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Selection for resistance to acequinocyl in *Amblyseius andersoni* (Chant) (Anactinotrichida: *Phytoseiidae*)

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Abstract: *Selection for resistance to acequinocyl in* Amblyseius andersoni (*Chant*) (*Anactinotrichida:* Phytoseiidae). The majority of pesticides used in pest control in orchards are found to be harmful to beneficial arthropods. However, the development of resistant predators and parasitoids could contribute to their enhanced use in crops where the use of pesticides is necessary. The goal of the current work was to select a line of predatory mite *Amblyseius andersoni* resistant to acequinocyl acaricide belonging to a group of mitochondrial complex III electron transport inhibitors. A selective dose used in experiments was that causing 55–65% mortality of phytoseiid gravid females. A laboratory population had a nine-fold increase in resistance to acequinocyl after two selection cycles, and over 30-fold increase after four cycles. This selected population of *A. andersoni* also developed medium cross-resistance to fenpyroximate.

Key words: predatory mites, pesticides, selection, acequinocyl, fenpyroximate

INTRODUCTION

Integration of a biocontrol agent (BCA) into most agricultural systems will not be successful unless the natural enemy can survive chemical treatments in these crops (Zalom and Irigaray 2010). Thus, studies on the side-effect of pesticides on beneficial organisms as well as attempts of the selection of natural enemies resistant to synthetic insecticides and acaricides are essential for effective integrated pest management (IPM). Among the beneficial arthropods, phytoseiid mites play an important role as predators of many herbivores, especially spider mites (McMurtry and Croft 1997, Nomikou et al. 2001). While spider mites develop resistance to a number of pesticides within a short period of time, predacious mites react not so promptly (Overmeer and van Zon 1981, Hull and Beers 1985). Enhancing the phytoseiid survival after pesticides application may result in more effective control of phytophagous insects and mites (Fournier et al. 1985, Solomon et al. 1993).

Amblyseius andersoni (Chant) is a phytoseiid species commonly found on apple and peach trees in many European countries (Koveos and Broufas 2000, Stojnić et al. 2014). It was reported as an important predator of orchards' key pests, i.e. the European red spider mite (*Panonychus ulmi* (Koch)) and two-spotted spider mite (*Tetranychus urticae* Koch) (Ivancich-Gambaro 1986, McMurtry and Croft 1997, Fiedler 2009, Szabo and Penzes 2013). The resistance of *A. andersoni* to various pesticides was previously confirmed in laboratory and field investigations. Ioriatti et al. (1992) reported the *A. andersoni* strain from San Michele all'Adige (Italy) resistant to dithianon. Females of the Italian strain treated with another fungicide – mancozeb – also survived, but showed significantly reduced fecundity. Whilst Duso (1992) observed a high sensitivity of *A. andersoni* to the pyrethroids used in Italian orchards, Bonafos et al. (2007) have found strains of this predator resistant to deltamethrin and lambda-cyhalothrin in the vineyards of south-western France. Another *A. andersoni* strain from France was resistant to organophosphates, namely chlorpyriphos-ethyl (Bonafos et al. 2007). It was previously proved that, in *A. andersoni*, at least two genes responsible for pesticide resistance are operating, one causing the formation of highly insensitive acetyl cholinesterase and one encoding for an enzyme able to hydrolyse various organophosphates (Anber and Overmeer 1988, Anber and Oppenoorth 1989). Still, we do not know much about the resistance potential of *A. andersoni* to mitochondrial electron transport inhibitors (METI) (James 2002, Rodrigues and Torres 2007), an important group of acaricides. One of them – acequinocyl – is an active substance of the naphtoquinones class. Its mode of action is to inhibit electron transfer in the mitochondria of spider mites via inhibition of the Q_0 centre by acting as a structural analogue of ubiquinone (Kinoshita et al. 1999). Acequinocyl has been designated as a “reduced-risk” pesticide with low pest resistance potential and lower toxicity to non-target organisms (Tiwari 2013). However its impact on *A. andersoni* has not been studied so far. The present study was set up to assess the resistance potential of commercially available *A. andersoni* to acequinocyl (mitochondrial complex III electron transport inhibitor) and the cross-resistance of the *A. andersoni* population produced via selection pressure in the laboratory to another acaricide, fenpyroximate (mitochondrial complex I electron transport inhibitor).

MATERIAL AND METHODS

Stock colony of *Amblyseius andersoni*

The stock colony of *A. andersoni* was initiated with specimens (Anderline Pro Bioline AgroSciences Ltd., United Kingdom) obtained from Hortico SA Company (Wrocław, Poland). The colony was then maintained in the laboratory of the Section of Applied Entomology (Warsaw University of Life Sciences – SGGW) in an environmental test chamber (25 ±0.5°C, 70 ±10% RH, 16L : 8D photoperiod). Using a fine paintbrush, the predators were placed in breeding containers (18 × 15 × 7 cm). In each container a tile of black plastic (15 × 1 cm) was resting on a sponge (16 × 13 × 4.5 cm) dipped in water. Wet tissue paper strips (1 cm wide) formed a barrier around the tile. On the tissue paper a ring of insect glue (fruit tree grease Vitax®) was laid as a barrier to prevent predators escape. Several roof-shaped pieces of transparent plastic with black sewing threads underneath were placed on the tile as shelters where the phytoseiids could deposit their eggs. *Amblyseius andersoni* was fed with cattail pollen (Nutrimite®) and reared in these conditions for at least four generations before the start of the experiment.

Acaricides and bioassay unit

Two commercial METI acaricides were used: Kanemite®150 SC with acequinocyl as an active ingredient (14.42%;

150 g·L^{-1}) (Cheminova Deutschland GmbH & Co. KG, Germany) and Otrus®05 SC with fenpyroxymate (5.02%; 51.2 g·L^{-1}) (Nichino Europe Co., United Kingdom). Each bioassay unit consisted of a detached been leaf placed upside-down on a Plexiglas plate (10 × 5 × 0.3 cm) covered with four--times folded wet filter paper. The four--centimetre diameter ring of sticky barrier (Vitax®) was provided on each leaf, to prevent the predators from escaping.

Toxicity tests

The method of Sato et al. (2000) was modified for selection studies. Four days before the test a mass of *A. andersoni* deutonymphs was transferred to a separate breeding container (prepared as described previously). After two days, males were added to the container for 24 h, to provide mating opportunities to the newly emerged females. Only gravid females were then chosen for the experiments. Ten *A. andersoni* females were transferred on each bean leaf arena before planned sprayings. At least 400 females were used in each selection. As according to Irigaray and Zalom (2006), pesticides mixed with distilled water were applied using a 200-millilitre hand sprayer held 30 cm away from the leaf surface. The untreated controls were sprayed with distilled water alone. Females were left on the bean leaves for three days after spraying, incubated in the environmental test chamber (25 ±0.5°C, 70 ±10% RH, 16L : 8D). The next day after spraying, cattail pollen was provided into arenas as food for survivors. Individual mite survival was determined 72 h after treatment by light touching each specimen with a fine brush. Phytoseiids unable to react were considered dead.

The maximum concentration of tested acaricides was used, corresponding to those suggested by producers for field application: 561 mg ai·L^{-1} for acequinocyl (recommendations from 2014–2015, nowadays 295–280 mg ai·L^{-1}) and 128 mg ai·L^{-1} for fenpyroximate. To determine LC_{50} values of the selected and initial populations of *A. andersoni* a blank control and seven acequinocyl or three fenpyroximate doses were prepared with a one-half serial dilution series.

Selection for resistance

According to Sato et al. (2000), a selective pesticide concentration (initiating selective pressure towards predator's resistance to tested pesticide) is that causing mortality from 55 to 65% of treated individuals. Therefore, 72 h after each spraying, females from the combination in which 35–45% of treated individuals survived were transferred to a new breeding container (prepared as described previously). Females and their progeny were rearing in the container kept in the environmental test chamber (25 ±0.5°C, 70 ±10% RH, 16L : 8D) and fed with cattail pollen. The second selection spraying was after 7–9 days, which corresponded to the period required for reaching maturity of the progeny (F1) of selected females. The spraying procedure was similar to that described in the "Toxicity tests" section. As there was a decrease in female fecundity in the F1 generation, further spraying intervals were adopted from 21 to 27 days. This period, corresponding to the required time for approximately three generations, allowed a sufficient number of individuals (in F4, F7, F10) to be tested.

Cross-resistance tests

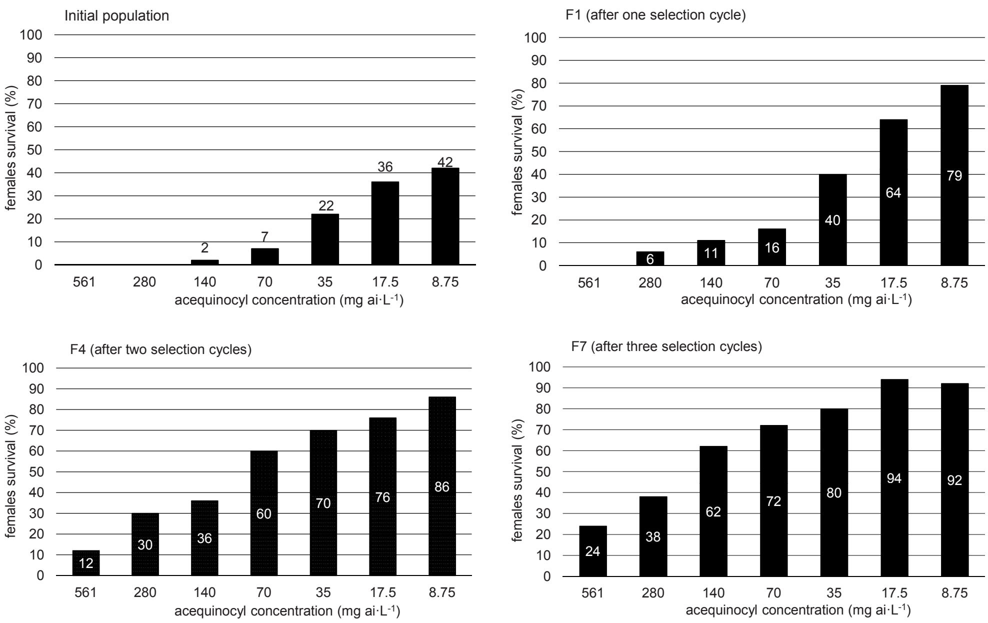
To determine the effect of cross-resistance of the selected *A. andersoni* population to fenpyroximate, females in the acequinocyl selected F11 generation as well as those in the initial population were sprayed with three concentrations of this acaricide (procedure described in the "Toxicity tests" section).

Data analyses

The concentration-response data were subjected to probit analysis (LeOra Software 1994). The response of each generation was considered different if the 95% confidence limits at the LC_{50} did not overlap. The survival values of females were corrected by Abbott's formula (1925). The Mann–Whitney test for comparing the survival of females in the initial and selected populations was used. The significance level for all analyses was 0.05. Statistical elaboration was performed in the statistical software package PAST version 2.02 (Hammer et al. 2010). Percentage data were arcsine transformed for ANOVA. Resistance ratios (RR) were obtained by dividing the LC_{50} values of the selected populations by the LC_{50} value of the initial population.

RESULTS AND DISCUSSION

Integration pesticides and the biocontrol of mites in agricultural systems should comprise practices such as: use the least disruptive pesticides for pest control, monitoring for spider mites, monitoring for predators (including phytoseiids), use of economic thresholds, considering predator-prey ratios before applying pesticides and employing releases of pesticide-resistant predators when augmentation is needed (Hoy et al. 1982, Zalom and Irigaray 2010). In augmentative biological control, naturally or artificially selected natural enemies that are resistant to pesticides can be used. In the current work, the initial (commercially available) population of *A. andersoni* was exposed to four selection cycles to determine the development of acequinocyl resistance. The subsequent steps of this process resulting in mortality changes of tested females are shown in Figure 1. These data were used to estimate the LC_{50} values of each generation (the table). In the initial population, the lethal acequinocyl concentration causing a 50% mortality of the tested *A. andersoni* females was 8.78 $mg \cdot L^{-1}$. In the selected F1 generation, the LC_{50} value increased to 25.34 $mg\ ai \cdot L^{-1}$ showing a threefold increase of resistance to acequinocyl compared to the initial population. After four selections for resistance, the LC_{50} value increased from 8.79 to 267.07 $mg\ ai \cdot L^{-1}$, and the resistance ratio increased by 30.4-fold (the table). The selected *A. andersoni* strain with the 30.4-fold increase of resistance to acequinocyl was named the AAP4 population. Salman et al. (2015) have shown a similar increase (32.75-fold) in resistance developed with six-time continuous selection with acequinocyl in another phytoseiid mite population – *Phytoseiulus persimilis* Athias-Henriot. Our selection was not conducted continuously, still, a significant increase in resistance was obtained. This is consistent with



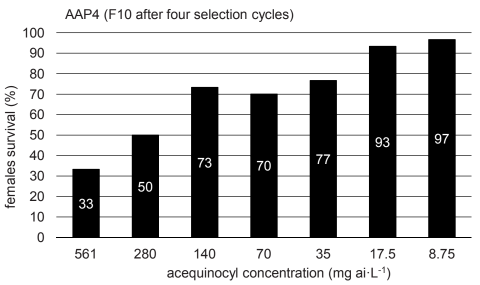


FIGURE 1. Females survival of particular *Amblyseius andersoni* populations treated with different concentrations of acequinocy

the findings of other authors who reported that the selections for pesticide resistance in *Phytoseiidae* might be efficient despite the fact that the intervals between selections are longer than one generation (Sato et al. 2000). The laboratory selection for resistance changed the susceptibility of *A. andersoni* to acequinocyl. The survival of females in the initial population and the selected AAP4 population treated with different concentrations of acequinocyl is shown in Figure 2. The significance of differences in survival of *A. andersoni* females of both populations was compared in combination with 8.75 to 140 mg ai·L^{-1} because no female in the initial population survived treatments with the pesticide in the concentration 561 mg ai·L^{-1} (a dose recommended for orchard sprayings in 2014–2015) and 280 mg ai·L^{-1} (currently recommended). Thus it can be stated that the initial population of *A. anderosni* was highly susceptible to acequinocyl. In comparison, 33% of females of the selected AAP4 population survived spraying with the highest concentration of acequinocyl and 50% the treatment with half

TABLE. Resistance ratio and LC_{50} levels determined after selection with acequinocyl from *Amblyseius andersoni* populations

Treatment	Formulation	Population	*n*	Slope ±*SE* (0.95% CL)	LC_{50} (mg ai·L^{-1}) (0.95% CL)	*RR**
Acequinocyl	Kanemite 150SC	initial population	1 200	1.61 ±0.35	8.78	–
		select-1 (F1)	800	1.64 ±0.38	25.34	2.88
		select-2 (F4)	800	1.20 ±0.30	80.45	9.16
		select-3 (F7)	400	1.25 ±0.32	183.43	20.89
		AAP4 (F10)	400	1.17 ±0.30	267.06	30.41
Fenpyroximate	Ortus 05SC	initial population	400	0.93 ±0.2	3.35	–
		AAP4	400	1.46 ±0.34	40.32	12.03

*Resistance ratio = the LC_{50} value of resistance population / LC_{50} value of the susceptible population.

of that dose (280 mg ai·L^{-1}). In the other combinations a significantly higher survival was observed in the selected population compared to the initial ($p < 0.03$). The biggest difference in females survival was noted when the concentration of 140 mg·L^{-1} of aquinocyl was used. With this treatment only 2% of females in the initial population and 73% in the selected AAP4 strain survived (Fig. 2). There is scarce information about toxicity to phytoseiid mites pesticides that similarly to acequinocyl inhibit electron transport complex III preventing the utilization of energy by mites cells. One of these substances is bifenazate, which in the full-field rate was moderately to highly toxic to *A. andersoni* and some other phytoseiids, i.e. *Galendromus occidentalis* Nesbitt and *Neoseiulus fallacis* Garman, causing 37–81% mortality of predators on hops grown in the US (James 2002). In contrast to bifenazate, acequinocyl seems to be a promising candidate for use in integrated mite management programs where *P. persimilis* or *Amblyseius womersley* Schicha are the major natural enemies. In laboratory surveys, 88% of *P. persimilis* females and 86% of *A. womersley* survived spraying with acequinocyl at 150 mg·L^{-1} (Kim and Seo 2001, Kim and Yoo 2002); however, the populations of phytoseiid mites tested by these authors originated from crops where various pesticides had been previously used, and so could be more resistant to acequinocyl than the *A. andersoni* population tested in the current study. Puchalska and Piotrowska (2016) demonstrated that acequinocyl was slightly or moderately toxic to *Typhlodromus pyri* Scheuten individuals that previously were in contact with pesticides and highly toxic to specimens that had never been under pesticide pressure.

The AAP4 *A. andersoni* population showed a medium-level development of cross-resistance to fenpyroximate (a 12.03-fold increase) – the table. Statistical analysis performed to compare the survival of *A. andersoni* females in the initial population and AAP4 population treated with the highest dose of fenpyroximate (128 mg ai·L^{-1}) did not show significant differences ($p = 0.0940$) – Figure 3. However, spraying with half

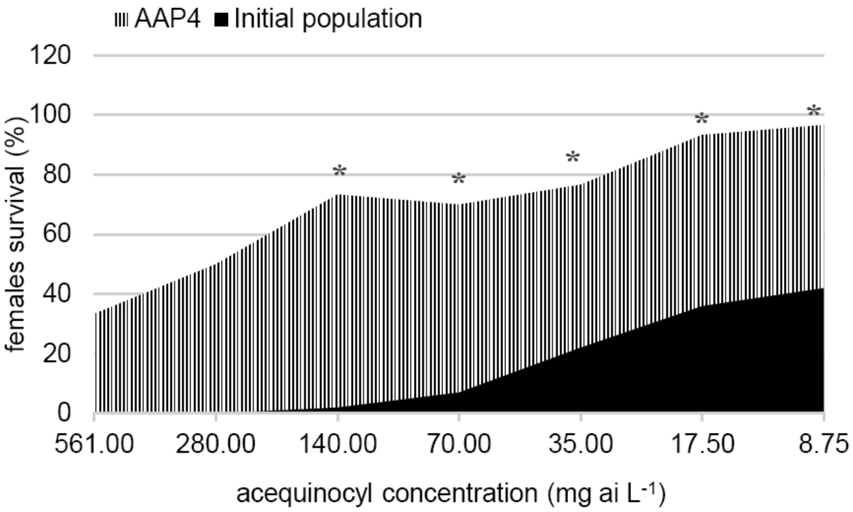


FIGURE 2. Survival of *Amblyseius andersoni* females in the initial population and laboratory selected AAP4 population treated with different concentrations of acequinocyl (an asterisk means a significant difference between populations; Mann–Whitney test; significance level 0.05)

of the dose (64 mg ai·L^{-1}) nine times more females in the acequinocyl resistant population (AAP4) survived than in the initial one (p = 0.0007). Difference was also shown in both populations' survival when sprayed with 32 mg·L^{-1} of fenpyroximate (p = 0.0261), with 14% of females in the initial population and 47% in the AAP4 population surviving (Fig. 3). The development of cross-resistance to fenpyroximate was observed despite the fact that fenpyroximate and acequinocyl bind to the mitochondrial system at different sites (complex I for fenpyroximate and complex III for acequinocyl) (Kinoshita et al. 1999). In field observations, the toxicity of fenpyroximate was assessed as slightly to moderately harmful to *A. andersoni* and *Euseius stipulatus* (Athias-Henriot) occurring in apple orchards in Portugal (Rodrigues and Torres 2007). It can be supposed that the acequinocyl and fenpyroximate impact on the AAP4 strain of *A. andersoni* in field conditions will be less harmful than shown in these studies; laboratory results give higher mortality rates because of a higher exposure of females to pesticide under experimental bioassay than in field conditions where phytoseiids may disperse to untreated plant surfaces (Bonafos et al. 2007). Nevertheless, field trials verifying this hypothesis are needed.

CONCLUSIONS

Amblyseius andersoni is one of the most promising BCAs of spider mites occurring in European fruit crops (Caccia et al. 1985, Ivancich-Gambaro 1986, Croft et al. 1993, Fiedler 2009, Szabo and Penzes 2013), therefore the development of the predator strain resistant to acaricides commonly used in such crops

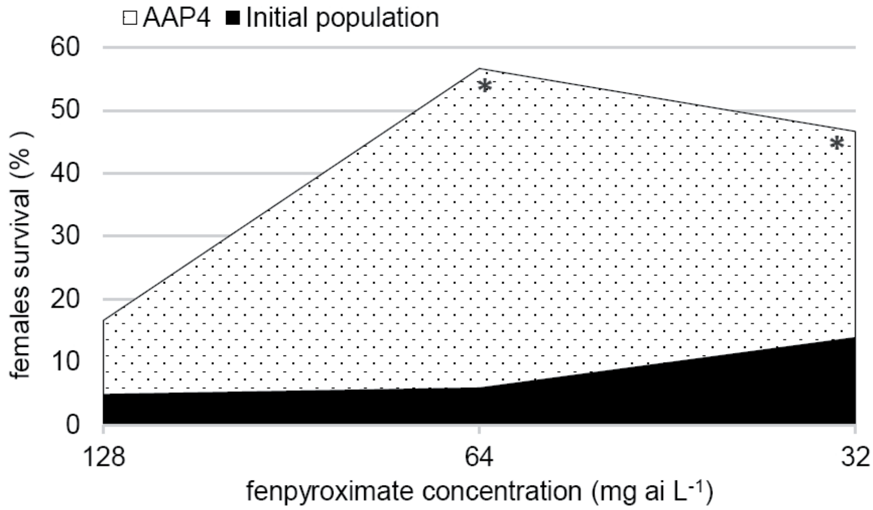


FIGURE 3. Survival of *Amblyseius andersoni* females in the initial population and laboratory selected AAP4 population treated with different concentrations of fenpyroximate (an asterisk means a significant difference between populations; Mann–Whitney test; significance level 0.05)

may increase its implementation in IPM systems. Current work has proved that *A. andersoni* specimens commercially available as Anderline Pro product, are highly susceptible to some METI type acaricides such as acequinocyl and fenpyroximate, which limits the chance of using these pesticides in crops where *A. andersoni* were introduced. The treating gravid females of the predator with a selective pesticide concentration causing mortality from 55 to 65% of the tested individuals seems to be an effective method of selecting an *A. andersoni* strain resistant to acequinocyl. The value of resistance rate increases with subsequent pesticide treatments. During four selection cycles lasting for 10 generations, it is possible to achieve an over 30-fold increase in *A. andersoni* resistance to acequinocyl. It has been shown that the population selected for resistance to acequinocyl is more resistant than the initial (commercial) population to another pesticide, fenopyroximate. To assess the durability of resistance to acequinocyl in the selected AAP4 strain, further investigation providing the information whether the resistance is monogenic or polygenic and whether the resistance genes are dominant or recessive will be conducted.

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Streszczenie: *Selekcja* Amblyseius andersoni (*Chant*) (*Anactinotrichida*: Phytoseiidae) *w kierunku odporności na acekwinocyl*. Większość pestycydów stosowanych w zwalczaniu szkodników w sadach jest szkodliwa dla pożytecznych stawonogów. Wyselekcjonowanie drapieżców i parazytoidów odpornych na chemiczne środki ochrony roślin mogłoby przyczynić się do ich lepszego wykorzystania w uprawach, w których stosowanie pestycydów jest konieczne. Celem niniejszej pracy była selekcja linii drapieżnego roztocza *Amblyseius andersoni* odpornej na acekwinocyl – akarycyd należący do grupy inhibitorów transportu elektronów w kompleksie III mitochondrialnego łańcucha oddechowego. Jako dawkę selekcyjną zastosowano stężenie pestycydu powodujące śmiertelność 55–65% zapłodnionych samic drapieżcy. U wyselekcjonowanej laboratoryjnie linii odnotowano dziewięciokrotny wzrost oporności na acekwinocyl po dwóch cyklach selekcyjnych i ponad 30-krotny wzrost po czterech cyklach. Wyselekcjonowana populacja

A. andersoni rozwinęła również umiarkowaną oporność krzyżową na fenpiroksymat.

Słowa kluczowe: drapieżne roztocza, pestycydy, selekcja, acekwinocyl, fenpiroksymat

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