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Selection for resistance to spiromisifen in the predatory mite *Metaseiulus occidentalis* (Acari: Phytoseiidae)

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ABSTRACT

A heterogeneous Base colony of *M. occidentalis* was derived from 22 different colonies that included strains resistant to organophosphates, sulfur, carbaryl, abamectin and permethrin, as well as strains with no documented pesticide resistance. The susceptible (S) Base colony was selected 14 times with increasing concentrations of spiromesifen resulting in a 4.4-fold increase in LC_{50} compared with the original Base colony. Concentration-mortality lines for the reciprocal crosses between the resistant (R) and S colonies (R♀ x S♂ and S♀x R♂) were close to the susceptible Base colony. Availability of a spiromesifen-resistant strain of *M. occidentalis* could be useful in developing integrated mite management programs in greenhouses and plastic houses.

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INTRODUCTION

Many crop plants are attacked by spider mites (Acari: Tetranychidae). Spider mites are widespread agricultural pests, causing severe damage on a variety of greenhouse and field crops as they use their stylet-like mouthparts to suck out the contents of plant cells, causing reduced yields and even plant death [14, 21]. The two-spotted spider mite, *Tetranychus urticae*, is extremely polyphagous with a cosmopolitan distribution [18]. It is known to feed on over 900 host species including over 150 economically important ornamental and food crops, including grass and broadleaved crops. Because of its wide distribution and wide host range, the two-spotted spider mite is the most economically important phytophagous mite in the world [12]. Pesticides are the primary method of control of *T. urticae*. However, populations develop resistance to acaricides and the chemicals leave residues on fruits. Population increases of spider mites related to the use of synthetic compounds have been documented extensively. Van Leeuwan *et al.* [20] reported mite resistance to over 95 acaricidal/insecticidal active ingredients.

Fortunately, there are predators of these plant parasites, including mites in the family Phytoseiidae (Chelicerata: Arachnida: Acari: Gamasida: Phytoseiidae). Approximately 2,000 phytoseiid species have been named, but many may remain unidentified because they occur in less-sampled habitats and cryptic species may be common. The predatory mite *Metaseiulus occidentalis* (= *Galendromus* or *Typhlodromus*) (Nesbitt) (Acari: Phytoseiidae) is an important natural enemy of spider mites in the western USA where it can control spider mite species in apples, almonds, pears, strawberries, grapes, cotton, and other crops [11]. It is adapted to hot, dry conditions and tolerates low relative humidities. It has been genetically selected for resistances to carbaryl and pyrethroids and also has become resistant to sulfur and organophosphate insecticides in the field [10, 17].

It is often the case that natural enemies cannot, by themselves, provide adequate control of pests in vegetable crops. Augmentative biological control with predatory mites (Acari: Phytoseiidae) can be effective in many cases to control spider mites, broad mites, thrips, and other small arthropod pests, but sometimes selective pesticides need to be used to suppress the spider mite populations or insect pests. Many pesticides are highly toxic to phytoseiids, so pesticide selectivity is important in reducing spider mite populations without disrupting the predatory mite populations.

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Genetic improvement of natural enemies has been discussed as a method of enhancing the biological control of arthropod pests for over seven decades [4]. Genetic improvement of phytoseiids has proven to be practical and cost effective when the trait(s) limiting efficacy can be identified, the improved strain retains its fitness, and methods for implementation are developed [4]. *M. occidentalis* responded to laboratory selection with abamectin, an insecticide-acaricide-nematicide derived as a fermentation product of *Streptomyces avermitilis* [5], carbaryl [17] and the pyrethroid permethrin [7].

The research reported here aimed to determine whether populations of *M. occidentalis* could be artificially selected for resistance to spiromesifen, an acaricide used in mite management programs in vegetable production. Spiromesifen is a tetronic acid derivative that has recently been commercialized as an acaricide with a novel mode of action (inhibition of acetyl-CoA-carboxylase) [15]. It is highly effective against all relevant phytophagous mite species, including mite populations resistant to other acaricides [1,2].

MATERIALS AND METHODS

Population of Tetraychus urticae:

The colony of *T. urticae* was established at the Department of Entomology and Nematology, University of Florida, on pinto beans, *Phaseoulus vulgaris* L. (Fabales: Fabaceae) in a greenhouse since 1992 and served as prey for *M. occidentalis,* an obligatory predator.

Populations of Metaseiulus occidentalis:

A heterogeneous Base colony of *M. occidentalis* was developed in September 2014 prior to selections by combining 30 to 35 females from each of 22 different colonies maintained in the Department of Entomology and Nematology, University of Florida, some of which had been in culture for more than 30 years. The colonies included strains resistant to organophosphates, sulfur, carbaryl, abamectin and permethrin, as well as strains with no documented pesticide resistances. After the colonies were combined, the new Base colony was reared for one generation before selection began. After the first selection, the Base colony was maintained separately for comparison with the Selected colony. Base and Selected colonies were reared on paraffin-coated paper squares resting on water-soaked cotton and fed daily all life stages of *T. urticae,* brushed with a mite-brushing machine from pinto beans. Colonies were reared at 25 $\pm$ 0.5 $^{\circ}$C under continuous light.

Chemical tested:

Spiromesifen belongs to a new class of pesticides, the keto-enols, with a commercial formulation called Oberon®' (suspension concentrate, 240 g/l a.i), produced by Bayer CropScience. The keto-enols are derivatives of tetronic acid (spirocyclic tetronic acids) and are reported to act through inhibition of lipid biosynthesis. Spiromesifen is an effective acaricide [13] but its toxicity to predatory mites (Phytoseiidae) has not been reported.

Selection for resistant M. occidentalis:

Excised pinto bean leaflets (2.2 cm in diameter) were placed on wet cotton in plastic trays to discourage run off, and upper surfaces are sprayed with the formulated pesticide (Oberon) at the required concentration. The leaf discs were allowed to dry for one hour and held at 25 ± 0.5 °C for 48 h after which the number of actively moving females were recorded. Five females were tested in each leaf disc (350 to 700 females in each selection).

The first 5 selections were performed by placing an average of 500 females on pinto bean leaf discs (5 females per disc) and spraying them with 0.96 gm/l a.i spiromesifen with aerosol sprayers (Preval, Chicago Aerosol, Coal City, Illinois) under a fume hood. Selections 6–14 were made by spraying spiromesifen at a concentration of 1.92 gm/l a.i After 48 hrs, survivors (females that could walk when touched with a fine camel's hair brush) were recorded and placed on a new paraffin-coated paper squares resting on water-soaked cotton and fed daily all life stages of *T. urticae*. Adults were left to deposit eggs for 48 hrs before their removal. The new adult females were subjected to a new selection.

Mode of Inheritance:

The obtained resistant (R) and the susceptible (Base) colonies were crossed to produce reciprocal F_1 female (RS or SR) progeny. Crosses were made in the laboratory with 250 virgin females and 250 males each for R x S and S x R crosses and 170 virgin females and 170 males each for R x R and S x S crosses. Virgins were obtained by isolating deutonymphal females. Five females and 5 adult males were placed on pinto bean leaf discs for crosses: R x R, R x S, S x R and S x S. Once parental females had emerged and mated, the mated females were moved to clean bean leaf discs (5 per disc) each day for 8 days. The eggs that these parental females deposited were held for about 10 days at 25 ± 0.5°C under constant light until the F_1 adults appeared. F_1 females were tested using the Preval aerosol sprayers to obtain concentration mortality lines. Preliminary tests were conducted to determine appropriate ranges so that mortality for crosses treated with different concentrations of spiromesifen ranged from 8 to 97 %.

Concentration-mortality lines:

Concentration-mortality lines were estimated by exposing F_1 progeny females (R x R, R♀ x S♂, S♀ x R♂, S x S) to a series of concentrations of spiromesifen and a water control. We used concentrations that ranged between 5.76 gm/l and 0.12 gm/l a.i of the formulated form of spiromesifen. Solutions were made fresh each test date. Five females were placed on each leaf disc for a total of at least 100 females for each concentration (20 replicates). Tests were conducted as described above in which females placed on the leaf discs then sprayed to drip. Females were held at 25 + 1 °C under continuous light for 48 hr. Survivors were scored after 48 hr as described previously. Only concentrations that gave mortalities between 96% and 4% were considered. Mortalities were subjected to Abbott's correction then analyzed with the probit option of the SAS program (SAS 9.4).

RESULTS AND DISCUSSION

Preliminary tests showed that spiromesifen at a concentration of 0.96 gm/l (results not shown) was suitable for starting selection on the heterogeneous Base colony of *M. occidentalis* because it allowed 18 % of adult females to survive.

Figure 1 shows survival rates of gravid adult females of *M. occidentalis* after 14 selections with two rates of spiromesifen. Survival after the second selection was doubled (42 %) and the third selection resulted in 63% survival. Because the fourth and fifth selections did not result in an increase in survival rates, we changed to a higher concentration (1.92 gm a.i /liter) in the sixth selection and 25.5 % survival was obtained. The increase in survival was slower with the 1.92 gm a.i/liter than that obtained with the previous rate with only a 10 % increase in the survival rate with each successive selection. After six selections at 1.96 gm a.i/liter, the survival rate of *M. occidentalis* was 75 %. No increase in survival was obtained in the 12^{nd}, 13^{th} and 14^{th} selections using spiromesifen.

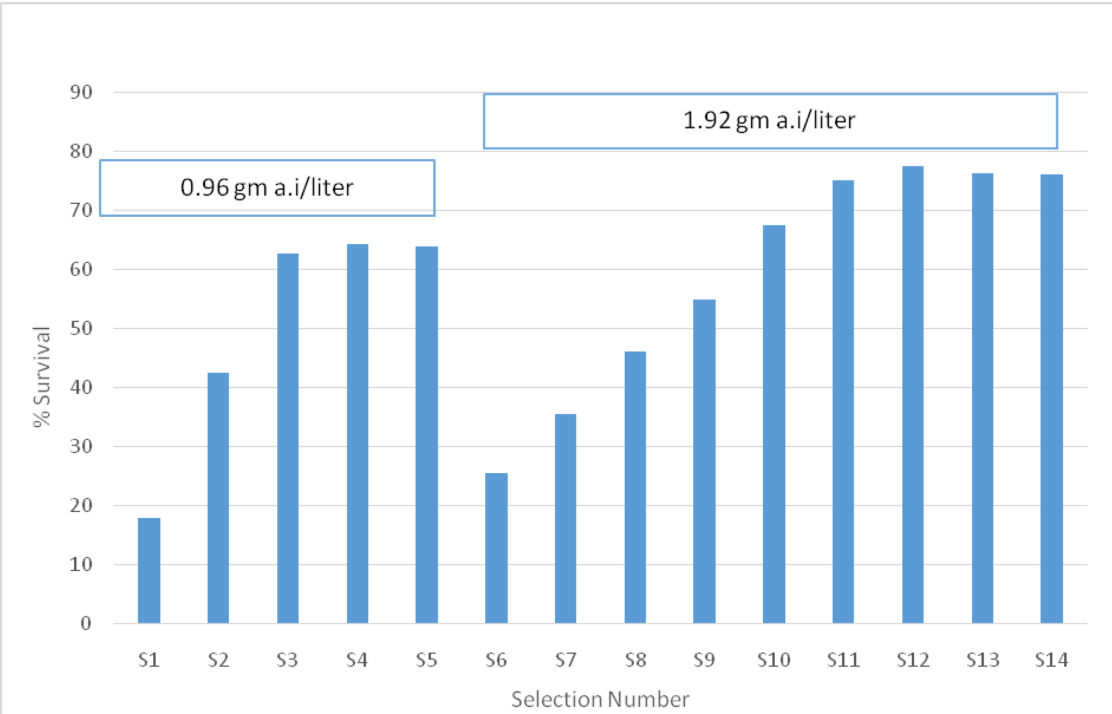

Fig. 1: *Metaseiulus occidentalis* survival after 14 selections using two rates of spiromesifen.

The LC_{50} value obtained for the Selected strain after 14 selections is about 4.4-fold greater than that of the Base strain from which it was derived (Table 1) and the LC_{90} value for the Selcted strain is about 2.7-fold greater than that of the Base colony**.** The Selected colony may have a sufficiently high level of resistance that it could survive low spiromesifen application rates in the field (field application rates range from 1.92–2.88 gm a.i. /liter), although this needs to be confirmed with field tests. Also, more research is required to confirm that the resistance levels are stable in the absence of continued selection with spiromesifen.

The reciprocal F_1 female progeny of R♀ x S♂ and S♀ x R♂ crosses gave LC_{50} values of 1.20 and 0.72, respectively. They are significantly lower than the LC_{50} of the Selected strain considering the 95% CL values (Table 1). In fact, the LC_{50} values of the reciprocal crosses were not significantly different from that of the Base colony, which had an LC_{50} of 0.76 gm a.i./liter. (Table 1, Fig. 2). This suggests that spiromesifen resistance is recessive or, possibly, determined by multiple genes [19]. Inheritance in *M. occidentalis* is complicated by the fact that the males are haploid and inherit (as far as we know) their resistance genes only from their mother (parahaploidy) while females are diploid. Clearly, spiromesifen resistance does not seem to be determined by a single dominant gene. Hoy and Ouyang [5] reported a gradual and modest shift in concentration-mortality line was obtained after 20 selections with increasing concentrations of abamectin.

Table 1: Concentration-mortality data for resistant, susceptible and reciprocal F_1 females of *M. occidentalis* treated with spiromesifen.

Cross	Slope ± SE	LC_{50} (gm/l)	95% CL	LC_{90} (gm/l)	95% CL
R	1.03 ± 0.1	3.37	3.09 –3.65	5.49	5.05-6.10
F_1 RS (R♀ x S♂)	1.30 ± 0.20	1.20	0.93 –1.50	2.88	2.37-3.84
F_1 SR (S♀ x R♂)	1.53 ± 0.35	0.72	0.44 –1.06	2.16	1.61-3.64
S (Base colony)	1.72 ± 0.33	0.76	0.54 –1.03	2.04	1.60-3.01

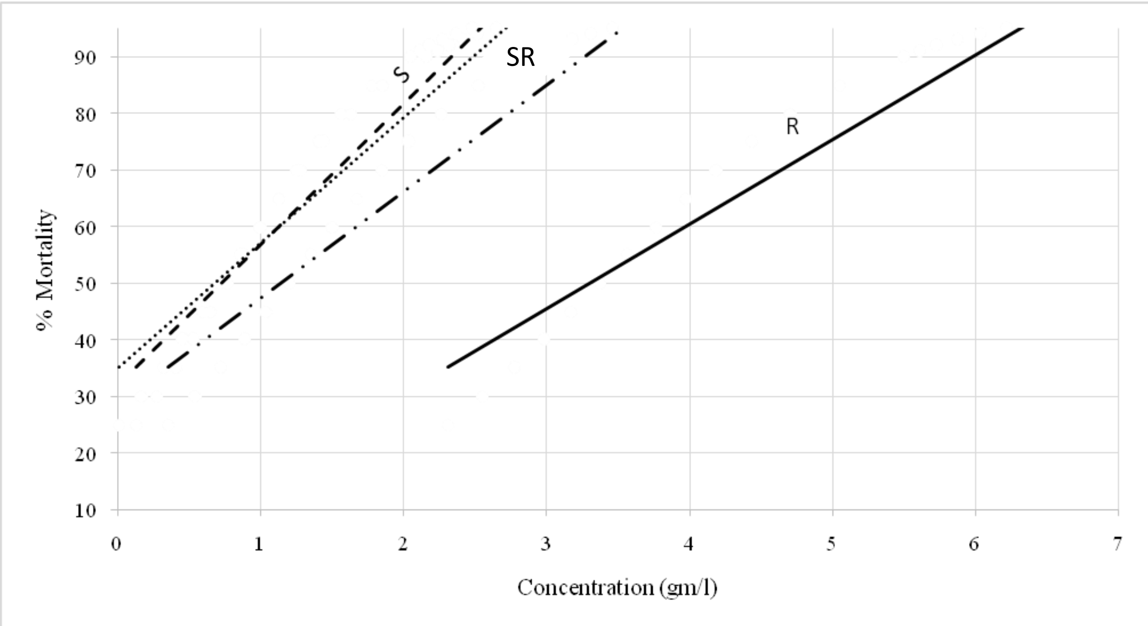

Fig. 2: Mode of inheritance of spiromesifen resistance in *M. occidentalis*; concentration-mortality lines of the reciprocal F_1 females compared to the Base (S) and Selected colonies (R).

M. occidentalis has been subjected to intensive and thorough researches for both artificially and naturally induced pesticide resistances, including carbaryl, permethrin and abamectin [17,7,5]. These researches yielded resistant strains of this predator to abamectin, a natural product produced by *Streptomyces avermitilis* [5,16], organophosphates (azinphosmethyl, diazinon and phosmet), carbamates (carbaryl, benomyl and propoxur), pyrethroids (permethrin) and sulfur [17,8,9,6,7]. In the case of carbaryl resistance, the resistance appears to have been determined by a major semidominant gene [17], but the permethrin resistance appears to be determined quantitatively [7].

Finally, more research is required to test the efficiency of the selected spiromisefen-resistant strain of *M. occidentalis* under greenhouse conditions and to determine if the resistance is stable in successive generations.

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