garden pebble moth (Jones et al., 1988). This compound is present in several crucifers as carbohydrate or lactic acid esters (Jones et al., 1988), but any role in natural resistance of crucifers to insects has not been reported. Sinapic acid is a feeding stimulant for *Pieris brassicae* larvae (Van Loon, 1988).

Although several details are still unresolved, the results outlined above demonstrate that most cruciferous insects respond to inhibitory compounds. These compounds seem to preclude the utilization of some plants which contain highly stimulatory glucosinolates, e.g. *Iberis umbellata*. This plant contains a stimulatory glucosinolate pattern, but the content of cucurbitacins preclude its utilization by several flea beetle species (Nielsen, 1978a; Nielsen et al., 1979a).

## 5. Prospects for breeding for resistance to insects

Most of the available information deals with mechanisms used by insects to discriminate between different species of crucifers, but differences in acceptability of cultivars within species are probably determined by the same factors. The prospects for resistance breeding of course depend on 1) whether resistance is based on antixenosis and 2) whether the resistance is based mainly on chemical differences between resistant and susceptible plants.

Antixenosis has been described as an important mechanism of resistance in crucifers against several insects. Resistance in *Brassica* varieties and in radish against cabbage and turnip root flies is at least partially due to low oviposition rates on resistant cultivars (Alborn et al., 1985; Birch, 1988; Ellis et al., 1980; Ellis & Hardman, 1988; Gagnebin & Freuler, 1988). Low preference for oviposition also seems to be involved in resistance of *Brassica* varieties to lepidopterous pests (Dunn & Kempton, 1976). Resistant glossy varieties of cauliflower and cabbage are preferred for oviposition by the diamond back moth (*Plutella xylostella*) (Lin et al., 1984), but factors on the plant surface have a negative effect on larval behaviour (Eigenbrode et al., this volume). Susceptible cabbage cultivars are colonized by larger numbers of onion thrips than resistant cultivars (Stoner & Shelton, 1988).

There are only few examples where chemical differences between resistant and susceptible cultivars of cruciferous plants have been documented. Variations in release rates of volatile hydrolysis products of glucosinolates explained about half of the variation in oviposition rates of cabbage root flies on radish cultivars (Ellis et al., 1980). A susceptible cultivar of chinese cabbage contained higher levels of extractable non-glucosinolate oviposition stimulants than a resistant cultivar of kale (Alborn et al., 1985). No studies have yet determined the relative importance of the resistance mechanisms outlined above: 1) inadequate content of glucosinolates and their hydrolysis products 2) inadequate content of other stimulatory compounds, and 3) presence of inhibitory compounds. At least in ovipositing Diptera and Lepidoptera, it seems to be important to search for resistance factors on the plant surface (Städler, 1986).

When the chemicals involved have been identified, quantitative chemical analyses may be a valuable alternative or supplement to the present screening methods. However, the identification of the compounds involved is a difficult task, and reliable quantitative analytical methods for several compounds may not be available for many years.

A distinction between the three mechanisms outlined above could probably be made without a detailed knowledge of the compounds involved. Extracts from resistant and susceptible cultivars could be divided into fractions with and without glucosinolates (Alborn et al., 1985; Nielsen et al., 1979b; 1989; Schöni et al., 1987), and the content of stimulatory and inhibitory activity in different fractions could be evaluated. This information could be important for making decisions on which lines should be selected for breeding purposes. If for example, resistance in one breeding line is based on inadequate glucosinolate content and in another line on high levels of inhibitory compounds, any progeny containing intermediate levels of both types of compounds might be more acceptable than both parents. It is a common experience that breeding and selection work does not

always lead to improvement of resistance, and that selection for resistance may even lead to higher susceptibility or vice versa (Ellis et al., 1980; Ellis & Hardman, 1988; Freuler et al., this volume). Maybe some of these drawbacks could be avoided, if resistance mechanisms were recognized by performing simple experiments with plant extracts and fractions as outlined above.

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Résumé

Effets de substances végétales sur l'acceptation de la plante-hote par quelques insectes se nourrissant de crucifères: comment cette information peut-elle faciliter la sélection pour la résistance?

La reconnaissance de la plante-hote par les insectes inféodés aux crucifères est régi par
1) les glucosinolates et leurs produits hydrolysés,
2) d áutres substances stimulantes présentes dans les plantes-hotes et
3) des substances inhibitrices.
Un apercu du role de ces facteurs chez différents insectes est présenté. La résistance chez les crucifères cultivés est souvent basée sur l'ántixénose (non-préférence), mais les véritables mécanismes mis en jeu les substances concernées sont, pour la plupart, inconnus. Il est important de savoir le type de facteur de résistance présent chez les différentes lignées sélectionnées, afin de pouvoir adopter la meilleures méthode de sélection et de croisement.

References

ALBORN, H., KARLSON, H., LUNDGREN, L., RUUTH, P. & STENHAGEN, G., 1985. Resistance in crop species of the genus *Brassica* to oviposition by the turnip root fly, *Hylemya floralis*. Oikos 44: 61-69.

BIRCH, A.N.E., 1988. Field and glasshouse studies on components of resistance to root fly attack in swedes. Ann. appl. Biol. 113: 89-100.

DUNN, J.A. & KEMPTON, D.P.H., 1976. Varietal differences in the susceptibility of Brussels sprouts to lepidopterous pests. Ann. appl. Biol. 82: 11-19.

ELLIS, P.R., COLE, R.A., CRISP, P. & HARDMAN, J.A., 1980. The relationship between cabbage root fly egg laying and volatile hydrolysis products of radish. Ann. appl. Biol. 95: 283-289.

ELLIS, P.R. & HARDMAN, J.A., 1988. Investigations of the resistance in cruciferous crops to cabbage root fly at Wellesbourne. IOBC/WPRS Bull. 1988/XI/3: 63-64.

FEENY, P., 1983. Coevolution of plants and insects. In: D.L. WHITEHEAD and W.S. BOWERS (eds.), Natural Products for innovative Pest Management. Pergamon Press, Oxford, pp. 167-185.

FENWICK, G.R., HEANEY, R.K. & MULLIN, W.J., 1983. Glucosinolates and their breakdown products in food and food plants. CRC crit. Rev. Fd. Sci. Nutr. 18: 123-201.

FINCH, S., 1978. Volatile plant chemicals and their effect on host plant finding by the cabbage root fly (*Delia brassicae*). Entomol. exp. appl. 24: 350-359.

FINCH, S. & SKINNER, G., 1982. Trapping cabbage root flies in traps baited with plant extracts and with natural and synthetic isothiocyanates. Entomol. exp. appl. 31: 133-139.

GAGNEBIN, F. & FREULER, J., 1988. Progress in the studies of resistance to cabbage root fly (*Delia radicum*) in cauliflower. IOBC/WPRS Bull. 1988/XI/3: 67-69.

GÖRNITZ, K., 1956. Weitere Untersuchungen über Insekten-Attraktivstoffe aus Cruciferen. Nachr.Bl. Dt. Pfl.Schutz-dienst. N.F. 10: 137-147.

JONES, T.H., COLE, R.A. & FINCH, S., 1988. A cabbage root fly oviposition deterrent in the frass of garden pebble moth caterpillars. Entomol. exp. appl. 49: 277-282.

JONES, T.H. & FINCH, S., 1987. The effect of a chemical deterrent, released from the frass of caterpillars of the garden pebble moth, on cabbage root fly oviposition. Entomol. exp. appl. 45: 283-288.

LARSEN, L.M., NIELSEN, J.K., PLÖGER, A. & SØRENSEN, H., 1985. Responses of some beetle species to varieties of oilseed rape and to pure glucosinolates. In: H. SØRENSEN (ed.), Advances in the Production and Utilization of cruciferous Crops with special emphasis to oilseed Rape. Dr. W. Junk Publ., Dordrecht, pp. 230-244.

LERIN, J., 1980. Influence des substances allelochimiques des cruciferes sur les insectes. Acta Oecol., Oecol. Gener. 1: 215-235.

LIN, J., DICKSON, M.H. & ECKENRODE, C.J., 1984. Resistance of *Brassica* lines to the diamondback moth (Lepidoptera: Yponomeutidae) in the field, and inheritance of resistance. J. econ. Entomol. 77: 1293-1296.

MA, W.C., 1972. Dynamics of feeding responses in *Pieris brassicae* Linn. as a function of chemosensory input: A behavioural, ultrastructural and electrophysiological study. Meded. Landbouwhogeschool Wageningen 72-11: 162 pp.

MA, W.C. & SCHOONHOVEN, L.M., 1973. Tarsal contact chemosensory hairs of the large white butterfly *Pieris brassicae* and their possible role in oviposition behaviour. Entomol. exp. appl. 16: 343-357.

MITCHELL, B.K., 1978. Some aspects of gustation in the larval red turnip beetle, *Entomoscelis americana*, related to feeding and host plant selection. Entomol. exp. appl. 24: 540-549.

NAIR, K.S.S. & MCEWEN, F.L., 1976. Host selection by the adult cabbage maggot, *Hylemya brassicae* (Diptera: Anthomyiidae): Effect of glucosinolates and common nutrients on oviposition. Can. Entomol. 108: 1021-1030.

NAYAR, J.K. & THORSTEINSON, A.J., 1963. Further investigations into the chemical basis of insect-host plant relationships in an oligophagous insect, *Plutella maculipennis* (Curtis) (Lepidoptera: Pyralidae). Can. J. Zool. 41: 923-929.

NIELSEN, J.K., 1978a. Host plant discrimination within Cruciferae: Feeding responses of four leaf beetles (Coleoptera: Chrysomelidae) to glucosinolates, cucurbitacins and cardenolides. Entomol. exp. appl. 24: 41-54.

NIELSEN, J.K., 1978b. Host plant selection of monophagous and oligophagous flea beetles feeding on crucifers. Entomol. exp. appl. 24: 562-569.

NIELSEN, J.K., 1988. Crucifer-feeding Chrysomelidae: Mechanisms of host plant finding and acceptance. In: P. Jolivet, E. Petitpierre & T.H. Hsiao (Eds.), Biology of Chrysomelidae. Kluwer Akad. Publ., Dordrecht, pp. 25-40.

NIELSEN, J.K., 1989a. Host plant relations of *Phyllotreta nemorum* L. (Coleoptera: Chrysomelidae). II. Various defensive mechanisms in plants and their role in defining the host plant range. J. appl. Entomol. 107: 193-202.

NIELSEN, J.K., 1989b. Plant chemicals influencing host plant specificity in *Ceutorhynchus* species feeding on Cruciferae. Proc. 7th int. Symp. Insect-Host Plant Relationships, Budapest (in press).

NIELSEN, J.K., DALGAARD, L., LARSEN L.M. & SØRENSEN, H., 1979a. Host plant selection of the horseradish flea beetle *Phyllotreta armoraciae* (Coleoptera: Chrysomelidae): Feeding responses to glucosinolates from several crucifers. Entomol. exp. appl. 25: 227-239.

NIELSEN, J.K., KIRKEBY-THOMSEN, A.H. & PETERSEN, M.K., 1989. Host plant recognition in monophagous weevils: specificity in feeding responses of *Ceutorhynchus constrictus* and the variable effect of sinigrin. Entomol. exp. appl. 53: 157-166.

NIELSEN, J.K., LARSEN, L.M. & SØRENSEN, H., 1979b. Host plant selection of the horseradish flea beetle *Phyllotreta armoraciae* (Coleoptera: Chrysomelidae): Identification of two flavonol glycosides stimulating feeding in combination with glucosinolates. Entomol. exp. appl. 26: 40-48.

RENWICK, J.A.A. & RADKE, C.D., 1988. Sensory cues in host selection for oviposition by the cabbage butterfly, *Pieris rapae*. J. insect. Physiol. 34: 251-257.

RENWICK, J.A.A., RADKE, C.D. & SACHDEV-GUPTA, K. 1989. Chemical constituents of *Erysimum cheiranthoides* deterring oviposition by the cabbage butterfly, *Pieris rapae*. J. Chem. Ecol. 15: 2161-2169.

ROTHSCHILD, M., ALBORN, H., STENHAGEN, G. & SCHOONHOVEN, L.M., 1988. A strophanthidin glycoside in Siberian wallflower: A contact deterrent for the large white butterfly. Phytochemistry 27: 101-108.

SCHÖNI, R., STÄDLER, E., RENWICK, J.A.A. & RADKE, C.D., 1987. Host and non-host plant chemicals influencing the oviposition behaviour of several herbivorous insects. In: V. LABEYRIE, G. FABRES and D. LACHAISE (eds.), Insects-Plants: Proc. 6th int. Symp. Insect-Plant Relationships, Dr.W. Junk Publ., Dordrecht, Boston, Lancaster, pp.31-36.

SCHOONHOVEN, L.M., 1972. Plant recognition by lepidopterous larvae. Symp. R. Soc. Lond. 6: 87-99.

SÖMME, L. & RYGG, T., 1972. The effect of physical and chemical stimuli on oviposition in *Hylemya floralis* (Fallén) (Dipt., Anthomyiidae). Norsk ent. Tidsskr. 19: 19-24.

STÄDLER, E., 1986. Oviposition and feeding stimuli in leaf surface waxes. In: B. JUNIPER & R. SOUTHWOOD (eds.), Insects and the plant surface. Edward Arnold Publ., London, pp. 105-121.

STONER, K.A. & SHELTON, A.M., 1988. Role of non-preference in the resistance of cabbage varieties to the onion thrips (Thysanoptera: Thripidae). J. econ. Entomol. 81: 1062-1067.

TANTON, M.T., 1977. Response to food plant stimuli by larvae of the mustard beetle *Phaedon cochleariae*. Entomol. exp. appl. 22: 113-122.

TEROFAL, F., 1965. Zur problem der Wirtsspezifität bei Pieriden (Lep.). Unter besonderer Berücksichtigung der einheimischen Arten *Pieris brassicae* L., *P. napi* L. und *P. rapae* L. Mitt. münch. Entomol. Ges. 55: 1-76.

USHER, B.F. & FEENY, P., 1983. Atypical secondary compounds in the family Cruciferae: tests for toxicity to *Pieris rapae*, an adapted crucifer-feeding insect. Entomol. exp. appl. 34: 257-265.

VAN LOON, J.J.A., 1988. Sensory and nutritional effects of amino acids and phenolic plant compounds on the caterpillars of two *Pieris* species. Ph.D. thesis, Agricultural University, Wageningen, 211 pp.

WALLBANK, B.E. & WHEATLEY, G.A., 1979. Some responses of the cabbage root fly (*Delia brassicae*) to allyl isothiocyanate and other volatile constituents of crucifers. Ann. appl. Biol: 91, 1-12.

ZOHREN, E., 1968. Laboruntersuchungen zu Massenanzucht, Lebensweise, Eiablage und Eiablageverhalten der Kohlfliege *Chortophila brassicae* Bouché (Diptera: Anthomyiidae). Z. angew. Entomol. 62: 139-188.

# TECHNIQUES FOR EXPLOITING THE RESISTANCE TO CARROT FLY IN THE CULTIVATED CARROT

P.R. ELLIS AND J.A. HARDMAN
Institute of Horticultural Research, Wellesbourne,
Warwick, CV35 9EF, Great Britain.

Summary

The resistance to carrot fly, Psila rosae (F.), identified in several commercially-acceptable carrot cultivars at Wellesbourne, has been exploited by:

1) Recurrent selection within partially-resistant cultivars;
2) Crossing of partially-resistant cultivars followed by selection of the least damaged lines;
3) Production of a range of inbreds in a single seed descent programme;
4) The breeding of male sterile lines of the resistant cultivar 'Sytan'.

The most resistant lines arising from this breeding work have been included in a joint hybrid scheme set up between the Institute of Horticultural Research-Wellesbourne (IHR-W) and 5 seed companies to develop new cultivars.

## 1. Introduction

The carrot fly, Psila rosae (F.), remains a severe pest of carrot and related crops despite the use of insecticides. The development of resistant cultivars of carrot, Daucus carota L., offers a relatively cheap, long lasting and environmentally-acceptable method of control which could complement other methods and reduce the growers' use of insecticides. The resistance of carrots and related plants has been researched at Wellesbourne for nearly 20 years with two main strategies:

a) the resistance to carrot fly in wild umbelliferous species, particularly those belonging to the genus Daucus, to which the cultivated carrot belongs;
b) the resistance to carrot fly in cultivated carrot material, the subject of this paper.

Partial levels of resistance have been discovered in a wide range of carrot cultivars and breeders' lines evaluated at Wellesbourne over several seasons (Ellis, Hardman, Jackson & Dowker, 1980; Ellis, Hardman & Dowker, 1982a; Ellis, Hardman & Dowker, 1982b; Ellis, Hardman & Dowker, 1987). The highest levels of resistance exist in several Nantes and fodder types of carrot. In collaborative trials carried out under the auspices of the International Organisation for Biological Control (IOBC), the cultivars representing the extremes of the range of resistance have been evaluated at centres in seven countries to investigate possible genotype/environment interactions. These studies showed that the cultivars performed consistently in small and large scale experiments and that there was not any indication of the existence of races of carrot fly which might overcome the resistance (Ellis & Hardman, 1981; Philipsen, 1988).

The partial resistance can lead to a reduction of as much as 50% in

carrot fly damage and a 50% reduction in the numbers of carrot fly pupae remaining in the soil after cropping (Ellis, Freeman & Hardman, 1984). The resistance complements insecticidal control of carrot fly, leading to significant reductions in the doses of chemicals required to provide satisfactory control (Thompson, Ellis, Percivall & Hardman, 1980). By combining appropriate sowing and harvest dates for the crop and the use of partially-resistant carrot cultivars, carrot fly damage can be reduced to minimal levels (Ellis & Hardman, 1988).

All these studies have indicated the potential contribution of partial resistance to controlling carrot fly. However, to be of commercial value in a distinct cultivar, a higher level of resistance is required and therefore an extensive programme of breeding work has been carried out at Wellesbourne, involving close collaboration between entomologists and plant breeders. The breeding work is described in this report.

## 2. Recurrent selection within cultivars

At Wellesbourne, two partially-resistant carrot cultivars have formed the basis of recurrent selection work:

### Cv.'Long Chantenay'

This cultivar was the least damaged amongst a group of Chantenay and Autumn King types of carrots evaluated at Wellesbourne in 1971-72. At harvest, roots were graded into four groups according to the amount of carrot fly damage - grade 1, <5% root surface damaged; grade 2, 5-25% damaged; grade 3, 26-50% damaged; and grade 4, >50% damaged (Ellis, Wheatley & Hardman, 1978). Progenies raised from each of these four groups showed a slight response to selection when tested in replicated trials in 1973. Family selection was practised subsequently, based on the assessment of damage at harvest, and the least damaged 10-20% of the roots of families were selected for seeding. Four cycles of selection were completed, seed being produced in 1972 (1st cycle), 1974 (2nd cycle), 1976 (3rd cycle) and 1978 (4th cycle). The full complement of generations was tested in 1979 and 1980 but there was no marked overall improvement in resistance from one cycle of selection to another. The results of these experiments and of earlier tests on the different generations of material suggested that non-genetic factors interacted with resistance to carrot fly attack. For example, in more than half of the experiments, a negative linear relationship was established for the family means between carrot fly damage and root density at harvest. This line of breeding has consequently been abandoned.

### Cv.'Sytan'

During the period in which recurrent selection was being tried with cv.'Long Chantenay', a search was in progress for cultivars with higher levels of resistance. In 1975, the cultivar 'Sytan'was grown alongside 30 other cultivars representing various types of carrots in trials at Wellesbourne and at Cawood (Yorkshire). 'Sytan' was found to be the least damaged cultivar at both sites and significantly more resistant than any other cultivar tested previously. Because of the initial success with the 'Long Chantenay' programme (see above), it was decided to repeat tests on 'Sytan' to confirm its resistance and then attempt a recurrent selection programme on this promising cultivar.

Confirmatory experiments at Wellesbourne showed that this cultivar was consistently less damaged than all others tested previously (Ellis, Freeman & Hardman, 1984). In an attempt to increase levels of resistance in this cultivar, 15 rows of 'Clause's Original Sytan' (DC 76356) were sown in May 1979 at Wellesbourne resulting in the production of 2500 roots. Carrot fly attack was severe and the 100 least damaged roots of good agronomic quality were saved for seeding; 67 first generation families were secured in 1980. At the same time that this work was in progress at Wellesbourne, colleagues at the Institute for Horticultural Plant Breeding (IVT), Wageningen in The Netherlands were also working with this same cultivar and it was decided to set up a collaborative programme to facilitate the exchange of breeding material for comparisons in the two countries. In 1981, ten half-sib families from IHR-W were grown alongside nine IVT first generation inbreds from 'Sytan' in field experiments in both countries; the parent cultivar was included for comparative purposes. The overall level of carrot fly attack was higher at Wellesbourne with only 2% undamaged roots, than in The Netherlands, where 47% of the roots were undamaged. A significant effect of plant density was revealed at Wellesbourne, requiring an adjustment for root numbers in the statistical analysis of the data. There were no significant differences in damage between the IVT selections but certain IHR-W families were significantly less damaged than others. Each Institute retained promising roots from the least damaged selections for further studies. At Wellesbourne in 1982, all 67 first generation half-sib families, including the ten forming part of the exchange programme, were grown in a field experiment alongside parental material. Most of the families were less damaged than the parental stock and 11 half-sib selections which had the least damage were multiplied in polytunnels and glasshouse compartments. The plants grown on in the polytunnels constituted a simple multiplication of the selections whereas those roots grown on in the glasshouse were selected for minimal carrot fly damage. The eleven families, consisting of first and second generation selections, were evaluated at Wellesbourne and in The Netherlands in 1984. Similar results were obtained at both Institutes and there were significant differences between the parents and selections, the latter being less damaged. Ranking of the families at each site showed that there was good agreement in the performance of selections (Table 1). Roots of the four most promising families were selected for seeding at Wellesbourne. Despite the consistent results obtained at the two Institutes the response to selection was slight and further cycles of selection were not attempted.

Table 1. Carrot fly attack (expressed as % undamaged roots) to half-sib families of cv. 'Sytan' grown at Wellesbourne and at Wageningen in 1984 (Ranking of genotypes in parentheses).

| Generation | Seed stock number | Wellesbourne | Wageningen |
|---|---|---|---|
| Parent | DC 76356 | 5 (8) | 30 (7) |
| | DC 79001 | 6 (7) | 27 (8) |
| Half-sib second generation families | DC 83647 | 8 (6) | 32 (5=) |
| | DC 83659 | 9 (5) | 34 (4) |
| | DC 83646 | 10 (4=) | 41 (3) |
| | DC 83645 | 10 (4=) | 44 (2) |
| | DC 83663 | 13 (2) | 32 (5=) |
| | DC 83644 | 18 (1) | 45 (1) |

3. Crosses between cultivars

One way of exploiting resistance to insects in plants is to combine different sources of resistance in a single cultivar. To achieve this objective with carrots, promising cultivars which had been identified at Wellesbourne and confirmed in subsequent experiments were crossed with the most promising partially-resistant cultivar 'Sytan'; the cultivars and the years of crossing are given in Table 2.

Table 2. Production of crosses between cv. 'Sytan' and seven other cultivars possessing partial resistance to carrot fly attack

| Cultivar | Carrot type | Seed stock number | Year of production $F_1$ | $F_2$ |
|---|---|---|---|---|
| 'Vertou' LD | Nantes | DC 76344 | 1978 | 1980 |
| 'St Valery' | Half long | DC 75221 | 1978 | 1980 |
| 'Long Chantenay' | Chantenay | DC 74436 | 1978 | 1980 |
| 'Vita Longa Flakee Improved' | Autumn King | DC 70200 | 1978 | 1980 |
| 'Gelbe Rheinische' | Fodder | DC 75242 | 1978 | 1980 |
| 'Berlicum Berjo' | Berlicum | DC 74026 | 1983 | - |
| 'Berlicum Newmarket Red Cored' | Berlicum | DC 74033 | 1983 | - |

Each of the principal maincrop carrot types were represented in this programme, with the objective of incorporating some level of resistance into all of them. In certain cases, the $F_1$ families were grown on to produce $F_2$'s but in others insufficient time and labour were available to complete further generations. In 1981, the parent population, the $F_1$ and the $F_2$ families from crosses between cv.'Sytan' and five other cultivars were evaluated in a field experiment at Wellesbourne. As expected, the least damaged progenies were those which had been bred from crosses involving the most resistant parent cultivars, namely 'Sytan' x 'Gelbe Rheinische' and 'Sytan' x 'Vertou' (Table 3). The least damaged roots from the most promising root stocks were grown on for seeding.

Table 3. Carrot fly attack (expressed as % marketable roots) to families representing crosses between five carrot cultivars at Wellesbourne in 1981

| Generation | | % marketable roots |
|---|---|---|
| Parent | 'Sytan' | 9.4 |
| | 'Vertou' | 6.5 |
| | 'Long Chantenay' | 4.2 |
| | 'Vita Longa Flakee Improved' | 2.0 |
| | 'Gelbe Rheinische' | 14.1 |
| $F_1$ | 'Sytan' x 'Vertou' | 5.8 |
| | 'Sytan' x 'Long Chantenay' | 2.8 |
| | 'Sytan' x 'Vita Longa Flakee Improved' | 4.4 |
| | 'Sytan' x 'Gelbe Rheinische' | 7.4 |
| $F_2$ | 'Sytan' x 'Vertou' | 4.5 |
| | 'Sytan' x 'Long Chantenay' | 4.1 |
| | 'Sytan' x 'Vita Longa Flakee Improved' | 4.6 |
| | 'Sytan' x 'Gelbe Rheinische' | 10.6 |

4. Production of inbreds

Because of the failure to achieve a marked response in the recurrent selection programme (outlined above), it was decided to attempt to enhance resistance levels by developing inbreds through a single seed descent programme, a technique proposed by Professor Jinks and colleagues at the University of Birmingham (Jinks, 1981). The choice of cultivars from which to develop inbred lines was decided after consultation with agricultural advisory officers and carrot breeders from the private and public sectors. The cultivar 'Sytan' was selected because it represented the most resistant commercially-acceptable carrot known at the time and represented the carrot group, Nantes, which, overall, contained the most promising resistant material. The cultivar 'Long Chantenay' was chosen as the second parent because it represented the most widely grown group of maincrop carrots grown in Britain at the time. The development of resistant varieties of Chantenay carrots would be particularly valuable as they are carrots which are vulnerable to carrot fly attack for many months during the autumn and winter when control of the pest is particularly difficult.

The single seed descent programme was begun in August 1981 when 1920 $F_2$ plants resulting from the 'Sytan' x 'Long Chantenay' cross were raised in closely-spaced, 9 cm square pots in the glasshouse with the aim of producing plants with minimum numbers of secondary umbels. The temperature was maintained between 5-10°C from November 1981 to March 1982 to induce bolting and then raised to 15°C in April to promote vigorous growth. The plants grew rapidly in the spring, most of them attaining a height of 2 m, and almost all bolted. Flowering occurred over a short period during May-June during very warm weather. The plants were self-pollinated using blowflies placed in cellophane bags which separately enclosed the primary umbel of each plant; all side umbels were removed. The minimum temperature

in the glasshouse was increased to 20°C in July to ripen seed in time for the beginning of the next cycle of inbreeding. However, many plants failed to produce seed and only 35% produced viable seed. The $F_3$ plants were sown on 23 August 1982, together with additional $F_2$ seed to gap-up missing $F_3$ plants. The programme was continued during 1983-85, progress in the production of inbreds being listed in Table 4. At the $F_5$ stage, the breeding lines were bulked to provide sufficient seed for evaluation experiments. In order that the 753 inbreds should be reduced to manageable numbers for tests against carrot fly, two preliminary evaluations were carried out. Firstly, short rows of all inbreds were sown in seed trays in the glasshouse which was maintained at 20°C. The cultivar 'Sytan' was included in every tray to serve as a standard. The numbers of emerged seedlings and their size after three weeks were recorded. It was possible to derive information from these records on the viability and vigour of each inbred and to relate these details to those obtained for 'Sytan'. Only those breeding lines which were as large and as vigorous as 'Sytan' were chosen for further study. The second test was to analyse in seedlings from each inbred the concentration of chlorogenic acid, a chemical related to the resistance of carrot cultivars to carrot fly attack (Cole, 1985). Once again, the cultivar 'Sytan' was included for comparative purposes and the results for each inbred were related to this standard. A list was drawn up to rank the inbreds according to their chlorogenic acid content and those with the least concentration (i.e. the most resistant inbreds) were chosen for further testing. These preliminary tests reduced the numbers of candidate inbreds for field experiments from 753 to 66.

Table 4. Progress at Wellesbourne in the development of carrot inbreds from an initial population of 1920 $F_2$ lines in a single seed descent programme

| Breeding line situation | Year | | | | | |
|---|---|---|---|---|---|---|
| | 1982 | 1983 | 1984 | 1985 | 1986 | 1987 |
| Continuing | 1920 | 1274 | 1040 | 504 | 146 | 0 |
| Abandoned | 0 | 458 | 644 | 866 | 1057 | 1163 |
| Vegetative | 0 | 188 | 236 | 111 | 15 | 4 |
| Completed | 0 | 0 | 0 | 439 | 702 | 753 |

The most promising inbreds chosen as a result of the two preliminary tests were included in field experiments at Wellesbourne in 1986 and 1988 and were grown alongside the standard cultivar 'Sytan'. Five inbreds identified in 1986 and another three in 1988 combined vigour and a higher level of resistance to carrot fly than the standard partially-resistant cultivar 'Sytan'.

5. Male sterile lines of 'Sytan'

In 1983, a backcrossing programme was started to develop male sterile lines of several 'Sytan' selections. The first pair crosses to transfer male sterility from a 'Chantenay' male sterile line were made in June and ripe seed was obtained in August. Plants were raised from these pair crosses in 1984 and most were shown to have maintained sterility. Sixty first backcrosses were obtained by crossing the pair crosses with the 'Sytan' maintainers. The resulting families were scored for sterility and fertile ones were discarded. Further cycles of backcrossing were achieved in 1985, 1986 and 1987, resulting in the production of six male sterile lines of 'Sytan'.

6. Joint hybrid scheme with five seed companies

At the end of 1986, it was decided to seek support from seed companies to exploit the resistance that existed in the advanced breeding lines. A letter was sent to the British Society of Plant Breeders inviting expressions of interest in developing in a joint programme new carrot hybrids possessing resistance to carrot fly. Eventually five companies joined the scheme which formulated the following objectives;

1) The exchange of male sterile lines and production of $F_1$ hybrids in 1988 and 1989.
2) The evaluation of the $F_1$ hybrids by the seed companies in 1989 and 1990 to identify material which was agronomically promising.
3) The screening of the most promising lines for carrot fly resistance at Wellesbourne in 1990 and in following years.
4) The seed companies to develop new cultivars and be responsible for the submission of the material in trials to determine their distinctiveness, uniformity and stability.

For this programme the following advanced breeding lines were chosen: the four most resistant selections of 'Sytan', five of the most promising single seed descent lines identified in 1986 and the six male sterile lines of 'Sytan'. It is envisaged that, in a second phase of the programme, the remaining single seed descent lines identified in 1988 and certain of the most advanced lines bred from crosses between Daucus carota x Daucus capillifoliius will be submitted to the joint programme.

7. Résumé

Techniques pour l'exploitation de la résistance des carottes à la mouche (Psila rosae (F.))

A Wellesbourne, la résistance ont été identifiés dans plusieurs cultivars de carotte commercialement acceptable à la mouche de la carotte, Psila rosae (F.) a été exploitée comme suit:

1) Sélection répétée parmi les cultivars prometteurs;
2) Croisement de cultivars prometteurs, et puis sélection des familles les moins attaquées;
3 Production d'une série de lignées consanguines dans un programme de "descendance partant d'une seule graines";
4) Sélection de lignées avec stérilité mâle de cultivars avec résistance partielle.

Les familles les plus résistantes issues de ces techniques de sélection ont été inclués dans un programme d'hybridation en collaboration avec cinq firmes de production de semences dans le but de développer de nouveaux cultivars.

## 8. References

COLE, R.A., 1985. Relationships between the concentration of chlorogenic acid in carrot roots and the incidence of carrot fly larval damage. Ann. Appl. Biol. **106**: 211-217.

ELLIS, P.R., FREEMAN, G.H. & HARDMAN, J.A., 1984. Differences in the relative resistance of two carrot cultivars to carrot fly attack over five seasons. Ann. Appl. Biol. **105**: 557-564.

ELLIS, P.R. & HARDMAN, J.A., 1981. The consistency of the resistance of eight carrot cultivars to carrot fly attack at several centres in Europe. Ann. Appl. Biol. **98**: 491-497.

ELLIS, P.R. & HARDMAN, J.A., 1988. Non-insecticidal contributions to an integrated programme for the protection of carrots against carrot fly. In: Integrated Control in Field Vegetable Crops. IOBC/WPRS Bull. XI/1, pp. 33-39.

ELLIS, P.R., HARDMAN, J.A. & DOWKER, B.D., 1982a. Screening of Japanese carrot cultivars for their susceptibility to carrot fly. Tests of Agrochemicals and Cultivars No. 3 (Ann. Appl. Biol. **100** Suppl.), pp. 94-95.

ELLIS, P.R., HARDMAN, J.A. & DOWKER, B.D., 1982b. Screening of carrot cultivars for their susceptibility to carrot fly, 1976-1980. Tests of Agrochemicals and Cultivars No. 3 (Ann. Appl. Biol. **100** Suppl.), pp. 96-97.

ELLIS, P.R., HARDMAN, J.A. & DOWKER, B.D., 1987. Screening of carrot cultivars and breeding lines for their susceptibility to carrot fly at Wellesbourne in 1983. Tests of Agrochemicals and Cultivars No. 8 (Ann. Appl. Biol. **110** Suppl.), pp. 136-137.

ELLIS, P.R., HARDMAN, J.A., JACKSON, J.C. & DOWKER, B.D., 1980. Screening of carrots for their susceptibility to carrot fly attack. J. natn. Inst. Agric. Bot. **15**: 294-302.

ELLIS, P.R., WHEATLEY, G.A. & HARDMAN, J.A., 1978. Preliminary studies of resistance to different carrot cultivars in relation to root length. Ann. Appl. Biol. **88**: 159-170.

JINKS, J.L., 1981. The genetic framework of plant breeding. Phil. Trans. R. Soc. Lond. B **292**: 407-419.

PHILIPSEN, H., 1988. Resistance to carrot fly (Psila rosae) in two carrot cultivars - Sytan and Danvers - evaluated in field plots. Report of the 4th meeting Eucarpia/IOBC/WPRS Working Group on Breeding for Resistance to Insects and Mites. IOBC/WPRS Bull. 1988/XI/3, pp. 59-62.

THOMPSON, A.R., ELLIS, P.R., PERCIVALL, A.L. & HARDMAN, J.A., 1980. Carrot fly. Integrated control using insecticides with carrot cultivars of differing susceptibilities. Rep. natn. Veg. Res. Stn. for 1979, p. 30.

# EVALUATION OF DAUCUS SPECIES AS SOURCES OF RESISTANCE AMONGST UMBELLIFEROUS PLANTS TO CARROT FLY ATTACK

J.A. HARDMAN AND P.R. ELLIS
Institute of Horticultural Research, Wellesbourne,
Warwick, CV35 9EF, Great Britain.

## Summary

Ten species of Daucus and eight subspecies of wild carrot were evaluated amongst a total of 132 umbelliferous species of plants for resistance to carrot fly (Psila rosae (F.)) attack at Wellesbourne. The levels of resistance were determined by recording the emergence of adult carrot fly from plants exposed to a field population of the pest. Several species demonstrating high levels of resistance which are also compatible with the cultivated carrot were identified. These species may have potential as parents in programmes designed to breed carrot cultivars resistant to the carrot fly.

## 1. Introduction

The study of the Umbelliferae began at Wellesbourne in 1972 as part of the project to investigate the host range of the carrot fly (Psila rosae (F.)) and to search for resistant plant species which could be used as parents in a breeding programme. A special effort was made to collect Daucus species, as many of them are crossed readily with the cultivated carrot which belongs to the same genus. The genus Daucus contains 22 species divided into several taxonomic sections; the section Daucus which includes 13 subspecies of D. carota and 11 other species, which can all be hybridised with the cultivated carrot (Heywood, 1983); and ten more distant relatives which belong to the sections Meoides, Anisactis, Leptodaucus, Chrysodaucus, Platyspermum and Pseudoplatyspermum which are mainly incompatible with cultivated carrot (Heywood, 1978). Prior to 1972 the only reports of carrot fly hosts amongst Daucus species were those listing the cultivated carrot D. carota ssp. sativus (Hoffm.) first observed in 1802 (Machray, 1814), and wild carrot, D. carota ssp. carota (L.) Thell. (for example, Crosby & Leonard, 1918). More recently de Ponti and colleagues evaluated the resistance to carrot fly of 70 accessions of wild carrot obtained from various botanic gardens or collected on expeditions in The Netherlands and Israel. They found that the levels of resistance were surprisingly low and, on average, no higher than that of the cultivated carrot grown alongside them in the same experiment. Seven of the wild accessions were selected for further study but were not used in the main carrot fly breeding programme and have not been reported on since (de Ponti, Freriks, Steenhuis & Inggamer, 1981). In this paper we report on the evaluation of the resistance in wild Daucus species to carrot fly attack at Wellesbourne.

## 2. Materials and Methods

Daucus species and subspecies were collected by the authors from the wild or were obtained from botanic gardens, universities, specialist growers or plant breeders. In most cases, supplies of seed were very limited and it was necessary to multiply stocks in polytunnels at Wellesbourne. All seedstocks were maintained in refrigerated cold stores at about 5°C.

The resistance of these species to carrot fly was evaluated by exposing the plants to a natural population of the fly in field experiments and then determining the numbers of adult carrot fly produced per plant.

1981-83 experiments

Seed was sown in 15 cm clay half pots containing seed compost and maintained outdoors in a bird-proof cage. Seedlings were pricked out in 7.5 cm diameter clay pots containing John Innes compost and grown on in an unheated glasshouse. Prior to exposure to carrot fly, the pots were sunk to rim level in plots consisting of a single 10-plant row with the plants spaced 10 cm apart. Plots were spaced 75 cm apart and the three blocks 1.2 m apart to prevent carrot fly larvae migrating from species to species. Plots were watered to aid plant establishment and weeded as necessary during the experiment.

Plant species exposed to first generation carrot fly attack were lifted in July; those exposed to second and third generations were lifted the following April. The ten pots constituting a plot were removed from the soil and the plants, together with the surrounding soil, were transferred to clear, gussetted 30 x 60 cm polyethylene bags. These bags were placed in a cool glasshouse or Tygan$^{R}$-screened house to await the emergence of the adult carrot flies. A yellow water trap containing a solution of Teepol$^{R}$ in water (20 ml/l) was placed in each bag to trap the flies and the sides of the bag were held apart by a stake. The bags were closed to prevent insects escaping and only opened once a month to record the numbers of carrot flies trapped.

1984-1989 experiments

For these experiments, plants were raised by two different techniques. Those species which were in short supply, frost susceptible, of known poor emergence or slow-growing were raised using the technique described above. However, at the time of transplanting the plants were removed from their pots and arranged in plots consisting of two 5-plant rows, spaced 10 cm apart with 10 cm between plants. The plots were spaced 75 cm apart and the blocks 1.2 m apart. Following exposure to the flies, the plant foliage was trimmed down to ground level. Each plot was then enclosed in a 60 cm diameter, 30 cm high galvanised iron ring covered with a muslin top secured by a band of expanded curtain wire. A 'Marigold yellow' water trap was introduced into each ring, containing a solution of Teepol$^{R}$ in water (20 ml/l) and a preservative Campden$^{R}$ tablet. Ring cages were examined weekly and the numbers of carrot fly trapped or free inside each cage were recorded. The foliage inside the cages was trimmed down regularly to maximise insect trapping.

Plant species with abundant supplies of seed and which were known to germinate, emerge and grow rapidly were sown directly in the field in a well-prepared seed bed. Plots consisted of either two rows 50 cm long and spaced 15 cm apart, or of three rows 30 cm long and 15 cm apart; this configuration facilitated caging under the rings. Plots were spaced and caged as above.

## 3. Results

A total of 18 species and subspecies of Daucus were tested from 1972-89, many species being tested on several occasions, particularly if they supported only few insects. The numbers of carrot flies produced on all species were compared with the numbers from the cultivated carrot cv.'Danvers Half Long 126' which was included in every experiment (Table 1). There were great differences betweeen the various species ranging from D. carota azoricus, which supported more carrot flies per root than did the cultivated carrot and as many as the highly susceptible arable weed Aethusa cynapium, to D. broteri which supported no carrot fly. Amongst the umbellifers tested were 8 subspecies of Daucus, all of which supported appreciable numbers of carrot fly. Two species, D. capillifolius and D. maximus, belong to the same section of the genus as the cultivated carrot, the Daucus section; they contrasted in their resistance to carrot fly, D. maximus supporting almost half as many flies as the susceptible cultivar 'Danvers Half Long 126' and D. capillifolius supporting only 4% of the numbers produced on the susceptible cultivar. Six other species, two of which belong to other sections of the genus, were highly resistant to carrot fly.

## 4. Discussion

The traditional method of evaluating the resistance of umbelliferous crops to carrot fly at Wellesbourne and elsewhere has been to grow the accessions in rows in randomised block field experiments in areas where the pest is known to be present in high numbers. The plant roots are then graded for carrot fly damage at harvest, as described in Ellis, Wheatley & Hardman (1978) for example. In these experiments, it has been possible to estimate the percentage of undamaged roots for each accession or a mean damage grade, according to the proportion of root surface attacked by carrot fly. This method is suitable for plants such as carrot and parsnip which have a swollen tap root on which damage by maggots is observed and scored easily. However, this method is not suitable for plants such as wild Daucus species which do not have a swollen tap root but which possess a thin, tough main root and many much-divided side roots; carrot fly larvae do not enter these roots easily but tend to browse on the epidermis. The damage resulting from this feeding activity usually appears as small lesions or scars rather than the characteristic mines which occur on tap roots. Small lesions can be caused by many different organisms (for example, nematodes, Collembola, other soil-inhabiting insects, or even by scratching the root epidermis) and therefore scoring these lesions as carrot fly damage could be very misleading. For this reason, an alternative method was required for evaluating the resistance of these wild umbelliferous plants to carrot fly; the method described in this paper, whereby adult insect productivity is measured following exposure of the plants to the pest in the field, meets the requirements. We believe this method provides the most accurate assessment of a plant species' ability to serve as a host of the carrot fly.

All the available evidence indicates that the wild carrot, D. carota ssp. carota is unlikely to provide high levels of resistance to carrot fly: the five accessions originating in Great Britain (Warwickshire), Iran and Turkey and tested at Wellesbourne in the 1972-73 season were highly susceptible to carrot fly attack (Hardman & Ellis, 1982) and the accessions originating in The Netherlands and Israel which were tested by de Ponti, Freriks, Steenhuis & Inggamer (1981) were not resistant enough to form the

basis of a breeding programme. In the wild, this carrot subspecies probably acts as a reservoir for carrot fly which infest umbelliferous crops grown in the vicinity. The other subspecies of D. carota tested at Wellesbourne offer little in the way of material resistant to carrot fly. Indeed, several of the subspecies tested supported as many and, in certain cases, more carrot fly than the highly susceptible cultivated carrot. We have to look further afield for promising sources of resistance. Within the section Daucus of the genus, the north African species D. capillifolius is highly resistant and furthermore it is compatible with the cultivated carrot. This wild species has been the subject of an intensive research programme at Wellesbourne to develop advanced breeding lines of carrot possessing resistance to carrot fly (Ellis & Hardman, in prep. 1990). Some other species from the section Daucus which were tested in this series of experiments were equally resistant and one of them, D. broteri, has so far failed to support any carrot flies in field experiments. It is not yet known whether this species is compatible with the cultivated carrot. Three of the species tested belong to other sections of the genus and therefore are believed to be less closely related to cultivated carrot; these species do not have the same numbers of chromosomes as D. carota ssp. carota and are incompatible with this species (Heywood, 1983). Specialised techniques will be required to secure crosses between the cultivated carrot and incompatible species but, with current advances in biotechnology and particularly genetic engineering, there are prospects for utilising these wild plants as donors of resistance genes.

This evaluation of Daucus species has shown that there is a wide range of ability within the genus to serve as hosts of carrot fly and that certain species which are compatible with the cultivated carrot and also support few carrot flies have great potential in the development of resistant carrot cultivars.

## 6. Résumé

Dix espèces de Daucus parmi un total de 132 espèces d'ombellifères testées à Wellesbourne ont été évaluées quant à leur résistance à la mouche de la carotte (Psila rosae (F.)). Le niveau de résistance a été déterminé en enregistrant le nombre d'insectes adultes produit sur plantes exposées à une population naturelle du ravageur. Plusieurs espèces démontrant un niveau élevé de résistance et une compatibilité avec la carotte cultivée ont été identifiées. Ces espèces possèdent un potential important comme parents dans des programmes de sélection de cultivars de carotte résistance à la mouche de la carotte.

## 7. References

CROSBY, C.R. & LEONARD, M.D. (1918). Manual of Vegetable-garden Insects. New York: Macmillan, xv+391pp. (p. 181).

ELLIS, P.R., WHEATLEY, G.A. & HARDMAN, J.A. (1978). Preliminary studies of resistance in carrots to carrot fly attack. Ann. Appl. Biol. 88: 159-170.

HARDMAN, J.A. & ELLIS, P.R. (1982). An investigation of the host range of the carrot fly. Ann. Appl. Biol. 100: 1-9.

HEYWOOD, V.H. (1978). Multivariate taxonomic synthesis of the tribe Caucalideae. In: CAUWET-MARC, A.-M. & CARBONNIER, J. (eds) Actes du 2 ème

Symposium International sur Perpignan, May 1977, Les Ombelliferes "Contributions Pluridisciplinaires à la Systématique", pp. 726-736.

HEYWOOD, V.H. (1983). Relationships and evolution in the Daucus carota complex. Israel J. Bot. 32: 51-65.

MACHRAY, J. (1814). On the gooseberry caterpillar, and on the worms which infest carrots and onions. Memoirs Caledonian Hort. Soc. 1: 272-275.

PONTI, O.M.B. de, FRERIKS, J.C., STEENHUIS, M. & INGGAMER, H. (1981). Improving the resistance of carrot and onion to respectively carrot fly and onion fly by recurrent selection. In: Breeding for Resistance to Insects and Mites, IOBC/WPRS Bulletin 1981/iv/1: pp. 59-62.

## Susceptibility of Daucus species and subspecies to carrot fly attack at Wellesbourne

| Species or subspecies | Section of genus | Geographical origin | Total nos. of roots exposed | Total nos. of adult carrot fly emerged from roots | Mean nos. of carrot fly per root |
|---|---|---|---|---|---|
| Daucus carota azoricus Franco | Daucus | Mediterranean | 30 | 145 | 4.8 |
| [Aethusa cynapium (fool's parsley) | - | - | 248 | 993 | 4.0] |
| Daucus carota gummifer Hooker fil. | Daucus | Coastal Britain & Iberia | 82 | 285 | 3.5 |
| Daucus carota sativus (Hoffm.) Arc. (cv. 'Danvers Half Long 126') | Daucus | Cultivated | 1315 | 3425 | 2.6 |
| Daucus carota carota L., wild carrot | Daucus | Europe & SW Asia | 57 | 123 | 2.2 |
| Daucus muricatus (L.) L. | Platyspermum | W Mediterranean | 374 | 574 | 1.5 |
| Daucus carota commutatus (Paol.) Thell. | Daucus | Mediterranean | 476 | 596 | 1.2 |
| Daucus carota hispanicus (Gouan) Thell. | Daucus | Mediterranean | 42 | 44 | 1.0 |
| Daucus carota maritimus (Lam.) Batt. in Batt. & Trabut | Daucus | W Mediterranean | 207 | 212 | 1.0 |
| Daucus maximus (Desf.) Ball | Daucus | Mediterranean | 30 | 29 | 1.0 |
| Daucus carota drepanensis (Arc.) Heywood | Daucus | Mediterranean | 30 | 31 | 1.0 |
| Daucus carota gadecaei (Rouy & Camus) Heywood | Daucus | N W France | 78 | 70 | 0.9 |
| Daucus capillifolius Gilli | Daucus | N Africa | 115 | 6 | 0.05 |
| Daucus littoralis Sibth. & Sm. | Daucus | E Mediterranean | 121 | 6 | 0.05 |
| Daucus pusillus Michaux | Leptodaucus | S America | 410 | 17 | 0.04 |
| Daucus involucratus Sibth. & Sm. | Daucus | Greece & Aegean | 283 | 9 | 0.03 |
| Daucus gracilis Steinh. | Daucus | N Africa | 52 | 1 | 0.02 |
| Daucus glochidiatus (Labill.) Fisch. Mey. et Ave-Lall. | Anisactis | Australia | 286 | 4 | 0.01 |
| Daucus broteri Ten. | Daucus | N E Mediterranean | 10 | 0 | 0.0 |

# ATTEMPTS TO CORRELATE CARROT LEAF SURFACE COMPOUNDS STIMULATING OVIPOSITION WITH CARROT FLY ANTIXENOSIS RESISTANCE.

ERICH STÄDLER, WALTER KOCH AND HANS-RUEDI BUSER.
Eidg. Forschungsanstalt, CH-8820 WÄDENSWIL - SWITZERLAND.

Summary
Field experiments confirmed that the carrot cultivar "Danvers" is more severely attacked by the carrot fly than the cultivar "Sytan". In the laboratory females laid fewer eggs around the field-collected leaves of "Sytan" than of "Danvers". They also preferred leaf surface extracts from "Danvers" applied on filter paper surrogate leaves compared with the same extracts from "Sytan". This showed that compounds from the leaf surface are involved in carrot resistance based on antixenosis for oviposition.
The stimulatory compounds of the carrot leaf surface were isolated, identified and quantified. It was confirmed that the two attractive propenylbenzenes, trans-asarone and trans-methylisoeugenol, do occur at consistently higher concentrations in the less preferred cultivar "Sytan". Thus these compounds do not seem to be related to oviposition preference. The concentration of the other compounds analysed, furanocoumarins and polyacetylenes, which stimulate oviposition, were found to be little different between the carrot cultivars investigated. However, the pattern of stimulatory compounds was very different in another umbellifer host, celeriac. The differences in the patterns of stimulating and deterring compounds in the leaf surface of host plants influencing oviposition warrants further research.

Résumé
Des essais en champs ont confirmé que le cultivar de carotte "Danvers" est plus sévèrement attaqué par la mouche de la carotte que le cv. "Sytan". En laboratoire, les femelles ont pondu moins d'oeufs à la base des feuilles provenant de cultures de "Sytan" que sur celles provenant de champs de "Danvers". Les femelles préfèrent également les extraits de feuilles de "Danvers" appliqués sur des feuilles artificielles à ceux de "Sytan". Il est donc démontré que les substances de surface du feuillage sont impliquées dans la résistance antixénotique de la carotte à la ponte
Les substances stimulantes de la surface du feuillage de la carotte ont été isolées, identifiées et quantifiées. Il a été confirmé que deux propénylbenzènes attractives, le trans asarone et le trans-méthyl isoeugénol, sont régulièrement présents à des concentrations nettement supérieures dans le cv. "Sytan", moins apprécié. Ces substances ne semblent donc pas être liées à la préférence d'oviposition. La concentration des autres substances analysées stimulant la ponte, des furanocoumarines et polyacétylènes, ne diffèrent que peu entre les cv. de carottes étudiés. Néanmoins, le

contenu des substances stimulantes est très différent chez le céleri-ravi, une autre plante-hôte de la famille des Ombellifères.
Les différences dans la palette de substances, stimulant et dissuadant l'oviposition à la surface du feuillage des plantes-hôtes nécessitent des recherches plus approfondies.

## Introduction

Berüter & Städler (1971) isolated and identified the first oviposition stimulant for the carrot fly *(Psila rosae)* from a total leaf extract. This compound, trans-methylisoeugenol, was found by Guerin & Städler (1982) and Guerin et al (1983) together with another propenylbenzene, trans-asarone, also to be attractive in the field. Following these discoveries Visser & dePonti (1983) and Guerin & Städler (1984) tried to establish if the well substantiated carrot cultivar resistance (Ellis et al, 1978, 1984, Ellis & Hardman, 1981) was related to the occurrence of these stimulating compounds. Both studies, Visser & dePonti (1983) for trans-methylisoeugenol, and Guerin & Städler (1984) for trans-methylisoeugenol and trans-asarone found, if anything, a negative correlation. This implied that means the less damaged variety "Sytan" contained more of these two stimulatory or attractive propenylbenzenes than the preferred variety "Danvers".

Städler & Buser (1984) isolated additional oviposition stimulants from the carrot leaf surface and identified them as furanocoumarins and polyacetylenes. The artificial mixture of the seven compounds identified was shown to be as stimulatory as the total surface raw extract. These promising results prompted us to try again to relate the newly discovered compounds of the leaf surface extracts of different cultivars with their relative antixenosis.

## Materials and methods

*Plants:* The seeds were kindly supplied by P.R. Ellis and J.A. Hardmann, Institute of Horticultural Research, Weilesbourne, Warwick/UK.

Experiment in 1982/83: The carrot cultivars "Sytan" and "Danvers" and a celeriac "Tropa" were sown 6 July 1982 and the leaves harvested for extraction on 20 September 1982.

Experiments in 1988: On 25 April four carrot cultivars ("Sytan", "Danvers", "Nandor", "Nanthya") were sown in the field at the research station and harvested on 10 August at which time carrot fly damage was evaluated. The second sowing with the carrot cultivars "Sytan", "Danvers" and "Nandor" was arranged next to the first experiment on 13 July and harvested 26 October. The collection of leaves for the oviposition tests and the extraction of compounds were done immediately prior to harvesting of field experiments.

*Extraction and fractionation techniques:* Experiment in 1982/83: The raw extracts were obtained by dipping the undamaged leaves into two successive baths of $CH_2Cl_2$ (Städler & Buser, 1984). The combined extracts were concentrated and stored in a freezer until they were tested in the oviposition test during the following years.

Experiments in 1988: The basics of the methods have been described by Städler & Buser (1984). For these experiments the silicagel purification was simplified. A column (67 x 11.6 mm, silicagel 63-200 mesh, Merck "Kieselgel 60") was eluted successively with 20 ml portions of 0, 5, 100 % $Et_2O$ in

hexane and 100 % MeOH. The 100 % $Et_2O$ fraction contained the previously identified stimulants and was analysed and quantified using GC-MS. The mass counts from the GC-MS were calibrated using pure samples of the different compounds (sources listed in Städler & Buser, 1984).

We collected and extracted leaves sampled on 25 July 1988 from the field experiment in which yield and larval damage to roots had been recorded. In addition to the two cultivars "Danvers" and "Sytan" the leaves of the cultivars "Nandor" and "Nanthya" as well as those of the celeriac cultivar "Tropa" (extracted in 1982) were analysed. From each cultivar two different samples of 100 g each were taken which were extracted and purified separately along with a solvent control.

*Insects and bioassays:* The flies were obtained from our continuous laboratory culture (Städler, 1971) and kept in 100 x 100 x 100 cm screen cages in a controlled environment room providing a 16 h day, beginning at 0600 h and maintained at 21 ± 1 °C and 80 % ± 5 % r h. Oviposition stimulation was tested with leaves collected from the same carrots as above during the period 4 July to 2 August and from a second sowing (13 July 1988) during the period 19 September to 6 October. Two to three fresh leaves were inserted in the center of an oviposition dish (Städler, 1971). The assays with the extracts and fractions were performed using filterpaper surrogate leaves treated with the extracts and attached to the oviposition dish (Städler, 1977). The different treatments were repeated four times and arranged in alternating fashion in a circle on the floor of the same cage. The eggs were counted after 1, 3 or 4 days and the positions of the dishes was switched for the following oviposition period.

## Results

*Larval damage assessments in the field:* In the first experiment harvested on 10 August there was a significant difference in the percentage of slightly and severely attacked carrots between "Danvers" and "Sytan" (Fig.1). The variety "Nandor" was like "Danvers" more attacked than "Sytan". In the second sowing the differences in attack between the cultivars were smaller and not significant. However the same trends were found, in the sense that again "Danvers" had the highest percentage of damaged roots.

*Oviposition on leaves in the laboratory:* In the first period the cultivars "Danvers" and "Nandor" were compared, and in accordance with the damage recorded on the roots, there was no difference. On the "Danvers" dishes 652 eggs and on "Nandor" 669 eggs were laid. The oviposition experiments in the second period comparing 1) "Danvers" and "Sytan" and 2) "Sytan" and "Nandor" showed that the leaves of "Danvers" were more stimulatory than those of "Sytan". The difference between "Sytan" and "Nandor" was however not significant (Table 1).

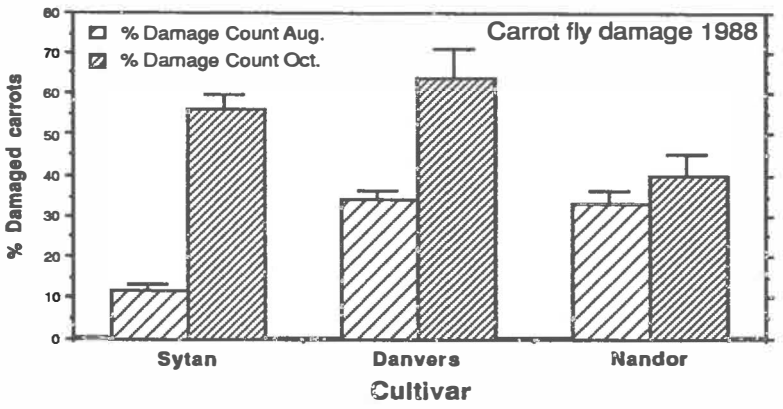


Fig. 1. Carrot fly damage in two field experiments sown at Wädenswil.
- means ± SE of the % damaged carrots are shown.
- 20 carrots sampled per each of 5 repetitions. Slightly and clearly damaged carrots were counted and expressed as %.
- The counts were analysed using the Friedman test. The test was significant for the first experiment harvested in August p=0.015. Wilcoxon test for difference between Sytan and Danvers significant p=0.0422; difference between Danvers and Nandor not significant. The Friedman test for the October harvest was not significant p=0.129.

*Oviposition on extracts:* It was known from earlier experiments that the leaves of carrot cultivars may stimulate different levels of oviposition (Guerin & Städler 1984). It was therefore postulated that physical characters of the leaves, like colour, shape, surface characteristics etc., or chemical factors of the leaf surface could explain the observed oviposition preference. As can be seen from a very extensive comparative experiment with raw surface extracts in 1983 (Table 2), the two varieties "Danvers" and "Sytan" did in fact yield extracts which stimulated oviposition in the same relation to real leaves. In both test periods significantly more eggs were laid around the surrogate leaves treated with the "Danvers" extract.

Table 1. Stimulation of oviposition by field collected leaves in the laboratory in dual choice experiments.

| Experimental period | Replicates dishes | Total number of eggs "Danvers" | "Sytan" | "Nandor" | Significance* |
|---|---|---|---|---|---|
| 4 July - 2 Aug. 1988 | 8 | 652 | | 669 | NS |
| 19 Sept.- 6 Oct. 1988 | 8 | 2766 | 2209 | | p = 0.0421 |
| 19 Sept.- 6 Oct. 1988 | 8 | | 2396 | 3116 | NS |

* Wilcoxon signed ranks test.

Table 2. Stimulation of oviposition by raw leaf surface extracts on surrogate leaves in dual choice experiments.

| Experimental period | Replicates dishes | Total number of eggs "Danvers" | "Sytan" | Significance* |
|---|---|---|---|---|
| 7 July - 15 July 1983 | 24 | 6131 | 4634 | $p < 0.025$ |
| 22 July - 25 July 1988 | 20 | 2766 | 2209 | $p < 0.01$ |

* Friedman test with repetitions.

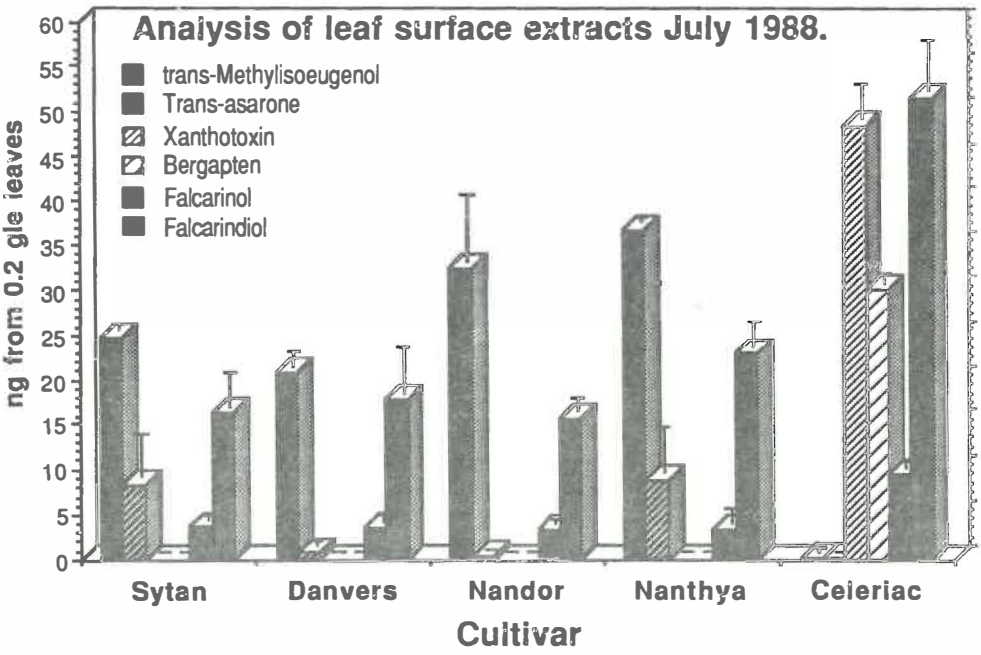


Fig. 2. Analytical data of the surface extracts of four carrot and one celeriac cultivar.
- Means ± SE of two independent analyses of leaves are given.
- The equivalents of 0.2 grams of leaves were injected into the GC-MS and analysed.

*Analysis of extracts:* Since stimulatory compounds extracted from the leaf surface were at least partly responsible for the observed oviposition preference we tried to quantify the compounds isolated and identified previously (Städler & Buser 1984). The differences in leaf contents of the cultivars "Sytan" and "Danvers" were not related to the stimulatory activity of the original raw extracts (Fig.2). The concentration of the two

propenylbenzenes, trans methylisoeugenol and trans-asarone were higher in the less severely damaged "Sytan" than in "Danvers". The concentrations of furanocoumarins in all carrot extracts were very low, in fact below the detection threshold for the tested samples relating to 0.2 gle. The differences in the contents of the two polyacetylenes were clearly not significantly different. The greatest difference was found between the celeriac variety and the four carrot cultivars. The carrot leaves all contained substantial amounts of propenyl-benzenes and little or no furanocoumarins. In contrast the celeriac was found to have little propenylbenzenes and substantial amounts of furanocoumarins. The highly stimulatory polyacetylenes did occur in all extracts in comparable amounts, celeriac having the highest content of falcarindiol.

## Discussion

The differences recorded in damage to the two carrot cultivars "Danvers" and "Sytan" again confirm the well established cultivar preference of the carrot fly (Ellis & Hardman, 1981; Ellis et al, 1984, 1987). The laboratory oviposition experiments, using detached leaves from field grown carrots, confirmed that oviposition preference or antixenosis (Kogan 1986) is involved in the observed resistance of certain cultivars in the field. As has been pointed out by Guerin & Städler (1984) and Guerin & Ryan (1984) this does not exclude the existence of antibiosis in the roots of the varieties tested. In fact using artificial inoculations we showed that the roots of carrot cultivars differ in susceptibility (Guerin et al, 1981), an observation recently confirmed by Maki & Ryan (1989).

Our experiments with crude leaf surface extracts showed that chemicals do play an important role not only in stimulation of oviposition but also in the carrot fly's preference for certain cultivars. This supports the view that the leaf surface is an important interphase between plants and phytophagous insects (Städler, 1986; Städler & Roessingh 1989). It remains to be seen wether physical characteristics alone are of little or no importance for host plant selection by the carrot fly.

The analytical data obtained from the crude leaf surface extracts are not easily interpreted. Propenylbenzenes do not seem to be not responsible for the observed preference since the less susceptible "Sytan" always contained more methylisoeugenol and asarone. This confirmed the results of Visser & dePonti (1983) and Guerin & Städler (1984). The low concentrations of the furanocoumarins recorded in all four carrot cultivars were unexpected in view of our earlier results (Städler& Buser 1984). The fact that the samples of the celeriac contained substantial amounts of these stimulating compounds (bergapten, xanthotoxin) excludes the possibility of an error due to a purification or analytical procedure. The polyacetylenes falcarinol and falcarindiol were found in all samples confirming their crucial role in oviposition stimulation (Städler& Buser, 1984, Städler, 1986). Recently Maki et al (1989) found that these compounds also play a role in larval behavior.

The question remains why we were not able to correlate the fly's preference with the analytical data from the stimulants identified in the leaf surface. It could be that any differences were masked by the high variability of the leaf surfaces contents of a a single cultivar. Otherwise the leaf surface compounds of a cultivar could be specifically related to the age of the leaves sampled. This seems unlikely because Ellis et al (1987) found that the

difference in resistance between "Danvers" and "Sytan" was remarkably stable over several seasons and within a single season and to a large degree independent of sowing and harvest time. However this conclusion is based on the recorded damage at the time of harvest and not actual oviposition preference. So the time of analysis may still be important and worthy of future investigation.

An additional reason for the difficulties in relating root damage to the concentration of compounds stimulating oviposition may be that not all stimulants have yet been identified. Such a compound could be chlorogenic acid. Its content in the carrot roots has been found early in the season to be negatively correlated with resistance (Cole, 1985, Cole et al, 1987). Data on the contents of chlorogenic acid in leaves of different cultivars are not yet available. But it is known that chlorogenic acid does also occur in carrot leaves and may have an effect on oviposition, as has been shown for another insect herbivore of carrots, the butterfly *Papilio polyxenes* (Feeny et al. 1988). Chlorogenic acid is not likely to occur in our $CH_2Cl_2$ surface extracts. It is also not known wether other surface extraction methods using more polar solvents would yield new, not yet identified oviposition stimulants.

Deterrents or repellents also occur in carrot leaves (Städler, 1971/72) and could, if present on the leaf surface, reduce carrot fly oviposition as well. Since this aspect has not been tested at all with crude extracts, such compounds could also be involved in oviposition. Again it has to be remembered that $CH_2Cl_2$ extracts will contain little or no highly polar compounds of the leaf surface. Thus such compounds, which could be involved in the oviposition preference of the carrot fly, should clearly be included in future investigations.

A further explanation for the results reported in this paper may be that we tried to correlate oviposition preference with individual compounds. In fact Städler & Buser (1984) & Städler (1986) found that the patterns of compounds was very important and that the components interact in a synergistic or additive manner. Thus it is possible that the female flies prefer specific patterns and not just high concentrations of single components. Very different patterns can indeed be acceptable for the carrot fly as is evident from the analytical result obtained for celeriac. Celeriac is a highly preferred host, but has a very different pattern of stimulating compounds in its leaf surface than the carrot cultivars tested in this work.

In conclusion, it is clear that at present no explanation for resistance or susceptibility can be based on the leaf surface compounds identified which stimulate oviposition. The analysis of carrot leaf surface compounds should be extended systematically to other cultivated Umbelliferae also taking into consideration leaf age. Additional investigations of other compounds are needed which may stimulate and/or inhibit oviposition. Despite the fact that our knowledge is still elementary, it is clear that oviposition preference or antixenosis of carrots is partly responsible for the observed differences between carrot cultivars. It is therefore important to study the stimulation of carrot fly oviposition in greater detail in order to apply fundamental knowledge of host plant selection to the development of less susceptible crops of umbellifers.

References

Berüter,J.& Städler,E. 1971. An oviposition stimulant for the carrot rust fly from carrot leaves. Z.Naturf. 26b, 339-340.

Cole,R.A. 1985. Relationship between the concentration of chlorogenic acid in carrot roots and the incidence of carrot fly larval damage. Ann.appl.Biol. 106, 211-217.

Cole,R.A., Phleps,K., Ellis,P.R.& Hardman,J.A. 1987. The effects of time of sowing and harvest on carrot biochemistry and resistance of carrots to carrot fly. Ann.appl.Biol.110, 135-143.

Ellis,P.R.& Hardman,J.A. 1981. The consistency of the resistance of eight carrot cultivars to carrot fly attack. Ann.appl.Biol. 98, 491-497.

Ellis,P.R., Freeman,G.H.& Hardman,J.A. 1984. Differences in the relative resistance of two cultivars to carrot fly attack over five seasons. Ann.appl.Biol. 105, 557-564.

Ellis,P.R., Freeman,G.H., Dowker,B.D., Hardman,J.A.& Kingswell,G. 1987. The influence of plant density and position in field trials designed to evaluate the resistance of carrots to carrot fly (Psila rosae) attack. Ann.appl.Biol. 111, 21-31.

Ellis,P.R., Hardman,J.A., Cole,R.A.& Phleps,K. 1987. The complementary effects of plant resistance and the choice of sowing and harvest times in reducing carrot fly (Psila rosae) damage to carrots. Ann.appl.Biol. 111, 415-424.

Ellis,P.R., Wheatley,G.A.& Hardman,J.A., 1978. Preliminary studies of carrot susceptibility to carrot fly attack. Ann.appl.Biol. 88, 159-170.

Feeny,P., Sachdev,K., Rosenberry,L.& Carter,M., 1988. Luteolin 7-O-(6''-O-malonyl)-ß-D-glucoside and trans-chlorogenic acid: oviposition stimulants for the black swallowtail butterfly. Phytochem. 27, 3439-3448.

Guerin,P.M.& Ryan,M.F. 1984. Relationship between root volatiles of some carrot cultivars and their resistance to the carrot fly, Psila rosae. Entomol.exp.appl. 36, 217-224.

Guerin,P.M.& Städler,E. 1982. Host odour perception in three phytophagous Diptera - A comparative study. In: Proc.5th Int.Symp.Insect-Plant Relationships, Visser,J.H.& Minks,A.K., eds., Pudoc Wageningen, 95-105.

Guerin,P.M.& Städler,E. 1984. Carrot fly cultivar preferences: some influencing factors. Ecol.Entomol.9, 413-420.

Guerin,P.M., Gfeller,F.& Städler,E. 1981. Carrot resistance to the carrot fly - contributing factors. IOBC/WPRS Bull. 1981 IV/1, 63-65.

Guerin,P.M., Städler,E.& Buser,H.R. 1983. Identification of host plant attractants for the carrot fly, Psila rosae. J.chem.Ecol. 9, 843-861.

Kogan,M. 1986. Plant defense strategies and host-plant resistance. In: Ecological Theory and Integrated Pest Management Practice, Kogan,M. ed., J.Wiley& Sons N.Y., 83-134.

Maki,A.& Ryan,M.F. 1989. Root-mediated effects in carrot resistance to the carrot fly, Psila rosae. J.chem.Ecol. 15, 1867-1882.

Maki,A., Kitajima,J., Abe,F., Stewart,G.& Ryan,M.F. 1989. Isolation, identification and bioassay of chemicals affecting non-preference carrot root resistance to the carrot fly larva. J.chem.Ecol. 15, 1883-1897.

Städler,E., 1971. An improved mass-rearing method of the carrot rust fly, Psila rosae (Diptera: Psilidae). Can.Ent. 103, 1033-1038.

Städler,E. 1971/1972. Über die Orientierung und das Wirtswahlverhalten der Möhrenfliege, Psila rosae F. (Diptera: Psilidae) Z.angew.Entomol. 69, 425-438. 70, 29-61.

Städler,E. 1977. Host selection and chemoreception in the carrot rust fly (Psila rosae F., Dipt. Psilidae): Extraction and isolation of oviposition stimulants and their perception by the female. Coll.Int.CNRS Comportement des Insectes et Milieu Trophique 265, 357-372.

Städler,E. 1986. Oviposition and feeding stimuli in leaf surface waxes. In: Insects and the Plant Surface. Juniper,B.E.& Southwood,T.R.E. eds., Edward Arnold London, 105-121.

Städler,E.& Buser, H.-R. 1984. Defense chemicals in leaf surface wax synergistically stimulate oviposition by a phytophagous insect. Exper.40, 1157-1159.

Visser,J.H.& De Ponti, O.M.B., 1983. Resistance of carrot to carrot fly, Psila rosae. Progress Report 1979/1981 C.E.C. Program on Integrated and Biological Control. C.E.C. Luxembourg 214-224.

## Workshop 2

## PROTECTED and OUTDOOR VEGETABLE CROPS: BEANS and LETTUCE

# STABILITY OF PARTIAL RESISTANCE TO BLACK BEAN APHID IN A FABA BEAN CULTIVAR INFESTED WITH SEVERAL FUNGAL DISEASES

C. P. W. ZEBITZ[1], B. BÖHNKE, H. KEHLENBECK and G. KRAL
Institute for Plant Pathology and Plant Protection
Hannover University
Herrenhäuserstr. 2, 3000 Hannover 21, FRG.

Summary
The changes in host plant quality of the partially resistant faba bean cultivar 'BOLERO' to *Aphis fabae* was studied when the plants were stressed with either *Pythium ultimum*, *Fusarium solani*, *Botrytis fabae* or *Uromyces viciae-fabae*. Compared to healthy and diseased plants of susceptible cv. 'DIANA' the antixenotic resistance of cv. 'BOLERO' was decreased by *U. viciae-fabae* and slightly affected by *B. fabae*. Mean relative growth rate (MRGR) and intrinsic rate of natural increase, $r_m$, of the aphids were taken to determine alterations of antibiotic resistance properties of cv. 'BOLERO'. Antibiosis was almost nullified when the plants were diseased with *B. fabae* or *U. viciae-fabae*. The root pathogens *P. ultimum* and *F. solani* did not affect the resistance level. Possible consequences for further research are discussed.

## 1. Introduction

The growing of partially resistant faba bean cultivars could reduce the occasions as well as the concentrations required when insecticides need to be applied against *Aphis fabae* Scop. in the field. This is particularly relevant to situations where beneficial arthropods play a role in integrated pest management (YAKTI & POEHLING, 1988; YAKTI, 1989). Although genetically fixed resistance can only be affected to a limited extent (KOGAN, 1975) by environmental factors, we must consider the plant as part of a more or less complex pathosystem surrounded by in an even more complex ecosystem. Thorough investigations have been carried out mainly on the effects of different abiotic factors on the stability of resistance, but very little work has been done on biotic factors which may affect resistance (FRITZSCHE *et al.*, 1987).

Although there is no evidence, it is conceivable that certain parasites exploiting the same host might be capable of inducing physiological reactions in the plant which will alter its level of resistance to another parasite. This paper attempts to answer the following questions: (i) are diseases really capable of changing the resistance level of a host plant to an aphid? and, if this could be proved true, (ii) which forms of resistance are affected? and (iii) how severe are the effects?

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[1] To whom correspondence should be addressed

## 2. Materials and methods

The studies focussed on the pathosystem of *Vicia faba* L., and involved the partially resistant cultivar 'BOLERO' (var. *minor*) which exhibits antixenosis and antibiosis to *A. fabae*, and the susceptible cv. 'DIANA' (var. *minor*). The aphids were reared on the cultivar 'CON AMORE' (var. *major*).

The root pathogens *Pythium ultimum* Trow and *Fusarium solani* (Mart.) Appel et Wallr. were obtained from their respective culture media and inoculated in the pot soil to obtain 15 - 20 % root infestation 14 days after germination of the test plants. Broad bean rust *Uromyces viciae-fabae* (Pers.) Schroet. was inoculated in the plant using a suspension of 50,000 uredospores per ml whereas chocolate spot disease *Botrytis fabae* Sard. was inoculated as homogenated mycelium in 14 days old plants.

Non-preference was assayed in dual choice tests as well as in multiple choice tests. Assays for spatial dispersion of individuals on test plants and dual choice tests were done with fourth instar larvae and young apterous virginoparae from the laboratory culture. Multiple choice tests were carried out in a flight chamber (1.20 m diam, 1 m height) using alatae from the field. A preference index (PI) was calculated using the formula $PI = (N_A - N_B)/(N_A + N_B)$, where N is the number of individuals on a leaf either indexed A or B depending on which were being compared. Thus, PI values of +1 or -1 indicate preference for one of the leaves tested.

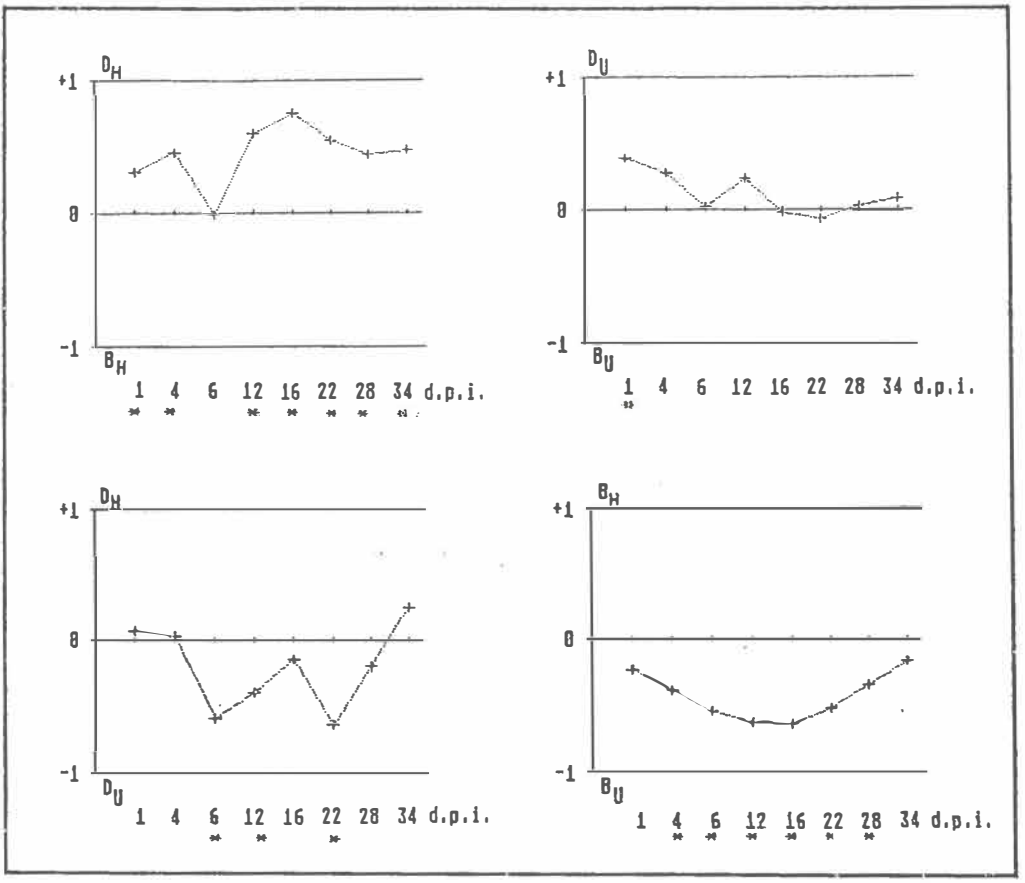
Antibiotic resistance was assessed by recording the development time, the weight of adults at the start of larviposition, survival and the fecundity of caged virginoparae. From these parameters a mean relative growth rate (MRGR) was derived according to the formula employed by RADFORD (1967) and the intrinsic rate of natural increase ($r_m$), using WYATT & WHITE's formula (1977).

Studies with root pathogens were started when plants were two weeks old, while assays with leaf diseases began when the first symptoms were visible. The tests were carried out under glasshouse conditions using supplementary light (16/8 h L/D) and a temperature range of 20 to 25 °C.

## 3. Results

The leaf disease *B. fabae* had no effect on antixenotic resistance of cv. 'BOLERO' in dual choice tests. In contrast to this perthotrophic fungus the biotrophic broad bean rust significantly enhanced the attractiveness of infested leaves (Fig. 1 C-D). For a period of at least four weeks diseased leaves of cv. 'BOLERO' clearly lost their resistant properties (Fig. 1 B) which were apparent in healthy leaves (Fig. 1 A). If given free access to healthy or diseased plants these shifts could also be observed in the spatial distribution of the aphids (Table 1). The attractiveness of the three uppermost fully expanded leaves and the growing point, normally the preferred parts of a plant by an aphid, was diminished thus benefitting the inoculated leaves.

Figure 1. Preference-index (PI) revealed after dual choice assay between healthy and rust-diseased (*Uromyces viciae-fabae*) leaves of faba beans at different days post inoculation (dpi). (D: cv. 'DIANA', B: cv. 'BOLERO', H: healthy, U: diseased; *: stat. signif. different from expectation at $p \leq 0.05$, $X^2$-test).




Preference for cv. 'DIANA' by alatae was not adversely affected by broad bean rust when the insects were allowed to chose between healthy and diseased plants of both cultivars (Table 2A). In contrast to these findings the winged females preferred chocolate spot diseased bean plants. They still favoured cv. 'DIANA' but the non-preference resistance level appeared to be reduced (Table 2B).

Table 1. Relative (%) spatial dispersion of *Aphis fabae* after free access to healthy and diseased faba beans 12 (A) and 26 (B) days post inoculation (dpi) with *Uromyces viciae-fabae* in no-choice tests. (IL: infested leaves, MS: middle section, UL: three uppermost completely expanded leaves)

| | | cv. 'DIANA' healthy | cv. 'DIANA' infested | cv. 'BOLERO' healthy | cv. 'BOLERO' infested |
|---|---|---|---|---|---|
| A | IL | 9.3 | 25.3 | 14.5 | 18.1 |
| | MS | 3.8 | 18.0 | 19.5 | 10.9 |
| | UL | 28.9 | 10.7 | 27.6 | 27.1 |
| | Apex | 58.0 | 46.0 | 38.4 | 43.9 |
| B | IL | 5.4 | 12.4 | 1.5 | 20.0 |
| | MS | 6.1 | 6.8 | 17.7 | 28.8 |
| | UL | 10.0 | 19.3 | 35.0 | 5.6 |
| | Apex | 78.5 | 61.5 | 45.8 | 45.6 |

Table 2. Relative (%) dispersion of alate *Aphis fabae* on healthy and diseased faba bean plants infected with (A) *Uromyces viciae-fabae* and (B) *Botrytis fabae* in flight-chamber assay (multiple choice).

| | host | dpi | cv. 'DIANA' healthy | cv. 'DIANA' diseased | cv. 'BOLERO' healthy | cv. 'BOLERO' diseased |
|---|---|---|---|---|---|---|
| A | *Evonymus europaeus* | 8 | 34.7 | 42.9 | 14.2 | 8.2 |
| | *Vicia faba* | 20 | 44.2 | 22.5 | 16.2 | 17.1 |
| B | *Vicia faba* | 10 | 29.6 | 34.5 | 11.1 | 27.8 |

dpi: days post inoculation

Although in certain cases both *U. viciae-fabae*- and *Botrytis fabae*-infections resulted in a decreased level of antixenotic resistance, the outcome of host plant root infections with *Pythium ultimum* was different. The dual choice tests revealed a strong non-acceptance effect on the aphids exerted by all leaves of diseased plants. Any properties specific to the cultivars were suppressed. Moreover the aphids remained restless, making no attempt to settle down if confined to leaves of diseased plants. Washing the leaf

surface with water, 30 % ethanol and 70 % ethanol and subsequent comparison with non-treated leaves in dual choice tests revealed that water and 30 % ethanol washings could nullify the earlier observed adverse effect on the aphids. In these tests comparisons were only made between treated and untreated leaves of healthy and diseased plants.

The possible effect of diseases on the antibiotic resistance can best be elucidated by comparison of parameters related to population dynamics.

The MRGR, derived from weight gain during preimaginal development and time elapsed from birth until start of larviposition, reflects the quality of food. Whereas both root pathogens did not change the resistance level, *B. fabae* as well as *Uromyces viciae-fabae* exerted a strong effect on the resistance of cv. 'BOLERO'. Aphids reared on diseased leaves of this cultivar nearly reached the same MRGR as those bred on the susceptible cultivar 'DIANA' (Table 3). Splitting up the MRGR into its relevant components revealed that the increased weight of adults was largely responsible for the enhancement of the derived parameter. In addition to increased weight gain accelaration of preimaginal development augmented MRGR of aphids on rust-diseased leaves.

Like the MRGR, the $r_m$-values were also enhanced if the aphids were reared on leaves diseased with chocolate spot or broad bean rust almost resulting in the disappearance of antibiotic resistance in cv. 'BOLERO' (Table 4). *Fusarium solani* and *P. ultimum* slightly increased the resistance level or had no effect.

Table 3. Mean relative growth rates (MRGR) of *Aphis fabae* reared on healthy or diseased faba bean plants. Resistance index is given for cv. BOLERO, data from healthy and diseased cv. 'DIANA' resp. were taken as 100 %.

| | *Pythium ultimum* | | *Fusarium solani* | | *Botrytis fabae* | | *Uromyces viciae-fabae* | |
|---|---|---|---|---|---|---|---|---|
| cv. DIANA healthy | 0.464 | | 0.483 | | 0.340 | | 0.408 | |
| cv. BOLERO healthy | 0.442 | 95 % | 0.405 | 84 % | 0.277 | 81 % | 0.313 | 77 % |
| cv. DIANA diseased | 0.475 | | 0.462 | | 0.335 | | 0.418 | |
| cv. BOLERO diseased | 0.431 | 91 % | 0.381 | 83 % | 0.307 | 92 % | 0.401 | 96 % |

Table 4. Mean intrinsic rates of natural increase ($r_m$) of *Aphis fabae* reared on healthy or diseased faba bean plants. Relative values are given for cv. 'BOLERO', data from healthy and diseased cv. 'DIANA' were taken as 100 %.

| | *Pythium ultimum* | | *Fusarium solani* | | *Botrytis fabae* | | *Uromyces viciae-fabae* | |
|---|---|---|---|---|---|---|---|---|
| cv. DIANA healthy | 0.351 | | 0.423 | | 0.322 | | 0.414 | |
| cv. BOLERO healthy | 0.335 | 95 % | 0.392 | 93 % | 0.249 | 77 % | 0.318 | 77 % |
| cv. DIANA diseased | 0.335 | | 0.417 | | 0.298 | | 0.398 | |
| cv. BOLERO diseased | 0.303 | 90 % | 0.352 | 85 % | 0.274 | 92 % | 0.382 | 96 % |

## 4. Discussion

The main conclusions of this study are that (i) the most pronounced effect on antixenotic resistance was exerted by the broad bean rust in dual choice tests, (ii) alatae were only slightly affected in host finding and host acceptance, and (iii) taking MRGR and $r_m$-values *B. fabae* and *U. viciae-fabae* had a marked effect on the level of antibiosis resistance. Thus, the first two questions in the introduction have been answered.

The noticeable acceptance of rust-diseased leaves by *A. fabae* is supported by the enhanced MRGR and, ultimately, by increased $r_m$-values. THROWER & THROWER (1966) reported the existence of a strong metabolic sink induced by the fungus and it seems probable that this either facilitates the setting up of the physical sink by the aphids or it improves food quality. As aphids can strengthen the sink effect by aggregation at a feeding site (WAY & CAMMELL, 1970) broad bean rust might mimic a big aphid colony. This implies that it should be an effect dependent on rust pustule density but up to now we have not been able to prove this theory.

Altered host plant quality of *Botrytis*-infested leaves might be due to degradation of plant tissue by the fungus. However, the significantly increased MRGR and $r_m$-values found on chocolate spot diseased leaves did not coincide with an increasing acceptance by aphids. The root pathogens either did not alter host plant quality like *P. ultimum*, or they slightly decreased quality like *F. solani* but the resistance level of cv. 'BOLERO' was left unchanged. This can be explained by the nature of host plants which overcome loss of root biomass by increasing the efficiency of the remaining, uninfected, roots.

The attempt to rank the diseases according to their effect on host plant resistance to *A. fabae* might identify those diseases which we should include in further investigations on stability of resistance. Broad bean rust was found to rank highest, followed by *B. fabae* and ending with the root pathogens.

From the aphid nutrition and the rust from the plants points of view the fungus is quite similar to the aphids; both 'parasites' will induce a sink of metabolites in infested leaves. The perthotrophic fungus *B. faba*, however, does not induce sinks but is capable of decomposing the leaf tissue and may using it as a food resource. Moreover, chocolate spot diseased plants were able to compensate for the destroyed tissue by improving their physiological fitness (Kehlenbeck and Zebitz, unpublished). Both factors might profit the aphids but seem to be not as efficient as the effects induced by the rust.

In conclusion, it can be stated that those members of a plant pathosystem which exhibit close similarity with the insect in the exploitation of food resources or other requisites should be monitored for possible interactions affecting host plant resistance to the animal. Furthermore, detailed physiological and biochemical investigations of these interactions might help to elucidate the mechanisms of resistance.

Résumé

**La stabilité de la résistance partielle d'un cultivar de fève infecté avec plusieurs maladies fongiques envers le puceron noir, *Aphis fabae*.**

Les modifications de la qualité de plante hôte du cultivar de fève 'Bolero' à résistance partielle envers *Aphis fabae* ont été étudiées sur des plantes infectées par un des agents fongiques suivants: *Pythium ultimum, Fusarium solani, Botrytis fabae* et *Uromyces viciae-fabae*. Comparée aux plantes saines et malades du cultivar sensible 'Diana', la résistance antixénotique du cultivar 'Bolero' a été réduite par *U. viciae-fabae* et légèrement affectée par *B. fabae*. La mesure des modifications des propriétés de la résistance antibiotique chez le cultivar 'Bolero' a été déterminée par le taux moyen de croissance relative (MRGR) et le taux intrinsèque d'accroissement naturel ($r_m$) des pucerons. L'effet d'antibiose a quasiment disparu des plantes atteintes par *B. fabae* ou *U. viciae-fabae*. Les maladies de la racine, *P. ultimum* et *F. solani* n'ont pas influencé le niveau de résistance. Les conséquences possibles pour les recherches ultérieurs sont discutées.

## 5. References

YAKTI, R. & POEHLING, H.-M. (1988). Zum Einfluss eines Insektenwachstumsregulators (DSC 24 300 I) auf die Entwicklung von *Aphis fabae* unter besonderer Berücksichtigung von Nebenwirkungen auf Blattlausprädatoren. Med. Fac. Landbouww. Rijksuniv. Gent 53/3a, 1033-1043.

YAKTI, R. (1989). Einfluss von Sortenresistenz, Prädatoren und Juvenilhormonanaloga auf die Populationsentwicklung von *Aphis fabae* Scop. an *Vicia faba* L. Ph. D. thesis, Hannover University, 131 pp.

KOGAN, M. (1975). Plant resistance in pest management. In: Metcalf, R. L. & Luckmann, W. H. (Eds.): Introduction to insect pest management. John Wiley & Sons, New York, 103-146.

FRITZSCHE, R., DECKER, H., LEHMANN, W., KARL, E. & GEIßLER, K. (1987). Resistenz von Kulturpflanzen gegen tierische Schaderreger. Springer Verlag, Berlin, 356 pp.

RADFORD, P. J. (1967). Growth analysis formulae - Their use and abuse. Crop Sci. 7, 171-175.

WYATT, I. J. & WHITE, P. F. (1977). Simple estimation of intrinsic increase rates for aphids and tetranychid mites. J. appl. Ecol. 14, 757-766.

THROWER, L. B. & THROWER, S. L. (1966). The effect of infec- tion with *Uromyces fabae* on translocation in broad bean. Phytopathol. Zeitschrift 57, 267-276.

WAY, M. J. & CAMMELL, M. (1970). Aggregation behaviour in relation to food utilization by aphids. In: Watson, A. (Ed.): Animal populations in relation to their food resources. Blackwell Scientific Publisher, Oxford, 229-247.

# SOME FURTHER STUDIES OF THE VICIA FABA VARIETY "BOLERO" SHOWING PARTIAL RESISTANCE TO APHIS FABAE

H.M.POEHLING[1], B.TENHUMBERG[2], R.YAKTI[2] and C.PRÜTER[2]
[1]Institute of Plant Pathology, University of Göttingen, Göttingen FRG
and [1]Institute of Plant Diseases and Plant Protection, University of Hannover, Hannover FRG

Summary

1. Effects of temperature and humidity on the level of resistance
The level of resistance in the Vicia faba variety "Bolero" was strongly influenced by temperature compared with the more susceptible cultivar "Diana". The main differences between intrinsic rates of increase ($r_m$) could be observed at 20 °C whereas at 14 °C aphids developed at about the same rate on both cultivars. As with temperature, changes in soil humidity or light intensity affected aphid performance on both varieties in different ways. Resistance of "Bolero" was increased by drought stress and with increasing ligth intensity.

2. Combination of natural enemies, insecticides with low initial efficacy (JHA, reduced rates) and partial resistance for aphid control
The aphid predators Coccinella septempunctata and Chrysoperla carnea had a higher predation rate on the resistant cultivar. Additionally the efficiency of substances like pyriproxiphen or fenoxycarb which normally show only low aphicidal activity at reduced rates could be increased by using the resistant variety as host plant. It appears that combinations of resistance, natural enemies and selective insecticides of low dose rates could be an interesting approach for an environmentally beneficial aphid control.

## 1. Introduction

During recent years numerous studies have been performed by our working group on partial resistance of Vicia faba varieties to Aphis fabae. The first results of screening experiments and investigations on the mechanism of resistance of the variety "Bolero" were published elsewhere (Morvan et al. 1988).

This paper reports investigations of the stability of resistance in relation to changing abiotic conditions (temperature, humidity and light intensity) and, on a more practical approach, about the attempt to integrate resistance, natural enemies and reduced dosages of insecticides with low efficacy for an ecologically safer control of Aphis fabae.

## 2.1 Part 1

Effects of temperature, humidity and light intensity on the level of resistance.

Methods:

Aphids were confined on bean leaves using clip cages. Weight increase, relative growth rates, time from birth to adult and reproduction rates were recorded. From this data intrinsic rates of increase (Wyatt and White 1977) were calculated. All experiments were performed in controlled environment rooms with a light period of 16 hours. In the temperature experiments only aphid development was measured on plants of the same age under field conditions.

Results - temperature:

Aphid development was strongly affected on the susceptible variety "Diana" as well as on the partial resistant "Bolero" (Table 1). Aphids reached higher adult weights at low temperatures and the time from birth to adult was prolonged. Differences, however, between both varieties became significant only at temperatures ≥ 15°C. The reproduction rate generally increased with temperature. A comparison of the two varieties indicated virtually a levelling off at low temperatures. Above 15 °C the production of offspring increased considerably on the susceptible variety. From these data the intrinsic rate of increase could be calculated, showing clearly the relation between temperature and the level of resistance.

Table 1 Adult weight and intrinsic rates of increase ($r_m$) of Aphis fabae at different temperatures on the Vicia faba varieties "Diana" (D) and "Bolero" (B)

| | 13.6 °C* | | 17 °C | | 20 °C | | 26 °C | |
|---|---|---|---|---|---|---|---|---|
| | D | B | D | B | D | B | D | B |
| w | 1180 | 1150 | 1170 | 800 | 970 | 560 | 950 | 610 |
| $M_d$ | 46.6 | 43.3 | 54.89 | 23.86 | 41.5 | 30.26 | 49.78 | 39.3 |
| d | 14 | 12 | 10 | 10 | 7 | 9 | 6 | 7 |
| $r_m$ | 0.210 | 0.203 | 0.302 | 0.239 | 0.401 | 0.286 | 0.491 | 0.395 |
| $\Delta$ $r_m$ in % (Diana =100) | | - 3.4 | | - 20.9 | | - 28.7 | | - 19.6 |

w = adult weigth in ug
$r_m$ = intrinsic rate of increase (Wyatt & White 1977)
= c ($\log_e M_d$/d)
$M_d$ = effective fecundity
d = time from birth to the onset of reproduction in days
c = correcting constant 0.754
* = field experiment (mean temperature 13.6 °C)

Results - humidity:

To quantify a possible relation between soil humidity and resistance, bean plants supporting feeding aphids were submitted to a moderate drought stress at a temperature level of 26 °C. It became obvious that reduced soil humidity influenced aphid performance negatively on both varieties. On the resistant variety in paticular, however, the reproduction rate was much more strongly affected than on the susceptible one. Drought stress at this temperature increased the level of resistance. This is well documented by a comparison of $r_m$ - values (Table 2).

Results - light intensity:

At low light intensities, the effective fecundity ($M_d$) on both varieties increased. However the time of development was reduced only on "Bolero" which resulted in a large increase of the intrinsic rate of increase on this variety (Table 3).

Table 2 Intrinsic rate of increase ($r_m$) of Aphis fabae at different soil humidities

| | control | | drought | |
|---|---|---|---|---|
| | D | B | D | B |
| $M_d$ | 49.8 | 39.3 | 50.9 | 22.4 |
| d | 6 | 7 | 6 | 8 |
| $r_m$ | 0.491 | 0.395 | 0.494 | 0.293 |
| $\Delta r_m$ in % (Diana =100) | | - 19.5 | | - 40.7 |

Table 3 Intrinsic rate of increase ($r_m$) of Aphis fabae at different soil humidities

| | control | | drought | |
|---|---|---|---|---|
| | D | B | D | B |
| $M_d$ | 49.8 | 39.3 | 50.9 | 22.4 |
| d | 6 | 7 | 6 | 8 |
| $r_m$ | 0.491 | 0.395 | 0.494 | 0.293 |
| $\Delta r_m$ in % (Diana =100) | | - 19.5 | | - 40.7 |

Satisfactory explanations for the observed phenomena cannot be given at present. First attempts to derive correlations between the level of resistance under different conditions and the distribution of amino acids in leaf extract or aphid honeydew did not provide clear results. One major problem is that the distribution of various components like amino acids or phenols could not be followed from their origin in the plant via the phloem to the aphid body. The "missing link" has until now been the failure to get enough phloem sap for biochemical analysis from Vicia faba via the stylets of Aphis fabae by stylet cutting technics (HF- microcautery), a technique which works very well for instance with cereal aphids. Only when our present data can be completed by a quantitative evaluation of phloem substances, will it be possible to discuss differences in phloem loading, phloem uptake by the aphids and resorption processes in greater detail to find at least the key to solve the "puzzle".

## 2.2 Part 2

Combination of natural enemies, insecticides of low initial efficacy (JHA, reduced dose rates) and partial resistance.

One important aim of integrated plant protection is the "intelligent" combination of different "natural" control strategies, like natural enemies, specific culture methods, resistance etc., to lower the need for chemical control.

In recent years, different studies (Dunn and Kempton 1969, Lowe 1975, Acreman and Dixon 1985, v. Emden 1986 - to mention only some ) have shown that the effects of partial resistance of plants to aphids could be enhanced by natural enemies. In addition chemical control on resistant varieties could be achieved with considerably reduced insecticide dosages compared with susceptible controls.

In the case of Vicia faba an interesting approach has been to study the influence of resistance on the efficiency of natural enemies or "soft" insecticides.

To quantify the effects of single regulation factors we worked under semi-field conditions. In small plots of 1 $m^2$, plants were artificially infested with aphids (5 aphids/plant) and, after a few days, larvae of Coccinella septempunctata or Chrysopa carnea, important aphid predators, were added. As insecticides, the slow acting juvenoids Pyriproxiphen and Fenoxycarb, not normally used for aphid control because of their low efficiency, were sprayed on the same date. In some plots they were applied alone as control agents, while in others in combination with aphid predators.

It is obvious that resistance alone reduced aphid density by only about 60 % (table 4). The absolute number of aphids remaining on the resistant plants was far beyond any acceptable threshold. Coccinella septempunctata reduced aphid density very much with a significantly higher predation rate on the resistant variety. The juvenoid Pyriproxiphen (similar results were obtained with Fenoxycarb) also

limited aphid population growth at both dose rates tested, the normal recommended 0.02 % and a reduced rate of 1/10 of the recommended concentration. The reduced rate was particularly efficient on the resistant variety and on this variety absolute aphid densities nearly fell below critical densities of 100 aphids/plant. A combination of both factors nearly eliminated the aphids, but it has to be mentioned that the recommended dose rate of the juvenoid caused strong toxic effects on the Coccinellid larvae, through an inhibition of pupal moult. These effects were negligible at the reduced rate.

Table 4 Influence of Coccinella septempunctata and the JHA Pyriproxiphen on the development of Aphis fabae on the Vicia faba varieties "Diana" (s) and "Bolero" (r). Hannover 1986 (Efficiency - % Abbott)

| | aphids/plant Diana | Bolero | Bolero rel. (Diana=100%) |
|---|---|---|---|
| 19.6. untreated | 35 | 32 | 89 % |
| 14.7. (15 d) | | | |
| untreated | 2512 | 1584 | 63 % |
| + 1 $L_1$ C.sep./plant | 501 (79.6) | 178 (88.7) | 36 % |
| + JHA 0.02 % | 255 (89.8) | 102 (93.5) | 40 % |
| + JHA 0.002 % | 402 (83.9) | 125 (92.1) | 31 % |
| + (C.sep. + JHA 0.02 %) | 104 (95.9) | 20 (98.7) | 18 % * |
| + (C.sep. + JHA 0.002 %) | 159 (93.7) | 25 (98.4) | 16 % |

* strong toxic effects on Coccinella larvae ($L_4$--> pupal moult)

Table 5 Influence of Chrysopa carnea and the JHA Pyriproxiphen on the development of Aphis fabae on the Vicia faba varieties "Diana" (s) and "Bolero" (r). Hannover 1987 (Efficiency - % Abbott)

| | aphids/plant Diana | Bolero | Bolero rel. (Diana=100%) |
|---|---|---|---|
| 24.6. untreated | 43 | 34 | 79 % |
| 5.7. (11 d) | | | |
| untreated | 1778 | 1122 | 63 % |
| + 1 $L_1$ C.car./plant | 1258 (29) | 501 (55) | 40 % |
| + JHA 0.002 % | 524 (70) | 286 (75) | 54 % |
| + (C.sep. + JHA 0.002 %) | 281 (84) | 112 (90) | 40 % |

The benefits of using the resistant variety became obvious, too, when efficiencies were calculated. The highest efficiencies could always be measured on the resistant variety. The same tendencies occurred when Chrysopa carnea was used as aphid predator (Table 5).

These experiments are described in more detail by Yakti 1989 and Yakti and Poehling 1988. They show that the usefulness of partially resistant varieties, even with low levels of resistance, can be very high in integrated systems. It would to be valuable to continue these investigations further with other insecticides, especially with more selective aphicides.

Résumé

Nouvelles études sur la résistance partielle de Vicia faba, variété "Bolero", à l'égard d'Aphis fabae

1. Effets de la température et de l'humidité sur le degré de résistance. Le degré de résistance de Vicia faba variété "Bolero", est fortement influencé par la température, comparé au cultivar sensible "Diana". Les plus grandes différences entre les taux intrinsèques de croissance ($r_m$) sont observées à 20 °C, alors que de développement des pucerons à 14 °C est presque identique sur les deux cutivars.
Comme pour le facteur température, les variations de l'humidité du sol et de l'intensité lumineuse influencent le dévelppement des pucerons chez les deux variétés dans des proportions différentes. La résistance de "Bolero" est renforcé par l'effet de la sécheresse et par l'augmentation de l'intensité lumineuse.

2. Combinaison d'ennemis naturels, d'insecticides compatibles, mais à efficacité réduite (IGR,é dose ) et de résistance partielle pour lutter contre les pucerons. Les prédateurs Coccinella septempunctata et Chrysoperla carnea ont un taux de prédation plus élevé sur cultivar résistant. L'efficacité de substances comme le pyriproxiphène ou le fenoxycarbe qui ne montrent normalement qu'une faible activité aphicide à dose réduite, est augmentée sur la variété résistance. Il semble que la combinaison de résistance, ennemis naturels et insecticides sélectifs à faible doses peut apporter une contribution intéressante à une lutte contre les pucerons respectueuse de l'environnement.

## 3. References

Acreman, T.M. and Dixon, A.F.G. (1985). Developmental patterns in wheat and resistance to cereal aphids. Crop protection, 4, 322-328.

Dunn, J.A. and Kempton, D.P.H. (1969). Resistance of rape (Brassica napus) to attack by the cabbage aphid (Brevicoryne brassicae L.). Annals of Applied Biology, 64, 203-212.

van Emden, H.F. (1986). The interaction of plant resistance and natural enemies: Effects on populations of sucking insects. In: "Interactions of Plant Resistance and Parasitoids and Predators of Insects". Ed. by Boethel, D.J. and Eikenbary, R.D., Ellis Horwood, Chichester, UK, pp 138-150.

Lowe, H.J.B. (1975). Crop resistance to pests as a component of integrated control systems. Proceedings of the 8th British Insecticide and Fungicide Conference, 95, 87-92.

Morvan, Y., Borgemeister, C. und Poehling, H.M. (1988). Ansiedlung, Nahrungsaufnahme und Entwicklung von Aphis fabae an anfälligen und teilresistenten Sorten von Vicia faba. Entomol. Gener., 13, 207-220.

Wyatt, I.J. and White, P.F. (1977). Simple estimation of intrinsic increase rates for aphids and tetranychid mites. J. appl. Ecol., 14, 757-766.

Yakti, R. (1989). Einfluß von Sortenresistenz, Prädatoren und Juvenilhormonanaloga auf die Populationsentwicklung von Aphis fabae Scop. an Vicia faba L. Dissertation, Universität Hannover

Yakti, R. und Poehling, H.M. (1988). Effizienz von Blattlausprädatoren (Coccinelliden- und Chrysopidenlarven) und einem Insektenwachstumsregulator (JHA) gegenüber Aphis fabae Scop. (Homoptera, Aphididae) an resistenten und anfälligen Sorten von Vicia faba L.. Mitt. der Deutschen Gesellschaft für allgemeine und angewandte Entomologie (DGaaE), 6, 37-43.

# RESISTANCE IN LETTUCE TO *MACROSIPHUM EUPHORBIAE* AND *UROLEUCON SONCHI*.

KEES REININK[1] and FRANS L. DIELEMAN[2]
1) Institute for Horticultural Plant Breeding (IVT), P.O.Box 16, 6700 AA Wageningen, The Netherlands; 2) Department of Entomology, Agricultural University, P.O.Box 8031, 6700 EH Wageningen, The Netherlands

## Summary

With a labour efficient test, in which the weight of eight day-old aphids was used as a criterion of plant resistance, it was possible to identify lettuce genotypes with a high level of resistance to *M.euphorbiae* or to *U.sonchi*. Genotypes with low aphid weights showed little or no reproduction in subsequent tests. High levels of resistance to both aphid species were found in butterhead cultivars. Of the crisphead cultivars tested only "Marbello" showed a high level of resistance to *M.euphorbiae*, while none showed resistance to *U.sonchi*. Several lettuce genotypes combined resistance to both *M.euphorbiae* and *U.sonchi*, but others were only resistant to *M.euphorbiae*. This indicates that the resistances to these two aphid species are at least partly based on different factors. Within groups of genotypes with resistance to *Nasonovia ribisnigri*, *Myzus persicae* or *Pemphigus bursarius* large differences in resistance to *M.euphorbiae* and *U.sonchi* were found, indicating that the resistances to different aphid species are at least partly independent. A free reproduction test, in which aphids were not confined to leaf cages, confirmed genotypical differences in resistance identified in previous tests. The level of resistance to both aphid species decreased with plant age.

## Introduction

Host plant resistance of lettuce (*Lactuca sativa*) to leaf aphids has been studied in a cooperative project between the Institute for Horti-cultural Plant Breeding and the Department of Entomology of the Wageningen Agricultural University since 1973. Until 1987 this work was restricted to the resistance of lettuce to *Nasonovia ribisnigri* and to *Myzus persicae*. Results have been presented in previous meetings of the working group (Dieleman & Eenink, 1981; Eenink & Dieleman, 1977; 1981; Reinink & Dieleman, 1988). Two types of resistance were found: monogenic complete resistance to *N.ribisnigri* (Nr-gene) (Eenink *et al.*, 1982a; 1982b) and partial resistance to *M.persicae* (Eenink & Dieleman, 1977; 1980; Reinink *et al.*, 1988; 1989).

Lines with the Nr-gene or with partial resistance to *M.persicae* were also tested for resistance to *Macrosiphum euphorbiae* (Reinink & Dieleman, 1989). The Nr-gene had no effect on the resistance of lettuce to *M.euphorbiae*. In lines with partial resistance to *M.persicae* some resistance to *M.euphorbiae* was found, but other cultivars with little resistance to *M.persicae* also showed partial resistance to *M.euphorbiae*. Also in further experiments with two different clones of *M.euphorbiae*

no clear relationship between resistance of lettuce to *M.euphorbiae* and to *M.persicae* was found (Reinink *et al.*, 1989). Therefore new screening experiments were done to study the genotypical variation of resistance to *M.euphorbiae* in lettuce. Another leaf aphid species, *Uroleucon sonchi*, was also included in this new screening procedure because this leaf aphid species is also frequently found on lettuce in the Netherlands.

This paper discusses the results obtained for both aphid species in screening and evaluation experiments.

## Materials and methods

Ninety lines and cultivars of lettuce were screened for resistance to *M.euphorbiae* (clone WMe1) and to *U.sonchi* (clone WUs1) in separate weight tests. In these tests the weight of eight day-old aphids was used as a criterion of plant resistance. This test method requires much less labour and takes less time than the reproduction test that was used in most previous research (Reinink & Dieleman, 1989, Reinink *et al.*, 1988; 1989) and thus makes it possible to screen more genotypes. Each plant was infested with a total of 18 larvae less than 24 h old. The larvae were distributed in six leaf cages. These cages were attached to the underside of three leaves. After seven days the surviving aphids were counted and weighed. The 90 genotypes include modern butterhead cultivars for glasshouse and for outdoor production, old butterhead cultivars from the CGN-collection, modern crisphead cultivars for glasshouse and for outdoor production, old crisphead cultivars and lines with partial resistance to *M.persicae* (Reinink *et al.*, 1988; 1989), complete resistance to *N.ribis-nigri* (Reinink & Dieleman 1989) or with resistance to the root aphid, *Pemphigus bursarius* (Dunn, 1974; Dunn & Kempton, 1980).

Based on the results of the weight test for *M.euphorbiae*, 30 genotypes were selected for a second evaluation, in which the reproduction test was used. Of these genotypes, 21 produced low aphid weights and nine produces high aphid weights in the test with *M.euphorbiae*. In separate experiments these 30 genotypes were tested with *M.euphorbiae* and with *U.sonchi*. The reproduction test followed the standard procedure described earlier (Reinink & Dieleman, 1989; Reinink *et al.*, 1988; 1989). The criterion of resistance was the number of larvae produced by apterous females which were 16 days old at the end of the test.

The next experiment was a free reproduction test to evaluate the results of the previous screens with aphids that were not confined to leaf cages. Four genotypes were selected that had shown large differences in resistance to *M.euphorbiae* and to *U.sonchi*. (Table 1).

The effect of plant age was also tested in this experiment. Three plant ages were included: at the end of the experiment plant ages were 38, 44 and 50 d after sowing for *M.euphorbiae* and 43, 49 and 55 d for *U.sonchi*. Half of the plants were infested with five one day-old larvae of *M.euphorbiae*, the other plants with five one day-old larvae of *U.sonchi*. After 14 d for *M.euphorbiae* and 19 d for *U.sonchi* the total number of aphids per plant was counted.

All experiments were done in an environmentally-controlled glasshouse

at a constant temperature of 20°C and a minimum daylength of 16 hours, achieved when necessary by additional illumination.

**Table 1. Genotypes selected for a free reproduction test and their scores in the weight and reproduction tests. (M: *Macrosiphum euphorbiae*; U: *Uroleucon sonchi*; R: resistant; S; susceptible)**

| No | Name | Weights (mg) M | Weights (mg) U | No of larvae M | No of larvae U | Classification M | Classification U |
|---|---|---|---|---|---|---|---|
| 1 | Charan | 0.4 | 1.3 | 0 | 2 | R | R |
| 2 | Marbello | 0.6 | 2.0 | 2 | 34 | R | S |
| 3 | Avoncrisp | 2.0 | 1.9 | 21 | 16 | S | S |
| 4 | Batavia Chou de Naples | 2.1 | 2.0 | 39 | 33 | S | S |

## Results and discussion

The results of the weight tests with *M.euphorbiae* and *U.sonchi* are presented in Tables 2 and 3. The highest level of partial resistance to both aphid species was found in butterhead cultivars. With one exception (Marbello) crisphead cultivars had little resistance to *M.euphorbiae*. No crisphead cultivar was found with a high level of resistance to *U.sonchi*.

**Table 2: Frequency distributions of groups of lettuce genotypes based on the results of a weight test with *Macrosiphum euphorbiae*.**

| Group | Weight class (mg) <0.4 | -0.8 | -1.2 | -1.6 | -2.0 | -2.4 | N | Mean |
|---|---|---|---|---|---|---|---|---|
| Butterhead cultivars: | | | | | | | | |
| Modern, glasshouse | 2 | 3 | 5 | 3 | - | - | 13 | 0.9 |
| Modern, outdoor | 1 | 3 | 2 | 3 | - | - | 9 | 0.9 |
| Old | - | 8 | 13 | 14 | - | 1 | 36 | 1.1 |
| Crisphead cultivars: | | | | | | | | |
| Modern, glasshouse | - | 1 | - | 3 | 1 | - | 5 | 1.3 |
| Modern, outdoor | - | - | - | 2 | 5 | - | 7 | 1.7 |
| Old | - | - | 2 | 6 | - | 2 | 10 | 1.5 |
| Lines with resistance to: | | | | | | | | |
| *Myzus persicae* | - | 3 | 1 | 3 | - | - | 7 | 1.1 |
| *Nasonovia ribisnigri* | - | - | 1 | 2 | 1 | - | 4 | 1.4 |
| *Pemphigus bursarius* | - | - | 1 | 4 | 1 | - | 6 | 1.4 |

The correlation between the weight data of both species for individual lettuce genotypes was rather low ($r = 0.58$, $P<0.01$).

The results of the genotypes selected for resistance to *M.persicae*, *N.ribisnigri* or *P.bursarius* did not indicate a pronounced effect

of these resistances on *M.euphorbiae* or *U.sonchi*: within these groups a large variation in resistance to *M.euphorbiae* and *U.sonchi* was found. Two of the six lines previously selected for high resistance to *M.persicae* (Reinink *et al.*, 1988) also showed high levels of partial resistance to *M.euphorbiae* and to *U.sonchi* which shows that a combination of resistances is possible.

**Table 3: Frequency distributions of groups of lettuce genotypes based on the results of a weight test with *Uroleucon sonchi*.**

| Group | Weight class (mg) | | | | | | N | Mean |
|---|---|---|---|---|---|---|---|---|
| | ≤0.4 | -0.8 | -1.2 | -1.6 | -2.0 | -2.4 | | |
| Butterhead cultivars: | | | | | | | | |
| Modern, glasshouse | - | 2 | 4 | 5 | 2 | - | 13 | 1.2 |
| Modern, outdoor | - | - | 1 | 7 | 1 | - | 9 | 1.4 |
| Old | - | 1 | 13 | 18 | 4 | - | 36 | 1.3 |
| Crisphead cultivars: | | | | | | | | |
| Modern, glasshouse | - | - | - | - | 1 | 4 | 5 | 2.0 |
| Modern, outdoor | - | - | - | - | 3 | 4 | 7 | 2.1 |
| Old | - | - | - | 5 | 4 | 1 | 10 | 1.6 |
| Lines with resistance to: | | | | | | | | |
| *Myzus persicae* | - | 2 | 1 | 3 | 1 | - | 7 | 1.1 |
| *Nasonovia ribisnigri* | - | - | 2 | 1 | 1 | - | 4 | 1.3 |
| *Pemphigus bursarius* | - | - | 1 | 2 | 3 | - | 6 | 1.6 |

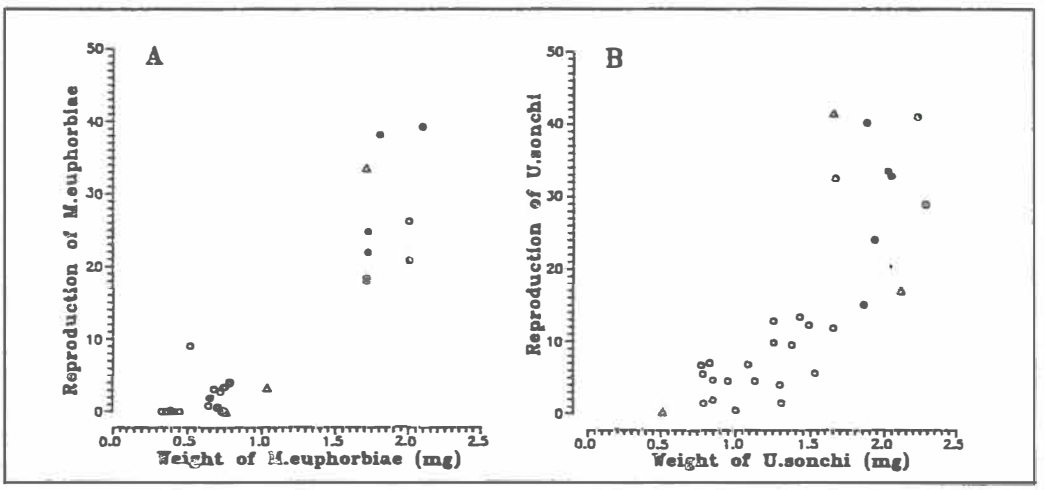



**Figure 1. Relationship between reproduction (number of larvae produced per apterous adult during test period) and weight (mg) of eight day-old individuals of *Macrosiphum euphorbiae* (A) and *Uroleucon sonchi* (B) on 30 lettuce genotypes. (o: butterhead type; ●: crisphead type; Δ: other lettuce types)**

Figure 1 shows the relationship between weight and reproduction of *M.euphorbiae* (Fig. 1A) and *U.sonchi* (Fig. 1B). Genotypes with a low aphid weight supported little or no aphid reproduction. On genotypes with high resistance to *M.euphorbiae* the larval mortality and larval period were greatly increased. Figure 1 shows that for both aphid species the relation between aphid weight and reproduction was negligible above a weight of about 1.5 mg. In the range of high aphid weights significant differences between genotypes could be detected with the reproduction test that were not found with the weight test. Reproduction means of the two aphid species on the 30 lettuce genotypes were significantly correlated ($r=0.72$, $P<0.01$). However, some genotypes combine resistance to *M.euphorbiae* with a high susceptibility to *U.sonchi* (e.g. "Marbello"). The reverse combination was not found.

Figure 2 shows the results of the free reproduction test for *M.euphorbiae* and *U.sonchi*. The resistance of "Charan" and "Marbello" to *M.euphorbiae* and of "Charan" to *U.sonchi*, detected in the weight and the reproduction tests, was confirmed in this free reproduction test. Also the higher level of resistance to *U.sonchi* in "Avoncrisp" in comparison to the other two susceptible cultivars, shown in the reproduction test, was confirmed in the free

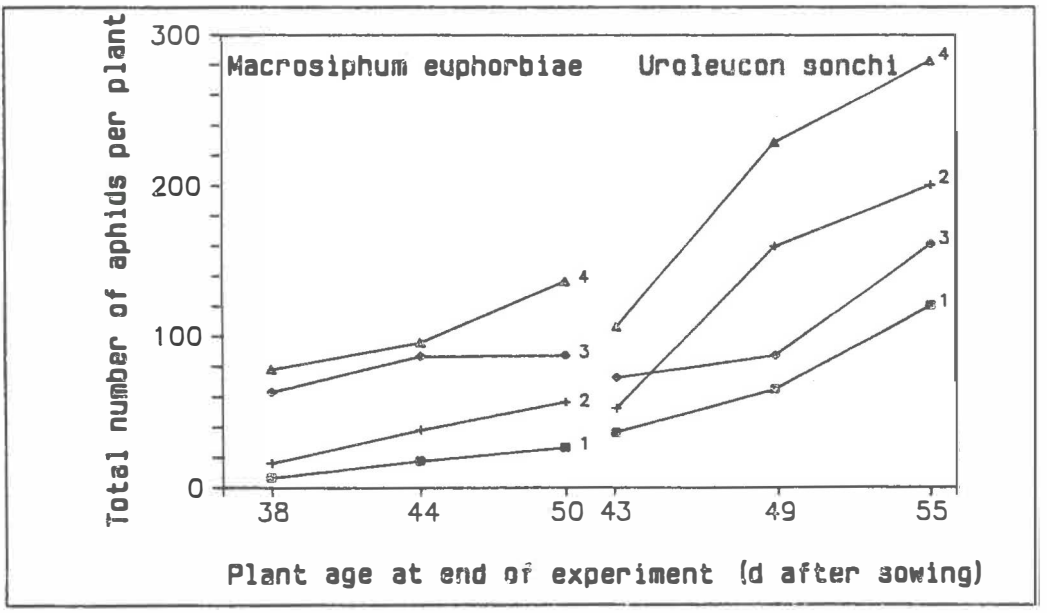



**Figure 2. Number of aphids counted on plants of three ages in a free reproduction test. *Macrosiphum euphorbiae* was counted 14 d and *Uroleucon sonchi* 19 d after inoculation. For cultivar names see Table 1.**

reproduction test. Resistance to both aphid species decreased with plant age. For *U.sonchi* a small (5% of the total sum of squares) but significant interaction between plant age and resistance was found. For *M.euphorbiae* no such interaction was found.


## Résumé

**La résistance de la laitue à *Macrosiphum euphorbiae* et *Uroleucon sonchi*.**

Grâce à un test efficace, consistant à utiliser le poids des pucerons âgés de 8 jours comme critères des plantes, il a été possible d'identifier des génotypes de laitue à haut niveau de résistance envers *Macrosiphum euphorbiae* et *Uroleucon sonchi*. Les génotypes ayant produit des pucerons de faible poids ont montré, dans des tests subséquents, qu'ils empêchaient ou diminuaient la reproduction de ceux-ci. Des hauts niveaux de résistance aux deux espèces de pucerons ont été trouvés chez des cultivars de laitue pommée. Parmi les cultivars testés de laitue frisée, seul "Marbello" a montré un niveau élevé de résistance envers *M.euphorbiae*, alors qu'aucun cv. n'a été trouvé résistant à *U.sonchi*. Plusieurs génotypes de laitue ont montré une résistance combinée aux deux espèces *M.euphorbiae* et *U.sonchi*, mais d'autres uniquement à *M.euphorbiae*. Ceci indique que les résistances aux deux espèces sont basées, au moins partiellement, sur des facteurs différents. Parmi des groupes de génotypes à résistance envers *Nasonovia ribisnigri*, *Myzus persicae* ou *Pemphigus bursarius*, de grandes différences de résistance à *M.euphorbiae* et *U.sonchi* ont été observées indiquant que les résistances aux différentes espèces de pucerons sont, en tout cas partiellement, indépendantes. Un essai de reproduction libre, dans lequel les pucerons n'étaient pas confinés dans des cages foliaires, a confirmé les différences génotypiques de résistance identifiées par des essais antérieurs. Le niveau de résistance aux deux espèces de pucerons a faibli au fur et à mesure que le végétal vieillisait.


## References


DUNN, J. A. (1974). Study on inheritance of resistance to root aphid, *Pemphigus bursarius*, in lettuce. Annals of Applied Biology **76**, 9-18.

DUNN, J. A. & KEMPTON, D.P.H. (1980). Susceptibility to attack by root aphid in varieties of lettuce. Tests of Agrochemicals and Cultivars, No 1, (Annals of Applied Biology **94**, Supplement) pp. 56-57.

DIELEMAN, F.L. & EENINK, A.H. (1981). Resistance of lettuce to *Myzus persicae*: factors influencing the host-parasite relationship. SROP/WPRS Bull. **1981/IV**, 25-28.

EENINK, A.H. & DIELEMAN, F.L. (1977). Resistance in *Lactuca* L. to *Myzus persicae* (Sulzer). SROP/WPRS Bull. **1977/3**, 61-66.

EENINK A.H. & DIELEMAN, F.L. (1977). Screening *Lactuca* for

resistance to *Myzus persicae*. Netherlands Journal of Plant Pathology **83**, 139-151.

EENINK A.H. & DIELEMAN, F.L. (1980). Development of *Myzus persicae* on a partially susceptible genotype of lettuce (*Lactuca sativa*) in relation to plant age. Netherlands Journal of Plant Pathology **86**, 111-116.

EENINK, A.H. & DIELEMAN, F.L. (1981). Resistance of lettuce to *Nasonovia ribisnigri*: research on the occurrence of differential interactions between host and aphid genotypes and on the inheritance of resistance. SROP/WPRS Bull. **1981/IV**, 41-46.

EENINK A.H., GROENWOLD R. & DIELEMAN, F.L. (1982a). Resistance of lettuce (*Lactuca*) to the leaf aphid *Nasonovia ribisnigri* 1. Transfer of resistance from *L.virosa* to *L.sativa* by interspecific crosses and selection of resistant breeding lines. Euphytica **31**, 291-300.

EENINK A.H., DIELEMAN F.L. & GROENWOLD, R. (1982b). Resistance of lettuce (*Lactuca*) to the leaf aphid *Nasonovia ribisnigri* 2. Inheritance of the resistance. Euphytica **31**, 301-304.

REININK, K. & DIELEMAN, F.L. (1988). Progress in the studies of resistance to aphids in lettuce. SROP/WPRS Bull. **1988/XI/3**, 3-4.

REININK K. & DIELEMAN, F.L. (1989). Comparison of sources of resistance to leaf aphids in lettuce (*Lactuca sativa* L.). Euphytica, **40**, 21-29.

REININK K., DIELEMAN F.L. & GROENWOLD, R. (1988). Selection of lines of lettuce (*Lactuca sativa* L.) with a high level of partial resistance to *Myzus persicae* (Sulzer). Euphytica **37**, 241-245.

REININK K., DIELEMAN, F.L., JANSEN J. & MONTENARIE A.M. (1989). Interactions between plant and aphid genotypes in resistance of lettuce to *Myzus persicae* and *Macrosiphum euphorbiae*. Euphytica, **43**, 215-222.

**Appendix: RESULTS FOR INDIVIDUAL LETTUCE GENOTYPES**

WM : *Macrosiphum euphorbiae*: mean aphid weight in micrograms
RM : *Macrosiphum euphorbiae*: mean reproduction per adult
WU : *Uroleucon sonchi*: mean aphid weight in micrograms
RU : *Uroleucon sonchi*: mean reproduction per adult

**Modern butterhead cultivars, glasshouse type**

| | Name | Firm | GM | RM | GU | RU |
|---|---|---|---|---|---|---|
| 1 | Bistro | ENZA | 743 | 3.28 | 1666 | 32.58 |
| 2 | Brenado | Bruinsma | 976 | | 1078 | |
| 3 | Columbus | Bruinsma | 1077 | | 1160 | |
| 4 | Deci-Minor | Rijk Zwaan | 1295 | | 1419 | |
| 5 | Nanda | Sluis & Groot | 1119 | | 1497 | |
| 6 | Norden | ENZA | 779 | 3.89 | 1652 | 11.92 |
| 7 | Pallas | Leen de Mos | 1349 | | 1278 | |
| 8 | Panvit | Sluis & Groot | 962 | | 1015 | |

| | | | | | | |
|---|---|---|---|---|---|---|
| 9 | Pascal | Leen de Mos | 389 | 0.16 | 768 | 6.83 |
| 10 | Ravel | Rijk Zwaan | 1292 | | 1279 | |
| 11 | Salina | Leen de Mos | 327 | 0.00 | 847 | 2.08 |
| 12 | Sitonia | ENZA | 726 | 0.17 | 778 | 5.58 |
| 13 | Sorbon | Sluis & Groot | 865 | | 1234 | |

**Modern butterhead cultivars, outdoor type**

| | Name | Firm | GM | RM | GU | RU |
|---|---|---|---|---|---|---|
| 14 | Benita | Nunhem | 1275 | | 1702 | |
| 15 | Charan | Sluis & Groot | 358 | 0.00 | 1302 | 1.67 |
| 16 | Cindy | Nickerson Zwaan | 448 | 0.04 | 1527 | 5.75 |
| 17 | Index | Jos Huizer | 1129 | | 1215 | |
| 18 | Mondian | Sluis & Groot | 1176 | | 1514 | |
| 19 | Ovation | ENZA | 642 | 0.80 | 1250 | 10.00 |
| 20 | Reskia | Rijk Zwaan | 409 | 0.00 | 1000 | 0.67 |
| 21 | Soraya | Rijk Zwaan | 1240 | | 1561 | |
| 22 | Tannex | Jos Huizer | 1514 | | 1341 | |

**Modern crisphead cultivars, glasshouse type**

| | Name | Firm | GM | RM | GU | RU |
|---|---|---|---|---|---|---|
| 24 | Kelly | Leen de Mos | 1622 | | 2012 | |
| 25 | Marbello | Bruinsma | 649 | 1.90 | 2024 | 33.67 |
| 27 | Catona | Sluis & Groot | 1500 | | 2286 | |
| 28 | Tanja | Rijk Zwaan | 1424 | | 2110 | |
| 33 | Bastion | ENZA | 1528 | | 1816 | |

**Modern crisphead cultivars, outdoor type**

| | Name | Firm | GM | RM | GU | RU |
|---|---|---|---|---|---|---|
| 23 | Crispino | Rijk Zwaan | 1647 | | 2208 | |
| 26 | Nabucco | Royal Sluis | 1488 | | 2080 | |
| 29 | Saladin | Rijk Zwaan | 1714 | 18.57 | 1878 | 40.42 |
| 30 | Itacka | Rijk Zwaan | 1723 | 22.19 | 2288 | 29.00 |
| 31 | El Toro | Nunhem | 1589 | | 1980 | |
| 32 | Zodiac | ENZA | 1724 | 25.00 | 1932 | 24.20 |
| 34 | Nr 702 | Leen de Mos | 1802 | 38.33 | 2230 | 41.17 |

**Genotypes with partial resistance to *Myzus persicae***

| Genebank Nr. | | Name | GM | RM | GU | RU |
|---|---|---|---|---|---|---|
| 35 | PIVT 42 | Batavia la Brillante | 1209 | | 1263 | |
| 36 | PIVT 47 | Batacer | 1314 | | 1394 | |
| 37 | PIVT 180 | Iceberg | 1573 | | 1705 | |
| 38 | PIVT 227 | Liba | 677 | 3.12 | 785 | 1.67 |
| 39 | PIVT 339 | Plenos | 1169 | | 987 | |
| 40 | PIVT 364 | PI169497 | 758 | 0.00 | 510 | 0.25 |

## Cultivars with resistance to *Pemphigus bursarius* (R6)

| Genebank Nr. | | Name | GM | RM | GU | RU |
|---|---|---|---|---|---|---|
| 41 | PIVT 30 | Avoncrisp | 2005 | 21.03 | 1856 | 15.17 |
| 42 | PIVT 31 | Avondeviance | 1239 | | 1193 | |
| 43 | PIVT 146 | Grand Rapids | 1371 | | 1853 | |
| 44 | PIVT 16 | Ardente | 1384 | | 1761 | |
| 45 | | Nusette (ENZA) | 1099 | | 1300 | |
| 46 | | Sabine (ENZA) | 1208 | | 1537 | |
| 47 | PIVT 1091 | Webbs Wonderfull (susceptible control) | 1534 | | 1599 | |

## Old cultivars from the CGN genebank

| Genebank Nr. | | Name | GM | RM | GU | RU |
|---|---|---|---|---|---|---|
| 48 | PIVT 36 | Batavia Blonde de Paris | 1092 | | 1452 | |
| 49 | PIVT 87 | Batavia Chou de Naples | 2093 | 39.46 | 2047 | 33.00 |
| 50 | PIVT 1095 | Reine des Glaces | 1202 | | 1935 | |
| 51 | PIVT 45 | Batavia R. Grenobloise | 1188 | | 1158 | |
| 52 | PIVT 1446 | Bautzener Dauer | 1235 | | 1442 | |
| 53 | PIVT 77 | Bibb | 782 | 4.11 | 845 | 4.92 |
| 54 | PIVT 59 | Blonde d'Eté | 1440 | | 1596 | |
| 55 | PIVT 61 | Blonde de Prieure | 936 | | 1379 | |
| 56 | PIVT 72 | Brune d'Hiver | 1523 | | 1147 | |
| 57 | PIVT 74 | Brune Percheronne | 719 | 2.71 | 947 | 4.75 |
| 58 | PIVT 84 | Cazard | 1455 | | 1272 | |
| 59 | PIVT 90 | Cordon Rouge | 1112 | | 1340 | |
| 60 | PIVT 92 | Craquerelle du Midi | 1645 | | 1146 | |
| 61 | PIVT 104 | Deer Tongue | 1714 | 33.71 | 1653 | 41.58 |
| 62 | PIVT 114 | Dippe's Futura | 1179 | | 1580 | |
| 63 | PIVT 117 | Du Bon Jardinier | 701 | 0.54 | 1425 | 13.58 |
| 64 | PIVT 595 | Brilliant | 961 | | 1101 | |
| 65 | PIVT 51 | Blackpool | 749 | 3.43 | 1371 | 9.67 |
| 66 | PIVT 137 | Gotte à Graine Blonde | 1467 | | 1374 | |
| 67 | PIVT 140 | Gotte Jaune d'Or | 1187 | | 1344 | |
| 68 | PIVT 141 | Gotte Lente à Monter | 2003 | 26.38 | 1487 | 2.42 |
| 69 | PIVT 160 | Grosse Blonde d'Hiver Bourguignonne | 1234 | | 1004 | |
| 70 | PIVT 161 | Grosse Bl. Paresseuse | 1316 | | 1540 | |
| 71 | PIVT 164 | Grosse Brune têtue | 1049 | | 1299 | |
| 72 | PIVT 194 | Kagraner Sommer | 1378 | | 1613 | |
| 73 | PIVT 198 | Kampioen | 985 | | 1371 | |
| 74 | PIVT 286 | Maikonig | 988 | | 989 | |
| 75 | PIVT 297 | Merveille des Quatre S. | 517 | 9.07 | 1288 | 4.25 |
| 76 | PIVT 309 | Monstrueuse Ronde d'Eté | 1356 | | 1688 | |
| 77 | PIVT 177 | Hjerter Es | 731 | 0.00 | 1125 | 4.70 |
| 78 | PIVT 326 | Passion Blanche à Graine Noire | 1240 | | 1029 | |
| 79 | PIVT 330 | Passion Verte d'Hiver | 1145 | | 1543 | |
| 80 | PIVT 130 | Geduld | 1137 | | 1179 | |
| 81 | PIVT 344 | Premice | 1442 | | 1781 | |
| 82 | PIVT 351 | Proeftuin's Blackpool | 703 | 0.69 | 1081 | 7.00 |
| 83 | PIVT 24 | Attraction | 950 | | 1174 | |

**Controls**

| | Genebank Nr. | Name | GM | RM | GU | RU |
|---|---|---|---|---|---|---|
| 84 | PIVT 280 | *Lactuca virosa* (Nr) | 1006 | | 864 | |
| 85 | PIVT 255 | *Lactuca serriola* | 1471 | | 1211 | |
| 86 | 85996 | (Nr-line 1) | 1382 | | 1106 | |
| 87 | 82356 | (Nr-line 2) | 1574 | | 1878 | |
| 88 | 82330 | (Nr-line 3) | 1713 | 18.20 | 1253 | 13.00 |
| 89 | 841363 | (line 4; *M.pers.* res.) | 742 | 0.13 | 825 | 7.17 |
| 90 | PIVT 313 | Taiwan (*M.pers.* susc.) | 1032 | 3.35 | 2113 | 17.25 |

# INHERITANCE OF RESISTANCE IN LETTUCE TO LEAF APHIDS.

KEES REININK[1] and FRANS L. DIELEMAN[2]
1) Institute for Horticultural Plant Breeding (IVT), P.O.Box 16, 6700 AA Wageningen, The Netherlands; 2) Department of Entomology, Agricultural University, P.O.Box 8031, 6700 EH Wageningen, The Netherlands.

## Summary

Almost complete resistance to *Nasonovia ribisnigri* is under control of one dominant gene (Nr-gene). This gene also increases the level of resistance to *M.persicae*. Partial resistance to the aphid species *Myzus persicae*, *Macrosiphum euphorbiae* and *Uroleucon sonchi* is inherited quantitatively, indicating oligo- or polygenic inheritance. The expression of partial resistance is dependent on environmental conditions. Dominance effects in the $F_1$ and $F_2$ are either small or absent, indicating a mainly additive inheritance. For *M.persicae* some reciprocal $F_1$'s were tested and no indications of cytoplasmic inheritance were found. Estimates of heritability in the $F_2$ for the quantitative resistances indicated that in most crosses selection for an increased level of resistance has good prospects. The resistances to *M.persicae*, *M.euphorbiae* and *U.sonchi* could have some genes in common, but other genes seem to be specific for only one leaf aphid species. Combining resistance to *N.ribisnigri* and a high level of partial resistance to *M.persicae* or to *M.euphorbiae* is feasible because of the effect of the Nr-gene on *M.persicae* and the availability of modern lettuce cultivars with a very high level of partial resistance to *M.euphorbiae*.

## Introduction

Leaf aphids cause problems in lettuce production because they spread virus diseases, cause reduced or abnormal growth and when present in the harvested heads render the product unmarketable. In the present situation damage can only be prevented by means of repeated applications of pesticides when weather conditions are favorable for aphid reproduction. As an alternative to this situation host plant resistance of lettuce to leaf aphids has been studied in the Netherlands. A complicating factor in this research has been that lettuce is a good host plant for many aphid species (Blackman & Eastop, 1984). Breeding cultivars with resistance to all of these species may be difficult, unless resistance factors can be identified that provide resistance to more than one aphid species. Four leaf aphid species were included in the research: *Nasonovia ribisnigri*, *Myzus persicae*, *Macrosiphum euphorbiae* and *Uroleucon sonchi*. Detailed information on the relative importance of these species in field situations is lacking. However, in the Netherlands (as probably elsewhere in NW Europe) *N.ribisnigri* is by far the most common leaf aphid on lettuce. Averaged

over the years the incidence of *M.persicae* and of *M.euphorbiae* could be of about the same size, but *M.persicae* is a more efficient virus vector. *U.sonchi* is thought to be less important than the other three species, but is still frequently found on lettuce (Mentink & Prinsen, 1987). *U.sonchi* is highly conspicuous because of the brown colour of the larvae and the shiny black colour of adults.

In this paper a general presentation is given of the results of studies on the genetics of host plant resistance of lettuce to the four leaf aphid species mentioned above. Some of the results have been published elsewhere, but most of the information on the inheritance of resistance to *M.persicae*, *M.euphorbiae* and *U.sonchi* is new.

## Development of test methods

Screening for resistance in lettuce to leaf aphids was sometimes carried out using natural infestations in the field (Dunn & Kempton, 1980), but in most tests controlled infestation and experimenting was used. Three types of tests have been used in breeding research in the Netherlands. Firstly, a macrotest or free reproduction test was used to evaluate large numbers of plants (Eenink & Dieleman, 1977). In this test each plant is infested with a number of aphids of mixed ages and development. The aphids are allowed to move freely over the plants and after 2-4 weeks the number of aphids per plant is scored. A second test which has been used is a reproduction test (Reinink *et al.*, 1988). A few synchronized larvae less than one day-old are confined to leaf cages. A day before the aphids start reproducing one apterous aphid is left in each cage to determine the production of larvae per adult over a period of about nine days. In most tests each plant had six cages clipped to the underside of three different leaves. Larvae are counted and removed every 2-3 days to avoid density effects. The reproduction test is very accurate but requires a high input of labour. Therefore the number of plants that can be handled with this test is limited.

For studies on the inheritance of nearly complete resistance the macrotest, although being less accurate, was quite satisfactory (Eenink & Dieleman, 1983; Eenink *et al.*, 1982b). However, for the study of the inheritance of intermediate levels of resistance (partial resistance; PR) the results of the macrotest are too inaccurate. The reproduction test also is less suited for the study of the genetics of PR because its labour requirement does not allow the testing of large segregating populations. Therefore, in later research a third test method was used: a weight test (Reinink & Dieleman, in press). In this test 3-5 synchronized larvae less than 24 hours old are put in each of six leaf cages per plant. The cages are attached to the underside of three different leaves. After six days for *M.persicae* and seven days for *M.euphorbiae* and *U.sonchi* the surviving aphids are counted and weighed. At this time the first adults are found on susceptible plants. All the aphids from one plant are weighed together. The calculated mean aphid weight is used as the criterion of resistance. This weight test is more sensitive to differences in resistance than the macrotest and requires less labour input than a reproduction test.

With this test it was possible to obtain detailed information on the inheritance of PR in lettuce to several aphid species.

## Inheritance of resistance to *Nasonovia ribisnigri*

In field tests lettuce cultivars showed differences in the attack of *N.ribisnigri* (Dunn & Kempton, 1980). The inheritance of this type of resistance has not been studied because later the attention shifted towards nearly complete resistance found in the wild species *Lactuca virosa*. Through interspecific crosses the resistance was transferred to the cultivated lettuce (Eenink *et al.*, 1982a). The inheritance of this resistance was tested both in the wild species (Eenink & Dieleman, 1983) and, after transfer, in the cultivated species (Eenink *et al.*, 1982b). In both studies it was found that the resistance is governed by one dominant major gene, later named the Nr-gene. The high level of resistance conferred by this gene permits the use of the macrotest in breeding research and practice. Furthermore the presence of the Nr-gene in plants can be observed quite easily by the absence of growth disturbances after inoculation of seedlings with *N.ribisnigri*.

## Inheritance of resistance to *Myzus persicae*

Screening of 645 *Lactuca* genotypes for resistance to *M.persicae* with a macrotest revealed large genotypical variation in resistance to *M.persicae* (Eenink & Dieleman, 1977). In contrast to the results for *N.ribisnigri* no complete resistance was found. Wild *Lactuca* spp. did not show a higher level of PR than cultivated lettuce. A further increase in resistance was obtained by crossing PR cultivars identified in the previous screen and selecting progeny with high resistance, using the macrotest (Reinink *et al.*, 1988). It was suggested that the inheritance of PR to *M.persicae* was controlled by a small number of genes with relatively large effects because a relatively high number of lines with increased resistance was found. All screening and selection experiments were done with clone WMp1 of *M.persicae*, which grows and reproduces very well on lettuce. Evaluation of some of the selected lines with other clones of *M.persicae* showed that the resistance was almost complete to other clones of *M.persicae* (Reinink *et al.*, 1989).

Because the procedure used in the selection experiments did not allow a detailed genetical analysis, a new project was started to study the inheritance of PR in lettuce to *M.persicae*. $F_1$ and $F_2$ populations of three crosses between PR cultivars and of two crosses between PR cultivars and Norden, a highly susceptible butterhead cultivar, were evaluated using the weight test. Two control genotypes were used: PIVT313 ("Taiwan"), a highly susceptible cos type, and "Line 4", a breeding line with a high level of resistance obtained from the cross PIVT47x227 (Reinink *et al.*, 1988; 1989). Figure 1 shows the results of an experiment in which all parents and available $F_1$'s were tested together and the results of a series of five experiments in which each experiment contained one $F_2$ population together with the two parents,

the $F_1$ and the controls. Table 1 presents information on the deviations of the mean values of the $F_1$ and $F_2$ from the parental mean and heritability estimates in the $F_2$ generation.

**Table 1. Mean weights (mg) of *Myzus persicae*: deviations of the $F_1$ and $F_2$ means from the parental mean and estimates of heritability ($h^2$) in the $F_2$ generation (**: sign. at P=0.01; ***: sign. at P=0.001; PR: partially resistant; S: susceptible).**

| $P_1$ x $P_2$ | Type | $\bar{F}_1-\bar{P}$ a) | $\bar{F}_1-\bar{P}$ b) | $\bar{F}_2-\bar{P}$ b) | $h^2$ b) |
|---|---|---|---|---|---|
| PIVT47 x 42 | PRxPR | 0.01 n.s. | 0.02 n.s. | 0.01 n.s. | 0.33 |
| PIVT47 x 227 | PRxPR | -0.05 *** | not tested | -0.04 *** | 0.61 |
| PIVT364 x 47 | PRxPR | 0.09 *** | 0.01 n.s. | 0.00 n.s. | 0.34 |
| Norden x 47 | SxPR | 0.03 n.s. | 0.00 n.s. | -0.03 ** | 0.44 |
| Norden x 227 | SxPR | -0.02 n.s. | 0.00 n.s. | 0.01 n.s. | 0.39 |

a) obtained in one experiment; b) obtained in five separate expts.

In the experiment in which all parents were grown together the mean weights on the PR parents were 26-45% lower than on Norden, the susceptible parent, and 40-55% lower than on the susceptible control. Significant deviations of $F_1$ or $F_2$ from the midparent value, indicating dominance effects occurred consistently in the cross PIVT47x227, where resistance to *M.persicae* was dominant. The $F_1$ of the cross PIVT364x47 exceeded both parents in mean aphid weight in the first test, indicating overdominance of susceptibility. However, in the experiment with both $F_1$ and $F_2$ no significant deviations were found for this cross. The $F_2$ of PIVT227 x Norden showed a small negative deviation, but no deviations from the midparent value were detected for the $F_1$ in both tests. In general, resistance is intermediately inherited and dominance effects seem to be of minor importance. From the two crosses of the SxPR type reciprocal $F_1$'s were available. Reciprocal $F_1$'s had equal mean aphid weights, which means that PR in lettuce to *M.persicae* is not cytoplasmically inherited.

The results for the controls and for those parents that were included in several experiments show that the mean aphid weight on a certain genotype varies with environmental conditions. It was found that mean aphid weights increased with increasing natural daylength and light intensity.

Heritability estimates in the $F_2$ population ranged from 0.31 to 0.61. Contrary to expectations the highest heritability was not found in the crosses with the largest differences in resistance between parents (SxPR), but in the cross PIVT227x47. Also in previous research (Reinink *et al.*, 1988) this cross showed very promising results. In fact "Line 4", the PR control used in these experiment, was selected from this cross. All $F_2$'s showed a unimodal distribution indicating the absence of major gene effects. The fact that the PR x PR crosses did not show smaller heritability estimates than the crosses between genotypes with large differences in resistance (SxPR) could mean that the PR genotypes that were used did not represent the extremes of resistance to

*M.persicae*. The segregation in the PRxPR crosses shows that the PR parents differ in genes for resistance and that in an $F_2$ population lines with a superior combination of resistance genes can be found.

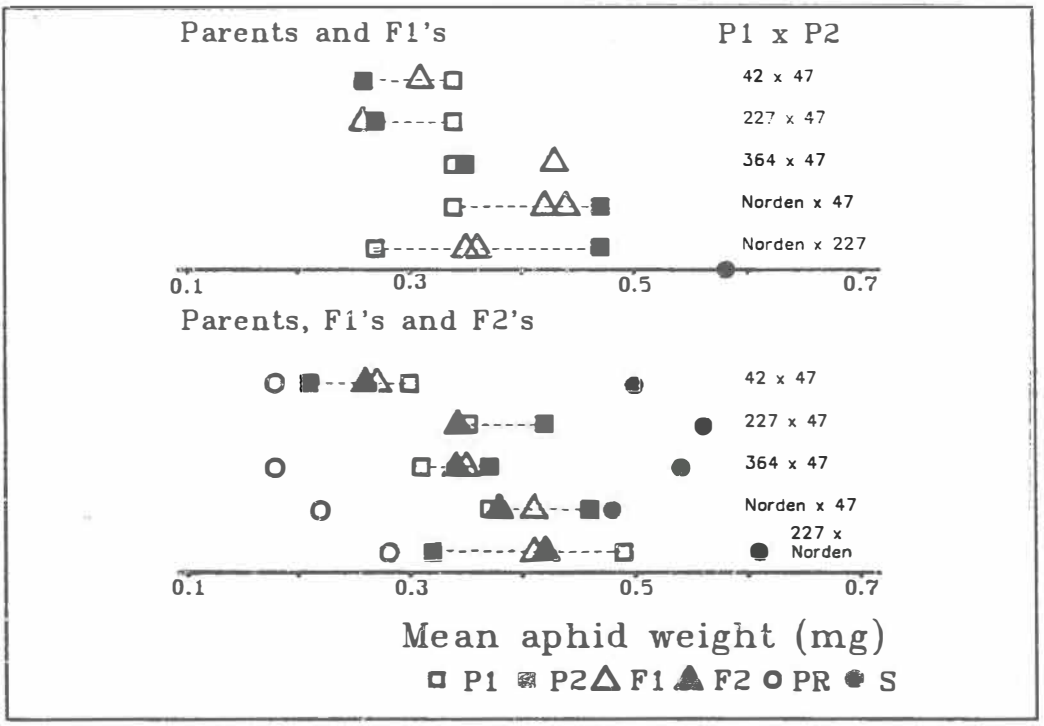



**Figure 1. Mean weight of *Myzus persicae* on parental and $F_1$ genotypes of five crosses tested in one experiment (above) and on parental, $F_1$, $F_2$ and control genotypes tested in five separate experiments (below). (PR: partially resistant; S: susceptible)**

The conclusion of this research is that PR in lettuce to *M.persicae* is quantitatively inherited and that the expression of PR is dependent on environmental conditions. Dominance effects were found in some cases but do not seem to be very important. No cytoplasmic inheritance was found. The estimates of heritability in the $F_2$ populations of PRxPR crosses show that a further accumulation of resistance genes is possible. In all crosses except PIVT227x47 heritability estimates were below 0.5. Therefore line selection in the $F_3$ or later populations should be preferred to selection of individual plants in the $F_2$.

## Inheritance of resistance to *Macrosiphum euphorbiae*

No previous reports on the inheritance of resistance in lettuce

to *M.euphorbiae* are available. Based on the results of a screening of 90 lettuce genotypes for resistance to *M.euphorbiae* and *U.sonchi* (Reinink & Dieleman, in press) four genotypes were selected for genetic studies (Table 2). These genotypes were crossed with each other in one direction. In a first test the six $F_1$'s and four parental genotypes were tested. In each of five following experiments 16 plants of each parent and 32 plants of each $F_2$ were tested both against *M.euphorbiae* and *U.sonchi*. In all experiments the weight test was used to measure the level of resistance.

Figure 2 shows the results of the experiment with parents and $F_1$'s and the averaged results of the five experiments in which $F_2$'s and parents were tested. Table 3 presents information on the deviations of the $F_1$ and $F_2$ from the midparent value and heritability estimates in the $F_2$ generation. Aphid weights on the susceptible parents were between three and four times higher than on resistant parents. In the first test $F_1$'s were intermediate between their parents, except in crosses 1x4 and 3x4 which showed relatively slight dominance effects of opposite sign. The positive deviation of $F_1$(3x4) was not confirmed by the results of the $F_2$ population. For the deviations of the $F_2$'s from the parental mean no significances are given because of statistical difficulties owing to the occurrence of genotype x environment interactions. For the same reason the estimates of heritability are only rough. In all crosses the $F_2$ was close to the midparent value. Estimates of heritability in the $F_2$ population were very low in crosses with parents of the same resistance type (SxS and PRxPR) and high in crosses with parents of different resistance type. This indicates that the parents that were chosen represent the extremes of resistance to *M.euphorbiae*. A further increase of resistance above the level found in parent 1 (Charan) cannot be expected with the test method used in this research. The frequency distributions of the $F_2$ populations indicated a quantitative inheritance without detectable major gene effects.

For *M.euphorbiae* it can be concluded that PR in lettuce is inherited quantitatively and dominance effects seem to be of minor importance. Selection for resistance in crosses between PR and susceptible genotypes should not be too difficult because estimates of heritability are high in these crosses. Any increases in resistance above the level of cultivar Charan seem unlikely, because no segregation was detected in the cross between the two resistant parents.

**Table 2. Number, name, plant type and resistance level of parental genotypes selected to study the inheritance of resistance in lettuce to *Macrosiphum euphorbiae* and *Uroleucon sonchi*. The resistance classification is based on previous research (Reinink & Dieleman, in press).**

| No | Name | Type | Resistance classification *M.euphorbiae* | Resistance classification *U.sonchi* |
|---|---|---|---|---|
| 1 | Charan | butterhead | resistant | resistant |
| 2 | Marbello | crisphead | resistant | susceptible |
| 3 | Avoncrisp | crisphead | susceptible | susceptible |
| 4 | Batavia Chou de Naples | crisphead | susceptible | susceptible |

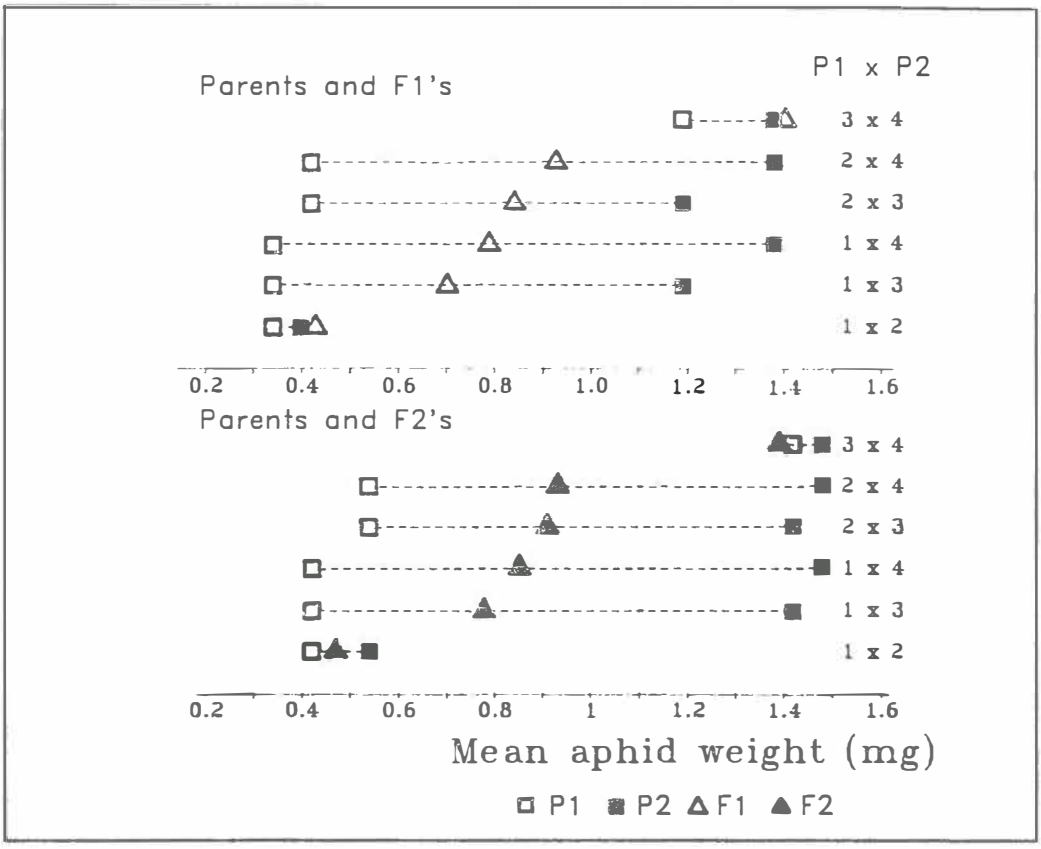



**Figure 2. Mean weight of *Macrosiphum euphorbiae* on parents and $F_1$'s of six crosses tested in one experiment (above) and on parents and $F_2$'s (average values from five experiments, below).**

**Table 3. Mean weights (mg) of *Macrosiphum euphorbiae*: deviations of $F_1$ and $F_2$ means from the parental mean and estimates of heritability ($h^2$) in the $F_2$ generation for six crosses. (*: sign. at P=0.05; PR: partially resistant; S: susceptible).**

| $P_1$ x $P_2$ | Type | $\bar{F}_1-\bar{P}$ a) | $\bar{F}_2-\bar{P}$ b) | $h^2$ b) |
|---|---|---|---|---|
| 1 x 2 | PRxPR | 0.02 n.s. | -0.01 | 0.00 |
| 1 x 3 | PRx S | -0.06 n.s. | -0.14 | 0.70 |
| 1 x 4 | PRx S | -0.07 * | -0.10 | 0.76 |
| 2 x 3 | PRx S | 0.04 n.s. | -0.07 | 0.69 |
| 2 x 4 | PRx S | 0.02 n.s. | -0.08 | 0.72 |
| 3 x 4 | S x S | 0.11 * | -0.04 | 0.21 |

a) obtained in one experiment; b) means of five expts.

## Inheritance of resistance to *Uroleucon sonchi*

The experiments described above for *M.euphorbiae* were also used to study the inheritance of resistance in lettuce to *U.sonchi*. Figure 3 shows the results of the experiment with parents and $F_1$'s and the averaged results of the five experiments in which $F_2$'s and parents were tested. Table 4 presents information on the deviations of the $F_1$ and $F_2$ from the midparent value and heritability estimates in the $F_2$ generation. Again no significances are given for the $F_2$ deviations because of the occurrence of genotype x environment interactions.

**Table 4. Mean weights (mg) of *Uroleucon sonchi*: deviations of $F_1$ and $F_2$ means from the parental mean and estimates of heritability ($h^2$) in the $F_2$ generation for six crosses. (*: sign. at P=0.05; **: sign. at P=0.01; PR: partially resistant; S: susceptible).**

| $P_1$ x $P_2$ | Type | $\bar{F}_1-\bar{P}$ a) | $\bar{F}_2-\bar{P}$ b) | $h^2$ b) |
|---|---|---|---|---|
| 1 x 2 | PRx S | -0.06 n.s. | -0.18 | 0.36 |
| 1 x 3 | PRx S | 0.12 * | -0.09 | 0.51 |
| 1 x 4 | PRx S | 0.19 ** | -0.01 | 0.48 |
| 2 x 3 | S x S | 0.06 n.s. | -0.09 | 0.34 |
| 2 x 4 | S x S | 0.14 ** | -0.07 | 0.28 |
| 3 x 4 | S x S | 0.08 n.s. | -0.03 | 0.14 |

a) obtained in one experiment; b) means of five expts.

The weight of *U.sonchi* on the susceptible parents was about twice as high as that on the resistant parent. Relatively small but significant deviations of the $F_1$ population from the midparent value towards higher susceptibility were found in three crosses. However, all deviations of the $F_2$ were towards higher resistance. Again the importance of dominance effects seem to be limited. The highest estimates of heritability were found in the crosses with the most extreme parents: 1x3 and 1x4. The size of the heritabilities indicates that selection for resistance in these crosses is possible and that preferably a line selection method should be used. Two reasons can be given for the smaller size of the heritabilities compared to those obtained with *M.euphorbiae*. Firstly, the mean residual variation was higher for *U.sonchi* than for *M.euphorbiae* and thus with equal genetic variances heritabilities will be lower for *U.sonchi*. Secondly, the resistant cultivar Charan seems to be less extreme for resistance to *U.sonchi* than for *M.euphorbiae*. The frequency distributions of the $F_2$ populations showed a quantitative inheritance of resistance to *U.sonchi* without detectable major gene effects.

The conclusion for *U.sonchi* is that PR is quantitatively inherited. Again dominance effects seem to be of minor importance. Heritability estimates indicate that with line selection good results can be expected.

## Combined resistance to several aphid species

To summarize: for *N.ribisnigri* a major gene resistance, and for each of the other three leaf aphid species, high levels of quantitatively inherited PR were found. Introduction of the Nr-gene into new lettuce

cultivars is relatively easy and will provide nearly complete resistance to the most common leaf aphid. The prospects of breeding cultivars with combined resistance to several leaf aphid species depend on whether the quantitative resistances are independent of each other. To combine several polygenic resistances will require much time and labour. Obtaining combined resistance will be easier when genes can be found that provide multispecific resistance.

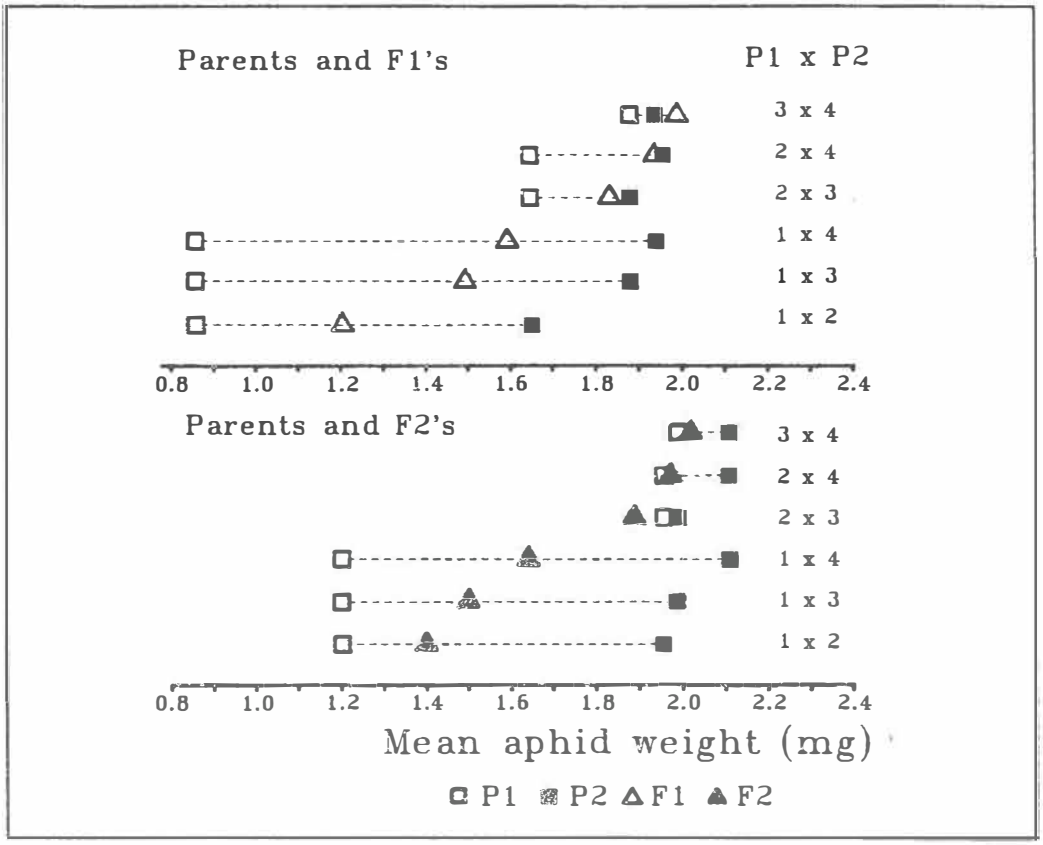



**Figure 3. Mean weight of *Uroleucon sonchi* on parents and $F_1$'s of six crosses tested in one experiment (above) and on parents and $F_2$'s (average values from five experiments, below).**

In segregating $F_2$ populations the Nr-gene, which gives nearly complete resistance to *N.ribisnigri*, also increased the level of PR to *M.persicae* (Reinink & Dieleman, 1989). In contrast the Nr-gene had no effect on the reproduction of *M.euphorbiae* in these experiments. In screening tests lines with the Nr-gene did not show a high level of resistance to *U.sonchi*. Therefore the introduction of the Nr-gene

into modern lettuce cultivars will reduce the problems with *N.ribis-nigri* and *M.persicae*, but will have no effect on *M.euphorbiae* and *U.sonchi*.

Some of the selected lines with an increased level of PR to *M.persicae* also showed high levels of resistance to *M.euphorbiae* and *U.sonchi* (Reinink & Dieleman, in press). On the other hand cultivars of the crisphead type identified as having a high level of PR to *M.persicae* were very susceptible to both *M.euphorbiae* and *U.sonchi*. The lines with high levels of PR to *M.persicae* showed no resistance to *N.ribisnigri* (Reinink & Dieleman, 1989). The results for *M.euphorbiae* and *U.sonchi* show that most of the crisphead cultivars are highly susceptible to both species and many butterhead cultivars have a high level of PR to *M.euphorbiae* and a moderate level of PR to *U.sonchi* (Reinink & Dieleman, in press). However, other cultivars, both of the crisphead and the butterhead type, show a high level of PR to *M.euphorbiae* and high susceptibility to *U.sonchi*. Correlations between resistance scores for both species in the $F_2$ populations that were discussed above were rather low (r=0.22-0.46). Therefore the resistance to *M.euphorbiae* and to *U.sonchi* must at least partly be controlled by different genes.

In conclusion, the monogenic resistance to *N.ribisnigri* also increases the level of resistance to *M.persicae*. The quantitative resistances to the other three leaf aphid species could share some resistance genes because in certain studies the results for *M.persicae* and *M.euphorbiae* or *M.euphorbiae* and *U.sonchi* were correlated. However other genes seem to be specific for only one leaf aphid species. Therefore, to obtain combined resistance it is necessary to test with all leaf aphid species of interest. For practical breeding this limits the possibilities of combining resistance to all aphid species in one genotype. On the other hand, a combination of resistance to *N.ribisnigri* with a high level of PR to *M.persicae* or to *M.euphorbiae* seems feasible regarding the effect of the Nr-gene on *M.persicae* and the availability of modern lettuce cultivars with a very high level of PR to *M.euphorbiae*.

**Résumé**

**L'hérédité de la résistance de la laitue aux pucerons du feuillage**

Une résistance quasi complète à *Nasonovia ribisnigri* est contrôlée par un gène dominant (Nr-gène), qui augmente également le niveau de résistance envers *Myzus persicae*. La résistance partielle aux espèces de pucerons *M.persicae*, *Macrosiphum euphorbiae* et *Uroleucon sonchi* est un charactère quantitatif indiquant une hérédité oligo- ou polygénique. La manifestation de la résistance partielle dépend des conditions d'environnement. La faiblesse ou l'absence d'effets dominants en $F_1$ et $F_2$ indique une hérédité essentiellement additive. Quelques $F_1$ reciproques ont été testés avec *M.persicae* sans mise en évidence d'une hérédité cytoplasmique. Les estimations de l'héritabilité des résistances quantitatives en $F_2$ montrent qu'il existe, pour la pluspart des croissements, de bonnes perspectives de sélection pour une amélioration du niveau de résistance. Les résistances à *M.persicae*, *M.euphorbiae* et *U.sonchi* pourraient avoir quelques gènes communs, mais d'autres gènes semblent être spécifiques à une seule espèce de puceron. La combination de la résistance à *N.ribisnigri* et d'une résistance partielle élevée à *M.persicae* ou à *M.euphorbiae* est envisageable

grâce à l'effet du Nr-gène sur *M.persicae* et à la disponibilité de cultivars modernes de laitue montrant une résistance partielle élevée envers *M.euphorbiae*.

**References**


BLACKMAN R.L. & V.F. EASTOP (1984). Aphids on the Worlds's Crops: An Identification Guide. John Wiley & Sons, Chichester, U.K., 466 pp.

DUNN, J. A. & KEMPTON, D.P.H. (1980). Susceptibility to attack by top aphids in varieties of lettuce. Tests of Agrochemicals and Cultivars, No 1, (Annals of Applied Biology **94**, Supplement) pp. 58-59.

EENINK A.H. & DIELEMAN, F.L. (1977). Screening *Lactuca* for resistance to *Myzus persicae*. Netherlands Journal of Plant Pathology **83**: 139-151.

EENINK A.H. & DIELEMAN F.L. (1983). Inheritance of resistance to the leaf aphid *Nasonovia ribisnigri* in the wild lettuce species *Lactuca virosa*. Euphytica **32**: 691-695.

EENINK A.H., GROENWOLD R. & DIELEMAN, F.L. (1982a). Resistance of lettuce (*Lactuca*) to the leaf aphid *Nasonovia ribisnigri* 1. Transfer of resistance from *L.virosa* to *L.sativa* by inter-specific crosses and selection of resistant breeding lines. Euphytica **31**: 291-300.

EENINK A.H., DIELEMAN F.L. & GROENWOLD, R. (1982b). Resistance of lettuce (*Lactuca*) to the leaf aphid *Nasonovia ribisnigri* 2. Inheritance of the resistance. Euphytica **31**: 301-304.

MENTINK, P.J.M. & J.D. PRINSEN (1987). Bladluizen, elk jaar een probleem. Groenten & Fruit, 12 June 1987, 2pp.

REININK K. & DIELEMAN, F.L. (1989). Comparison of sources of resistance to leaf aphids in lettuce (*Lactuca sativa* L.). Euphytica **40**:, 21-29.

REININK K. & DIELEMAN, F.L. Resistance in lettuce to the leaf aphids *Macrosiphum euphorbiae* and *Uroleucon sonchi*. Ann. Appl. Biology **125**: in press.

REININK K., DIELEMAN F.L. & GROENWOLD, R. (1988). Selection of lines of lettuce (*Lactuca sativa* L.) with a high level of partial resistance to *Myzus persicae* (Sulzer). Euphytica **37**: 241-245.

REININK K., DIELEMAN, F.L., JANSEN J. & MONTENARIE A.M. (1989). Interactions between plant and aphid genotypes in resistance of lettuce to *Myzus persicae* and *Macrosiphum euphorbiae*. Euphytica, **43**: 215-222.

# CHARACTERIZATION OF *MYZUS PERSICAE* BIOTYPES WITH DIFFERENT AGGRESSIVENESS TO LETTUCE.

F.L. DIELEMAN[1] and K. REININK[2]

[1]Department of Entomology, Agricultural University, P.O. Box 8031, 6700 EH Wageningen, The Netherlands. [2]Institute for Horticultural Plant Breeding (IVT), P.O. Box 16, 6700 AA Wageningen, The Netherlands.

## SUMMARY

Morphometric analysis gave a good group separation within a set of glasshouse reared *M. persicae* clones, according to the clone specific aggressiveness to lettuce. The analysis of canonical variates shows also morphological differences within each category. No correlation was found between clone specific esterase pattern and aggressiveness, and the best lettuce adapted clone is the most sensitive to organophosphorus insecticides. In spite of the differences in host plant adaption, the tested clones showed an equal performance on an artificial diet. The conspicuous resistance differences of selected lettuce genotypes could not be realized on leaf discs.

## INTRODUCTION

The green peach aphid, *M. persicae*, has a world-wide distribution and is a pest of many important crops (Mackauer & Way, 1976). Resistance to this polyphagous aphid has been found in the primary host, peach (Massonie & Monet, 1988) and in some of the secondary host plants, sugar beet (Lowe & Singh, 1985), potato (Radcliffe *et al.* , 1988) and lettuce (Reinink *et al.* , 1988).

A field population of *M. persicae* may display a wide range of genetic variation: host plant adaption (Weber, 1986), insecticide resistance (Takada, 1979), temperature adaption and virus transmission (Tamaki *et al.* , 1982). As a result of the complex life cycle and natural or artificial selection, the biotype frequency may change rapidly in space and time. Although theoretically the spring migrants are the preferred morphs to test for host plant resistance (Frazer, 1972), in practice mainly field selected and glasshouse cultured virginoparae are used. Characterization and recognition of biotypes is a prerequisite for obtaining comparable test

results and reliable field evaluation.

A limited set of glasshouse reared biotypes of *M. persicae* has been used in our test procedure and some aphid-plant genotype interaction was found (Reinink *et al.* , 1989). In this paper we present the preliminary results on the discrimination between aphid clones by morphometric methods, esterase isoenzyme pattern, and differences in aggressiveness to some food substrates.

## MORPHOMETRIC VARIATION

Apterous virginoparae of 0.8 - 0.9 mg (exept for clone 8) were selected from a standardized glasshouse culture of *M. persicae* clones on oilseed rape, and slide-mounted. The sample size was 12 aphids per clone and 12 characters were measured per individual adult (Blackman & Paterson, 1986). The morphometric data were analysed by the method of canonical variates (SAS Institute, 1982), and grouped by clone number or category of aggressiveness to lettuce (Fig. 1.).

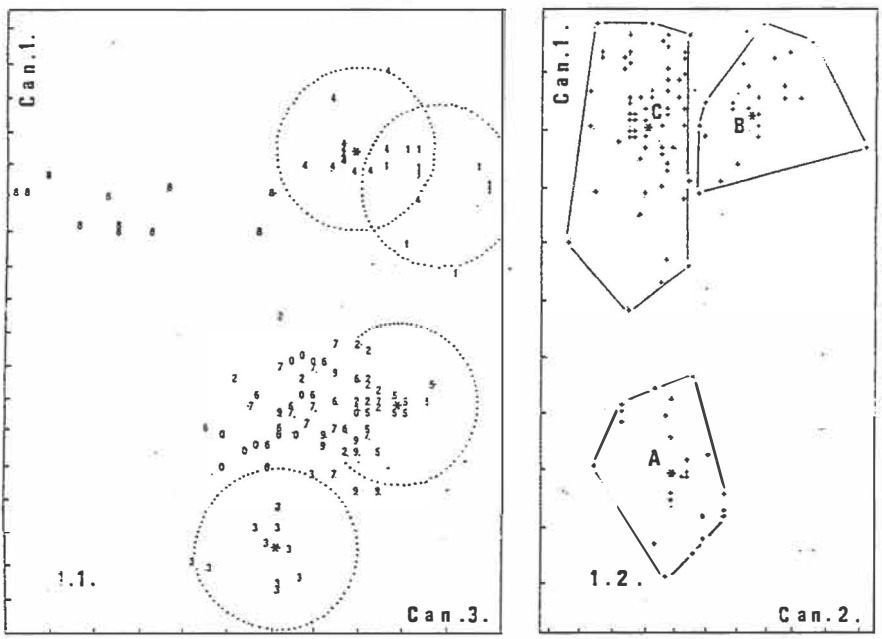



Fig. 1.1. Plot of individual aphids grouped per clone number.

1.2. Plot of individual aphids grouped per category of aggressiveness.

* Group centroids.

Figure 1.1. shows that the most aggressive clones (1 and 4) were rather close but separated from the rest. Clone 3 and 5 are the holocyclic clones, both showing an intermediate aggressiveness to lettuce. Clone 3 especially seems to be morphologically very different from the others. The plot shows a scattered group of clones in the catergory of non-aggressive aphid genotypes. Clone 8 is an androcyclic clone, like clone 4, but non-aggressive and very different from the others in several morphological and biological characters.
Figure 1.2. shows a plot of the same morphometric data but grouped in 3 categories of aggressiveness to lettuce: A = the most aggressive clones (1 and 4), B = the intermediate group (clone 3 and 5) and C = the non-aggressive clones (2, 6, 7, 8, 9, 0). This method discriminates surprinsingly well between the 3 categories, A, B and C. The group separation suggests a morphologically closer relationship within a category than could be concluded from plot 1.1.

The method of canonical variates is a handsome tool to confirm a morphological relationship within and between groups, when the group separation is based on other criteria. A disadvantage is the influence of environmental factors, temperature and host plant, on some morphological characters. This method was successfully used to separate biotypes of *Schizaphis graminum* (Inayatullah *et al.* , 1987), morphs of *Metopolophium dirhodum* (Hand, 1986), and was also used by Blackman (Blackman, 1987; Blackman & Patterson, 1986) to separate *M. antirrhinii* and *M. nicotianae* from the *Myzus* group.

## ESTERASE ISOENZYME PATTERN

For the electrophoretic study of esterase isoenzymes a standard method was used on a 7.5% vertical polyacrylamide gel, with an equivalent of 0.5 - 0.8 aphid per well (Loxdale *et al.*, 1983). Clone 1 can be easily recognized by the low staining intensity of band E 3, 4 (Fig. 2. lane 1). This finding is in agreement with the relative sensitivity of clone 1 to organophosphorus insecticides. Clone 3, 4 and 5 show a very similar isoenzyme pattern but different from the others. All the clones show the E 7 band which is missing in an esterase pattern from *Myzus antirrhinii* (French-Constant *et al.*, 1988). There is no relationship between esterase pattern and clone specific aggressiveness. In general, until now the detected enzyme polymorphism in aphids has been much lower than would be expected from the wide range of variation in biological characters.

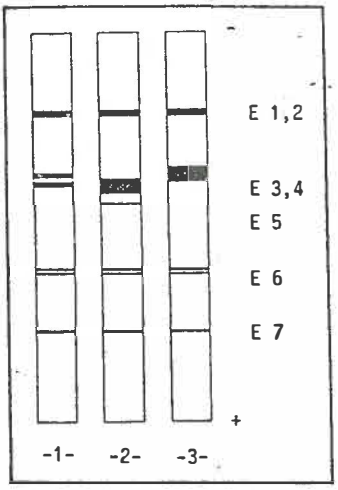



Fig. 2. Esterase isoenzymes
1 = Clone 1, 2 = Clone 3, 4 and 5
3 = Clone 2,6, 7 and 9.

## AGGRESSIVENESS

Using our standard test procedure, newly born larvae were transferred from oilseed rape to the plants or substrates to be tested. The weight of the first generation apterous adults was used as a criterion for biotype performance (Table 1).

Table 1: Adult weight of 6 *M. persicae* biotypes (µg)

| | 1 | 2 | 3 | 4 | 5 | 6 |
|---|---|---|---|---|---|---|
| art. diet | 417 ± 54 | 446 ± 37 | 466 ± 44 | 443 ± 22 | 464 ± 47 | 501 ± 43 |
| oilseed rape | 794 ± 91 | 893 ± 100 | 844 ± 78 | 753 ± 39 | 943 ± 138 | 810 ± 77 |
| tobacco | 553 ± 70 | 702 ± 54 | 642 ± 58 | 537 ± 100 | 664 ± 77 | 574 ± 62 |
| broad bean | 309 ± 75 | 377 ± 74 | 545 ± 95 | 369 ± 60 | 392 ± 43 | 527 ± 87 |
| lettuce - S | 632 ± 31 | 341 ± 91 | 476 ± 106 | 562 ± 66 | 554 ± 124 | 312 ± 86 |
| lettuce - PR | 317 ± 61 | * | 271 ± 69 | 282 ± 98 | 194 ± 54 | * |

S: susceptible; PR: partially resistant; *: 100% mortality

The neutral substrate, an artificial diet (Harrewijn, 1983), did not show clear differences in nutritional requirement between the biotypes. In spite of the weight reduction of about 50% in

comparison to oilseed rape, the adult stage was reached in the same time. The large differences in food plant quality for *M. persicae* are well known, but we found only minor differences between biotype weight per food plant. There is a slight tendency for a lower weight of clone 1 on the tested plants: oilseed rape, tobacco, broad bean, radish, potato and sweet pepper. On lettuce just the reverse was found, which indicates the more specialized nature of clone 1. The adult weight on the lettuce genotypes, S and PR, is in agreement with the separation into categories of aggressiveness.

The use of a test set of selected lettuce genotypes seems to be a valuable and reliable method of recognizing different classes of aggressiveness. The discrimination between clones within a class requires other methods.

## TEST PROCEDURE

The most aggressive clone 1 was found to be also the most reliable test clone. However, the frequency distribution of *M. persicae* clones in field populations, and the variation within and between populations is not fully known. Our standard resistance test, a glasshouse test, is a reliable but not a very valuable test method and for practical purposes it needs some simplification. A bioassay making use of leaf discs instead of whole plants could provide a suitable method. Excised leaflets have been also used in the selection of *M. persicae* resistant potato clones (Sams *et al.*, 1975).

A resistance test on whole plants and corresponding leaf discs was carried out with 3 aphid clones (1, 2 and 3) and 3 lettuce genotypes with differing levels of resistance. The 3 cm diameter leaf discs, with 5 newly born larvae per disc, were kept on moist filter paper. After 4 days they were transferred to fresh leaf discs. The weight of the pre-reproductive apterous adults was used as a criterion for resistance.

Figure 3 shows that the aphid performance on the leaf discs was much better than on the corresponding plants. Also the larval development period on the leaf discs of the partial resistant genotype was shorter and less variable in comparison to whole plants. The striking difference in resistance between the lettuce genotypes shown by whole plants was lost on the leaf discs. The forced senescence of the leaf discs apparently overrules the differences in resistance.

This finding suggests that nutrients and environmental factors affecting the nutritional quality of the plant may play an important role in the expression of *M. persicae* resistance in lettuce.

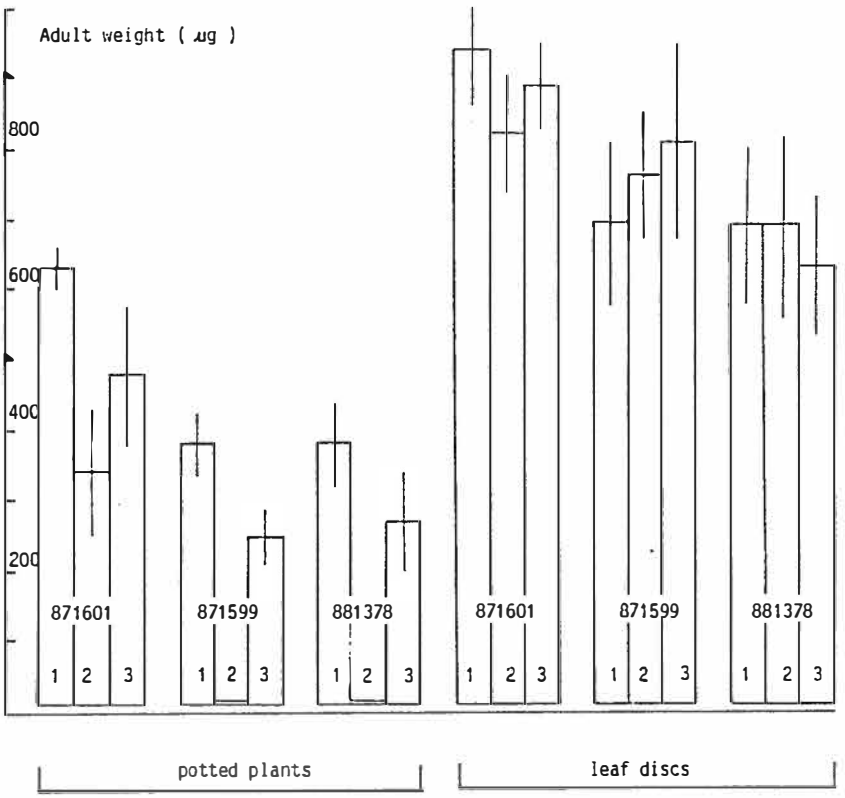



Fig. 3. Adult weight of 3 clones of M. persicae (1, 2 and 3) on 3 lettuce genotypes (871601, 87599, 881378). Comparison of weight on leaves of potted plants and corresponding leaf discs.

## RÉSUMÉ

**Caractérisation des biotypes de *Myzus persicae* montrant divers degrés d'agressivité envers la laitue.**

Des analyses morphométriques ont permis une bonne différenciation en groupes d'une série de clones de *M. persicae* élevés en serre, selon leur "agressivité" spécifique vis-à-vis de la laitue. L'analyse canonique révèle également des différences morphologiques dans chaque catégorie. Aucune corrélation n'a été observée entre les bandes-estérase spécifiques aux clones

et leur agressivité. Le clone le mieux adapté à la laitue s'est révélé être le plus sensible aux esters phosphoriques. Malgré leurs différences dans l'adaption à la plante-hôte, les clones étudiés ont montré des performances indentiques sur milieu artificiel. Les différences marquées de résistance de génotypes de laitue sélectionnés n'ont pu être mises en évidence sur disques foliaires.

Acknowledgements

We are grateful to Theodoor Heijerman for the computer calculations and to Hannenke van Heest for rearing and mouting the aphids.

## REFERENCES

BLACKMAN, R.L. (1987). Morphological discrimination of a tobacco-feeding form from *Myzus persicae* (Sulzer) (Hemiptera: Aphididae), and a key to New World *Myzus* (Nectarosiphon) species. Bull. ent. Res. 77, 713-730.

BLACKMAN, R.L. and PATERSON, A.J.C. (1986). Separation of *Myzus* (Nectarosiphon) *antirrhinii* (Macchiati) from *Myzus* (N.) *persicae* (Sulzer) and related species in Europe (Homoptera: Aphididae). Systematic Entomol. 11, 267-276.

FRENCH-CONSTANT, R.H. BYRNE, F.J., STRIBLEY, M.F. and DEVONSHIRE, A.L. (1988). Rapid identification of the recently recognized *Myzus antirrhinii* (Macchiati) (Hemiptera: Aphididae) by polyacrylamide gel electrophoresis. The Entomologist 107, 20-23.

FRAZER, B.D. (1972). Population dynamics and recognition of biotypes in the pea aphid (Homoptera: Aphididae). Can. Entomol. 104, 1729-1733.

HAND, S.C. (1986). The use of multivariate morphometric methods in the separation alate morphs of the rose-grain aphid, *Metopolophium dirhodum*. Ann. appl. Biol. 109, 19-31.

HARREWIJN, P. (1983). The effect of cultural measures on behaviour and population development of potato aphids and transmission of viruses. Med. Fac. Lanbouww. Rijksuniversiteit Gent 48/3, 791-799.

INAYATULLAH, C., WEBSTER, J.A. and FARGO, W.S. (1987). Morphometric variation in alates of greenbug (Homoptera: Aphididae) biotypes. Ann. Entomol. Soc. Am. 80, 306-311.

LOWE, H.J.B. and SINGH, M. (1985). Glasshouse tests and field selection for heritable resistance to *Myzus persicae* in sugar beet. Ann. appl. Biol. 107, 109-116.

LOXDALE, H.D., CASTAÑERA, P. and BROOKES, C.P. (1983). Electrophoretic study of enzymes from cereal aphid populations. 1. Electrophoretic techniques and staining systems

for characterizing isoenzymes from six species of cereal aphids (Hemiptera: Aphididae). Bull. Ent. Res. 73, 645-657.

MACKAUER, M. and WAY, M.J. (1976). *Myzus persicae* Sulz., an aphid of world importance. In: V.L. Delucchi (ed.), Studies in biological control program 9. Cambridge University Press, Cambridge 51-119.

MASSONIE, G. and MONET, R. (1988). Résistance de pêcher au puceron vert du pêcher, *Myzus persicae* Sulzer. OILB, Bulletin SROP 11/3, 31-32.

RADCLIFFE, E.B. TINGEY, W. GIBSON, R.W. VALENCIA, L. and RAMAN, K.V. (1988). Stability of green peach aphid (Homoptera: Aphididae) resistance in wild potato species. J. Econ. Entomol. 81, 361-367.

REININK, K., DIELEMAN, F.L. and GROENWOLD, R. (1988). Selection of lines of lettuce (Lactuca sativa L.) with a high level of partial resistance to *Myzus persicae* (Sulzer). Euphytica 37, 241-245.

REININK, K., DIELEMAN, F.L., JANSSEN, J. and MONTENARIE, A.M. (1988). Interactions between plant and biotypes in resistance of lettuce to *Myzus persicae* and *Macrosiphum euphorbiae*. Euphytica 43, 215-222.

SAMS, D.W., LAUER, F.I. and RADCLIFFE, E.B. (1975). Excised leaflet test for evaluating resistance to green peach aphid in tuber-bearing Solanum germplasm. J. Econ. Entomol. 68, 607-609.

TAKADA, H. (1979). Esterase variation in Japanes populations of *Myzus persicae* (Sulzer) (Homoptera: Aphididae), with special reference to resistance to organophosphorus insecticides. Appl. Ent. Zool. 14, 245-255.

TAMAKI, G., NAWROCKA, B., FOX, L. ANNIS, B. and GUPTA, R.K. (1982). Comparison of yellow holocyclic and green anholocyclic strains of *Myzus persicae* (Sulzer): Transmission of beet Western Yellows virus. Environ. Entomol. 11, 234-238.

WEBER, G. (1986). Ecological genetics of host plant exploitation in the green peach aphid, *Myzus persicae*. Entomol. exp. appl. 40, 161-168.

# RESISTANCE OF LETTUCE TO THE APHID *NASONOVIA RIBISNIGRI.*

## Are Electrical Penetration Graphs (EPGs) helpful in finding the origin of resistance ?

IR. MAARTEN VAN HELDEN, Department of Entomology, Agricultural University, POBox 8031, 6700 EH Wageningen, The Netherlands.

**ABSTRACT:**

The Electrical Penetration Graphs (EPG) of aphid probing activity by *Nasonovia ribisnigri* on resistant and susceptible lettuce lines showed clear differences in penetration behavior. Aphids seemed to be able to discriminate between resistant and susceptible plants before they reached a sieve element. The aphids were capable of reaching sieve elements in the resistant plants and mechanical barriers did not seem to be involved.

### 1. INTRODUCTION:

EPG recording of aphid penetration activities shows many different waveform patterns some of which have been correlated with the position of the stylet tips in the plant (Tjallingii, 1985, 1990). The different patterns distinguished up till now are: A: the aphid makes electrical contact by penetrating the plant cuticle; B: Salivary sheath formation; C: tissue penetration activities including intracellular punctures (potential drops) and salivary sheath formation; $E_{(pd)}$: Sieve element ingestion (if > 8 min); F: Special type of stylet movement; G: Xylem feeding. Pattern A, B and C often overlap in time and therefore will be grouped together here as pattern ABC. In this study the pattern indication '$E_{(pd)}$ > 8 min' is still used. Recently Tjallingii (1990) described a new pattern and introduced the indication 'E2' for this pattern. At present little is known about the activities of the aphid during penetration before a sieve element is reached (mainly pattern C). It is still unknown whether and (if so) where the aphids sample small volumes of plant sap before the sieve element is reached. On artificial diet a small intake of radioactive diet during pattern ABC was shown by Tjallingii (1978b). During pattern ABC the many intracellular punctures that take place are recorded as potential drops (pd) (Tjallingii, 1985). It is unknown whether these are used for sampling the constituents of the plant. The gustatory receptors of aphids are exclusively located in the pharyngeal cavity (Wensler and Filshie, 1969), so uptake is necessary to sample the plant quality.

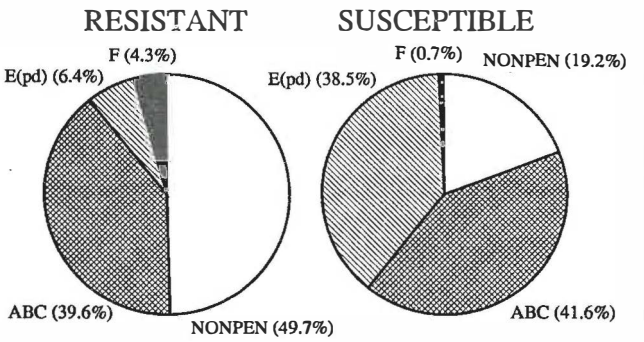



Fig. 1. Summed duration of EPG patterns on resistant (left) and susceptible lettuce lines during 3-hour recordings. ABC= Patterns A,B and C together; E(pd)=E2; F=F; NONPEN= Non penetration time

## 2.MATERIALS AND METHODS:

EPG recordings were performed as described by Tjallingii (1985) with a DC amplifier. Alate females of *Nasonovia ribisnigri* reared on *Lactuca sativa* 'Taiwan' were used for EPG recording. After the gold wire was attached to the aphid it was placed on a 'Taiwan' lettuce plant for at least 3 hours. Then it was transferred to the susceptible or resistant lettuce where the EPG was recorded during the following 3 hours. In total 18 recordings per cultivar were made.

## 3.RESULTS:

Frequencies and summed durations of the EPG patterns differed between resistant and susceptible genotypes (fig. 1). During a 3-hour recording period the number of penetrations was higher on the resistant cultivar (fig 2b.) but most were short penetrations that did not reach a sieve element (fig 2a.). Less aphids reached the phloem on the resistant cultivar (fig 3a.). From access to the plant (start of the EPG recording) it takes more time for aphids to reach a sieve element on the resistant line (fig 3b). Yet, seen from the start of an eventual "successful" penetration, aphids reached the sieve element on the resistant line at least as fast as on the susceptible genotype (fig 3c, only 4 aphids reached a sieve element on the resistant line). The EPGs were too short to show clear dif-

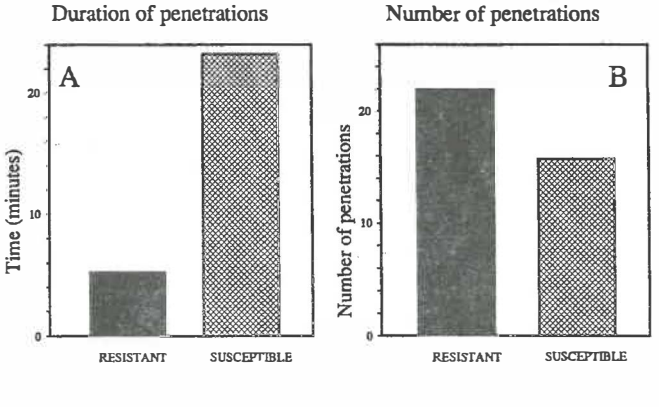



Fig. 2. Mean duration (a) and mean number (b) of penetrations during 3-hour recordings on resistant and susceptible lettuce

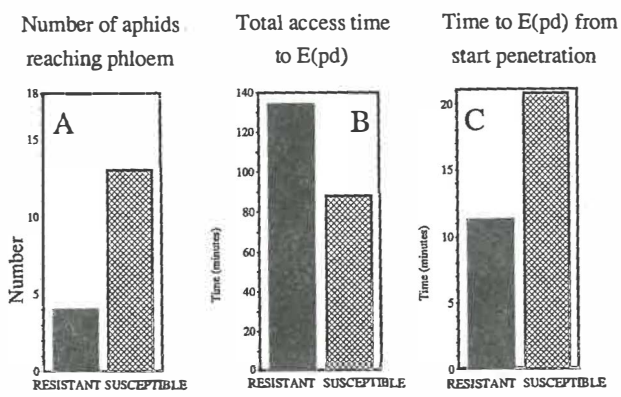



Fig 3. A. Number of aphids reaching a sieve element (= showing E(pd)) during recording; B. mean time needed to reach sieve element from start of experiment; and (C) from beginning of the penetration in which sieve element is reached.

ferences in the duration and/or frequency of the 'E(pd)>8 min' pattern (phloem feeding, Tjallingii, 1985): few aphids reached a sieve element on the resistant line and most of these E(pd) patterns occurred late in the recording period and were ended by the end of the 3-hour recording period.

## 4. CONCLUSIONS:

*Nasonovia ribisnigri* is able to discriminate between resistant and susceptible lettuce genotypes from clues perceived before a sieve element is reached. These differences are probably not caused by mechanical barriers (e.g. difference in pectin methylation, Dreyer and Campbell, 1984) since the sieve elements can be reached equally fast on both lines. Longer EPGs are needed to see if there is a difference in the duration of phloem ingestion (E(pd)). This would suggest the presence of a resistance factor in the phloem sap. Experiments should focus on correlating wave form features during the ABC pattern with the exact position of the stylet tips in the plant tissue and the possible intake of small volumes of sap which can be tasted by the pharyngeal chemoreceptors. Which cells are punctured by the aphid, does it ingest sap from these cells? Simultaneous recordings of EPGs and myograms of the food-pump muscles and/or radio-isotope experiments will be tried to get more information. When a chemical substance correlated with resistance can be found, the position of this substance in the plant can provide new clues on aphid behavior. Other causes of resistance (plant volatiles or non-chemical stimuli) still can not be excluded.

**RÉSUMÉ:**

**La résistance de la laitue (*Lactuca sativa*) au puceron *Nasonovia ribisnigri*. Les enregistrements électriques de pénétration sont-ils utiles pour découvrir l'origine de la résistance ?**

Les enregistrements électriques de pénétration lors des piqûres de sondage du puceron *Nasonovia ribisnigri* montrent clairement des différences de comportement de pénétration entre des lignées de laitue resistantes et sensibles. Le puceron semble capable de distinguer entre les plantes résistantes et sensible avant de parvenir au phloème. Cependant, ce puceron est apte à atteindre le phloème des plantes résistantes, et des barrières mécaniques ne semblent donc pas être impliquées.

**LITERATURE:**

DREYER, D,L. & B.C. CAMPBELL (1984). Association of the degree of methylation of intercellular pectin with plant resistance to aphids and with induction of aphid biotypes. Experienta 40: 224-226.

TJALLINGII, W.F. (1978a): Mechanoreceptors of the aphid labium. Entomol. exp. appl. 24.: 731-737.

TJALLINGII, W.F. (1978b): Electronic recording of penetration behavior by aphids. Entomol. exp. appl 24.: 721-730 .

TJALLINGII, W.F. (1985): Stylet penetration activities by aphids. Thesis Agricultural University Wageningen, The Netherlands.

TJALLINGII, W.F. (1990): Continuous recording of stylet penetration activities by aphids. XVIII international congress of Entomology. Proceedings symposium "Mechanisms of Aphid-Plant Genotype interactions". In press.

WENSLER, R.J. & B.K. FILSHIE (1969): Gustatory sense organs in the food canal of aphids. J. morph.129: 473-492.

## Workshop 3

## PROTECTED and OUTDOOR VEGETABLE CROPS: CUCURBITS, TOMATOES and SWEET POTATOES

# INVOLVEMENT OF VOLATILE AND NON-VOLATILE CHEMICAL FACTORS IN CUCURBITS IN OVIPOSITION HOST SELECTION OF THE PICKLEWORM MOTH, DIAPHANIA NITIDALIS (STOLL.) (LEPIDOPTERA:PYRALIDAE)

J. K. PETERSON and K. D. ELSEY
U. S. Department of Agriculture, ARS, U. S. Vegetable Laboratory,
2875 Savannah Highway, Charleston, SC 29414, USA

Summary
Studies with caged gravid females of the pickleworm showed that leaves of yellow squash contain chemicals which stimulate egglaying. Small (MW <500), non-volatile, highly polar and water-soluble compounds obtained via various separatory techniques were responsible for 'in vitro' egglaying. At least 5 such compounds were present. Addition of volatile compounds from whole leaves increased oviposition further. The volatiles extracted from whole leaves could be substituted by volatiles present in leaf glandular trichome exudates.

## 1. Introduction

The pickleworm is an important pest of cucurbit vegetables in the southeastern United States, in particular since the larvae feed on the fruits. Among cucurbit species, as well as varieties and cultivars, large variations exist in levels of infestations in the field (Corley, 1973; Day et al.. 1961; Pulliam, 1979; Quisumbing & Lower, 1975). Screening for antibiosis to the larvae showed little, if any, resistance to feeding (Wehner et al., 1985). However, resistance to oviposition exists. For example, glabrous mutants in both muskmelon and cucumber were non-preferred hosts (Day et al., 1961; Elsey & Wann, 1982). Apparently, resistance in these cases was due to lack of plant hairs, since near-isogenic lines of glabrous and pubescent cucumber plants were used in these tests (Elsey & Wann, 1982). Subsequently, it was shown that the mechanism of resistance to the pickleworm in Cucurbita moschata species was oviposition non-preference (Elsey, 1985). 'In vitro' studies using ethanol extracts of cucurbit leaves sprayed on glasswool pads resulted in oviposition. Preference was shown in the order: squash, cucumber and watermelon (Elsey & McFadden, 1981). Since chemical factors were suspected to be involved in oviposition preference, studies were undertaken to isolate these stimulants. Yellow squash leaves were used since these provide most preferred oviposition sites.

## 2. Materials and Methods

Extraction of squash leaves: Recently expanded leaves of yellow squash (cultivar Early Straightneck Yellow Summer Squash), approximately 2/3 of full size, were removed from the plant, cut in pieces and homogenized in hot 95% ethanol. After one repetition, the combined extracts were filtered through paper depth filters under pressure in a stirring cell. The resulting filtrate was condensed to a small volume under vacuum and water was added. The

resulting extract was partitioned against hexane (3X) to remove lipoidal material including volatile components. Further partitionings were performed against dichloromethane and ethyl acetate, respectively.

The aqueous fraction was filtered through a series of molecular surface membrane filters; the smallest pore size having a nominal molecular weight cut-off of 500.

Molecular filtration was followed by low pressure column chromatography using polyamide as the stationary phase. Eluents were: water, 100% methanol and methanol:formic acid--995.5. Elution was monitored at 280 nm.

The most active fraction from the polyamide column (water fraction) was applied to sheets of Whatman 3 MM (46 x 57 cm) and developed in the descending mode using tert-butanol:acetic aid:water--3:1:1. After the sheets were dried, the sides were sprayed with ninhydrin and 12 bands were distinguishable. The bands were extracted and bioassayed.

Thin-layer chromatography was carried out on various types of plates, but mostly on silica gel. The best performing solvent systems for silica gel were: n-butanol:acetic acid:ether:$H_2O$-9:6:3:1 (system 1) and iso-propanol:butanone:acetone:methanol:water:ammonia-40:20:20:1:14:5 (system 2).

Anion exchange chromatraphy was performed using a mixture of primary and secondary amines ($NH/NH_2$ solid phase extraction material). Eluting solutions: 50% ethanol, water; and 0.5 N, 2.0 N, 10 N and 20 N formic acid.

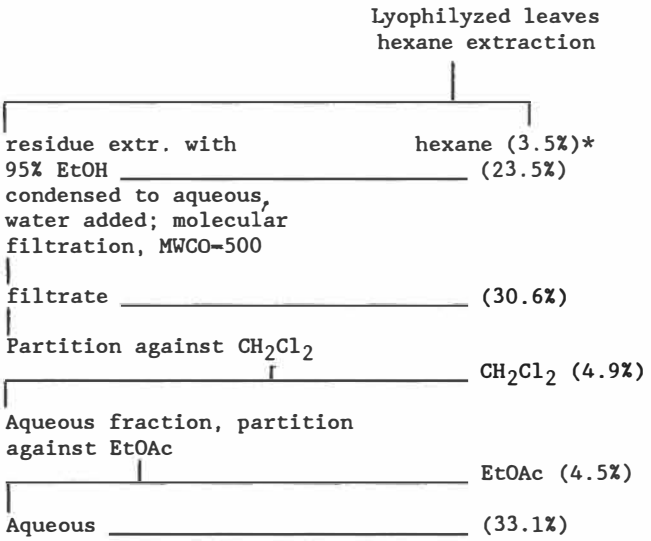
The active polyamid fraction (water fraction) was hydrolyzed in 6 N HCl at 95C for 6 hrs, the precipitate was centrifuged and the supernatant was bioassayed after removal of the HCl.

Bioassays: Pads of building insulation glasswool were used as a substrate for oviposition. Circular pads (diameter = 9 cm, thickness = 2-3 cm) were either sprayed with testing material or dipped in solutions. After the solvents were completely evaporated, the pads were suspended from the top of wire screen cages (120 x 90 x 90 cm), which contained ca. 50 pairs of 3-day old moths. The cages were located in a dark, humidified room. Testing material was placed in the cages in the late afternoon and removed the next morning after which the eggs were counted. Volatiles were tested in various ways; initially airstreams directed via whole squash plants into the glass pads, volatiles desorbed from resinous adsorption materials or small whole leaves sandwiched between glasswool pads gave good results. However, resinous material obtained from glandular trichomes of squash leaves and sandwiched between pads gave similar results. Data are presented using the last mentioned approach.

## 3. Results and Discussion

After extraction and partitioning procedures, as shown in Figure 1, all activity was retained in the aqueous fraction. When the last aqueous fraction was passed through a series of molecular filters, the activity of the filtrates increased after successive filtrations (Table 1). However, the activity of the supernatants increased as well. Although no simple explanation is possible, the results show that most, if not all, active molecules have a nominal molecular weight below 500.

Fig. 1. Bioassays of various fractions obtained by extracting and partitioning.




*Average percentage of total egglaying and corrected for "background".

Table 1. Bioassays of fractions obtained by filtration through membrane molecular filters.

| | | |
|---|---|---|
| Prefiltrate, aqueous | 600* | |
| Filter (MWCO) | Supernatant | Filtrate |
| 20 K | 250 | 863 |
| 5 K | 399 | 1225 |
| 1 K | 502 | 1750 |
| 500 | --- | 800 |

*Average number of eggs per pad (6 experiments).

The results from the polyamide chromatography are listed in Table 2. The activity in the aqueous fraction was higher than in the pre-column matrix; the other fractions had negligible activity. Further fractionation of the aqueous eluent showed that all activity eluted in the first 240 ml. Much activity appeared before the absorption at 280 nm raised above base line.

Table 2. Polyamide column chromatography monitored at 280 nm.

| Eluents | Volume (ml) | Activity* |
|---|---|---|
| $H_2O$ | 1320 | 120 |
| MeOH | 548 | 21 |
| MeOH/HCOOH-995/5 | 560 | 14 |

*Percent activity relative to pre-column matrix; numbers represent averages of 4 bioassays.

Table 3. Paper chromatography and bioassay results of the water fraction derived from a polyamide column.

| Band | Rf | $\bar{x}$ % eggs rel. to control* |
|---|---|---|
| 1 | 0 - 11.1 | 24 |
| 2 | 11.1 - 19.5 | 24 |
| 3 | 19.5 - 26.5 | 20 |
| 4 | 26.5 - 34.7 | 54 |
| 5 | 34.7 - 40.5 | 30 |
| 6 | 40.5 - 47.9 | 43 |
| 7 | 47.9 - 50.8 | 36 |
| 8 | 50.8 - 57.7 | 31 |
| 9 | 57.7 - 63.8 | 25 |
| 10 | 63.8 - 68.3 | 35 |
| 11 | 68.3 - 76.2 | 28 |
| 12 | 76.2 - 100 | 59 |
| Controls | --- | 100 |
| Recombined | --- | 227 |
| Blanks | --- | 15 |

*Average of 4 independent bioassays, material derived from 4 chromatograms.

Paper chromatograms of the aqueous fraction derived from polyamide column chromatography were sprayed with ninhydrin, on strips of 2 cm wide on both sides of the chromatograms. Colors were various hues and intensities of blue, purple and pink, and no yellow color was observed. Twelve distinguishable bands were cut, extracted with 50% ethanol and bioassayed. All bands were assayed with 0.5 g DW equivalent (i.e. material derived from 0.5 g DW leaf material). Also tested was material of all bands recombined at 0.5 g DW equivalent each. Controls were 0.25 g DW equivalent of the aqueous polyamide fraction. Blanks were pads that received 50% ethanol only. All treatments were present in one cage, the assay was repeated four times and results were averaged. Activity was spread all over the chromatograms, however, many bands were not much above background activity. Only the most active regions were further separated.

Numerous thinlayer chromatography (TLC) efforts were undertaken using cellulose, silica and C-18 plates. For brevity, only one of such efforts is reported here. Band number twelve of the paper chromatograms was used on silica plates (most active region) and developed with system 1. One active region was found which had migrated very close to the solvent front. From various such efforts, 5 fairly clean compounds were obtained. Bioassay results from TLC bands showed that apparently single compounds show little activity; full activity could be recovered however when several TLC bands were recombined.

Results of ion exchange chromatography of the aqueous fraction eluted from the polyamide column are shown in Table 4.

Table 4. $NH/NH_2$ ion exchange chromatography of the aqueous fraction of the polyamide column.

| Eluent | Volume (ml) | Number eggs (% control) |
|---|---|---|
| 50% EtOH | 65 | 34 |
| $H_2O$ | 65 | 26 |
| 0.5 N HCOOH | 83 | 46 |
| 2.0 N HCOOH | 385 | 11 |
| 10 N HCOOH | 160 | 38 |
| 20 N HCOOH | 104 | 13 |

The large fraction eluted with 2.0 N formic acid showed no significant activity, but was the only fraction with high absorbence at 280 nm. No strongly ionic material was present.

Hydrolysis of the aqeuous polyamide fraction resulted in approximately equal or some cases higher activity than the non-hydrolyzed material.

Trichome exudates sandwiched between glasswool pads increased egglaying by an average of 50%. It is not suspected that these volatiles are oviposition stimulants, but rather aid the moths in finding host plants at night. Our bioassays do not distinguish between these possibilities.

## 4. Conclusions

Stimulation of oviposition by the pickleworm moth [Diaphania nitidalis (Stoll.)] on leaves of yellow squash (Cucurbita pepo L.) is caused by chemical factors. At least five compounds were present, which gave some stimulation individually. However, oviposition at high levels required a combination of these compounds. The molecules are non-volatile, highly polar, soluble in water and can carry a charge. The compounds were stable, and strong hydrolysis did not change activity. Their molecular weights are below 500.

Addition of volatile compounds, derived from glandular trichome exudates, increased oviposition considerably. It is assumed that the volatiles are used 'in vivo' to locate host plants rather than serve as oviposition stimulants since the insects oviposit at night. Our bioassays do not distinguish between these possibilities. It is hypothesized that oviposition preference by the pickleworm moth is based on the presence of both volatiles and the described polar molecules in leaves of host cucurbits.

Résumé

Le rôle des facteurs chimiques volatiles et non volatiles chez les cucurbitacées sur le choix de l'hôte pour la ponte par la pyrale du concombre, Diaphania nitidalis (STOLL.) (LEPIDOPTERA: PYRALIDAE)

Des expériences en cages avec des femelles gravides de la pyrale du concombre ont montrè que les feuilles de la courge jaune contiennent des substances stimulant la ponte. Des substances légères (P.M. < 500) non volatiles, fortement polaires, solubles à l'eau et obtenues par diverses techniques de séparation sont responsables de la ponte "in vitro". Au moins 5 composés ont été mis en évidence. En y ajoutant des composés volatiles provenant de feuilles entières, la ponte est augmentée. Ces composés volatiles de feuilles entiéres peuvent être remplacés par des volatiles provenant d'exsudats de trichomes glandulaires des feuilles.

## 5. References

1. CORLEY, W. L., 1973. Response of muskmelon botanical varieties to pickleworm infestation. HortScience 13:286-287.

2. DAY, A., NUGENT, P. E. & ROBINSON, J. F., 1961. Resistance of squash varieties to the pickleworm and the value of resistance to insecticide control. J. Econ. Entomol. 54:1191-1197.

3. ELSEY, K. D., 1985. Resistance mechanisms in Cucurbita moschata to pickleworm and melonworm (Lepidoptera: Pyralidae). J. Econ. Entomol. 78:1048-1051.

4. ELSEY, K. D. & MCFADDEN, T. L., 1981. Pickleworm: Oviposition on an artificial surface treated with cucurbit extract. J. Econ. Entomol. 74:473-474.

5. ELSEY, K. D. & WANN, E. V., 1982. Differences in infestation of pubescent and glabrous forms of cucumber by pickleworms and melonworms. HortScience 17:253-254.

6. PULLIAM, T. L., 1979. The effect of plant types and morphology of the cucumber, Cucumis sativus L., on infestation by the pickleworm, Diaphania nitidalis (Stoll.). M.S. Thesis, North Carolina State University.

7. QUISUMBING, A. R. & LOWER, R. L., 1975. Screening cucumber cultivars for pickleworm resistance. HortScience 10:147. (Abstr.).

8. WEHNER, T. C., ELSEY, K. D., & KENNEDY, G.G., 1985. Screening for antibiosis to pickleworm. HortScience 20:1117-1119.

# DEVELOPMENT OF A METHOD TO TEST RESISTANCE TO WESTERN FLOWER THRIPS (FRANKLINIELLA OCCIDENTALIS) IN CUCUMBER.

CHRIS MOLLEMA[1], GREET STEENHUIS[1] AND PAUL VAN RIJN[2]
1. Institute for Horticultural Plant Breeding (I.V.T.),
P.O.-Box 16, 6700 AA WAGENINGEN, THE NETHERLANDS.
2. University of Amsterdam, Dept. of Pure and Applied Ecology,
Section Population Biology, Kruislaan 302, 1098 SM AMSTERDAM.

## Summary
Western flower thrips (WFT) is the most important pest of many glasshouse crops in the U.S.A., Canada and Europe. Chemical and biological control of this past are difficult in both vegetable and ornamental crops, particularly in cucumber. Therefore, breeding of resistant varieties may provide a solution to this problem. For this purpose more knowledge about the biology of the WFT is required. In a preliminary investigation a test method was developed based on characters describing population growth of WFT. This method will be used to search for plants on which population growth is completely or partially reduced.

## Introduction

### 1. Status of the pest.
Since the early nineteen eighties the western flower thrips (Frankliniella occidentalis) has been a major problem in glasshouses. In different parts of the world (U.S.A., Canada and Europe) this pest damages many vegetable as well as ornamental crops. The spectrum of host plants of western flower thrips (WFT) is very wide and it has been found on 219 species from 59 genera (Brødsgaard, 1989). Although WFT prefers flowers, it also feeds on leaves and stems. As WFT is a vector for tomato spotted wilt virus (TSWV), host plant losses may result from the direct effects of feeding, or by infection by the virus (Robb, 1989). Amongst vegetable crop species, cucumber (Cucumis sativus) is threatened most, because chemical and biological control in this crop often fail.
Chemical control of WFT is difficult because of its hiding behaviour and its resistance to many pesticides. The most effective insecticide Dichlorvos is phytotoxic, resulting in damage to leaves and to fruit abortion (Mantel & van de Vrie, 1988). Moreover, chemical control disturbs the biological control of spider mites (Tetranychus urticae), glasshouse whiteflies (Trialeurodes vaporariorum) and leafminers (Liriomyza sp.). The predatory mites Amblyseius cucumeris or Neoseiulus barkeri are used for the biological control of WFT. However, pollen is probably needed to sustain these predator populations, and this is not present in parthenocarpic cucumber. Hence these mites have to be introduced repeatedly in large numbers. Other biological control methods are still in a developmental stage e.g. the use of insect pathogenic fungi or predatory Orius species (Brodsgaard, 1989). The problem of WFT on cucumber could also be solved by the use of resistant varieties. Therefore, a project was started to develop a method to test the resistance to WFT in this crop.

### 2. Population growth of pest insects.
The development of the test method was based on a knowledge of population growth of pest insects. Using this strategy, it is assumed that host plant resistance results in reduced values for the population growth of the insects. In general,

population growth of pest insects is based on three life history parameters: generation time, reproduction and mortality (Birch, 1948; Krebs, 1982). To find a resistant host plant genotype, each of these parameters has to be measured. Unless the existence of an absolute resistant genotype can be demonstrated, the resistance will be expressed by adverse values for these parameters. The relative impact on unlimited population growth of prolongation of the generation time, decrease of reproduction or increase of mortality, has been demonstrated (Lewontin, 1965; Hulspas-Jordaan & van Lenteren, 1989). Decisions depend on the genetic variation in the host plant genotypes concerning such changes, and the calculated impact on population growth. Only then can the most appropriate parameter(s) be selected for further development of a test method. The final test method must be suitable for a breeding programme.

3. The lack of knowledge about WFT.

Although WFT has been one of the major insect pests of the last decade, very little is known about its biology (Mantel, 1989). The life cycle of WFT can be summarized as follows: an egg stage (inside the plant tissue, not visible), two larval stages (L1 and L2; both feeding), two pseudopupal stages (Prepupa and Pupa; both not feeding). The adults emerge from the pupae. Since WFT is a haplo-diploid organism (females are diploid; males are haploid), unfertilized females produce (male) offspring.

There are a few life history studies concerning WFT, but none of them relates to cucumber. Recently, Trichilo & Leigh (1988) reported reduced reproductive fitness of WFT on a cotton variety with resistance to the spider mite T.urticae. The manipulation of WFT is difficult, as these insects are small (adults are 1.1 mm), very mobile and flexible, and escape very easily from experimental arenas. Therefore, some orienting experiments were needed to learn how WFT could be handled best and how a reliable mass-rearing could be established. The main purpose of this study was to design experiments by which the important characters mentioned above could be measured on cucumber.

## Methods and results

The mass-rearing of WFT.

Mass-rearing of WFT was developed on flowering cucumber plants. Since these plants must not be treated with any chemicals, a susceptible variety ("Autumn Green") with resistance to powdery mildew was used. The mass-rearing was situated in an isolated greenhouse. The heavily infested plants were put into a cage to prevent invasion by other pest insects. The WFT was collected in a glass tube by an aspirator.

The measurement of the developmental period.

To measure the WFT parameters, it is necessary that the thrips are confined to a part of the plant from which they could not escape. The methods developed for the two-spotted spider mite (T.urticae) (de Ponti, 1977) were not suitable, because WFT escaped very easily from leaf cages and floating leaf discs in petri dishes. Therefore, leaf discs (1.5 cm diameter) were used, which were laid upside down on a little tap water in a well of a tissue culture plate. To prevent escape of the egg laying females, the multiwell was covered by a lid from which the ventilation rests were removed. By the time the second larval stage was reached, this lid was replaced by a thin (14 µm) transparent plastic film, as this stage in particular frequently tries to escape. The multiwells were placed in an incubator maintained at 25°C, 60 % R.H. and a 16:8 (L:D) photoperiod. In an

experiment in which the influence of the temperature on the developmental period was studied, the multiwells were placed at 12°C, 15°C, 20°C, 25°C, 30°C, 32.5°C or 35°C respectively. The leaf discs were observed by transmitted light using a magnification of 20 times.
To determine the duration of the different developmental stages, five WFT females (collected randomly from the culture) per leaf disc were allowed to lay eggs for a period of five hours. After this period the females were removed carefully. Two to four days later, the eggs hatched and the number of L1 larvae was counted. The L1 larvae were isolated on new leaf discs. These discs were observed daily and the moments of transition to later stages were recorded. The latter events were distinguished by the remnants of cuticles which were deposited on the surface of the leaf disc. As soon as the L2 stage was reached, the larvae were transferred to new leaf discs. During the L2 stage, these discs were refreshed once more. The experiment was continued until the adults emerged.
On the susceptible cucumber variety "Corona" at 25°C the following values were measured:

| stage | duration in days |
|---|---|
| Egg stage | 2.7 |
| L1 stage | 2.4 |
| L2 stage | 5.0 |
| Prepupal stage | 1.1 |
| Pupal stage | 2.2 |
| Egg to adult | 13.4 |
| Egg to egg | 15.1 |

The optimal temperature for WFT development is 30°C. At 35°C none of the eggs hatched. The pre-adult survival at 25°C was 75 %.

The measurement of reproduction.
To measure the reproduction, late L2 larvae were collected from the culture and kept on leaf discs until emergence. The young females were isolated on new leaf discs each together with one male. After one day, the males were removed and the females were transferred to new leaf discs. The leaf discs were replaced daily and the number of hatched larvae per disc was recorded until reproduction stopped.
From the second till the fourth day after emergence, the WFT females produced on average 4.5 eggs per day. The life history parameters recorded, resulted in a calculated intrinsic rate of population increase of 0.15 per day, implying that at 25°C the WFT populations doubled in 4.5 days.

## Discussion and conclusion

Although Robb (1989) found some survival even at 35°C, the observed optimal temperature is in agreement with her results. Because of the sharp decline of the rate of development, and the increased mortality at higher temperatures, the measurements can be carried out most satisfactorily at 25°C.
In conclusion, the method developed can be used for measurements of WFT parameters important for their population increase. When compared to values found

on a standard susceptible host plant genotype, each parameter enables us to express resistance in different genotypes. The success of this method depends on the possibility of maintaining leaf discs in multiwells and whether such a system can be repeated and is consistent with whole plants in vivo.


## Résumé

**Développement d'une méthode pour tester la résistance au thrips de Californie (Frankliniella occidentalis) chez le concombre**

Le thrips de Californie est le ravageur le plus important sur de nombreuses cultures sous serre aux E.U., au Canada et en Europe. La lutte chimique et biologique contre ce ravageur est difficile aussi bien dans les cultures légumières qu'ornementales, et particulièrement sur le concombre. Aussi, la sélection de variétés résistantes pourrait apporter une solution à ce problème. A cet effet, l'amélioration des connaissances sur la biologie du thrips de Californie est nécessaire. Dans une phase de recherche préliminaire, une méthode expérimentale a été développée prenant pour base les caractéristiques de la croissance de la population du thrips. Elle sera utilisée pour détecter les plantes sur lesquelles cette croissance est réduite ou annihilée.


## References


Birch, L.C., 1948. The intrinsic rate of natural increase of an insect population. J. Anim. Ecol. 17, 15-26.

Brødsgaard, H.F., 1989. Frankliniella occidentalis (Thysanoptera; Thripidae) a new pest in Danish glasshouses. A review. Tidsskr. Planteavl 93, 83-91.

Hulspas-Jordaan, P.M. & van Lenteren J.C., (1989). The parasite-host relationship between Encarsia formosa (Hymenoptera: Aphelinidae) and Trialeurodes vaporariorum (Homoptera: Aleyrodidae). XXX. Modelling population growth of greenhouse whitefly on tomato. Wageningen Agricultural University Papers, 89.2, 1-54.

Krebs, C.J., 1982. Ecology. The experimental analysis of distribution and abundance. Harper & Row, New York. 678 pp.

Lewontin, R.C., 1965. Selection for colonizing ability. In: H.G. Baker & G.L. Stebbins (eds.), The genetics of colonizing species. Academic Press, New York. pp. 77-94.

Mantel, W.P. & van de Vrie M., 1988. The Western Flower Thrips, Frankliniella occidentalis, a new thrips species causing damage in protected cultures in the Netherlands. Ent. Ber., Amst. 48, 140-144.

Mantel, W.P., 1989. Bibliography of the western flower thrips Frankliniella occidentalis (Pergande) (Thysanoptera: Thripidae). IOBC/WPRS Bull. XII/3, 29-66.

Ponti, O.M.B. De, 1977. Resistance in Cucumis sativus L. to Tetranychus urticae Koch. 2. Designing a reliable laboratory test for resistance based on aspects of the host-parasite relationship. Euphytica 26, 641-654.

Robb, K.L., 1989. Analysis of Frankliniella occidentalis (Pergande) as a pest of floricultural crops in California greenhouses. Ph.D.-Thesis University of California, Riverside. 135 pp.

Trichilo, P.J. & Leigh T.F., 1988. Influence of resource quality on the reproductive fitness of flower thrips (Thysanoptera: Thripidae). Ann. Entomol. Soc. Am. 81, 64-70.

# INVESTIGATION OF THE RESISTANCE IN TOMATO TO THE LEAFMINER LIRIOMYZA TRIFOLII.

CHRIS MOLLEMA & MAAIKE VAN DER SCHOOT
Institute for Horticultural Plant Breeding (IVT),
P.O.-Box 16, 6700 AA WAGENINGEN, THE NETHERLANDS.

## Summary

The leafminer Liriomyza trifolii is an important pest of tomato. For several reasons, the development of resistant varieties is desirable. Therefore, certain components of resistance to this leafminer were investigated in about 50 Lycopersicon accessions. A test method was developed, based on models for the population growth of pest insects. Some accessions of L.hirsutum showed complete resistance, while one accession of L.cheesmanii had a high level of partial resistance.

## Introduction

For approximately 10 years the Florida leafminer Liriomyza trifolii has been an important pest in Dutch greenhouses (Minkenberg & van Lenteren, 1986). As a result of its resistance to many pesticides, chemical control of this insect is difficult. Although natural parasitism by Hymenopterous parasites occurs frequently, the impact on the increase of the leafminer populations is limited. Hence, monitoring of the pest is needed and, if necessary, extra (commercially produced) parasites must be introduced. Such parasites are expensive and this procedure is time-consuming, so biological control is not widely practiced yet. The most effective way of controlling L.trifolii at present is complete disinfection of the empty greenhouse before the start of a new crop. However, in the near future the chemicals used for disinfection may be prohibited in The Netherlands. Therefore another solution to the problem is required. This may be provided by the introduction of resistant varieties.
It is assumed that the population growth of the leafminer is completely or partially reduced on a resistant tomato variety. Reduction of an insect population growth is the result of the derangement of important life-history component(s). According to models describing the unlimited increase of insect populations, the three most important components are: the developmental period, reproduction and survival (Lewontin, 1965; Hulspas-Jordaan & van Lenteren, 1989). Since it is unknown which of these components of leafminer life history are varying on Lycopersicon accessions, they should all be measured. The main purpose of this study was to determine the genetic variation in some Lycopersicon accessions concerning L.trifolii components that have a negative affect on their population growth. Decisions depend on the extent of such genetic variation. Only then can the most suitable component be selected for further development of a method for a breeding programme.

## Methods and results

The leafminers were reared on the susceptible cultivar Moneymaker. The experiments were carried out in a greenhouse maintained at 17 - 22°C and 16 hours photophase. For each tomato accession six replicates were used.

| genotype | average # small mines | average larval survival in % |
|---|---|---|
| **Group A** | | |
| Moneymaker | 20 | 95 |
| Sonatine | 22 | 79 |
| Pando | 22 | 93 |
| L.esc. PI124036 | 17 | 100 |
| L.esc. PI124036 | 40 | 79 |
| L.esc. PI140403 | 23 | 84 |
| L.esc. PI140403 | 22 | 63 |
| L.hirs. G1.1560 | 1 a | 0 a |
| L.hirs. G1.1290 | 1 a | 0 a |
| L.hirs. PI126445 | 1 a | 0 a |
| L.hirs. PI127826 | 1 a | 0 a |
| L.hirs. glabr. PI134417 | 0 a | - a |
| L.pimp. G1.1077 | 7 | 73 |
| L.pimp. G1.562 | 18 | 100 |
| L.pimp. G1.564 | 10 | 63 |
| L.cheesm. PI266375 | 11 | 69 |
| L.cheesm. LA1448 | 4 a | 50 |
| L.cheesm. LA1448 | 19 | 18 a |
| L.cheesm. PI266875 | 14 | 71 |
| L.esc. PI120272 | 24 | 76 |
| L.esc. PI128230 | 7 | 89 |
| **Group B** | | |
| Moneymaker | 12 | 100 |
| Sonatine | 14 | 96 |
| Pando | 17 | 96 |
| L.hirs. PI126445 | 5 a | 4 a |
| L.hirs. PI308182 | 0 a | 0 a |
| L.hirs. PI308182 | 1 a | 0 a |
| L.hirs. PI127826 | 0 a | 0 a |
| L.hirs. PI127827 | 3 a | 83 |
| L.hirs. G1.1257 | 0 a | - a |
| L.hirs. G1.1297 | 0 a | - a |
| L.hirs. glabr. G1.1561 | 1 a | 25 a |
| L.hirs. glabr. G1.1562 | 3 a | 33 a |

Table I.
Average number of small mines and larval survival of L.trifolii on Lycopersicon accessions. Moneymaker, Sonatine and Pando were used as susceptible controls of L.esculentum. The accessions were evaluated in two groups: "A" and "B". Values indicated with "a" differ significantly from susceptible controls.

The measurement of developmental period and pre-adult survival.
Tomato plants with at least three full grown leaves were placed separately in plant cages. Two leafminer females for each plant (collected randomly from the mass-rearing) were allowed to lay eggs during one day. One week later the number

of small mines were recorded per plant (see table I). To collect the pupae which fell from the leaves, plants were laid on their sides in a tray. From the eleventh day after egg laying the pupae were collected and recorded daily. Larval survival per plant is expressed as the number of pupae in proportion to the number of small mines (see table I). The pupae were stored in an incubator at 25°C and 85 - 90 % R.H. until emergence of the adult flies. Emergence was recorded daily. Neither pupal survival per plant (= the number of emerged flies in proportion to the number of pupae) nor the duration of the developmental period was significantly different from the susceptible controls.
Three accessions of L.peruvianum, three of L.chilense and six of L.parviflorum/L.chmielewski were evaluated in a group of five plants per genotype. These groups were placed separately in an insect cage in which 10 leafminer females were allowed to lay eggs for one week. Later the number and size of the mines was estimated per plant. In this experiment one accession of L.chilense (Gl.1556) and four L.parviflorum/ L.chmielewski accessions (LA735, LA1045, Gl.74455 and Gl.771496 respectively) showed little or no symptoms.

The measurement of reproduction and adult survival.
The reproduction of young females of L.trifolii was determined on five Lycopersicon accessions and two hybrids from a cross between L.esculentum and L.hirsutum. For this purpose, one recently-emerged female was put inside a leaf cage together with two males. The leaf cage was transferred daily to a new leaflet of the same plant until the female died. Two days after egg-laying, the number of eggs was recorded by observing the leaves under a stereo microscope using transmitted light. Six plants per accession were tested and for each plant the reproduction and lifespan (longevity) of two females was recorded. The results are shown in table II. On all except two L.hirsutum accessions, no egg were laid, while on Moneymaker at least 27 eggs per female were laid.

| genotype | average # eggs per female | female longevity in days |
|---|---|---|
| Moneymaker | 43 | 8 |
| L.esc. PI128230 | 44 | 6 |
| L.hirs. Gl.1560 | 3 a | 4 a |
| L.hirs. Gl.1290 | 8 a | 4 a |
| L.cheesm. LA1448 | 15 a | 5 a |
| Fl (Moneymaker x L.hirs. Gl.1560) | 16 a | 5 a |
| Fl (Moneymaker x L.hirs. Gl.1290) | 13 a | 5 a |

Table II.
The reproduction and longevity of L.trifolii on seven Lycopersicon genotypes. Values indicated with "a" differ significantly from the susceptible control "Moneymaker".

## Discussion

These experiments show that most L.hirsutum accessions are completely resistant to L.trifolii, while L.cheesmanii LA1448 is partially resistant. The time taken for the doubling of the leafminer population on Moneymaker is 5.4 days, on L.cheesmanii LA1448 10.4 days and is indefinite on most L.hirsutum accessions. These results are in agreement with those of Webb et al (1971) and Schuster et

al (1979) who demonstrated resistance in L.hirsutum to two other leafminer species (L.munda and L.sativae respectively). When their and our results are taken together, it can be concluded that the L.hirsutum accessions PI127826 and PI126445 have the highest levels of resistance to leafminers. The partial resistance shown in L.cheesmanii is supported by Laterrot et al (1987) who reported that another accession of L.cheesmanii (LA1401) also had a high level of resistance to L.trifolii and L.sativae.
If the number of small mines (table I) corresponds to the number of eggs laid, the experiments indicate that L.hirsutum is less accepted for egg laying. This is partly caused by anti-biotic factors against the egg laying female, as her longevity is also significantly reduced (see table II). However, on many L.hirsutum plants no eggs were laid at all. This implies that anti-xenosis may play a role as well. Since the females used were reared on Moneymaker, the latter phenomenon may be the result of the shift in host plant species.
The values for total reproduction and longevity on the hybrid plants (table II) suggest an intermediate inheritance of the resistance.
Further development of a test method for breeding programmes should be based just on larval or adult survival, or on the number of eggs laid.

**Résumé**

**Recherches sur la résistance de la tomate à la mouche mineuse Liriomyza trifolli**

La mouche mineuse Liriomyza trifolii est un ravageur important de la tomate. Plusieurs raisons plaident en faveur d'un développement de variétés résistantes. Quelques composantes de la résistance à cette mineuse ont donc été étudiées sur 50 lignées de Lycopersicon. Une méthode expérimentale a été développée en se basant sur un modèle de croissance de populaton des ravageurs. Quelques lignées de L.hirsutum ont montré une résistance complète, alors qu'une lignée de L.cheesmanii possédait un niveau élevé de résistance partielle.

**References**

Hulspas-Jordaan, P.M. & van Lenteren J.C., 1989. The parasite-host relationship between Encarsia formosa (Hymenoptera: Aphelinidae) and Trialeurodes vaporariorum (Homoptera: Aleyrodidae). XXX. Modelling population growth of greenhouse whitefly on tomato. Wageningen Agricultural University Papers, 89-2, 1-54.

Laterrot, H., Bordat M., Renand M. & Moretti A., 1987. Various levels of leafminer resistance in Lycopersicon genus. Report of the Tomato Genetics Cooperative 37, 47-49.

Lewontin, R.C., 1965. Selection for colonizing ability. In: H.G. Baker & G.L. Stebbins (eds), The genetics of colonizing species. Academic Press, New York. pp. 77-94.

Minkenberg, O.P.J.M. & van Lenteren J.C., 1986. The leafminers Liriomyza bryoniae and L.trifolii (Diptera: Agromyzidae), their parasites and host plants: a review. Wageningen Agricultural University Papers 86-2, 1-50.

Schuster, D.J., Waddill V..H., Augustine J.J. & Volin R.B., 1979. Field comparisons of Lycopersicon accessions for resistance to the tomato pinworm and vegetable leafminer. J. Amer. Soc. Hort. Sci. 104, 170-172.

Webb, R.E., Stoner A.K. & Gentile A.G., 1971. Resistance to leaf miners in Lycopersicon accessions. J. Amer. Soc. Hort. Sci. 96, 65-67

# HONEYDEW EXCRETION AS A POSSIBLE TOOL TO SCREEN TOMATO RESISTANCE TO VIRUS TRANSMISSION BY *Bemisia tabaci*

M.J. Berlinger, R. Dahan, Orah C. Berlinger and Sara Mordechi.
Entomology Laboratory, Agricultural Research Organization (ARO)
Gilat Regional Experiment Station, Mobile Post Negev 85-280, Israel

Summary

The existing methods, used for screening whitefly resistance, have been developed for species that cause direct damage. These methods are not refined enough for detecting resistance to virus-transmission, which requires almost a complete prevention of feeding. It was suggested that the feeding should be measured indirectly through the amount of honeydew excretion. If excretion is quantitatively correlated with feeding, then the amount of excreted honeydew must be correlated with the ability of virus transmission. If this is confirmed, honeydew excretion could be a useful tool for estimating levels of plant resistance to virus transmission. In laboratory experiments virus-transmission (% infested plants) was found to be correlated with feeding time (r=0.96), and feeding time with the amount of excreted honeydew (r=0.96). In the field experiment virus-susceptible, but whitefly-resistant wild *Lycopersicon* plants, became infected more slowly and thus later by the virus in comparison with tomato control plants. The results of further experiments, supporting this hypothesis, are presented and discussed.

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## Introduction

The tobacco whitefly *Bemisia tabaci* (Gennadius) is a potential limiting factor in tomato production (Avidov, 1956; Johnson *et al.*, 1982). It injures both open-field and greenhouse tomatoes by causing direct damage-through sucking the plant's sap and excreting honeydew which renders the fruit unmarketable; it also causes indirect damage by adult whiteflies transmitting virus diseases (Duffus, 1987). Most of the whitefly-borne plant viruses and all whitefly-borne tomato viruses, are transmitted by *B. tabaci* (Duffus, 1987). *B. tabaci* is known in all tropical and sub-tropical countries of the world (Mound and Halsey, 1978). Recently it has expanded its range to the temperate regions of Western-Europe and USA (Fransen, 1989; and personal communications), where it prevails mainly in greenhouses. Crop losses due to *B. tabaci*- borne viruses are increasing accordingly (Duffus, 1987). Although the tomato is not its preferred host, *B. tabaci* is able to transmit efficiently several virus diseases to this crop in various countries (Ponti de, *et al.*, -in press).

Tomato yellow leaf curl virus (TYLCV), the most harmful tomato disease in the Middle East, is transmitted solely by *B. tabaci* (Cohen and Nitzany, 1966). From the moment the symptoms appear, about three weeks after inoculation, plant-growth ceases and no further fruit-set occurs. The younger the plant is infected, the greater is the economic damage. Thus, any delay in the infection time due to a decrease in the speed of virus spread, will reduce crop loss. The Economic Injury Level (EIL) for virus transmission is rather low, but the incidence of virus-infected plants is directly related to the whitefly population density and to the whitefly feeding time. TYLCV cannot be controlled directly. Since breeding for virus resistance is not yet sufficiently advanced, the majority of growers try to restrict virus transmission by an attempt to control the vector. This can be done either by chemical control measures which are aimed at the reduction of the whitefly density, the efficacy of which is limited (Berlinger *et al.*, 1988); or by using the plant's self-defence mechanisms against the vector, i.e. 'vector resistance', to reduce the whitefly's feeding time. Accordingly, the breeding of resistant plants is being aimed at minimizing the whitefly feeding-time on the plants. Then, even virus-susceptible tomato plants will not become infected as long as the whitefly feeding period does not exceed the time required to transmit the virus, which is about 3-4 h. Several attempts to minimize virus transmission through the use of insect-vector resistant cultivars show promise (summarised in Berliner and Dahan, 1987). Plant resistance to whiteflies has been found in various wild *Lycopersicon* spp. (Berlinger, 1986). All hitherto used methods to evaluate tomato-

resistance levels to whiteflies (Berlinger and Dahan, in press; Berlinger and de Ponti, 1981; Berlinger et al.,1984) have been aimed at screening plant resistance to *Trialeurodes vaporariorum* which causes direct damage. The level of resistance, required to prevent direct damage by whiteflies to the tomato, is considerably lower than the level required to prevent virus transmission. Therefore, the methods used to screen plant resistance to *T. vaporariorum* are unsuitable for the detection of vectors-resistance.

The objective of this research is to develop reliable, convenient, and cheep methods to detect plant-resistance levels which are sufficiently high to minimize whitefly feeding upon the plant, and thus prevent virus transmission. Since virus transmission is closely related with feeding time, and feeding time with honeydew production, it is proposed that honeydew excretion may be a good measure of the potential to transmit the virus.

## Methods

The whiteflies were reared on young cotton plants (cv. SJ-2) in a cage (65x55x56 cm) in a semi-conditioned glasshouse (20-30$^0$C, 50-60% RH).

Laboratory experiments were conducted under constant conditions (27±1$^0$C, 50-60% RH, 120 µEinstein/ cm$^2$). To quantify the excreted honeydew, whitefly adults were confined on the underside of a tomato leaflet by means of a clip-on-cage (a modified Munger-cell). The leaf could be detached from the plant if kept in close contact with moist filter paper. The duration of the tests was 4 or 24 h. The honeydew, collected on a filter paper (Whatman No. 1), was sprayed with Ninhydrin (0.2%), which stained the amino acids of the honeydew blue and allowed the recorder to count droplet number, measure their area, or rate them (0-9). To determine the total sugars- the honeydew was collected on a microscope glass cover slide and washed off by 1 ml of distilled water to which 2 ml of Anthrone (0.2%) were 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.

The proportion of plants infected with TYLCV was also used as a criterion to determine levels of plant resistance to the vector. In laboratory experiments tomato seedlings, reared in a 'speedling' tray were introduced into a cage together with viruliferous whitefly adults. The final percentage of infected plants was evaluated. In field tests the investigated plants were planted during July-August, when the main virus infection occurs. The proportion of infected plants was recorded weekly.

## Results and discussion

The following are the first results of the project.

### Preliminary Experiments

Preliminary experiments showed that without food the whiteflies did not excrete honeydew and did not survive more than 24 h. There was no distinct difference in honeydew production, whether the whiteflies were confined on a detached or on an intact leaflet, or whether confined on the lower or upper side of the leaflet. Both white filter paper and microscope-slide cover glasses, were suitable for collecting honeydew. On the filter paper the honeydew drops could be stained blue, with Ninhydrin, and then easily counted or evaluated by measuring their diameter, their surface area or by visual rating (0-9). The main advantage of the filter paper was that the whiteflies did not stick to their own excretion, which was absorbed by the paper. The main advantages of using the glass plates were two fold: 1) the honeydew drops could be seen through the glass and counted during the experiment, 2) the honeydew could be quantified by washing it off, staining its sugars with Anthron, determining the optical density with a spectrophotometer, and finally comparing the readings with a pre-prepared calibration curve.

### The effect of whitefly density on honeydew production

The optimal number of whiteflies per experimental-cage was investigated (Table 1). The total amount of honeydew increased, within 4 h, from 2.2 to 5.3 µg sugar with the density of whiteflies increasing from 1 to 10 per replicate (Experiment A). After a 24 h period (Experiment B), the total amount of honeydew increased as well, from 18.9 to 132.0 µg sugar, while the number of whiteflies increased from 1 to 30 per replicate. However, the amount of honeydew per whitefly in both experiments (2.2 and 18.9 µg sugar) was highest, after 4 and 24 h respectively, when only a single whitefly was tested per replicate. Any increase in the number of whiteflies per replicate was followed immediately by a marked decrease of honeydew produced per female. This reduction in the amount of honeydew per female may be the result of a disturbance or competition among the whiteflies in the cell. However, for practical reasons it was decided to use 5 whiteflies per replicate in the following experiments.

Table I. The amount of honeydew (µg sugar) produced in 4 (Experiment A) and 24 h (Experiment B) by a varying number of whiteflies (1-30) per replicate. The experiment was performed with 10 replicates.

| Density of whiteflies per replicate | Experiment A: Honeydew (µg sugar) produced in 4 h | | Experiment B: Honeydew (µg sugar) produced in 24 h | |
|---|---|---|---|---|
| | per replicate | per whitefly | per replicate | per whitefly |
| 1 | 2.2 | 2.2 | 18.9 | 18.9 |
| 3 | 3.2 | 1.0 | 46.8 | 15.9 |
| 5 | 5.0 | 1.0 | 87.0 | 17.4 |
| 10 | 5.3 | 0.5 | 110.8 | 12.3 |
| 20 | - | - | 114.9 | 5.7 |
| 25 | - | - | 126.9 | 5.1 |
| 30 | - | - | 132.0 | 4.4 |

Honeydew excretion and its frequency

It took 30-40 min before the first honeydew drops were recorded (Table II, replicates 5 and 6). Then the total number of drops, as well as the number of replicates in which honeydew was produced, increased with time. After 70 min, honeydew was noticed in all replicates, whereas the number of drops continued to increase. In some replicates the number of drops exceeded the number of whiteflies, probably because excreting whiteflies changed their sucking site. Nevertheless, the maximum number of excreting whiteflies could not exceed 10 (No. of whiteflies). This was observed when the "% of excreting whiteflies" was calculated. The percentage of excreting whiteflies was in the same order of magnitude as the increasing rates of TYLCV infected plants, found by Berlinger, Orah C. (unpublished) and Cohen and Nitzany (1966). This similarity in percentages with time, may be the result of the time needed for each individual whitefly to reach the phloem tissue in the leaves.

Other factors affecting honeydew excretion

The amount of honeydew production was positively and directly influenced by temperature, in the range of 21-31°C (R=0.99).

TYLCV-infected whiteflies (+V) produced less honeydew (38.4 µg sugar) than "healthy" (-V) virus-free whiteflies (54.1) after 4 h (Table III).

Table II. Accumulative number of excreted drops with time, their total number, the number of replicates with honeydew excretion, the maximum number and percentage of excreting whiteflies, compared with the % of TYLCV infected plants*).

The experiment was performed with 8 replicates of 10 whiteflies (n).
The honeydew drops were counted cumulatively every 10 min, during 30-180 min.

| Rep. | (n) | 30 | 40 | 50 | 60 | 70 | 80 | 90 | 100 | 110 | 120 | 130 | 140 | 150 | 160 | 170 | 180 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | (10) | 0 | 0 | 0 | 1 | 2 | 3 | 3 | 4 | 5 | 5 | 6 | 7 | 7 | 8 | 8 | 9 |
| 2 | (10) | 0 | 0 | 0 | 1 | 1 | 2 | 2 | 2 | 5 | 6 | 8 | 10 | 10 | 11 | 11 | 11 |
| 3 | (10) | 0 | 0 | 0 | 0 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 2 | 4 | 5 | 5 | 6 |
| 4 | (10) | 0 | 0 | 0 | 2 | 2 | 3 | 3 | 3 | 3 | 3 | 4 | 8 | 8 | 8 | 8 | 9 |
| 5 | (10) | 0 | 2 | 4 | 5 | 5 | 5 | 6 | 8 | 8 | 8 | 9 | 12 | 12 | 12 | 13 | 13 |
| 6 | (10) | 0 | 2 | 3 | 5 | 5 | 5 | 5 | 5 | 5 | 6 | 6 | 10 | 10 | 10 | 10 | 10 |
| 7 | (10) | 0 | 0 | 0 | 0 | 1 | 3 | 3 | 3 | 3 | 3 | 3 | 4 | 4 | 4 | 5 | 6 |
| 8 | (10) | 0 | 0 | 0 | 1 | 1 | 1 | 1 | 1 | 2 | 2 | 2 | 3 | 3 | 3 | 4 | 4 |
| Total No. of Drops | | 0 | 4 | 7 | 15 | 18 | 23 | 24 | 27 | 32 | 34 | 39 | 56 | 58 | 61 | 64 | 68 |
| No. of replicates with honeydew | | 0 | 2 | 2 | 6 | 8 | 8 | 8 | 8 | 8 | 8 | 8 | 8 | 8 | 8 | 8 | 8 |
| Maximum No. of excreting whiteflies | | 0 | 4 | 7 | 15 | 18 | 23 | 24 | 27 | 32 | 34 | 39 | 54 | 56 | 58 | 60 | 64 |
| Proportion of excreting whiteflies (%) | | 0 | 0.05 | 0.09 | 0.19 | 0.23 | 0.29 | 0.30 | 0.34 | 0.40 | 0.43 | 0.49 | 0.68 | 0.70 | 0.73 | 0.75 | 0.80 |
| Proportion of plants infected with TYLCV | | 0 | | | 0.20 | | | | | | 0.60 | | | | | | 0.73 |

Table III. The influence of virus, in the plant or in the whitefly, on honeydew production (µg sugar) by whiteflies.
The experiment was performed with 10 replicates of 5 whiteflies per replicate.
+V = Virus infected plants / viruliferous whiteflies
-V = Healthy plants / virus free whiteflies

| The Plants | Experiment Duration (h) | Whiteflies -V | Whiteflies +V |
|---|---|---|---|
| -V | 4 | 54.1 | 38.4 |
| +V | 4 | 37.2 | 25.0 |
| -V | 24 | 220.7 | --- |
| +V | 24 | 157.2 | --- |

When tested for 24 h, healthy whiteflies produced significantly more honeydew (220.7 µg-sugar) when fed on healthy plants, compared with the control group fed on virus-infected tomato plants (157.2 µg sugar).

The number of whiteflies that ensure TYLCV infection

Since not every viruliferous whitefly is able to infect a plant within 24 h, it was important to find out the minimum number of whiteflies which will ensure a 100% infection rate of the control plants. It was found that 5 viruliferous whiteflies per replicate, confined on a healthy tomato plant, for 24 h, will infect the plant with TYLCV (Table IV).

The effect of vector-resistance on virus spread

All previously tested *Lycopersicon* accessions were found to be virus-susceptible, but showed high levels of vector resistance. The two most whitefly-resistant *L. pennellii* accessions were selected, according to previous laboratory experiments (Berlinger and Dahan, in press). They were compared in the field, for vector resistance, with a tomato cultivar. At a time that all tomato plants were already TYLCV infected only 5% of one of the *L. pennellii* accessions was infected and none of the second accession. The most vector resistant *L. pennellii* was selected and crossed with a susceptible tomato and thereafter back-crossed. Only two of the $F_2$ progeny plants (No. 53, and 67) did not become TYLCV infected as a result of their high level of vector resistance (Table V). This was expressed by the high leaf tackiness (2.8% sucrose) followed by a low amount of honeydew

excretion (6.8 and 13.2 µg sugar). All other accession became infected by the virus. These results verified the hypothesis that vector-resistance might be efficient in the prevention of virus spread and that it is also inherited by the progeny, although at different levels.

Table IV. Honeydew production (µg sugar) and proportion of virus infected plants (%) resulting from different numbers of viruliferous whiteflies (0->50 in Experiment A and 0- 30 in Experiment B). The experiment was performed in 10 replicates with a varying number of whiteflies per replicate.

| Experiment A | | | Experiment B | | |
|---|---|---|---|---|---|
| No. white-flies/ replicate | Honeydew (µg sugar) per replicate | TYLCV infection (%) | No. white-flies/ replicate | Honeydew (µg sugar) per replicate | TYLCV infection (%) |
| 0 | 0 | 0 | 0 | 0 | 0 |
| 1 | 1.1 | 17 | 5 | 33.4 | 100 |
| 5 | 12.7 | 100 | 20 | 187.1 | 100 |
| 10 | 24.3 | 85 | 30 | 262.8 | 100 |
| 15 | 33.6 | 85 | | | |
| >50 | -- | 100 | | | |

## Concluding Remarks

The economic damage of TYLCV is directly related to the density of whiteflies. This fact, along with the statements of our hypothesis, is the reason why even partially-resistant plants will have a significant impact on the reduction of virus transmission, as observed by various authors in other pest-crop systems (Jones, 1987).

From the results presented here it seems that the amount of honeydew may be used as a criterion to estimate the probability of virus transmission, as with aphids transmitting potato leaf curl virus (v.d. Heuvel and Peters, in press) and as a tool in plant selection for vector-resistance. For long-term pest management, resistant cultivars should be considered as the best and cheapest plant protection measure for the grower. Since no

Table V. Whitefly survival, the leaf-tackiness index, and honeydew exuded in relation to TYLCV-infection of BC1F2 hybrid lines* (Tomato x *L. pennellii*).
Leaf-Tackiness (%-sucrose)** is a measure of resistance level in the *L. pennellii* accession (Dahan, 1985; Plage, 1975).

| Accession | Survival (%) | Leaf-Tackiness (%-sucrose)** | Honeydew (µg Sugar) | TYLCV (Yes+/No-) |
|---|---|---|---|---|
| *L. pennellii* | 0.0 | 4.2 | 0.4 | - |
| 53* | 23.2 | 2.8 | 6.8 | - |
| 67* | 94.3 | 2.8 | 13.2 | - |
| 5* | 100.0 | 1.9 | 39.1 | + |
| 36* | 100.0 | 1.6 | 33.0 | + |
| 33* | 76.0 | 1.4 | 35.3 | + |
| 11* | 100.0 | 1.2 | 60.3 | + |
| 3* | 94.2 | 1.0 | 48.8 | + |
| 41* | 100.0 | 0.8 | 39.5 | + |
| 8* | 100.0 | 0.4 | 68.3 | + |
| 69* | 95.3 | 0.2 | 21.2 | + |
| Tomato | 94.2 | 0.0 | 59.3 | + |

pest-resistant tomato cultivar is as yet commercially available, any progress made toward the goal of developing whitefly-resistant, or even partially-resistant plants, appears to have the potential to add a very significant dimension to the control of whitefly damage.

Indeed, it is still doubtful whether resistance to the pest could solve the problem of virus transmission. At the same time it is also not yet clear if the recently-developed TYLCV-tolerant cultivars will solve the problem on their own. Therefore, it is likely that the integration of whitefly-resistance, together with the TYLCV-tolerance will be more useful in achieving resistance of longer duration.

Resistance to the whitefly vector is expected to protect the plants at the same time against the whole range of semi-persistent whitefly-borne viruses. Since the level of vector-resistance must be higher than required to prevent direct damage, it goes without saying that any vector-resistant cultivar will be resistant also to whiteflies causing direct damage.

Previous experience showed that the same plant material comprises resistance to both whitefly species (Berlinger, 1980; Berlinger, unpublished; Berlinger and de Ponti, in preparation; Dahan, 1985). Therefore, any method useful for one of the whitefly species, may be also applicable for other species. The same methods are expected to be useful for evaluating plant resistance in other crops (cotton, cucumber, melon, sweetpotato, cassava, etc.) and certain other sucking insects which excrete honeydew.


## Resume

EXCRETION DE MIELLAT EN TANT QUEMOYEN DE SELCTION POSSIBLE DE LA RESISTANCE A LA RESISTANCE A LA TRANSMISSION DE VIRUS PAR Bemisia tabaci SUR TOMATE

Les méthodes usuelles utilisées dans la selection pour la résistance aux mouches blanches ont été développées pour des espèces provoquant des dégâts directs. Ces méthodes ne sont pas assez fines pour une détection de la résistance à la transmission du virus, qui exige la suppression quasi totale de la nutrition. Il a été suggéré de déterminer le niveau de nutrition, de façon indirecte, en ce basant sur la quantité de miellat excrété. Si l'excrétion est corréllée quantitativement avec la nutrition, la quantité de miellat produit doit alors être corrélée avec la capacité de transmettre le virus. En cas de confirmation, l'excrétion de miellat pourrait etre une méthode utile pour déterminer le niveau de résistance des plantes à la transmission du virus. Des essais en laboratoire ont montré que la transmission du virus (pourcent de plantes infectées) est corrélée avec la durée de prise de nourriture (r=0.96) et que cette durée elle-même est corrélée la quantité de miellat produit (r=0.96). En plein champ, des lignées de *Licopersicon* sauvages, sensibles au virus mais résistant à la mouche blanche, ne sont que légèrment et tradivement virosées. Des résultats d'autres essais, soutenant cette hypothèse, sont présentés et discutés.


## References


Avidov, Z. 1956. Bionomics of the tobacco whitefly (*Bemisia tabaci* Gennad.) in Israel. Ktavim 7: 25-41.

Berlinger, M.J. 1986. Host plant resistance to *Bemisia tabaci* . Agriculture, Ecosystems & Environment 17: 69-82.

Berlinger M.J. and R. Dahan 1987. Breeding for resistance to virus transmission by whiteflies in tomatoes. Insect Sci. Applic. 8: 783-784.

Berlinger M.J. and R. Dahan (in press) The importance of plant resistance in the control of whiteflies and whitefly-borne viruses in tomato and the development of screening methods. Proc. Intern. AVRDC, Tainan, Taiwan.

Berlinger, M.J. and O.M.B. de Ponti 1981. Methods for testing resistance to whiteflies in tomato and related species. Bull. IOBC/WPRS 1981/IV/1: 115-118.

Berlinger, M.J., R. Dahan and E. Shevach-Urkin 1984. Resistance to the tobacco whitefly, *Bemisia tabaci* , in tomato and related species: a quick screening method. Bull. IOBC/WPRS 1984/VII/4; 39-40.

Berlinger, M.J., R. Dahan and S. Mordechi 1988. Integrated pest management of organically grown tomatoes in Israel. Applied Agricultural Research 3: 233-238.

Cohen, S. and F.E. Nitzany 1966. Transmission and host range of the tomato yellow leaf curl virus. Phytopathology 56: 1127-1131.

Dahan, R. 1985. *Lycopersicon pennellii* as a source of resistance to the tobacco whitefly *Bemisia tabaci* in tomato. 55+II pp. M.Sc. Ben Gurion University Of The Negev, Beer Sheva.

Duffus, J.E. 1987. Whitefly transmission of plant viruses. in: K.F. Harris [Ed.] Current Topics in Vector Research. Springer-Verlag New York Inc. Vol. 4: 73-91.

Fransen, J.J. 1989. List of pests in order of importance in relation to European countries. Bull. IOBC/WPRS 1989/XII/3: 7-8.

Heuvel, J.F.J.M. v. d. and D. Peters (in press). Transmission of potato leafroll virus related to the honeydew excretion of Myzus persicae (Sulz.).

Johnson,M.V., N.C. Toscano, H.T. Reynolds, E.S. Sylvestris, K. Kido and E.T. Natwick 1982. Whiteflies cause problems for southern California. California Agriculture 36: (9/10), 24-26.

Jones, A.T. 1987. Control of virus infection in crop plants through vector resistance: a review of achievements, prospects and problems. Ann. appl. Biol. 111: 745-772.

Mound, L.A. and S.H. Halsey 1978. Whitefly of the world. British Museum (Natural History), John Wiley & Sons, Chichester and New York, NY.

Plage, R.F. 1975. Host plant resistance to the greenhouse whitefly *Trialeurodes vaporariorum* (Westw.) in the tomato (*Lycopersicon esculentum* Mill.) and related species, especially *Solanum pennellii* Correll. Ph.D. thesis, Cornell University, Ithaca, N.Y. 67 pp.

Ponti, O.M.B. de; L.R. Romanov and M.J. Berlinger (in press) Whitefly - Plant Relationship: Plant resistance. in: D. Gerling (ed.) WHITEFLIES: their Bionomics, Pest status and Management. Intercept, Dorset, U.K.

# DESIGNING A RELIABLE TESTING METHOD FOR DETERMINING RESISTANCE TO THE GLASSHOUSE WHITEFLY (*TRIALEURODES VAPORARIORUM*) IN TOMATO

W.A. VAN GIESSEN AND C. MOLLEMA
Centre for Plant Breeding Research CPO
P.O.Box 16, 6700 AA WAGENINGEN, THE NETHERLANDS.

Summary
The lack of a reliable and relatively fast test method for resistance to whitefly (*Trialeurodes vaporariorum* Westw.) in tomato has so far hindered the breeding of resistant varieties by plant breeders. Available tests contradicted each other, possibly caused by an underestimation of the complexity of the plant-insect interactions involved in resistance. Our approach therefore is to measure not just one, but a number of life history components of whitefly population growth per tomato genotype. These are taken together in a computer simulation model with which the rate of increase of the population can be calculated. In this way resistance can be quantified, rather than qualified. A sensitivity analysis of the different components together with an analysis of the available genetic variation for these components can lead to a simplification of the test method. Possibly the same test can be used for breeding for resistance to whitefly in other crops, such as cucumber, eggplant and sweet pepper or for resistance to other insect pests.

## 1. Introduction

The glasshouse whitefly, *Trialeurodes vaporariorum* Westw.), is a serious pest of outdoor and glasshouse tomatoes (*Lycopersicon esculentum* Mill.) in Europe. Chemical control is possible though not always sufficiently effective. Biological control with the parasitic wasp *Encarsia formosa* Gahan is, at least in the Netherlands and Great Britain, widely used in greenhouses and is generally successful (van Lenteren & Woets, 1988). A third solution, which is gaining more and more attention, is breeding for resistance to pest insects. Evidently, control through insect resistance is not hazardous to the environment as is the use of insecticides. Furthermore, even partial resistance could be very effective if it were complementary to biological control (de Ponti, 1978). Although at the IVT, de Ponti and co-workers have developed tomato varieties with a substantial level of resistance to whitefly (de Ponti *et al.*, 1975), a release of these lines was hindered through the lack of a fast and reliable test method for determining resistance. Our goal is to develop such a test, based on a computer model that simulates the growth of a whitefly population on tomato.

## 2. Breeding for resistance

At the IVT, de Ponti and co-workers started a research program in 1972 to investigate the possibility of breeding for resistance to whitefly in tomato. They found high levels of resistance only in species related to *L. esculentum*: *Solanum pennellii*, *L. hirsutum*, and *L. hirsutum glabratum*. A breeding programme was started with the latter species which included interspecific crosses. Selection for resistance was done in separate glasshouse compartments by counting the number of empty pupal cases after a fixed period from inoculation. Later selection took place in a single normal glasshouse with plants of different genotypes placed randomly. This resulted in the production of a number of backcross lines possessing resistance close to or better than that of the resistant parent. To prepare the release of these lines to commercial plant breeders, three different tests were carried out to confirm their resistance. Unfortunately the results of these tests contradicted each other completely. As a result the intended release was cancelled and further research was necessary. These contradictions may be partly due to an underestimation of the complexity of the interactions between host plants and herbivorous insects. In most tests a single factor is used to measure the level of resistance. Resistance can be defined as "any reduction in population growth of the population of a target insect as influenced by the host plant, compared to an existing situation or to standard variety" (Berlinger, 1986). Insect resistance is the sum effect of such influences, some of which have relatively more impact on population growth than others. Prolongation of the generation time for example has a relatively more profound effect on growth than a comparable reduction in fecundity (Lewontin, 1965). Similar results were obtained for the glasshouse whitefly by using a state variable, temperature driven simulation model (Hulspas-Jordaan & van Lenteren, 1989).

## 3. Concept test method

The life cycle of the glasshouse whitefly consists of several stages (egg, a number of larval stages, a pupal and an adult stage), but can roughly be subdivided into a pre-adult and an adult stage. Principal life history characteristics are reproduction, survival and developmental rates, which together determine the intrinsic rate of increase of the insect population (Birch, 1948). To investigate which factors contribute to changes in population growth and to what extent, four parameters are measured. A number of whitefly females are anaesthetized and confined in a leaf cage, clipped to the underside of a plant approximately 6 weeks old. These females are allowed to lay eggs for a fixed period of time. When the leaf cage is removed the number of females that have survived is recorded. The number of eggs laid by these females and the resulting adults developing

from these eggs are counted. The average developmental period from egg to adult is determined by daily counts of emerged adults.

concept test method

| | stage | parameter | unit |
|---|---|---|---|
| A | adult | oviposition rate | # eggs/♀/day |
| B | adult | survival | fraction/day |
| C | pre-adult | survival | fraction |
| D | pre-adult | developmental period | # days |

▼

Population Growth Simulation Model

▼

Relative Resistance: $R_i = \frac{r_c - r_i}{r_c} \cdot 100\ \%$

These four measurements are fed into a computer model that simulates population growth. It calculates the intrinsic growth rate of a hypothetical whitefly population. The growth rate ($r_i$) found for a particular genotype i can then be compared with the rate ($r_c$) found for a variety of known susceptibility (e.g. cv 'Moneymaker'). With these two values the relative resistance ($R_i$) for that genotype can be calculated:

| | parameters | | | | | |
|---|---|---|---|---|---|---|
| material | A | B | C | D | $r_i$ | $R_i$ |
| cv 'Moneymaker' | 3.3 | 0.96 | 0.94 | 30 | 0.081 | 0 |
| cv 'Counter' | 3.1 | 0.94 | 0.89 | 30 | 0.074 | - 9 |
| Backcross 82207 | 3.0 | 0.96 | 0.64 | 30 | 0.069 | - 15 |
| *L.hirsutum* | 2.0 | 0.79 | 0.49 | 29 | 0.019 | - 77 |

The model, which is still under development, can also be used to carry out a sensitivity analysis of the four parameters. After an analysis of the genetic variability for the different parameters, it should be possible to distill a test that is fast and still as reliable as the elaborate test described above. When such a test for mass screening of varieties on whitefly resistance is available, further development of the existing resistant lines into commercial varieties will be feasible for private breeders. In the future, the same test might be used for breeding for resistance to whitefly in other crops, such as cucumber, eggplant and sweet pepper or for resistance to other pest insects.

Résumé
Obtention d'une méthode sûre pour tester la résistance de la tomate á la mouche blanche des serres (*Trialeurodes vaporariorum*)

Jusqu'à aujourd'hui, une méthode sûre et assez rapide pour tester la résistance de la tomate à la mouche blanche des serres faisait défaut aux sélectionneurs. Les méthodes existantes donnaient des résultats contradictoires car elles sous-estimaient probablement la complexité des interactions ravegeur/plante-hôte mises en jeu dans les phénomènes de résistance. Notre approche consiste donc à ne pas mesurer un seul, mais bien toute une série de facteurs régissant la dynamique des populations de la mouche blanche sur chaque génotype de tomate. Ces facteurs sont intégrés dans un modèle de simulation par ordinateur, permettant de calculer le taux de croissance de la population. La résistance peut de ce fait être "quantifiée" plutôt que "qualifiée". Une analyse de la sensibilité des différentes composantes, combinée avec une analyse de la variation génétique disponible de celles-ci, peut conduire à une simplification du test de résistance. Il est possible que la même méthode puisse être appliquée à d'autres cultures pour la recherche de résistance à la mouche blanche, (telles que concombre, aubergine et poivron), et qu'elle soit adaptable à d'autres ravageurs.

## 4. Literature cited

BERLINGER, M.J., 1986. Host plant resistance to *Bemisia tabaci*. Agriculture, Ecosystems and Environment 17: 69-82.

BIRCH, L.C., 1948. The intrinsic rate of natural increase of an insect population. J. Anim. Ecol. 17: 15-26.

HULSPAS-JORDAAN, P.M. & LENTEREN, J.C. VAN, 1989. The parasite-host relationship between *Encarsia formosa* (Hymenoptera: Aphelinidae) and *Trialeurodes vaporariorum* (Homoptera: Aleyrodidae). XXX. Modelling population growth of greenhouse whitefly on tomato. Wageningen Agricultural University Papers 89 (2): 1-54.

LENTEREN, J.C. VAN, & WOETS, J., 1988. Biological and integrated pest control in greenhouses. Ann. Rev. Entomol. 33: 239-269.

LEWONTIN, R.C., 1965. Selection for colonizing ability. In: H.G. Baker & G.L. Stebbins (eds.), The genetics of colonizing species. Academic Press, New York, pp. 77-94.

PONTI, O.M.B. DE, 1978. Status of resistance breeding in relation to integrated pest control. II. Arthropods. Working paper presented at the 8th session of the FAO Panel of Experts on Integrated Pest Control, September 1978, Rome.

PONTI, O.M.B. DE, PET, G. & HOGENBOOM, N.G., 1975. Resistance to the glasshouse whitefly (*Trialeurodes vaporariorum* Westw.) in tomato (*Lycopersicon esculentum* Mill.) and related species. Euphytica 24: 645-649.

# RESISTANCE FACTORS AGAINST DIABROTICA BALTEATA LE CONTE IN THE PERIDERM OF SWEETPOTATO [IPOMOEA BATATAS L. (LAM.)]

J. K. PETERSON and J. M. SCHALK
United States Department of Agriculture, ARS, U.S. Vegetable Laboratory,
2875 Savannah Highway, Charleston, SC 29414, USA

Summary
Periderm tissue extracts of the sweetpotato cultivar 'Regal' were tested for reduction of growth and survival of second instar larvae of the banded cucumber beetle. Assayed were hexane, 100% methanol, 50% methanol, and water extracts (sequential extractions). The 100% methanol extract accounted for almost all reduction in growth. Subsequently, the 100% methanol extract was further fractionated on a Sephadex LH-20 column. Two fractions showed high activity; the most active fraction was further separated by HPLC methods. One of the peaks reduced larval growth to approximately 26% at 0.6 mg per ml diet.

## 1. Introduction

The banded cucumber beetle (BCB), Diabrotica balteata Le Conte, causes great losses of sweetpotatoes in the southern U. S. (Rolston, 1977). BCB larvae eat small round holes through the root periderm of sweetpotato and form enlarged cavities under the surface. Previously, feeding studies were performed using the resistant cultivar 'Regal' and a control non-resistant breeding line, SC 1149-19 (Schalk and Creighton, 1989; Schalk et al., 1986). Second and third instar larvae were exposed to whole potatoes (periderm in tact) and the tissues of the cortex and stele. Subsequent weights of emerged adults and the number of survivors was significantly reduced when second instar larvae were exposed to sweetpotatoes with intact skin (Regal). The same effect, but reduced, was found when the larvae were exposed to cortical tissue, and the stelar tissue had no effect. The number of days until eclosion was reduced when larvae were fed any of the three tissues of 'Regal'; the effect was most pronounced when the larvae were exposed to whole sweetpotatoes.

When third instar larvae were exposed to the same treatments, most measured parameters showed changes in the opposite direction.

Given the above observations, efforts were initiated to isolate chemical factors which interfere with growth and development of second instar larvae.

## 2. Materials and Methods

Freshly harvested sweetpotatoes of the resistant cultivar 'Regal' were cleaned by gently scrubbing under flowing water. Non-blemished periderm was scraped off with a scalpel knife, dried at 50C overnight and stored at -20C until use. Extractions were performed sequentially with hexane (4 x 24 hrs), methanol (4 x 24 hrs), 50% aqueous methanol (2 x 24 hrs) and water (4 x 24 hrs), using 15 ml solvent per gram dry tissue. The extracts were dried under vacuum at 45C.

Column chromatography was performed on the most active fraction (100% methanol) using Sephadex LH-20 as stationary phase. Step-gradient elutions were performed using ethyl acetate-methanol mixtures. Five fractions were collected and bioassayed. The most active fraction (fraction 3) was subjected to high-pressure liquid chromatography (HPLC).

HPLC separations were conducted using a C-18 column and water/methanol/acetonitrile gradients. Three fractions were collected; fraction 3 was a pure compound and dose-response assays were initiated.

Bioassays were conducted on extracts and chromatography fractions in the following way: measured amounts of extract were pipetted into small plastic cups, the solvents were evaporated, and the residues were mixed with 4 ml of defined agar based diet (Schalk and Peterson, 1989). After solidification the diet was cut in half and one half was placed in another cup. To each cup, only one second instar larva was added since cannibalism was suspected.

All bioassay data are preliminary and not subjected to statistical analyses, since the bioassays were solely used to identify active extracts and chromatography fractions.

## 3. Results and Discussion

Of the four crude extracts, the 100% methanol extract accounted for almost all activity. The results were variable quantitatively, since problems of insolubility and instability in air and light were encountered.

Fractions derived from Sephadex column chromatography of the 100% methanol extract showed high activity (Table 1). Fraction 2 gave anomalous results; after 5 days 75% of the larvae had died, but the remaining ones showed a relative growth of 134%. This phenomenon may be explained by assuming that growth inhibitory compounds while decaying in light reach low concentrations, at which levels the compounds promote growth. Some indication was exhibited in an HPLC fraction (Table 2). The most active fraction from the Sephadex column (fraction 3) was subsequently further fractionated into 3 fractions, two of which showed similar spectra in UV. Fraction 3 was a pure compound and tested for dose-response activity (Table 2). At 0.6 mg (actual compound weight) per ml diet, larval growth was inhibited by approximately 76%. However, at 0.036 mg per ml, growth was promoted by 74%.

Table 1. Bioassays of eluted fractions from a Sephadex column*.

| Fraction | % Relat. Growth | % Dead |
|---|---|---|
| 1 | 104 | 17 |
| 2 | 134 | 75 |
| 3 | 48 | 83 |
| 4 | 56 | 58 |
| Control | 100 | 25 |

*Second instar larvae, growth after 5 days, concentrations: 8 mg dry weight equivalent (i.e. extract derived from 8 mg dry periderm) per ml diet.

Table 2. Bioassays of HPLC fraction 3.*

| $mg.ml^{-1}$ | rel. % growth |
|---|---|
| 0.600 | 25.6 |
| 0.150 | 103 |
| 0.036 | 174 |
| 0.009 | 101 |
| 0.000 | 100 |

*Concentrations were actual compound weights per ml diet. Growth measured after 5 days exposure.

## 4. Conclusions

Preliminary results obtained from feeding experiments with second instar larvae of Diabrotica balteata Le Conte showed that several compounds exist in the periderm of sweet potato which interfere with growth and survival. The limited amount of data also indicated that some compounds may promote growth when present at very low concentrations.

Résumé:
Les facteurs de résistance au chrysomèle Diabrotica balteata Le Conte, dans le périderme des racines de la patate douce [(Ipomoea batatas L. (Lam.)].

L'influence des extraits de périderme de patate douce du cultivar "Regal" sur la réduction de la croissance et la survie du deuxième stade larvaire du chrysomèle a été expérimenté. Ont été testés des extraits à l'héxane, au méthanol 100%, au méthanol 50% et à l'eau (extractions séquentielles). L'extrait au méthanol 100% assure la plus importante réduction de croissance et il a été fractionné, par la suite, dans une colonne de Sephadex LH-20. Deux fractions ont montré une grande activité; la plus active a été analysée par HPLC. Le composé de l'un des "pics" a réduit la croissance larvaire d'environ 26%, à la concentration de 0,6 mg par ml de milieu nutritif.

## 5. References

1. ROLSTON, L. H., 1977. Cooperative Research Program submitted to the USDA-ARS. Control of the soil insect complex attacking sweet potato by insecticidal and cultural methods with emphasis on host plant resistance. LSU, Baton Rouge, LA.

2. SCHALK, J. M. & CREIGHTON, C. S., 1989. Influence of sweet potato cultivars in combination with a biological control agent (Nematoda: Heterorhabditis heliothidis) on larval development of the banded cucumber beetle (Coleoptera: Chrysomelidae). Environ. Entomol. 18(6). In press.

3. SCHALK, J. M., JONES, A., & DUKES, P. D., 1986. Factors associated with resistance in recently developed sweet potato cultivars and germplasm to the banded cucumber beetle, Diabrotica balteata Le Conte. J. Agric. Entomol. 3(4):329-334.

4. SCHALK, J. M., & PETERSON, J. K., 1989. A meridic diet for banded cucumber beetle larvae, Diabrotica balteata Le Conte. Submitted to J. Agric. Entomol.

# BREEDING FOR MULTIPLE INSECT RESISTANCE IN SWEETPOTATO

J. M. Schalk, A. Jones and P. D. Dukes

U. S. Vegetable Laboratory, 2875 Savannah Highway,

Charleston, South Carolina 29414.

## SUMMARY

The development of a mass selection breeding program insures wide genetic diversity, high root yield and root quality with resistances to 10 major insects pests in single plant selections. The U. S. Vegetable Laboratory has released 8 breeding lines and 6 cultivars with multiple resistance to wireworms, Diabrotica, flea beetles and grubs.

## PROGRAM

Sweetpotatoes are damaged or destroyed by a group of insects which have become a major limiting factor in their production. The larval stages of these pests actively feed on storage and feeder roots. Insecticides, until recently were the first line of defence in reducing damage by these pests, but since the removal of the persistent chlorinated hydrocarbons, for environmental and human health reasons, and their replacement with less persistent compounds, such as organophosphate and carbamates, effective soil insect control in sweetpotatoes is not reliable. An alternative to insecticides is the development of insect resistant plants. This requires no additional action by the farmer as the control is genetically programmed within the plant. Most sweetpotatoes produced in the U. S. are generally similar and vary in susceptibility to insect problems, however most are susceptible. Therefore, in 1966 scientist at the U. S. Vegetable Laboratory, Charleston, SC initiated a mass selection breeding program to develop horticulturally acceptable breeding clones and cultivars with resistance to insects and diseases (4, 5, 6, 13, 14, 16, 17, 18, 19, 21).

The program emphasizes the development of sweetpotatoes with the good horticultural qualities, and resistance to insects and diseases. Cuthbert (4) identified the insects responsible for different kinds of damage to sweetpotato by exposure of roots to known larval species. However, changes in the appearance of injury during subsequent root growth may cause injury by several different species to appear similar at harvest. An example is the damage resulting from wireworm, Diabrotica and Systena (WDS) which cannot be easily determined at harvest and has been grouped into a complex called WDS (4). The original holes are usually shallow but when larvae of

the WDS complex penetrate the vascular cambium the damage can be considerably deepened by subsequent growth. Feeding by sweetpotato flea beetle larvae (Chaetocnema confinis Crotch) leaves narrow channels or grooves just under the skin (periderm). Injury from grubs (Plectris aliena Chapin, and Phyllophaga ephilida (Say)) is easily recognized as they gouge broad shallow areas in the roots.

Mass Selection: Sweetpotatoes are uniquely different from most other vegetables in that classical pedigree breeding procedures are difficult to follow. The sweetpotato, a hexaploid, has 90 chromosomes with a complex quantitative inheritance. Plants are clonally propagated and pedigree records are of limited value, as it is not necessary to reproduce a particular genotype. Mass selection procedures which combine rapid generation turnover with high selection pressure provides a sound basis for crop improvement. A breeding program should involve both long and short term goals with one or more mass selection populations to provide new parental types and to assure the development of a wide genetic base. The mass selection program moves from one sexual generation to next by advancing from true seed each year. In this process selected plants are not used in the next cycle as only the seed is used from them. Thus seeds from selected plants can be bulked to start the next cycle (15).

The exact number of cultivars needed to start a mass selection program for long-term objectives (resistance to future pests) should include as wide a genetic base as possible. For instance, one of our mass selection populations was started with 350 plant collections from 17 different countries. These plant were open pollinated and about 3000 seedlings (grown from true seed) started in the greenhouse with 700 representing as near as possible all the various sources moved to a trellis area (for open pollination by arthropods). Seed from about 200 were used to start the next cycle. In the third cycle the number selected as seed parents was reduced to about 100 among 700 trellised plants. For short-term goals, as few as 6 plants can be used, this will result in a narrow genetic base and should provide a better chance for rapid advancement of a particular objective (for example insect resistance) (15).

After at least three cycles of intercrossing it becomes necessary to change to a two year cycle to better evaluate for yield, sprouting, and storage traits. Seedling can be evaluated in the greenhouse for disease resistance and horticultural characters; vine cuttings can be transplanted to the field for evaluation of insect resistance (appropriate controls included) and further horticultural selections in nonreplicated plots. About 150 of the best selections can be stored and evaluated for keeping quality and for palatability. In the spring the remaining selections can be rated for bedding traits. The best 75-100 selections are then planted on trellises in four or five replications. These are pollinated by arthropods and seeds collected and labeled. During the same season, vine cuttings from the plant beds are used to plant replicated field trails. Data are collected on horticultural characteristics and insect susceptibility or resistance, and the best 25 or 30 selections identified. Seed from these are used to start the next cycle of selection (15).

New plant collections can be introgressed into the mass selection population through the seed increase nursery (Jones et al. 1986).

Polycross nursery: This nursery, although similar to the mass selection nursery the number of plant entries is usually limited to no more than 30 advanced lines. These plants are replicated four times in each of two locations each year. It is a limited number of advanced lines, which are randomly crossed by a natural population of arthropods, to meet our most important short-term objectives. In this case the development of insect resistant sweetpotatoes. Each time a new line is added to the polycross, one of the previous entries is dropped (15).

Seedlings from the polycross usually provide a major source of potential cultivars and are screened for the many essential traits as in the mass selection breeding program. Selections made in the first year are tested in the second year seedling trails and in the advanced line trails for 2 more years. The best selections may be vegetatively increased for submission into the national regional trails (National Sweetpotato Collaborators Group) were they are evaluated for horticultural qualities and insect resistance (15).

## Conclusions

The researcher team at the U. S. Vegetable Laboratory has developed 8 breeding clones, and 6 cultivars with multiple resistance to the WDS complex, sweetpotato flea beetle, and grubs (6, 17, 18, 20, 22).

SCHALK, JONES, DUKES

Resume

Selection chez la patate douce pour la resistance multiple aux insectes

Le developpement d'un programme de croisements/selection de masse assure une grande diversite genetique, un rendement et une qualite de racines eleves, ainsi que des resistances envers 10 insectes ravageurs importants, dans les selections de plants individuels. Le laboratoire maraicher de Charleston aux E.-U. a rendu disponibles 8 lignees selectionnees et 6 cultivars montrant des resistances multiples aux larves de taupins, Diabrotica, altises et vers blancs.

Table 1. U. S. cultivars developed from 1971-1989 with resistance or susceptibility to soil insects.

| Cultivar | Reaction | | |
|---|---|---|---|
| | WDS[a] | Flea beetle[b] | Grubs[c] |
| Beauregard (27) | S[d] | S | I |
| Caromex (3) | I-S | I | S |
| Carver (30) | I | R | S |
| Eureka (28) | S | R | I |
| Excel (21) | R | R | R |
| Georgia Jet (10) | S | S | S |
| HiDry[e] (9) | R | S | - |
| Jasper (12) | I-S | I-R | S |
| Jewel (26) | S | R | S |
| Painter (8) | I-S | S | S |
| Pope (2) | I-S | I | I |
| Redmar (24) | I-S | S | S |
| Rojo Blanco (29) | I | I | S |
| Regal (19) | R | R | R |
| Resisto (18) | R | R | R |
| Southern Delite (20) | R | R | R |
| Sumor (7) | R | I | R |
| Travis (11) | S | R | S |
| Vardaman (1) | S | S | S |

[a]Wireworms (Conoderus falli Lane, C. vespertinus Fabricious), Diabrotica (D. balteata LeConte, D. undecimpunctata howardi Barber), Systena (S. frontalis Fabricius, S. blanda Melsheimer, S. elongata Fabricious).

Table 1 Continued

[b] *Chaetocnema confinis* Crotch.

[c] *Plectris aliena* Chapin, *Phyllophaga ephilida* (Say).

[d] S= susceptible, I= intermediate, R= resistant.

[e] High dry matter cultivar (starch).

Literature Cited

1. Allison, M. L., G. R. Ammerman, J. W. Garner, H. L. Hammett, C. Singleton, F. T. Withers, and H. D. Palmetree 1981. Vardaman, a new early maturing sweetpotato variety for Mississippi. Miss. Agric. For Exp. Stn., Inf. Sheet 1305.

2. Collins, W. W. 1982. 'Pope' Sweetpotato. HortScience 17:265.

3. Collins, W. W., D. T. Pope and D. C. H. Hsi. 1979. 'Caromex' Sweetpotato. HortScience 14:646.

4. Cuthbert, F. B. 1967. Insects affecting sweetpotato. Handbook no. 329. USDA, Wash. DC.

5. Cuthbert, F. P. Jr., and A. Jones. 1972. Resistance in sweetpotato to Coleoptera increased by recurrent selection. J. Econ. Entomol. 65:1655-58.

6. Cuthbert, F. P. Jr., and A. Jones. 1978. Insect resistance as an adjunct or alternative to insecticides for control of sweetpotato soil insects. J. Am. Soc. Hortic. Sco., 103:443-45.

7. Dukes, P. D., M. G. Hamilton, A. Jones and J. M. Schalk. 1987. 'Sumor' a multi-use sweetpotato. HortScience 22:170-71.

8. Graves, B. 1976. 'Painter', a new sweetpotato variety. Veg. Grow. News 30 (11), 1.

9. Hamilton, M. G., P. D. Dukes, A. Jones and J. M. Schalk. 1985. 'HiDry' sweetpotato. HortScience 20:954-55.

10. Harmon, S. A. 1974. 'Georgia Jet' an early yielding high quality sweetpotato cultivar for Georgia. Ga., Agric. Exp. Stn., Res. Rep. 193.

11. Hernandez, T. P., R. J. Constantin, H. Hammett, W. J. Martin, C. Clark, L. H. Rolston. 1981. 'Travis' sweetpotato. HortScience. 16:574.

12. Hernandez, T. P., T. P. Hernandez, R. J. Constantin and W. J. Martin. 1974. 'Jasper', a soil rot-resistant sweetpotato variety. La. Agric. Exp. Stn. Cir. 100

13. Jones, A. 1965. A proposed breeding procedure for sweetpotato. Crop. Sci. 5:191-92.

14. Jones, A., P. D. Dukes, and F. P. Cuthbert Jr. 1975. W-13 and W-178 sweetpotato germplasm. HortScience. 10:533.

15. Jones, A., P. D. Dukes, and F. P. Cuthbert Jr. 1976. Mass selection in sweetpotato: Breeding for resistance to insects and diseases and for horticultural characteristics. J. Am. Soc. . Hort. Sci. 101:701-04.

16. Jones, A., P. D. Dukes, and J. M. Schalk, 1986. Sweetpotato breeding. In 'Breeding Vegetable Crops' Ed. M. J. Bassett. AVI Publishing Co. Inc. Westport Connecticut chap. 1.

17. Jones, A. P. D. Dukes, J. M. Schalk, M. A. Mullen. M. G. Hamilton, D. R. Patterson and T. E. Boswell. 1980. W-71, W-115, W-119, W-125, W-149, and W-154 sweetpotato germplasm with multiple insect and disease resistance. HortScience. 15:835-36.

18. Jones, A. P. D. Dukes, J. M. Schalk, M. G. Hamilton, M. A. Mullen, R. A. Baumgardner, D. R. Patterson, and T. E. Boswell 1983. 'Resisto' sweetpotato. HortScience. 18:2512-13.

19. Jones, A., P. D. Dukes, J. M. Schalk, M. G. Hamilton, M. A. Mullen, R. A. Baumgardner, D. R. Patterson and T. E. Boswell.

1985. 'Regal' sweetpotato. HortScience 20:781-82.

20. Jones, A., P. D. Dukes, J. M. Schalk, M. G. Hamilton, and R. A. Baumgardner. 1987. 'Southern Delite' sweetpotato. HortScience. 22:329-30.

21. Jones, A., P. D. Dukes, J. M. Schalk, M. G. Hamilton. 1989. 'Excel' sweetpotato. HortScience 24:171-72.

22. Jones, A., J. M. Schalk and P. D. Dukes. 1979. Heritability estimates for resistance in sweetpotato to soil insects J. Am. Soc. Hort. Sci. 104:424-26.

23. Jones, A., J. M. Schalk and P. D. Dukes. 1987. Control of soil insect injury by resistance in sweetpotato. J. Am. Soc. Hort. Sci. 112:195-97.

24. Maryland AES 1971. Mimeo Rel. Notice. Jan. 19. Redmar.

25. Maryland AES 1972. Mimeo Rel. Notice. Jan. 17. Goldmar.

26. Pope, D. T., L. W. Nielson, and N. C. Miller. 1971. 'Jewel' a new sweetpotato variety for North Carolina. N. C., Agric. Exp. Stn. Bull. 442.

27. Rolston, L. H., C. A. Clark, J. M. Cannon, W. M. Randell, E. G. Riley, P. W. Wilson, and M. L. Robbins. 1987. 'Beauregard' sweetpotato. HortScience 22:1338-39.

28. Scheuerman, R. W., T. P. Hernandez, W. J. Martin, C. Clark, R. J. Constantin and H. Hammett. 1981. 'Eureka' sweetpotato. HortScience 16:689.

29. Whatley, B. T. and B. R. Phills 1977. 'Rojo Blanco' sweetpotato. HortScience 12:265.

30. Whatley, B. T., and B. R. Phills 1977. 'Carver' sweetpotato. HortScience 12:266.

## Workshop 4

## FRUIT and CEREAL CROPS: RASPBERRIES and CEREALS

# RESISTANCE TO THE VIRUS VECTOR APHID AMPHOROPHORA IDAEI IN RASPBERRY

A.N.E. BIRCH and A.T. JONES
Scottish Crop Research Institute,
Invergowrie, Dundee DD2 5DA, Scotland

Summary
Field cage, glasshouse and laboratory tests have been used to quantify antixenosis (alate and apterous aphid settling) and antibiosis (7 days reproduction) in a range of raspberries containing minor and major gene resistance. Responses of the main aphid biotypes to different resistant genotypes have also been studied. Currently we are examining in more detail probing behaviour using EPG and olfactory responses, in relation to leaf chemistry.

## 1. Introduction

The large raspberry aphid, Amphorophora idaei, is an important pest of raspberry and is the main vector of four viruses commonly infecting raspberries in Europe. Chemical control of A. idaei is not very effective in decreasing virus transmission, and no sources of immunity to these viruses have been found in Rubus. Several genes for resistance to A. idaei have been identified, some differing in their effectiveness against the four known biotypes of A. idaei in the UK (Jones, 1988). When tested under field conditions, aphid resistance was shown to be very effective in controlling numbers of A. idaei and also in preventing virus infection. Currently more than 80% of the UK raspberry hectarage is planted with cultivars containing either minor (polygenic) or major (single gene) resistance to A. idaei. The extensive use of resistance genes to A. idaei is likely to pose a selection pressure on the aphid. In some localities in the UK an increase has been found in biotype 2, which can colonise raspberries containing the resistance gene $A_1$. The most recently introduced gene, $A_{10}$, confers resistance to all four known biotypes of A. idaei, but the mechanism(s) of resistance due to this and the other 13 reported resistance genes are unknown. To counter the potential threat of further A. idaei biotype development, studies have been started to gain a more fundamental understanding of the mode of action of different A. idaei-resistance genes and their interactions with aphid biotypes.

## 2. Progress

Aphid behaviour and reproduction has been studied on raspberry cultivars containing no resistance to A. idaei (S), partial resistance controlled by minor genes (r) and strong resistance controlled by major gene $A_1$ (resistance to

biotypes 1 and 3) and $A_{10}$ (resistance to all four biotypes). Several different types of assessment have shown consistent differences in levels of aphid resistance, for mature plants, seedlings and also excised leaves (Birch & Jones, 1988).

Infestation tunnel assessments (antixenosis, antibiosis)

Potted plants were exposed to artificially high numbers of biotype 1 A. idaei alatae. The numbers of alatae settling after 40 h was used as a relative measure of antixenosis shown by the cultivars. Table 1 shows that even under the very high inoculum pressure, significantly fewer aphids settled on cultivars with major gene resistance, and to a lesser extent, on those with minor gene resistance than on the susceptible control, cv. Malling Jewel. A subset of these plants, after removal of alatae, was transferred to a glasshouse. Five days later the numbers of nymphs that developed was counted. Table 1 shows that nymph production was lowest respectively on the cultivars containing genes $A_{10}$, $A_1$ and minor gene resistance. Nymph development was also slowest on cultivars containing the $A_{10}$ gene.

Table 1. Levels of antixenosis and antibiosis to biotype 1 Amphorophora idaei in raspberry cultivars differing in resistance.

| Cultivar and resistance class | | Alatae settling after 40 h | No. nymphs after 5 days |
|---|---|---|---|
| Malling Jewel | S | 337 | 492 |
| Norfolk Giant | r | 239 | 298 |
| Glen Prosen | $A_1$ | 135 | 181 |
| Malling Landmark | $A_1$ | 104 | 40 |
| Autumn Bliss | $A_{10}$ | 111 | 1 |
| Joy | $A_1 + A_{10}$ | 98 | 6 |

An unexpected difference in the level of antibiosis expressed against biotype 1 was found between two cultivars reported to contain gene $A_1$, Glen Prosen and Malling Landmark. Further tests were carried out to explore this difference, using seedling and excised leaf bioassays.

Laboratory and glasshouse assessments

In further tests, the settling of A. idaei biotypes 1 and 2 (apterae) were compared on young plants and excised leaflets floating on water (floating leaf test) over 48 h. Table 2 shows that in general there was good agreement between screens using young plants and the simpler floating leaf test. The 48 h floating leaf test detected both moderate and strong levels of resistance to biotypes 1 and 2 of A. idaei (Table 3).

Table 2. Percentage of A. idaei biotypes 1 and 2 remaining on young plants and floating leaflets of four raspberry cultivars after 48 h.

| Cultivar and resistance class | | Young plants biotype 1 | Young plants biotype 2 | Floating leaf biotype 1 | Floating leaf biotype 2 |
|---|---|---|---|---|---|
| Malling Jewel | S | 75 | 75 | 95 | 95 |
| Glen Clova | r | 41 | 64 | 84 | 91 |
| Malling Landmark | $A_1$ | 20 | 75 | 30 | 75 |
| Autumn Bliss | $A_{10}$ | 10 | 10 | 0 | 5 |

Table 3. Percentage of A. idaei biotypes 1 and 2 remaining on floating leaflets of raspberry cultivars differing in resistance to A. idaei.

| Cultivar and resistance class | | % A. idaei remaining after 48 h biotype 1 | biotype 2 |
|---|---|---|---|
| Malling Jewel | S | 100 | 97 |
| Glen Clova | r | 90 | 87 |
| Glen Prosen | $A_1$ | 50 | 68 |
| Glen Moy | $A_1$ | 40 | 74 |
| Malling Landmark | $A_1$ | 20 | 87 |
| Delight | $A_1$ | 5 | 66 |
| Joy | $A_{10}$ | 20 | 40 |

The difference in settling response of biotype 1 to resistance gene $A_1$ in SCRI cvs Glen Prosen and Glen Moy compared with IHR-East Malling cvs Delight and Malling Landmark was confirmed using this test. Recent testing of seedling progeny from a Glen Prosen x susceptible raspberry cross indicates that the resistance in Glen Prosen may be due to minor genes and not to gene $A_1$. This is possible, as the standard plant breeders screen does not readily distinguish between major gene resistance and strong minor gene resistance.

## Settling and probing behaviour studies

Visual evaluation of settling and superficial probing behaviour of biotype 1 A. idaei on Malling Jewel (susceptible) and Autumn Bliss ($A_{10}$) revealed differences during the first 20 minutes on each host. Aphids were more restless and made more short (<0.5 min) probes on Autumn Bliss than on Malling Jewel. On Malling Jewel, 70% of the total time was spent making long probes (>3 min), compared with only 25% of the total time on Autumn Bliss. These differences are now being investigated in detail, using an Electrical Penetration Graph (EPG) system.

Alate A. idaei respond to raspberry leaf volatiles and various olfactometer tests are currently being used to quantify aphid responses to leaf odours. Raspberry leaf surface chemistry is also being examined, to identify chemical factors involved in settling and probing responses of A. idaei.

## 3. Résumé

Des essais en cages au champ, en serre et en laboratoire ont été effectués pour quantifier l'antixénose (degre d'etablissement des ailés et des aptères) et l'antibiose (reproduction en 7 jours) dans une série de framboisiers contenant un gène mineur et majeur de résistance. La réponse des 2 biotypes principaux de puceron aux divers génotypes résistants a aussi été examinée. Actuellement, nous examinons plus en détail le comportement de "sondage" des pucerons, par l'utillisation de l'enregistrement électrique de pénétration (EPG) et l'étude de la réponse olfactive en relation avec la composition chimique foliaire.

## 4. References

BIRCH, A.N.E. & JONES, A.T. 1988. Levels and components of resistance to Amphorophora idaei in raspberry cultivars containing different resistance genes. Ann. appl. Biol. 113: 567-578.

JONES, A.T. 1988. The influences of cultivating new raspberry varieties on the incidence of viruses in raspberry crops in the UK. Aspects of appl. Biol. 17: 179-186.

# INVESTIGATIONS OF THE MECHANISMS OF RESISTANCE OF THE GENUS RIBES TO THE GALL MITE CECIDOPHYOPSIS RIBIS

ROMEO HERR
Vorderer Alter Berg 7, D-7507 Pfinztal, Federal Republic of Germany

## Summary

The gall mite Cecidophyopsis ribis is the most serious pest in the black currant. The red currants, the gooseberry, Ribes ussuriense and R.nigrum sibiricum are resistant to gall mite and were partly chosen as donors in breeding for gall mite resistance. The nature of this resistance is not well understood. Primarily information about the type of resistance were obtained by infestation experiments in the field. Resistant and susceptible Ribes plants were inoculated by tying big buds taken from black currants on young shoots of the test plants in spring. The behaviour of the gall mite was recorded one and four weeks following inoculation. The experiments showed that there were both antixenosis (the gall mites entered the bud to a lower extent) and antibiosis effects (the gall mites entered the bud but died inside). The phenolic contents of Ribes buds, especially of the susceptible black currants are rather high. So it is possible that phenolics are involved in mite-plant-relationships. The phenolic compounds of currants and gooseberry were analysed using HPLC. Not a single phenolic substance was correlated with resistance but there exist characteristic phenolic patterns for susceptible and for resistant plants.

## Introduction

The black currant gall mite Cecidophyopsis ribis (Westwood, 1869), Eriophyidae, Acari, is the most serious pest in black currant (Ribes nigrum L.) in Europe. The infested buds change to galls ("big buds") induced by sucking of mites. Furthermore the mite is the vector of reversion disease which is the most serious problem of black currant (MASSEE 1952, KRCZAL 1976).

In spring the mites emigrate from old desiccating galls and, after a short period of migration, they enter young buds. The invasion starts at the end of April, and the period of most intensive invasion occurs during and after the blossoming of black currant. Six weeks after entering a young bud egg-laying begins. The infested buds change to galls. By the next spring the population in the big bud has increased to about several thousands of mites (COLLINGWOOD & BROCK 1959, SMITH 1961).

West-European black currant cultivars are more or less susceptible to gall mite. Red currants (Ribes sativum Syme, R.petraeum Wulfen, R.spicatum Robson, R.multiflorum Kit.) and gooseberries (R.uva-crispa L.) are resistant. Breeding programms were carried out to transfer resistance to gall mite from gooseberry to black currant. Furthermore it was possible to transfer resistance from Ribes ussuriense Jancz. and R.nigrum ssp. sibiricum to black currant (ANDERSON 1971, KNIGHT et al. 1974).

In this presentation the type of resistance of six Ribes cultivars to the gall mite Cecidophyopsis ribis will be classified as "antixenosis" (i.e. the mites did not enter the bud) or "antibiosis" (i.e. the mites entered the bud but died inside). Furthermore the results of the HPLC separation of the phenolic compounds extracted from Ribes buds will be discussed.

## Infestation experiments

The Ribes bushes were planted out for field experiments in Stuttgart-Hohenheim (South-West Germany) in 1984 - 1986. The test plants were inoculated in 1987 and 1988 five times between 20 April and 20 May by tying two big buds taken from

black currant. The inoculated shoots were examined in June, about 3 weeks after the last inoculation. For examination, the shoots were cut from each cultivar and dissected in the laboratory. The attack of gall mites at the meristems of the young buds was registered as:

"frequency of attack" : relative proportion of young buds attacked by mites,
"intensity of attack" : number of mites per attacked bud
and "attack" : frequency x intensity.

Table 1 gives the results of the infestation experiments for 1988 (in 1987 the results were similar).

Table 1: Infestation of meristems of young Ribes buds in spring and formation of big buds in winter following inoculation with C.ribis

| | Gall mites at meristems<br>frequency x intensity = attack | | | proportion of survivors (%) at the meristems in June | no. and % of big buds/bush in winter |
|---|---|---|---|---|---|
| red currants | | | | | |
| Fay's Prolific | 0.31 | 3.2 | 1.00 | 0 | 0 |
| Rondom | 0.31 | 2.5 | 0.77 | 0 | 0 |
| black currant | | | | | |
| Goliath | 0.40 | 4.1 | 1.64 | 90 | 46 (5%) |
| Titania | 0.20 | 1.7 | 0.34 | 100 | 38 (4%) |
| hybrids | | | | | |
| Ni 289 | 0.04 | 1.8 | 0.07 | 0 | 0 |
| Josta | 0.04 | 2.6 | 0.11 | 0 | 0 |
| gooseberry | | | | | |
| Weiße Triumph | 0.00 | 0.0 | 0.00 | (no mites) | 0 |

Ni 289 : black currant x R.ussuriense
Josta : tetraploid hybrid from black currants x gooseberries

The gall mites have to enter the centre of the young buds and reach the meristem for feeding, oviposition and gall induction. The gooseberry Weiße Triumph was free of attack (Tab.1), this type of resistance may be called antixenosis. The attack on the buds of the hybrids Ni 289 and Josta was significantly lower than the attack observed on the black and the red currants; the two hybrids showed some degree of antixenosis.

After successful entry into the centre of the bud the mites can survive only an the meristems of black currants (proportion of survivors: 90 - 100%, Tab.1). On the red currants and the hybrids the mites within the buds were dead by June, i.e. 3 weeks after the last date of inoculation. The effect of high and early mortality on the meristems may be called antibiosis. On those cultivars which showed antibiosis in June no big bud could be found in the following winter (Table 1). Red currant buds which were attacked in the meristems changed dramatically: by May the whole bud inclucing the meristems were deformed, dark brown and dead. The deformation and browning of the attacked meristems seemed to be a result of a hypersensitive reaction. On the dead meristem no gall mites can survive.

Analysis of phenolic compounds of Ribes buds

The Ribes buds were collected in winter between November and January. The phenolic compounds were extracted from the buds in a SOXHLET-device with acetone-methanol. The extract was purified by column-chromatography on polyamid.

To facilitate the final HPLC analysis the extract was hydrolysed with 2 N HCl (100°C, 40 min.) or 2% NaOH (20°C, 90 min.). The HPLC was carried out with a reversed-phase-column, gradient elution (5% formic acid - methanol-butanol) and UV (280 nm) detection. Table 2 shows just 7 of the 21 resulting peaks of the HPLC chromatogramm.

Tab.2: HPLC separation of phenolic compounds in Ribes buds after acid or alkaline hydrolysis (mg/g dry weight)

| | cinnamic acids | | flavonols | | unknown substances | | |
|---|---|---|---|---|---|---|---|
| | caffeic | p-coumaric | myricetin | quercetin | | | |
| $t_R \longrightarrow$ | 7:00 | 10:40 | 14:40 | 18:10 | 6:15 | 9:40 | 15:20 |
| red currants | | | | | | | |
| Fay's Prolific | 0.4 | 0.2 | 0.1 | 0.5 | 3.6 | 0.1 | - |
| Rondom | 0.4 | 0.2 | 0.1 | 0.5 | 2.8 | 0.1 | - |
| black currants | | | | | | | |
| Goliath | 0.4 | 0.4 | 1.2 | 2.4 | 1.6 | 0.5 | 1.2 |
| Titania | 0.3 | 0.7 | 1.2 | 3.8 | 2.7 | 0.4 | 2.4 |
| hybrids | | | | | | | |
| Ni 289 | 0.9 | 0.5 | 1.1 | 4.0 | 2.7 | - | 0.6 |
| Josta | 0.3 | 0.2 | 0.6 | 3.5 | 3.8 | 0.2 | 0.05 |
| gooseberry | | | | | | | |
| Weiße Triumph | 0.1 | 0.01 | 0.1 | 1.5 | 3.2 | 0.1 | - |

$t_R$ : retention time (minutes)
concentration of unknown substances were calculated as quercetin

Usually the susceptible black currants had high concentrations of phenolic substances, especially flavonols. The phenolic pattern of the resistant hybrid Ni 289 was very similar to that of black currants expect for two peaks of unidentified substances: $t_R$ 9:40, 15:20 min. The resistant red currants and the resistant gooseberry had similar or mostly lower concentrations of phenolic substances except for one peak: $t_R$ 6:15 min. (Tab.2). The phenolic contents of the resistant Josta is influenced genetically both by the susceptible black currant and the resistant gooseberry: the concentration of cinnamic acids and flavonols in the Josta buds are similar to the concentration in black currant. However, the peaks 6:15, 9:40 and 15:20 seem to be influenced by dominant genes of the gooseberry.

Characteristic phenolic patterns exist for susceptible plants: high concentrations of flavonols, of peaks 9:40 and 15:20 and low concentrations of peak 6:15. The characteristic pattern for a resistant plant is: low concentration of flavonols or peaks 9:40 or 15:20 or high concentration of peak 6:15. It seems that no single substance is correlated with resistance. It is only a combination of substances that leads to different degrees and different types of resistance. The mode of action and the effect of the phenolics on the mite-plant-relationships are not known. According to the results presented in table 2 it should be possible to test Ribes genotypes in breeding programms by chromatography of bud phenolics in order to obtain information on the resistance or susceptibility of the plant.

Résumé
Recherche sur le mécanisme de la résistance du genre Ribes à l'ériophyide du cassis Cecidophyopsis ribis
L'ériophyide du cassis, Cecidophyopsis ribis, est le ravageur le plus important

du cassis. La groseille rouge, la groseille à maquereau, Ribes ussuriense et Ribes nigrum sibiricum sont résistants à cet acarien et ont partiellement servi de donneurs lors de la sélection pour la résistance à ce ravageur. Les facteurs de cette résistance ne sont pas bien connus. Les premières informations sur le type de résistance ont été obtenues par des essais d'infestation au champ. Des plants résistantes et sensibles de Ribes ont été inoculées en fixant au printemps de grands bourgeons provant du cassis à de jeunes pousses de plantes à tester. Le comportement de l'ériophyide a été observé une et quatre semaines après l'infestation. On peut démontrer des effects d'antixénose (les acariens ne pénètrent le bourgeon que dans une faible mesure) et d'antibiose (les acariens pénètrent, puis meurent à l'intérieur du bourgeon). Le contenu phénolique des bourgeons de Ribes, spécialement de ceux du cassis, est relativement élevé. Il est possible que ces substances soient impliquées dans la relation ravageur-plant hôte. Les composés phénoliques du cassis et de la groseille rouge et à maquereau ont été analysés par HPLC. Aucune substance phénolique n'est corrélée avec la résistance, mais il y a un spectre de phénols caractéristique des plantes sensibles et résistantes.

References

Anderson, M.M. 1971: Resistance to gall mite (Phytoptus ribis Nal.) in the Eucoreosma section of Ribes
Euphytica 20: 422 - 426

Collingwood, C.A. & Brock, A.M. 1959: Ecology of the black currant gall mite (Phytoptus ribis Nal.)
The Journal of Horticultural Science 34: 176 - 182

Knight, R.L., Keep, E., Briggs, J.B. & Parker, J.H. 1974: Transference of resistance to black-currant gall mite, Cecidophyopsis ribis, from gooseberry to black currant
The Annals of Applied Biology 76: 123 - 130

Krczal, H. 1976: Investigations on the effects of reversion disease on crop and growth of black currant
Acta Horticulturae 66: 91 - 97

Massee, A.M. 1952: Transmission of reversion of black currants
Annual Report of East Malling Research Station for 1951: 162 - 165

Smith, B.D. 1961: Population studies of the black currant gall mite (Phytoptus ribis Nal.)
Annual Report of the Agricultural and Horticultural Research Station Long Ashton for 1960: 120 - 124

# The role of hydroxamic acids in the resistance of maize to insects

Stephen Morse
Biology Department, Building 44, The University,
Southampton SO9 5NH, U. K.

## Summary

A number of stress factors, including damage and drought, were found to influence the concentration of hydroxamic acid (Hx) in maize. Stress generally caused an increase in the levels of Hx relative to unstressed plants. However, the growth rate of Rhopalosiphum padi aphids was apparently unaffected by the level of Hx in the host leaf tissue.

## 1. Introduction

The benzoxazin-3-one group of hydroxamic acids (Hx) occur in many Gramineae, and have been implicated in resistance to insect pests as well as to a wide range of fungal and bacterial pathogens (Niemeyer, 1989). They are normally present in the plant tissue as glucosides and are enzymically hydrolysed upon tissue damage to release the toxic aglucone. The glucosides are located mostly in the phloem parenchyma, but they also occur in leaf mesophyll cells. The commonest Hx in maize and wheat is 2,4-dihydroxy-7-methoxy-1,4-benzoxazin-3-one (DIMBOA). It has been shown in wheat that a good negative correlation exists between aphid growth rate and Hx concentration (Bohidar et al., 1986). In maize, Hx has been negatively correlated with damage by the first generation of the European corn borer (Ostrinia nubilalis).

The aim of the work reported here was to determine the effects of some stress factors on the concentration of Hx in maize, and to study the relationship between Hx concentration and the growth of some insect herbivores.

## 2. Method

Hydroxamic acid determinations in maize tissue were carried out using the colorimetric method described by Bohidar *et al.* (1986), and a HPLC method based on that decribed by Niemeyer *et al.* (1989).

The maize cultivar used for most of the experiments was LG 11 (Elsoms seeds, U. K.), a temperate hybrid, and most of the work took place in a glasshouse maintained at approximately 20 centigrade and a 16 hour photoperiod.

## 3. Results

The concentration of Hx in each maize leaf was found to show an approximately logarithmic decline with leaf age, with higher levels normally being found in the young tissue. Artificial damage (crushing) inflicted on a maize leaf was found to increase the concentration of Hx in the whole plant ($F = 4.759$ ; d.f. = 1,96 ; $P<0.05$), and in leaves which had been isolated and supplied with a nutrient solution ($F = 12.24$ ; d.f. = 1, 21 ; $P<0.01$). The concentration of Hx in drought stressed plants was also found to increase relative to controls ($F = 12.612$ ; d.f. = 1, 108 ; $P<0.001$), largely due to an increase in total Hx. However, abcissic acid, a plant hormone produced under conditions of drought stress, was found to decrease significantly the concentration of Hx in isolated maize leaves relative to those supplied only with water ($F = 17.404$ ; d.f. = 1, 14 ; $P<0.01$). An auxin solution had no apparent effect.

Aphids (*Rhopalosiphum padi*) caged on a maize leaf artificially damaged by crushing, showed significantly higher mortality than those caged on undamaged controls (G-statistic, with Williamsons correction = 9.3079 ; d.f. = 1 ; $P<0.005$). Aphids caged on a maize leaf which had previously been infested with a colony of aphids also showed a significantly lower relative growth rate in the first 24 hours compared to controls ($F = 4.627$ ; d.f. = 1,32 ; $P<0.05$). By the use of HPLC, the DIMBOA glucoside has been found in the honeydew of *R. padi* aphids feeding on maize.

Investigations of the growth rate of *R. padi* on maize varieties having different concentrations of Hx in their leaf tissue has, however, failed to find a significant negative link between the two. For example, the relative growth rate of the aphids on B49, a variety having a high level of Hx, was found to be much higher than on B36, a variety low in Hx (Figure 1).

## 4. Discussion

The concentration of Hx within maize tissue can be influenced by stress factors such as drought and mechanical damage. Under such conditions, Hx levels usually increase ; this could be interpreted as an adaptive strategy to increase defence at a time of vulnerability to herbivores and pathogens. Leaf damage inflicted artificially or by aphids was found to have a detrimental effect on subsequent aphid survival and/or growth rate, but this may not necessarily be due to increased levels of Hx. Indeed, the growth rate of R. padi on a number of maize varieties does not seem to be negatively correlated with Hx concentration within the leaf tissue, indicating that for this plant species resistance factors are much more complex.

Future work will examine the growth rate of other insect herbivores on maize varieties having different levels of Hx in their leaf tissue.

MORSE

Résumé

Le rôle des acides hydroxamiques dans la résistance du maïs aux insectes.

Un certain nombre de facteurs de stress, dont des dommages physiques causés aux plantes et la sécheresse, influence la concentration des acides hydroxamiques (Hx) dans le maïs. Le stress amène généralement à une augmentation du niveau des Hx par rapport aux plantes indemnes. Toutefois, le taux de croissance du puceron Rhopalosiphum padi n'a apparemment pas été affecté par le niveau des Hx dans les tissus de la plante-hôte.

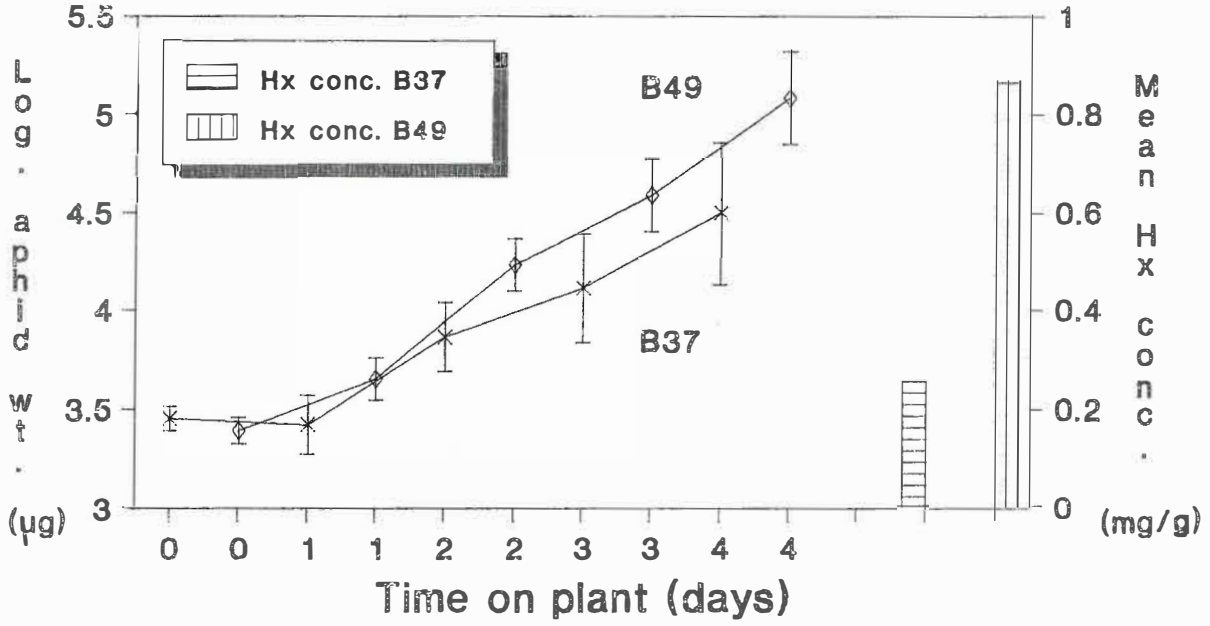
## References

BOHIDAR, K., WRATTEN, S. D. and NIEMEYER H. M., 1986. Effects of hydroxamic acids on the resistance of wheat to the aphid Sitobion avenae. Annals of Applied Biology 109: 193 - 198.

NIEMEYER, H. M., 1988. Hydroxamic acids (4-hydroxy-1,4-benzoxazin-3-ones), defence chemicals in the Gramineae. Phytochemistry 27: 3349 - 3358.

NIEMEYER, H. M., PESEL, E., COPAJA, S. V., BRAVO, H. R., FRANKE, S. and FRANCKE, W., 1989. Changes in hydroxamic acid levels of wheat plants induced by aphid feeding. Phytochemistry 28: 447 - 449.

## Figure 1. Growth of *R. padi* on 2 maize varieties, B37 and B49.




**The aphid growth is displayed in terms of log (base e) mean aphid weight in micrograms and 95% confidence limits.**

# THE RESISTANCE OF WHEAT TO THE APHIDS SITOBION AVENAE AND RHOPALOSIPHUM PADI IN RELATION TO LEVELS OF HYDROXAMIC ACIDS.

DEBORAH J. THACKRAY
Department of Biology, Building 44, The University, Southampton, SO9 5NH, UK

## SUMMARY

Within hexaploid and tetraploid Triticum material total plant concentrations of hydroxamic acids (Hx) explained a significant proportion of the variation in intrinsic rate of natural increase ($r_m$) of the cereal aphid Sitobion avenae. However, for diploid Triticum and Aegilops taxa, the relationship was not significant. Significant correlations were also found between taxa with respect to resistance to the aphid Rhopalosiphum padi. Although the concentrations of Hx in whole plants declined during seedling growth, concentrations of Hx in newly-emerging leaves remained high in plants of all ages, including in the emerging flag-leaves of mature plants. Aphid infestation and artificial damage to the plant were followed by increases in Hx concentrations in some situations, but these were rapid and appeared to be short-lived. Significant correlations were also found between resistance to S. avenae and Hx concentrations in the flag leaves of mature Chilean and UK wheat plants.

## 1. INTRODUCTION

The possibility of exploiting inherited, low-level resistance in wheat has prompted the screening of many British cultivars and breeding lines of hexaploid wheat, Triticum aestivum L., but resistance levels have not varied markedly (Lowe, 1982). The progress of plant breeders in the search for resistant genes is still restrained by the absence of a reliable, rapid and convenient assay for resistance, and by the lack of information on the mechanisms of resistance when it is found and on its genetic basis.

Hydroxamic acids (Hx) occurring in cereal extracts, in particular the compound 2,4-dihydroxy-7-methoxy-1,4-benzoxazin-3-one (DIMBOA), have been shown to be involved in the resistance of cereals to bacteria, fungi and several insects including the aphid species Metopolophium dirhodum (Wlk.), Schizaphis graminum (Rond.) and Rhopalosiphum maidis (Fitch) (Niemeyer, 1988). Sitobion avenae (F.) has been investigated in this context only in preliminary work by Bohidar, Wratten & Niemeyer (1986) who found that 96 % of the variance in the resistance of seedlings of six cultivars was explained by concentrations of Hx.

A two-year project at Southampton aimed to assess antibiotic resistance to the cereal aphids S. avenae and Rhopalosiphum padi (L.) in relation to levels of Hx in a wide genetic range of cultivars and species of the genus Triticum and to attempt to explain the residual variation by studying environmental and temporal factors affecting Hx concentrations.

## 2. MATERIALS AND METHODS

Seedling plants were mainly used for the following reasons. Firstly, the concentration of Hx for the total plant is highest at seedling emergence and declines with age in most wheats (so that quantification of lower concentrations becomes increasingly less accurate). Secondly, whilst S. avenae can be particularly damaging to mature plants during grain-ripening, it and R. padi can be important pests of young seedlings in the autumn, as vectors of BYDV.

A total of 20 lines were investigated in the study including representatives of hexaploid, tetraploid and diploid Triticum species. The methods used for producing test insects and plants for assessment of Hx concentration and aphid performance were similar to those used by Bohidar et al. (1986). The intrinsic rate of natural increase ($r_m$; Birch, 1948) was used as a measure of pre-reproductive development time and subsequent fecundity for Sitobion avenae introduced onto plants at the early two-leaf

stage (G.S. 11-12; Zadoks, Chang & Konzak, 1974), about 7 days after seedling emergence. At the same time concentrations of Hx were determined in uninfested plants of the same cohort. The method of Hx extraction followed that of Bohidar et al. (1986) and was based on the colorimetric absorption of a hydroxamic acid-ferric chloride complex. Hx were also assessed in whole plants of a variety of ages and individual assessments were made of leaves of different ages in two-, three-, four- and five-leaved plants.

Since the measurement of aphid performance used in the major screening of 20 lines was made over a considerable period of time (at least 14 days), fluctuations in Hx levels and in environmental conditions could have contributed to the variation found in the relationship between $r_m$ and concentrations of Hx. The relationship between aphid performance and Hx levels was therefore re-assessed in six lines under carefully controlled environmental conditions, the mean relative growth rate (mrgr) being used so as to considerably shorten the length of the assessment period required to give a reliable measure of aphid performance on each line. The mrgr for each aphid species on each cultivar was calculated following the method of van Emden (1969) as:

$$\frac{\log \text{final weight} - \log \text{initial weight}}{3 \text{ days}}$$

The mrgr of S. avenae was also used in experiments designed to study the effect of damage and environmental changes on aphid performance and Hx levels.

## 3. RESULTS

The relationship between concentrations of Hx and $r_m$ for hexaploid and tetraploid Triticum material is shown in Fig. 1. Values for concentrations of Hx ranged from 1.98 to 27.15 m mole/kg dry weight and were particularly high in some of the tetraploid wheats such as Triticum durum cv. SNA3, Triticum turgidum and Triticum dicoccon. Values for $r_m$ ranged from 0.140 to 0.309, the highest values (indicating the least resistance) being associated predominantly with the hexaploid wheats and the lowest values with some of the tetraploid wheats. The total concentration of Hx in the plant explained a significant proportion of the variation in intrinsic rate of increase of S. avenae on hexaploid and tetraploid Triticum material ($\log y = 0.61 - 0.15 \log x$; $r = -0.59$; $P < 0.01$).

Although total plant concentrations of Hx declined rapidly during the early seedling growth period, concentrations of Hx were highest in the emerging leaves of seedlings of T. durum cv. SNA3 and T. aestivum cv. Likay but declined sharply as the leaves aged (Fig. 2). At all plant ages, the last leaf to emerge had the highest concentrations of Hx. The newly emerging flag-leaf of Triticum aestivum cv. Mission had relatively high concentrations of Hx (10.8 m mole/kg dry weight), which declined rapidly during anthesis to 1.1 m mole/kg dry weight (Thackray, 1988).

Plants grown in John Innes No. 2 compost with added nitrogen fertiliser had higher Hx concentrations and produced lower mrgr values than those with a reduced nitrogen supply. Aphid infestations and artificial damage to the plant were followed by increases in Hx concentrations in some situations but these were rapid and short-lived (Thackray, Morse & Leech, 1988).

When mean relative growth rate was used as a measure of performance for S. avenae on six selected cultivars under controlled environmental conditions, the correlation between aphid performance and Hx levels in the oldest leaf of seedlings was very strong ($r = -0.69$; $P < 0.05$)(Fig. 3). Aphid mrgr and the Hx concentrations of flag-leaves of the same cultivars were also strongly correlated.

## 4. CONCLUSIONS

Although resistance to aphids in modern wheat is generally low, a wide range of resistance can be seen when the range of genetic plant material screened is increased. Hx appear to explain 35 % of the resistance to

FIGURE ONE

The relationship between $r_m$ of Sitobion avenae and [Hx] in 7-day-old seedlings of hexaploid and tetraploid Triticum

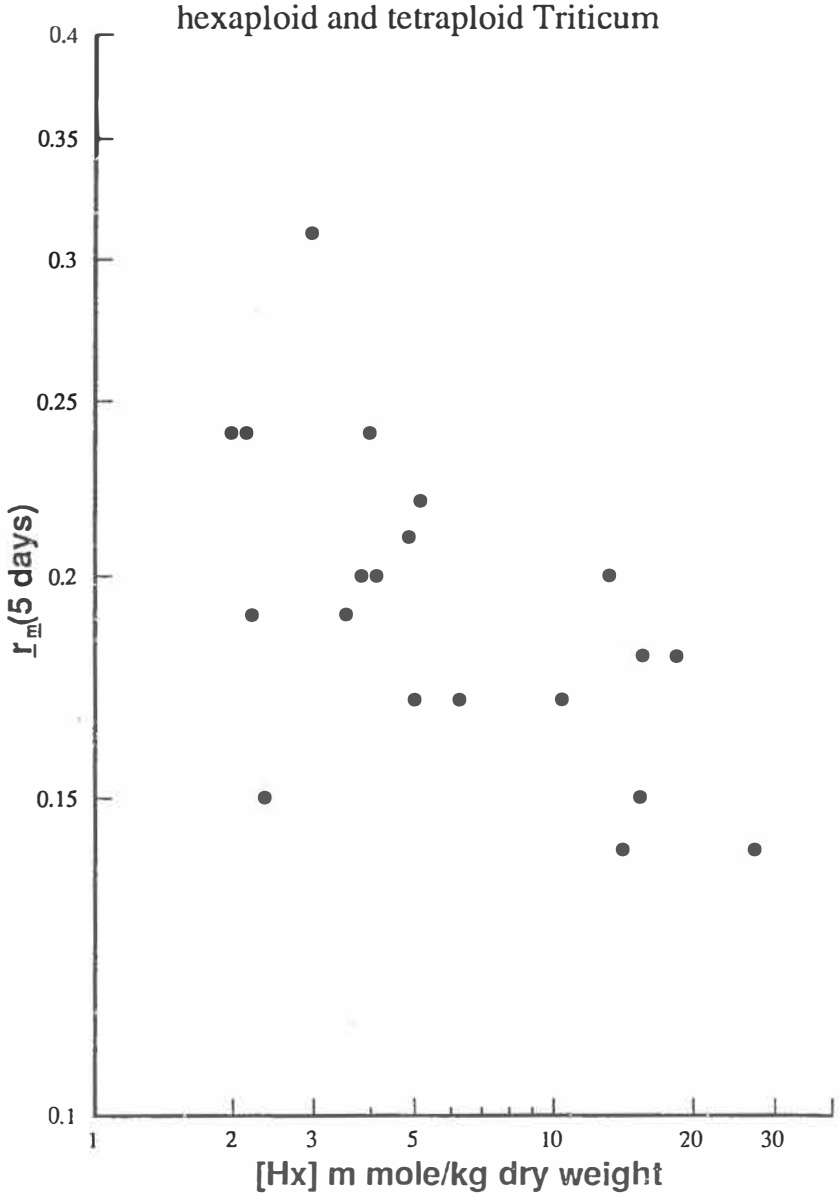


FIGURE TWO

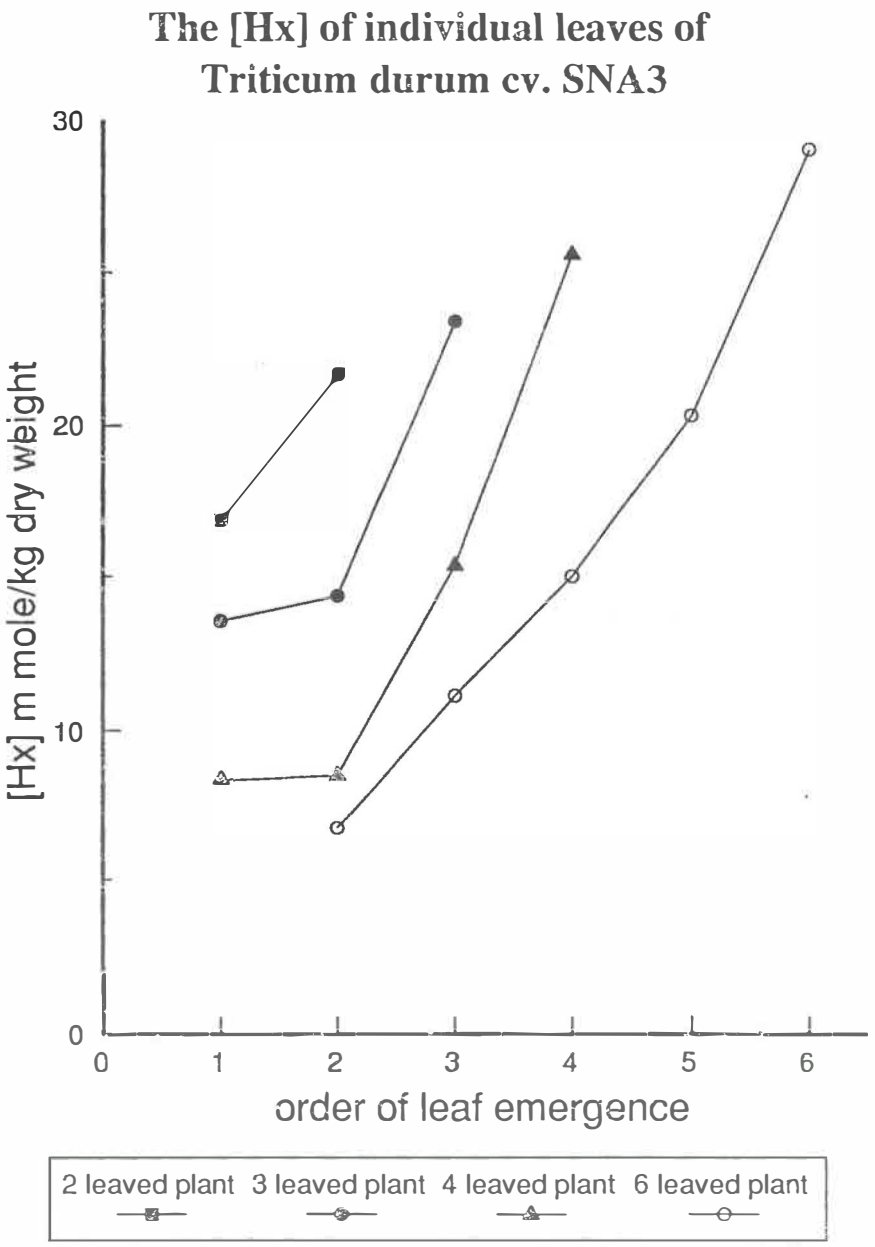
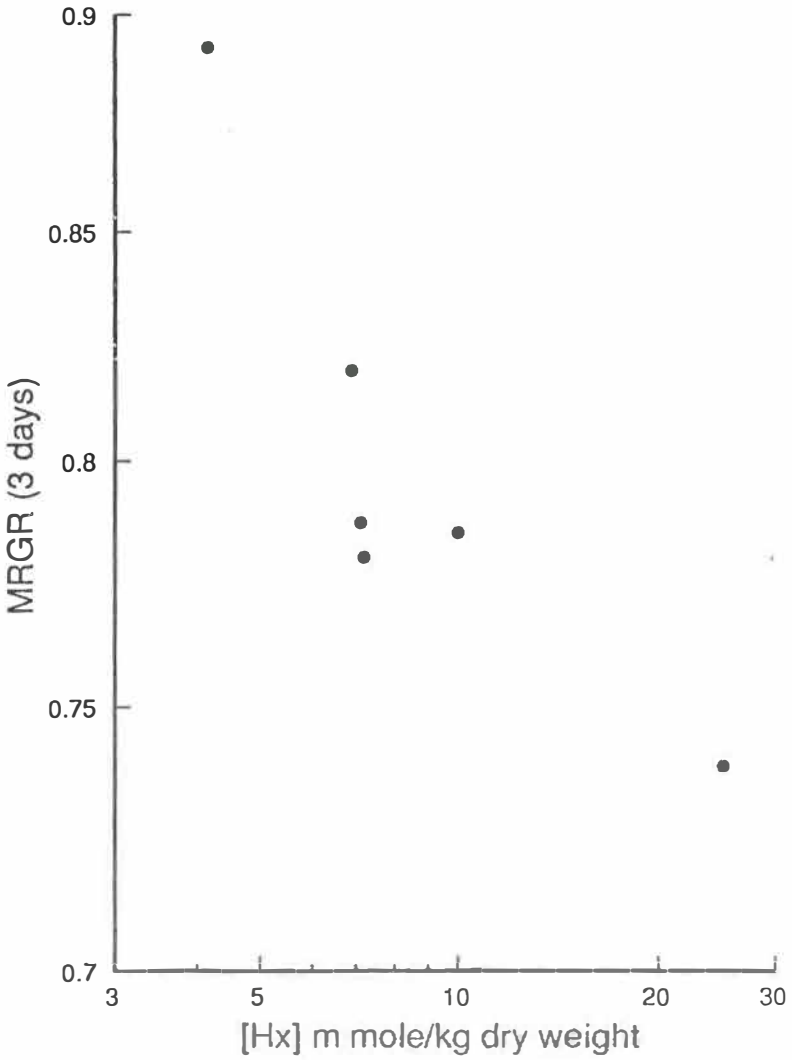

The [Hx] of individual leaves of
Triticum durum cv. SNA3
[Hx] m mole/kg dry weight
30
20
10
0
0
1
2
3
4
5
6
order of leaf emergence
2 leaved plant
3 leaved plant
4 leaved plant
6 leaved plant

FIGURE THREE

**The relationship between MRGR of Sitobion avenae and [Hx] in the first leaf of 7-day-old seedlings of 6 cultivars**



Sitobion avenae in a selection of 20 seedling tetraploid and hexaploid wheats when $r_m$ is the measure of aphid performance. When environmental conditions are more strictly controlled and mrgr over three days used, 47 % of the resistance to S. avenae is explained by Hx in the oldest leaf of six seedling wheats. The localisation of Hx in areas of sensitive new growth has important implications for the plant's resistance to aphid damage.

From this study, a useful matrix of data giving the taxanomic pattern of resistance within Triticum has emerged and it is hoped that the increasing knowledge of the behaviour of Hx within the plant might lead to the use of hydroxamic acid analysis in plant breeding programs, both as a standard when selecting promising lines for resistance to cereal aphids and also in the isolation of resistant genes.

## 5. RESUME

LA RESISTANCE DU BLE ENVERS LES PUCERONS SITOBION AVENAE ET RHOPALOSIPHUM PADI EN RELATION AVEC LE NIVEAU DU CONTENU EN ACIDES HYDROXAMIQUES.

Les concentrations d'acides hydroxamiques (Hx) parmi des plantes entières de Triticum héxa- ou tétraploïdes expliquent une proportion significative de la variation du taux intrinsèque d'accroissement naturel ($r_m$) du puceron des céréales Sitobion avenae. Cependant, la corrélation n'est pas significative pour les taxons diploïdes de Triticum et Aegilops.

Des corrélations significatives entre des taxons et la résistance au puceron de Rhopalosiphum padi ont également été mises en évidence. Bien que les concentrations totales en Hx déclinent pendant la croissance des jeunes plants, celles des feuilles nouvellement formées restent élevées chez les plantes de tout âge, y compris dans la feuille-étendard sur les plantes matures.

Expérimentalement, l'infestation par des pucerons ou un dommage artificiel infligé à la plante ont été suivis par des augmentations de concentrations en Hx dans quelques cas; néanmoins, ces réactions étaient rapides et apparemment de courte durée. Des corrélations significatives ont aussi été trouvées entre la résistance à S. avenae et les concentrations en Hx dans les feuilles-étendards de plantes matures de blé du Chile et du Royaume Uni.

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## 7. REFERENCES

BIRCH, L.C. (1948). The intrinsic rate of natural increase of an insect population. Journal of Animal Ecology 17, 15-26.

BOHIDAR, K., WRATTEN, S.D. & NIEMEYER, H.M. (1986). Effects of hydroxamic acids on the resistance of wheat to the aphid Sitobion avenae. Annals of Applied Biology 109, 193-198.

EMDEN, H.F. VAN. (1969). Plant resistance to Myzus persicae induced by a plant regulator and measured by aphid relative growth rate. Entomologia Experimentalis et Applicata 12, 125-131.

LOWE, H.J.B. (1982). Resistance to aphids. Report of the Plant Breeding Institute for 1981 99-101.

NIEMEYER, H.M. (1988). Hydroxamic acids (4-hydroxy-1,4-benzoxazin-3-ones) defence chemicals in the Gramineae. Phytochemistry 27, 3349-3359.

THACKRAY, D.J. (1988). The role of hydroxamic acids in resistance of Gramineae to aphids. Proceedings of 1988 BCPC Conference - Pests and Diseases, 1167-1172.

THACKRAY, D.J., MORSE, S. & LEECH, C. (1988). The role of hydroxamic acids in wheat and maize in conferring resistance to aphids. Aspects of Applied Biology 17, part 2. pp 225-227.

ZADOKS, J.C., CHANG, T.T. & KONZAC, C.F. (1974). A decimal code for the growth stages of cereals. Weed Research 14, 415-421.

# CEREAL RESISTANCE TO APHIDS - 15 YEARS OF RESEARCH IN POLAND

SEWERYN M. NIRAZ/1/ AND ZBIGNIEW T. DABROWSKI/2/
1) Agricultural and Teachers University (WSRP), Department of Biochemistry, ul. Prusa 12, 08-110 Siedlce, Poland.
2) Warsaw Agricultural University (SGGW-AR), Department of Applied Entomology, ul. Nowoursynowska 166, 02-744 Warszawa, Poland.

Summary
During the period 1976-1989 the following research projects were investigated on cereal resistance to aphids: (1) large scale screening of spring and winter wheat, winter rye and barley cultivars; (2) the role of nutritional elements; (3) studies on constitutive aphid resistance in winter wheat; (4) mechanism of constitutive versus inducible resistance in winter wheat (5) detoxicating mechanisms in cereal aphids. The aim of the study was to find resistant cereal cultivars amongst existing germplasm and to develop a new strategy for the rational screening and breeding of cereals for resistance.

## 1. Introduction

Cereal aphids are some of the most important pests of cereals in Poland. In an integrated programme, besides chemical and biological methods varietal resistance offers great potential for the control of aphids.

The aim of our studies was to find resistant cereal cultivars amongst existing germplasm and to develop a new strategy in the rational screening and breeding of cereals for resistance.

## 2. Large scale screening

During the period 1976-1979 a total of 1429 spring wheat varieties, 4232 varieties of winter wheat, 2350 varieties and lines of winter rye and 2300 lines and varieties of barley were screened for resistance to two aphid species: Sitobion avenae(F.) and Rhopalosiphum padi (L) (Dąbrowski et al., 1979).

The following four indices were used to describe the effects of a wheat variety on aphid biology: /1/ number of aphids/plant counted at least four times during the growing season using a natural field infestation; /2/ number of aphids/plant using an artificial infestation of the same initial population; /3/ number of larvae/caged female/day measured during 10 consecutive days; and /4/ number of embryos in females of known age reared on various cultivars under field and insectory conditions.

Most of the varieties studied were susceptible, a few had

partial resistance but none were found to be absolutely resistant. 7-12 varieties showing various level of susceptibility were selected for more advanced studies.

## 3. The role of nutritional elements

The influence of nutritional elements on selected varieties of winter wheat possessing different levels of susceptibility to Sitobion avenae(F.) and Rhopalosiphum padi (L) was examined (Niraz, Dąbrowski, 198●). The results obtained during those investigations enabled following conclusions to be drawn:

1. During the period of aphid attack high susceptibility of winter wheat to the aphids was related to high contents in leaves of: chlorophyll a and b; total and reducing sugars as well as sucrose; total and soluble nitrogen; total phosphorus; total and soluble proteins; protein and free amino acids, especially the essential amino acids; total phenolics; as well as magnesium and manganese.

2. Highest susceptibility was associated with a high ratio of total nitrogen, total proteins, total phosphorus to the total sugar contents in the leaves of winter wheat during heading and flowering.

3. Low susceptibility of winter wheat to aphid pests is related to high contents of monophenols in the leaves during the period of aphid attack as well as with a high ratio of monophenols to polyphenols during the stages of heading and/or flowering.

4. The quality and quantity phenolic contents is the element of highest importance.

5. The phenolics are located mainly in the epidermis, schlerenchymis and veins. Moreover, the least susceptible variety was characterized by possessing the thickest schlerenchymis layer, which contained the phenolic compounds. This layer might also have been a mechanical obstacle to the pest.

6. Observations of the effects on wheat metabolism of aphid feeding showed that:

- an increase of oxydoreductases activity (catalase, peroxidase and polyphenol oxidase) was observed when aphids fed on the leaves of the least susceptible wheat variety, Atlas 66.
- an increase in hydrolitic enzymes activity: α-amylase, acid phosphatase as well as lyases: TAL and PAL was observed in the leaves of the most susceptible variety, Bezostaya 1.

A study of the shape and the size of the cells, layers and the tissues showed that the least susceptible variety is better adapted from the anatomical point of view to repel the aphids. For instance a lower number of stomata on the under side of leaves and a higher density of hairs on the upper side, or awns on the ears of moderately resistant cultivars.

## 4. Studies on constitutive aphid resistance in winter wheat

Prior to the late seventhies we did not know too much about the physiological and biochemical background of cereal resistance to pests. Therefore it was important under these circumstances to

identify homologies and dissimilarities so that breeding of resistant cultivars could be rationalised.

Our more detailed studies on aphid resistance in winter wheats started in 1981 with the selection of moderately resistance winter wheats from domestic germplasm. Most attention was paid to the so-called constitutive (natural) resistance, especially to the role of different concentrations of prevously selected nutritional elements and secondary plant metabolites in the mechanism of non-preference resistance (antixenosis), and antibiosis (Niraz et al., 1987).

The research dealt with the influence of chemical content upon the level of aphid resistance in certain winter wheat varieties. Two aspects could be discriminated in the research:

I. Selection of winter wheat varieties with differentiated levels of total resistance, non-preference (antixenosis) and antibiosis mainly to S. avenae, which is one of the most important cereal aphid in Poland.

II. The influence of the content of:
   a) attractants and feeding stimuli (soluble proteins, free aminoacids, sucrose)
   b) repellents (phenolic compounds, hydroxamic acids and indole alkaloids)
   c) structural substances (hemicelluloses, pectins, lignin)
   d) plant pigments (chlorophylls a and b, carotenes, flavonols) on aphid resistance of certain wheat varieties.

During the period 1981-1988, 20 cultivars were tested. Varieties: Asta, Grana and Saga were generally more resistant than Dana, Liwilla and Emika.

The studies showed that cereal aphid resistance in wheat was mainly due to antibiosis (Table 1), antixenosis being diminished (Table 2). A higher level of antixenosis in some varieties resulted from the dark green colour of the plants conditioned by varietal differences in the content of yellow - orange to green pigments (Table 3). A high antibiosis level of hydroxamic acids (Fig. 1), indole alkaloids (Fig. 2) and total phenols (Fig. 3) (2) existed, as well as a higher value for the of "toxicity index" expressing the free phenols content to free amino acids content ratio; (Table 4). The proposed toxicity index and colour coefficient make a very useful criterion in the process of preselection of aphid resistant cultivars (Niraz et al., 1987).

The resistant varieties strongly influenced the biology of the grain aphid. The flag leaves of Asta and Saga varieties had a negative effect on development of the grain aphid population (Leszczyński, 1987). The variations in aphid behaviour could be related to differences in the quantitative and qualitative composition of nutritional and/or allelochemical components of wheat tissue. Flag leaves exerted a stronger antibiotic effect to apterous S.avenae than the ears. Aphids on ears of all varieties tested developed faster and deposited a higher number of offspring than on leaves. The highest population of the grain aphid during most of the season occurs on the ears. Therefore, it is important to find factors which could protect the ears against the aphid and in consequence might reduce crop losses. The most resistant var., Asta, was characterized by very low numbers of S. avenae on ears. The reason for this may have been because of the presence of awns on the ears of Asta. The other varieties tested did not have awns (Leszczyński, 1987). The light brown colour forms of S. avenae that are very common in field conditions in Poland are very sensitive to

contact stimuli. The presence of awns on ears, in addition to biochemical and anatomical characteristics of the host-plant, could compose an important morphological source of winter wheat resistance to cereal aphids. In this case secondary antixenosis that occurs when aphids move from leaves to ears may be more important than antibiosis, because caged aphids develop as fast on Asta as on some varieties without awns (Table 5).

The variations in resistance of wheat varieties indicate the possibility of breeders combining different types of resistance in new lines with greater resistance to the grain aphid than those found hitherto. Further work on the biochemical basis of wheat resistance to S. avenae is in progress and may lead to the identification and understanding of the nature of the resistance.

## 5. The mechanism of constitutive resistance, versus inducible

The proposed analysis of the mechanisms causing only constitutive (passive) resistance in certain varieties, indicates the complicated nature of the aphid resistance process. It also indicates considerable potential (a combination of the lack of acceptance and antibiosis in one variety, which offer great promise for future aphid plant protection).

In this connection it seemed to us reasonable to proceed with further studies of the induced resistance mechanism (in progress in our laboratory) which together with the present results, should explain the biochemical basis of the aphid resistance as well as permitting research results to be put into practice.

It has been observed that under the influence of S.avenae feeding in the flag leaves of partially resistant varieties the contents of total phenols, p-dihydroxyphenols and flavonols slighty decreased, whereas that of susceptible varieties markedly increased. During the same time peroxidase activity oscillated between the values for control plants. Partially resistant varieties were observed to show an increase in the activity of isoperoxydases mainly with the molecular weight above 28 daltons, whereas in the tissues of susceptible varieties the activity increase was found in the fraction with lower molecular weight (Niraz et al., 1988).

Under the influence of the pest attack an increase of the activity of phenylalanine ammonia-lyase (PAL) and tyrosine ammonia-lyase (TAL) in partially resistant varieties was observed. These changes were strictly correlated with the increase of free L-phenyloalanine and L-tyrosine content (Ciepiela, 1989).

These preliminary results indicate that the resistant and susceptible cultivars differ genetically and this is expressed by different level of oxydative enzymes and secondary plant substances in their tissues.

## 6. The detoxicating mechanisms in cereal aphids and their adaptations to host plants

Phytophagous pests secrete enzymes into the tissues of host

in vivo, and thus decompose the tissues. Then they excrete or incorporate in their own metabolism harmful plant substances which become thoroughly detoxicated.

Our research proves the existance of detoxicating enzymes in cereal aphids (Niraz et al., 1988). For instance, grain aphid is able to defend itself effectively from phenols. This has been demonstrated by the activity of peroxidase, polyphenol oxidase and β-glucosidase both in the saliva of an aphid and in its body. These enzymes may act jointly in detoxicating free phenols and those bounded in cell walls to inactive melanin. But this mechanism seems to be effective only in those plants which contain small amounts of phenols in their tissues (Urbańska, Niraz, 1989). Greater concentration of phenols does not induce but inhibits the activity of insect enzymes. Though many toxic phenols are oxidized to quinones in vitro, grain aphid increases the activity of peroxidase as a protection only against catechol and m- and o-coumaric acid, whereas many other plant phenols inhibit the activity of both peroxidase and polyphenol oxidase in grain aphid.

Presumably a very important detoxicating enzyme in S.avenae is glutathione-S-transferase which condenses various toxins with glutathione and thus facilitates metabolism of those toxins. Inactivity of tannase in the pest's saliva and in its body shows that grain aphid does not hydrolyze tannins which seem to perform a very important function in plant protection against the insect (Urbańska, Niraz, 1989).

Acknowledgement

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Résumé

La résistance des céréales aux pucerons: 15 ans de recherche en Pologne

De 1976 à 1989, les recherches suivantes ont été entreprises en matiére de résistance des céréales aux pucerons: 1) controles à grande échelle de cultivars de blé de printemps et d'automne, de la seigle d'automne de l'orge. 2) détermination du rôle des éléments nutritifs, 3) études de la résistance constitutive du blé d'automne envers les pucerons 4) étude comparative de la résistance constitutive et induisible du blé d'automne et 5) étude de mécanismes de détoxication chez les pucerons des céréales.
Le but de l'étude était de trouver des cultivars de céréales résistants parmi les germeplasmes existants et de développer une nouvelle stratégie pour le tri rationnel et les croisements de céréales, en vue de leur résistance.

References

CIEPIELA, A.1989. Akumulacja i przemiany fenyloalaniny i tyrozyny w kłosach odpornych i podatnych odmian pszenicy ozimej, indukowane żerowaniem mszycy zbożowej (Sitobion avenae /F./). Zesz. Probl. Post. Nauk Roln. (In press, summary in english).

DABROWSKI, Z.T., PIECHOTA, J., BIELAK, B., PLASKOTA, E., LEGUTOWSKA, H., 1979. The mechanisms conditioning differences in susceptibility of cereals to aphids. Final report on investigations in project nr 02.P.14. Part.I. Entomological experiments. Warsaw Agricultural University, Entomology Dept.: 1-224.

LESZCZYNSKI, B., 1987. Winter wheat resistance to grain aphid Sitobion avenae /Fabr./ (Hom., Aphididae). Insect Sci. Applic., 8/2/:251-254.

LESZCZYNSKI, B., WRIGHT, L.C., BAKOWSKI, T., 1989. Effect of secondary plant substances on winter wheat resistance to grain aphid. Entomol. exp. appl. 52: 135-139.

NIRAZ, S., DABROWSKI, Z. T., 1980. The mechanisms involved in sensivity of winter wheats to aphids. Summaries of papers of the IX Congress EUCARPIA. 16-20 September, Leningrad, USSR. Symposium II: Resistance of plants to pests: 42-43.

NIRAZ, S., LESZCZYNSKI, B., CIEPIELA, A., URBANSKA, A., 1987. The importance of various plant chemical compounds to constitutive aphid resistance in winter wheats.Rocz.Nauk Rol.Ser.E. 17/18:61-75.

NIRAZ, S., URBANSKA, A., MARCINIUK, M., MATOK, H., BAKOWSKI, T., ZIELINSKA, M., LESZCZYNSKI, B., 1988. Zmiany w zawartości związków fenolowych w pszenicy ozimej pod wpływem mszycy zbożowej (Sitobion avenae F.). Materiały XXVIII Sesji Naukowej Instytutu Ochrony Roślin w Poznaniu: 26-29 (Summary in english).

NIRAZ, S., URBANSKA, A., CZYŻEWSKA, D., LESZCZYNSKI, B., 1988. Some physiological and biochemical relations between cereals and aphids. (In:) Endocrynological Frontiers in Physiological Insect Ecology. Proc. of the Int. Conf., 7-12 September 1987, Szklarska Poręba, F. Sehnal, D. Denlinger, A. Zabża eds., Wrocław Technical University Press, vol 1: 123-128.

URBANSKA, A., NIRAZ, S.,1989. Study on phenol detoxicating enzymes enzymes in grain aphid. Abstracts of lectures and posters of the 7-th International Symposium on Insect-Plant Relationships. 3-8 July, Budapest, Hungary, P-55.

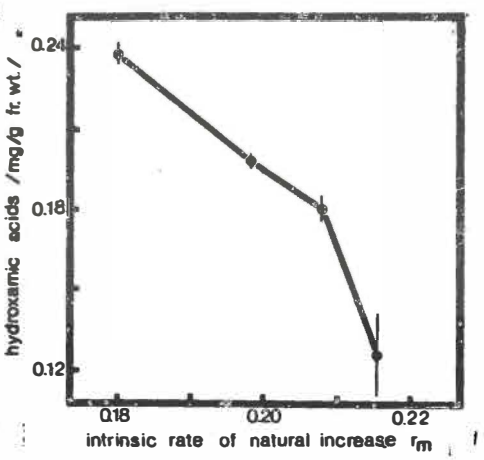


Fig.1. A comparison of hydroxamic acids content with the antibiotic influence on intrisic rate of natural increase ($r_m$); ( Leszczyński et al., 1959 )

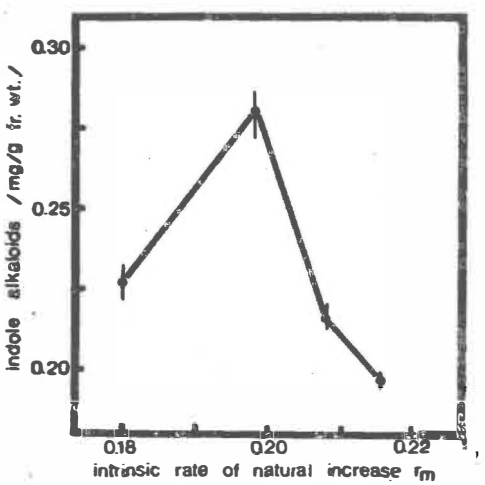


Fig.2. A comparison of indole alkaloids content with the antibiotic influence on intrisic rate of natural increase ($r_m$): (Leszczyński et al.,1959)

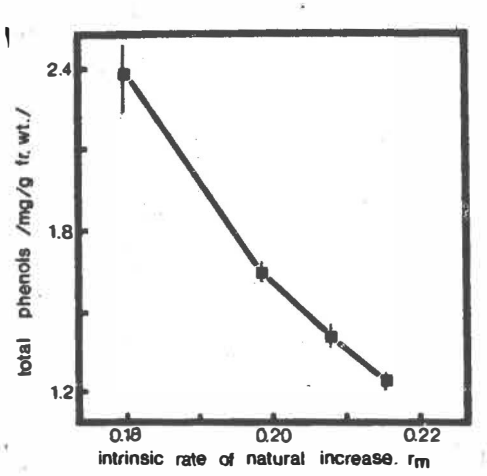


Fig.3. A comparison of total phenols content with the antibiotic influence on intrisic rate of natural increase ($r_m$); (Leszczyński et al.,1989)

Table 1. A comparison of resistance /total aphid index per plot/ and antibiosis /intrinsic rate of natural increase $r_m$/ in four wheat cultivars to cereal aphids /Niraz et al., 1987/

| Variety | Resistance | Antibiosis |
|---|---|---|
| Resistant | | |
| Grana | 324.7 a | 0.2150 |
| Saga | 387.7 a | 0.1997 |
| Susceptible | | |
| Dana | 519.0 b | 0.2260 |
| Liwilla | 821.7 c | 0.2298 |

Values not followed by the same letter are significantly different at 5% level

Table 2. A comparision of resistance /total aphid index per plot/ and antixenosis /aphid index during migration per plot/ in four wheat cultivars to cereal aphids /Niraz et al., 1987/

| Variety | Resistance | Antixenosis |
|---|---|---|
| Resistant | | |
| Grana | 324.7 a | 19.3 a |
| Saga | 387.7 a | 31.3 a |
| Susceptible | | |
| Dana | 519.0 b | 109.0 b |
| Liwilla | 821.7 c | 121.7 c |

Values not followed by the same letter are significantly different at 5% level

Table 3. A comparison of antixenosis and "colour coefficient" /the content of yellow-orange pigments to green pigments/ of four wheat cultivars /Niraz et al., 1987/

| Variety | Antixenosis | Colour coefficient |
|---|---|---|
| Resistant | | |
| Grana | 19.3 a | 0.55 a |
| Saga | 31.3 a | 1.10 c |
| Susceptible | | |
| Dana | 109. b | 0.68 b |
| Liwilla | 121.7 c | 0.72 b |

Values not followed by the same letter are significantly different at 5% level

Table 4. A comparison of resistance and values of the toxicity index of four wheat cultivars /free phenols/free amino acids ratio/ /Niraz et al., 1987/

| Variety | Resistance | Toxicity index |
|---|---|---|
| Resistant | | |
| Grana | 324.7 a | 0.123 c |
| Saga | 387.7 a | 0.071 b |
| Susceptible | | |
| Dana | 519.0 b | 0.066 b |
| Liwilla | 821.7 c | 0.057 a |

Values not followed by the same letter are significantly different at 5% level Correlation coefficient r= 0.9689 /significant at 1% level/

Table 5. The resistance of six wheat cultivars to the grain aphid Sitobion avenae in free choice and caged tests under field conditions /Leszczyński, 1987/.

| Variety | No. aphids/plant/season | | No. alate adults/plant/ 2 first weeks | Prereproductive period (days) | | No. offspring female/day | | Intrinsic rate of increase $r_m$ (female offspring/female/day) | |
|---|---|---|---|---|---|---|---|---|---|
| | Ear | Flag leaf | Flag leaf | Ear | Flag leaf | Ear | Flag leaf | Ear | Flag leaf |
| Asta | 1.94a | 0.62a | 0.047a | 10.45b | 13.90a | 2.43a | 1.71a | 0.2305 | 0.1645 |
| SD | 0.19 | 0.14 | 0.007 | 2.45 | 2.90 | 0.75 | 0.88 | | |
| Grana | 2.88b | 0.34a | 0.030a | 11.25a | 11.65b | 2.50a | 2.34a | 0.2172 | 0.1984 |
| SD | 0.36 | 0.06 | 0.010 | 1.75 | 2.35 | 1.00 | 0.79 | | |
| Saga | 3.06b | 0.41a | 0.053a | 11.20a | 13.20a | 2.42a | 2.24a | 0.2102 | 0.1799 |
| SD | 0.31 | 0.04 | 0.017 | 1.80 | 1.80 | 0.83 | 1.64 | | |
| Dana | 3.63b | 1.53b | 0.097b | 9.75b | 11.65b | 2.84b | 2.42a | 0.2499 | 0.2071 |
| SD | 0.20 | 0.23 | 0.017 | 1.25 | 2.35 | 0.91 | 0.98 | | |
| Liwilla | 6.59c | 1.46b | 0.110b | 10.25b | 11.65b | 3.00b | 3.08c | 0.2505 | 0.2144 |
| SD | 0.89 | 0.29 | 0.010 | 1.25 | 1.65 | 0.70 | 0.83 | | |
| Emika | 7.17c | 1.62b | 0.177c | 9.95b | 12.55b | 3.61c | 2.78b | 0.2684 | 0.2080 |
| SD | 1.59 | 0.34 | 0.027 | 1.05 | 2.55 | 0.89 | 0.97 | | |

Values in the same column not followed by the same letter are significantly different according to Duncan's new multiple range test /p = 0.01/

x Migration of alate adults before heading

# GENERAL TOPICS

# PAST AND PRESENT APHID RESISTANCE WORK IN SWEDEN

J. WEIBULL. Weibullsholm Plant Breeding Institute, P.O. Box 520, S-261 24 Landskrona - SWEDEN.
Previous address: Swedish University of Agricultural Sciences, Dept. of Plant and Forest Protection, P.O. Box 7044, S-750 07 Uppsala - SWEDEN.

Summary
The work concerning resistance to the bird cherry-oat aphid (BCOA) (**Rhopalosiphum padi** L.) since 1987 is reviewed and emphasis is given to a method whereby large numbers of plants can be screened chemically with respect to their amino acid content. The recently initiated work regarding plant resistance to pea aphid (PA) (**Acyrthosiphon pisum** Harr.) and mustard aphid (MA) (**Lipaphis erysimi pseudobrassicae** Kalt.) is briefly summarized.

## 1. Mechanisms of resistance to BCOA in some oat and barley species

In a previous report (Weibull, 1988a) two wild cereal species were noted as exceptionally resistant: **Avena macrostachya** (Bal., ex Coss. et Dur.) and **Hordeum bogdani** Wil. The screening experiments were followed up by more detailed studies of aphid growth and behaviour on these highly interesting species, as well as chemical analysis of the free amino acids in the phloem sap and in the aphids' honeydew. These results have been reported elsewhere (Weibull, 1988b) and will only be summarized here.

Aphid growth was severely impeded on both species and the sizes of fullgrown aphids were only 30-50% of 'normal' individuals. In addition, development times were prolonged (between 2 and 4.5 days) and nymph production was drastically reduced. Aphids on **H. bogdani** were also much more restless, indicating a possible presence of phagodeterrents. Leaf sectioning did not reveal any obvious anatomical obstacles to stylet penetration.

The coupled analysis of both phloem sap and honeydew did not demonstrate any major differences in food utilisation between the wild and cultivated species. Generally the aphids were very good at removing the vital free amino acids from the plant sap and less than 10% was 'lost' in the excreta (one exception is noted in the paper).

Free amino acids were also analysed in phloem sap from some more oat and barley cultivars/species and these data were subjected to multivariate analysis (Weibull, 1988c). The results showed that there was no relationship between total amino acid content and resistance to BCOA but that all resistant genotypes had much higher concentrations of glutamic acid. The explanation may be that phloem sap having high glutamic acid content has a lower pH and is less suited as food for BCOA which, together with many other aphid species, prefers to feed on solutions of a slightly alkaline pH (unpublished data).

2. Screening for resistance to BCOA in Hordeum spontaneum

During the 'aphid year' 1988 more than 400 lines of H. spontaneum were screened under field conditions in an attempt to identify new valuable sources of resistance to be included in a breeding programme against BCOA. The colonisation and population build-up of aphids was very rapid and by the end of June the populations collapsed. This only allowed us to score the aphid numbers twice. Furthermore, a very high level of infestation might mask true genotypic differences as these are often quite small. Nevertheless, 30 lines have been selected for more extensive testing and this will be completed during the spring of 1990. This work is funded by the Plant Breeding Council (under the Swedish Council for Forestry and Agricultural Research, SCFAR).

3. Rapid screening method for free amino acid composition

A prerequisite for efficient breeding work is that a reliable screening method for aphid resistance is available. This work on BCOA and its relationships to oats and barley has shown that part of the resistance found can be attributed to the nutritional quality of the plants, i.e. the composition of free amino acids. All previous analyses have been made on sap samples taken from severed aphid stylets - indeed a time-consuming and inefficient method! Work by plant physiologists during the mid-70's inspired us to investigate whether sap exuding from cut leaves really represents true phloem sap. If so, large numbers of plants could, with little labour, be sampled with respect to their phloem sap composition and no aphids would be needed. We collected phloem sap and leaf exudates from a large number of plants and correlated the amino acid composition of the two sample types. Leaves were cut and immersed in a weak EDTA-solution for about one hour. The results will soon be published (Weibull et al., in press) and the general conclusion is that leaf exudates can, in fact, be used as predictors of phloem sap composition. Amino acids present at high concentrations correlate better than those of low content. The larger the variation in amino acid composition between plants the better the correlation.

4. Resistance mechanisms against pea aphid and mustard aphid

Recently two projects were initiated to look at resistance mechanisms against to other aphid species, the pea aphid and the mustard aphid. The first study is funded by SCFAR and the second is part of an Indo-Swedish collaboration on Brassica oilseed crops financed by SAREC (Swedish Agency for Research Cooperation with Developing Countries). The pea aphid project is primarily concerned with the biochemical relationships between the aphid and the plant whereas the mustard aphid study deals mainly with aphid behaviour. In charge of the two projects are Mr J. Sandström and Mr D. Stephanson, respectively (Dept. of Plant and Forest Protection; address - see above).

## 5. Résumé

**Travaux anciens et actuels sur la résistance envers les pucerons en Suède**

Le travail concernant la résistance envers le puceron du merisiers à grappes (**Rhopalosiphum padi** L.) entrepris depuis 1987 est passé en revue, et une méthode permettant de trier chimiquement un grand nombre de plantes en se basant sur leur contenu en acides aminés est mentionné. Des travaux concernant la résistance des plantes au puceron vert du pois (**Acyrthosiphon pisum** Harr.) et au puceron du navet (**Lipaphis erysimi pseudobrassicae** Kalt.) ont été récemment entrepris.

## 6. Acknowledgements

The invaluable cooperation of Dr S. Brishammar (Head of the Biochemical Plant Pathology group) and Mr F. Ronquist is gratefully acknowledged, as well as skilful assistance by Mrs S. Walmer-Nordin and Mr. G. Melin.

## 7. References

WEIBULL, J., 1988a. Resistance in wild oat and barley species to bird cherry-oat aphid (**Rhopalosiphum padi** L.). WPRS Bulletin XI/3, pp. 33-34.

WEIBULL, J., 1988b. Resistance in the wild crop relatives **Avena macrostachya** and **Hordeum bogdani** to the aphid **Rhopalosiphum padi**. Entomol. exp. appl. 48:225-232.

WEIBULL, J., 1988c. Free amino acids in the phloem sap from oats and barley resistant to **Rhopalosiphum padi**. Phytochemistry 27:2069-2072.

WEIBULL, J., RONQUIST, F. & BRISHAMMAR, S., 1990. Free amino acid composition of leaf exudates and phloem sap. Plant Physiol. 91 (in press).

# THE AVAILABILITY OF (PARTIAL) RESISTANT CROPS: A FIRST PRINCIPLE IN INTEGRATED CONTROL.

C. MOLLEMA Institute for Horticultural Plant Breeding (IVT), P.O.-Box 16, NL-6700 AA Wageningen, The Netherlands.

**Summary**
Much research for integrated pest management concerns the optimalisation of chemical control or the introduction of natural enemies. Although host plant resistance has at least equal potentials, much less emphasis is given to investigations of insect-plant relationships. In contrast to other control measures, host plant resistance is not directed only to an increase of mortality rates, but rather intends to reduce the population growth of the pest organisms. Therefore, even low levels of resistance may be very effective. Strategies and prospects of plant breeding for resistance to insects are indicated.

## 1. Introduction

The two most important conditions for exponential increases of pest insect populations are: an unlimited amount of food (monocultures of host crops) and absence of natural enemies. The latter condition in particular has been the subject of many studies concerning integrated control. The first condition, however, has often been neglected, although manipulation of the insect's food resources (i.e. by breeding resistant cultivars) provides at least equivalent prospects for integrated crop protection.

## 2. Advantages of Host Plant Resistance

The effects of integrated control measures and host-plant resistance differ in principle. Integrated control measures are intended to increase the death rate of insect populations (e.g. by using traps, by releasing biological control agents or by selective chemical control). However, by breeding for resistance, the aim is to decrease birth rates rather than to increase death rates. According to population dynamics theories and simulation studies (Lewontin, 1965; Carter & Dixon, 1981; Hulspas-Jordaan & van Lenteren, 1989), such an approach affects population growth rates significantly (See Table 1). Even low levels of resistance in host plants can be very effective. Moreover, when host plant resistance alone does not provide a sufficient level of control, the characteristic slow development of the insect population on resistant cultivars will markedly facilitate other control measures. The high compatibility with other measures of control is one of the greatest advantages of host plant resistance as a basic component of integrated control.

**3. Research Items**

Breeding for resistance to insects has some fundamental prerequisites, which to some degree are similar to those needed in other fields of integrated control. First it is necessary to have methods for testing the resistance of host plants. If not available, research must first concentrate on the development of such methods. This includes the organization of reliable mass-rearing which can supply large numbers of (synchronized) insects at a certain time, and in which manipulation of diapause is possible. Also the presence of different biotypes of the insect species has to be determined.
Since-host plant resistance is expressed by population growth rates of the insects rather than by damage to the plants, an important

Table 1: Simulated effects of 10 % changes in developmental period, fecundity and mortality (of whiteflies on tomato) on the population density after a certain fixed period of time. In the simulation model, the effects were calculated relative to values that fitted very well with counts in the glasshouse. (After Hulspas-Jordaan & van Lenteren, 1989).

| Character | Relative Change | Effect on Population Density |
|---|---|---|
| Developmental Period | + 10 % | - 43 % |
| Fecundity | - 10 % | - 26 % |
| Mortality | + 10 % | - 4 % |

prerequisite to the development of test methods is the ability to measure life history components of the insects. Once resistance can be determined, research is continued by the search for genetic variation in host plant collections. To avoid confusion of non-preference (in choice situations) and resistance, the plant genotypes used in these experiments have to be isolated from other genotypes. In the next phase, the sources of resistance are genetically analysed, and eventually the level of resistance can be improved by crossing and further selection. The last step towards the introduction of a new cultivar is the incorporation of the resistance into commercial varieties.

It has to be mentioned that an almost inevitable problem in all these experiments is the growing of plants without the use of pesticides (which may interfere with detecting resistance) and without attacks before the start of the experiments.

De Ponti (1982) argued that these types of research are most successful when entomologists and plant breeders work closely together.

## 4. Prospects for the near future

At least three different strategies are currently practiced in the breeding for resistance to insect pests.
First, the conventional type of breeding by which genetic resources from host plants and their closely related species are used (e.g. de Ponti & Inggamer, 1984; Dickson et al., 1984; Ellis et al., 1988). This approach can exploit a reservoir of unknown useful genes, while modern techniques may be helpful in interspecific crosses.
Secondly, the introduction of genes for resistance from other plant species or even other organisms by genetic engineering will receive more and more attention. Three types of genes are studied at present: genes coding for toxins (from Bacillus thuringiensis; Vaeck et al., 1987), genes coding for proteinase inhibitors like Cowpea Trypsin Inhibitor (Hilder et al., 1987) and genes coding for enzymes that produce secondary metabolites like repellents or antifeedants (Pickett, 1985).
Thirdly, breeding for resistance without the use of insect bioassays is much easier. Breeding companies usually object to the introduction of pest insects into their selection fields. They prefer methods by which the resistance can be determined indirectly. This approach is only possible when the correlation between the resistance and the involved plant parameter is rather high. Cole (1984) showed a correlation between the concentration of certain phenolic acids and resistance to root aphids in lettuce. A higher correlation could probably be found by the use of (DNA) Restriction Fragment Length Polymorphisms (RFLP; see Tanksley et al. (1989) for a review).

In conclusion, host-plant resistance provides excellent prospects. It should be a basic ingredient in integrated control programmes. It is regrettable though, that relatively little attention has been paid to this aspect. Only a few groups in Europe are working in this field, which is in great contrast to the amount of researchers studying natural enemies. This contribution aims to stimulate researchers to include different host-plant genotypes in their experiments. In most cases they can be obtained free of charge.

## 5. Résumé

La disponibilité de cultures (partiellement) résistantes: premier principe de lutte intégrée.

Beaucoup de recherches en protection intégrée concernent l'optimisation de la lutte chimique ou l'introduction d'ennemis naturels. Bien que la résistance de plantes-hôtes ait au moins les mêmes perspectives, une bien plus faible importance est accordée aux études des relations insecte plante. Contrairement à d'autres méthodes de lutte, la résistance des plantes ne se limite pas à l'effet direct sur l'augmentation du taux de mortalité, mais réduit plutôt la croissance de la population du ravageur, de sorte que même un faible niveau de résistance peut être très efficace. Les stratégies et perspectives de la sélection de plantes résistantes aux insectes sont évoquées.

## 6. References

Carter, N. & A.F.G. Dixon (1981). The use of insect population simulation models in breeding for resistance.
SROP/WPRS Bulletin 1981/IV/1, 21-24.

Cole, R.A. (1984). Phenolic acids associated with the resistance of lettuce cultivars to the lettuce root aphid.
Ann. Appl. Biol. 105, 129-145.

Dickson, M.H., C.J. Eckenrode and A.E. Blamble (1984). NYIR 9602, NYIR 9605 and NYIR 8329 lepidopterous pest-resistant cabbage breeding lines.
HortScience USA 19, 311-312.

Ellis, P.R., J.A. Hardman and B.D. Dowker (1988). Exploitation of the resistance in carrots to the carrot fly at Wellesbourne.
SROP/WPRS Bulletin 1988/XI/3, 55-56.

Hilder, V.A., A.M.R. Gatehouse, S.E. Sheerman, R.F. Barker & D. Boulter (1987). A novel mechanism of insect resistance engineered into tobacco.
Nature 300, 160-163.

Hulspas-Jordaan, P.M. & J.C. van Lenteren (1989). The parasite-host relationship between Encarsia formosa (Hymenoptera: Aphelinidae) and Trialeurodes vaporariorum (Homoptera: Aleyrodidae). XXX. Modelling population growth of greenhouse whitefly on tomato. Wageningen Agricultural University Papers, 89.2, 1-54.

Lewontin, R.C. (1965). Selection for colonizing ability. In: H.G. Baker & G.L. Stebbins (eds.), The genetics of colonizing species. Academic Press, New York. pp. 77-94.

Pickett, J.A. (1985). Production of behaviour-controlling chemicals by crop plants.
Phil. Trans. R. Soc. Lond. B 310, 235-239

Ponti, O.M.B. de, 1982. Plant resistance to insects: a challenge to plant breeders and entomologists.
Proc. 5th int. Symp. Insect-Plant Relationships, Wageningen, 1982, Pudoc, Wageningen, pp. 337-347.

Ponti, O.M.B. de & H. Inggamer (1984). Resistance to the onion fly in Allium cepa and Allium fistulosum.
Synopsis 3rd Eucarpia Allium Symposium, Wageningen, pp. 21-23.

Tanksley, S.D., N.D. Young, A.H. Paterson and M.W. Bonierbale (1989). RFLP mapping in plant breeding: new tools for an old science.
Bio/technology 7, 257-263.

Vaeck, M., A. Reynaerts, H. Höffe, S. Jansens, M. de Beuckeleer, C. Dean, M. Zabeau, M. van Montague and J. Leemans (1987). Transgenic plants protected from insect attack.
Nature 328, 33-37.

# DISCUSSION

# REPORT ON DISCUSSION MEETING: FIFTH IOBC/WPRS EUCARPIA WORKING GROUP ON BREEDING FOR RESISTANCE TO INSECTS AND MITES

a. It was agreed that meetings of the Working Group should continue to be held every 3 years. Dr P.R. Ellis proposed that the next meeting could be held at the Institute of Horicultural Research, Wellesbourne, and a provisional date was set for September 1992.

b. The extent and scope for funding activities within the Working Group were discussed. Besides providing administration costs, these include limited subsidies towards travel and subsistence for members to attend Working Group meetings, costs of producing publications and also funds for running collaborative projects.

## Travel and subsistence

Financial help towards attending meetings was greatly appreciated by members, many of whom would otherwise be unable to attend.

## Publications

Presently the Working Group supports the publication of a Bulletin (every 3 years) and an Aphid Resistance Newsletter (every 12 months). The Bulletin was generally considered to be a very useful document, summarising the Group's meetings and activities. The Aphid Resistance Newsletter was considered by some Working Group members to overlap with the existing Bulletin and the Aphidologists Newsletter. Opinions were expressed that it may be possible to combine the two Aphid Newsletters, to cover Europe and USA, or to incorporate the Aphid Resistance Newsletter into the Bulletin, but with widened interests (i.e. not restricted to aphid resistance). Some members considered that a good newsletter should be published annually, and should be primarily used for messages and as a forum for thoughts and new ideas.

## Collaborative projects

These require a minimum of 3 countries and can be centred on a crop, pest or discipline. The Carrot Fly subgroup involving participants from the UK, Denmark, Netherlands, Switzerland and Poland over 2 years (3 meetings) was considered to have been a very useful and successful collaborative programme.

Areas for new collaborative projects were considered:

a. Lettuce aphids: (Proposed by Drs Kees Reinink and Frans Dielman). A pest status survey of lettuce aphid spp. in several countries was

proposed. This could be extended to study the consistency of lettuce cultivars differing in susceptibility to several aphid spp. in those countries.

b. Western Flower Thrips: (Proposed by Dr Chris Mollema). It was proposed that this important pest should be carefully monitored in several countries, together with occurrences of possible predators and parasites. This could be extended to evaluate sources of resistance in cucumbers and chrysanthemum genotypes.

c. Plant surface chemistry in relation to insect behaviour: (Proposed by Dr Erich Städler). It was proposed that several groups already investigating brassica plant surface chemistry should collaborate by testing extracts and chemicals from susceptible and resistant plants, initially on root fly oviposition behaviour. This could be extended to also include studies on gall midge, leaf feeding beetles, and brassica butterflies.

d. Electron Penetration Graph (EPG) and stylet cutting: (Proposed by Maarten van Helden). It was proposed to link several laboratories now using EPG to study aphid and whitefly feeding behaviour. Possible areas of collaboration could include optimisation of standardised methodologies to study host plant resistance to a selected aphid species. This could be extended to study feeding pattern differences in biotypes of one species from different countries.

e. New developments in molecular biology and genetic manipulation: (Proposed by Dr G.C. van Blokland). Since this is a new and rapidly developing area affecting the Working Group, it was proposed that guest speakers who are leading experts should be invited to one or more meetings to discuss the implications and applications of this technology with Working Group members.

The meeting closed with a vote of thanks from all members to Dr Jost Freuler, his wife and colleagues for hosting a most enjoyable and stimulating meeting, and to Dr P.R. Ellis for helping to organise and chair the meeting.

**A.N.E. BIRCH**