The possibility of *Nasonovia ribisnigri* resistance breaking biotype development due to plant host resistance: a literature study

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Abstract: After 15 years of breeding, the first lettuce, *Lactuca sativa*, variety with resistance to the lettuce leaf aphid *Nasonovia ribisnigri* was released in 1996. Many varieties with this insect resistance are now currently available. Due to selection pressure and a high degree of host specificity, the insect may evolve into a resistance breaking biotype. Comparable insect-host relationships are reviewed for possible similarities to the aphid-host relation as a gene-for-gene interaction. However the closest comparable aphid-host relation, aphid resistance to lettuce root aphid *Pemphigus bursarius*, has already survived for over 40 years. The sequential release of single resistance genes, combined with careful monitoring and testing of insect population biotypes found on different hosts, is the most effective gene deployment strategy for insect resistance in a host. The use of chemicals, refuge crops and the reduction of the alatae phase will slow down the development of the insect towards a resistance breaking biotype.

Keywords: *Lactuca sativa*, lettuce, leaf aphid, *Nasonovia ribisnigri*, host resistance, Nr insect resistance, insect pests, pest resistance, biotypes, lettuce root aphid, *Pemphigus bursarius*, resistance strategies.

Introduction

It took lettuce breeders of Leen de Mos, now Nunhems, 15 years of breeding to develop the *Nasonovia ribisnigri* lettuce aphid resistant lettuce variety Dynamite (Van der Arend, 1999). Since the release of this variety in 1999 many more Nasonovia resistant lettuce (Nr) varieties have been introduced by the breeding company Nunhems Seeds.

Four insect resistance mechanisms are commonly described. 1) Antibiosis: when aphids are allowed a choice to feed on plants of different lines, susceptible and resistant plants differ for rate of aphid increase. 2) Certain internal characteristics of a resistant plant may cause adverse effects on the insects that feed on it. The aphid migrates to another plant. 3) Tolerance: plants are able to repair the insect injury so that plant development is not reduced as a result of supporting an insect population living on it. 4) Antixenosis: the insect avoids the plant because it is an undesirable host due to certain plant characteristics (e.g. surface texture). The success of host plant resistance strategy will be challenged by the occurrence of resistance-breaking insect biotypes. Biotype refers here to a population of insects that damage plant varieties that are resistant to other populations of the same species.

Insects biotypes known in different crop pest systems

Several insect resistance breaking biotypes have evolved in other crops then lettuce. Twelve insect-host relations are used in this paper (Table 1).

Aphids in Lettuce

Peach-potato aphid (Myzus persicae) on several hosts including lettuce

Several clones of *M. persicae* showed very different levels of aggressiveness on lettuce. Differences between lettuce lines in aphid reproduction increased with increasing aggressiveness of the aphid clone, which means that aggressive clones are most effective for selection purposes. No evidence was found for clone-specific plant genotype reactions, suggesting that lines resistant to one clone will also be resistant to other clones of *M.persicae,* although not necessarily at the same level (Reinink, 1989).

Table 1. References used with several insect-host relations and existing biotypes.

Insect	Name	Host	Reference
Greenbug aphid	*Schizaphis graminum*	Wheat & Sorgum	Curvetto, 1998; Kindler, 1999 Porter, 2000; Kindler, 2001
Russian wheat aphid	*Diuraphis noxia*	Wheat	Kiriac, 1990; Shufran, 1997 Zsuzsa, 2001
Hessian fly	*Mayetiola destructor*	Wheat	Naber, 2000
Gall midge	*Orseolia oryzae*	Rice	Katiyar, 2000
Brown planthopper	*Nilaparvata lugens*	Rice	Heinrichs, 2001; Huang, 2001
Leaf midge	*Dasineura tetentsi*	Black current	Hellqvist, 2001
Large raspberry aphid	*Amphorophora idaei*	Raspberry	Jones, 2000
Phylloxera	*Daktulospaira vitifoliae*	Grapes	Martinez-Peniche, 1999 Omer, 1999
Rosy apple aphid	*Dysaphis plantaginea*	Apple	Rat-Morris, 1998
Woolly apple aphid	*Eriosoma lanigerum*	Apple	Young, 1982
White fly	*Bemisia tabaci*	Tomato	Nombela, 2001
Acyrthosiphon aphid	*Acyrthosiphon kondoi*	Alfalfa	Zarrabi, 1995

Lettuce root aphid (Pemphigus bursarius)

Lettuce root aphid, exhibits a host-alternating lifecycle, overwintering as eggs on the primary host plant (poplar) before migrating in summer to the secondary host plant, mainly annual *Compositae* including lettuce. A proportion of the population does not produce return migrants (sexuparae) in the autumn but remains in the soil and overwinters as asexual apterae, even after the annual plants have died in early winter. Overwintered asexual populations produce alatae in July, which are able to colonise other lettuce plants, indicating that they were not sexuparae. Clones can therefore persist indefinitely as both asexual apterae and alatae without the need to return to the poplar and undergo the sexual phase of the life cycle (Philips, 1999). Striking varietal differences in susceptibility to attack by the lettuce root aphid were first found in lettuces grown at Wellesbourne, UK in 1955. Subsequent work has confirmed that several varieties show differences in resistance. Immigrant winged forms of *P.bursarius* showed no preference for colonising any particular variety of lettuce and it seems that resistance to attack results from antibiosis (Dunn, 1960). Since then the single dominant root aphid resistance gene Ra, linked to the downy mildew resistance gene Dm6, is widely used by the lettuce breeders and is still active.

Lettuce leaf aphid (Nasonovia ribisnigri)

The lettuce leaf aphid has a comparable lifecycle as *P. bursarius*. It has a sexual phase in winter on the primary host *Ribes* (gooseberry, currants) and an asexual phase in summer on the secondary hosts lettuce and chicory and various wild plants. The fundatrix (basic ancestor), emerging from winter egg, feed on the primary hosts and by parthenogenesis and vivipary produces foundation colonies from which, in May and June, appear the winged aphids, which migrate, to the secondary hosts. The aphids then establish colonies, comprising individuals from several successive generations, which colonise neighbouring plants. In autumn, the sexuparous individuals appear, male and female, which migrate back to the primary hosts. Each mated female lays a winter egg on the primary host. In warm regions, overwintering may also occur on the secondary host (INRA, 2003). Resistance, governed by the same single dominant gene Nr, to this aphid was found in 1978 in several *Lactuca virosa* accessions. Different coloured clones, as an example of variability, are known but no clone-specific plant genotype reactions were found (Reinink, 1989).

Results

General

In this paper 15 insect biotype developments are evaluated (Table 2), described by many authors. Most of these evaluations support a gene for gene system independently of the resistance type dominant, recessive or both (mixed). No evidence is found that resistance-breaking biotypes are active in lettuce. For *M. persicae* differences in aggressiveness appears i.e. it is unlikely to be a gene-for-gene system.

Table 2. Biotype developments in several insect-host relations.

Insect	Name	Host	# Resistance Genes/type	# Breaking biotypes
Greenbug aphid	*Schizaphis graminum*	Wheat & Sorgum	Many/dominant	Many
Russian wheat aphid	*Diuraphis noxia*	Wheat	Several/	Several
Hessian fly	*Mayetiola destructor*	Wheat	27/mixed	Many
Gall midge	*Orseolia oryzae*	Rice	5/mixed	5
Brown planthopper	*Nilaparvata lugens*	Rice	10/mixed	Many
Leaf midge	*Dasineura tetentsi*	Black current	1/dominant	1
Large raspberry aphid	*Amphorophora idaei*	Raspberry	3/dominant	2
Phylloxera	*Daktulospaira vitifoliae*	Grapes	2	Several
Rosy apple aphid	*Dysaphis plantaginea*	Apple	1/dominant	1
Woolly apple aphid	*Eriosoma lanigerum*	Apple	1/dominant	Several
White fly	*Bemisia tabaci*	Tomato	1/dominant	1
Acyrthosiphon aphid	*Acyrthosiphon kondoi*	Alfalfa	1/dominant ca partial	1
Potato aphid	*Macrosiphum euphorbiae*	Lettuce (others)	Partial*	0
Peach-potato aphid	*Myzus persicae*	Lettuce (others)	Partial	0
Lettuce root aphid	*Pemphigus bursarius*	Lettuce	>2/dominant*	0
Lettuce leaf aphid	*Nasonovia ribisnigri*	Lettuce	1/dominant	0

* Known from other papers/research.

Conclusions

Insect population

- Monitoring the insect population biotypes is very important (Ratcliffe, 2001).
- Variation in host plant performance among populations of a phytophagus insect pest is a potential threat to the durability of host plant resistance. Virulent biotypes may overcome the protective properties of formerly resistant cultivars (Sardesai, 2000; Heinrichs, 2001).
- Insect populations from different parts of the world exhibit considerable biotypic variation (Shufran, 1997).
- Pest biotypes are host-based races (Martinez-Peniche, 1999; Omer, 1999).

- Biotypes of the insects are evolving as a result of selection pressure exerted by large scale growing of resistant cultivars (Kindler, 1999; Naber, 2000).
- The wide spread planting of one variety (monocrop) is decreasing the genetic diversity of a crop. As a result some insect species have overcome the resistance of certain varieties.
- Wild susceptible relative plants may also have a potentially important role in driving the development, and in harbouring unknown biotypes (Kindler, 1999).
- Obligatory sexual reproduction limits the development of possible resistant breaking lines.
- Aggressive clones, resulting in increased reproduction, are most effective for selection purposes in breeding programs (Reinink, 1989).

Genetic plant resistance
- If single genes govern the resistance to different biotypes a gene-for-gene interaction may be active is in place or likely to evolve (Jones, 2000).
- The sequential release of single resistance genes is equivalent to pyramiding resistance genes (Porter, 2000).
- The widespread use of one resistance gene is decreasing the genetic diversity of a host. As a result some insect species will break the resistance gene (Heinrichs, 2001).
- Resistant plant germplasm has geographical limits because of variation in agro-ecosystems of insect populations (Zsuzsa, 2001).
- Resistance genes that act by killing the insect are more selective towards a resistance breaking biotype.
- Resistance due to Antibiosis will put high pressure on biotype development.
- Resistance due to Antixenosis will put little pressure on biotype development.
- Different levels of combined resistance components exist in different lines.
- Complete tolerance will put no pressure on biotype development.

Discussion & Strategic proposals

General
- Resistance breaking biotypes are to be expected where a gene for gene interaction is found or anticipated.
- Resistance should be combined with cultural practices e.g. use of crop rotation to break the pest life cycle, remove or destroy plant debris, weeds or other sources of pest infestation.
- Chemicals give extra protection next to genetical, cultural, mechanical, biological and seasonal protection.
- Use of predators is not acceptable for lettuce. The consumer must tolerate insects and/ or predators in the vegetable plant product.

Insect population
- Monitor the insect population for biotype variation, before and after the deployment of resistant cultivars.
- Biotype testing will show variation between the insect populations especially populations collected from widely dispersed growing areas.
- Aggressive clones are most effective for selection purposes.
- Monitoring the resistant crops for emergence of resistance breaking biotypes.
- Use of susceptible cultivars for at least 20% of the growing area to offer refugees to the main avirulent biotype in the insect population. On these refugees the insect should be left alone or only treated with chemical insecticides (Sloderbeck, 1997 and Kerlin, 2002).

- Use of multiline cultivars or tolerant cultivars that minimises biotype selection is possible in some crops but not in lettuce because of production and quality issues.
- Inspect the wild plant relatives of the host for possible new biotypes.
- Reduction of males or females in the mating population.
- Removal of the winter host to reduce to limit the areas where the insects can survive winter conditions.
- Stimulating insect survival through sexual phase without a cloning alatae phase, will be less stimulating for new biotype development.
- Distract the insect by using winter host scent on the summer host.

Genetic plant resistance

- Genebank testing to find new sources with putative new resistance genes.
- Gene rotating where varieties with different resistance genes are used in different cropping seasons to minimise selection pressure on given resistance genes.
- Geographical deployment by planting varieties with different resistance genes in adjacent cropping areas.
- Use of cultivars with different insect resistance genes.
- Stimulate migration of the insect by using deterrent genes.
- Use of cultivars with different types of insect resistance genes.
- Develop horizontal resistance, a type of resistance that is expressed equally against all biotypes by combining several resistance components.
- Use of tolerant varieties. The consumer must tolerate insects in the vegetable plant product. This is not acceptable for lettuce. It may be acceptable in e.g. potatoes.

A strategy to prevent the evolution of *Nasonovia ribisnigri* resistance breaking biotypes

N. ribisnigri and *Pemphigus bursarius* can avoid the ecological dead-end that would occur through local path extinction. Clones can indefinitely overwinter as both asexual apterae and alatae without the need to return to the winter host and undergo the sexual phase of the lifecycle.

Immigrant winged forms of *N. ribisnigri* and *P. bursarius* show no preference for colonising any particular variety of lettuce and it seems that resistance to attack results from antibiosis after landing on the secondary host.

The leaf aphid *N. ribisnigri* depends on lettuce to survive and a resistance breakdown can certainly not be ruled out. *N. ribisnigri* has long phases in its life cycle with many cloning parthenogenic phases and aphids are not killed by the resistance gene but forced to migrate to susceptible plants (migration is the only diminishing biotype development factor). When a greater area is planted with Nasonovia resistance lettuce varieties possessing the gene Nr, with antibiosis as the mode of resistance, biotype development is stimulated.

Therefore it is to be expected that eventually Nasonovia biotypes will develop (Baenziger, 2001). Especially if the Nr gene is not ‘protected’ by combining its use with other means of control.

Several means should be used to protect the resistance gene and keep it effective as long as possible.

- Chemical control. Growers should always use chemicals in a Nasonovia resistant crop twice. The first time when plants start heading and the second time 10 days before harvesting. In this way 2 objectives are reached. 1) Possible new biotypes of Nasonovia are killed and 2)

The harvested lettuce head will be clean of aphids. Not using any chemicals means an attack on the endurance of the resistance gene.

- Monitoring. Attention should be paid to growers that use Nasonovia resistant varieties. Special care should be taken when complaints emerge towards aphids found in a resistant variety. Is Nasonovia the attacking aphid? Is the lettuce variety/plant Nasonovia resistant?
- Resistance breeding. If a new biotype of *N.ribisnigri* is likely to occur the breeding program needs to start searching as soon as possible for a new resistance source (gene).

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