Project title:	Selection of strains of predatory mites that can survive applications of insecticides required for SWD control
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Project leader:	Name: David Buss Address: NIAB EMR, New Road, East Malling, Kent ME19 6BJ
Report:	Final Report, 2017 Compiled by Jean Fitzgerald e-mail jean.fitzgerald@emr.ac.uk
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Key staff:	Jerry Cross (NIAB/ EMR) Jean Fitzgerald (NIAB/ EMR) Phil Brain (NIAB/ EMR) Adrian Harris (NIAB EMR)
Location of project:	NIAB/ EMR
Industry Representative:	Marion Regan (Hugh Lowe Farms Ltd.)
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GROWER SUMMARY

Headline

- Research has shown that some *Neoseiulus cucumeris* and *Amblyseius andersoni* predatory mites are likely to survive field applications of spinosad (Tracer) and cyantraniliprole (Exirel).

Background and expected deliverables

Britain and the rest of Europe currently rely extensively on predatory mites for the control of mites and thrips on soft fruit crops. Of the 3,981 ha of strawberries grown in the UK in 2012, 2,567 ha were treated with *Neoseiulus cucumeris* (primarily for thrips control), 2,417 ha with *Phytoseiulus persimilis* (for control of two-spotted spider mite) and 239 ha with *Neoseiulus californicus* in protected crops (Garthwaite *et al*., 2013). This represented a 20 fold increase since 2001. Likewise, 83% of UK grown raspberries were treated with *Phytoseiulus persimilis* in 2012 and 57% with *Neoseiulus cucumeris*.

The effective use of predatory mites relies on careful integration with crop protection products to maintain their numbers in the crop, as predatory mites are generally considered to be more vulnerable to these products than pest species. This Integrated Pest Management (IPM) has worked very successfully until now. However, this situation is changing with the establishment of Spotted Wing Drosophila (SWD - *Drosophila suzukii*) in the UK.

The four principal soft fruit crops grown in the UK in terms of area, strawberries (38%), blackcurrants (22%), raspberries (15%) and grapevines (15%) are all vulnerable to SWD (Cini *et al* 2012) and outbreaks of SWD could lead to increased use of crop protection products as no IPM solution for this pest currently exists. The most effective product groups for SWD control are organophosphates, spinosyns, synthetic pyrethroids and anthranilic diamides. Organophosphates are used for SWD control in the USA, but their use in Europe is gradually declining and they are no longer approved in the UK. Synthetic pyrethroid products are thought to be harmful to most predatory mites, while the spinosyns and diamides have mixed effects on different mite species.

Therefore if growers spray against SWD they risk incurring further crop damage through other pests if biocontrol agents such as predatory mites are killed. One possible method to minimise this risk could be the selection of predatory mites for resistance to specific

products. If selection effectively increased tolerance to the products it may then be possible to rear these strains commercially and make them available to growers when required.

The initial aim of this project was to attempt to develop phytoseiid mites resistant to crop protection products that growers could use whilst controlling SWD, to allow biological control of spider mites and thrips to continue effectively. However, it was not possible to rear sufficient numbers of *Amblyseius andersoni* or *Neoseiulus cucumeris* under laboratory conditions to develop strains with greater tolerance to spinosad. Based on these results and following discussion with AHDB, the focus in the final 6 months of the project switched to study the effects of three products (currently approved for use in the UK and capable of controlling SWD) on two species of predatory mite widely used in biocontrol programmes. Such information will help growers to make more rational decisions on choice of product for SWD control.

Summary of the project and main conclusions

In the initial project work to develop strains of predatory mites resistant to products that may be used in integrated pest management programmes, *Amblyseius andersoni* and *Neoseiulus cucumeris* were chosen as they are commercially available for control of a range of important pests and are considered “native” species for authorisation for any subsequent use on non-glasshouse crops.

Commercially available *A. andersoni* and *N. cucumeris* were obtained and assessed for their susceptibility to spinosad (Tracer) and lambda-cyhalothrin (Hallmark). In laboratory residual bioassay tests, the predatory mites were found to be highly susceptible to lambda-cyhalothrin, but much less so to spinosad.

Using residual doses of spinosad, the lethal dose required to kill 50% of individuals tested (LD50) was obtained for both mite species. This was 2.6 time field rate for *N. cucumeris* and 4.1 times field rate for *A. andersoni*. However, mortality in residual assays is likely to be less than from topical assays where the mite is covered with product.

Since *N. cucumeris* showed some tolerance to spinosad in residual assays the selection programme commenced with this product. However, after the population had been selected twice, there was no significant decrease in mortality when the mites were exposed to field rate doses of spinosad in residual assays.

A major problem was encountered during this project as it was found difficult to sustain sufficient numbers of mites for onward selections. The use of Nutrimite, a pollen derived from Typha plants and sold for mite rearing, was used in initial rearing studies. Experiments comparing a range of pollens, both with and without additional prey mites, showed that fresh Typha pollen was the most effective food source for rearing these predators. However, it was still not possible to produce sufficient mites for onward selections so this work was terminated.

To provide growers with more information on the effects of three products (commonly used for control of SWD) on *N. cucumeris* and *A. andersoni*, topical applications of lambda-cyhalothrin, cyantraniliprole and spinosad were made to the adult mites and the outcome assessed. A topical application is one where the product is applied direct to the predator rather that to leaf tissue where it can be picked up by the predator. For *A. andersoni* the LD50 (the dose required to kill 50% of tested individuals) for lambda-cyhalothrin after 48 hours was 0.17 field rate, for cyantraniliprole was 2.2 times field rate and for spinosad was 3.6 times field rate. For *N. cucumeris* an LD50 for lambda-cyhalothrin was not derived, but the LD50 after 48 hours for cyantraniliprole was 0.5 times field rate and for spinosad was around field rate. These results suggest that some mites are likely to survive field rate applications of spinosad and cyantraniliprole; effects on immatures and potential effects on fecundity or effects of multiple applications were not addressed in this study.

Financial benefits

UK horticulture utilises Integrated Pest Management for control of many pests. However, if increased crop protection product usage is required for new threats such as spotted wing drosophila then predator numbers may be reduced and other pests such as two-spotted spider mite are likely to increase. One potential solution to this problem is to develop predators that are tolerant of such products and capable of establishment in regimes with higher product usage. This strategy has been successful for the use of pyrethroid resistant *Phytoseiulus persimilis* in the Dutch chrysanthemum market, which allows spraying against capsids without loss of spider mite control (Simon Jones, Certis Europe, personal communication). Should such a strategy be successful in soft fruit for SWD control, this economically damaging pest could be controlled without incurring further pest damage.

Action points for growers

- The use of synthetic pyrethroids for SWD control can be highly damaging to *N. cucumeris* and *A. andersoni* and if used at the wrong time of the season, can incur further crop damage from other insect pests.
- Spinosad and cyantraniliprole have been found to be considerably less damaging to *N. cucumeris* and *A. andersoni* and allow survival of some of these mites.
- Where possible, when aiming to use crop protection products for SWD control, choose spinosad and cyantraniliprole at those times of the season when predatory mite establishment, feeding and breeding are pivotal to the control of other pests and rely on synthetic pyrethroids for control at other times of year when predator activity is less important.

SCIENCE SECTION

1. Introduction

Predatory mites are currently important in the control of crop pests such as spider mites, but are vulnerable to various insecticides. Whilst this can be managed by choice of insecticide, the withdrawal of some insecticides, and increased use of others to control new pest species, such as *Drosophila suzukii*, will make IPM more difficult. The overall objective of this project is to develop strains of predatory mites resistant to selected insecticides for use in integrated pest management.

Choice of predatory mite species is important. The choice is wide; for instance; Bioline currently produce 7 predatory mites suitable for use on strawberries or raspberries; *Amblyseius andersoni*, *A. barkerii*, *A. montdorensis*, *Hypoaspis miles, Neoseiulus californicus*, *N. cucumeris*, and *Phytoseiulus persimilis*. However, regulatory restrictions on non "native" species such as *Neoseiulus californicus* reduce their potential, as they cannot be released into outdoor crops even if they are collected from a UK source. Differences in ease of culture and commercial viability also apply.

The greatest threat to phytoseiid mites is likely to come from increased use of pyrethroids and spinosad. The pyrethroid lambda cyhalothrin is recommended for SWD control in many countries, and in a trial of various insecticides in the Trento region of Italy only lambda cyhalothrin gave adequate control of this pest (Grassi *et al* 2012). However, pyrethroids are generally highly toxic to predatory mites (for example, Solomon *et al* 1993, Bostanian & Belanger 1985). The toxicity of spinosad to predatory mites is unclear in the literature (Jones *et al* 2004, Villanueva and Walgenbach, 2005, Cuthbertson *et al* 2012), and probably varies between life stages. Given its usefulness to soft fruit growers (43% of strawberry acreage was sprayed with spinosad in 2012, Garthwaite *et al* 2013) it would be valuable for growers to have spinosad compatible predators available. Cyantraniliprole has been reported to be relatively safe to predatory mites (Kaplan *et al* 2012; You *et al* 2016) with the latter study suggesting that it may be repellent to *N. cucumeris*.

Reports of small populations of predatory mites that have survived insecticide treatments show the potential for considerable increases in resistance. For example, natural field selection of *Typhlodromus pyri* produced organophosphate resistant populations capable of pest control (Solomon *et al* 1993), whilst populations of *Amblyseius longispinosus* in China have been reported showing a 25-30 times resistance level (Zhao *et al* 2013). Similar cases

have been reported for pyrethroids in populations of *Amblyseius andersoni* and *Typhlodromus pyri* in French vineyards (Bonafos *et al* 2007) and *Neoseiulus californicus* in Brazilian citrus groves with, in this last case a 24 fold deltamethrin resistance ratio compared to susceptible controls (Poletti & Omoto 2005). Field selection of *Typhlodromus pyri* at East Malling produced a population of *Typhlodromus pyri* with increased levels of resistance to pyrethroids (Solomon & Fitzgerald, 1993). However, naturally occurring tolerance of insecticides at these levels is comparatively rare. Even when present, resistant populations are diluted by immigration of susceptible mites as soon as selection pressure is eased. For reliable control growers would require a readily available source of pesticide resistant predatory mites for release into crops. The aim of this project was to determine if resistance to pesticides used for control of SWD can be developed in phytoseiid mites in laboratory selection programmes, and to obtain information on the toxicity of spinosad, lambda cyhalothrin and cyantraniliprole on *N. cucumeris* and *A. andersoni*.

2. Materials and methods

2a) Choice of species for selection for resistance to insecticides

As discussed in the previous Annual Reports for this project, the native species *Amblyseius andersoni,* which gives good control of spider mites, and *Neoseiulus cucumeris,* which is widely used for control of thrips and tarsonemid mites, were chosen for the initial selection work.

2b) Mite sources

Mites were purchased from UK commercial suppliers and used for bioassays and subsequent selections and also in the bioassays to determine the dose that would kill 50% of tested individuals (LD50). Except where mentioned the mites were obtained from the same supplier.

2c) Optimising culture conditions

Predatory mites were cultured using a modified version of the method of Overmeer (1985). Rearing arenas (Figure 1) consisted of plastic tiles on water saturated foam in plastic boxes half filled with water and detergent, with filter and tissue paper around the edge to minimise escapes and increase humidity on the plate. Cotton wool fibres under coverslips served as shelter and oviposition sites. As a further guard against cross contamination and loss of mites, a sticky gel (Oecotak, Oecos Ltd., Kimpton, UK) was placed around the paper barrier in the boxes. Work in Year 1 showed that humidity needed to be increased for effective

rearing of the mites, so culture boxes were closed with lids containing a 2 cm hole covered in gauze. This increased humidity but not to such a level that the pollen used as food became mouldy. Cultures were reared in CT rooms set to 24 ^{o}C, on a 16 hr light/ 8 hour dark cycle with air circulation, but without additional humidification. In the first two years of the project predator populations were fed with Nutrimite (Biobest, Westerlo, Belgium), a commercially available pollen source from *Typha*, marketed for feeding predatory mites (Annual Reports for 2014; 2015).

Figure 1. Mite rearing arena without lid

This method appeared to maintain populations but did not lead to substantially increased numbers in the earlier experiments. To test this, in Year 3, an experiment was set up to follow numbers of *A. andersoni* produced over time on a culture plate using Nutrimite as a food source. Two replicate plates were set up with 15 males and 15 females on each. Numbers of immatures and adults present were recorded over 100 days.

As reported in the Annual Report for Year 1, information on rearing methods used commercially is commercially sensitive and not available outside each company so it is not possible for us to use these techniques. In Year 1 of this project a population of mites selected with spinosad was sent to a biocontrol company for rearing, but they were unable to maintain it. Therefore we focused on improving the method in use at NIAB EMR. Throughout the project researchers around the world were contacted to obtain information on mite rearing methods, including Gerben Messelink (PPO Bleisweijk, Wagenenin), Carlo Duso (University of Padua), Gilberto Moraes (University of Sao Paulo, Brazil), Irina Goleva

(Hohenheim University), and Andreas Walzer (Division of Plant Protection, Universität für Bodenkultur, Vienna). Discussion with Jan Holshof (Biotus, Finland) again confirmed most of the protocol used at NIAB EMR, but he suggested honey water as a food supplement. Further consultation and a visit to the mite rearing laboratory at the University of Padua led to a number of small modifications to the rearing method including an increased rearing temperature, but the most significant factor appeared to be the type of pollen used as a rearing diet. As a result of this consultation in Year 3 an experiment was set up to test the effects of different types of pollen on population increase. In addition a culture of *Tyrophagus putrescentiae* was set up, using the same culture techniques as above and fed with Nutrimite. This mite is used commercially as a food source for *N. cucumeris* and was used in addition to one of the pollens in the feeding tests.

Treatments used in the experiment were:

1. Nutrimite (*Typha*) pollen (Biobest, Westerlo, Belgium),
2. Dwarf bean pollen (from Bias Labs, Kirkcaldy, UK)
3. *Typha* pollen collected and kindly supplied by Dr Marie Stephanie Tixier of University of Montpellier
4. Collected *Typha* pollen as above with *Tyrophagus putrescentiae*

Three replicates of each treatment were applied to *N. cucumeris*, and 2 replicates of Treatments 1, 2 and 4 to *A. andersoni*. Ten males and 10 females were confined on a culture plate and fed with the relevant food type. Numbers of adults, immatures and eggs were counted at regular intervals for 34 days for *N. cucumeris* and 23 days for *A. andersoni*. Counts were analysed using GLM with the Poisson distribution & log-link function. A combined analysis, using all pollens and times was done, together with snapshot analysis at each recording day separately. There was evidence of over dispersion in the results for both species, so all analyses were adjusted for this.

Further modifications were also made to the culture technique in Year 3, with a comparison made of population growth over time when fresh Typha pollen was added to the culture plates either once or twice each week; it is possible that the pollen becomes unusable by the mites within a few days, although an inspection suggested that the pollen had not deteriorated visually. Six plates were set up as described above. 50 adult *N. cucumeris* were placed on each plate. Three plates were fed once a week and three plates were fed twice each week. Numbers of eggs, immatures and adults on each plate were recorded every 2-3 days for 20 days. Cumulative numbers of mites recorded in the sticky barrier were

also recorded to determine if higher numbers of mites were caught in the barrier if the culture plate became less suitable for the mites over time.

2d) Application of insecticide for residual bioassays and selection

The choice of insecticide for selection was based on those recommended for SWD control in countries already with SWD infestations, which included pyrethroids, spinosad, neonicotinoids and organophosphates. Given the phasing out of neonicotinoids and the low probability of authorisation for organophosphates, we focused on spinosad (formulated as Tracer; Dow Agrosciences Ltd., Hitchin, UK), and a pyrethroid, lambda cyhalothrin, formulated as Hallmark (Syngenta UK Ltd., Cambridge, UK).

Results from Year 1 showed that, as reported in the literature and product information, lambda cyhalothrin was very damaging to *A. andersoni*, with 100% mortality after 24 hours with a dose equivalent to 0.1 of the field rate and 76% mortality at 0.01 of the field rate. It was therefore decided to focus on selection with spinosad, which had given 28% mortality at a dose equivalent to the field rate in these assays.

As determined in Year 1 it was not possible to use NIAB EMR's Burkard sprayer for topical application of insecticides for selection tests, where it was necessary to retain mites not killed by the pesticide for further rearing, as the spray action of the machine blew the mites from the dish. Consequently another technique was developed, using a modified method of Sato *et al* (2000) which is a residual assay. Residual effects of pesticides are likely to be lower than effects of direct topical application.

Filter papers were soaked in pesticide solution, placed in a 9 cm Petri dish and left to dry. Water soaked paper was used as a control. Oecotak, a sticky gel, was used around the paper to ensure the mites stayed within the dish. Shelters consisting of coverslips over cotton threads were provided (Figure 2). Mites were added and held on the paper at 20°C and mortality was assessed after 24 hours by touching the mites with a fine brush; those that did not respond were recorded as dead.

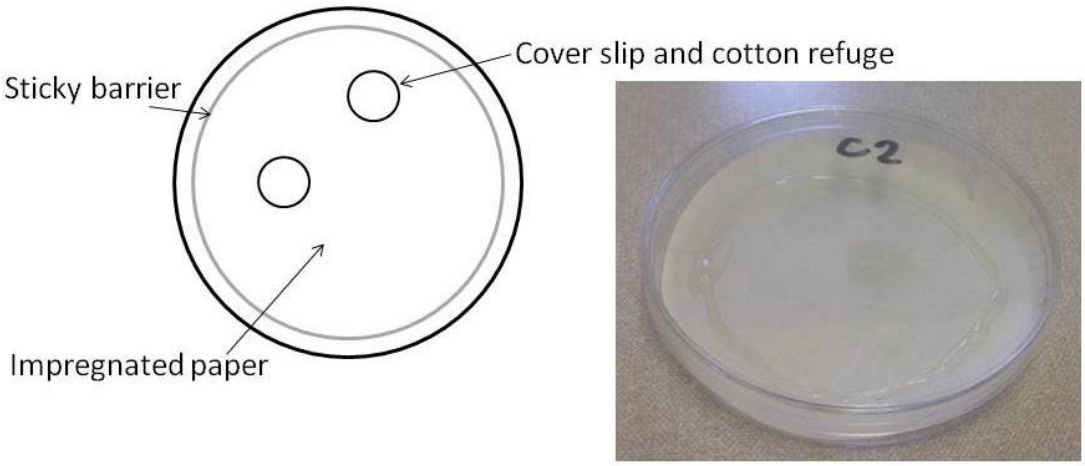

Figure 2. Design of the system used for exposing mites to insecticide, based on a 9cm Petri dish with pesticide impregnated paper and a sticky barrier.

2e) Determination of residual LD50 of spinosad for *N. cucumeris* and *A. andersoni*

Mites were assayed using the method described above with different rates of spinosad. These assays were done over several days and there were different total numbers of replicates for each rate. This has been taken into account in the analysis of the results. For *N. cucumeris* the rates used were 0.5, 1, 2 and 3 times the field recommended rate of 150 ml/ha. For *A. andersoni* the rates were 0.1, 1 and 2 times the field rate. Controls were water-dipped filter papers. Mites were kept on the treated paper at 20°C and mortality was recorded after 24 hours. Data were analysed using a GLM with binomial distribution and a logit-link function. For *A. andersoni* results should be treated with caution as only three doses were tested. Two populations of *A. andersoni* were compared; one collected from a cherry orchard at East Malling, and the second from a commercial biocontrol supplier.

2f) Selection for resistance; *N. cucumeris*

The selection concentration of spinosad, based on previous bioassays, was 0.15 ml/l Tracer. This corresponds to the field dose.

N. cucumeris (a mixture of males and females) were added to each dish and mortality was assessed after 24 hours, as above. Survivors were transferred to a new rearing arena. Survivors from the control (water) treated papers were also cultured on a separate arena, to be used as a comparison for future assessments of susceptibility. All survivors were cultured with collected *Typha* pollen and *Tyrophagus putrescentiae* as described above.

2g) Determination of topical LD50 of spinosad, cyantraniliprole and lambda cyhalothrin for *A. andersoni* and *N. cucumeris*

In these bioassays, a different technique, tested successfully in other projects at NIAB EMR, was used. Adult mites were placed on their backs on double-sided sticky tape on glass microscope slide, using a fine paintbrush. Ten mites were placed on each slide; once on their back these mites were not able to move off the slide and survived for over 48 hours. The mites were then sprayed under the Burkhard sprayer with different concentrations of the test pesticides. Doses were based on recommended field rates for the pesticides (150 ml/ha for spinosad; 900 ml/ha for cyantraniliprole (cherry rate) and 90 ml/ha for lambda cyhalothrin). Initial 'ranging shot' assays with low numbers of mites made it possible to narrow the range of concentrations used in the final assays. Once the slides had dried they were placed into boxes containing damp paper tissues to maintain humidity, and held at 20°C. Mortality of the mites was assessed 24 and 48 hours after pesticide application by inspecting the mites under a stereomicroscope. There were 4 or 5 replicate slides for each concentration (i.e. 40 or 50 mites per concentration), and appropriate water controls.

The mortality vs log(Dose) relationship was modelled for each set of data using a standard dose response curve of the form %Mortality = %Control Mortality + (100-%Control Mortality)*((Dose/LD50)^B)/(1+(Dose/LD50)^B). The %Control Mortality was included as a parameter as Control Mortality was present in all cases. The model was fitted using a GLM with a Poisson distribution and a non-linear link. Fiducial limits for the LD50's were estimated using Fieller's Theorem.

3. Results

3c) Optimising culture conditions

The population development of *A. andersoni* fed on Nutrimite on two culture plates is shown in Figure 3. The total population decreased by *c*. 50% after the first 35 days and very few eggs were laid.

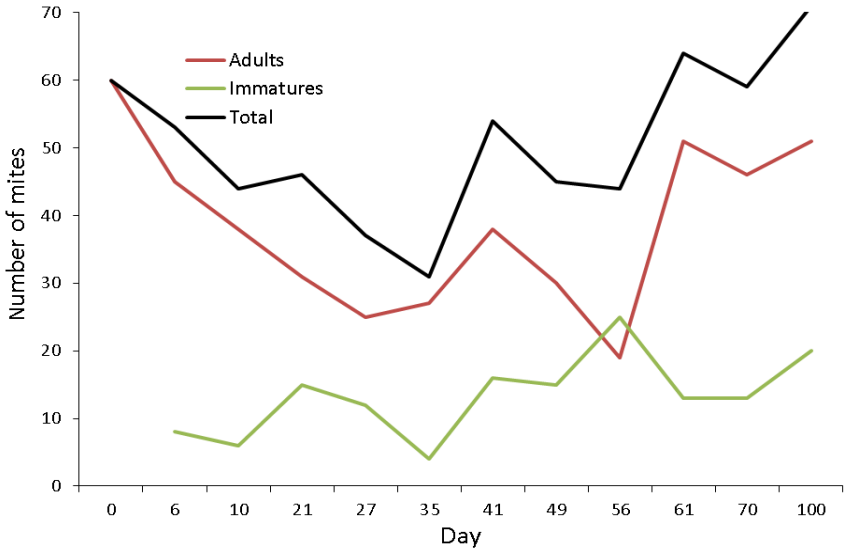

Figure 3. Population development of *A. andersoni* over time fed on Nutrimite; totals from two culture plates

After 100 days the total population was only slightly more than that established initially, indicating that Nutrimite was not satisfactory as a diet for increasing populations of this species.

T. putrescentiae were successfully reared using our standard culture conditions as described above, with numbers increasing 5 fold in 12 days. This indicates that this prey species could be reared on arenas if needed to supplement the food source for *N. cucumeris* or *A. andersoni*.

Diet analysis: *N. cucumeris*. In the combined analysis there was no evidence that adding *T. putrescentiae* to *Typha* pollen increased population development of *N. cucumeris* so the counts from treatments with *Typha* pollen alone and *T. putrescentiae* plus *Typha* pollen were pooled in a further analysis. There was a significant effect of food type on population development of *N. cucumeris*, with numbers increasing and maintained when the mites were fed on fresh *Typha* pollen (Figure 4), and numbers decreasing rapidly when fed on

Nutrimite. Results of the statistical analysis are shown in Table 1. Mean population sizes of *N. cucumeris* when fed on a pollen type showing the same letter on a particular date (i.e. in rows) are not significantly different.

Table 1: Effect of different food types on population development of *N. cucumeris* (mean numbers of actives plus eggs)

Days after set up	Pollen type			Significance
	Typha	Bean	Nutrimite	
3	37.0 a	23.0 b	16.0 b	0.002
7	29.5 a	25.3 a	13.7 b	0.02
10	34.5 a	25.7 a	8.7 b	0.008
14	42.0 a	20.0 b	4.7 c	0.006
17	40.5 a	18.5 b	3.3 c	<0.001
20	36.8 a	15.0 b	3.0 c	<0.001
24	35.7 a	14.5 b	0.0 c	0.012
31	38.0 ns	20.0 ns	-	0.224

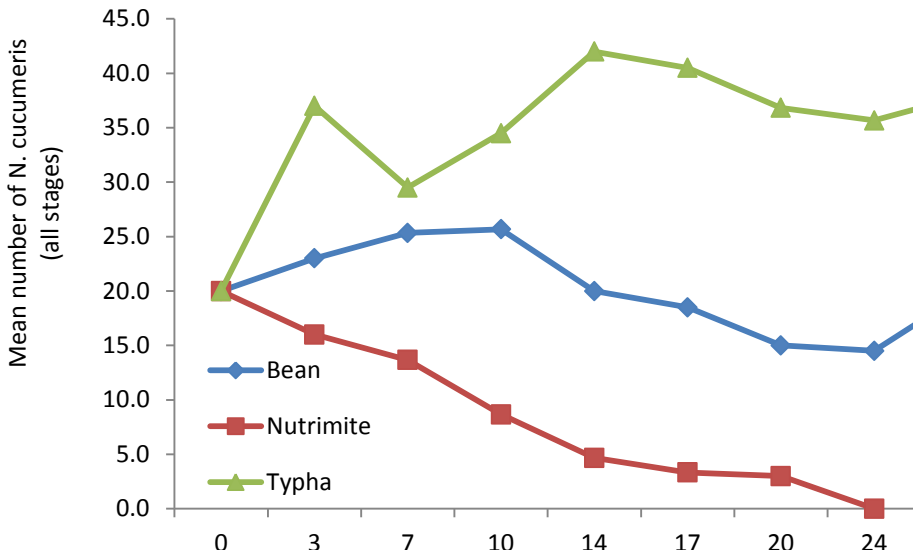

Figure 4. Population development of *N. cucumeris* (mean numbers of actives plus eggs) on different food types (results of statistical analysis are shown in Table 1)

Diet analysis: *A. andersoni.* In the combined analysis there was evidence that the replicates within a food type had different profiles over time (hence overdispersion). After adjusting for

the overdispersion in the analysis there were no significant differences between food types on population development. In the snapshot analysis on each day separately, adjusting for overdispersion there were significant differences only on day 20 (p=0.024) (Figure 5), with higher numbers of *A. andersoni* where the food type was fresh *Typha* pollen.

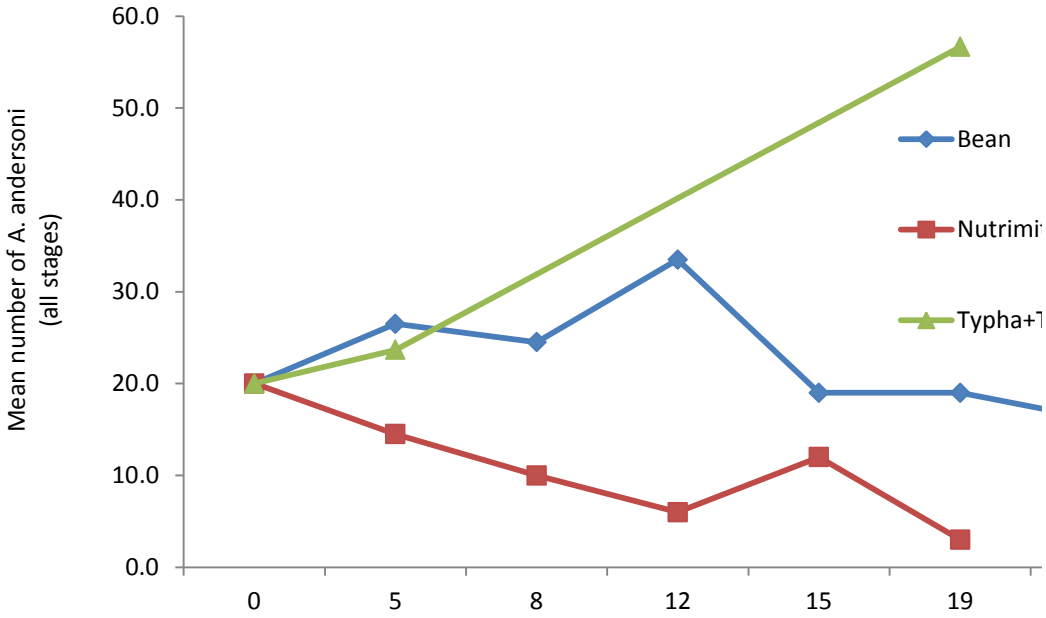

Figure 5. Population development of *A. andersoni* (actives plus eggs) on different food types (results of statistical analysis are shown in Table 1)

Further modification of culture method. Feeding *N. cucumeris* on freshly frozen *Typha* pollen twice each week was more effective than feeding once a week. The former treatment maintained numbers of mites for over 27 days, but numbers did not increase substantially over that time (Figure 6). There was no evidence of mass movement of mites off the plate; only adults were recorded in the sticky barrier and numbers caught were constant over time, but around half of the adults reared eventually were lost to the culture over 27 days.

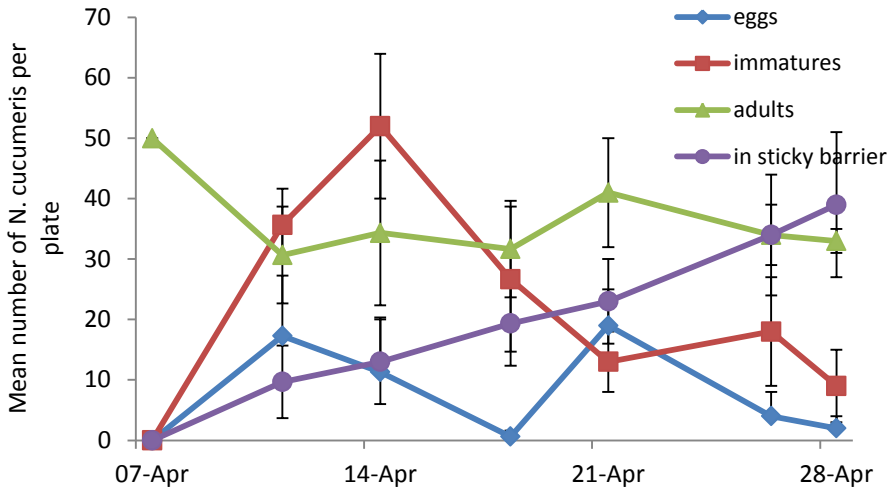

Figure 6. Population development of *N. cucumeris* when fed fresh *Typha* pollen twice each week

On the plates that were fed once each week, numbers of adults present had decreased to a mean of around 5 after 27 days. Because of the short period of time remaining in the project it was agreed in discussion with AHDB that it was impractical to assess further possible modifications to the rearing technique to enable a substantial pesticide selection experiment to be undertaken and this work was terminated.

3e) Determination of residual LD50 of spinosad for *N. cucumeris* and *A. andersoni*

N. cucumeris: There was no evidence of differences in mortality on any assay date. There was a significant effect of spinosad concentration (Figure 7).

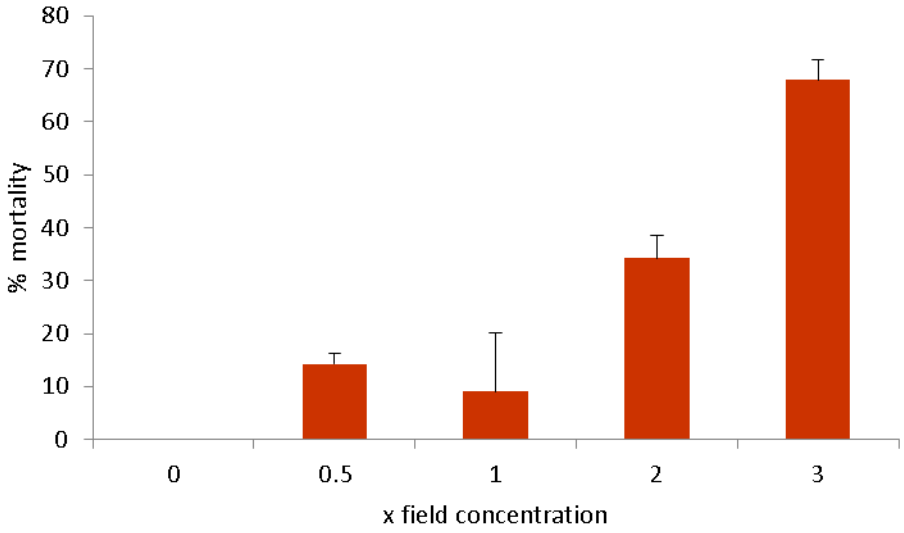

Figure 7: Effect of increasing concentration of spinosad on mortality of *N. cucumeris* in residual assays

The logit analysis of the results gave an estimated LD50 for this strain of *N. cucumeris* as 2.6 x the field concentration (lower 95% 2.1 x field concentration; upper 95% 3.8 x field concentration).

A.andersoni: There was a significant effect of concentration and also a significant difference between strains ($p<0.001$) of *A. andersoni* in their responses to spinosad applications (Figure 8). Mortality was significantly higher in populations collected from the cherry orchard at East Malling. A standard dose response curve was fitted with control mortality. The best model was:

$$\%Mortality = C + (1 - C)\frac{\left(\frac{Dose}{\mathrm{LD50}}\right)^{B}}{1 + \left(\frac{Dose}{LD50}\right)^{B}}$$

Where: C = Control Mortality

B = Slope of logit of adjusted %mortality

LD50 = Concentration that kills 50% of susceptible insects

The estimated LD50 for *A. andersoni* collected from cherry was 0.04 of the field rate (SE 0.04) and from the commercial supplier was 4.1 times the field rate (SE 10.9). However, because of the low number of mites tested these results should be viewed with caution.

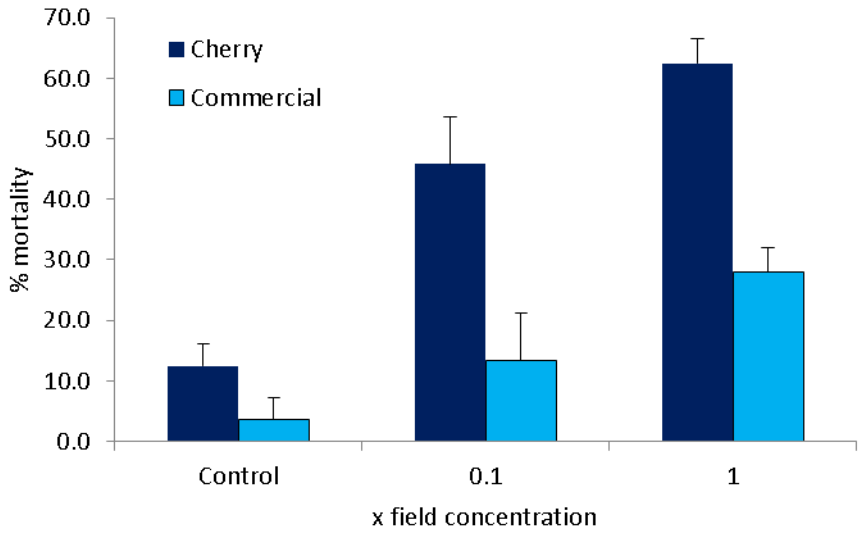

Figure 8: Effect of 0.1 and 1 times field rate concentrations of spinosad on *A. andersoni* in residual assays

3f) Selection for resistance in *N. cucumeris*

In the selection experiment there was no significant difference in percentage mortality at field rate doses of spinosad after the population had been selected twice (Figure 9).

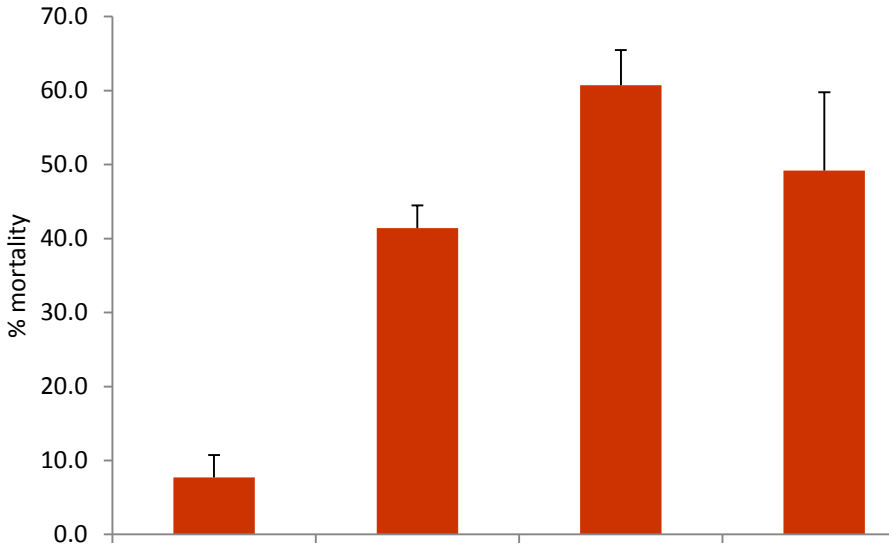

Figure 9: Mortality of *N. cucumeris* in residual bioassays with field rate spinosad before and after population selection

3g) Determination of topical LD50 for spinosad, cyantraniliprole and lambda cyhalothrin on *A. andersoni* and *N. cucumeris*

Amblyseius andersoni: calculated LD50s for the different insecticides are shown in Table 2. Application of 0.25 of the field rate of lambda cyhalothrin caused 80% mortality of adult *A. andersoni* after 48 hours. At 0.13 field rate mortality was 28% after 48 hours; control mortality after 48 hours was 8%. The dose required to give 50% mortality was calculated to be 0.2 x field rate at 24 hours and 0.17 times field rate after 48 hours (Table 2). This confirms the toxicity of this pyrethroid to this species of predatory mite. In contrast, field rate of spinosad gave only 20% mortality and 22% of treated mites survived 5 times field rate. Calculated LD50 for spinosad was 4.8 times field rate after 24 hours and 3.6 times field rate after 48 hours (Table 2); the residual LD50 after 24 hours in earlier assays (Section 3e) was estimated as 4 times field rate. For cyantraniliprole, field rate applications gave 12.5% mortality and 2.5 times field rate applications gave 70% mortality. The calculated LD50 for this product was over 2 times field rate after 24 and 48 hours.

Table 2: Calculated LD50 for three pesticides for *A. andersoni* in topical laboratory assays. Dose of 1 = field rate

			confidence limits		
			lower	upper	Control Mortality
		LD50	95%	95%	(%)
cyantraniliprole	24 hr	2.37	2.18	2.70	3.39
	48 hr	2.17	1.95	2.45	5.85
spinosad	24 hr	4.75	3.83	8.05	4.71
	48 hr	3.64	2.45	5.78	7.19
lambda	24 hr	0.20	0.17	0.23	3.65
cyhalothrin	48 hr	0.17	0.14	0.20	7.88

Neoseiulus cucumeris: calculated LD50s for two of the tested insecticides are shown in Table 3. For lambda cyhalothrin a dose of 0.06 times field rate gave 60% mortality after 48 hours; this result highlights the damaging effects of applying a pyrethroid to biocontrol agents. It was decided not to continue to test more dilute doses of the pesticide to obtain an

LD50 for this product. In the residual bioassay with spinosad, with low numbers of mites, the estimated LD50 was over 2 times field rate, whereas in this topical assay, the LD50 was estimated as around field rate, highlighting the different responses in residual and topical application. For cyantraniliprole, after 48 hours the estimated LD50 was around half field rate, suggesting that tolerance to this product was less than for spinosad.

Table 3: Calculated LD50 for two pesticides for *N. cucumeris* in topical laboratory assays. Dose of 1 = field rate

			confidence limits		
			lower	upper	Control Mortality
		LD50	95%	95%	(%)
cyantraniliprole	24 hr	1.68	0.61	7.55	10.76
	48 hr	0.51	0.14	0.91	18.65
spinosad	24 hr	1.08	0.67	1.47	18.08
	48 hr	0.93	0.52	1.27	26.44

These results suggest that applications of spinosad to control spotted wing drosophila may be compatible with IPM programmes based on the use of phytoseiid mites, since not all adults would be killed by field rate applications. However, effects on immature life stages are likely to be greater; in Year 1 of this project a residual assay with field rate spinosad gave 29% mortality of adults and 74% mortality of immatures. Also, there may be behavioural effects (cyantraniliprole has been reported to be repellent to predatory mites; You *et al* 2016), or effects on fecundity, which may affect the survival of populations of mites after pesticide applications.

4. Discussion

A limiting factor for mass rearing of insecticide-selected mites for this project has been a method to give large scale increases in population. Although the method used maintained population levels over the short term it did not increase numbers sufficiently. Consultation suggested that one factor might have been the diet used. Nutrimite, a commercially available form of pollen derived from *Typha* reeds, is a standard food in mite rearing and has been shown to be an adequate diet under the right circumstances (eg. Nomikou et al 2001). Both *A. andersoni* (McMurty & Croft, 1997) and *N. cucumeris* (Goleva et al 2015)

can be reared on pollen, but different pollens have different effects on developmental success (Goleva et al 2015). Pollen, however, is very difficult to obtain in large quantities, which is why Nutrimite was used in the first instance, as *Typha* reeds produce much larger quantities of pollen than, for example, bean plants. It is also likely, however, that the method of collection, and quality of the source plants, are important. The results presented here suggest that *Typha* pollen can potentially be a good food source under the right collection and storage regimes, but more work would be needed to develop a robust laboratory rearing method.

Results from the residual bioassays indicated that the strain of *N. cucumeris* tested was more tolerant to spinosad than the two strains of *A. andersoni* tested. The results of the bioassays also indicate that, even at twice field rate, dry residues of spinosad were only moderately toxic to *N. cucumeris* giving around 35% mortality in the laboratory bioassays and with some survivors at 3 times field rate. This suggests that *N. cucumeris* populations may survive if introduced after applications of spinosad. Results also indicated that there are likely to be differences between strains of the same species for tolerance to spinosad.
LD50 values were estimated for both *N. cucumeris* and *A. andersoni* for spinosad in these residual assays. These values were based on low numbers of mites and only a few different concentrations of the insecticide so should be treated with caution, but were 2.6 times field rate for *N. cucumeris* and 4.1 times field rate for *A. andersoni*.

There was no apparent increase in tolerance of *N. cucumeris* to spinosad after two residual selections at field rate with small numbers of mites.

In the topical applications of spinosad, as expected, the LD50 for *N. cucumeris* was lower than that derived from the residual assays and was around field rate dose, suggesting that this species would not be completely eliminated from crops treated with this product. For the strain of *A. andersoni* tested the topical LD50 was around 4 times field rate. The topical LD50 for cyantraniliprole was also higher than field rate doses of this product. Further testing of this product would be beneficial, in particular because of its reported repellency to *N. cucumeris*. Lambda cyhalothrin was damaging to both *N. cucumeris* and *A. andersoni* even at very low rates and should not be used where biocontrol with predatory mites is an important strategy.

Conclusions

- The rearing method was modified after testing different food sources for the predatory mites, to enable mites to be produced in larger quantities. However, even with more frequent application of food, this modified culture technique did not produce the large numbers of mites required for multiple insecticide selections
- Discriminatory concentrations were derived and then applied to the mites in attempts to increase the tolerance of populations of *A. andersoni* and *N. cucumeris* to spinosad. However, results with *N. cucumeris* showed no significant increase in tolerance after two selections with the equivalent of field rate of spinosad
- For both *Amblyseius andersoni* and *Neoseiulus cucumeris* an LD50 for spinosad was derived in residual assays, but with low numbers of mites. Results suggested that the dose required to kill 50% of the individuals tested was over 2 times the field rate for both species. This indicates that spinosad is relatively harmless to *N. cucumeris* and *A. andersoni*, and that released populations may survive field rate of this insecticide. Potential effects of the product on fecundity were not assessed
- Direct topical applications of spinosad indicated that the LD50s for this product for both *N. cucumeris* and *A. andersoni* were greater than field application rates
- Direct topical applications of cyantraniliprole indicated that the strain of *A. andersoni* tested was more tolerant than the *N. cucumeris* strain tested, with an LD50 of greater than twice field application rate
- Residual and topical applications of lambda cyhalothrin were toxic to *N. cucumeris* and *A. andersoni*.

Knowledge and Technology Transfer

A summary of the project and initial results were presented at the AHDB meetings on November 26th 2014 and November 25th, 2015.

References

Bonafos, R., E. Serrano, P. Auger, and S. Kreiter. (2007). Resistance to deltamethrin, lambda-cyhalothrin and chlorpyriphos-ethyl in some populations of *Typhlodromus pyri* Scheuten and *Amblyseius andersoni* (Chant) (Acari: Phytoseiidae) from vineyards in the south-west of France. Crop Protection 26: 169-172.

Bostanian, N.J., Belanger, A. (1985). The toxicity of three pyrethroids to *Amblyseius fallacis* (garman) acari. Phytoseiidae and their residues on apple foliage. Agriculture, Ecosystems & Environment 14: 243-250.

Cini, A., Ioriatti, C., Anfora, G. (2012). A review of the invasion of *Drosophila suzukii* in Europe and a draft research agenda for integrated pest management. Bulletin of Insectology 65: 149-160.

Cuthbertson, A.G., Mathers, J.J., Croft, P., Nattriss, N., Blackburn, L.F., Luo, W., Northing, P., Murai, T., Jacobson, R.J., Walters, K.F. (2012). Prey consumption rates and compatibility with pesticides of four predatory mites from the family Phytoseiidae attacking *Thrips palmi* Karny (Thysanoptera: Thripidae). Pest Management Science 68: 1289-95.

Garthwaite, D.G., Hudson, S. Barker, I., Parrish, G., Smith, L. & Pietravalle, S. (2013). Pesticide Usage Survey report 251. Soft fruit in the United Kingdom. London. Defra.

Goleva, I., Rubio Cadena, E.C., Ranabhat, N.B., Beckereit, C., Zebitz, C.P. (2015). Dietary effects on body weight of predatory mites (Acari, Phytoseiidae). Experimental & Applied Acarology 66(4): 541-53.

Grassi, A., Palmieri, L., Giongo, L., (2012). *Drosophila* (*Sophophora*) *suzukii* (Matsumura), new pest of soft fruits in Trentino (North-Italy) and in Europe.- IOBC/WPRS Bulletin 70: 121-128.

Jones, T., Scott-Dupree, C., Harris, R., Shipp, L., Harris, B. (2004). The efficacy of spinosad against the western flower thrips, *Frankliniella occidentalis*, and its impact on associated biological control agents on greenhouse cucumbers in southern Ontario. Pest Management Science 61: 179-185.

Kaplan, P., Yorulmaz, S., Ay, R. (2012). Toxicity of insecticides and acaricides to the predatory mite *Neoseiulus californicus* (McGregor) (Acari: Phytoseiidae). International Journal of Acarology 38: 699-705.

McMurtry, J.A., Croft, B.A. (1997). Life-styles of phytoseiid mites and their roles in biological control. Annual Review of Entomology 42: 291–321.

Nomikou, M., Janssen, A., Schraag, R., Sabelis, M.W. (2001). Phytoseiid predators as potential biological control agents for *Bemisia tabaci*. Experimental & Applied Acarology 25(4): 271-91.

Overmeer, W.P.J (1985). Rearing and handling. In: World crop pests: spider mites. Their biology, natural enemies and control, Vol. 1B. Eds. Helle, W. & Sabelis, M.W. Elsevier Science Publishers: 161-170.

Poletti, M. Omoto, C. (2005). Variabilidades inter e intraespecífica na suscetibilidade de ácaros fitoseídeos à deltametrina em citros no Brasil. ManejoIntegrado d e Plagas y Agroecología (Costa Rica) 75: 32 -37.

Sato, M.E., Miyata, T., Kawai, A., Nakano, O. (2000). Selection for resistance and susceptibility to methidathion and cross resistance in *Amblyseius womersleyi* Schicha (Acari: Phytoseiidae). Applied Entomology and Zoology 35: 393-399.

Solomon, M.G., Easterbrook, M.A., Fitzgerald, J.D. (1993). Mite-management programmes based on organophosphate-resistant *Typhlodromus pyri* in UK apple orchards. Crop Protection 12: 249–254.

Solomon, M.G., Fitzgerald, J.D (1993). Orchard selection for resistance to a synthetic pyrethroid in organophosphate—resistant *Typhlodromus pyri* in the UK. Biocontrol Science and Technology 3: 127-132.

Villanueva, R.T., Walgenbach, J.F. (2005). Development, oviposition, and mortality of *Neoseiulus fallacis* (Acari: Phytoseiidae) in response to reduced-risk insecticides. Journal of Economic Entomology 98: 2114-2120.

You, Y., Lin, T., Wei, H., Zeng, Z., Fu, J., Liu, X., Lin, R., Zhang, Y. (2016). Laboratory evaluation of the sublethal effects of four selective pesticides on the predatory mite *Neoseiulus cucumeris* (Oudemans) (Acari: Phytoseiidae). Systematic and Applied Acarology 21: 1506-1514.

Zhao, H., Yi, X., Deng, Y., Hu, M., Zhong, G., Wang, P. (2013). Resistance to fenpropathrin, chlorpyriphos and abamectin in different populations of *Amblyseius longispinosus* (Acari: Phytoseiidae) from vegetable crops in South China. Biological Control 67: 61-65.

