Toxicity of Selected Acaricides in a Glass-vial Bioassay to Twospotted Spider Mite (Acari: Tetranychidae)

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Abstract. Twospotted spider mite, *Tetranychus urticae* Koch, feeds on epidermal cells of foliage, destroys photosynthetic cells, and reduces yield, fiber quality, and seed germination of cotton, *Gossypium hirsutum* L. With a short life cycle, prolific fecundity, an arrhenotokous reproduction, and an ability to expeditiously digest and detoxify xenobiotics, twospotted spider mite has the propensity to develop resistance to insecticides. Despite mobility, small size, and difficulties associated with handling of twospotted spider mites, this study demonstrated that the 20-ml glass-vial bioassay is a useful technique to evaluate contact toxicity of acaricides against adult mites in a laboratory. A colony of twospotted spider mites was maintained on pinto beans, *Phaseolus vulgaris* L. in a greenhouse. Abamectin with LC_{50} (95% CL) of 0.014 (0.01-0.02) µg per vial was 1,006 time more toxic than spiromesifen with a LC_{50} of 14.086 (7.592-42.371) µg per vial. The LC_{50} values of spiromesifen and propargite were comparable. Bifenazate was 10 times more toxic than dicofol to twospotted spider mite. The order of toxicity of acaricides tested against twospotted spider mite adults was abamectin > bifenazate > dicofol > propargite = spiromesifen. These data are useful for developing baseline contact toxicity for adult twospotted spider mite and monitoring tolerance to acaricides used on cotton in Central Texas.

Introduction

The tiny, herbivorous twospotted spider mite, *Tetranychus urticae* Koch, is the sixth most damaging pest of cotton, *Gossypium hirsutum* L., in the United States, especially in the mid-South region, and caused an estimated loss of 57,441 bales of cotton in 2011 (Adamcyzyk and Lorenz 2012, Williams 2012, Gore et al. 2013). Twospotted spider mite feeds on mesophyll cells on the bottom surface of cotton leaves and significantly impacts plant growth and reduces crop yield, fiber quality, germination rate, and oil content of seeds (Wilson 1993; Sadras and Wilson 1996, 1997a,b). Spider mites also reduce photosynthetic rate, stomatal conductance, transpiration efficiency, and chlorophyll content of cotton (Bondada et al. 1995, Reddall et al. 2004).

Suppression of twospotted spider mite is primarily limited to the use of insecticides. Its short life cycle, prolific production of progeny, and arrhenotokous reproduction facilitate rapid development of resistance to insecticide (Van Leeuwen et al. 2010). Grbic et al. (2011) found the genome of the spider mite comprised of gene families involved in digestion and detoxification of xenobiotics, resulting in

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rapid development of resistance to insecticides. It is, therefore, important that concerted effort be made to evaluate toxicity of insecticides currently available for control of spider mites on cotton. However, a universally accepted bioassay technique for toxicity studies is lacking because of the mobility, small size, and difficulties associated with handling of twospotted spider mite. Twospotted spider mite is less than 0.5 mm long and best seen with the aid of a microscope (Steinkraus et al. 2003).

Several workers reported on techniques to gather baseline data on toxicity of acaricides to twospotted spider mite. Kabir et al. (1993) evaluated six bioassay methods to test toxicity of propargite and fenbutatin oxide against twospotted spider mite and European red mite, *Panonychus ulmi* Koch. They found the precision of the test varied with the acaricide and species of mite. Dennehy et al. (1993) described a micro-immersion bioassay that involved drawing mites into a small pipette under vacuum and immersing them in test solutions for 30 seconds. Hoffmann et al. (1997) reported using a 40-ml glass vial to determine toxicity of acaricides against red-legged earth mite, *Halotydeus destructor* (Tucker); they mixed acaricides in water and used a drying rack to process the vials containing test solution. Pierce and Godfrey (2008) dipped leaf disks (3.5 cm diameter) in water solutions of acaricides and placed two treated disks stacked together on a bed of 1% agar in a 100-mm Petri dish. They placed the spider mites on the top leaf disk while the bottom leaf disk served as a barrier between the moist agar and top leaf disk. Kanga et al. (2010) used a 20-ml glass-vial bioassay for monitoring resistance to organophosphorous and pyrethroid insecticides in an ectoparasitic *Varroa* mite, *Varroa destructor* Anderson & Trueman, which is two to three times larger than twospotted spider mite.

The 20-ml glass-vial technique developed by Plapp et al. (1987) for detecting resistance in tobacco budworm, *Heliothis virescens* (F.), adult moths in cotton is commonly used in the United States for determining resistance to insecticides for a wide variety of insects such as moths, boll weevils, stink bugs, fleahoppers, thrips, aphids, and whiteflies (Bayoun et al. 1995; Prabhaker et al. 1996; Snodgrass 1996; Willrich et al. 2003; Pietrantonio et al. 2007; Lopez et al. 2008a,b; Miller et al. 2010). No report, however, exists on the use of this bioassay technique to evaluate toxicity of insecticides to twospotted spider mite. The objective of this study was to demonstrate the use of 20-ml glass-vial bioassay for assessing contact toxicity of acaricides against twospotted spider mite raised in a greenhouse. Secondly, we sought to characterize the toxicity of acaricides for monitoring tolerance of twospotted spider mite in Central Texas.

Materials and Methods

Pinto beans, *Phaseolus vulgaris* L. (Dwarf Horticulture, Bush), were grown in plastic trays (56 X 28 X 5 cm) in a greenhouse. Leaves infested naturally with spider mite colonies on greenhouse-grown cotton plants were removed and placed randomly on young pinto bean plants. Pinto beans were infested regularly with spider mites until the plants were infested. Spider mites spin webs of silk and cover the entire plant surface, and masses of mites congregate on tips of bean foliage (Fig. 1). A 4-liter milk carton was cut into a funnel and placed near the clusters of mites on the top canopy of the bean plants (Fig. 2). The spider mites being phototropic travelled to light and formed a ring around the rim of the milk carton. The milk carton with spider mites around the rim was taken to a laboratory.

Fig. 1. A cluster of twospotted spider mites hanging before dispersal from the tip of a bean plant.

Technical-grade acaricides (abamectin, bifenazate, dicofol, propargite, and spiromesifen) were purchased from ChemService, West Chester, PA., and stored in a freezer. Stock cultures were prepared by weighing the chemical in a Sartorius® balance (Model LA120S) sensitive to 0.1 mg. About 1 g of each chemical was weighed and mixed with acetone (assay 99.5%). Various concentrations of the chemical were prepared using the serial dilution procedure. All acaricide solutions were kept in a freezer for 30 days, and fresh solutions were prepared thereafter.

Vial test procedures for adult mites were similar to those described by Plapp et al. (1987, 1990), Kanga and Plapp (1992), Kanga et al. (1995), Snodgrass (1996), Willrich et al. (2003), and Miller et al. (2010). One-half milliliter of various concentrations of chemical prepared in acetone was pipetted into a 20-ml scintillation vial put onto a hot dog roller with the heating element removed. Samples were processed until the acetone was evaporated. Using a fine camel-hair brush, five spider mite adults were carefully removed from the rim of the milk carton and placed into each vial. The vial was capped, turned upside down, and placed in a 10 X 10 scintillation vial cardboard slot. The number of dead mites was assessed 24 hours later and compared to a nontreated check. Spider mites that did not move after being probed with a dissecting needle were considered dead. Post-treatment exposure time and criteria for determining mortality of twospotted spider mite varied with bioassay method (Kabir et al. 1993). Kabir et al. (1993) found that 24 hours of exposure and scoring moribund mites as dead were the most satisfactory criteria for precisely estimating LC values. The criterion for post-treatment exposure time and determination of mortality used in this study were in agreement with the recommendations of Kabir et al. (1993).

Fig. 2. A 4-liter milk jug cut into a funnel was placed on the top of bean plants infested with twospotted spider mites. The mites congregated at the rim of the jug.

SAS (2008) was used for probit analyses. A goodness-of-fit statistic for the Pearson χ^2 was used to determine the approximation of the data to the probit model. Failure of 95% fiducial limits to overlap was used as a criterion to separate lethal concentration values between insecticides (Robertson and Preisler 1992).

Results and Discussion

Table 1 shows dosage mortality data and lethal concentration values for 24-hour response of twospotted spider mite relative to contact toxicity of acaricides. The *p*-values in the goodness-of-fit statistic for the Pearson χ^2 indicated a good fit with the probit model for all the acaricides tested. Finney (1971) reported that in basic binary bioassays, comparisons are made at the LC_{50} because this is the most stable point for comparison and easily estimated. Abamectin, with LC_{50} of 0.014 (0.01-0.02) µg per vial, was significantly most toxic to twospotted spider mite.

Table 1. Dosage Mortality Data and Lethal Concentration Values (LC_{50}s) for 24-hour Response of Adult *T. urticae* to Selected Acaricides in a Glass-vial Bioassay

Chemical	N	Slope ± SE	χ^2	$P > \chi^2$	LC_{50}[a]	95% CL
Abamectin	323	3.15 ± 0.47	0.24	4.17	0.014d	0.01-0.02
Bifenazate	205	3.71 ± 0.54	0.29	1.11	0.111c	0.097-0.134
Dicofol	200	11.24 ± 2.97	1.06	0.30	1.086b	0.827-1.227
Propargite	237	4.57 ± 0.69	2.11	0.55	6.860a	5.007-8.682
Spiromesifen	329	1.25 ± 0.28	0.30	2.40	14.086a	7.592-42.371

[a]LC_{50} values were calculated using the Proc Probit procedure (SAS 2008). LC_{50} values followed by the same lowercase letter in a column are not significantly different based upon lack of overlap of 95% confidence limits.

The least toxic acaricides to twospotted spider mite were spiromesifen with LC_{50} of 14.086 (7.592-42.371) µg per vial and propargite with LC_{50} of 6.86 (5.007-8.682) µg per vial. Toxicity of spiromesifen was comparable to that of propargite. Abamectin was 1,006 and 490 times more toxic than spiromesifen and propargite, respectively. Abamectin was 78 times more toxic than dicofol with LC_{50} of 1.086 (0.827-1.227) µg per vial. Abamectin was eight-fold more toxic to twospotted spider mite than bifenazate with LC_{50} of 0.111(0.097-0.134) µg per vial. Bifenazate was 10 times more toxic than dicofol to twospotted spider mite. Propargite was six times more toxic compared to dicofol. The order of toxicity of acaricides tested against twospotted spider mites in this study was abamectin > bifenazate > dicofol > propargite = spiromesifen.

The data are in agreement with those of other researchers (Dunbar et al. 1989, Hilton and Dybas 1989, Keillor and Godfrey 2001, Jimenez et al. 2004, Shelton et al. 2006) who reported that abamectin provided superior control of twospotted spider mite on cotton. Pierce and Godfrey (2008) found a cotton leaf bioassay of spiromesifen at the highest dosage of 100 ppm killed only 14% of the spider mites. However, an earlier report by Jimenez et al. (2004) found that spiromesifen provided control of early and mid-season spider mites similar to abamectin but was superior to that of other acaricides. Shelton et al. (2006) reported that control of spider mites by spiromesifen was comparable to that of abamectin. Ashley et al. (2006) reported that propargite killed a significant portion of a mixed-stage population of twospotted spider mite on peanut, *Arachis hypogaea* L., in Virginia. Dunbar et al. (1989) found that propargite and dicofol provided superior residual control of spider mites compared to abamectin. Younis and Ibrahim (1996) reported that in Minia, Egypt, propargite did not effectively control twospotted spider mite. There is considerable variation in the efficacy of propargite against twospotted spider mite as reported by several workers, probably because of environmental conditions, sampling methodology, and host plant characteristics. However, data of almost all workers indicated abamectin provided effective control of twospotted spider mite on cotton. Data presented here are useful baseline information for determining tolerance of twospotted spider mite to acaricides used in Central Texas.

Conclusions

Despite mobility, small size, and difficulty handling of twospotted spider mite, the glass-vial bioassay is a useful technique for evaluating contact toxicity of

acaricides against adult twospotted spider mite. The order of toxicity of acaricides against adult twospotted spider mite was abamectin > bifenazate > dicofol > propargite = spiromesifen. Baseline contact toxicity data will be useful in a resistance management program *vis-à-vis* twospotted spider mite in Central Texas.

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