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Project leaders:	**G.M. Tatchell** **HRI Wellesbourne** **Warwick** **CV35 9EF** **I. Denholm** **IACR-Rothamsted** **Harpenden** **Herts AL5 2JQ**
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Key Workers:	**N Kift (HRI Wellesbourne)** **M Barber (IACR-Rothamsted)** **G Moores (IACR-Rothamsted)**
Location:	**HRI Wellesbourne and IACR-Rothamsted**
Project Co-ordinator:	**Mr David Barney, Bourne Salads Ltd., Tunnel Bank, Cherryholt Road, Bourne PE10 0DJ** **Mr Ian Gillot, Ian Gillot Consultancy Services, 26 Sandhurst Road, Wokingham RH40 3JD**
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3. PRACTICAL SECTION FOR GROWERS

Objectives and Background

Owing to their outstanding efficiency, insecticides exert intense selection for insect pests genetically adapted to tolerate greater exposure than others. This may arise from an improved ability to detoxify insecticides, or differences in the sensitivity of insecticide target sites. Under selection, such individuals increase gradually in frequency in populations to a point at which control treatments are less effective than before and eventually fail. Indeed, reports by growers and advisors of insecticide treatments losing efficiency, or requiring shorter intervals between applications, often provide the first suspicions of resistance.

Resistance to insecticides in the currant-lettuce aphid, *Nasonovia ribisnigri*, has recently been confirmed in laboratory assays of samples from the UK (FV 210). This follows its detection in southern France and Spain (Rufingier *et al*., 1997) though no further research on its incidence or management is being done in southern Europe (N. Pasteur pers. comm.). The implications of these laboratory results for the control of this pest in the field require urgent attention.

The peach-potato aphid, *Myzus persicae*, also occurs commonly on lettuce, as well as a number of other crops, and has been known for many years to be resistant to insecticides both in the UK and elsewhere.

The HDC Salads R&D Group identified effective aphid control as their top priority for research and development. The present work will result in improved crop protection strategies by providing a scientific basis to assist with the choice of insecticides that in turn should prevent any further increase in chemical usage, and may even reduce the number of treatments applied. This in turn will improve the environmental acceptability of control strategies and improve product quality.

Lettuce crops receive on average five applications of insecticide (MAFF Pesticide Usage Survey for 1995), though those grown in mid-summer, or where the recently introduced seed treatments of imidacloprid are used are likely to have different treatment regimes. There is currently a very limited number of insecticides available for the control of aphids on lettuce, and this could conceivably diminish further as a consequence of a current review on the use of organophosphate (OP) insecticides in UK agriculture and horticulture. At present, however, the outcome of this review is uncertain.

The unrestrained use of insecticides without taking account of the levels and mechanisms of resistance present within aphid populations could accelerate the development of resistance leading to the complete loss of effective chemicals, including the recently introduced imidacloprid seed treatment. There are also few new insecticides likely to become available for aphid control on lettuce in the near future. Two potential candidates, acetamaprid (Aventis; a foliar rather than systemic insecticide) and thiamethoxam (Syngenta), act at the same target site as imidacloprid, raising the possibility of cross-resistance between all three products. Excessive reliance on imidacloprid as a seed treatment could therefore threaten the efficacy of these chemicals also. Further escalation of resistance in *N. ribisnigri*

should be avoided at all costs, through management of resistance to insecticides both available currently and those that may be introduced in the future, if growers are to continue to produce quality lettuce free of aphids that meets the demands of processors and retailers.

In response to these concerns, HDC have commissioned a new three year project (FV 210a) to investigate further the incidence, implications and management of insecticide resistance in UK populations of *N. ribisnigri*. The report summarises results achieved during the first phase of FV 210a and outlines work planned during the remainder of the project.

Summary of Results

In the laboratory, bioassays were conducted on eight field populations of *N. ribisnigri* sent to Rothamsted from around the UK during 1999. The main bioassay used against *N. ribisnigri* entailed placing winged adults on leaf discs cut from lettuce that had previously been dipped into insecticide solutions and allowed to dry. Mortality was scored three days later. The compounds tested were cypermethrin, pirimicarb, heptenophos and imidacloprid. As in project FV 210, the 'ROTH' (now referred to as Nr1A) population of *N. ribisnigri*, originating from HRI Wellesbourne and subsequently cultured at Rothamsted, was used as a fully susceptible reference strain. The *N. ribisnigri* strain 1312 (now referred to as Nr2A), shown to exhibit c.10-fold resistance to pirimicarb in project FV 210, was used as a resistant reference strain for laboratory bioassays and in the field experiments reported later. Each of these populations has now been established as a clone derived from a single female, in order to eliminate unwanted genetic variation and ensure the consistency of their responses over time.

The results of the bioassays demonstrated the presence of four distinct modes of resistance in the field strains tested: (1) fully susceptible populations; (2) pirimicarb-resistant populations; (3) pyrethroid-resistant populations and (4) populations resistant to both pirimicarb and pyrethroids. This indicates that there are at least two mechanisms of resistance present in the field: one conferring resistance to pirimicarb and one to pyrethroids. There was little or no evidence of resistance to the OP, heptenophos, or the neonicotinoid, imidacloprid. Three of the field populations exhibiting responses of interest have since been cloned, and work is underway to investigate the cross-resistance patterns in more detail.

Biochemical tests re-confirmed an association between resistance and a highly active esterase enzyme, analogous to but not identical to that implicated in insecticide resistance in *M. persicae*. Preliminary investigations have shown the increase in esterase activity to be caused by an increase in production of an existing esterase rather than the presence of a new one.

A rapid biochemical test for diagnosing the presence of the esterase in individual aphids would be a valuable tool for resistance management. Attempts to develop such an assay based on measurements of total esterase activity were only partly successful. While the majority of susceptible and resistant individuals can be distinguished by this means, the method is not 100% reliable. Further work is now

being undertaken to develop a rapid diagnostic test in the form of an immunoassay specific to the esterase implicated in resistance.

None of the strains examined showed evidence of an altered target-site (so-called MACE) mechanism that has been reported for *N. ribisnigri* from southern Europe, and which is currently spreading through the UK in *M. persicae*. This mechanism has the potential to confer total immunity to pirimicarb and triazamate.

The implications of resistance for the field performance of insecticides were investigated in a field experiments at HRI Wellesbourne. Pirimicarb, deltamethrin and heptenophos all reduced significantly the numbers of both susceptible (Nr1A) and resistant (Nr2A) *N. ribisnigri*, as compared to untreated controls, two days post treatment. There was no difference in the reduction in aphid numbers achieved by any of the insecticides. Six days post treatment all three insecticides significantly reduced the number of *N. ribisnigri* as compared to the untreated control irrespective of the aphid development stage examined. However, pirimicarb tended to be less effective against the resistant aphids (Nr2A), and significantly less effective when adults or young nymphs alone were examined. Similarly, residues of pirimicarb were less effective against resistant than susceptible aphids one and two days after the insecticide had been applied. These findings imply that the relatively low levels of pirimicarb resistance detected in UK populations to date reduce the residual life of pirimicarb rather than its initial efficacy as a foliar spray.

Action Points for Growers

Based on results to date and experience with other pests, we can advance the following action points and tentative recommendations.

- Growers should be aware that difficulties with controlling aphids on lettuce may relate to resistance to insecticides in *M. persicae, N. ribisnigri* or (conceivably) both species

- Suspected cases of resistance should be reported immediately to advisors or technicians, who should in turn seek specialist advice if deemed necessary. If *M. persicae* is implicated, insects can be sent to Rothamsted for rapid characterisation of resistance status. Within project FV210a, Rothamsted can accommodate limited testing of *N. ribisnigri* as well.

- Make every effort to apply insecticides according to agreed recommendations, ensuring correct timing and maximum coverage of plants. Without specialist advice, do not deviate from recommended application rates as this will not assist with defeating resistance and could exacerbate the problem.

- Never follow up a suspected control failure with a repeated application of the same product. At present there is no evidence for a consistent association between resistance to pirimicarb and pyrethroids in *N. ribisnigri*. Thus, for the time being it appears possible to alternate these insecticide classes. OPs appear less strongly resisted than pirimicarb but may be affected by the same mechanism, ie. there is a danger that OP use will select for pirimicarb resistance. If so, it would be preferable to use OPs after rather than prior to the use of

pirimicarb. Imidacloprid still appears unaffected by resistance but, due its high persistence, constitutes a major resistance risk in both *N. ribisnigri* and *M. persicae*. Suspicions of imidacloprid failing should be reported immediately to advisors or the manufacturer. Rothamsted has the facilities to confirm or refute resistance to imidacloprid in either species.

Practical and Financial Benefits from Study

The cost-benefits of this research are considerable. The value of the UK lettuce crop is £64.3 M (MAFF Basic Horticultural Statistics for the UK). The widespread development of resistance to insecticides could make it impossible to grow lettuce in the UK that meet the quality standards of freedom from aphid infestation demanded by processors and retailers so leading to the total collapse of the industry. However, through knowledge and management of resistance, the life of available insecticides will be prolonged and time will also be provided for the development of alternative control strategies (aphid resistant varieties, biological control, aphid behavior modifying chemicals etc.).

The industry would benefit from more detailed knowledge of the extent and level of resistance to insecticides in aphid populations that infest lettuce in the UK and so be better informed when selecting chemical control agents to ensure that they will be effective and will not exacerbate the problems of insecticide resistance. Such knowledge should also assist with identifying needs and opportunities for introducing new insecticides that could contribute to resistance management strategies.

The industry would benefit from the development of tools for rapid detection of resistance to insecticides in aphid populations and hence guide the selection of control agents and the preservation of existing products. These monitoring techniques could be implemented at a central location, or could equally be conducted by trained personnel (eg. pest management advisors) in regional laboratories.

Improved attention to resistance management will avoid the environmental impact of applying ineffective products.

Consumers will benefit by having quality produce to which the minimum of insecticides has been applied to achieve effective aphid control.

4. SCIENCE SECTION

4.1. INTRODUCTION

The effective control of aphids on the foliage of outdoor lettuce is essential to ensure the marketability of this crop. In the UK, lettuce is colonised by a complex of aphid pests including the currant-lettuce aphid, *Nasonovia ribisnigri* (Mosley), the peach-potato aphid, *Myzus persicae* (Sulzer), and the potato aphid, *Macrosiphum euphorbiae* (Thomas). *Nasonovia ribisnigri* is often the major pest and is much more specific to lettuce than the other two species, which attack several other crop and non-crop plants (Ellis *et al.*, 1995). On ecological grounds, *N. ribisnigri* is therefore a primary candidate for the selection of resistance to insecticides, which remain the mainstay of its control in the UK and elsewhere in Europe.

In contrast to *M. persicae*, for which the incidence and causes of resistance are well documented (reviewed by Field *et al*, 1997), little has been reported regarding the status of resistance in *N. ribisnigri*. Published bioassay data from project FV210 relating to UK field populations of *N. ribisnigri* showed widespread but varied levels of resistance to pirimicarb and lower, varied levels of resistance to pyrethroids and organophosphates (Barber *et al*., 1999). Resistance was correlated with an intensely-staining esterase band disclosed by polyacrylamide gel electrophoresis (PAGE) but no direct link was established. Bioassays conducted in France with field strains originating from southern France and Spain exhibited a maximum of 12-fold resistance at LC_{50} to the organophosphate (OP) acephate and 660-fold to the cyclodiene endosulfan (Rufingier *et al.*, 1997). Maximum levels of resistance to the pyrethroid deltamethrin (28-fold) and the carbamate pirimicarb (19-fold) were intermediate to these extremes. Laboratory selection experiments using French field populations of *N. ribisnigri* have since shown that endosulfan resistance can result from glutathione-S-transferase detoxification, and pirimicarb resistance from modified acetylcholinesterase (MACE) (Rufingier *et al.*, 1999).

The present study was initiated following the confirmation of insecticide resistance in *N. ribisnigri* in the UK (project FV210) and the growing need to control the problem. We report here on bioassays conducted with representative chemicals from relevant insecticide classes against two laboratory strains, including one considered likely to exhibit baseline susceptibility to the chemicals, and eight field strains. Confirmation of the correlation between elevated esterases and resistance, and screening for modified acetylcholinesterase (AChE, the target site of OPs and carbamates) was undertaken to gain information on resistance mechanism(s) in this species. The implications of resistance were investigated by evaluating the efficacy of insecticide treatments against susceptible and resistant clones in field and glasshouse experiments.

4.2. MATERIALS AND METHODS

Aphid strains and rearing methods

The two laboratory 'standard' clones maintained in culture throughout the study are: Nr1A, a clone derived from the long-standing susceptible 'ROTH' strain initially established at HRI Wellesbourne in 1994 and transferred to Rothamsted in

1995; and Nr2A, a clone originating from '1312', collected in 1997 from a site in Kent experiencing control problems with pirimicarb (Barber *et al*., 1999).

Eight field strains collected during 1999 from the north (2 strains), west (1 strain), south (2 strains), east (2 strains) and southeast (1 strain) of England were tested with one chemical from each of four chemical classes: cypermethrin (pyrethroid), heptenophos (OP), pirimicarb (carbamate) and imidacloprid (neonicotinoid). These were initially all cultured at Rothamsted where, after bioassays, they were either discarded or cloned depending on the results obtained.

All strains of *N. ribisnigri* were reared parthenogenetically in the laboratory on whole plants of *Lactuca sativa* c.v. 'Webb's Wonderful', without exposure to insecticides, at 21ºC with a 16:8h (L:D) photoperiod. Plants were changed regularly and new ones re-infested to avoid host plant deterioration and excessive crowding of aphids.

Insecticides

Formulated insecticides used for leaf-dip bioassays were cypermethrin ('Cythrin', 200g/l EC); heptenophos ('Hostaquick', 55g/l EC); and pirimicarb ('Aphox', 500g/kg SG) (soluble granules). For leaf-dipping, all formulations were diluted to the required concentration in distilled water containing 0.01% 'Agral' (Zeneca Agrochemicals), a non-ionic surfactant added to improve leaf-wetting and to compensate for the loss of formulant at low insecticide concentrations. Imidacloprid was applied topically as technical material (>99% purity; Promochem Ltd.) diluted to the required concentrations in acetone.

Bioassays

Leaf-dip bioassays Leaf discs (35mm diameter) cut from lettuce (*Lactuca sativa* cv. 'Webb's Wonderful') were dipped in insecticide solution for 20s, placed upside down on an agar bed (25mm in depth) in disposable plastic containers (30mm high), and allowed to air-dry. Alate adult *N. ribisnigri* of the required strain (10 per container) were placed on the treated leaf surface and confined by applying a ring of fluon to the exposed lip of the container. Leaf discs dipped in water plus Agral were used as controls. Bioassay containers were covered with a fine mesh lid and stored upright in a constant environment facility at 20ºC under ambient daylight conditions.

Topical application bioassay For bioassays with imidacloprid, alate adults were placed on untreated leaf-discs in containers (10 per container) as described above, and dosed individually with a 0.25µg droplet of insecticide in acetone, with acetone alone used as a control. Treated aphids were stored as described above.

Design and analysis of bioassays Dose-response bioassays against the two standard strains were conducted using three batches of aphids (i.e. 30 insects) at at least five insecticide concentrations. Each bioassay was conducted at least twice in order to confirm the responses recorded in project FV210. Adults incapable of co-ordinated movement of legs (after gentle prodding if necessary) were scored as dead. All bioassays were scored at intervals 48h and 72h following initial exposure to insecticide. 1999 field strains were tested at least once, over 2 - 4 concentrations with two batches of 10 alate adults per concentration in order to compare responses to 'indicator' chemicals and identify strains of interest. The latter were tested

further using three replicates and up to 6 concentrations. Owing to this low number of insects and the possibility of genetic heterogeneity within strains, no attempt was made to fit probit lines to these data.

Polyacrylamide gel electrophoresis (PAGE)
Electrophoretic patterns of non-specific esterases in individual aphids after homogenisation in sucrose/Triton X-100 (1.6%) were analysed using 7.5% polyacrylamide gel slabs containing 0.2% Triton X-100 and a discontinuous buffer system (Davis, 1964) run at 250V for 1.5h. Gels were rinsed in distilled water for 30min then stained in 0.2M phosphate buffer, pH 6.0, containing 5mM Fast Blue RR, 1% acetone and 0.6 mM 1-naphthyl acetate, then fixed and stored in 7% acetic acid.

Radiolabelling 10 aphids of Nr1A and Nr2A were homogenised in 25µl sucrose/Triton X-100. The mass homogenate was centrifuged at 1100g for 10 s and the supernatant taken. 10µl of each supernatant was run on an Ornstein-Davis gel as described above. 2µl diisopropylfluorophosphate (DFP) was added to the remaining 15µl of each supernatant and centrifuged at 1100g for 10 s. After a 30 min incubation at 24ºC, 10µl of each supernatant was run on an Ornstein-Davis gel as described above. After rinsing in distilled water for 30 min, the gel was soaked in 1M sodium salicylate solution for 30 min. The gel was then dried for 3hrs and placed next to X-ray film (Fuji medical X-ray film) at -80ºC for 5 weeks before developing.

Total esterase assays
Total esterase assays were initially based on the methods of Grant *et al*. (1989). Single aphids were homogenised in 20µl phosphate buffer (pH 7.0, 0.02M, containing 0.1% Triton X-100) in individual wells of a 96-well microplate. A separate sample of the same buffer containing 1.5mM Fast Blue RR salt was filtered, and 1-naphthyl acetate in acetone was added to give a final substrate concentration of 1mM. 200µl of this was added to each homogenate and mixed. Reactions were monitored for 10 min at a wavelength of 450nm using a Molecular Devices Thermomax kinetic plate reader.

Subsequent total esterase assays using the fluorometric substrates carboxyfluorescein diacetate (CFD) and 7-acetoxy-4-methylcoumarin (AMC) involved homogenising single aphids (CFD) or a 5% fraction of a single aphid (AMC) in 10µl phosphate buffer (pH 7.0, 0.02M, containing 0.1% Triton X-100) in individual wells of a 96-well opaque microplate. Substrates in acetone were added to a separate sample of the pH7 buffer to give a final substrate concentration of 2µM (CFD) or 1mM (AMC). 190µl of the appropriate substrate was added to each homogenate and the reactions monitored for 30 min. Both assays were read using a Perkin Elmer Luminescence Spectrometer using excitation wavelengths of 485nm (CFD) or 260nm (AMC) and emission wavelengths of 530nm (CFD) or 472nm (AMC).

AChE assays
Assays characterising the sensitivity of AChE to pirimicarb and heptenophos were based on the methods of Moores *et al*. (1988) for rapid diagnosis of AChE sensitivity in single insects from field populations.

Diagnosis of AChE insensitivity to pirimicarb in individual aphids Single aphids (8 per field strain) were homogenised in 20µl phosphate/Triton buffer (pH 7.5, containing 0.1M phosphate and 0.1% Triton X-100) in separate wells of a 96-well microplate, and left for 30 min at 4ºC to enhance AChE solubilisation. Buffer (100µl), homogenate (50µl) and DTNB (50µl) were equilibrated in a fresh microplate, using duplicate samples of homogenate to give an uninhibited control synchronised with one inhibition reaction. Assays were started by the addition of ATChI in buffer (100µl) with and without a diagnostic concentration of pirimicarb, to give a final substrate concentration of 0.5mM, a final DTNB concentration of 15µM and a final pirimicarb concentration of 10^{-5}M. Assays were monitored for 20 min by a Thermomax microplate reader (Molecular Devices), utilising SOFTmax software that subsequently fitted linear regressions to successive absorbence readings taken at 405 nm from each well.

The same assay method was used to characterise the sensitivity of AChE to heptenophos in Nr1A and a field strain exhibiting the highest level of heptenophos resistance. However, rather than a single diagnostic concentration of heptenophos, a serial dilution ranging over seven, 3-fold dilutions from a final concentration of 1mM to 0.46µM was administered to eight individual aphids from both strains.

Field efficacy of insecticides against resistant and susceptible *N. ribisnigri*
A field experiment at HRI Wellesbourne aimed to determine the survival of clones of *N. ribisnigri,* known to be resistant or susceptible to pirimicarb, exposed to field-rate applications of pirimicarb, deltamethrin or heptenophos.

Plant material Lettuce, cv. Saladin, was sown on July 1 1999 in modules and grown in a screened glasshouse until being planted in the field on 22 July 1999. Plants were spaced 30 cm apart in rows 45cm apart. Each plot was two rows wide and 3m long and comprised 20 plants. Each plot of 20 plants was covered with an insect-proof cage the day following planting. Four days after planting, those plants that had failed to establish were replaced. In total 48 plots of 20 plants were planted and covered.

Clones of *N. ribisnigri* The two clones of *N. ribisnigri* used in this experiment were the susceptible Nr1A and the pirimicarb-resistant Nr2A whose integrity had been confirmed previously at Rothamsted by electrophoresis. Each clone was transferred to HRI Wellesbourne, and numbers bulked up, on lettuce cv. Saladin in separate buildings to avoid contamination between clones.

Infestation of plants Aphids from stock culture plants were transferred to pieces of lettuce leaf in a Petri dish. Each piece of lettuce was infested with approximately 10 *N. ribisnigri* of either Nr1A or Nr2A. The cage was removed from an individual plot, a piece of *N. ribisnigri*-infested leaf was inoculated onto each plant in the cage and then the plot was immediately re-covered. These aphids were allowed to establish for five days before the first sample was taken and plots treated with insecticide.

Insecticide treatments Treatments were applied with a knapsack sprayer as follows:
A. Untreated control
B. Pirimicarb at 0.5 g/l at an application rate of 600 l/ha
C. Heptenophos at 840 ml/ha at an application rate of 600 l/ha
D. Deltamethrin at 250 ml/ha at an application rate of 600 l/ha

Each cage was removed for insecticide application and then replaced. The total spray volume used for each treatment was 4 l.

Assessment of aphid numbers Samples of six plants were taken from each plot two days before treatment, and two and six days after treatment with insecticide. Plants were placed individually in labelled bags and returned to the laboratory where all aphids present were counted. Aphids were identified as adults, old nymphs (3^{rd} or 4^{th} instar) or young nymphs (1^{st} or 2^{nd} instar).

A sub-sample of adult aphids from the untreated control plots from the plants collected six days after insecticide treatment were tested at Rothamsted to confirm their identity by electrophoresis.

Experimental design and statistical analysis The experiment was a randomised block design with six replicates. Two adjacent plots of 20 plants, one infested with Nr1A and the other with Nr2A, were treated as paired plots in a split plot design, so giving a total of 48 cages.

Counts (totals from six plants per plot) were analyzed within a generalized linear model, using a log-linear model assuming a Poisson error structure. The pre-treatment counts were used as a co-variate to adjust for any differences in aphid numbers between plots prior to insecticide application. The log-linear analysis ignored the split-plot design to simplify interpretation. This may influence the significance of the effect of insecticide or aphid clone within the analysis. However, as the response of interest related to the aphids inoculated onto plants rather than the plants themselves, it was reasonable to assume that any effect on significance was minimal.

Persistence of insecticide residues against resistant and susceptible *N. ribisnigri*

Sixty 18-day old lettuce plants, cv. Saladin, (sown 13 October 1999 and treated on 1 November 1999) were sprayed using a knapsack sprayer with one of the four following treatments:
A. No insecticide
B. pirimicarb at 0.5 g/l at 600 l/ha,
C. deltamethrin at 250 ml/ha at 600 l/ha
D. heptenophos at 840 ml/ha at 600 l/ha

These plants were then transferred to a cold frame and covered at night to protect plants from frost. Twelve plants from each of the four treatments (untreated and 3 insecticides) were arranged in five randomised blocks in the cold frame to account for any spatial variation in weathering.

Immediately after treatment (day 0) and then one, two and four days after treatment, ten plants of each treatment (2 from each of the 5 blocks in the cold frame) were taken into a heated glasshouse with supplementary lighting (12-24°C, 16:8 L:D). Of these ten plants, five were inoculated with ten adult winged pirimicarb-resistant *N. ribisnigri* (Nr2A) and five were inoculated with ten adult winged susceptible *N. ribisnigri* (Nr1A). All aphids were confined on the underside of one leaf in a single clip cage. The numbers of aphids alive and dead in each clip cage were recorded after 48 hours and mortality was expressed as the proportion of aphids that were recovered that were dead. This proportion was arcsin transformed for analysis of variance.

4.3 RESULTS

Screening of field strains

Bioassays Responses of the field strains varied considerably. However, four different modes of resistance could clearly be distinguished between the eight populations. These were: (1) no resistance, ie. overall responses similar to those of Nr1A; (2) pirimicarb resistance of a similar level to that identified in Nr2A in project FV210; (3) cypermethrin resistance at levels both equal and greater than that seen in some field strains in project FV210, and (4) both pirimicarb and cypermethrin resistant individuals. Figure 1 shows the variability seen in the responses of laboratory and field clones to cypermethrin. In comparison, responses to heptenophos and imidacloprid were relatively homogeneous and yielded little or no evidence for resistance to these chemicals.

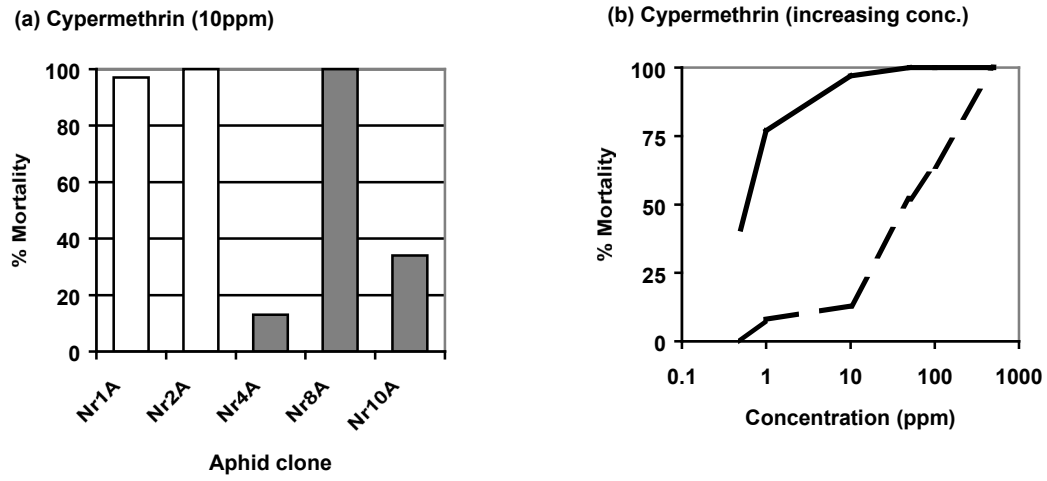

Figure 1. (a) Percentage mortality at 10ppm cypermethrin of the two laboratory clones (clear bars) and three clones derived from field populations (shaded bars). (b) The response of the laboratory susceptible clone Nr1A (solid line) and the field clone Nr4A (dashed line) to an increasing concentration of cypermethrin.

AChE screening As with standard strains, a discriminating concentration of 10^{-5}M pirimicarb and a range of concentrations of heptenophos disclosed no evidence of MACE-type resistance to the chemicals in individuals from field strains.

Esterase banding Strains exhibiting insecticide resistance consistently showed a heavily staining esterase band on gels treated with 1-naphthyl acetate (figure 2). Preliminary results with radiolabelled gels suggested that the elevation of esterase activity in the resistant aphids was due to an over production of the esterase rather than the presence of an mutant, more active form of the esterase.

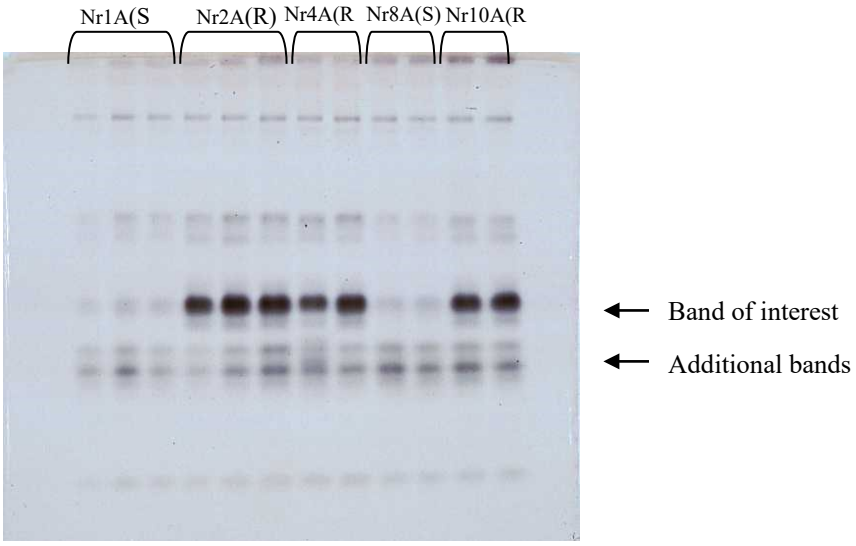

Figure 2. Esterase banding patterns in five strains of *Nasonovia ribisnigri* showing both the esterase associated with resistance and additional bands with a stain intensity which is equal between strains (S, susceptible R, resistant).

Total esterase assessment

Levels of esterase activity using the substrate 1-naphthyl acetate in Nr1A and Nr2A did not differ sufficiently enough to provide an accurate diagnostic technique for screening field populations. The gel shown in figure 2 suggests that additional esterases present in equal amounts in all of the aphids run on the gel may contribute to the incomplete separation of susceptible and resistant aphids. The fluorometric substrate CFD appeared to bind to a different esterase from that of interest and AMC appeared to bind to the aphid AChE. Thus, neither substrate proved sufficiently specific to diagnose differences in the esterase implicated in resistance.

Field efficacy of insecticides against resistant and susceptible *N. ribisnigri*

Electrophoresis of aphids on each plot at the end of the field experiment indicated that susceptible (Nr1A) or resistant (Nr2A) clones were present as expected. There were two exceptions in which cages infested with susceptible aphids had one or two aphids exhibiting a dark band, suggesting they were resistant. The confinement of individual clones within the cages was generally very effective.

All three insecticides reduced significantly the numbers of both susceptible (Nr1A) and resistant (Nr2A) *N. ribisnigri* two days post treatment irrespective of the aphid developmental stage assessed (Table 1a). There was no difference in the reduction in aphid numbers achieved by any of the insecticides.

Six days post treatment, all three insecticides again reduced significantly aphid numbers compared to the untreated control irrespective of the aphid development stage examined. However, pirimicarb tended to be less effective against the

resistant aphids (Nr2A), and significantly less effective when adults or young nymphs alone were examined (Table 1b).

Table 1a The proportional reduction in the numbers of *N. ribisnigri,* compared to untreated controls, two days after treatment with insecticides

Aphid development stage	Insecticide	Aphid clone		Significance (S vs R)
		Susceptible (Nr1A)	Resistant (Nr2A)	
Total aphids	deltamethrin	0.787	0.825	n.s.
	pirimicarb	0.757	0.778	n.s.
	heptenophos	0.734	0.711	n.s.
Adults and large nymphs	deltamethrin	0.789	0.788	n.s.
	pirimicarb	0.807	0.732	n.s.
	heptenophos	0.724	0.755	n.s.
Adults only	deltamethrin	0.783	0.811	n.s.
	pirimicarb	0.773	0.746	n.s.
	heptenophos	0.744	0.817	n.s.
Small nymphs	deltamethrin	0.785	0.850	n.s.
	pirimicarb	0.716	0.811	n.s.
	heptenophos	0.741	0.679	n.s.

Table 1b The proportional reduction in the numbers of *N. ribisnigri,* compared to untreated controls, six days after treatment with insecticides

Aphid development stage	Insecticide	Aphid clone		Significance (S vs R)
		Susceptible (Nr1A)	Resistant (Nr2A)	
Total aphids	deltamethrin	0.863	0.817	n.s.
	pirimicarb	0.897	0.674	n.s.
	heptenophos	0.647	0.639	n.s.
Adults and large nymphs	deltamethrin	0.778	0.855	n.s.
	pirimicarb	0.861	0.788	n.s.
	heptenophos	0.600	0.768	n.s.
Adults only	deltamethrin	0.649	0.586	n.s.
	pirimicarb	0.807	0.231	$p<0.05$
	heptenophos	0.390	0.390	n.s.
Small nymphs	deltamethrin	0.916	0.762	n.s.
	pirimicarb	0.919	0.508	$p<0.05$
	heptenophos	0.674	0.447	n.s.

Persistence of insecticide residues against resistant and susceptible *N. ribisnigri*

Levels of mortality were very variable between treatments in this experiment. Generally mortality due to deltamethrin or heptenophos was low and it was difficult to draw any conclusions. However, the mortality of pirimicarb-resistant *N. ribisnigri* (Nr2A) was significantly less than susceptible aphids (Nr1A) one and two days after application (Table 2).

Table 2 The mean percentage mortality of pirimicarb-susceptible (Nr1A) or resistant (Nr2A) *N. ribisnigri* when place on lettuce plants up to four days after treatment with a range of insecticides. Values that differ significantly between susceptible and resistant clones are shown in bold italics.

Days after treatment	Untreated		deltamethrin		pirimicarb		heptenophos	
	Suscept. (Nr1A)	Resist. (Nr2A)	Suscept. (Nr1A)	Resist. (Nr2A)	Suscept. (Nr1A)	Resist. (Nr2A)	Suscept. (Nr1A)	Resist. (Nr2A)
0	6.0	6.0	19.2	19.0	74.0	55.0	24.4	19.8
1	8.8	6.2	***15.2***	***78.0***	***42.0***	***17.2***	10.8	10.0
2	14.8	8.4	4.0	4.0	***30.8***	***6.4***	4.0	0.0
4	11.0	4.6	2.0	14.0	2.8	19.2	4.6	6.0

4.4 DISCUSSION

The monitoring of UK field populations of *N. ribisnigri* over 1999 has demonstrated varied levels of resistance, similar to those identified in project FV210, which can be categorised into four phenotypic modes: (1) susceptible to all insecticides tested; (2) resistant to pirimicarb; (3) resistant to pyrethroids; (4) resistant to pirimicarb and pyrethroids. Modes 2, 3 and 4 have consistently been associated with a heavily staining esterase band on PAGE gels and preliminary results suggest this is related to an over-production of the esterase rather than the production of a different esterase. Examples of field strains exhibiting these different modes have been identified and cloned for more detailed cross-resistance work.

The screening of field strains found no modified acetylcholinesterase (MACE) which is consistent with the low levels of resistance seen in the bioassays. If a MACE population is found in the UK or is acquired from abroad, further AChE characterisation will be undertaken to optimise conditions for detecting it in field populations.

Although the resistance factors recorded in FV210 appear somewhat low to account for control problems, difficulties with relating laboratory bioassay data to field performance are well recognised (e.g. Sawicki, 1987). There are examples of substantially increased tolerance in bioassays causing little or no reduction in field control efficacy (Farnham *et al.*, 1984) and, conversely, examples of resistance barely detectable in bioassays having a major impact in the field (Dennehy & Granett, 1984). However, field experiments have demonstrated that the levels of resistance seen in *N. ribisnigri* are proving to be problematic in aphid control on lettuce. In the field cage experiments, the numbers of *N. ribisnigri* two days after application were reduced equally irrespective of whether they were resistant or susceptible to insecticides or the insecticide applied. However, six days after insecticide application on plots treated with pirimicarb there were significantly more aphids on plots infested with the pirimicarb-resistant clone (Nr2A) than the susceptible clone (Nr1A). These data suggest that direct contact with the spray application, represented in the data two days post application, may cause a different response to contact with residues of pirimicarb, represented by the six day post application data. This was supported when the persistence of insecticide residues

was examined in the laboratory. Further experiments will be directed at confirming the results to date under different environmental conditions and towards unraveling the difference between the direct impact of spray application as opposed to residues on the mortality of susceptible and resistant *N. ribisnigri*. In addition, experiments will include a clone of *N. ribisnigri* resistant to pyrethroids, while studies with heptenophos will cease.

Owing to the non-specific nature of the substrates used, a rapid diagnostic for elevated esterase levels has yet to be found for use with *N. ribisnigri*. It is suspected that 1-naphthyl acetate binds to other esterases, in addition to the one of interest, which are present in equal quantities in all strains tested thus masking a distinct difference between susceptible and resistant individuals. Similarly, the fluorometric compound AMC appeared to bind to the aphid AChE as well as the esterase of interest while CFD appeared not to bind to the esterase at all. The next step in the production of a rapid diagnostic resistance test will therefore be the purification of the elevated esterase leading to an esterase specific immunoassay.

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