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# Breeding for Resistance to Insects and Mites

# Amélioration des Plantes pour la Résistance contre les Insectes et les Acariens

editors:

P.R. Ellis & S. Derridj

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IOBC / WPRS

**Working Group "Breeding for Resistance to Insects and Mites"**

OILB / SROP

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

**In association with EUCARPIA**

# PROCEEDINGS of a MEETING

at

**Dundee, Scotland**
14-17 September 1998

Edited by P.R. Ellis & S. Derridj

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## PREFACE

The eighth meeting of the Working Group was held at the West Park Centre, Dundee, Scotland, 14-17 September 1998. The local organiser was Dr Nick Birch who was helped by colleagues from his team at the Scottish Crop Research Institute, Invergowrie, Dundee. A total of 26 participants from 7 different countries attended. The first two days included lecture and poster sessions. The sessions included papers on:-

- Mechanisms of host plant resistance and techniques.
- Sources of resistance, breeding and testing.
- Host plant resistance, pest biotypes and IPM.
- Round table discussions on Project Groups.
- Round table discussions on new areas of collaboration, biotechnology, etc.

On the following two days visits were made to local sites of interest, a winery and several participants toured the laboratories at the Scottish Crop Research Institute. Reports were given of progress with Project Groups and a long discussion took place concerning the Project Group on Tritrophic Interactions. This will be linked with a similar group in the USA and will include work on genetically modified crops.

The papers presented at this meeting are published in this IOBC Bulletin.

The Convenor of the Working Group, Bob Ellis, from Horticulture Research International, Wellesbourne, Warwick, UK, retired following ten years in the post and handed over to Nick Birch from the Scottish Crop Research Institute, Invergowrie, Dundee, Scotland. His address and contact numbers are:-

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The next meeting of the Working Group will be held in Sweden at Svalov in 2001 where the local organiser will be Dr Inger Ahman.

# REPORT ON THE GENERAL DISCUSSIONS AT THE MEETING

## Project Groups

1. Carrot fly. Collaboration was successful, objectives achieved and results published in 1981.

2. Lettuce aphids. Collaboration was successful, objectives achieved and results published in 1993.

3. Western Flower Thrips. The project group met in Arnhem, The Netherlands 17-19 October 1994.

4. Plant Surface Chemistry. Nick Birch presented a report on progress. Future collaboration will be expanded to include participants from France, the UK and Switzerland. Nick Birch will continue to act as coordinator.

5. EPG and Stylet Cutting. Collaborators were from The Netherlands and the UK. Coordination of the group has been passed form Maarten van Helden to Freddy Tjallingii who will be organising further workshops.

6. Tritrophic Interactions. Marcele Dicke, the coordinator was not present at the meeting. However, the project group was discussed at length, particularly in relation to risk assessment and safe deployment of genetically modified organisms (GMOs) in the presence of natural enemies of pests. The group will include scientists from Scotland, England (HRI Wellesbourne and IACR Rothamsted), The Netherlands and Switzerland. The group will interact with a similar project group operating in the USA. John Trumble will provide names of US entomologists willing to participate in collaboration on this topic.

## Round table discussions

Stuart Gordon from SCRI presented details of the CRAFT project called RACER which is supported by the small and medium company enterprise (SME) in collaboration with the EU. The project covered topics such as the use of molecular markers in resistance breeding, the identification of resistance-breaking biotypes of pests and the role of private industry funding for these subjects. As a preliminary it is essential that sources of resistance are first identified and appropriate bioassays developed for the evaluation of resistance.

## Triennial meetings

It was agreed that the next meeting of the Working Group would be held in Sweden, probably in 2001 and that Dr Inger Ahman would act as local organiser. Information will be circulated 18 months prior to the meeting.

## Convenor

Dr Nick Birch took over the position of Convenor from Dr Bob Ellis who retired after holding the post for 10 years.

## Publications

Dr Bob Ellis will edit the proceedings of this Dundee meeting and Dr Sylvie Derridj will provide French versions of the abstracts of papers.

The Aphid Resistance Newsletter has been discontinued.

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# CONTENTS

**Session 3 – HPR, Pest Biotypes and Integrated Pest Management**

# Session 1 – Mechanisms of Host Plant Resistance and Techniques

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# Role of avocado idioblast cells in resistance to herbivorous insects

**Cesar Rodriguez-Saona & John Trumble**
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**Abstract:** The effect of avocado idioblast oil cells on larval survival and growth of a generalist herbivore, *Spodoptera exigua* was examined. Activity was recorded using no-choice artificial diet bioassays initiated with cohorts of newly hatched larvae, at concentrations similar to those found naturally in avocados (2% of tissue volume). After 9 days, the idioblast cells significantly reduced *S. exigua* larval weight and increased larval mortality at concentrations below those found in avocados.

Our results suggest that the presence of idioblast oil cells may explain partially why *S. exigua*, a polyphagous herbivore and a major pest of many agricultural crops, is not adapted to feed on avocados.

**Key words:** *Spodoptera exigua*, avocado, *Persea americana* idioblast oil cells

## Introduction

Avocados, *Persea americana* Mill., contain specialized idioblast oil cells which are cells that markedly differ from other constituents of the same tissue in form, structure, and contents. The cells are scattered throughout the avocado tissues and constitute approximately 2% of the tissue volume (Cummings & Schroeder, 1942). In addition, the cells have been reported to contain an oil that differs from other lipids found in the fruit mesocarp (Platt & Thomson, 1992). However, the role of these avocado idioblast oil cells in plant protection against herbivores has not been investigated.

Therefore, our work has focused on testing the effects of these cells on a generalist insect herbivore the beet armyworm, *Spodoptera exigua* (Hübner). *S. exigua* was chosen because it has an extensive host range and it is an important pest of many agricultural crops; however, it is not adapted to feed on avocados.

## Methods

We tested the effect of the idioblast oil cells on the survival and growth of *S. exigua* larvae using no-choice artificial diet bioassays. Avocado idioblast oil cells were isolated as described by Rodriguez-Saona & Trumble (1996). Six concentrations were assayed: 0, 0.6, 1.2, 1.7, 2.3, and 2.8% of cells in artificial diet. Treatments were prepared by mixing the oil cells with artificial diet to produce a total of 100 g. Control diets (artificial diet alone) were prepared as described by Patana (1969). Control and treated diets were poured into 30 ml plastic cups (7 g of diet/cup). One neonate was placed in each cup, and cups were held in an environmental chamber. Fifteen neonates were tested at each concentration, and assays were repeated 4 times. Mortality and larval weights were recorded after 9 days.

## Conditions During Experiments

*Spodoptera exigua* larvae used in all experiments were maintained on artificial diet at 28 ± 2 °C and a L 14: D 10 photoperiod. The colony was originally collected from Orange County, California in 1982, and had new genetic material added within 12 months before the study. All neonates were used within 12 hours of eclosion. All experiments were conducted in environmental chambers at 28 ± 2 °C, a relative humidity of 75%, and L 14: D 10 photoperiod with fluorescent lighting.

## Results

The avocado idioblast oil cells affected *S. exigua* survival and growth at concentrations below those found in avocado tissues (2% of cells/tissue volume; Table 1). At 2% of cells in diet, larval mortality was about 45% after 9 days.

In addition, larval growth was inhibited by more than 70% compared to the controls at a concentration of 2% of idioblast cells in diet.

Table 1. Effects of avocado idioblast oil cells on the survival and growth of *S. exigua* larvae.

| % Oil Cells in Artificial Diet | *S. exigua* 9-day % Mortality | *S. exigua* 9-day Larval Weight |
|---|---|---|
| Control | 5 | 115.3 a |
| 0.6 | 20 | 78.4 b |
| 1.2 | 37 | 70.2 b |
| 1.7 | 42 | 32.1 c |
| 2.3 | 47 | 27.8 c |
| 2.8 | 60 | 21.7 c |

Different letters indicate statistical differences between treatments (Tukey's pairwise comparisons, $P < 0.05$).

## Discussion

*Spodoptera exigua* feeds on more than 35 host plants around the world (Steiner 1936), but avocados are not listed as a suitable host plant. The present results indicate that the idioblast oil cells, which are located randomly in all avocado tissues, detrimentally affect *S. exigua* survival and growth. Therefore, their presence in the avocado tissues might be one possible reason why this polyphagous herbivore has not adapted to feed on avocados.

Furthermore, our results indicate that *S. exigua* may break down the cells after ingestion, thus releasing the toxic insecticidal components that have been identified from the avocado oil cells. Rodriguez-Saona *et al.* (1997) reported that a compound commonly known as persin from the avocado oil cells inhibited *S. exigua* growth at concentrations of 200 µg/g or higher. Later, Rodriguez-Saona *et al.* (1998) identified a group of alkylfurans present in the avocado idioblast oil that were toxic to *S. exigua* larvae. This group of compounds has received the common name

of avocadofurans. Currently, we are investigating the physiological and behavioral effects of the avocado idioblast cells and the cell oil on an insect that is adapted to feed on avocados, the omnivorous looper, *Sabulodes aegrotata*.


## Résumé

L'effet des cellules idioblastiques de l'avocat a été étudié sur la survie des larves et la croissance d'un insecte généraliste phytophage, *Spodoptera exigua*. L'activité des cellules a été observée par des expérimentations en condition de non-choix sur milieu artificiel sur des cohortes de larves néonates, à des concentrations similaires à celles trouvées naturellemnt chez l'avocat (2% du volume tissulaire). Aprés 9 jours, à des concentrations inférieures à celles trouvées dans l'avocat, les cellules idioblastiques réduisent significativement le poids des larves et augmentent leur mortalité. Nos résultats suggérent que la présence des cellules idioblatiques puisse expliquer en partie la raison pour laquelle *S. exigua*, qui est un insecte polyphage et le ravageur de beaucoup de cultures, ne soit pas adapté à l'avocat pour s'alimenter.


**Mots clés:** *Spodoptera exigua*, avocat, *Persea americana*, idioblaste.

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# Biochemical composition of leaf surfaces and influence on plant resistance studies

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**Abstract:** Chemicals present on the leaf surfaces of plants provide information for insects and influence their behaviour. Plant evaluation by contact and its acceptance are the crucial steps prior to ovipositing or injuring the plant by test-biting. Water soluble primary and secondary metabolites and mineral cations occur on the leaf surface. They provide the insect with information concerning the plant and leaf sites, plant physiology, and plant specificity. Their presence is the result of a dynamic equilibrium between the leaf tissues and the leaf surface.

Leaf surface carbohydrates promote oviposition of a generalist (*Ostrinia nubilalis)* and a specialist (*Cydia pomonella*) Lepidoptera. After alighting, both insects sample the leaf surface with the ovipositor. Carbohydrate, malic acid and cations are perceived by tarsal and ovipositor sensillae of *O. nubilalis.* Glucosinolates present on the leaf surface at several positions are negatively correlated with *O. nubilalis* oviposition.

These results suggest new ways of obtaining plant resistances, based on changing contact cues for host plant selection.

**Key words:** host selection, oviposition, leaf surface, metabolites, *Ostrinia nubilalis, Cydia pomonella.*

## Introduction

The survival of phytophagous insects depends initially on plant selection for oviposition and then for feeding. Recognition and acceptance of the plant by the insect happens after alighting when the insect is in contact with the leaf surface. Are there on the leaf surface biochemical stimuli that could help insects "evaluate" the nutrient value of the plant and could explain repellence or acceptance? In this study we focused on Lepidoptera and their oviposition behaviour which happens without any contact with internal plant tissues. The significance of leaf surface biochemistry in host plant selection was investigated.

## Methods

### *Identification of metabolites*

Substances were collected from the leaf surface, excluding the cuticular layer, by a light spraying of the leaf surface with ultra-pure water. Collection of internal leaf fluid was avoided by sealing the wounded leaf parts in liquid parafilm (Fiala *et al.*, 1990). Collection was followed by filtration of the water samples through a 0.22 µm filter to exclude microorganisms. Chemical analysis was done for water soluble carbohydrates (fructose, glucose, sucrose), polyols and organic acids after derivatization by GLC, and for the 20 free amino acids, alcaloids and glucosinolates by HPLC. Substances were identified by mass spectrometry.

***Origin of metabolites***

Incorporation of $^{13}CO_2$ into soluble carbohydrates of corn leaves *in situ* was performed to demonstrate the photosynthetic origin of metabolites found on the leaf surface. Repenetration of carbohydrates into the leaf through the cuticle was observed by following $^{14}C$-labelled carbohydrates deposited in small water droplets on corn and lettuce leaf surfaces over 24 hours.

***Localization of carbohydrates on the leaf surface***

X-ray microanalysis associated with scanning electron microscopy was used to visualize sugar complexes (oses-silver) on the leaf surface of several plant species.

***Cuticle permeability***

Cuticle permeability to metabolites was investigated on isolated cuticle with the method described by Stammitti *et al.*, 1996.

***Bioassays on insects***

Experiments with Lepidoptera species were done on plants grown hydroponically with a nutrient solution in greenhouses. The insects were given the choice between plants in greenhouse compartments. Eggs and eggs per egg mass were noted as well as their localization on the plant. Plants were sampled at the time of insect release for chemical analysis of the leaf surface. Correlations were obtained between insect oviposition site preference and chemical composition of the leaf surface (Derridj *et al.*, 1989). An artificial substrate (nylon cloth) impregnated with one or several compounds, was used to perform binary choices on isolated females. Electrophysiogical recordings were carried on tarsal and ovipositor sensillae of *O. nubilalis*. Substances tested included carbohydrates, some organic acids, proline and cations.

## Results

***Origin of metabolites***

As early as 30 min. after pulsed $^{13}CO_2$ labelling, $^{13}C$-labelled carbohydrates appeared on corn leaves. Compared to non-labelled molecules their proportions increased until 3 and 6.5 hours (Derridj *et al.*, 1996). $^{14}C$ labelled sugars deposited on corn leaf surface partly repenetrated into the leaf in 15 min, increasing slowly until 24 hours, 35 % of which repenetrated after deposition. The phenomenon varied according to the molecule and the plant species.

***Role of the cuticle on the leaf surface metabolite composition***

Earlier experiments showed that there are movements of molecules from both sides of the cuticle. Using isolated laurel cuticle we observed that movements were selective and linked to several parameters, such as the chemical and physical characters of the molecules, plant species and unknown characters of the cuticle. The distribution of carbohydrates on the leaf surface of corn, lettuce, and cherry laurel was rather diverse according to the plant species. They can be scattered uniformly over the leaf (lettuce) or concentrated along the epidermal walls e.g. on corn leaves.

***Significance of the chemical information given by the leaf surface***

Primary and secondary metabolites were present on the leaf surfaces. Soluble carbohydrates (fructose, glucose, sucrose), the 20 free amino acids, organic acids like malic acid, trans-aconitic acid, fumaric acid were found in quantities varying from 5 to 500 ng per $cm^2$. Secondary

metabolites like pyrrolizidinic alcaloids on Senecio sp. and glucosinolates (about 12) on Brassica napus leaves were found at levels of 1 ng per $cm^2$.

Information provided by the leaf surface metabolites is complex and precise. Plant species can be identified by both primary and secondary metabolites. Groups of approximately 6 free amino acids can be used to discriminate between plant species like corn, sunflower, leek, tansy ragwort (Derridj *et al.*, 1996), and between Senecio species (Soldaat *et al.*, 1996). Primary metabolites also give information on plant physiology. They can vary according to time of day, the leaf (age and surface) (Fiala *et al.*, 1990, Derridj, 1996), and the plant's adaptation to environmental conditions, such as atmospheric $CO_2$ .

***Insect behaviour and chemicals from the leaf surface***

Observations were carried out on the lepidopteran generalist, *Ostrinia nubilalis* and on the specialist species such as *Choristoneura fumiferana*, *Cydia pomonella* and *Tyria jacobaea*. In both generalist and specialist cases soluble carbohydrates were highly correlated with the insect site preference for oviposition. *Ostrinia nubilalis* and *C. pomonella* were tested on artificial substrates, using isolated soluble carbohydrates. The disruption of carbohydrate ratios or increase of their concentrations disturbed or suppressed insect preferences. Cations like $Na^+$, $Ca^{++}$, $K^+$ may interact with carbohydrates, and high cation concentrations had an adverse effect on *O. nubilalis*. A negative correlation was found between glucosinolates and oviposition on the *B. napus* leaf surface for this insect.

***Insect perception of the chemicals***

Sensilla on the tarsae and on the ovipositor of *O. nubilalis* were sensitive to the leaf surface washings and to compounds like carbohydrates, malic acid, proline (Derridj *et al.*, 1996) and cations (Derridj *et al.*, 1997). Qualitative variations in substances detected were perceived by different types of sensilla. Similar results were observed for *C. fumiferana* (Robert, P. J. personal communication ).

## Discussion

***Possible applications in resistance breeding and biological control***

This is the beginning of studies of the activity of water soluble metabolites present on the leaf surfaces. Many other substances are present on the leaf surfaces and are probably important for the recognition and acceptation of the plant by insects (generalists and specialists) before ovipositing and feeding. Can plants be selected for the production of modified signal molecules that could influence insect behaviour, without changing important plant attributes? Possible advantages include: 1) avoiding yield loss and reduced quality associated with the accumulation of foreign substances e.g. toxins, and; 2) use of a battery of plant genes to alter external signals, thus possibly slowing down insect adaptation to these changes in signals.

## Résumé

Les substances biochimiques présentes à la surface des feuilles donnent une information aux insectes et influencent leur comportement. L'évaluation de la plante par contact et son acceptation sont des étapes cruciales avant la ponte ou l'attaque de la plante. Les métabolites primaires et secondaires et les cations minéraux sont présents à la surface des feuilles. Ils informent l'insecte

sur la plante (physiologie, espèce et le site). Leur présence et le résultat d'un équilibre dynamique entre les tissus et la surface des feuilles.

Les sucres de surface de feuille ont une incidence sur la ponte d'un lépidoptère généraliste (*Ostrinia nubilalis*) et spécialiste (*Cydia pomonella*). Après atterrissage de ces deux insectes ceux-ci examinent la surface de la plante avec leur ovipositeur. Les sucres solubles, l'acide malique, les cations sont perçu au niveau des sensilles gustatives situées sur les tarses et l'ovipositeur de *O. nubilalis*. Les glucosinolates présents à la surface de différents niveaux foliaires du colza sont négativement corrélés avec la ponte d'*O. nubilalis*.

Ces résultats suggèrent de nouvelles pistes de recherche de résistance basée sur les stimuli de contact qui agissent lors de la sélection de la plante hôte par l'insecte.

**Mots clés:** Sélection de la plante hôte, ponte, surface foliaire, métabolite.

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# A rapid method to test resistance to spider mite (Tetranychus urticae) in cucumber

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**Abstract:** The spider mite (*Tetranychus urticae*) is an important pest of many greenhouse crops. Chemical and biological methods are being used to control the mite population, but both are expensive. Host plant resistance is an attractive alternative but up to now, screening tests were laborious. A simple method to test for resistance was developed for cucumber. Using this method, information about the degree of resistance to spider mite can be obtained from single plants. This greatly improves the efficiency of selection in a breeding programme.

**Key words**: *Tetranychus urticae*, cucumber, host plant resistance

## Introduction

Spider mite (*Tetranychus urticae*) is an important pest of many ornamental and vegetable greenhouse crops. Chemical and biological methods are being used to control the mite population, but both are expensive. Host plant resistance is an attractive alternative but up to now, screening tests were laborious. De Ponti (1980) described two methods for determining the resistance of plants, one using whole plants in a greenhouse and the other using small leaf disks in the laboratory. The first method is space consuming and takes about three months, but is not very labour intensive. The second method determines the oviposition rate on small leaf disks after a period of adaptation to the plant to be tested; this takes less time (3.5 weeks) but is very laborious. Van Impe & Hance (1993) described a leaf disk method with synchronized female spider mites; this test takes only 12 days but requires refreshing of the leaf disks every day which makes it very laborious.

We developed a laboratory method which requires less labour, time and greenhouse space than the above described procedures.

## Methods and Materials

One or two leaf disks (Ø 49mm) were cut from a fully-expanded leaf of each plant to be tested. The disks were placed upside down in a tray lined with filterpaper, moistened with a 10 ppm benzimidazole solution such that the disks nearly floated. This prevented the mites from moving to another disk. The trays were covered to maintain a high relative humidity, but a small opening made to prevent condensation on the disks.

Three non-synchronized adult female mites from the mass rearing were placed on each disk. The disks were incubated at 25°C for 24 hours. After this period, the mites were removed and the eggs produced were counted. The disks were further incubated at 25°C for about 10 days until the

next generation of adults was present and could be counted. The ratio of the number of adults to the number of eggs (% reproduction) was used as a measure for the susceptibility of the plant.

The method was evaluated by comparing the results obtained in this study with those from earlier tests, using the procedures described by De Ponti (1977). Eight cucumber genotypes were chosen: six genotypes, coded as 9103, 9104, 9140, 9143, 9145 and 9160 were accessions from different research programmes. 'Vetobit' is a bitter cultivar and 'G6' is a non-bitter gynoecious, homozygous breeding line of the Dutch slicer type.

For the laboratory test, seeds were sown in January 1996 and nine plants of each genotype were potted and grown in the greenhouse at a 23C/19C day/night regime. Four weeks later, the first true leaf was cut from the plant and two leaf disks were punched from each leaf. The disks were arranged in the trays according to a randomized block design with one plant of each genotype per block.

The 'De Ponti' spider mite tests were performed in the spring of 1994. The acceptance and the oviposition rate were determined on each genotype using 10 replicates. For adaptation, 20 deutonymphs were put on the first leaf, and is petiole was covered with 'tanglefoot' and its to prevent the nymphs from moving to the other plant parts. Ten days later, the remaining mites were counted (a measure of acceptance) and five of them were put on small leaf disks (Ø 22mm), punched from the second leaf. The disks were incubated as described above. After three days, the eggs were counted (oviposition rate).

The plants were transplanted to larger pots and arranged in a randomized block design. The plants were widely spaced to prevent them from touching each other and the third leaf of each plant was inoculated with 10, non-synchronized female spider mites. Six weeks later, damage to the top three leaves was estimated on a scale from zero to five (damage index). The relative leaf size of each accession was determined by measuring three representative leaves and expressing that area as the mean of all the accessions. This coefficient was used to correct the damage index for leaf size.

## Results

The results obtained with both testing procedures are presented in Table 1. In the 'De Ponti' test, acceptance was not a good measure at all for the visually-scored damage which is considered to be comparable to spider mite damage in commercial cucumber growing. The omit, oviposition rate with the adapted females was significantly correlated with relative damage (Table 2). In the new laboratory test with non-adapted females, the number of eggs, produced by the three female spider mites on each disk averaged 30 and did not differ between genotypes. After hatching, nymphs died rapidly on disks from the genotypes 9103, 9104 and 9140. On the other genotypes, the mites died during the resting stages. The reproduction percentage was highly significantly correlated with the oviposition rate and the visually-scored damage in the 'De Ponti' test.

## Discussion

The newly developed laboratory method gave results similar to the oviposition and damage tests reported by De Ponti (1977). The former thus proved to be a good predictor of damage from spider mite infestation in the greenhouse and required less labour and time than the latter tests. The main difficulty in the test was to keep the leaf disks in good condition. This problem can be

avoided by using young leaves from healthy growing plants. Furthermore, the use of benzimidazol is important to prevent bacterial infection.

The new method covers the entire period from egg to adult stage. Van Impe & Hance (1993) followed the oviposition during 12 days but did not consider the full period after hatching of the eggs. We observed that on the resistant genotypes, nymphs died during one of the resting stages and those which survived, reached the adult stage at a later date than on susceptible genotypes.

Because as many leaves per plant as desired can be tested independently, the method can be used to select single plants in segregating generations which is of great importance in a breeding programme.

Table 1. Results of the 'de Ponti' and 'new' spider mite tests with eight cucumber accessions

| | 'De Ponti' spider mite test | | | | | new method |
|---|---|---|---|---|---|---|
| Accession | Acceptance[a] | Oviposition[b] | Damage[c] | Leaf size Coefficient | Damage rel. to leaf size | % Reproduction[d] |
| 9140 | 11.8 | 8.3 | 1.4 | 0.6 | 0.8 | 0.2 |
| 9103 | 11.6 | 10.8 | 1.8 | 1.1 | 2.0 | 0.2 |
| 9104 | 10.2 | 10.3 | 1.8 | 0.9 | 1.6 | 5.1 |
| 9145 | 10.4 | 17.9 | 3.4 | 0.7 | 2.4 | 17.7 |
| 9143 | 10.7 | 24.0 | 3.7 | 0.8 | 3.1 | 38.1 |
| 'Vetobit' | 9.2 | 23.0 | 3.3 | 1.2 | 4.0 | 57.9 |
| 9160 | 7.4 | 20.4 | 4.2 | 1.4 | 5.7 | 87.8 |
| G6 | 12.4 | 29.0 | 4.9 | 1.3 | 6.2 | 88.0 |
| LSD | 2.8 | 4.0 | 0.9 | | | 8.4 |

[a]: acceptance: average number of surviving mites after 12 days (max. 20)
[b]: oviposition: average number of eggs per adapted female
[c]: damage: mean of three visually scored leaves per plant, scale 0 - 5;
0 = no damage; 5 = leaf entirely damaged and webs present
[d]: % reproduction: percentage adult spider mites from number of counted eggs

Table 2. Coefficients of correlation between different measures for resistance to spider mite of eight cucumber accessions

| | Oviposition | Rel. dam | % reprod. |
|---|---|---|---|
| Acceptance | -0.12 | -0.32 | -0.39 |
| Oviposition | | 0.85 | 0.85 |
| rel.damage | | | 0.98 |

$r_{0.01} = 0.834$

## Résumé

Le tétranyque tisserand (*Tetranychus urticae*) est un ravageur important pour beaucoup de cultures sous serre. Des méthodes de contrôle chimique et biologique sont utilisée pour limiter les populations d'acariens, mais elles sont toutes chères. L'utilisation de plante résistantes est une alternative intéressante, mais jusqu'à présent, les tests de criblage étaient fastidieux.

Une méthode simple a été mise au point pour tester la résistance du concombre. Des disques foliaires sont prélevés sur la première vraie feuille à l'emporte-pièce; sur chaque disque, on note la reproduction de trois acariens adultes non adaptés. Avec cette méthode, on peut obtenir une information sur le niveau de résistance à partir d'une seule plante par génotype testé. Le rendement de la sélection dans un programme d'amélioration est ainsi fortement augmenté.


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# The ideal glucosinolate profile for pest resistance in oilseed rape

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**Abstract:** The results of research into the responses of oilseed rape pests (i.e. seed weevil, *Ceutorhynchus assimilis*, cabbage stem flea beetle, *Psylliodes chrysocephala*, pollen beetle, *Meligethes aeneus*, and brassica pod midge, *Dasineura brassica*) to glucosinolates in rape are summarised. Volatile glucosinolate catabolites, such as isothiocyanates, are important cues to the orientation of these insects. Glucosinolates stimulate biting by cabbage stem flea beetle, but their presence is not a prerequisite to feeding.

Two strategies are suggested for improving the glucosinolate content of oilseed rape for pest resistance. The first involves rape lines with low constitutive but high induced glucosinolate levels. The second involves rape lines with a high proportion of glucosinolate types that do not catabolise to isothiocyanate, particularly the higher alkenyl isothiocyanates.

**Key words**: *Brassica napus*, glucosinolate, isothiocyanate, *Ceutorhynchus assimilis*, *Psylliodes chrysocephala*

## Introduction

Semiochemistry of the major U.K. pests of oilseed rape, *Brassica napus,* (seed weevil, *Ceutorhynchus assimilis*, cabbage stem flea beetle, *Psylliodes chrysocephala*, pollen beetle, *Meligethes aeneus*, and brassica pod midge, *Dasineura brassicae*) has been extensively studied at IACR-Rothamsted (Pickett *et al.*, 1995). The secondary metabolite chemistry of crucifers (Brassicaceae) such as oilseed rape is characterised by the presence of glucosinolates, important chemical mediators of insect-plant interactions. However, most studies of insect responses to glucosinolates have tested sinigrin (2-propenylglucosinolate), which is not found in oilseed rape in appreciable amounts (Milford *et al.*, 1989), and the catabolic product 2-propenyl (allyl) isothiocyanate.

This paper summarises research at Rothamsted on the responses of oilseed rape pests to glucosinolates and glucosinolate catabolites produced in the commercial rape crop. Plant breeding (Giamoustaris & Mithen, 1996) and genetic engineering (Hallahan *et al.*, 1992) can be used to alter the glucosinolate composition of oilseed rape. Our knowledge of the behaviour of oilseed rape pests is used to speculate how best the glucosinolate content of the crop may be altered to reduce its susceptibility to pests.

## Responses of the rape pests to glucosinolates

Crucifer-feeding insects show long range orientation to volatile glucosinolate catabolites as well as close range (feeding and oviposition) responses to intact glucosinolates (Chew, 1988). To determine how the seed weevil orientates to the volatile products of oilseed rape, coupled GC-electroantennography and coupled GC-single cell recording was used to identify which components of air entrainment extracts from oilseed rape (cv. Willi) elicited an electrophysiological response

from the seed weevil at natural physiological concentrations (Blight *et al.*, 1995). From over 250 compounds detectable in the extract, 25 were found to be electrophysiologically active, seven of which were attractive when tested in a linear track olfactometer (Bartlet *et al.*, 1997). Of these, five (three nitriles and two isothiocyanates) were glucosinolate catabolites.

Field trapping experiments have confirmed the attraction of the seed weevil to isothiocyanates and nitriles (Smart & Blight, 1997). Attraction of the cabbage stem flea beetle, pollen beetle and pod midge to isothiocyanates from oilseed rape has also been demonstrated in the field (Bartlet *et al.*, 1992; Murchie *et al.*, 1997a; Smart *et al.*, 1995). Thus, glucosinolate catabolites appear to be of particular importance to the orientation of the seed weevil and the other major pests of oilseed rape. This is further evidenced by the large proportion of receptors on the antennae of the seed weevil and the cabbage stem flea beetle that respond to isothiocyanates from rape (Blight *et al.*, 1989).

The cabbage stem flea beetle is the only oilseed rape pest whose feeding behaviour has been studied in detail. In choice tests with 40 different plants, its feeding was restricted to glucosinolate-containing species (Bartlet & Williams, 1991). Gustatory sensilla on the antennae responded to glucosinolates, glucobrassicin (3-indolylmethylglucosinolate) evoking the most spikes (Isidoro *et al.*, 1998; Ziesmann pers. comm.). When added to agar, glucobrassicin was also the most effective glucosinolate at stimulating feeding (Bartlet *et al.*, 1994). However, the presence of glucosinolates is not a prerequisite for feeding as cabbage stem flea beetles will feed avidly on agar plus sucrose and feeding rates on various breeder's lines of oilseed rape did not correlate with their glucosinolate content (Bartlet *et al.*, 1994; Bartlet *et al.*, 1996).

The role of glucosinolates in determining host-plant range is unclear, as all plants rejected in the choice tests have been found to contain feeding deterrents (Bartlet, 1995). A starved flea beetle would probably feed on a rape plant even if all the glucosinolates were removed, a situation that has been demonstrated with *Phyllotreta cruciferae*, a related species of crucifer-specialist (Bodnaryk & Palaniswamy, 1990).

Oviposition responses of oilseed rape pests to glucosinolates have not been studied and may have potential for control, since it has been demonstrated that decreasing the ovipositional stimuli for pollen beetles reduces oogenesis (Hopkins & Ekbom, 1996).

## Altering the glucosinolate content of rape to minimise pests and disease

Our research has demonstrated that glucosinolates and glucosinolate catabolites are important cues to host selection by cruciferous pests, aiding both orientation to and recognition of the host plant. A reduction in the glucosinolate content of oilseed rape should make it more difficult for rape pests to orientate to the crop, although it would not be expected to dramatically affect the feeding rates of those insects that do succeed in locating the crop. However, whilst a reduction in the glucosinolate content of oilseed rape may decrease the recruitment of crucifer-feeders, it will also increase the plant's susceptibility to unadapted generalist-feeders and disease (Chew, 1988). Furthermore, generalists are more sensitive to changes in glucosinolate concentration than specialists (Blau *et al.*, 1978). This may explain why the seedlings of double low (low seed erucic acid and glucosinolate) cultivars of oilseed rape are more vulnerable to attack from generalist feeders but no less susceptible to specialists (Williams, 1989; Glen *et al.*, 1990). How can the glucosinolate content of oilseed rape be altered to make the crop less conspicuous to crucifer specialists without increasing vulnerability to other pest and disease problems?

*i)* ***Low constitutive/high induced glucosinolate levels***
The indolylglucosinolate content of oilseed rape increases in response to damage, usually resulting in a net increase in total glucosinolates (Birch *et al.*, 1992; Bartlet *et al.*, in press). This change may persist for several weeks (Bodnaryk, 1992). A rape plant with low constitutive but high induced glucosinolate levels would be unobtrusive to specialist insects in the absence of attack, but protected from generalist feeders and pathogens once damaged (Doughty *et al.*, 1995; Bartlet *et al.*, in press). Furthermore, there is evidence that other elements of the damage response ensure that induced plants are not more susceptible to crucifer-specialists (Bartlet *et al.*, in press).

*ii)* ***Reduced emission of glucosinolate metabolites***
Some glucosinolate types, such as the indolyls, do not metabolise to produce stable isothiocyanates (Pickett *et al.*, 1995). Increasing the proportion of these types in the glucosinolate profile of oilseed rape would reduce the emission of isothiocyanates, particularly alkenyl glucosinolates such as 3-butenyl isothiocyanate, that attract oilseed rape pests. However, the overall glucosinolate concentration of the plant would be maintained as protection from other herbivores and from disease. It should be kept in mind however that increasing the proportion of indolylglucosinolates in the plant may cause increased feeding as they are the most effective glucosinolates at stimulating the feeding of the cabbage stem flea beetle and other crucifer specialists (Bartlet *et al.*, 1994).

Both these strategies aim to produce rape lines that emit lower levels of attractants for crucifer pests. Such lines would be best used as part of an integrated pest management system, such as the stimulo-deterrent diversionary strategy (Miller & Cowles, 1990). This is because, even if isothiocyanate and nitrile emissions were minimised, a large oilseed rape crop could be located by other cues, such as methyl salicylate or colour (Smart *et al.*, 1995; Smart *et al.*, 1997; Bartlet *et al.*, 1997). However, insects could be diverted from the main crop if a highly attractive "trap" crop were planted in close proximity (Pickett *et al.*,1995).

Field trapping with allyl and 2-phenylethyl isothiocyanate caught significant numbers of *Platygaster subuliformis* and *Omphale clypealis*, parasitoids of the brassica pod midge (Murchie *et al.*, 1997a). Since natural enemies such as these can exert significant natural control of oilseed rape pests (Murchie *et al.*, 1997b) more information on the influence of glucosinolates on natural enemies of oilseed rape pests is required. This area is currently under investigation at Rothamsted.

## Résumé

Les résultats de la recherche des réponses des ravageurs (i.e. *Ceutorhynchus assimilis, Psylliodes chrysocephala, Meligethes aeneus, Dasineura brassica*) aux glucosinolate du colza sont résumés. Les catabolites volatils des glucosinolates, tel que les isothiocyanates, sont des indicateurs importants pour l'orientation de ces insectes. Les glucosinanates stimulent la morsure de *P. chrysocephala*, mais leur présence n'est pas nécessaire pour l'alimentation.

Deux stratégies sont proposées pour améliorer la teneur en glucosinolates du colza pour la résistance aux ravageurs. La première concerne des lignées de colza avec des taux faibles de glucosinolates constitutifs mais des taux élevés de glucosinolates induits. La deuxième concerne des lignées de colza avec une forte proportion de types de glucosinolates qui ne se catabolisent pas en isothiocyanate, particulièrement en alkenyl isothiocyanates les plus élevés.

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# Influence of leaf-vein characteristics on oviposition preference of the cotton leafhopper, Amrasca devastans

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**Abstract:** Oviposition preference of the cotton leafhopper, *Amrasca devastans* (Distant) was investigated in relation to leaf-vein characteristics of 12 host plants *viz*: okra, cotton, castor, cowpea, pigeonpea, sesame, egg plant, cluster bean, green gram, shoe flower, country mallow and portia tree. Observations on the oviposition of the leafhopper under no choice conditions were recorded on three occasions during the peak period of leafhopper incidence in the field. On all three occasions the leafhopper rejected green gram, shoe flower and portia tree for egg-laying thereby indicating non-acceptance of these plants for oviposition. For the remaining plants, the number of eggs per leaf differed significantly. Okra received the maximum number of eggs. Lateral veins of castor, cotton, cowpea, egg plant and country mallow leaves received more eggs in comparison to the main vein and sub-veins whereas in okra the sub-veins received the highest number of eggs followed by lateral and main veins. Correlations of trichome density with the number of eggs laid in different veins were non-significant. Likewise trichrome length had no influence on oviposition by the leafhopper. All types of veins in okra leaves were thicker than the rest of the host plants which might have stimulated more egg-laying in okra. Thickness of each category of vein *viz*: main vein (r = 60), lateral veins (r = 0.65) and sub-veins (r = 0.56) was found to be positively correlated with the number of eggs laid. Length of main and lateral veins were also found to be positively and significantly related to the number of eggs laid. It may be concluded from the present investigations that leaf vein thickness and length are crucial factor in influencing oviposition behaviour of the cotton leafhopper.

**Key words:** *Amrasca devastans*, cotton leaf-hopper, leaf-veins, oviposition, host range.

## Introduction

The cotton leafhopper, *Amrasca devastans* (Distant) is an important pest of several field crops including cotton, okra, egg plant, potato, tomato, sunflower, cluster bean (Butani & Jotwani, 1984), castor (Deshmukh *et al*, 1979), cowpea (Sagar & Mehta, 1982), green gram (Yein, 1982) and wild plants including country mallow (Atwal, 1976; Singh & Taneja, 1989). The potential of the leafhopper to inflict damage depends on its oviposition preference and subsequent population build up on different host plants. High pubescent character in cotton and okra has been found to confer resistance against leafhopper by reducing oviposition and feeding (Batra & Gupta, 1970, Singh, 1988) but it enhances the oviposition by other pests (Butler *et al*, 1986; Gannaway, 1994). Leafhopper females deposit eggs inside leaf veins (Agarwal et *al*, 1978) and it has been established in cotton and okra that a larger number of thicker veins (diameter > 1 mm) increases the oviposition response of leafhopper (Singh & Agarwal, 1988) but such information is lacking on other host plants. Therefore, investigations were made of the impact of leaf vein characteristics on oviposition preference of the leafhopper.

## Materials and methods

Among the 12 host plants, nine (okra, cotton, castor, pigeonpea, cowpea, cluster bean, green gram, egg plant and sesame) were grown at the Department of Entomology Research Farm in a randomised block design, replicated three times with a plot size of 18 sqm (4.5 x 4.0m). Sowing of the above host plants was done in the fourth week of June in 1995. The crops were raised according to recommended agronomic practices except the application of insecticides. Country mallow, portia tree and shoe flower were selected at 3 sites (3 plants/site) on the University Campus/Research Farm.

***Record of leafhopper eggs***

Ten leaves of each host plant were exposed to leafhopper females (30 adults) in muslin cloth cages (20 x 10cm) for 48 to 72 hours and then processed in lactophenol solution (Moffit & Reynolds, 1972) to stain the eggs laid inside veins. The eggs were counted in the different leaf veins (main, lateral and sub-veins) as suggested by Singh & Agarwal (1988) for cotton, okra, country mallow, shoe flower, portia tree and castor (Fig 1) while for non-malvaceous host plants *viz*: egg plant, sesame, cowpea, pigeonpea, green gram and cluster bean thick veins arising along the main vein were designated as lateral veins which further gave rise to sub-veins (Fig 2). There were no measurable sub-veins in sesame, pigeonpea and cluster bean. The observations on eggs in different leaf veins were recorded three times during the peak period of leafhopper activity.

***Measurements of morphological characteristics***

Density and length of trichomes on leaf veins (Batra & Gupta, 1970), and thickness and length of leaf veins (Singh & Agarwal, 1988; Singh & Taneja, 1989) were recorded from the same leaves as used for egg counting.

***Statistical Analysis***

The data were subjected to analysis of variance after appropriate transformations in randomized block design (Snedecor & Cochran, 1968). Simple correlation coefficients were worked out by using a standard product moment correlation coefficient formula to investigate the influence of various morphological factors on the oviposition.

## Results and Discussion

The leafhopper females laid eggs on the underside of leaf veins of various types. The different categories of veins *viz*: main vein (mid-rib), lateral veins and sub-veins were ascertained and counted on the leaves of different host plants as compiled in Table 1. There was only one main vein or mid-rib for each host plant leaf but lateral veins ranged from 4 in shoe flower to 18 in pigeonpea and sesame. Similarly, the number of sub-veins ranged from 3 in green gram to 47 in castor.

The leafhopper eggs were recorded on three occasions during the peak period of activity (Table 2). Green gram, shoe flower and portia tree leaves were found unacceptable for egg laying. In the remaining plants, the number of eggs per leaf ranged from 2.33 in egg plant to 69.50 in okra on 12.8.1995, 0.00 in pigeonpea to 93.66 in okra on 23.8.1995 and 0.00 in pigeonpea to 140.16 in okra on 6.9.1995. Okra received most eggs, followed by castor, cotton, egg plant, cowpea, country mallow, cluster bean, sesame and pigeonpea in descending order.

The distribution of eggs according to leaf vein categories are provided in Table 3. It is evident that lateral veins of castor, cotton, cowpea, egg plant and country mallow leaves received more eggs in comparison to main and sub-veins whereas in okra, sub-veins received most eggs followed by lateral veins and the main vein. Leaves of cluster bean and sesame were devoid of sub-veins. In these plants lateral veins received more eggs than the main vein. It may also be concluded that more eggs in lateral or sub-veins of different host plants were laid because of the higher number of veins in these categories. Otherwise on an individual vein basis, the main vein received more eggs. The same conclusion was drawn by Singh & Agarwal (1988) while working on cotton and okra. Therefore, leafhopper females are capable of selecting appropriate veins for oviposition.

***Impact of leaf vein characteristics***

The density of trichomes (number of trichomes on 3 mm long vein pieces) on the main vein, lateral and sub-veins of different host plants varied significantly. The number of trichomes on veins of country mallow (772 to 1324), pigeonpea (439 to 616) and egg plant (93 to 133) were higher than cotton (21 to 44), cowpea (2 to 3), cluster bean (13 to 34), sesame (21 to 49), green gram (6 to 8) and shoe flower (1 to 5). Leaf veins of castor and portia tree were free from trichomes. Trichome length also varied significantly on different host plants. The longest trichomes were observed for cotton followed by green gram and egg plant for all three types of veins. Correlation of trichome density on main vein ($r = -0.11$), lateral veins ($r = -0.09$) with oviposition were negative but non-significant. Similarly trichome length on different veins did not have any significant effect on oviposition by the leafhopper. But thickness and length of leaf veins may be providing appropriate size, stimulus and environment for placement of eggs (length = 0.658 mm and breadth = 0.162 mm) inside the leaf veins. It is evident from Table 4 that the thickness of leaf veins varied significantly among host plants. The thickness of the main vein ranged from 0.46 mm in pigeonpea and cluster bean to 2.18 mm in okra while the thickness of lateral veins varied from 0.22 mm in pigeonpea to 1.72 mm in okra. The thickness of sub-veins ranged from 0.18 mm in cowpea to 0.90 mm in okra. The thickness of each type of vein, i.e.: main vein ($r = 0.60$), lateral veins ($r = 0.65$) and sub-veins ($r = 0.56$) were found to be positively correlated with the number of eggs laid by leafhopper females. Similar conclusions were drawn by Singh & Agarwal (1988) while working on cotton and okra. These authors pointed out that a vein thickness of 0.7 to 1.1 mm is on the lower side for eliciting appropriate egg-laying response in leafhoppers. But in the present findings the leafhopper could lay eggs even in thinner veins (0.22 to 0.29 mm) on country mallow, sesame and cluster bean under no choice conditions. The length of veins of different types was also positively correlated with the number of eggs laid but the impact of length of sub-veins was non-significant. It may be concluded from the present investigations that leaf vein thickness and length are important factors in influencing the oviposition behaviour of the cotton leafhopper.

## Résumé

La préférence de ponte d'*Amrasca devastans* (Distant) a été étudié en relation avec les caractéristiques des nervures foliaires de 12 plantes hôtes viz: gombo, *Gossypium hirsutum, Ricinus communis, Vigna unguiculata, Sesamum indicum, Solanum melongena, Malva* sp.,. Les observations sur la ponte en condition de non choix ont été faites aux trois périodes d'incidence maximum de l'insecte au champ. Aux trois périodes l'insecte a refusé. pour pondre. Ceci indique

la non acceptation de ces plantes pour la ponte. Pour les autres plantes le nombre d'oeufs par feuille diffère significativement. Le gombo a reçu le maximum d'oeufs. Les nervures latérales des feuilles de. ont reçu plus d'oeufs que les nervures principales et secondaires alors que chez le gombo les nervures secondaires ont reçu la plus grande quantité d'oeufs suivies par la nervure latérale et principale. Les corrélations entre la densité de trichomes et le nombre d'oeufs déposés sur les différentes nervures ne sont pas significatives. La longueur des trichomes n'a pas d'lnfluence sur la ponte. Tous les types de nervures sur les feuilles de gombo sont plus épais que sur les autres plantes hôtes, ce qui a pu stimuler la ponte chez le gombo. L'épaisseur de chaque categorie de nervure: nervure principale (r = 0.60), les nervures latérales (r = 0.65) et les nervures secondaires (r = 0.56) et positivement corrélée avec le nombre d'oeufs déposé. La longueur des nervures principales et latérales est corrélée positivement avec le nombre d'oeufs déposé. Il peut être conclu de ces investigations que l'épaisseur et la longueur des nervures sont des facteurs cruciaux qui influencent le comportement de ponte de l'insecte.

## Acknowledgements

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Table 1. Number and category of leaf veins in different host plants.

| Host plant | Number of veins in each category | | | Total number of veins |
|---|---|---|---|---|
| | Main vein | Lateral veins | Sub-veins | |
| Okra | 1 | 6 | 26 | 33 |
| Castor | 1 | 6 | 47 | 54 |
| Cotton | 1 | 6 | 26 | 33 |
| Pigeonpea | 1 | 18 | 0 | 19 |
| Cowpea | 1 | 12 | 4 | 17 |
| Egg plant | 1 | 10 | 8 | 19 |
| Cluster bean | 1 | 10 | 0 | 11 |
| Sesame | 1 | 18 | 0 | 19 |
| Country mallow | 1 | 8 | 17 | 26 |
| Green gram | 1 | 8 | 3 | 12 |
| Shoe flower | 1 | 4 | 16 | 21 |
| Portia tree | 1 | 6 | 6 | 13 |
| Total | 12 | 112 | 153 | 277 |

Table 2. Oviposition of cotton leafhopper on different host plants.

| Host plant | Average number of eggs/leaf | | |
|---|---|---|---|
| | 12.8.1995 | 23.8.1995 | 6.9.1995 |
| Okra | 69.50(8.39) | 93.66(9.72) | 140.16(11.87) |
| Castor | 0.00(1.00) | 66.00(8.18) | 44.66(6.75) |
| Cotton | 30.50(5.60) | 23.00(4.89) | 13.33(3.78) |
| Pigeonpea | 3.00(2.00) | 0.00(1.00) | 0.00(1.00) |
| Cowpea | 11.66(3.55) | 13.00(3.73) | 7.66(2.94) |
| Egg plant | 2.33(1.82) | 17.50(4.28) | 3.00(2.00) |
| Cluster bean | 3.00(1.99) | 6.16(2.67) | 8.83(3.13) |
| Sesame | 9.00(3.16) | 4.33(2.30) | 4.00(2.23) |
| Country mallow | 6.00(2.64) | 11.83(3.57) | 0.00(1.00) |
| Green gram | 0.00(1.00) | 0.00(1.00) | 0.00(1.00) |
| Shoe flower | 0.00(1.00) | 0.00(1.00) | 0.00(1.00) |
| Portia tree | 0.00(1.00) | 0.00(1.00) | 0.00(1.00) |
| Mean | 11.24 | 19.62 | 18.47 |
| S.Em. ± | (0.04) | (0.08) | (0.05) |
| CD (P = 0.05) | (0.13) | (0.26) | (0.16) |

Figures in parentheses are $\sqrt{n+1}$ values.

Table 3. Oviposition of cotton leafhopper in different veins of host plants.

| Host plant | Average number of eggs/leaf vein category (23.8.95) | | |
|---|---|---|---|
| | Main vein | Lateral veins | Sub-veins |
| Okra | 9.00(3.15) | 33.33(5.85) | 51.50(7.24) |
| Castor | 11.50(3.53) | 54.50(7.44) | 0.00(1.00) |
| Cotton | 2.00(1.73) | 13.83(3.84) | 7.66(2.93) |
| Pigeonpea | 0.00(1.00) | 0.00(1.00) | - |
| Cowpea | 2.83(1.93) | 10.16(3.34) | 0.00(1.00) |
| Egg plant | 4.50(2.51) | 13.00(3.74) | 0.00(1.00) |
| Cluster bean | 2.16(1.77) | 4.00(2.23) | - |
| Sesame | 1.33(1.51) | 4.33(2.30) | - |
| Country mallow | 3.16(2.04) | 7.66(2.94) | 1.00(1.41) |
| Green gram | 0.00(1.00) | 0.00(1.00) | 0.00(1.00) |
| Shoe flower | 0.00(1.00) | 0.00(1.00) | 0.00(1.00) |
| Portia tree | 0.00(1.00) | 0.00(1.00) | 0.00(1.00) |
| Mean | 3.04 | 11.73 | 6.68 |
| S.Em. ± | (0.09) | (0.05) | (0.07) |
| CD (P = 0.05) | (0.27) | (0.15) | (0.23) |

Figures in parentheses are $\sqrt{n+1}$ values

Table 4. Thickness and length of leaf veins in relation to oviposition of cotton leafhopper.

| Host plant | Main vein | | Lateral veins | | Sub veins | |
|---|---|---|---|---|---|---|
| | Thickness (mm) | Length (cm) | Thickness (mm) | Length (cm) | Thickness (mm) | Length (cm) |
| Okra | 2.18 | 9.77 | 1.72 | 3.98 | 0.90 | 1.39 |
| Castor | 0.97 | 11.92 | 0.53 | 7.56 | 0.23 | 2.34 |
| Cotton | 1.40 | 7.51 | 0.85 | 5.19 | 0.28 | 1.65 |
| Pigeonpea | 0.46 | 5.16 | 0.22 | 1.68 | - | - |
| Cowpea | 0.88 | 5.23 | 0.45 | 2.12 | 0.18 | 1.22 |
| Egg plant | 2.10 | 10.14 | 0.82 | 3.85 | 0.22 | 1.63 |
| Cluster bean | 0.46 | 5.38 | 0.29 | 1.73 | - | - |
| Sesame | 0.63 | 6.24 | 0.23 | 3.16 | - | - |
| Country mallow | 0.57 | 5.23 | 0.37 | 2.78 | 0.22 | 0.80 |
| Green gram | 0.72 | 7.08 | 0.41 | 3.48 | 0.19 | 1.55 |
| Shoe flower | 0.85 | 5.64 | 0.49 | 2.90 | 0.26 | 0.78 |
| Portia tree | 0.66 | 6.01 | 0.39 | 2.76 | 0.19 | 0.78 |
| S.Em ± | 0.01 | 0.15 | 0.01 | 0.05 | 0.01 | 0.05 |
| CD(P = 0.05) | 0.04 | 0.44 | 0.02 | 0.16 | 0.02 | 0.16 |
| Correlation coefficient (r) | 0.60* | 0.84* | 0.65* | 0.81* | 0.56 | 0.08 |

- Significant at 5% level

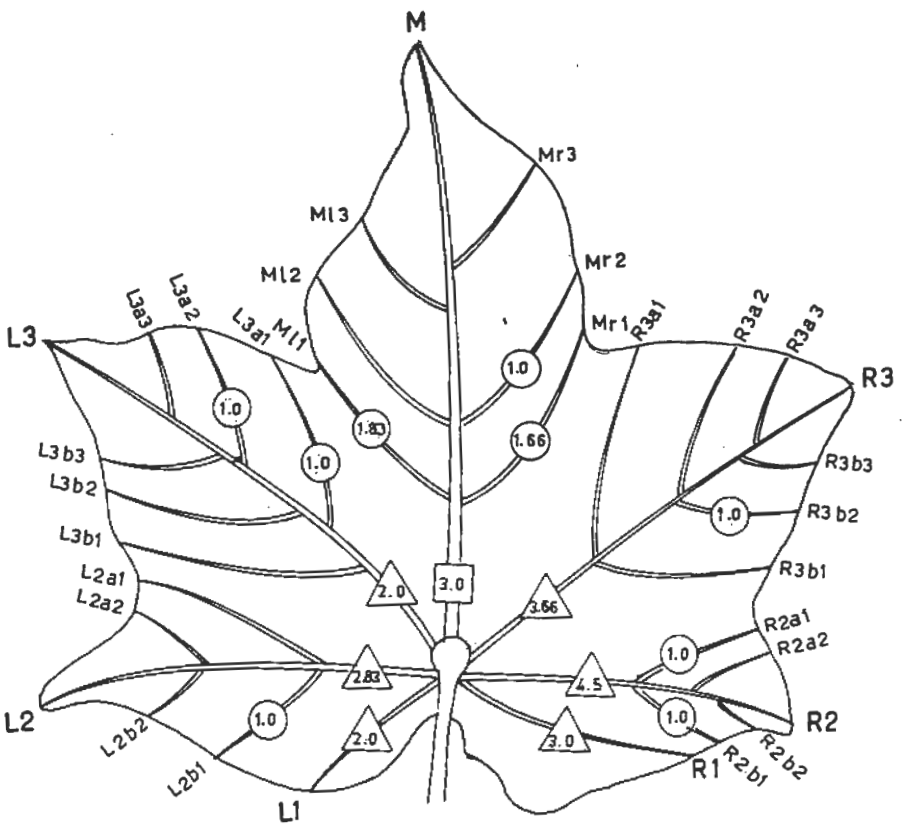


Figure 1. Vein pattern and distribution of leafhopper eggs in cotton leaf-veins. Number of eggs in main vein □, lateral veins Δ, and sub-veins O.

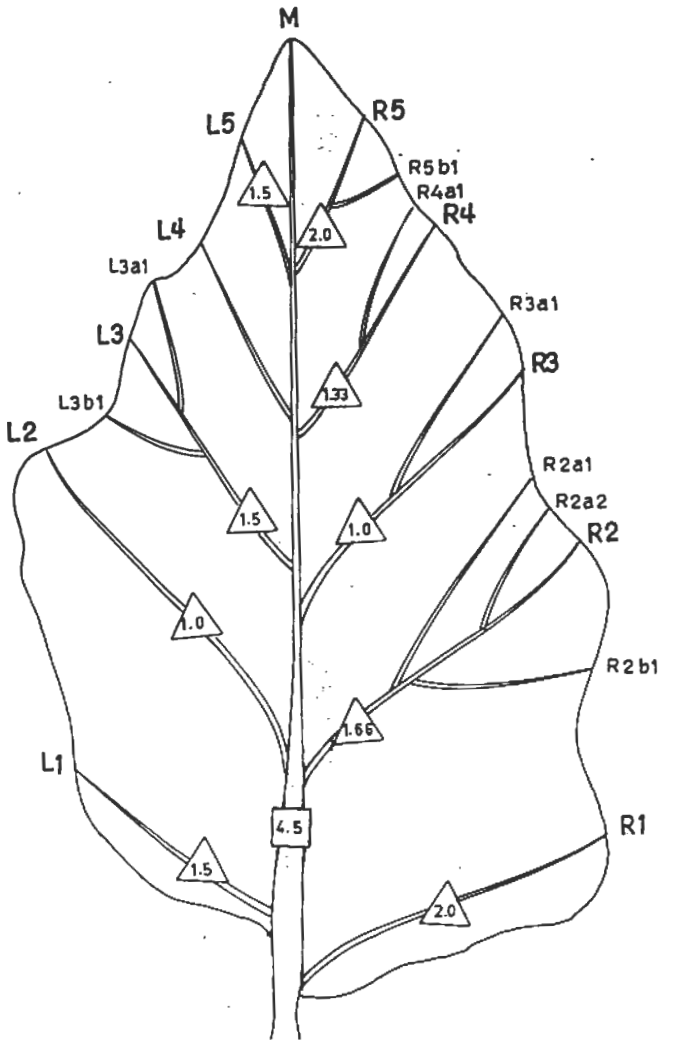


Figure 2. Vein pattern and distribution of leafhopper eggs in egg plant leaf-veins. Number of eggs in main vein□, lateral veins Δ.

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# Host-plant preferences in Delia radicum

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**Abstract:** The cabbage identification factor (CIF) is the most powerful stimulant chemical known for the cabbage root fly, *Delia radicum*. The amounts of CIF present on the leaf surfaces of 19 different plant species were quantified using electrophysiological recording of a tarsal sensillum of the fly. These data were compared with the oviposition preferences of the fly for the plant species. The results indicated that knowledge about the CIF content of plant leaf is of a limited value for the prediction of the fly's behavioural activity.

**Key words**: *Delia radicum*, cabbage root fly oviposition, cabbage identification factor (CIF), host-plant preference

## Introduction

Glucosinolates, present in Crucifers and in some other plant families, are known to be important oviposition stimulants for *Delia radicum* (Nair et al. 1976, Roessingh *et al.*, 1997)). No relation has been found, however, between glucosinolate concentrations in the leaves of some Crucifers and oviposition responses of *D. radicum* (Nair *et al.*, 1976). A new group of oviposition stimulants was isolated from cauliflower leaf surface extracts (Roessingh *et al.*, 1992). Results obtained by Baur *et al.*, (1996) and by Roessingh *et al.*, (1997) suggested that leaf extract fractions containing these compounds, named the cabbage identification factor "CIF", played a more prominent role in oviposition stimulation in *D. radicum* than glucosinolate containing fractions. The aim of this study was to evaluate in how far oviposition preferences in *D. radicum* correlate with the CIF contents in leaves. We measured CIF levels in leaf extracts from a range of wild crucifers and some other plants, and compared them with oviposition preferences in *D. radicum*.

## Methods

The plants used for testing were: *Allium porum* (Liliacae), *Alyssum alysoides* (Cruciferae), *Barbarea vulgaris* (Cruciferae), *Brassica napus* "Eurol" (Cruciferae), *Brassica rapa* "Hanko" (Cruciferae), *Capsella bursa-pastoris* (Cruciferae), *Cleome spinosa* (Capparidaceae), *Cochlearia officinalis* (Cruciferae), *Isatis tinctoria* (Cruciferae), *Lepidium campestre* (Cruciferae), *Lepidium sativum* (Cruciferae), *Raphanus raphanistrum* (Cruciferae), *Reseda luteola* (Resedaceae), *Rorippa islandica* (Cruciferae), *Rorippa silvestris* (Cruciferae), *Sinapis arvensis* (Cruciferae), *Sisymbrium officinale* (Cruciferae), *Thlaspi arvensis* (Cruciferae), *Tropaeolum majus* (Tropaeolaceae). These plants were chosen on the basis of the availability of data on oviposition preferences of *D. radicum* (Baur et al., unpublished results).

CIF concentrations were quantified by electrophysiological recording of the tarsal C5 sensillum. This sensillum contains a receptor neurone which is thought to be specifically sensitive

to CIF. The same leaf extracts which were previously used for oviposition preference tests by Baur et al. were analysed (unpublished data). Those extracts were made by dipping the leaves in $CH_2Cl_2$ and subsequently twice in MeOH for 5 seconds each. The MeOH fractions containing the oviposition stimulants (glucosinolates and CIF) were filtered through filter paper and evaporated to small volumes under vacuum at 45°C. Amounts and concentrations of samples are expressed in gle (gram leaf equivalent) or gle/ml respectively. One gle represents the amount of leaf surface extract from dipping 1 g of fresh leaf material.

Four to 5 gle of each leaf extract was diluted in 5 ml water and placed on a strong acid cation exchanger (DOWEX 50W X4) column which was conditioned with 1M HCl and subsequently washed neutral with distilled water. After loading the column with an extract, it was washed 4 times with 3 ml distilled water to remove the glucosinolates. The column was washed afterwards 5 times with 4 ml 3M ammonia. The collected fractions (containing CIF) were evaporated to dryness, diluted in 1 ml water, evaporated to dryness again, and diluted in 10 mM KCl to a concentration of 1 gle/ml to use them for electrophysiological recordings.

*Delia radicum* flies were reared following the method of Finch & Coaker (1969). Newly emerged female flies were kept isolated from the host plant and food, and used for electrophysiological recording 24 - 48 h after emergence.

The method used for electrophysiological recording was described earlier by Roessingh *et al.* (1992). Minor changes to the technique included always filling the indifferent electrode with saline and dissolving all stimuli in 10 mM KCl. Responses of the C5 sensillum were quantified by the number of spikes during the first second of stimulation.

## Results

The responses of the tarsal C5 sensillum to stimulation with the different plant extracts are shown in Figure 1. Analysis of the data using Spearman rank-order correlation coefficient showed a correlation $r_s = 0.56$ ($P < 0.02$) between oviposition preference and C5 response.

## Discussion

Electrophysiological recording of the C5 sensillum as a method for assessing the CIF levels in plant material is based on the assumption that the responses of the sensillum are highly specific to CIF (Roessingh *et al.* 1997). It should not be forgotten, however, that there are five receptor neurones, including one mechanoreceptor, associated with this sensillum (Isidoro *et al.*, 1994). Apart from the neurone which is sensitive to CIF, there is another neurone sensitive to certain glucosinolates (Roessingh *et al.*, 1997), while it is not known what the other neurones respond to. We can, therefore, not be certain that all the activity from the C5 sensillum is related to the presence of CIF in the stimulus solution, even after not removing the glucosinolates from the extracts. Despite this limitation, electrophysiological recording of the C5 sensillum, is at present the only feasible method to estimate CIF concentrations, as the amount of CIF present in *Brassica oleracea* leaves is estimated to be only 1 ng/gram leaf (Roessingh *et al.*,1997) and far too low to be measured otherwise.

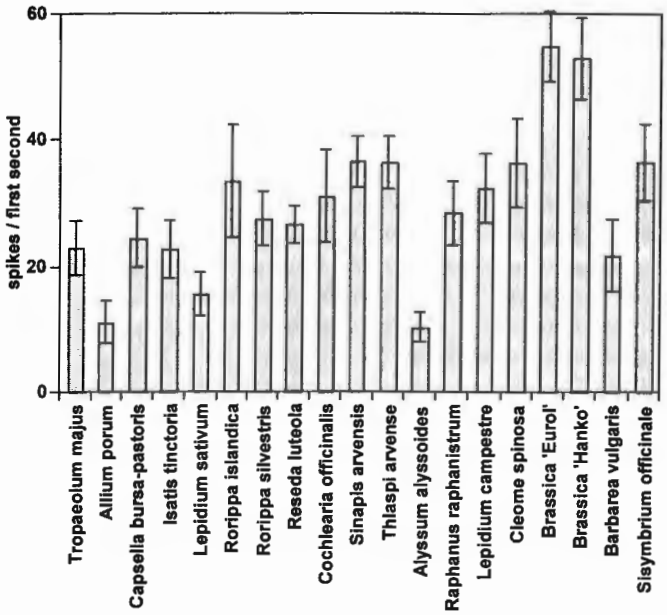


Figure 1. Mean responses (±SE) of the C5 sensillum of *Delia radicum* to stimulation with 1 gle/ml extract for 19 different plant species (N = 11). The plant species are arranged from left to right in an increasing oviposition preference order (Baur et al., unpublished data).

The electrophysiological data do not strongly correlate with the preference ranking for the *D. radicum* flies (Fig. 1). Apparently even the amount of CIF, the most powerful oviposition stimulant known, cannot fully explain the host preference of *D. radicum*. Some plants with comparable amounts of CIF (Fig. 1) and glucosinolates (Griffiths *et al.*, unpublished data), for example *Sinapis arvensis* and *Sisymbrium officinale*, elicit very different responses by the flies.

Results from work by Baur *et al.*, (1996) suggested that CIF concentrations can explain the oviposition preferences in *D. radicum*. This seems to be the case for different genotypes of swede (*Brassica rapa*, var. *rapa*). However, the results for different kale (*Brassica oleracea* var. *acephala*) genotypes were less convincing, and the authors suggested that interference of other chemical factors with the activity of oviposition stimulants might have played a role.

We found evidence for such interactions on the neurone's response to CIF. The C5 responses to the *Cleome spinosa* extract were significantly lower than after purification with HPLC. This suggests that CIF responses can be suppressed to some extent by other chemicals present in a less pure extract. We also found the responses of the C5 sensillum increased when samples were taken after each purification step when extracting CIF from rutabaga roots (unpublished results). Maybe differences between the plants in their levels of such chemicals obscure a clear correlation of the CIF levels with the oviposition preferences. There is also evidence for synergistic interactions at the behavioural level. *D. radicum* strongly prefers odour baited leaves over control leaves (De Jong & Städler, in preparation). Odour by itself is not enough to elicit oviposition, but synergizes with the response to simultaneously perceived sinigrin present on the leaves.

It seems that other chemicals can have an additional positive or negative influence on the fly's acceptance of a plant for oviposition. Knowledge about the CIF concentration in a plant, therefore, will give us only limited information about its antixenotic resistance to *D. radicum*.

**Résumé**

CIF est le plus puissant stimulant chimique de ponte connu chez la mouche du chou, *Delia radicum*. Nous avons quantifié les quantités de CIF présents à la surface des feuilles de 19 espèces de plante à l'aide de mesures électrophysiologiques de sensilles des tarses de la mouche. Ces résultats ont été comparés à la préférence des espèces de plante pour la ponte. Les résultats indiquent que la connaissance de la teneur des feuilles en CIF a une valeur limitée quant à l'activité comportementale de la mouche.

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## Session 2 – Sources of Resistance, Breeding and Testing

*Breeding for Resistance to Insects and Mites*
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# The making of the aphid resistant butterhead lettuce 'Dynamite'

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**Abstract:** In 1981 lettuce leaf aphid, *Nasonovia ribisnigri,* resistant material (Nr gene) was released to seed companies by IVT (now CPRO-DLO / Wageningen, Holland). In a backcross programme, using several recurrent parents with different characteristics, a combination of genetic characters was obtained.

The outdoor lettuce butterhead variety 'Dynamite' (LM-8021) possesses the *N. ribisnigri* resistance gene Nr, the *Bremia lactucae* resistance gene R18 and the LMV tolerance gene ga. It is also highly resistant to the lettuce root aphid, *Pemphigus bursarius,* and has some resistance to the common potato-aphid, *Macrosiphum euphorbiae,* and the brown lettuce aphid, *Uroleucon sonchi.* It has no resistance to the glasshouse-potato aphid, *Aulacorthum solani.*

'Dynamite' is medium sized, light green, semi-thick and slightly blistered. This variety is suitable for use in spring and early-summer croppings in north west Europe and also in autumn croppings in south European countries.

**Key words:** *Lactuca sativa, Lactuca virosa, Lactuca serriola, Nasonovia ribisnigri, Pemphigus bursarius, Macrosiphum euphorbiae, Aulacorthum solani, Uroleucon sonchi, Bremia lactucae,* Nas-resistance, leaf aphids, root aphids, aphid resistance, LMV, lettuce mosaic virus, host plant resistance, bioassays

## Introduction

In 1972 IVT (now CPRO-DLO) began investigations of the relationship between lettuce, *Lactuca sativa*, and the lettuce leaf aphid, *Nasonovia ribisnigri* (IVT letter of release 16th September 1980). This aphid is responsible for severe damage to lettuce heads. More than ninety per cent of all aphids found in lettuce in Europe are *N. ribisnigri*. As these aphids are mainly present on the younger leaves, colonizing the heart of the lettuce head, pesticides, especially contact ones, are less effective after the closing of the head. If *N.ribisnigri* is not present its place will not be taken by other aphids.

Leaf aphids suck plant juices from the phloem causing leaf curling, growth reduction and malformation. They cause heart rot and plants may wilt. Leaf aphids in general may also transmit virus diseases. While consumers are reluctant to buy lettuce contaminated with aphids, chemical spraying was, and has been, until now, the only control option

Bioassays were developed by IVT and in 1978 screening began of all varieties available at the Centre for Genetic Resources in the Netherlands (CGN) for resistance to *N. ribisnigri*.

Resistance to *N. ribisnigri* was found in six *Lactuca virosa accessions*, and shown to be governed by a single dominant gene, Nr (Eenink *et al.*, 1982b, Eenink & Dieleman, 1983). Because of sterility and incompatibility of an interspecific cross between the common lettuce *L. sativa* and *L. virosa*, *L. serriola* was used as a bridging species (Eenink *et al.*, 1982a) to transfer the gene from *L. virosa* accessions PIVT-280 and PIVT-cd72723 (Eenink *et al.*, 1982b) into *L. sativa*. Fertile *L. sativa* lines were obtained which were resistant to the lettuce leaf aphid. In 1981

these resistant lines possessing the dominant resistance gene, Nr, were offered and then released to five lettuce breeding companies.

A survey carried out under the auspices of the International Organisation for Biological Control (IOBC) in field-grown lettuce at 11 sites in six European countries showed that *N. ribis-nigri* was the most important pest and that the potato aphid, *Macrosiphum euphorbiae,* and the lettuce root aphid, *Pemphigus bursarius,* were also common pests. In the Netherlands, the brown lettuce aphid, *Uroleucon sonchi,* was also frequently found on lettuce (Reinink & Dieleman, 1993).

Partial resistance to the aphid species, *M. euphorbiae* and *U. sonchi,* is inherited mainly additively (Reinink *et al.*, 1995). The expression of partial resistance is dependent on environmental conditions. There are prospects for selecting for an increased level of resistance and combining the resistance traits in plant breeding programmes. The partial resistance to the three different species of aphid could have some genes in common (Reinink & Dieleman, 1990).

Resistance to the lettuce root aphid, *P. bursarius,* is governed by the Ra-gene (Dunn, 1974; Ellis *et al.*, 1994). Many lettuce varieties possess this gene and are therefore suitable parents as sources of resistance.

At Leen de Mos BV the first crosses in a backcross programme with the Nr material from IVT were made in 1982. It took another five years to discover that the Nr-resistance gene was linked with dwarfing and accelerated aging (designated: csv = compact snelle veroudering). The *N. ribisnigri* resistance was shown to be monogenetic dominant while the csv was monogenetic recessive. Each time we selected resistant, normal plants the offspring segregated for the csv plant type and the *N. ribisnigri* resistance. Progeny from csv-plants were 100% *N. ribisnigri* resistant and had the csv-plant type. Selecting for less dwarfing and less accelerated aging did not produce acceptable lines. In 1988 many crosses were made to break the Nr--csv link. In 1990, certain lines from a cross in the butterhead type showed *N. ribisnigri* resistance without the csv character. With this knowledge and appropriate material, AFLP markers for *N. ribisnigri* resistance were developed at Keygene/Wageningen. Using AFLP markers recombinants were discovered (Nr without csv) in 1992 in the crisphead type as well.

It is still not known what the mechanism is which causes the *N ribisnigri* resistance. The aphids penetrate the phloem in attempting to feed but quickly withdraw their stylets on resistant plants (Helden *et al.*, 1992; Helden & Tjallingii, 1993). Aphids will die of starvation rather than feed on this lettuce and they do not produce any offspring. After being placed on a susceptible plant, the starved aphids start feeding again. The resistance is not based on a toxic character because the Nr gene only affects *N.ribisnigri.* The resistance is probably due to a feeding deterrent or plants lack a stimulus that helps the aphid recognise its food.

| | | |
|---|---|---|
| 1981 | PIVT-Nr line * Butterhead-1 (Dm2,Nr--csv) (ga,Dm3+11) | PIVT-Nr line * Butterhead-3 (Dm2,Nr--csv) (R18,Ra) |
| 1985 | Line 24-685 * Butterhead-2 (Dm3+11,Nr--csv) (ga,Dm6/Ra) | |
| 1990 | Line 24-3888 * Line Br-10186 (Nr,no csv,ga,Dm3+11) (R18,Ra,Nr--csv) | |
| 1995 | | Line 71- 920132 (Nr,no csv,ga,R18,Ra) |
| 1996 | | Line 2-50519 |
| 1997 | | LM-8021 |
| 1998 | | Dynamite |

Figure-1: Pedigree of Dynamite (LM-8021)
Nr = *Nasonovia* resistant, csv = compact-accelerated-aging, Ra = root aphid resistant, ga = lettuce mosaic virus tolerant, Dm and R18 = downy mildew resistance genes.

## Materials and Methods

***Plant genotypes***
In all experiments susceptible and known resistant genotypes were used as controls to check the effectiveness of the plant material.

*Aphid biotypes*
In laboratory bioassays the red or brown/purple biotype of *N.ribisnigri* and *M.euphorbiae* were used *a priori*. These biotypes are easier to score than green or yellow biotypes. They were collected in the field from susceptible lettuce plants growing in selection plots. All aphids were reared on lettuce and maintained parthenogenetically for many generations. According to Reinink *et al.*, (1989), no clone-specific plant genotype reactions were to be expected. This means that lettuce lines resistant to one clone will also be resistant to other clones, allthough not necessarily at the same level.

***Laboratory bioassays***
Laboratory bioassays were used for the leaf aphids, *N. ribisnigri*, *M. euphorbiae* and *A. solani*. Two replicates of seven plants at the 4-5 leaf stage were inoculated by collecting aphids from susceptible lettuce plants. The aphids were freely scattered over all plants in the experiment. After 10-15 days the colonisation of plants was assessed. Winged aphids were not counted as colonisers but considered to be immigrants. *Nasonovia ribisnigri* was found on the apex and on younger

leaves of the plant, *M. euphorbiae* mainly found on the upper side of the older leaves, while *A.solani* was mainly found on the under surface of the oldest leaves.
A laboratory bioassay was used for the lettuce root aphid, *P. bursarius*. Two replicates of seven plants at the 4-5 leaf stage were transplanted in 0.5l pots (15 cm diam.) filled with coarse horticultural peat. One week after transplanting, plants were inoculated with aphids from susceptible lettuce plants by scattering the aphids on the soil in the pot. 30-35 days after inoculation the colonisation of the roots was assessed using 4 classes of aphid numbers.
The laboratory environment was set to simulate August conditions, providing root aphids with optimal conditions.

Downy mildew resistance was assessed in a controlled environment room maintained at a temperature of 15 °C, daylength of 12 hours, with a light intensity of 4000 lux. Trays of plants were covered and packed in plastic bags. One week after sowing, the plants in different trays were inoculated with a spore-suspension of several races of *B. lactucae* (fysio's NL-1 to NL-16) harvested from susceptible lettuce plants. Plants were scored on the 10th and 13th day after inoculation.

LMV-tolerance was investigated in the laboratory. Plants at the 4-5 leaf stage were mechanically inoculated with juice extracted from infested lettuce plants displaying symptoms of the disease. Test plants were assessed for symptoms of LMV 15-25 days after inoculation.

***Field bioassays***
Leaf aphid resistance was assessed in the field following natural infestation. On six sowing dates in April, June and July (early summer- early autumn harvest), at four different locations in The Netherlands (Maasdijk-1, Maasdijk-2, Breda, Sevenum), plants of four genotypes were transplanted at the 4-5 leaf stage in four plots, each consisting of 100 plants. A natural infestation of aphids was assessed by counting aphids every week during the growing period of the crop. The last count was made at harvest, on average on the 55th day after sowing. In addition, A.Ester, an independent reseacher at PAV/Lelystad carried out field experiments using two sowing dates (Ester, 1997; 1998).
The resistance of plant genotypes to the lettuce root aphid, *P. bursarius*, was assessed in field experiments.

## Results and Discussion

The results of experiments are presented in Tables 1-4 and in Figure 2. The results of the field experiment at four different sites in The Netherlands are shown in Table 1 and Figure 2.
The results of the field experiment on seven lettuce genotypes are shown in Table 2. Aphid numbers were scored in one of six classes using the following system:-
0 = no aphids, 1 = 1-10 aphids, 2 = 11-25, 3 = 26-50, 4 = 50-100, 5 = over 100 aphids. Mean class results were multiplied by 100/5=20 to give the disease incidence (0-100) (for formula see Table 4).

Table 1. Lettuce head weight, mean number and percentage distribution of three different species of leaf aphid on lettuce from four field locations in The Netherlands in 1997 (each figure is the mean of 480 plants).

| **Lettuce Genotype** | **Weight (g/head)** | *Nasonovia ribisnigri* | | *Macrosiphum euphorbiae* | | *Uroleucon sonchi* | |
|---|---|---|---|---|---|---|---|
| | | # | % | # | % | # | % |
| **LM-8019** | 401 | 0.3 | 0.3 | 1.2 | 15 | 2.3 | 10 |
| **Dynamite** | 430 | 0.3 | 0.3 | 1.8 | 22 | 4.1 | 18 |
| **LM-8017** | 393 | 45.3 | 47 | 2.2 | 27 | 7.6 | 33 |
| **Punch** | 401 | 51.2 | 53 | 3.0 | 36 | 9.1 | 39 |

Table 2. Lettuce head weight and mean aphid scores for seven lettuce genotypes grown in the field in The Netherlands in 1998 (each figure is the mean of 36 plants per genotype).

| **Lettuce Genotype** | **Type** | **Weight (g/head)** | *N. ribisnigri* | *M. euphorbiae* | *U. sonchi* |
|---|---|---|---|---|---|
| | | | mean class | mean class | mean class |
| **Dynamite** | butter | 325 | 0.4 | 0.9 | 0.2 |
| **Punch** | butter | 339 | 3.7 | 0.7 | 0.5 |
| **LM-8074** | butter | 357 | 4.3 | 0.5 | 0.5 |
| **Porto** | crisp | 343 | 3.1 | 0.1 | 0.2 |
| **Vetonas** | crisp | 347 | 0.5 | 0.3 | 0.3 |
| **Basic** | s.bowl | 333 | 4.6 | 0.5 | 0.7 |
| **LM-9608** | s.bowl | 400 | 0.5 | 1.6 | 0.7 |

The results of evaluating four lettuce genotypes against lettuce root aphid, *P. bursarius*, in the field are presented in Table 3.

Table-3: Numbers of plants of four lettuce genotypes tested against lettuce root aphid, *Pemphigus bursarius,* in the field in The Netherlands in 1997.

| Lettuce genotype | # plants tested | # plants with *P.bursarius* |
|---|---|---|
| **LM-8019** | 100 | 9 |
| **Dynamite** | 100 | 10 |
| **Punch** | 100 | 85 |
| **Franca** | 100 | 78 |

The results of testing seven lettuce genotypes against four aphid species in the laboratory are given in Table 4. The aphid damage score was calculated with the following formula in which x is the number of classes minus one and nx the number of plants scored in that class.
( 0*n0 + 1*n1 + 2*n2 + ....+ x*nx) / (x * (n0 + n1 + n2 + ... + nx))

Table 4. Damage scores and number of plants tested (in parentheses) for seven lettuce genotypes tested in the laboratory against four species of aphids from September 1997 - September 1998. (The number of classes used for scoring are indicated by [ ]).

| **Lettuce genotype (#plants scored)** | *N. ribisnigri* [2] | *P. bursarius* [4] | *M. euphorbiae* [2] | *A. solani* [2] |
|---|---|---|---|---|
| **Franca** | (27) 100 | (124) 84 | | |
| **Punch** | (41) 100 | (55) 81 | (41) 68 | (41) 100 |
| **Fortune** | (97) 97 | (326) 81 | (41) 78 | (27) 78 |
| **Reskia** | (55) 82 | (113) 80 | (13) 69 | (13) 100 |
| **Mirian** | (56) 100 | (48) **12** | (56) **36** | (28) 68 |
| **2-50519, source of Dynamite** | (308) **2** | (647) **33** | (238) **16** | (224) 95 |
| **Dynamite** | (42) **1** | (42) **36** | | |

The downy mildew tests showed that 'Dynamite' was resistant to *Bremia lactucae* races NL1, 2, 3, 4, 5, 6, 7, 10, 11, 12, 13, 14, 15 and 16. This resistance spectrum indicates the inheritance of R18 from its ancestor.

The LMV-trial showed that 'Dynamite' was tolerant to the LMV-LS1 virus.

The experiments showed that the butterhead lettuce variety 'Dynamite' (LM-8021) provides total protection against the lettuce aphid, *N. ribisnigri* (Tables 1, 2, & 4; Figure 2 ; Ester, 1997; 1998; van Melckebeke, 1998). This means that the heart of the plant will be aphid-free during its development. Although the number of aphids per head in the 1997 field experiment was low, nevertheless it is clear that 'Dynamite' provides considerable protection against *M. euphorbiae* and *U. sonchi* (Table 1; Figure 2)(Ester, 1997; 1998). These two species of aphids are found on

the oldest and thus the outer leaves. The results of the field experiment for *N. ribisnigri* and *M. euphorbiae* were supported by the results of the laboratory experiments (Table 4). 'Dynamite' and its predecessor line 2-50519 were highly resistant to both species. This variety was also highly resistant to *P. bursarius* (Tables 3 & 4) but not as high as the variety 'Mirian' that carries the Dm6--Ra gene. These findings support the theory that the Dm6--Ra gene is in fact a complex of several separate genes (e.g. 4 equivalents) of which the Dm6 on its own has a pleiotropical effect (e.g. 1 equivalent). R18, which is believed to be almost allelic with Dm6, has no root aphid equivalent. Combinations of R18--Ra are therefore less resistant than the Dm6--Ra combination. 'Mirian' was shown to be moderately resistant to *M.euphorbiae.*

**Aphid Distribution**

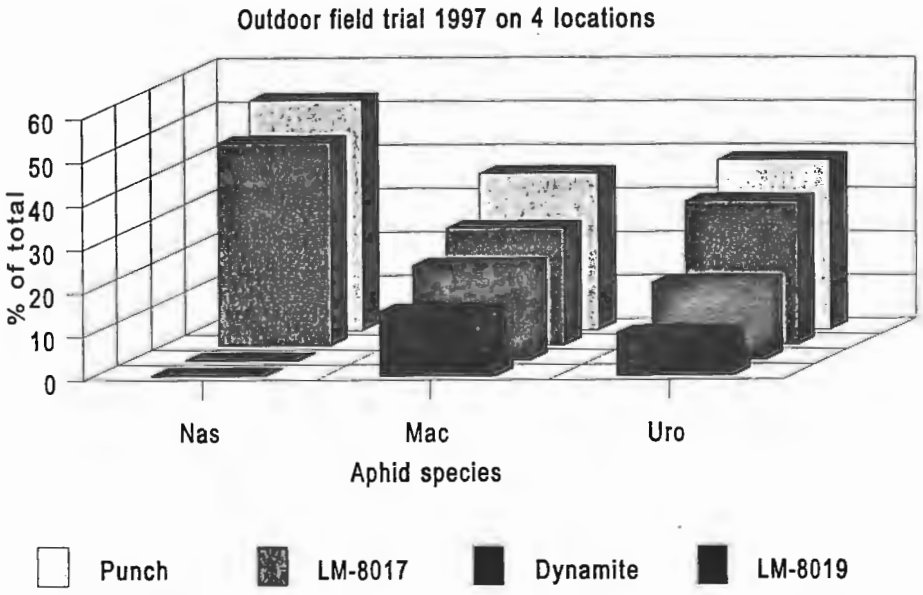


Figure 2. The distribution of three species of aphid on the foliage of four lettuce genotypes in the field in 1997 at four different locations in The Netherlands (each figure is the mean of 480 plants per genotype)(see also Table 1).

Nas = *Nasonovia ribisnigri,* Mac = *Macrosiphum euphorbiae* and Uro = *Uroleucon sonchi*

The results of the laboratory experiments indicated that 2-50519, the predecessor of 'Dynamite', is susceptible to *A. solani.* It would be expected that 'Dynamite' would not be resistant to this aphid.

It can be concluded that the introduction of lettuce varieties with resistance to aphids is an environmentally safe way to reduce both aphid damage and the use of insecticides in commercial lettuce production.

## Résumé

En 1981, l'IVT (maintenant CPRO-DLO / Wageningen, Hollande), a sorti du matériel résistant au puceron foliaire de la laitue *Nasanovia ribisnigri*. Une combinaison de caractères génétiques a été obtenue par un programme de rétrocroisement avec des parents récurrents possédant différents caractères.

La variété de laitue beurre de plein champ 'Dynamite' (LM-8021) possède les gènes Nr de résistance à *N. ribisnigri* , R18 de résistance à *Bremia lactucae* et ga de tolérance au LMV. Elle présente également une forte résistance au puceron des racines *Pemphigus bursarius* et une résistance modérée au puceron de la pomme de terre *Macrosiphum euphorbiae* ainsi qu'au puceron brun de la laitue *Uroleucon sonchi*. Elle n'est pas résistante au puceron de la laitue *Aulacorthum solani*.

'Dynamite' est de taille moyenne, vert clair, d'épaisseur moyenne et légèrement cloquée. Cette variété convient à des productions de début d'été dans les pays du Nord Ouest de l'Europe ainsi qu'aux productions d'automne des pays d'Europe du Sud.


**Mots clés:** *Lactuca sativa, Lactuca virosa, Lactuca serriola, Nasanovia ribisnigri, Pemphigus bursarius, Macrosiphum euphorbiae, Aulacorthum solani, Uroleucon sonch i, Bremia lactucae*, résistance Nas, pucerons foliaires, pucerons des racines, résistance aux pucerons, LMV, virus de la mosaïque de la laitue, résistance de la plante hôte, essais biologiques

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*Breeding for Resistance to Insects and Mites*
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# Exploiting the resistance in carrots and wild umbelliferae to the carrot fly, *Psila rosae* (F.)

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**Abstract:** Partial plant resistance to the carrot fly, *Psila rosae,* has been identified in several Nantes varieties of carrot, *Daucus carota*, which reduces larval damage to the roots and the number of pupae remaining in the soil after cropping by 50%. The resistance of the Nantes variety 'Sytan' was found to be consistent at 12 sites in 5 European countries over two seasons. The resistance has been shown to be based on antibiosis and associated with concentrations of chlorogenic acid in the roots.

The resistance complements cultural and chemical methods for controlling carrot fly. Thus, in field experiments over two seasons it was shown that carrots could be left in the ground for a longer period providing a greater sowing/harvesting interval with a resistant variety than a susceptible one because of the reduction in damage and the delayed development of insects on the resistant variety. In two seasons at two sites a partially-resistant variety required only one third of the dose of insecticide to provide a marketable crop compared with a susceptible carrot variety. In a breeding programme involving the variety 'Sytan', male sterile lines, inbreds and selections with improved levels of resistance were developed.

Much higher levels of resistance were identified in certain wild *Daucus* species and 15 years of crossing and selection produced lines with significantly higher levels of resistance than exists in the variety 'Sytan'. A range of resistant carrot material bred at Horticulture Research International has been released to seed companies for use in the production of improved carrot varieties. The partially-resistant variety 'Flyaway' was made available to amateur gardeners in 1993 and to commercial growers in 1995 as a result of this research.

A carrot fly screening service is offered by HRI to seed companies to evaluate breeding lines for their resistance to *P. rosae.*

**Key words**: Carrots, carrot fly, *Psila rosae*, host plant resistance.

## Introduction

The carrot fly, *Psila rosae* (F.), remains a severe pest of carrots despite all attempts to control its populations. To meet the stringent damage levels demanded by the supermarkets in the UK and Europe, commercial carrot production depends precariously on a few insecticides to control this pest. Growers have moved away from using carbamate and organo-phosphorus insecticides in recent years, partly because of the detection of insecticide residues in carrots following repeated sprays of these compounds but also because of accelerated biodegradation which reduced the effectiveness of soil-applied treatments (Suett, 1991). The insecticides used now include two carbamates; carbofuran and carbosulfan, three organophosphorus compounds; chlorfenvinphos, disulfoton and pirimiphos-methyl and three pyrethroids; deltamethrin, lambda-cyhalothrin and tefluthrin with specific off-label approval (SOLAs) (Anon., 1999). Granular formulations of the carbamates and organophosphorus compounds may be used at drilling. Mid-season sprays of

pyrethroids target the peak of the second generation of adult carrot flies in July/August and are timed according to forecasts of insect activity.

It is very difficult for the grower to avoid carrot fly attack because the pest is widely distributed in areas where carrots are grown and many wild host plants serve as reservoirs of the pest in the absence of carrot and related crops (Ellis *et al.*, 1992).

Few new insecticides have been registered for use against carrot fly in the last 10 years and investigations have concentrated on methods of using insecticides more efficiently and thereby prolonging their active life (Suett, 1991). In addition, forecasts that predict carrot fly emergence have been developed to target control treatments more accurately (Collier & Finch, 1988). The breeding of varieties of carrots resistant to carrot fly which could be used to supplement other measures in an integrated pest management programme is considered to be a promising approach. In this paper progress made in this work at HRI Wellesbourne and the future role of resistant varieties in commercial production of carrots are considered.

## Sources of resistance

Studies of the resistance in carrots and wild species have been in progress for more than 25 years (Ellis, 1999). A total of about 400 varieties collected from throughout the world have been screened and the majority of these are susceptible to carrot fly attack. However, in the 25-year period we have identified partial resistance in about 20 carrot varieties, most of which are Nantes types (Table 1) (Ellis, 1992). A knowledge of the susceptibility of carrot varieties may be helpful in itself to growers providing them with information on which varieties to avoid and which ones to select and grow if they have a carrot fly problem. The resistance provides a 50% reduction in damage to the roots and a 50% reduction in the number of insects remaining in the soil following removal of the crop (Ellis *et al.*, 1984). The resistance is largely based on factors which take affect after the female carrot flies have laid their eggs. So, the varieties which have been investigated are equally attractive to egg-laying. Once established, larvae take longer to develop and many die before reaching maturity. The partially-resistant varieties therefore have less damage, the development of attack is delayed and fewer insects remain in the soil. Research has shown that resistance is associated with the levels of chlorogenic acid in the roots, one of the phenolic acids present in many food crops (Cole, 1985). Low levels of chlorogenic acid are associated with high levels of resistance as the insect is believed to require this compound to develop normally (Cole, 1985). Certain of the partially-resistant carrots such as 'Sytan' and 'Flyaway' are reported to be sweeter than other varieties and it is quite possible that this characteristic is associated with low levels of chlorogenic acid.

## Consistency of resistance

The resistance in the variety 'Sytan' has been shown to be effective in more than 12 countries in the world (Ellis & Hardman, 1981) and, with the help of members of the Henry Doubleday Research Association, has been tested and shown to be resistant in many private gardens in the UK (Anon., 1982). Consistent performance at different sites in the presence of different populations of *P. rosae* is an important attribute if a variety is to be widely effective in helping to control the pest.The resistance is believed to be inherited in a complex fashion, probably involving several genes (Ellis, 1999). The resistance in the variety 'Sytan' is believed to have originated in Touchon types of Nantes carrots. A range of different varieties of carrot have been

bred from the Touchon type and many of them are believed to have inherited genes for resistance to *P. rosae.*

## Integrated pest management (IPM) of carrot fly

The partial resistance in 'Sytan' complements other approaches to reducing carrot fly attack. Thus, in field experiments over two seasons it was shown that carrots could be left in the ground for a longer period providing a greater sowing\harvest interval with the resistant variety than with a susceptible one. This occurred as a result of the reduction in damage and delayed development of the carrot fly on the resistant 'Sytan' (Ellis *et al.*, 1987). The resistance therefore offers growers a longer season for growing carrot crops free of damage. The resistance has also been shown to complement chemical control of carrot fly. In two seasons at both HRI Wellesbourne and at a site in Norfolk the partially-resistant 'Sytan' required only one third of the dose of insecticide to provide a marketable crop compared with the susceptible 'Danvers Half Long 126' (Thompson *et al.*, 1994). On a commercial holding in the late 1980s we showed that it was possible to grow a marketable crop without the use of any insecticide treatments. Carrots were grown in isolated pockets of land, 5km away from the nearest carrot crop and rotated so that a break followed the cropping with carrots. A fast-developing variety of carrot (16 weeks from sowing to reach maturity) was sown at the end of May, thus missing the end of the first generation of egg-laying by carrot fly and harvested early in October to avoid the build up of second generation damage. The crop yielded well and was free of carrot fly damage. It is this type of approach that organic growers could use to produce marketable crops. Crop covers can also be integrated with these methods to protect earlier-sown crops against the insect in their first few weeks of growth in late April and May. Minor variations in the timing of the generations of *P. rosae* which occur in different regions of a country can be predicted using the forecasts based on local weather conditions (Collier & Finch, 1988); in this way growers can accurately time the sowing and harvest of their crops.

## Breeding work at HRI Wellesbourne

The variety 'Sytan' has been the subject of an intensive breeding programme at HRI Wellesbourne which resulted in the production of a range of partially resistant inbreds (Ellis *et al.*, 1991), male sterile lines and families selected for increased resistance. Representatives of these different breeding lines were released to seed companies in either a joint hybrid scheme (in 1988 & 1989) or in the outright sale of 50 lines (1989). The first product of this collaboration has been the release of the variety 'Flyaway'.

## Resistance in wild carrot relatives

In order to identify higher levels of resistance to carrot fly, 130 different members of the Umbelliferae, the carrot family, have been evaluated in the field (Hardman & Ellis, 1982; Hardman *et al.*, 1990). Twenty seven of these species failed to support carrot fly development and a further 38 supported <0.5 insects per root. Certain common arable weeds such as fool's parsley, *Aethusa cynapium,* were better hosts than even the most susceptible carrot variety 'Danvers Half Long 126' which supported, on average, 13 carrot flies per root. The majority of umbelliferous herbs grown commercially or by home gardeners were susceptible to the pest. Our interest and

research has concentrated on those wild species which belong to the genus *Daucus*, the carrot, and especially *Daucus capillifolius*, a wild carrot which is found growing in the foothills of the Atlas Mountains in Tripolitania, Libya. This wild species is highly resistant to carrot fly as well as being easily hybridised with cultivated types. Fifteen years of breeding with this species produced a wide range of interesting carrot types possessing moderate resistance to the pest (Ellis *et al.*, 1993). Unfortunately the most advanced breeding lines have not turned out to be as promising as the wild parent as resistance levels have been eroded in the lengthy process of selection and crossing over many generations. Representatives of these families were also sold to seed companies.

## A screening service for seed companies and growers

For the last 8 years HRI Wellesbourne has offered seed companies a carrot fly screening service where breeding lines are evaluated against the insect (Ellis *et al.*, 1997). A group of four 0.5 ha fields were set aside for carrot fly studies when the Institute was established at Wellesbourne nearly 50 years ago. Consistently high populations of carrot fly have been maintained in this part of the farm ever since by growing carrots in the perimeter of each field each year to ensure that the flies always have host plants to lay their eggs on. In this way, field experiments are always subjected to high levels of attack. In all experiments standard resistant and\or susceptible varieties are grown for comparative purposes. As well as testing the latest breeding lines released by seed companies, popular commercial varieties have been included in order to assess their resistance to carrot fly. The results of the last 8 years of experiments are summarised in Table 2. All results for seed company lines are related to the standard partially-resistant 'Sytan' selection. The overall level of carrot fly attack varied from season to season and was very severe in 1991, 1992, 1996 and 1998. The attack at HRI Wellesbourne is likely to be more severe than on most growers' holdings or in gardens because of the management scheme to build up attack in the experimental field. In every season certain acccessions were much more severely damaged than 'Sytan'. At the beginning of the 1990s the most promising accessions were not significantly less damaged than the standard 'Sytan' but over the last five years considerable progress has been made in increasing the resistance of carrot breeding lines. Their resistance is significantly better than 'Sytan'. In 1997 and 1998 the most promising accession was 30 % better (Table 2). When these lines are released to the growers they should provide an even better base for integrated pest management programmes.

Table 1. The relative resistance of different commercially-acceptable carrot varieties to carrot fly at Wellesbourne 1991-98

| Carrot variety | Susceptibility ratio* |
|---|---|
| Bangor | 1.69 |
| Bertan | 1.24 |
| Bolero | 1.11 |
| Chantenay Red Cored | 1.36 |
| Danvers Half Long 126 | 1.90 |
| Express | 1.64 |
| Flyaway | 1.19 |
| Ingot | 2.00 |
| Maestro | 1.12 |
| Nairobi | 1.26 |
| Nada | 1.52 |
| Nandor | 1.12 |
| Nantucket | 1.05 |
| Narbonne | 1.26 |
| Narman | 1.17 |
| Natan | 1.70 |
| Navarre | 1.66 |
| Nerac | 1.51 |
| Newmarket | 1.55 |
| Parano | 1.11 |
| Primo | 1.52 |
| Sytan | 1.00 |

* Any value >1.2 is significantly more susceptible than 'Sytan'

Table 2. Resistance of carrot varieties and breeding lines to carrot fly, *Psila rosae,* at HRI Wellesbourne 1991 -1996. * Most resistant breeding lines in a season. ** An S.I. value <1.00 indicates improved resistance.

| Year | No. of entries | SED | Variety or breeding line* | Accession No. | % marketable roots | ** Susceptibility ratio |
|---|---|---|---|---|---|---|
| 1991 | 14 | 3.3 | 'Sytan' | DC 84022 | 12.0 | - |
| | | | 'Danvers Half Long 126' | DC 79002 | 1.2 | 1.90 |
| | | | Breeding line | 91-006 | 8.6 | 1.40 |
| 1992 | 8 | 4.3 | 'Sytan' | DC 84022 | 20.9 | - |
| | | | 'Danvers Half Long 126' | DC 79002 | 1.1 | 4.30 |
| | | | Breeding line | 92-006 | 16.5 | 1.26 |

| | | | | | | |
|---|---|---|---|---|---|---|
| 1993 | 21 | 3.5 | 'Sytan' | DC84022 | 64.0 | - |
| | | | 'Danvers Half Long | DC 79002 | 24.0 | 2.70 |
| | | | 126' | 93-001 | 41.9 | 1.70 |
| | | | 'Natan' | 93-004 | 65.1 | 0.98 |
| | | | Breeding line | | | |
| 1994 | 33 | 6.2 | 'Sytan' | DC 84022 | 68.0 | - |
| | | | 'Chantenay Red | DC 91210 | 32.0 | 2.13 |
| | | | Cored' | 94-028 | 62.0 | 1.09 |
| | | | 'Flyaway' | DC 92123 | 58.0 | 1.17 |
| | | | 'Narman' | DC 91212 | 54.0 | 1.26 |
| | | | Narbonne' | DC 91182 | 54.0 | 1.26 |
| | | | 'Nairobi' | 94-029 | 34.0 | 2.00 |
| | | | 'Ingot' | 94-024 | 68.0 | 1.00 |
| | | | Breeding line | | | |
| 1995 | 22 | 4.4 | 'Sytan' | DC 84022 | 64.0 | - |
| | | | 'Danvers Half Long | DC 79002 | 44.4 | 1.44 |
| | | | 126' | 95-021 | 34.4 | 1.86 |
| | | | 'Navarre' | 95-010 | 73.4 | 0.87 |
| | | | Breeding line | | | |
| 1996 | 17 | 2.8 | 'Sytan' | DC 84022 | 12.0 | |
| | | | 'Navarre' | 96-002 | 3.0 | 1.66 |
| | | | 'Bangor' | 96-015 | 2.8 | 1.69 |
| | | | 'Nerac' | 96-016 | 4.5 | 1.51 |
| | | | 'Newmarket' | 96-017 | 4.0 | 1.55 |
| | | | Breeding line | 96-006 | 17.9 | 0.72 |
| 1997 | 22 | 3.87 | 'Sytan' | DC84022 | 36.4 | - |
| | | | 'Flyaway' | 97-015 | 29.8 | 1.22 |
| | | | 'Resistafly' | 97-016 | 40.2 | 0.91 |
| | | | Breeding line | 97-010 | 61.2 | 0.59 |
| 1998 | 22 | 2.7 | 'Sytan' | DC84022 | 24.5 | - |
| | | | 'Bertan' | 98-015 | 19.8 | 1.24 |
| | | | 'Bolero' | 98-014 | 22.0 | 1.11 |
| | | | 'Maestro' | 98-002 | 21.8 | 1.12 |
| | | | 'Nanda' | 98-016 | 16.1 | 1.52 |
| | | | 'Primo' | 98-017 | 16.1 | 1.52 |
| | | | 'Danvers Half Long | DC96280 | 17.0 | 1.44 |
| | | | 126' | 98-012 | 35.4 | 0.69 |
| | | | Breeding line | | | |

**Résumé**

Une résistance partielle à la mouche de la carotte *Psila rosae* a été mise en évidence chez plusieurs variétés de carottes nantaises *Daucus carota*. Elle entraîne une réduction de 50% des dégâts larvaires sur les racines et une réduction de 50% du nombre de pupes restant dans le sol après la récolte.

La résistance de la variété nantaise ' Sytan' a été vérifiée sur 12 sites dans 5 pays européens pendant deux saisons de culture. La résistance est basée sur un effet d'antibiose; elle est liée à la concentration en acide chlorogénique dans les racines. La résistance vient en complément des méthodes culturales et chimiques de contrôle de la mouche de la carotte. Ainsi, dans des essais au champ pendant deux saisons, neuf combinaisons de dates de semis et de récolte ont produit plus de 75% de racine saines pour la variété ' Sytan', partiellement résistante, et seulement trois combinaisons de dates pour une variété résistante. Pendant deux saisons, sur deux sites, cette variété partiellement résistante n'a nécessité qu'un tiers de la dose insecticide utilisée sur une variété sensible pour obtenir une récolte commercialisable.

Un programme d'amélioration a conduit à l'obtention de lignées mâle stériles, d'autofécondations et à des sélections possédant un meilleur niveau de résistance. Certaines espèces de *Daucus* sauvages ont montré des niveaux de résistance très élevés et des croisements et sélections conduits sur 15 années ont abouti à des lignées présentant une résistance modérée. Une gamme de carottes résistantes sélectionnées à l'HRI a été fournie au compagnies grainières pour être utilisée dans la mise au point de variétés de carottes. La variété, 'Flyaway', sortie en 1993, est un des résultat de ce travail.

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# Plant resistance in rose cultivars to Frankliniella occidentalis: a commercial test

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**Abstract:** Host plant resistance to western flower thrips, *Frankliniella occidentalis*, was investigated in 20 rose cultivars. A simple test method was developed for assessing the resistance of rose cultivars in which two different parameters were recorded, (i) two types of feeding damage (silver and growth damage) on the leaves caused by thrips, and (ii) the number of larvae and adult thrips in rose flowers. The feeding damage was recorded four weeks after infestation with western flower thrips and the number of thrips was counted during the whole test period in flowers that were fully open. The rose cultivars differed significantly in the amount of silver damage that was caused by feeding thrips. Growth damage was very limited in the flowering plants used and was similar among the cultivars. The number of *F. occidentalis* adults and the number of larvae in the rose flowers differed significantly between cultivars. However, a high number of adults on a cultivar did not imply a high number of larvae and vice versa. The silver damage on the leaves was not found to be related to the thrips population in the flowers: possible reasons for the lack of this relationship are discussed.

**Key words:** host plant resistance; western flower thrips; *Frankliniella occidentalis;* roses; commercial test method

## Introduction

Since it was first found in The Netherlands, the western flower thrips (WFT) *Frankliniella occidentalis* (Pergande) (Thysanoptera: Thripidae) has been one of the most serious pests in the cultivation of roses (Mantel & Van de Vrie, 1988). Because of its extensive host range and severe feeding damage to plant tissue, it has world-wide pest status in many crops (Bradsgaard, 1989). Crop losses caused by direct feeding damage to flowers and leaves have been reported (Parella & Jones, 1987). Apart from the direct feeding damage, indirect damage is caused by wounding the tissue so that fungi and bacteria gain entry (Brødsgaard, 1989). WFT is also a vector of tomato spotted wilt virus (Peters *et al.*, 1991; Goldbach & Peters, 1994). Rose growers frequently use insecticides to control *F. occidentalis* in their crops, but increased tolerance or resistance to the chemical compounds (Brødsgaard, 1989 and 1994; Robb, 1989) has necessitated a search for alternative control methods. Biological control and host plant resistance are both alternative strategies for controlling WFT. Biological control is still unreliable (Van der Meer, 1995), sometimes reported to be successful, sometimes to be a failure (Brødsgaard, 1989). Host plant resistance has been identified in several vegetable and ornamental crops, such as cucumber (Mollema *et al.*, 1995; De Kogel, 1997), sweet pepper (Ferry & Schalk, 1991), chrysanthemum (De Jager *et al.*, 1993; De Jager, 1995) and in rose (Gaum *et al.*, 1994).

Rose breeders in the Netherlands require a simple, standard test-method to assess host plant resistance among rose selections and/or cultivars. A suitable method was developed a year ago

(De Jager *et al.*, 1997) for several other ornamental crops and is presently used in a standard (commercial) test. We present results of a resistance test for *F. occidentalis* which was developed in experiments involving 20 rose cultivars.

## Methods

***Test conditions***
The resistance test was performed in a glasshouse maintained at a relative humidity of 70-80%, a photoperiod of 20L: 4D and with the temperature set at 20°C. On sunny days when there were 13h sunshine, temperatures reached 31□C. Plants were shaded automatically above 350 watts/m².

***Plants***
Ten intact plants of each of 20 rose cultivars (code: A1-A6, B1-B8 and C1-C6) were arranged in the glasshouse in a randomised block design. All plants had only one stem and had just formed a flowerbud. After a further three days the insects were released on the plants.

***F. occidentalis***
A glass vial containing female *F. occidentalis* was placed at the base of the stem of each plant. *Frankliniella occidentalis* were used from a culture maintained on chrysanthemums.

***Feeding damage and thrips population***
Two types of feeding damage were recorded on the leaves of the test plants four weeks after thrips introduction: the highly characteristic silver damage and growth damage (for exact descriptions see De Jager, 1995; Robb, 1989). The extent of the silver damage to the leaf surface was determined according to a standard spot of 2mm²; the numbers of spots on all leaves of a plant were counted. The growth damage was recorded as the number of leaves on a plant with this kind of damage. As a parameter for the thrips population in the flowers, the numbers of larvae (larval stages 1 and 2) and the adults (male and female) in fully open flowers during the whole test period were counted.

## Results

The mean number of silver damage spots of 2mm² per cultivar and the mean number of leaves per cultivar with growth damage are shown in Table 1. Significant differences between the rose cultivars in one type of feeding damage, the silver damage, is shown (Table 1). Also the number of larvae and the number of adults in the flowers, which are used as parameters for the whole thrips population on the plants, differed significantly between the cultivars (Table 2).

Table 1. The mean number of silver damage spots (2mm²) and the mean number of leaves with growth damage on 10 plants of 20 rose cultivars with their respective standard errors (SE).

| Cultivar code | Silver damage | SE (silver) | Growth damage | SE (growth) |
|---|---|---|---|---|
| A5 | 1.30 a | 0.84 | 0.20 | 0.63 |
| A3 | 1.80 a | 1.58 | 0.00 | 0.00 |
| A2 | 2.40 a | 1.23 | 0.50 | 0.97 |
| C5 | 3.70 a | 2.09 | 0.80 | 1.48 |
| C2 | 4.00 a | 1.87 | 0.00 | 0.00 |
| A1 | 4.10 a | 3.14 | 0.20 | 0.63 |
| A6 | 4.10 a | 1.96 | 0.00 | 0.00 |
| A4 | 5.10 a | 1.07 | 0.00 | 0.00 |
| B2 | 7.40 a | 3.32 | 0.3 | 0.21 |
| B4 | 14.50 ab | 718 | 1.5 | 0.67 |
| B3 | 18.50 ab | 5.99 | 0.1 | 0.1 |
| B5 | 20.30 ab | 9.98 | 0.2 | 0.13 |
| B6 | 22.50 ab | 9.42 | 0.2 | 0.13 |
| C6 | 28.20 abc | 11.66 | 0.30 | 2.20 |
| B7 | 40.30 bc | 16.7 | 0 | 0.15 |
| B8 | 42.63 bcd | 11.8 | 0.3 | 0 |
| C1 | 43.40 bcd | 25.46 | 0.20 | 0.63 |
| C3 | 54.20 cde | 15.99 | 0.50 | 1.27 |
| C4 | 69.80 de | 14.35 | 0.40 | 0.97 |
| B1 | 76.90 e | 16.58 | 0.3 | 0.21 |

Significant differences (P=0.05) between cultivars (Kruskal-Wallis test followed by a multiple comparison) are indicated with different letters.

Table 2. The mean number of *F. occidentalis* adults and larvae in the flowers of 10 plants of 20 rose cultivars and their respective standard deviation (SD).

| Cultivar code | Mean # adults | SD (adulten) | Mean # larvae | SD (larvae) |
|---|---|---|---|---|
| A5 | 6.90 abcd | 4.41 | 24.80e | 38.82 |
| A3 | 5.10abcd | 5.49 | 22.00de | 24.54 |
| A2 | 8.10bcd | 5.47 | 21.40cde | 30.42 |
| C5 | 10.80d | 9.80 | 16.10abcd | 14.56 |
| C2 | 4.30abcd | 4.24 | 2.20a | 3.88 |
| A1 | 1.00a | 1.25 | 4.20ab | 5.57 |
| A6 | 4.40abcd | 2.55 | 7.10abc | 7.89 |
| A4 | 4.10abcd | 3.18 | 6.00ab | 5.10 |
| B2 | 3.20abc | 5.25 | 11.10abcd | 13.07 |
| B4 | 4.30abcd | 4.74 | 5.00ab | 10.77 |
| B3 | 3.20abc | 2.82 | 7.00abc | 19.10 |
| B5 | 1.60ab | 1.84 | 3.00ab | 3.43 |
| B6 | 2.80abc | 3.43 | 12.60abcd | 18.75 |
| C6 | 9.30cd | 10.70 | 4.20ab | 7.04 |

| | | | | |
|---|---|---|---|---|
| B7 | 4.60abcd | 3.50 | 10.40abcde | 8.10 |
| B8 | 9.88cd | 9.01 | 7.70ab | 10.45 |
| C1 | 8.20bcd | 10.13 | 3.40ab | 3.84 |
| C3 | 20.20e | 17.83 | 17.50bcde | 28.31 |
| C4 | 21.40e | 18.16 | 8.70abcd | 11.41 |
| B1 | 1.50ab | 1.18 | 4.20ab | 3.19 |

Significant differences (P=0.05) between cultivars (Kruskal-Wallis test followed by a multiple comparison) are indicated with different letters.

There was no correlation between damage (silver damage) on the leaves of rose cultivar and the size of the thrips population (the number of adults plus the number of larvae) in the flowers of the same cultivar (Figure 1).

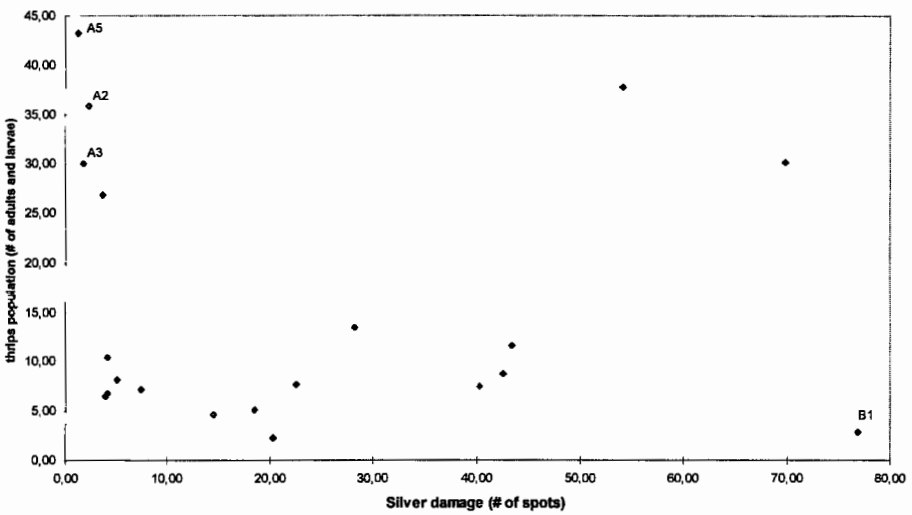


Figure 1. The relationship between *Frankliniella occidentalis* population (mean number of adults and larvae) in rose flowers and silver damage (mean number of 2 mm² spots) to plant leaves. Extremes are indicated: A2, A3, A5 and B1.

## Discussion

Significant differences were found between the cultivars in the thrips feeding damage on leaves as has also been reported by Van Dijken and Mollema (1992) and De Jager *et al.* (1997). Also the size of the population (number of larvae and number of adults) of *F. occidentalis* found in the flowers differed significantly among cultivars. This was reported too, for adult thrips, in flowers of rose cultivars by Gaum *et al.* (1994). Growth damage caused by feeding thrips did not differ among the cultivars. This was expected, because during the flowering stage there is almost no

growth or expansion of leaves for all cultivars and therefore, also no growth damage caused by thrips.

Feeding damage on leaves of rose cultivars was not correlated with the *F. occidentalis* population in the flowers of the same plants. This may be explained by the fact that western flower thrips feeds from pollen and is attracted to flowers of plants (de Jager, 1995). It is possible that different numbers of thrips were attracted to move from the leaves of a plant to its flowers. To determine this it would be necessary to correlate feeding damage leaf plants before flowering to the thrips population found in the flowers of the same cultivars during the flowering stage. In our test experiments certain cultivars (A2, A3 and A5, figure 1) were already infested with two other pest species, i.e. two spotted spider mite *(Tetranychus urticae)* and greenhouse whitefly *(Trialeurodes vaporariorum),* when offered by the client for the test. Although we tried to get rid of the insects mechanically during the test period, we could not manage this. Damage by these insects may have affected the silver damage caused by thrips and, therefore, the silver damage determined on the above mentioned cultivars could have been underestimated.


## Résumé

La résistance des plantes au thrips *Frankliniella occidentalis* a été testée sur 20 cultivars de rosier. Deux paramètres ont été utilisés dans une méthode simple pour évaluer la résistance des différents cultivars: (1) deux types de dégâts liés à la prise de nourriture par les thrips sur feuilles (taches argentées et troubles de croissance) et (2) le nombre de larves et d'adultes de thrips présent dans les fleurs. Les dégâts sur les feuilles ont été mesurés quatre semaines après l'infestation avec *F. occidentalis* et le dénombrement des thrips a été effectué pendant toute la durée de l'essai dans les fleurs épanouies.

Le niveau de dégâts par taches argentées est significativement différent entre les différents cultivars. La croissance des feuilles a été très peu affectée par la présence des thrips et très semblable pour l'ensemble des cultivars. Le nombre de *F. occidentalis* adultes et larves est significativement différent entre les cultivars. Toutefois, un nombre élevé d'adulte sur un cultivar n'implique pas un nombre élevé de larves sur ce même cultivar et *vice versa.* Nous n'avons pas trouvé de relations entre le niveau de dégâts par taches argentées sur les feuilles et la population de thrips dans les fleurs: quelques explications possibles sont discutées.


**Mots clés:** Résistance de la plante hôte, *Frankliniella occidentalis*, rosier, test commercial


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*Breeding for Resistance to Insects and Mites*
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# Eriophyid mites on Ribes – a complex story!

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**Abstract**: Eriophyid mites of the genus *Cecidophyopsis* colonise cultivated currant fruits (*Ribes* spp.). At present, the most damaging is the blackcurrant gall mite (*C. ribis*) on blackcurrant (*R. nigrum*). The importance, biology and taxonomy of blackcurrant gall mite and the other *Cecidophyopsis* mites that colonise currants are discussed in relation to their hosts. Until recently, taxonomic keys were confusing and in some cases inaccurate. The revision of the keys for the main mite species and the development of a molecular approach to assist with their identification are discussed. The latter has provided a rapid and cost effective method to identify mites associated with *Ribes*.

**Key words:** *Ribes*, currants, eriophyid mites, *Cecidophyopsis*, molecular determination, taxonomy.

## Introduction

Currant (*Ribes* spp.) production is a valuable horticultural enterprise in many parts of the world but the main concentration of production is in Europe and the Russian Federation (FAO, 1996). Blackcurrants (*R. nigrum*) are grown commercially in most temperate areas for both fresh fruit and processing. Similarly, redcurrants and whitecurrants (*R. rubrum* and closely related species) and gooseberry (*R. uva-crispa* L) are grown in similar locations. A small quantity of hybrid currants e.g. 'Jostaberry' (*R.* x *nidigrolaria* Bauer) are also grown, but this is largely a niche market.

Currants have a wide range of arthropod pests that cause direct damage to the plants or act as vectors of important virus or virus-like agents. Probably, the most important pest species on *Ribes* worldwide is the blackcurrant gall mite (*Cecidophyopsis ribis*) which can cause direct damage to buds as well as vector the agent for blackcurrant reversion disease (De Lillo & Dusco, 1996). Other *Cecidophyopsis* spp. have been identified on *Ribes* (Amrine & Stasny, 1994) but their role as virus vectors is uncertain. The eriophyid mites associated with commercial currant production are shown in Table 2.

## Mite Biology

Feeding by Eriophyid mites can frequently alter their host to provide a more suitable site for colonisation and reproduction and for development of their progeny. Such alteration has been categorised (Westphal & Manson, 1996) and is diverse in nature, ranging from slight modification to the hairs on the undersides of leaves as illustrated by the raspberry leaf and bud mite (*Phyllocoptes gracilis*) (Gordon & Taylor, 1976) to the spectacular nail gall on the leaves of *Tilia* spp. caused by *Phytoptus tiliae* (Westphal *et al.*, 1987) or bud galls of blackcurrant (caused by *C. ribis*).

Table 1. Major pests of Currants

| Pest | Blackcurrant | Red/whitecurrant | Gooseberry |
|---|---|---|---|
| Red currant blister aphid (*Cryptomyzus ribis*) | - | ** | - |
| Currant-lettuce aphid (*Nasonovia ribisnigi*) | - | - | * |
| Currant-sowthistle aphid (*Hyperomyzus lactucae*) | ** | * | - |
| Vine weevil (*Otiorhynchus sulcatus*) | ** | - | - |
| Black currant leaf midge (*Dasineura tetensi*) | ** | - | - |
| Common gooseberry sawfly (*Nematus ribensi*) | - | * | ** |
| Two-spotted spider mite (*Tetranychus urticae*) | *** | ** | ** |
| Eriophyid mites (*Cecidophyopsis* spp.) | **** | * | * |

**** major importance, widespread; *** very important, causing crop damage in most years; ** locally important; * rarely important

Eriophyid mites are very small, the adults averaging about 200µm in length, and it usually requires a microscope for their detection. Consequently most field biologists rely on symptoms to identify attack. Mites rely mainly on wind dispersal to move between plants but, movement on plants is probably a result of walking. Herr (1987; 1989) studied the emergence and dispersal behaviour of *C. ribis* on blackcurrant. He showed that time of emergence influenced the behaviour of the mites. When he exposed galled buds to raised temperature during the winter very few mites emerged, but exposing them to the same temperature in May, 50% of the mites within the buds emerged. He also observed that most of these mites climbed upwards, in the direction of the new foliage. The biology of *C. ribis* is probably the best understood, but they still require further clarification *viz.* method and efficiency of dispersal and colonisation, plant resistance mechanisms and the relationship between *C. ribis* and the reversion agent (Gordon *et al.*, 1994). Recent attempts to artificially infest blackcurrant buds with single *C. ribis* adults proved to be difficult with only one out of 79 'Daniels September' buds becoming colonised (S C Gordon, unpublished data). When it comes to the other Cecidiophyopsids, even less is known about their behaviour.

## Plant Damage

Galled blackcurrant buds resulting from *C. ribis* colonisation are typical of attack. Similarly, galls on redcurrant are typical of damage by *C. selachodon*. However, in recent years there has been an increase in reports of damage to blackcurrants by a 'non-gall forming' mite. The damage is confined to the buds and the mite can apparently cross species (Easterbook, 1980). Recent observation both in the UK (Fenton *et al.*, 1996; S.C. Gordon,

unpublished data), in the Pacific northwest of USA (K. Hummer, unpublished data)and in Tasmania (A.T. Jones and M. Williams, unpublished data) have shown that *C. grossulariae* can colonise blackcurrants. It is therefore highly probable that the 'non-gall forming' mite that Easterbrook was investigating was indeed the gooseberry mite.

Table 2. *Cecidophyopsis* mite species associated with commercial *Ribes* production and the type of damage caused (after Amrine *et al.*, 1994)

| Mite Species | Common Name | Hosts | Gall former | Bud/leaf damage | Virus vector |
|---|---|---|---|---|---|
| *Cecidophyopsis ribis* | Blackcurrant gall mite | Blackcurrants | ✓ | | ✓ |
| *Cecidophyopsis selachodon* | Redcurrant gall mite | Redcurrants | ✓ | | ? |
| *Cecidophyopsis grossulariae* | Gooseberry mite | Gooseberry<br>Blackcurrant<br>Hybrid berries e.g. Jostaberry<br>*R. curvatum* | | ✓ | ? |
| *Cecidophyopsis aurea* | - | *R. aureum* | ✓ | | ? |
| *Cecidophyopsis alpina* | - | Alpine currant (*R. alpinum*) | ✓ | | ? |

## Mite Identification

Eriophyid mites are found on a wide range of flowering plants, conifers and ferns throughout the world and most have co-developed with their hosts and show great host specificity. Many are found only on single plant species or genera (Oldfield, 1996). This appears to be so with *Ribes* eriophyids which are taxonomically very similar and difficult to separate because of their small size. Until Amrine *et al.*, (1994) reviewed the morphological features of the mites many researchers were unsure if *C. selachodon* on redcurrant was a distinct species or a 'non-gall forming' strain of *C. ribis* (e.g. Proeseler, 1973; Easterbrook, 1980). In their study, Amrine *et al.*, 1994, found it necessary to collect fresh mite specimens as much of the previous identification was based on missing or incomplete data, and in some instances the taxonomic descriptions were ambiguous or wrong. This confusion over identification led the United States Department of Agriculture (USDA) to enforce the destruction of many field plantings of *Ribes* in the Pacific Northwest and California in the early 1990s, as the mites found colonising the plants were incorrectly identified. Amrine *et al.*, (1994) also identified two new *Cecidophyopsis* species, one, *C. aurea* from galled buds of *R. aureum* in Poland, and the other, *C. alpina* collected from buds of *R. alpinum* in Finland.

## Molecular taxonomy

At the same time as the conventional taxonomy of the *Ribes* mites was being re-described, a group at SCRI developed a molecular method to separate and identify the mites using the polymerase chain reaction (PCR) to amplify the very small quantites of ribosomal DNA extracted from them. Using Restriction Fragment Length Polymorphism (RFLP) analysis they were able to show differences between mites collected from blackcurrant, redcurrant and gooseberry. Samples of these mites were also subjected to conventional taxonomic examination and proved to be *C. ribis*, *C. selachodon* and *C. grossulariae*, respectively (Fenton *et al.*, 1995). In addition, two samples of mites from alpine currant and *R. aureum* were molecularly identical, but differed from the other species examined. Further examination by conventional taxonomy showed these mites to be *C. alpina* and *C. aurea*. The reliability of this test has proved to be a useful tool to differentiate between the mites present on blackcurrant, redcurrant and gooseberry. The use of multiplex PCR analysis using mite rDNA has suggested that there may be another, possible new, mite species on redcurrant (Kumar *et al.*, 1999).

## Plant Resistance

Plant breeders have identified two major resistance genes to *C. ribis*; gene *Ce* from gooseberry (Knight *et al.*, 1974) and gene *P* (Anderson, 1971) from *R. nigrum* var *sibericum*, and other wild *Ribes* (Gordon *et al.*, 1994). In field trials of progeny containing one or the other of these resistance genes, the *Ce*-gene containing plants exhibited stronger resistance. Plants containing gene *P* became infested with mites, but the buds soon became necrotic. Further observations have shown that some gene *P* containing genotypes develop galled buds and become infected by reversion, indicating that mites can feed and survive under some conditions. As a consequence, breeding strategies are currently concentrating on *Ce* derived material.

## Discussion

The taxonomy of the eriophyid mites colonising *Ribes* is complex as the differentiating features are very minor, often difficult to locate and not easy to characterise. Until recently, the taxonomic keys have been confusing and inaccurate but the re-description of the genus by Amrine *et al.*, (1994) has improved their reliability. The parallel development of a molecular approach to species determination has provided another dependable method of identifying mites associated with currant that is more rapid and cost effective. Discovery of gooseberry mites on blackcurrant, especially those with Scandinavian or Russian parentage in the UK (Easterbrook, 1980), in the Pacific Northwest and in Tasmania is a cause for concern for breeders and pathologists, particularly if these mites can vector the reversion agent. The possible presence of yet another mite species on redcurrant further complicates the understanding of eriophyid mites on *Ribes* (Kumar *et al.*, 1999)

## Résumé

Les Eriophyides du genre *Cecidophyopsis* colonisent les fruits de la famille des groseilliers cultivés (*Ribes* spp.). Actuellement le plus destructif est *C. ribis* qui ravage le cassis (*R. nigrum*). L'importance, la biologie et la taxonomie de *C.ribis* et d'autres *Cecidophyopsis* qui colonisent les

cassis sont discutés en relation avec leurs hôtes. Il y a peu de temps encore, les clés taxonomiques portaient à confusion et dans certains cas étaient inadaptées. La révision des clés pour les espèces principales et le développement d'une approche moléculaire pour améliorer la détermination sont discutés. La dernière approche a fourni une méthode rapide, efficace et peu couteuse pour identifier les acariens inféodés à *Ribes*.

**Mots clés:** *Ribes*, groseilliers, eriophyides, *Cecidophyopsis*, détermination moléculaire, taxonomie.

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# Interpretation of aphid performance data in quantifying resistance

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**Abstract:** Measurement of the intrinsic rate of natural increase ($r_m$) is a widely used measure of aphid performance that can be derived by several different methods. This paper discusses the interpretation of $r_m$ values collected by different methods in the context of a programme to develop varieties of horticultural brassicas resistant to the cabbage aphid *Brevicoryne brassicae*. It is concluded that the most appropriate and practical method for assessing *B.brassicae* performance is to monitor development of a limited number of nymphs that are allowed to find their most favourable feeding site on the plant.

**Key words**: *Brevicoryne brassicae*, cabbage aphid, Brassica, rate of increase.

## Introduction

Several *Brassica* species have been shown to possess resistance to the cabbage aphid (*Brevicoryne brassicae*) (Singh *et al.*, 1994; Ellis & Farrell, 1995) and attempts are now being made to quantify the levels of resistance observed both between and within species of plant. This is necessary to determine the genetic basis of resistance through relating plant genotype to resistance phenotype.

Aphid population increase and growth rate of individuals are both important criteria or assessing resistance in host plants. The maximum possible rate of population increase, that occurs when there are no biotic restraints on the aphids, is the intrinsic rate of natural increase (Birch, 1948), and has been commonly given the notation $r_m$. As well as numerical increase, host suitability can be measured by individual weight gain over time. Thus aphid performance can be measured by either recording the growth rate of individuals (van Emden, 1969), counting the number of embryos in the mother at maturity (Adams & van Emden, 1972; Dewar, 1977), combining fecundity and developmental time (Birch, 1948; Laughlin, 1965; Wyatt & White, 1977) or counting aphid numbers in exponentially increasing aphid populations (Southwood, 1978; Verhs *et al.*, 1992; Guldemond *et al.*, 1998). Correlations between growth rates and $r_m$ values have been completed for some species (Kempton *et al.*, 1980; Leather & Dixon, 1984) but are not strongly related in other species, for example *Aphis fabae* (Wojechichekko-zyto & van Emdem, 1995) and *Aphis gossypii* (Guldemond *et al.*, 1998). No correlation of individual growth rate and numerical increase has been estimated/calculated for *B.brassicae*, and thus any discussion of $r_m$ for *B.brassicae* will have to be confined to direct measurement of numerical increase.

The use of different methods to obtain the same performance measure suggests that there is some confusion as how best to measure $r_m$. The aim of this study is to determine the most efficient method of $r_m$ determination so that differences in aphid suitability between individual

plants can be reliably obtained. To this end, $r_m$ was determined in two experiments, with either expanding aphid populations, or by studying individuals.

## Materials and Methods

***Experiment studying whole aphid population***

Five six-week-old *B.oleracea* plants (Brussels sprouts 'Oliver'), grown in a controlled environment room (19 ± 1°C, 70-80% RH), were each infested with a stable age distribution of 10 *B.brassicae* from the clone K3 (collected from a Brussels sprouts crop, Lincs, UK, 1997). The stable age distribution consisted of four 1st instars, two 2nd instars, three 3rd or 4th instars and one apterous adult (Deloach, 1974; Hughes, 1963). The first instars were born on the plant while all other aphids were transferred from culture to test plants. All adults used were 14 days old at the start of the experiment. The number of aphids on each plant was counted 1,3,6,8 and 9 days after establishment. Each plant was isolated in a plastic cylinder, with a mesh top, to restrain the movement of any alatae aphids that developed in the population. The natural log (ln) of aphid numbers was plotted against days after establishment for all five plants. If the populations on each plant had been increasing exponentially then the slope of the resulting straight line would be the $r_m$ value of the aphids on that plant (Southwood, 1978). An individual $r_m$ should therefore be obtained for each replicate plant.

***Experiment studying individual aphids***

Sixty six-week-old *B.oleracea* (brussels sprouts 'Oliver') were infested with either one, three or five newly born nymphs after allowing 14 day old adults to reproduce on the plants for one day. Any excess of nymphs and the adults were removed after 24 hours. Clip cages were removed to allow for the aphids to find the most favourable feeding site on each individual plant. Aphids were observed daily and the time of first reproduction was noted. Fecundity was then measured after a period equal to this pre-reproductive period, allowing for $r_m$ to be calculated by the method of Wyatt & White (1977) for each individual plant.

## Results

The results show clearly that more variation is seen when $r_m$ is measured using a stable age distribution than when using individual aphids (Table 1). No statistics can be performed on the variation between the two data sets as they were collected at different times. The $r_m$ values derived from stable age distributions of aphids were all the result of significant regressions of aphid numbers against days after infestation (Table 2). No differences were observed for the $r_m$ values calculated from plants infested with 1,3 or 5 nymphs, although there were significant differences in the pre-reproductive period ($F = 14.8$, 3,31 d.f., $p<0.001$) between these treatments (Table 1).

## Discussion

The results give $r_m$ values from two different experiments that cannot be directly compared. However, they are similar to those recorded in other studies (Deloach, 1974; Verma & Mahmook, 1988) on similar host plants.

The variability within each set of results is of primary concern to this study, as a consistent measure on the same plant type is vital if genetically determined resistance is going to be identified and quantified. For this reason the results from each experiment will be discussed separately.

The $r_m$ values obtained from using stable age distributions of aphids were all based on significant regressions, but they did not follow a straight line, as would be expected if exponential increase had been recorded. This is indicated in the amount of variability explained for each regression, which is above 90% for two of the five replicates only (Table 2). This deviation from a straight line suggests it was difficult to count aphids in situ reliably. This was due to the development of dense colonies of *B. brassicae* around the small and tightly-associated leaves at the growing point of *Brassica* plants. In addition, the variability may be compounded by mortality of adult or late instar nymphs, as well as possible development and movement of winged aphids. These factors would act to reduce artificially the rate of increase of the population, as the death or emigration of reproducing adults will reduce fecundity of the total population. Such occurrences make it difficult to use this method of measuring aphids to give an accurate measure of susceptibility between plants. The effect of the increasing population on the host plant may also be a factor that could be reducing the possibility of obtaining exponential increase. If plants that are used in experiments can only support relatively low numbers of aphids before adverse effects on the plant may limit its suitability, then this could be limiting population development without the experimenter knowing this. Such problems may be reduced if only a small number of adults are reproducing on a single plant.

There was less variability between replicates when individual aphids were studied, although it did vary between plants with 1,3 or 5 adult aphids on them. The lower variability may well be due to the defined number of individuals that can be followed accurately, whereas an exponentially increasing aphid population cannot be counted accurately without significant disturbance. The capacity to follow individual aphids allowed for the day of first reproduction, for each group, to be ascertained and also allowed for the $r_m$ to be calculated using fecundity over the pre-reproductive period only, as described by Wyatt & White, (1977).

Studying individuals additionally allowed for occasional adult mortality to be partially accounted for, as fecundity is recorded as reproduction per surviving adult. Thus the $r_m$ from a single aphid can be compared to that from 5 aphids. This does not account for any problems that may arise with adult mortality that occurs during the experimental period, but it is an improvement on the situation where migration and mortality cannot be readily checked, which occurs when studying increasing aphid populations.

The effect of crowding on aphid $r_m$ was investigated to a limited extent in this experiment, as 1.3 and 5 aphids were born on each of 20 plants each. Intrinsic rate of increase did not differ between these aphid densities, but single aphids had a significantly longer pre-reproductive period than those on plants inoculated with 3 or 5 aphids (Table 1). This suggests that crowding of *B.brassicae* had some positive effect on development in agreement with Hayamizu (1984). Results from this experiment suggest that the positive effects of crowding on *B.brassicae* occur rapidly, as nymphs born on plants supporting a single aphid were recorded as reproducing adults after a time equal to that of the initial pre-reproductive period. So these young aphids had grown more rapidly due to the presence of other aphids produced by the single adult. The $r_m$ values from single aphids were the most variable recorded. This suggests that a wide variation in reproduction, possibly resulting in differential positive effects of crowding, was observed on plants supporting a single insect.

## Conclusions

It is imperative that any variation observed in the insect-plant interaction is due to the quality of the host plant and not due to factors, such as aphid mortality or movement. To ensure that this is the case, the number of individuals that are alive and reproducing must be known, as must total fecundity. Whilst total fecundity can be counted using stable age distributions, it has been found that counting all *B.brassicae* accurately is particularly difficult as the population increases due to the development of dense colonies. This is not the case if a known number of nymphs are born on each plant without restriction. Both methods allow for comparisons of plants with different growth habits, as the aphid is free to choose the most favourable site in each situation. However, it will take longer to do any experiment if individual aphids are studied. This may be important if slow and fast growing plants are being compared as additional variation due to plant growth habits may effect the $r_m$ value. This means that where aphid movement and numbers can be recorded reliably (i.e. for species that do not form dense colonies) and plant growth habits are highly variable, then stable age distributions may be the most appropriate approach. However, with *B.brassicae*, the use of individual aphids allows for a less variable measure of $r_m$ to be obtained on individual plants.

## Résumé

La mesure du taux intrinsèque d'accroissement naturel ($r_m$) est un paramètre des performances des pucerons largement utilisé, qui peut être obtenu selon différentes méthodes. Cet article discute l'interprétation des valeurs de $r_m$ obtenues selon différentes méthodes dans le contexte d'un programme de développement de variétés de Brassica résistantes au puceron du chou.

Nous pensons que la méthode la plus pratique et la mieux adaptée pour tester les performances du puceron du chou est de suivre le développement d'un nombre limité de larves qui peuvent trouver le site alimentaire le plus favorable.

**Mots clés:** *Brevicoryne brassicae*, puceron du chou, Brassica, contournement de la résistance, taux intrinsèque d'accroissement

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Table 1. The intrinsic rate of increase of *B.brassicae* on *B.oleracea* as measured by studying individual aphids or populations with a stable age distribution

| Method of study | Number of Plants | $r_m$ value | Standard deviation | Total fecundity per adult | Days until first reproduction |
|---|---|---|---|---|---|
| Individual aphids | | | | | |
| 1 per plant | 16 | 0.1616 | 0.0013 | 28.06 | 13.6 |
| 3 per plant | 17 | 0.1905 | 0.0001 | 23.57 | 12.8 |
| 5 per plant | 18 | 0.1761 | 0.0009 | 18.83 | 11.56 |
| Stable age population | | | | | |
| | 5 | 0.2239 | 0.0035 | - | - |
| | | | | - | - |

Table 2. Relationship between the number of aphids recorded per population and day after infestation

| Plant type | Replicate no. | $r_m$ value (slope) | % variance accounted for | probability |
|---|---|---|---|---|
| Oliver | 1 | 0.2930 | 90.4 | 0.008 |
| Oliver | 2 | 0.2594 | 94.5 | 0.004 |
| Oliver | 3 | 0.2764 | 85.9 | 0.015 |
| Oliver | 4 | 0.0738 | 70.4 | 0.048 |
| Oliver | 5 | 0.2167 | 82.5 | 0.021 |

## Session 3 – HPR, Pest Biotypes and Integrated Pest Management

*Breeding for Resistance to Insects and Mites*
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# Resistance-breaking biotypes of rosy apple aphid, Dysaphis plantaginea, on the resistant cultivar 'Florina'

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**Abstract:** Surveys of the variability in the resistance of the apple cultivar 'Florina' to *Dysaphis plantaginea,* the rosy apple aphid, were carried out in European countries in 1995 and 1998. On more than 54 ha, three sources of resistance-breaking biotypes were found on three different sites, none of which spread to another tree nor developed on the same tree the next year. In 1998, no symptoms were recorded.

From the breaking-resistance sources, one line was isolated and reared. Its capacity for breaking resistance was confirmed in studies under controlled conditions by the presence susceptibility symptoms occurring on 'Florina' and by higher fecundity and adult survival on 'Florina' than a control line, while on the susceptible cv. 'Golden Delicious' its performances were similar to those of the control line. Risks induced by resistance-breaking aphid biotypes in orchards are discussed.

**Key words:** Rosy apple aphid, *Dysaphis plantaginea*, apple, resistance, resistance-breaking.

## Introduction

The fresh fruit cultivar 'Florina', from the INRA Research Station in Angers, is resistant to apple scab, *Venturia inaequalis* Cke., and to rosy apple aphid, *Dysaphis plantaginea* Pass., and tolerant to fireblight, *Erwinia amylovora* Burr., and to the European red mite, *Panonychus ulmi* Koch. (Lespinasse *et al.*, 1985). Both tolerance and antibiosis characterise the resistance to *D. plantaginea*, a major pest in European orchards (Rat-Morris, 1994).

The aim of this work was (i) to assess the stability of resistance to *D. plantaginea* through a survey of European orchards: variability among the aphid strains and resistance variability of 'Florina'; (ii) to test a resistance-breaking biotype under controlled conditions.

## Survey of European orchards

Two surveys were carried out in 1995 and 1998. A questionnaire was circulated widely to the different production areas in nine European countries requesting the following information: area cropped with 'Florina', market for the fruit purpose; aphicide treatments; number of *D. plantaginea* colonies and related symptoms on the trees.

'Florina' was grown in six countries, on more than 54 ha (Table 1). 'Florina' was grown as a cultivar for fresh fruit or juice production (Moselle, France) or as a pollinator (Nord, France).

This cultivar is valuable in breeding programmes and for organic production, but the fresh fruit quality is not good enough according to certain growers. The cultivar is not adapted for cultivation in the northern part of Europe.

In some areas, an aphicide programme is still used to control *D. plantaginea*, despite 'Florina's' resistance: the reasons for these treatments were because it was part of an experimental plot or a pollinator for a susceptible cultivar, or because its resistance was unknown. *D. plantaginea* is not a pest in some areas (Spain, Arges-Romania).

Table 1. Biodiversity in resistance of 'Florina' to rosy apple aphid, D. plantaginea : a survey of European orchards - 1995 & 1998

| Country | Surfaces | | Aphicides | Symptoms | *D. plantaginea* |
|---|---|---|---|---|---|
| **1995** | research | production | | | |
| Czech republic | 50 trees | 1 ha | ? | 0 | not present in this area |
| France | | | | | |
| (Anjou) | 1 ha | | 0 | + | on 1 tree, not spreading |
| (Hautes Alpes) | | 3 ha | 0 | 0 | |
| Italy | | | | | |
| (South Tyrol) | 0.8 ha | 4 ha | 0 | 0 | |
| (Northern Veneto) | | 17 000 trees (≈ 16 ha) | ? | ? | (trees from a nursery in South Tyrol) |
| Romania (Arges) | 1 ha | 10 ha | + | 0 | not present in this area |
| Spain (Catalonia) | 6 trees | | ? | ? | |
| Switzerland (Valais) | | 21 ha | 0/+ | + | 0n 2 trees, not spreading |
| **Total 1995** | **≈ 3 ha** | **≈ 55 ha** | | | |
| **1998** | | | | | |
| France | | | | | |
| (Anjou) | 1 ha | | 0 | 0 | |
| (Hautes Alpes) | | 3 ha | 0 | 0 | |
| (Moselle) | | 1 ha | + | 0 | |
| (Nord) | | 30 trees | + | 0 | |
| Germany | | | | | |
| (Ahrenbourg) | | few trees | 0/+ | 0 | Florina uninfested in an infested plot slight |
| (Dresden) | | 2 trees | + | ± | susceptibility |
| Italy | | | | | |
| (South Tyrol) | | 2 ha | | | |
| (Northern Veneto) | | 15 000 trees (≈ 14 ha) | | | |
| Switzerland (Valais) | | 21 ha | 0/+ | 0 | |
| **Total 1998** | **≈ 3 ha** | **≈ 51 ha** | | | |

Three populations were found in 1995, associated with symptoms of curled leaves. In France (research orchard, 1 ha, Angers) one colony developed on one shoot. In Switzerland (Valais) one

colony was found on one tree in a 5 ha production orchard, associated with severe leaf roll, and in a 6000 $m^2$ production plot, several colonies developed on one single tree. None of these three populations spread from the original tree to another, nor did they spread and increase on the same tree the next year.

There were no records of infestation on 'Florina' in 1998, and the cultivar was found uninfested in severely infested plots (France, Germany).

## Biology of resistance-breaking D. plantaginea under controlled conditions

### *Methods*

From the colonies found in Switzerland in 1995, one line of resistance-breaking *D. plantaginea*, called line M, was selected and reared on the susceptible cultivar 'Golden Delicious', on grafted, potted plants, 50-80 cm high, placed in a growth chamber (DL 16h; 20°C). A control line from Angers, called T, was reared in the same conditions.

Plant reactions were quoted on 'Florina' and 'Golden Delicious' in the same conditions, infested with young virginoparous females from lines M or T , placed on the upper side of the first leaf beneath the apex (5 aphids/plant, 10 plants/cv.).

Fecundity and survival for lines M and T were carried out on excised leaves of 'Florina' and 'Golden Delicious' in the growth chamber, using a method previously developed for control lines (Rat-Morris, 1993; 1994).

### Results

Leaf-roll symptoms occurred on 'Golden Delicious' for both lines M and control T. On 'Florina', there were no symptoms with line T as expected, while line M produced moderate leaf-roll.

Fecundity and adult survival of lines M and T were similar on the susceptible cv. 'Golden Delicious'; control line T fecundity and survival were strongly reduced. Performances of line M on 'Florina' were higher than those of control, but lower than those of both lines on 'Golden Delicious' (Table 2).

Table 2. Fecundity and adult survival of 2 *D. plantaginea* lines on excised leaves from the resistant apple cv. 'Florina' and the susceptible one 'Golden Delicious'; pre-adult development on 'Golden Delicious' (means on 20 leaves during 12 days, 1 female/leaf)

| ***D. plantaginea* line** | **Adult life on** | **Survival (days)** | **Total offspring** |
|---|---|---|---|
| M | 'Golden Delicious' | 10.55 ± 2.50 **a** | 22.35 ± 7.05 **a** |
| T (control) | 'Golden Delicious' | 10.75 ± 2.84 **a** | 22.55 ± 9.37 **a** |
| M | 'Florina' | 8.70 ± 2.40 **b** | 12.30 ± 4.53 **b** |
| T (control) | 'Florina' | 7.10 ± 3.80 **c** | 7.85 ± 6.42 **c** |

The same bold letter following results indicates no significant difference (t test)

In previous tests carried out on a different control line (Rat-Morris, 1994) over a period of 12 days resulted in the production of 42 larvae on 'Golden Delicious' and 26 on 'Florina' when Aphids were reared on 'Golden Delicious' during their pre-adult development, and 24 larvae on 'Golden Delicious' and 16 on 'Florina' when reared on 'Florina'. Line M can be compared with the

control line reared on Florina, while the same line reared on 'Golden Delicious' showed a higher fecundity on both cultivars. All the lines have shown a higher fecundity on 'Golden Delicious' than on 'Florina'.

## Discussion

The capacity for *D. plantaginea* line M to break the resistance of 'Florina' is confirmed and concerns the two aspects of resistance: (i) line M induces susceptibility symptoms on Florina, tolerance is then reduced; (ii) the gradient in fecundity fits well with a lower antibiosis effect of 'Florina' on line M than on control lines.

Breeding a resistant cultivar only makes sense if this resistance is stable in terms of space and sustainability, especially for a perennial crop such as apple. Planting up an orchard with a new cultivar represents a large investment for growers and so they cannot envisage changing cultivars in the short term.

There are numerous examples of resistance-breaking aphid biotypes (Campbell & Dreyer, 1990). Introducing resistant wheat cultivars to *Schizaphis graminum* Rond, which presents a sexual reproduction, led to the emergence of resistant-breaking biotypes (Puterka & Peters, 1990).

However, cases exist of long-lasting resistance to holocyclic aphids. For example, resistance in raspberry to *Amphorophora idaei* Borner has existed for more than 40 years (Birch *et al.*, 1993). The apple cultivars 'Northern Spy' and 'Winter Majetin' have been resistant to *Eriosoma lanigerum* (Hausmann) for more than 100 years (Auclair, 1989). 'Northern Spy', with its single dominant resistant gene *Er*, was used extensively as a parent in breeding programmes to obtain resistant apple rootstocks, especially the Malling-Merton series from the East Malling Research Station in the United Kingdom (Knight *et al.*, 1962). These rootstocks were propagated all over the world. *E. lanigerum* has only a parthenogenetic development out of North America, its native area. Yet, several resistance-breaking biotypes have been recorded in Australia (Sen upta & Miles, 1975), in South Africa (Gilliomee *et al.*, 1968) and in the USA (Rock *et al.*, 1974; Young *et al.*, 1982).

The complex heredity of the resistance of 'Florina' to *D. plantaginea* (Rat-Morris, 1994) favours its stability. However, some breaking-resistance sources have been found. Due to the biology of the aphid, which develop only as apterous forms on apple in spring and early summer and whose winged form fly away to its secondary host *Plantago* sp., such biotypes are not likely to colonise a broad area. Also, obligatory sexual reproduction limits the development of possible resistant lines.

Studying the behaviour of resistance-breaking biotypes will increase our understanding of the apple/*D. plantaginea* relationships and thus be a guide in breeding programmes.

## Résumé

Une enquête sur la variabilité de la résistance du cultivar de pommier Florina à *Dysaphis plantaginea* à été conduite dans zones de production en Europe en 1995 et 1998. Sur un total de plus de 54 ha, en 1995, seules trois sources de contournement de la résistance ont été trouvées, sur trois sites différents. Aucune des colonies ne s'est étendue d'un arbre à l'autre, ni sur le même arbre d'une année sur l'autre. Aucun symptôme n'a été observé sur Florina en 1998.

Une lignée a été isolée à partir d'une des sources de contournement de la résistance. Sa capacité à contourner la résistance a été confirmée par des études en conditions contrôlées. Elle

produit des symptômes de sensibilité sur Florina. La fécondité et la longévité des adultes sont supérieures sur Florina, comparées à celles d'une lignée témoin. Sur le cultivar sensible Golden Delicious, ses performances ne sont pas significativement différentes de celles de la lignée témoin. Les risques liés à la présence en verger de populations de pucerons contournant la résistance sont discutés.

**Mots clés:** *Dysaphis plantaginea*, pommier, résistance, contournement de la résistance

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# Ecological impact of transgenic potato expressing the anti-aphid snowdrop lectin on predatory ladybirds

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**Abstract:** An experimental potato line was genetically engineered to express the anti-aphid lectin from snowdrop (GNA), field-grown in mesh tunnels and assessed for possible tri-trophic interactions involving the insect resistance transgene (GNA lectin), target aphid pest (peach-potato aphid, *Myzus persicae*) and aphidophagous predator (adult 2-spot ladybird, *Adalia bipunctata*). A full-sib reciprocal cross design was used to mate ladybirds subjected to 3 different aphids diets, thus partitioning out effects of ladybird inter-family genetic variance, aphid diet and ladybird sex.

In no-choice feeding trials under laboratory conditions, ladybirds fed to excess on aphids from the transgenic GNA-expressing potatoes for an initial period of 12 days, then switched to optimal aphid diet (pea aphids, *Acyrthrosiphon pisum* from normal *Vicia faba)* until natural death, showed adverse effects on ladybird egg production, egg fertility and egg hatch. Adult longevity of female ladybirds was reduced by 51% after 12 days' feeding on *M. persicae* from GNA-expressing potatoes. The adverse effects on ladybirds' reproductive biology were reversed after switching to the optimal aphid diet for 2-3 weeks following feeding on aphids from GNA-expressing potatoes. The possible implications for risk assessment and field management of insect-resistant transgenic crops, to minimise adverse effects on natural enemies are discussed.

**Key words:** *Myzus persicae, Adalia bipunctata,* lectin, transgenic potato, tri-trophic interactions.

## Introduction

Transgenic crops genetically engineered for enhanced insect resistance should be compatible with other components of IPM for the pest resistance to be durable and effective. Extended laboratory studies were set up to study possible tri-trophic interactions involving a lectin-expressing transgenic crop (GNA-expressing potato, *Galanthus nivalis* agglutinin), a target pest aphid (peach-potato aphid, *Myzus persicae*) and a beneficial aphidophagous predator (2-spot ladybird, *Adalia bipunctata*). Plant lectins are considered to play a role in broad spectrum plant defence against pests and pathogens (Chrispeels *et al.,* 1991). Some plant lectins have been shown to be toxic towards insects, including sap-sucking hemipteran species (Powell *et al.,* 1993; Rahbe *et al.,* 1995). The snowdrop lectin, (GNA), is a mannose-binding lectin which is effective against several aphid species both *in vitro* (Down *et al.,* 1996) and when expressed in transgenic tobacco and potato (Gatehouse *et al.,* 1996), reducing aphid fecundity and retarding development. This type of partial resistance impacts on direct aphid feeding damage but is unlikely to be effective on its own against virus vector species. GNA also affects lepidopteran and coleopteran larvae when fed in artificial diet or delivered via a transgenic plant. Thus GNA expressed in transgenic potato

as a means of aphid resistance can also provide some protection against lepidopteran tomato moth larvae (Gatehouse *et al.,* 1997). Lectins are therefore considered a possible target for genetic engineering into crops for broader pest resistance. However, this broad-range protection may also have potential drawbacks. Lectins are also known to bind to insect gut cells, including those of aphids, and the lectin GNA can be present in aphids after feeding on GNA-expressing transgenic plants (Shi *et al.,* 1994). Thus, an anti-aphid lectin, expressed in leaves of a transgenic plant, could enter the food chain of aphidophagous insects, such as ladybirds, raising the potential for adverse effects on non-target beneficial insects if they are sensitive to the lectin.

## Methods

Risk assessment experiments were set up to compare the performance of ladybirds fed peach-potato aphids reared on transgenic potato plants expressing GNA, to ladybirds fed peach-potato aphids reared on control (non-transgenic) potato plants and a third group reared on pea aphids from non-transgenic *Vicia faba* beans (optimal ladybird diet). GNA-expressing transgenic potato (*Solanum tuberosum* L., cv. Desirée) lines contained a gene construct where the GNA coding sequence from clone LECGNA2 (Van Damme *et al.,* 1991) was expressed using the CaMV 35S promoter. Expression levels of GNA were estimated as 0.1 - 0.6% of total protein in leaves of transformed potato plants grown from tissue culture then under glasshouse conditions. 180 tubers each of control and GNA potatoes were planted in a randomised block design in four 12m long tunnels covered in Nicofence® aphid-proof mesh. Plants were fertilised and irrigated according to standard regimes. After 8 weeks growth, individually netted potato plants were infested with 100 virus-free adult *M. persicae.* Progeny from twelve singly mated isofemale $F_1$ 2-spot ladybirds, *Adalia bipunctata* (L.), collected from Glasgow, Scotland, were reared under controlled laboratory conditions. Eight males of progeny from each of six families and eight virgin female progeny from each of the other six families were fed separately (in excess to daily requirements), on one of three test diets (see below) in phase 1 (12 days) of the feeding trial and then used for replicated matings in six reciprocal crosses.

***Ladybird diet regimes during feeding trial, phase 1***

Diet 1- *M. persicae* from control potatoes ('control' aphid diet).
Diet 2- *M. persicae* from GNA potatoes ('GNA' aphid diet).
Diet 3- *Acyrthosiphon pisum* (Harris) from non-transgenic *V. faba* bean plants, ('optimal' aphid diet).

***Reciprocal crossing regime for ladybird matings, end of phase 1***

'GNA' diet male x 'optimal' diet female (cross type 1) and reciprocal cross (cross type 2)
'GNA' diet male x 'control' diet female (cross type 3) and reciprocal cross (cross type 4)
'Control' diet male x 'optimal' diet female (cross type 5) and reciprocal cross (cross type 6).
(Replication: Cross types 1-4, n=24 / cross. Cross types 5- 6, n=12 / cross).

In phase 2 (day 13 of feeding trial until natural adult death) all ladybirds (regardless of their diet during phase 1) were fed on 'optimal' aphid diet (diet 3, Methods), offered in excess to daily requirement. This was done a) to simulate the change of diet likely to be experienced by actively flying and foraging ladybirds under field (choice) conditions, and b) to measure the duration of any transient effect(s) caused by eating aphids from GNA-expressing transgenic potatoes during phase 1 (first 12 days) of the ladybird feeding trial.

## Results

***Aphid consumption (offered excess to daily requirements, no-choice tests)***

There was no significant difference between the cumulative (total) weight of aphids eaten during 12 days of phase 1 by ladybirds fed the 'control' aphid diet and those fed 'GNA' aphid diet (diets 1 and 2, Methods). Because aphids reared on GNA potatoes are smaller than those on control potatoes (Down *et al.*, 1996), more 'GNA' diet aphids (numbers of aphids eaten/adult ladybird) were consumed/ladybird than of 'control' aphids, although the total aphid biomasses of control and GNA aphids consumed did not differ significantly.

***Ladybird egg production and viability***

Egg production and viability (fertilisation, hatch rate) of individual, mated females from each of the six crosses was monitored daily for a further 28 days during phase 2, with all crosses fed on excess 'optimal' aphid diet. By the second week of phase 2 there was a significant effect of the initial (phase 1) aphid diet on ladybird fecundity (where diet designation refers to that consumed during phase 1 of the experiment). For example, in week 2 female ladybirds fed 'GNA' diet (cross type 2) produced a mean of 86 eggs/week, compared with 105 eggs/week for 'control' diet fed females from cross type 6. Greater aphid diet effects were seen in week 3, where 'GNA' aphid diet fed females (cross type 2) produced a mean of 64 eggs/week, compared with 101 eggs/week for 'control' aphid diet fed females from cross type 6. By week 4 the diet effect on female ladybird fecundity was not statistically significant across the 6 cross types, although the overall trend was similar to that observed in weeks 2 and 3.

For all cross types involving either the male or female ladybird fed on 'GNA' aphid diets in phase 1 (crosses 1-4), egg fertility (eggs showing embryonic development, as a percentage of total eggs) was significantly reduced, compared with crosses (types 5-6) involving ladybirds fed 'control' or 'optimal' aphid diets. The effect of 'GNA' aphid diet was most evident during the first week of egg production. Egg fertility was reduced from 95% in crosses involving 'control'- and 'optimal' diet-fed insects (types 5 and 6), to 71-85% for crosses involving insects fed on 'GNA' aphid diet (crosses 1-4).

Over the first 2 weeks of phase 2, all crosses involving a ladybird of either sex fed 'GNA' aphid diet produced eggs with significantly lower % hatch rates than crosses 5 and 6. In the first week, % hatch for females initially fed 'GNA' aphid diet (cross 2) was reduced to 69%, compared with 87% for the 'control' aphid diet females of cross 6. Males were also affected by the initial 'GNA' aphid diet, but to a lesser extent (74% hatch for cross 1, compared with 86% hatch for 'control' aphid diet males of cross 5. After 2 weeks feeding on 'optimal' aphid diet during phase 2, the effect of the initial 'GNA' aphid diet on either sex was not significant when compared with 'control' diet-fed insects. This indicated that the switch to 'optimal' diet during phase 2 reversed the adverse effects on egg hatch of the 'GNA' aphid diet eaten by ladybirds during the initial 12 days (phase 1).

***Ladybird adult longevity***

Female ladybirds fed on 'GNA' aphid diet during phase 1 died significantly sooner (mean female longevities for cross types 2 and 4 were 36 and 39 days respectively) than females fed on 'control' or 'optimal' aphid diets (mean longevities of cross types 5 and 6 were 55 and 74 days respectively), despite all six ladybird cross types switching to the 'optimal' aphid diet in phase 2.

This difference in female longevity was most marked when comparing females fed 'GNA' aphid diet (cross 2) with those fed 'control' aphid diet (cross 6), resulting in a 51% reduction in female lifespan. The effect of aphid diet on male ladybird longevity was less marked, but also significant in some comparisons (up to 10% reduction in male lifespan after 'GNA' aphid diet).

## Discussion

Our results from ladybird feeding trials under controlled environment conditions show that transgenic potatoes genetically-engineered to express the anti-aphid lectin GNA and grown under field conditions can adversely affect the longevity and reproductive biology of an important natural enemy, via aphids in its food chain. Although we only monitored oviposition for 28 days during phase 2, ladybirds will normally continue to lay eggs for 9-12 weeks, if suitable food and environmental conditions are available. The impact of reduced female ladybird longevity will impact not only on total fecundity, but also on the number of aphids consumed over the ladybird's life span. Each ladybird can consume up to 5,000 aphids in a normal life span, so a 50% in female ladybird longevity will inevitably reduce ladybirds' efficiency as biological control agents.

GNA lectin has been shown to be taken up from transgenic tobacco plants by feeding aphids, being detected in the honeydew of *M. persicae* by immunological assay (Shi *et al.*, 1994). Plant lectins have also been shown to bind to insect gut cells *in vivo,* including those of aphids . GNA and other lectins are taken up and accumulate in aphid tissues and can thus delivered to the predatory ladybird, where they bind to the ladybird's gut epithelium (Gatehouse, Birch *et al.*, unpublished data). It is still to be established whether GNA is having a direct (toxic) effect on ladybirds or if the effect of the transgenic host plant of the aphid is indirect, via decreased nutritional value of the aphids reared on GNA-expressing plants, or a combination of both effects (ongoing research).

A recent study on Bt-expressing corn, genetically engineered for resistance to target lepidoptera (European cornborer), has shown the potential for Bt toxicity to predatory lacewing larvae via prey (target or non-target lepidopteran larvae) in its food chain (Hilbeck *et al.*, 1998). Our results on ladybirds together with those on lacewings suggest that tri-trophic effects of anti-pest transgenes on non-target, beneficial predators may be more widespread than previously assumed, and so require careful testing prior to commercial release of transgenic crops.

The reversible nature of the adverse effects of a lectin-expressing transgenic crop on ladybird reproduction (but not longevity) observed in our extended laboratory tests suggests that under field conditions (as yet untested for GNA), it may be possible to reduce potentially harmful effects on aphid predators. This could be achieved by growing non-transgenic susceptible crop 'refuges' (e.g. inter-cropping or mixed plantings of aphid-susceptible and aphid-resistant plants), providing unaffected aphid populations within adult ladybirds' feeding range. Similar field management strategies using 'refuges' are currently being implemented with insect-resistant transgenic crops to reduce the rate of selection of resistance-breaking pest populations (Estruch *et al.*, 1997). Although we don't yet know how best to manage refuges and transgenic crops to optimise movement of natural enemies from the transgenic crop to the refuge (and *vice versa*) for feeding and mating, it is known that 2-spot ladybirds will preferentially avoid laying eggs near con-specific larvae, to avoid competition for food (aphids). It should be possible to manage aphid and ladybird densities in the transgenic crop and non-transgenic refuges to ensure ladybirds' intake of 'normal' aphids (from non-transgenic host plants) and to encourage matings between ladybirds from the two sites. We plan to model and test predictions of tri-trophic interactions so

that non-transgenic refuges can be structured and managed to minimise the impact of insect-resistant transgenic crops on ladybirds and other natural enemies.

Some pesticides currently used to control aphids are also likely to have harmful effects on beneficial natural enemies, including ladybirds. In some cases specific insecticides may be more toxic to ladybirds than a specific genetically engineered toxin (e.g. GNA), whilst in other pesticide v toxin comparisons this may not be true. In weighing up the potential risks posed by insecticidal transgene products relative to current or future pesticides, case-by case comparisons should be made so that the safest and most effective options for each crop and pest(s) are selected for use in future IPM.

The combined effect of partial resistance (transgenic and/or natural) with pest-regulating natural enemies potentially offers a more diversified and potentially durable approach to crop protection through IPM (Waage, 1997). Such an integrated approach is particularly important for pest resistance based on a single gene (vertical resistance), where selection pressure on the target pest for resistance-breaking traits can lead to the breakdown of the plants' genetic resistance (Birch *et al.*, 1997). The careful choice and use of anti-pest genes with targeted gene expression systems, the incorporation of pest and natural enemy refuges, the development of ecological modelling over realistic field scales and the inclusion of detailed and longer term tri-trophic interaction studies (under both laboratory and field conditions) will be crucial to the development of safe and durable pest-resistant transgenic crops for IPM.

## Résumé

Une lignée expérimentale de pomme de terre a été génetiquement modifiée pour exprimer la lectine anti-puceron de la perce neige (GNA). Elle a été cultivée au champ sous des tunnels grillages et evaluée dans des interactions tritrophiques incluant la résistance transgenique (GNA lectine), le ravageur cible (puceron vert du pêcher, *Myzus persicae*), et un prédateur du puceron (la coccinelle *Adalia bipunctata*). Des croisements consanguins réciproques ont été utilisés pour accoupler les coccinelles soumises à trois regimes de pucerons differents, afin de séparer les effets de la variance génétique entre familles, des régimes de pucerons et du sexe des coccinelles.

Des essais d'alimentation en laboratoire ont été poursuivis en conditions de non-choix. Les coccinelles ont été nourriés en excés de pucerons élèves sur plantes exprimant la « GNA » de la pomme de terre pendant une periode initiale de 12 jours, puis elles ont eu un régime alimentaire optimal (puceron du pois *Acyrthosiphon pisum* issu de *Vicia faba* normal) jusqu'a leur mort naturelle. Ce traitement à montre des effets négatifs sur la fertilité et l'eclosion des oeufs. La longevité des femelles adultes de coccinelles a été reduite de 51% apres 12 jours d'alimentation de *M. persicae* nourri sur des pommes de terre exprimant la « GNA ». Les effets negatifs sur la biologie de reproduction des coccinelles a été reversible après une alimentation de 2-3 semaines sur la source optimale de puceron. Sont discutées ensuite les implications possibles dans l'évaluation des risques et les méthodes culturales de plantes transgeniques résistantes aux insectes, afin de minimiser les effets négatifs sur les ennemis naturels.

**Mots clés**: *Myzus percicae*, *Adalia bipunctata*, lectine, pomme de terre transgenique, interactions tritrophiques.

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*Breeding for Resistance to Insects and Mites*
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# The need for understanding mechanisms of resistance: the example of celery

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**Abstract:** *Apium graveolens* L. (var 'Conquistador'), the related species *A. chilense* (A073), *A. nodiflorum* (A289) and *A. prostratum* (A230), as well as an F1 hybrid between celery 'T.U. 52-75' and *A. prostratum* and 23 backcross lines obtained by crossing the F1 hybrid back to the celery parent were examined for putative resistance to *Liriomyza trifolii*. These lines were tested using a no-choice design, so that maximum feeding and oviposition could be expected. Numbers of feeding punctures, mines, pupae, and adults were counted.

Of these accessions/lines, several appeared to have good-excellent leafminer resistance; of particular note was 91A-25. Compared to all plants except the wild types, this line had fewer feeding punctures, allowed much less larval development, and no leafminer pupae developed.

In order to document if the observed resistance was caused by the carcinogenic and mutagenic linear furanocoumarins, the amounts of psoralen, bergapten and xanthotoxin present in both leaves and petioles of each accession were quantified using high performance liquid chromatography. Line 91A-25, which offered excellent leafminer resistance was found to have nearly 450 µg/g fresh weight of linear furanocoumarins. This is some 25 times the levels known to cause an acute dermatitis in humans. However, for lines with less than 30 g/g of furanocoumarins, regression analyses indicated that substantial resistance was not due to these undesirable compounds. Because of the related nature of the lines tested, our results provided an indication of the inheritance patterns of linear furanocoumarins which could be developed. The implications for use in a breeding program for *A. graveolens* are discussed.

**Key words**: *Liriomyza trifolii*, *Apium graveolens*, host plant resistance, linear furanocoumarins

## Introduction

The technique of host plant resistance to control insects has proven effective for many crops (Eigenbrode & Trumble, 1994). Nonetheless, the value of this approach in integrated pest management programs has been generally understated. Use of this technique has reduced pesticide inputs, improved worker health and safety, and minimized the potential for environmental contamination. As a result of these benefits, and a continuing interest in reducing pesticide use (Trumble, 1998), there has been a renewed interest in breeding plants resistant to both insects and diseases.

In spite of the obvious advantages, some scientists have serious concerns regarding the application of this technique (Ames & Gold, 1990). How often do we breed for insect or disease resistance without knowing the mechanism? How often do we report the chemical responsible, but have no idea of the potential toxicity to humans? In the case of celery, which can produce the

carcinogenic and mutagenic linear furanocoumarins, such an approach may be not only dangerous, but unethical and irresponsible.

The phototoxic linear furanocoumarins (Fig. 1) are known to cross link DNA strands, leading to skin cancer, developmental mutations in insects (presumably humans as well), and a variety of other oncogenic, teratogenic and carcinogenic responses (Diawara & Trumble, 1997). Responses are exacerbated by the presence of UV light (hence the term 'phototoxic'). They probably evolved as protection against several physical and biological stresses, including UV light, insects, and pathogens (see ref. in Diawara & Trumble, 1997). Critical concentrations for an acute exposure dermatitis are approximately 18 μg/g fresh weight (Austad & Kavli, 1983). For chronic exposure dermatitis, (celery handlers, grocery store employees), only 9 μg/g are required.

Our objectives were threefold. First, we wanted to select *Apium* lines for resistance to the leafminer, *Liriomyza trifolii*. Second, we wanted to determine if any resistance observed was based on the mutagenic and carcinogenic linear furanocoumarins. Finally, we wished to document the relationships between the various forms of linear furanocoumarins: does selection against one influence levels of another? Does selection for low concentrations in petioles affect levels in leaves?

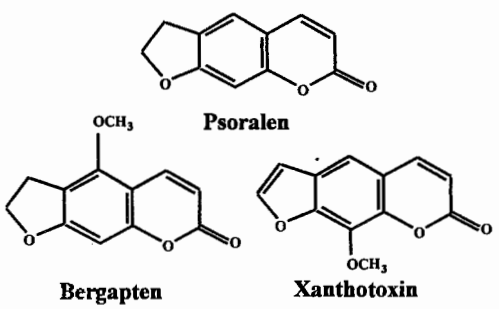


Fig.1 The phototoxic linear furanocoumarins in celery

## Methods

***Bioassay***

Because of: 1) the large number of plants, 2) the labor involved in the assays, and 3) a desire to minimize type 2 errors on post hoc tests, plants were split into four trials of 7 or 8 each. For purposes of comparison, each trial included a control accession (*A. graveolens* var 'Conquistador'). All accessions or lines were individually exposed to two mated pairs *of L. trifolii* for 2 hours; each accession/line was replicated 4 times within each trial. The data recorded or calculated included: number of feeding punctures/plant (as a measure of attractiveness); number of mines/plant (as a measure of attractiveness for oviposition); number of pupae/plant (as a measure of suitability for larval development); number of adults/plant (as a measure of suitability for complete development); the ratio of pupae/mine (as a measure of mortality in the larval stage); and the of adults/pupae (as a measure of mortality in the pupal stage). All insects from our laboratory colony were standardized by age. Plants were standardized to the extent possible by providing insects with equivalent leaf areas in which to oviposit.

*Furanocoumarin Analyses*
Two clones of each accession were split into leaf and petiole samples and prepared for analysis. The linear furanocoumarin contents were analysed using an HPLC with a reverse-phase column as described in Diawara *et al.* (1994) and Reitz *et al.* (1997). Specifically, we measured the μg/g fresh weight of psoralen, bergapten and xanthotoxin, the three most common furanocoumarins in celery (Diawara *et al.* 1994). We present data only for those accessions showing promise in the insect bioassays.

## Results and Discussion

Plant lines and accessions were initially chosen on the basis of impact on *L. trifolii* growth and development. The accessions showing the most promise for leafminer antixenosis and/or antibiosis were selected from Trials 1-4 (Fig. 2a-d). Fishers Protected LSD Test (not shown) was used to separate accession and line performance. Selected accessions/lines included 91A498-3, 5, 8, 9, 10, 12, 13, 16, 20, 23 and 25. Of particular note was 91A 498-25. Compared to all plants except the wild relatives of celery, this line had fewer feeding punctures, allowed much less larval development, and no leafminer pupae developed.

*Furanocoumain Analyses*
To determine if there was a consistent relationship between specific furanocoumarins in leaves and petioles, a simple regression was conducted for all combinations (excepting psoralen, which occurred in too few plants). These results are presented in Fig. 3.

Given that commercial celery often produces 30 μg/g of furanocoumarins in the leaves (Trumble, 1988, Diawara *et al.* 1994), and a concern that we not exceed the chronic dermatitis concentration of 9 μg/g in petioles (Siegelman *et al.*, 1987), the following accessions and lines were chosen as appropriate for additional immediate study: 91A498-8, 12, 13 and 23. However, those accessions with relatively high furanocoumarin contents (essentially above concentrations in current commercial celery) may still be suitable for further study, but would require additional backcrossing to celery and selection for furanocoumarin content reduction and improvement of horticultural traits. Results of the linear furanocoumarin analyses have been presented in Fig. 3.

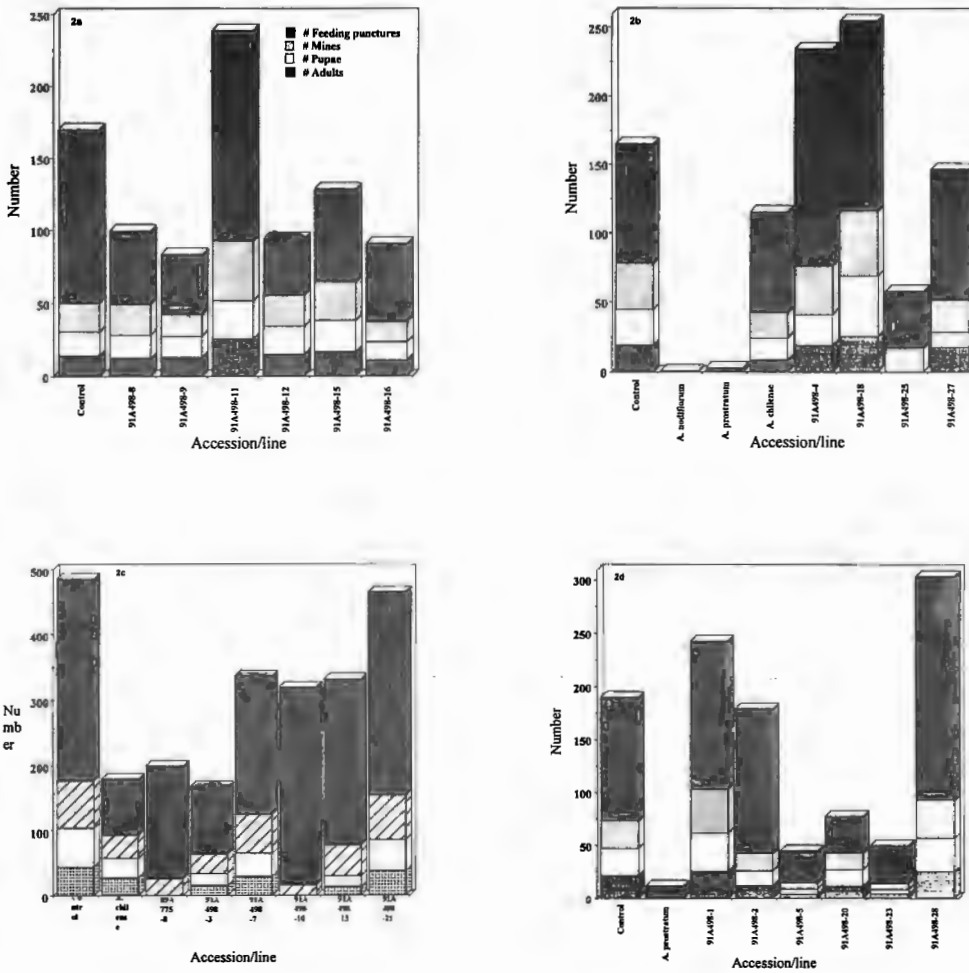


Figure 2. Trials 1-4 (2a, 2b, 2c, 2d) ,showing responses of *L. trifolii* of the best performing accessions and lines. The trials each included a control plant (*A. graveolens* var. 'Conquistador') and a wild relative (*A. chilense* or *A. nodiflorum*) to allow comparison on responses between trials

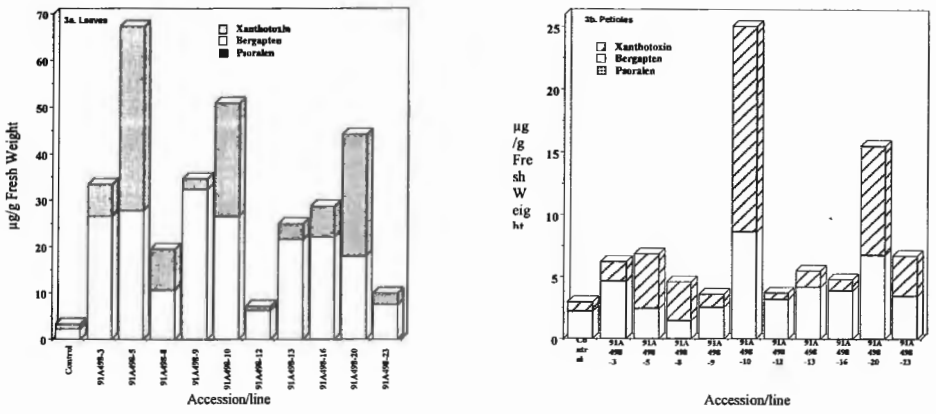


Figure 3. Linear furanocoumarin contents of leaves (3a) and petioles (3b) in selected accessions and lines of *Apium*. Line 91A-25 (not included in the figure), which offered excellent leafminer resistance, was found to have nearly 450 µg/g fresh weight of linear furanocoumarins. This is some 25 times the levels known to cause an acute dermatitis in humans.

Thus, even after additional backcrosses with *A. graveolens*, which would likely reduce the levels of linear furanocoumarins in the plants, the lines will need to be assayed for these undesirable compounds. Two additional caveats apply. First, the plants should be field tested to see if environmental conditions outside the greenhouse induce furanocoumarin production (see Diawara *et al.*, 1994). Second, the mechanism of resistance to leafminers for these lines is not known. Releasing a variety without knowing the mechanism for resistance assumes that there will be no consequences to the consumer. The consequences could be substantial of the potentially erroneous assumption that 'because resistance is not based on furanocoumarins the plants must be safe'.

## Résumé

La sélection classique des plantes par croisement a une longue histoire en ce qui concerne la lutte contre les insectes et les maladies. Sans conteste, les bénéfices pour l'humanité sont énormes. Néanmoins, beaucoup de scientifiques sont sérieusement inquiets de l'application de cette technique. Combien de fois sélectionnons nous des résistances vis à vis des insectes ou de maladies sans en connaître les mécanismes? Combien de fois impliquons nous les substances chimiques, mais sans avoir d'idée sur le potentiel de toxicité sur l'homme? Dans le cas du céleri une telle approche est non seulement dangereuse et irresponsable, mais également va à l'encontre de l'éthique scientifique.

*Apium graveolens* L. (var Conquistador), les genres voisins *A. chilense* (A073), *A. nodiflorum* (A289), et *A. prostratum* (A230), aussi bien que l'hybride F1 entre le célerie 'T.U. 52-75' et *A. prostratum* et 23 lignées obtenues par backcross en croisant l'hybride F1 au parent ont été

examinés en ce qui concerne la résistance putative vis à vis de *L. trifolii*. Ces lignées ont été testées en condition de non choix, situation dans laquelle on pouvait espérer le maximum d'alimentation et de ponte. Deux couples (mâle/femelle) de mouches par plante, ont été lachés pendant deux heures. Chaque test a été répété quatre fois avec trois plantes par répétition. Les piqûres de nourriture, les galeries, les pupes, et les adultes ont été dénombrés.

Parmi les croisements, plusieurs donnent une très bonne résistance au niveau des galeries; particulièrement la 91A-25. Comparée à toutes les plantes exceptées aux sauvages, cette lignée a moins de piqûres, perturbe nettement le développement larvaire et ne permet pas la formation des pupes.

Pour savoir si la résistance observée est due à des furanocoumarines linéaires carcinogènes et mutagènes, les taux de psoralène, bergaptène et xanthotoxine dans les feuilles et les pétioles de chaque croisement ont été mesurés par l'HPLC. La lignée 91A-25, qui est très résistante au niveau des galeries a presque 450 µg par g de matière fraîche de furanocoumarines linéaires. C'est 25 fois le taux connu qui cause une dermatite aiguë chez l'homme. Cependant pour les lignées qui ont moins de 30 g / g de furanocoumarines, des analyses de régressions indiquent que la résistance importante n'est pas due à ces composés indésirables. Nos résultats sur la nature des lignées testées, qui donnent une indication sur l'héritabilité des furanocoumarines linéaires pourraient permettre des développements. Les implications pour l'utilisation d'un programme de sélection d' *A. graveolens* sont discutées.

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# Larval development of Cacopsylla pyri (L.) [Homoptera : Psyllidae] on two resistant Pyrus genotypes

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**Abstract:** The development of *Cacopsylla pyri* larvae was observed on three *Pyrus* genotypes: cv. 'Katman' and NY 10355, identified as resistant to pear psylla, and cv. 'William's' pear as control. The study was conducted under controlled conditions, on trees grafted one year earlier. One psylla female was placed on each plant and removed after 50 eggs on average had been laid. To record *C. pyri* development, the number of different instars was counted each week on each plant.

On 'William's', larvae completed their development and adults started emerging after one month. Larval mortality was 68 %. On 'Katman' and on NY 10355, young larvae started feeding but all larvae died before completion of their development. In conclusion, under these experimental conditions, complete larval resistance to *C. pyri* was observed on 'Katman' and NY 10355.

**Key words:** *Cacopsylla pyri,* pear psylla, *Pyrus*, pear, host plant resistance.

## Introduction

*Cacopsylla pyri* is the most important insect pest in French pear orchards. Pear psylla produces copious amounts of honeydew that causes leaf necrosis and supports sooty moulds. This insect has developed resistance to different commonly used insecticides. Breeding for resistance to *Cacopsylla pyri* in pear trees offers an interesting alternative to chemical control.

Screening of 71 different genotypes of *Pyrus* has been done in field cages since 1996 at I.N.R.A. Beaucouzé and I.N.H., Angers, France. A dozen genotypes have been identified with a high level of resistance to pear psylla (not published). Two genotypes were selected for this study: 'Katman' and NY 10355. 'Katman' is a cultivar of *Pyrus communis* from Hungary. NY 10355 is a hybrid *Pyrus ussuriensis* x *Pyrus communis* from the United States (USDA). NY 10355 is known to be resistant to another psyllid : *Cacopsylla pyricola,* an important insect pest in American pear orchards.

## Material and methods

The development of *Cacopsylla pyri* larvae was observed on 'Katman' and NY 10355 in 1998. CV. 'William's', known to be sensitive to pear psylla, was the control. This study was conducted in a climatic chamber under controlled conditions: 12 h light / 12 h dark, temperature = 22 / 18°C, relative humidity = 65 / 85%. Trees, that had been grafted, one year earlier on *P. communis*

'Daytor-OHF 87', were grown in pots. Fourteen 'William's' twelve 'Katman' and fourteen NY10355 plants were used for this study.

One psylla female was placed on each plant within a sleeve of bridal veil. The female was removed after 50 eggs on average per plant had been laid (2 to 7 days). However, this value was exceeded on 'William's' because females tended to lay more eggs on that variety. At the beginning of the observations, an average of 76 eggs per plant were counted on 'William's', 53 eggs on 'Katman' and 46 eggs on NY10355.

To study larval mortality, the number of different instars was counted each week on each plant. However, it was difficult with a hand lens to distinguish between L.1 and L.2 and between L.3 and L.4. Therefore, the different instars were lumped into only four categories : young larvae (L.1 + L.2), intermediate larvae (L.3 + L.4), old larvae (L5) and adults.

## Results and discussion

Psylla development occurred normally on 'William's': the different instars appeared successively (Fig 1). The first adults started emerging after about one month and the latest emerged after about one and half months. Larval mortality was 68% and occurred mainly at the young larval stage (L.1 or L.2).

Larval development did not occur normally on 'Katman' and NY 10355. The results were similar for these two genotypes (Fig.). Eggs hatched, young larvae started feeding and produced small amounts of honeydew. Larval development then stopped : no larvae reached the 3rd instar. Larval mortality was 100% on both 'Katman' and NY 10355.

## Conclusion

Under the controlled environment conditions, complete resistance to *C. pyri* was observed in young plants of 'Katman' and NY 10355. All larvae died before completion of development. Feeding behaviour and movement of young larvae on these two genotypes is currently being studied. This should then indicate whether there is significant resistance occurring at the oviposition phase.

## Résumé

Le développement larvaire du psylle du poirier *Cacopsylla pyri* est étudié sur 2 *Pyrus* : Katman (*P. communis*) et NY10355 (*P. ussuriensis* x *P. communis*). Ceux-ci ont été identifiés comme étant résistants à *C. pyri* lors de criblages antérieurs. William's a été choisi comme témoin en raison de sa sensibilité. L'étude s'est déroulée en chambre climatisée sur 12 à 14 plantes. Les différents stades ont été dénombrés chaque semaine.

Sur William's les larves se développent et les adultes commencent à émerger au bout d'un mois. La mortalité larvaire est de 68 %.

Sur Katman et sur NY10355 les jeunes larves commencent à se nourrir et produisent de petites quantités de miellat. Le développement larvaire s'arrête ensuite, la mortalité larvaire atteint 100 %. Dans nos conditions expérimentales, Katman and NY 10355 sont donc totalement résistants à *C. pyri* ; toutes les larves meurent avant d'avoir terminé leur développement.

**Mots clés :** *Cacopsylla pyri*, développement larvaire, poirier, psylle, résistance.

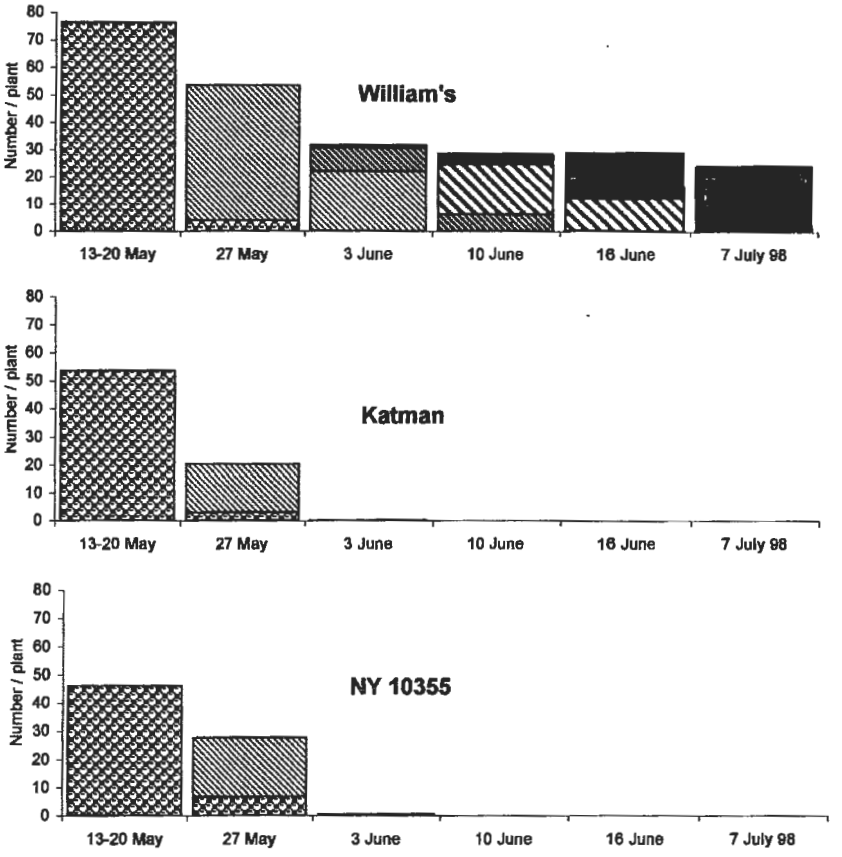


Fig. - Larval development of *Cacopsylla pyri* on William's, Katman et NY 10355 : average number of eggs, young larvae (L.1 + L.2), intermediate larvae (L3 + L.4), old larvae (L.5) and adults.

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