Project title: Selection of strains of predatory mites that can survive applications of insecticides required for SWD control

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GROWER SUMMARY

Headline

- Progress is being made to develop increased tolerance in *Amblyseius andersoni* and *Neoseiulus cucumeris* to Tracer (spinosad).

Background and expected deliverables

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

The effective use of predatory mites relies on their careful integration with spray programmes of traditional crop protection products to maintain their numbers in the crop, as predatory mites are generally considered to be more vulnerable to control products than pest species. This forms part of Integrated Pest Management (IPM) and has worked very successfully until now. However, this situation is changing with the arrival and establishment of spotted wing drosophila (SWD, *Drosophila suzukii*).

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 spotted wing drosophila (Cini *et al* 2012) and outbreaks of SWD will lead to increased use of crop protection products, as no IPM solution yet exists. The product groups which have been found to be effective against SWD around the world are organophosphates (eg. chlorpyrifos), spinonsyns (eg. spinosad) and synthetic pyrethroids (eg. deltamethrin, lambda-cyhalotrhin). Organophosphates are used for SWD control in the USA (for example, http://www.fruit.cornell. edu/spottedwing/pdfs/ BerrySWDinsecticide management.pdf), but their use in Europe is generally discouraged.

The arrival of SWD has presented growers with the dilemma of whether to ignore SWD damage, or spray against SWD and risk loss through other pests when predators are killed. One solution may be the selection of predatory mites for resistance to such products. These predators would be commercially available to growers when required.

In this project, we aim to develop insecticide resistant phytoseiid mites that growers can use whilst controlling SWD with crop protection products, to allow biological control of spider mites and thrips to continue.

Summary of the project and main conclusions

Amblyseius andersoni was selected as the initial subject as it gives good control of spider mites and is considered a “native” species for authorisation for any subsequent use on non-glasshouse crops.

Commercially available *A. andersoni* were obtained and assessed for their susceptibility to spinosad (Tracer) and lambda-cyhalothrin (Hallmark). They were found to be highly susceptible to lambda-cyhalothrin, but less so to spinosad and so selection commenced with spinosad. A population of selected mites was obtained and results indicated that tolerance to spinosad had increased.

Another predatory mite species, *Neoseiulus cucumeris*, again a widely used “native” species, was treated in the same way to derive a population of spinosad tolerant mites.

Later research focused on developing a method to increase the selected populations to derive large numbers for future work.

This work is ongoing.

Financial benefits

UK horticulture utilises Integrated Pest Management for control of many pests. However, if increased control product usage is required for new threats such as spotted wing drosophila, then predator numbers will be reduced, and other pests such as spider mites are likely to thrive. The answer to this is to develop predators that are already tolerant to control products and capable of release into regimes of higher product doses. A model for this would be 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).

Action points for growers

- When resistant strains have been successfully developed, discussion will be held with biocontrol companies to develop their commercialisation.

SCIENCE SECTION

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 insecticide for use in integrated pest management.

Choice of species is important. The choice is wide, for instance, Syngenta Bioline currently produce seven predatory mites suitable for use on strawberries or raspberries; *Amblyseius andersoni*, *barkerii*, *montdorensis*, *Hypoaspis miles, Neoseiulus californicus*, *cucumeris*, and *Phytoseiulus persimilis*. However, regulatory restrictions on non-“native” species such as *Neoseiulus californicus* reduce their potential, even if these are collected from a UK crop, whilst differences in ease of culture and commercial viability also apply.

The greatest threat to phytoseiid mites will come from increased use of pyrethroids and spinosad. Lambda cyhalothrin is recommended for SWD control in many countries, and indeed in a trial of various insecticides in the Trento region of Italy only lambda cyhalothrin gave adequate control (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 also be valuable for growers to have spinosad compatible predators available.

Reports of small populations that have survived insecticide treatments show the potential for considerable increases in resistance. For example, natural field selection of *Typhlodromus pyri* has produced organophosphate resistant populations capable of pest control (Solomon *et al.,* 1993), whilst *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, for *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). Careful field selection of *Typhlodromus pyri* at East Malling produced *Typhlodromus pyri* resistant to pyrethroids (Solomon & Fitzgerald, 1993).

However, naturally occurring tolerance of insecticides at these levels is comparatively rare, and very unlikely to generate sufficient numbers for bio control. Even when present, populations are diluted by immigration of susceptible mites as soon as selection pressure is eased. For reliable control growers will require a readily available source of pesticide resistant predatory mites for release into crops; generation of these mites is the aim of this project.

Materials and methods

Choice of species

Amblyseius andersoni was chosen as it gives good control of spider mites and is considered a “native” species for authorisation for any subsequent use on non-glasshouse crops. Species such as *Neoseiulus californicus*, which might have been potential candidates, and are found in UK orchards, would nonetheless have been unlikely to get regulatory approval for use, as they are officially “non-native”.

Neoseiulus cucumeris was chosen as it is recommended for control of thrips and tarsonemid mites and again is considered a “native” species for authorisation purposes.

Mite sources

Mites were purchased from a commercial supplier and used for initial trials and also subsequent selections.

A secondary source of *N. cucumeris* was also assessed. However, these proved very susceptible to spinosad (see below) and the biocontrol company was anyway ceasing production so this approach was discontinued.

Culture

Arenas

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. Cotton wool fibres under coverslips served as shelter and oviposition sites. As a further guard against cross contamination, a sticky gel (Oecotak, Oecos Ltd., Kimpton, UK) was placed along the ridge of the boxes. Any capture of mites by the gel was monitored and found to be minimal (data not shown).

Cultures were reared in CT rooms set to 20 ^{o}C, on a 16 hr light/ 8 hour dark cycle. Incubators had air circulation, but not humidification.

Predator populations were fed with Nutrimite (Biobest, Westerlo, Belgium), a commercially available pollen source from *Typha*, marketed for feeding predatory mites.

Figure 1. Mite rearing arena

Large-scale culture

Because the arena method was not producing sufficient mites for further selection a commercial company, the one which supplied the original stock, was approached to utilise their patented mite rearing method. This they kindly agreed to do and it was projected that greatly increased numbers should soon be available. As their method is commercially sensitive it cannot be described here. 50 selected (spinosad) mites were sent to the company.

Application of insecticide for bioassay

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 unlikelihood of authorisation for organophosphates, we focused on spinosad (in the form of Tracer, (Dow Agrosciences Ltd., Hitchin, UK), and a pyrethroid, lambda cyhalothrin, in the form of Hallmark (Syngenta UK Ltd., Cambridge, UK).

The initial proposal was to apply insecticide using a Burkard sprayer as has been used successfully on other species such as *Drosophila suzukii*. This machine passes a jet of spray droplets onto a surface below. However, it was found that the spray action of the machine blew the some of the mites from the dish. Consequently, another technique was developed, using a modified method of Sato *et al* (2000).

Papers were soaked in pesticide solution, placed in a 9cm Petri dish and left to dry. Water was used as a control and each treatment was replicated four times. Oecotak was used to ensure the mites stayed within the dish and shelters consisting of coverslips over cotton threads were provided (Figure 2). Mites (8 adults) were added and mortality was assessed after 24 hours, by touching the mites, with those that did not respond being counted as dead.

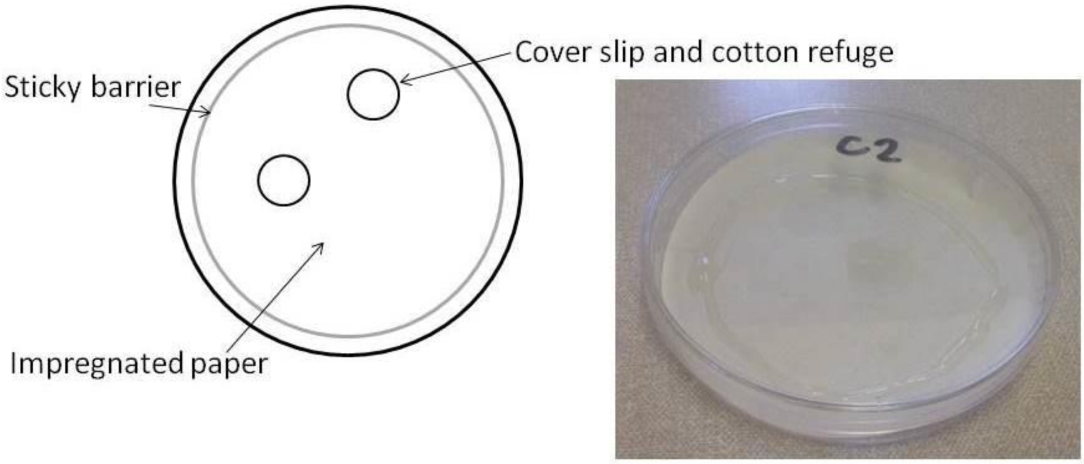

Figure 2. Design of the system used for exposing mites to insecticide, based on a 9cm Petri dish with pesticide impregnated paper and a sticky barrier

Initial bioassays aimed to determine a suitable dose for selection and were based on the recommended field dose. Thus lambda cyhalothrin (Hallmark) was tested at a field rate of 0.125 ml/l, 0.1x field rate (0.013 ml/l) and 0.01x field rate (0.0013 ml/l). Spinosad was tested at field rate (0.15 ml/ l) and 0.1x field rate (0.015 ml/l).

Selection for resistance

All trials used the same, commercially available, source populations. The method was similar to that used for bioassays above, except that controls were in duplicate, whilst selection dishes contained variable numbers of adults and immatures in order to maximise the number of mites exposed on each occasion. Survivors were transferred to the arenas described above for rearing.

Secondary selection of the selected population used the same discriminating dose.

Population monitoring

To assess if the insecticide exposure was impacting on long term survival *A. andersoni* were exposed to the selection procedure above but with or without insecticide. Both populations were then cultured using the arena method and their numbers monitored over time.

Individual cultures

To investigate the effect of selection on fertility individual male and female *A. andersoni* have been separated from the population and reared on "mini" arenas for one week. Ie. segments of a normal arena divided with wet filter paper and Oecotak, and supplied with a ovipostion site, cover and pollen. This trial is ongoing.

Tyrophagus putrescentiae culture

T. putrescentiae is a mite and pest of stored cereals. It is also supplied with some commercially produced predatory mites as a prey source to maintain populations during transport and storage. It was therefore considered valid to attempt to culture this mite to supplement the predatory mites cultures.

A culture method was developed based on Ree & Lee (1997). Mites were transferred to a pot containing 45 g ground oatmeal and 5 g yeast and this pot was stored within a larger box with a small mesh opening and wet tissue to maintain humidity.

Results

A. andersoni

Determination of selection dose for *A. andersoni*

Lambda cyhalothrin

The population of *A. andersoni* was found to be highly susceptible to lambda cyhalothrin, with 100% mortality at Field and 0.1x Field doses (0.125 and 0.0125 ml/l). A further ten-fold dilution (0.01x Field dose, 0.00125 ml/l) gave a mortality of 76% (Figure 3).

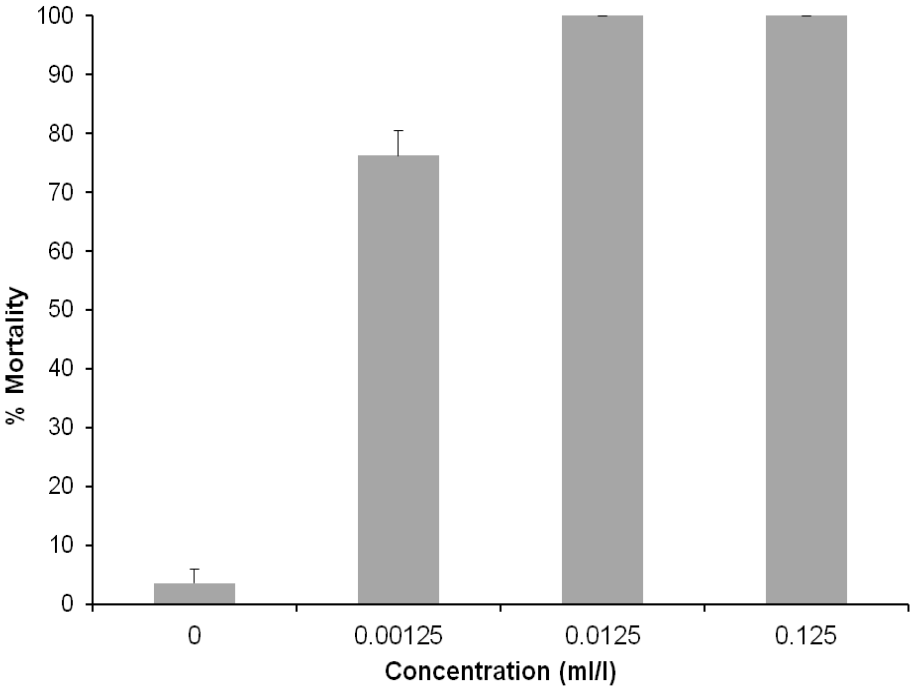

Figure 3. Mortality of *A. andersoni* adults from lambda cyhalothrin after 24 hours

Spinosad

The results of bioassays on *A. andersoni* with spinosad are given in Figure 4. A dose equivalent to that recommended for field use, 0.15 ml/l, gave 28% mortality after 24 hours. This dose was chosen for further resistance selection trials.

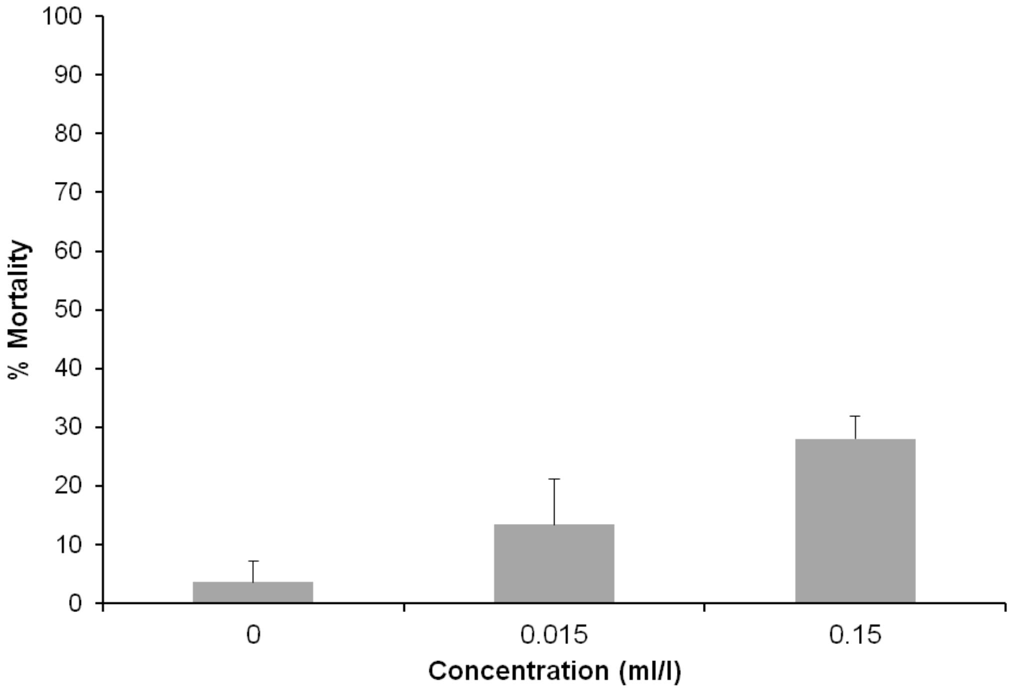

Figure 4. Mortality of *A. andersoni* adults from spinosad after 24 hours

Selection of *A. andersoni*

Because of the high susceptibility of the population to lambda cyhalothrin it was decided to focus on selection with spinosad.

Overall selection bioassays with the dose of 0.15ml/l were run on six separate occasions, exposing a total of 224 adult mites and 42 immatures with an average mortality rate of 29% for adults and 74% for immatures.

In addition a further two assays used a dose of 0.3 ml/l on 59 adult mites with an average mortality rate of 47%.

A second challenge of the population selected with spinosad (n=36) with the same dose gave an average mortality of 17%.

Population growth of *A. andersoni*

The population development of two control populations of *A. andersoni* and a population selected with spinosad (Tracer) are given in Figure 5a&b.

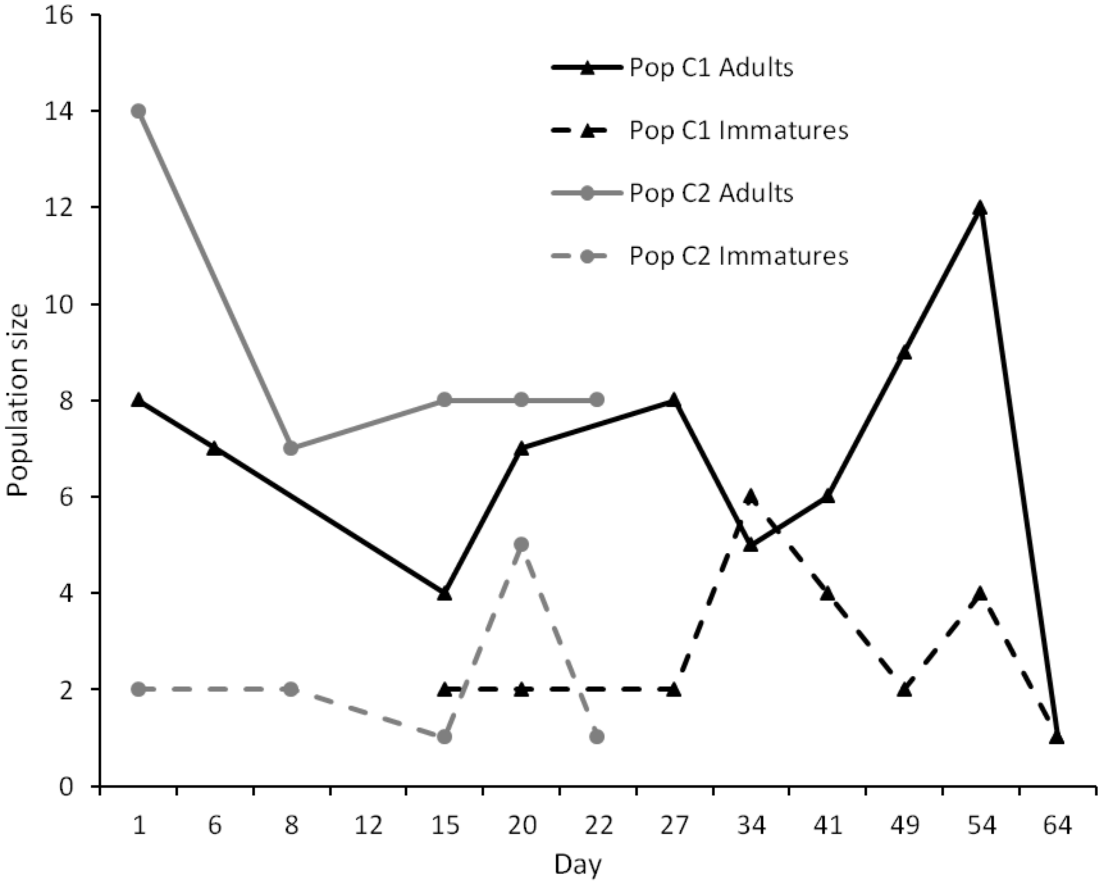

Figure 5a. Population development of *A. andersoni* (controls) over 64 days. Control populations 1 (black lines) and 2 (grey lines). Adults with solid lines, immature mites in dotted lines.

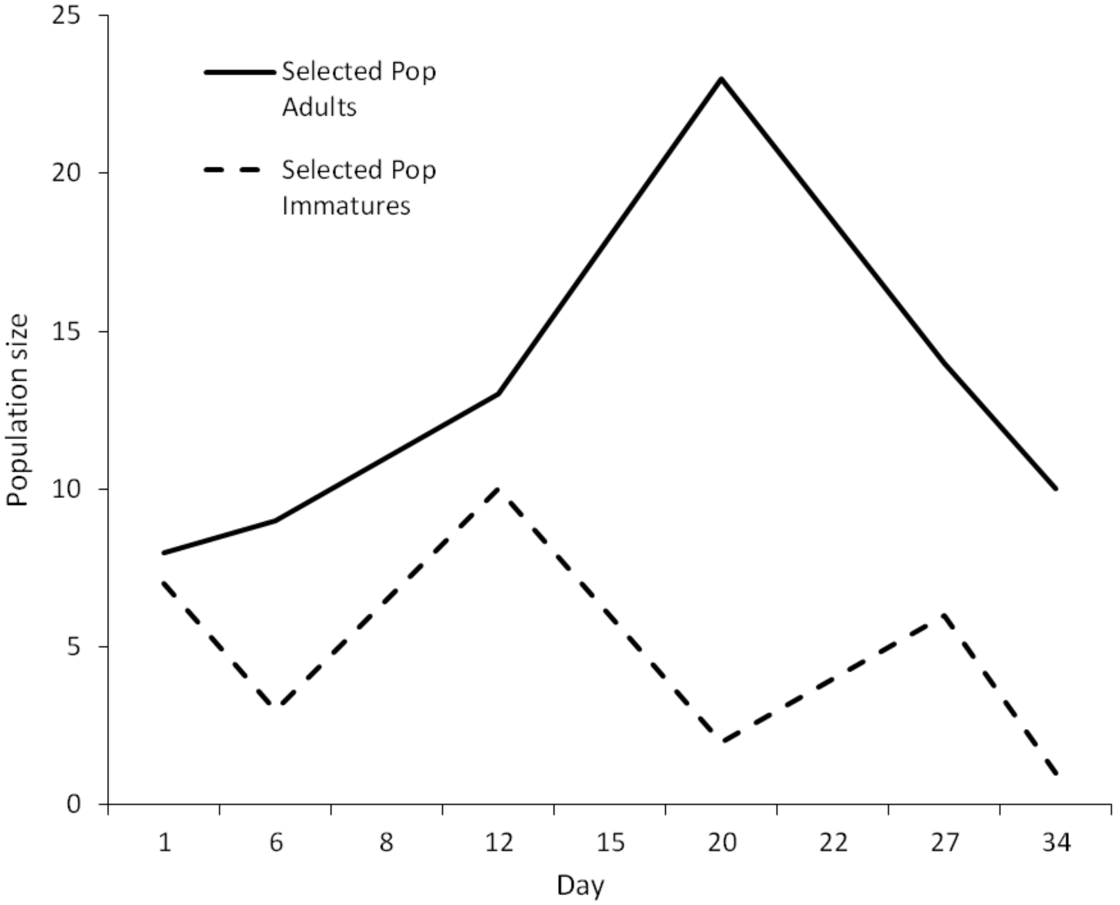

Figure 5b. Population development of *A. andersoni* selected with spinosad over 34 days. Adults with solid lines, immature mites in dotted lines

Large-scale cultures of *A. andersoni*

Unfortunately, the biocontrol company could not amplify the *A. andersoni* population sent to them, even though the original stock was from their cultures. Fecundity was sufficient to maintain numbers, but not increase the population, whereas the technique used should have led to rapid multiplication. Although details are not available it is known that various techniques were tried, including arenas with pollen very similar to section 2c (i) in the Methods section.

Individual arenas

This trial is ongoing, but initial results are displayed in Table 1.

Table 1. Production of eggs over one week of isolated female *A. andersoni*

	Females	**Eggs**	**Immatures**
Controls	16	5	0
Selected	14	6	6

N. cucumeris

Determination of selection dose for *N. cucumeris*

The results of bioassays on *N. cucumeris* with spinosad are given in Figure 6. A dose equivalent to that 2x recommended for field use, 0.3ml/ l, gave a predicted 38% mortality after 24 hours. This dose was chosen for further resistance selection trials.

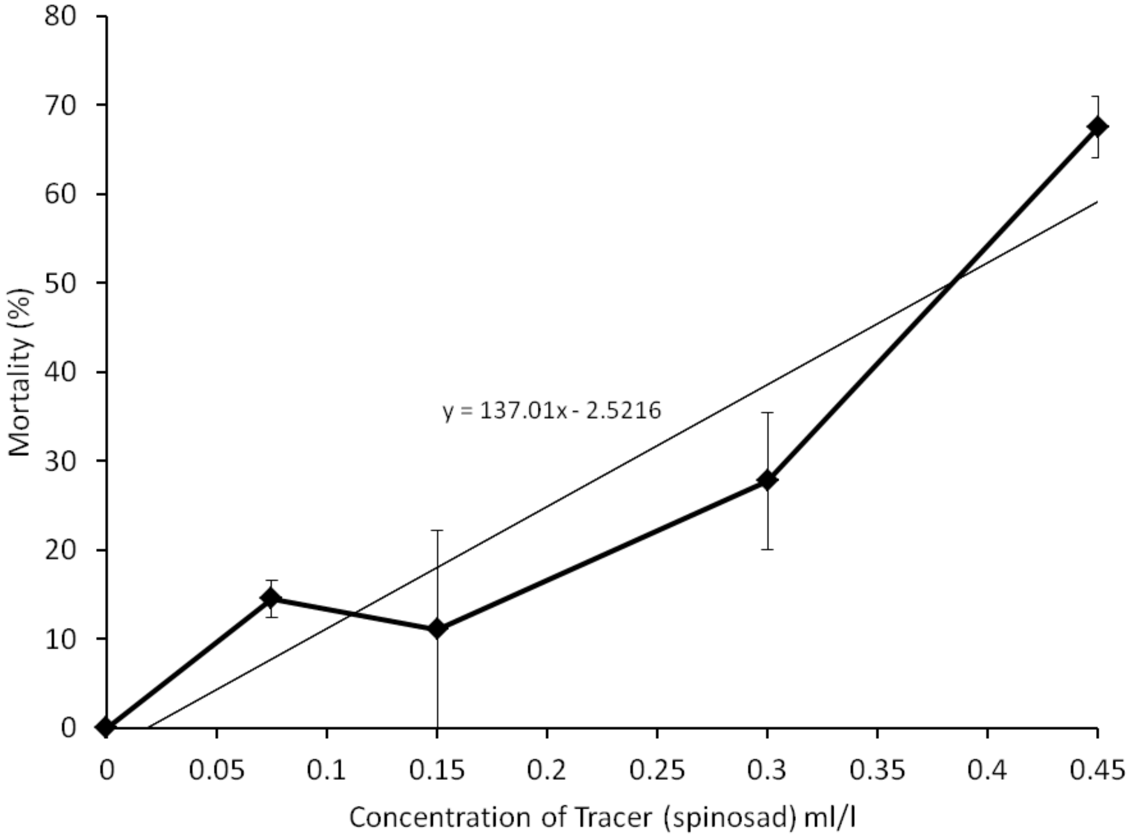

Figure 6. Mortality of *N. cucumeris* adults from spinosad after 24 hours

Selection of *N. cucumeris*

N. cucumeris (n=65) were challenged with a dose of 0.3ml/l spinosad (Tracer). The overall survival rate was 38.5% (males, 24%; females 50%). Survivors were transferred to fresh arenas for rearing.

Bioassay of *N. cucumeris* from biocontrol company B

N. cucumeris (n=43) were challenged with a dose of 0.3ml/l spinosad (Tracer), the same dose applied to the population from biocontrol company A. However, the mortality rate was considerably higher at 83% (males, 76%; females 94%).

As biocontrol company B was discontinuing production of *N. cucumeris* no attempt was made to culture this population.

Population growth of *N. cucumeris*

The population of selected *N. cucumeris* is given in Figure 7.

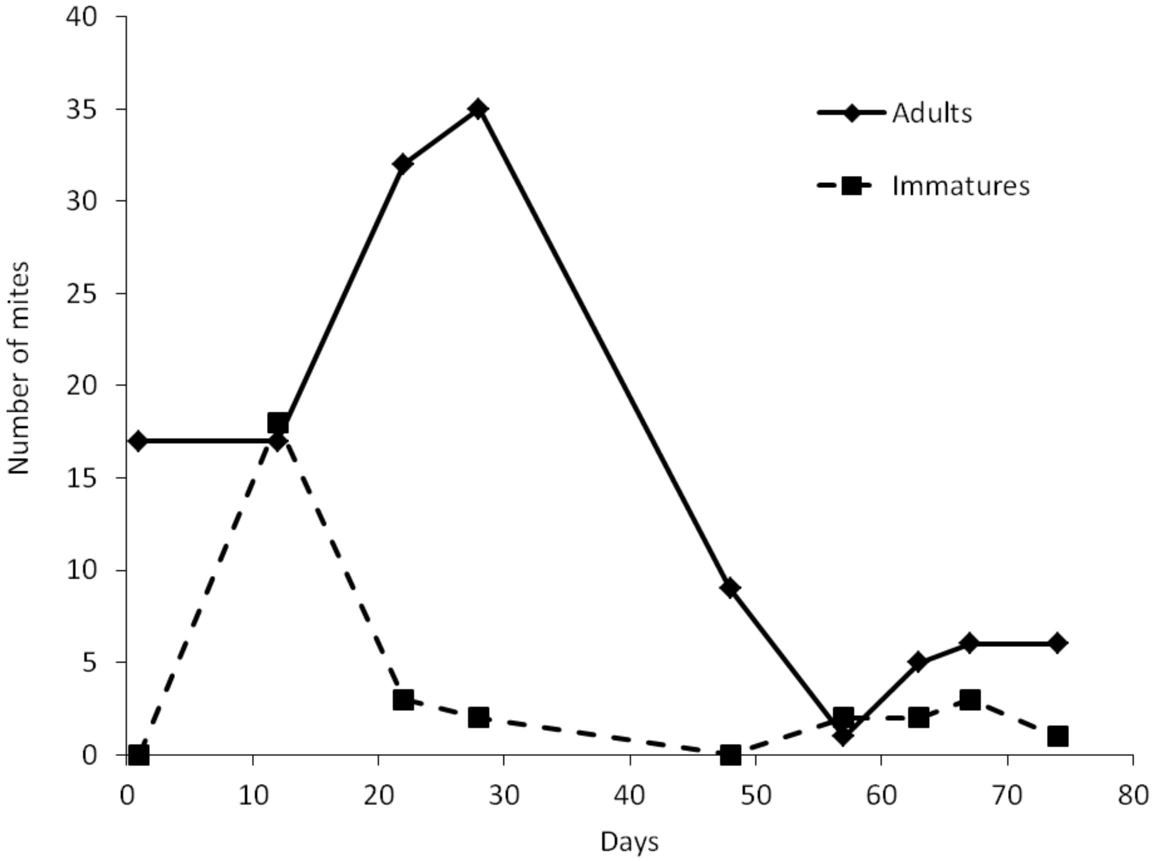

Figure 7. Population development of *N. cucumeris* adults selected with spinosad over 74 days.

Despite an initial fertility as shown by the appearance of immature mite stages, the population reduced to very low numbers within 60 days.

Discussion

Commercially available populations of *Amblyseius andersoni* were assessed for susceptibility to lambda cyhalothrin and spinosad to ascertain their suitability for development into insecticide tolerant strains. They were found to be extremely susceptible to lambda cyhalothrin, with 100% mortality at doses much lower than those likely to be found in the field. This is in agreement with trials on phytoseiid mites in the literature (for example, Solomon *et al*.,1993, Bostanian & Belanger, 1985).

In contrast, spinosad killed an average of 28% of adult mites at field rates. It was therefore felt that selection with spinosad offered a more likely route to successful selection in the short to medium term. The dose selected for selection was that equivalent to the field rate as this was both sufficiently toxic to remove susceptible individuals from the population and was relevant to the natural situation. Both adults and immatures were exposed to the selecting dose. On average, 29% of adults were killed, the survivors being cultured. In contrast 74% of immatures were killed. This differential effect on different life history stages has been found with other insecticides (for example, Kaplan *et al*., 2012). Mortality of the selected population of *A. andersoni* after a further application of the same selecting dose of spinosad was 17% in contrast to 28% for the unselected population (reported in year 1).

Following identification of this selecting dose, multiple assays (n=6) were run to derive a population of spinosad tolerant individuals. At the same time a similar process was undertaken with *Neoseiulus cucumeris*, which were found to require a higher selection dose. Again multiple assays (n=3) were run to derive a population of spinosad tolerant individuals. Survivors of both species were reared on standard arenas.

Although the rearing arenas could maintain population numbers over some months the population increases necessary for producing viable commercial strains of either species did not occur. Consequently the selected strain of spinosad tolerant *A. andersoni* were sent to a commercial biocontrol company to bulk up numbers. Again this proved difficult to achieve, even though this company supplied the original mites.

A population of *N. cucumeris* from another company was also investigated, but apart from appearing more sensitive to spinosad, the culture was still difficult to maintain over time, and as this company decided to discontinue selling the mite this culture was discontinued.

It was difficult to see why the populations did not thrive in the long term. Extensive discussion with different groups around the world yielded only small changes in technique compared to that used. The food provided was one possibility but *Amblyseius andersoni* have been shown to rear successfully with *Typha* pollen (for example, Ahmad *et al*., 2015).

Following discussion with representatives of the biocontrol company, EMR and the AHDB it was decided to determine any possible fertility effects of insecticide selection.

Assays were run on *A. andersoni* to select tolerant individuals and these were compared to individuals that had been through the same process but without insecticide. Initial data failed to show a negative effect on egg production of spinosad selection, in fact there was a slight increase in fertility. This is possibly because the smaller and weaker mites are more susceptible to pesticide exposure and thus selected mites are more fertile. A similar effect has been found with the mosquito *Aedes aegypti* when exposed to spinosad (Antonio *et al*., 2009). However, if spinosad tolerance had a fitness cost this would be expected to impact on population numbers over time, but both cultures could not be maintained over a sufficient period.

Initial cultures of single mites of both species have shown no difference in fertility, but this work is in its early stages. A new culture technique, using a prey mite *Tyrophagus putrescentiae* is also being developed. Also, a visit to the Department of Agronomy, Food, Natural Resources, Animals, Environment (DAFNAE) at the University of Padua, to one of the major mite research groups in Europe, is planned.

Conclusions

- For both *Amblyseius andersoni* and *Neoseiulus cucumeris* a discriminatory dose was derived and then applied to obtain spinosad tolerant populations.
- It has not proved possible, using current rearing techniques, to produce large quantities of mites for future selection.
- The rearing method is being modified to enable mites to be produced in large quantities

Knowledge and Technology Transfer

A summary of the project and initial results was presented at the EMR/AHDB Soft Fruit Day meeting on 25 November 2015.

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