

## COMPATIBILITY OF SPINOSAD WITH PREDACIOUS MITES (ACARI: PHYTOSEIIDAE) USED TO CONTROL WESTERN FLOWER THRIPS (THYSANOPTERA: THRIPIDAE) IN GREENHOUSE CROPS

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### ABSTRACT

Releases of predacious mites are recommended for use in greenhouse flower crops for suppression of western flower thrips, *Frankliniella occidentalis* (Pergande). Control from predacious mites alone, however, is not adequate and must be supplemented with the use of insecticides. The principal material currently used by growers in the northeastern United States for western flower thrips control is spinosad (Conserve®). In laboratory tests on direct toxicity, we found that fresh residues (2 h) of this material were not toxic to motile stages of *Neoseiulus* (= *Amblyseius*) *cucumeris* (Oudemans) (74 vs 78% survival for the treated group and the untreated water controls, respectively), the principal species of predacious mites used for control of western flower thrips, but did lower survival of *Iphiseius degenerans* (Berlese) (56 vs. 73% survival for the treated group and the untreated water controls, respectively). There were no differences for either species from exposure to older (24 h) residues. In contrast, using the same assay we observed 10 and 3% survival of first instar and adult western flower thrips. We found no indication of that either mite species was repelled by freshly dried (2 h post application) residues of this compound. Spinosad did, however, reduce oviposition of mites when confined in glass vials with pollen, a water source, and pesticide-treated foliage. Oviposition in the first 24 h period after confinement was not affected but in the second and third days, it was reduced by 48 and 76% for *N. cucumeris* and 41 and 70% for *I. degenerans*, compared with oviposition in the same periods by mites in untreated vials. These data indicate that the use of spinosad may not be compatible with releases of these predacious mites in a western flower thrips suppression program.

**Key Words:** *Frankliniella occidentalis*, *Neoseiulus* (= *Amblyseius*) *cucumeris*, *Iphiseius degenerans* (Berlese), pesticide compatibility

### RESUMEN

Liberaciones de ácaros depredadores son recomendadas para el uso en los cultivos de flores en los invernaderos para suprimir el trips occidental de flores, *Frankliniella occidentalis* (Pergande). Sin embargo, usando solamente ácaros depredadores para controlarlos, no es adecuado y debe ser suplementado con el uso de insecticidas. El material principal usado actualmente por los agricultores en el noreste de los Estados Unidos para el control del trips occidental de flores es spinosad (Conserve®). En pruebas del laboratorio sobre la toxicidad directa, nosotros encontramos que los residuos frescos (2 h) de este material no fueron tóxicos a los estadios móviles de *Neoseiulus* (= *Amblyseius*) *cucumeris* (Oudemans) (74 vs 78% sobrevivencia en el grupo tratado y en el grupo no tratado de control que recibió solo agua, respectivamente), la especie principal de ácaros depredadores usado para controlar el trips occidental de flores, pero bajo la sobrevivencia de *Iphiseius degenerans* (Berlese) (56 vs. 73% sobrevivencia en el grupo tratado y en el grupo no tratado de control que recibió solo agua, respectivamente). No hubo una diferencia en ambas especies expuestas a los residuos mas viejos (24 h). En contraste, usando el mismo bioensayo nosotros observamos 10 y 3% sobrevivencia del primer estadio y adulto del trips occidental de flores. No encontramos ninguna indicación que las dos especies de ácaros fueron repelidos por los residuos recién secados (2 h después de la aplicación) de este compuesto. Sin embargo, spinosad redujo la oviposición de ácaros confinados en ampollitas de vidrio con polen, una fuente de agua, y follaje tratado con pesticida. La oviposición no fue afectada en el primer periodo de 24 h después del sobrepardo pero en el segundo y tercer día, fue reducida a 48 y 76% en *N. cucumeris* y 41 y 70% en *I. degenerans*, comparada con la oviposición en los mismos periodos para los ácaros en las ampollitas no tratadas. Estos datos indican que el uso de spinosad puede ser incompatible con las liberaciones de estos ácaros depredadores en un programa de supresión del trips occidental de flores.

Most greenhouse crops in the northeastern United States and many other regions are affected by western flower thrips, *Frankliniella oc-*

*cidental* (Pergande) (Thysanoptera: Thripidae). This pest must be managed to prevent distortion of flowers and leaves by thrips feeding, and trans-

mission of tospoviruses (Yudin et al. 1986; Robb 1989; Daughtrey et al. 1997; Jacobson 1997; Lewis 1997). Producers of spring flower crops in the northeastern United States most often rely on application of pesticides for control of this pest, and currently spinosad, a "reduced-risk" material derived from a soil microorganism, is the most frequently used pesticide. This product currently provides excellent control, but western flower thrips has frequently developed resistance to pesticides, including abamectin, bifenthrin, chlorpyrifos, cyfluthrin, dimethoate, methomyl, and permethrin (Robb 1989; Immaraju et al. 1992; Robb et al. 1995; Jensen 2000).

To reduce the risk of resistance developing to spinosad, the potential for managing western flower thrips in greenhouse flower crops with predators has been investigated. The predators with most potential for use in short term flower crops are predatory mites, including *Neoseiulus* (= *Amblyseius*) *cucumeris* (Oudemans) and *Iphiseius degenerans* (Berlese) (Acari: Phytoseiidae) (Van Driesche et al. 1998). Efficacy of *N. cucumeris* in greenhouses varies among crops, being most effective in crops such as peppers, which have long production periods and provide pollen (a resource used by this predator) (de Klerk & Ramakers 1986). Efficacy of this species in floral crops has not been widely substantiated, despite its being recommended by natural enemy producers. In California, a single release of *N. cucumeris* on caged chrysanthemums at 2.5 mites per leaf reduced western flower thrips to about 2-7 per leaf over a three week test period (Hessein & Parrilla 1990), but this density was too high for commercial crops. Gill (1994) reported that releases of *N. cucumeris* in open rearing units ("sachets") lowered pesticides needed for western flower thrips control in bedding plants from 3.6 to 0.4 applications per crop, but thrips densities in control and treated greenhouses were not reported. In the United Kingdom, Bennison et al. (2001), obtained effective western flower thrips control on impatiens with weekly applications of higher releases of *N. cucumeris* (180 mites per m<sup>2</sup>, 3-4× the commercially recommended rate). Similarly, De Courcy Williams (2001) in the U.K. found that weekly releases of 200 *N. cucumeris* mites per m<sup>2</sup> reduced western flower thrips on cyclamen. Trials in Massachusetts with this higher rate found that it provided better control than the commercially recommended rate in short term spring flower crops, but even at this higher rate *N. cucumeris* alone did not strongly suppress western flower thrips (Van Driesche et al. 2005). Even low densities of western flower thrips may pose a risk to the crop by spreading tospoviruses such as impatiens necrotic spot virus (INSV). No information is available on the efficacy of *I. degenerans* in flower crops. While more effective per mite as a thrips control agent than *N. cucumeris*, this species is

much more expensive to rear and so has not been commercially popular, although it remains available.

Based on the partial effectiveness of biological control agents for western flower thrips in spring bedding plants and the frequency with which this thrips has evolved pesticide resistance, it may be useful to combine spinosad (to achieve high thrips suppression) and predatory mite releases (to reduce survival of potentially pesticide-resistant thrips that might survive pesticide applications). Spinosad has been reported to be generally compatible with predatory mites (Miles et al. 2003; Ahn et al. 2004; Kim et al. 2005). The objective of our study was to further assess spinosad compatibility with *N. cucumeris* and *I. degenerans*. For both mite species, we assessed (1) contact toxicity to mites vs thrips (adults and larvae), (2) repellency of residues of spinosad to mites, and (3) effects of residues on mite oviposition.

## MATERIALS AND METHODS

### Sources of Mites and Thrips

Western flower thrips used in all tests were from a colony reared in our laboratory (University of Massachusetts), started about 1997 with material from a laboratory colony from Texas A & M University (K. Heinz laboratory). Thrips were reared on excised bean leaves by the method of Doane et al. (1995) modified by Lim et al. (2001). *Neoseiulus cucumeris* mites were purchased from Koppert Biological Systems (Koppert B. V., The Netherlands), shipped in bran with grain mites (*Tyrophagus putrescentiae* [Schrank], Acarina: Acaridae) and quality of these mites was consistently high, with large numbers of live mites in containers. *Neoseiulus cucumeris* mites were used in experiments immediately upon receipt. *Iphiseius degenerans*, packed in sawdust, were purchased from Biobest N. V. (Westerlo, Belgium). Shipments of *I. degenerans* repeatedly arrived in poor condition with many mites being dead or moribund. Before being used in experiments, live mites were placed on castor bean (*Ricinus communis* L., Euphorbiaceae) leaves dusted with red apple pollen and allowed to feed for at least 16 h to ensure that only mites in good health were used in experiments.

### Experiment #1: Toxicity to Adult Thrips

Toxicity of spinosad at the full labeled rate (0.1198 g a.i./ml solution) to adult and larval thrips was tested as a positive control in view of the low toxicity that we expected to find for the beneficial mites. For thrips, we examined only the effects of 2 h-old residues.

For tests with adult thrips, excised leaves of young red kidney bean (*Phaseolus vulgaris* L., Fa-

baceae) plants produced in growth chambers were sprayed to the point of run-off with a hand held sprayer. Control leaves were sprayed with tap water. The nozzle of a hand powered spray bottle was held approximately 10 cm from leaf surfaces and a total of five sprays were applied to each leaf surface. Leaves were then allowed to dry at room temperature on the lab bench for 2 h at which time 2 x 1.5 cm leaf rectangles were cut and placed individually in 1-dram glass shell vials (Kimble Glass, Inc, Vineland, NJ). Vial caps had ventilation holes cut in them, and a small piece of Nitex screening (03-95/33) (Sefar America, Inc., Briarcliff Manor, NY) was held in place by the perforated cap. A disk (6 mm dia) of filter paper (#3 Qualitative) (Whatman Limited, Kent, U.K.) cut with a standard office hole punch was moistened with tap water and added to each vial to provide humidity.

Five adult female thrips from our laboratory colony were placed in each of 46 pesticide-treatment and 46 water-treatment vials. Vials were placed on trays and held for 24 h in a growth chamber at 22°C on a light cycle of 16:8 (L:D), and about 30% RH. After 24 h, thrips were checked for mortality.

#### Experiment #2: Toxicity to Larval Thrips

Spinosad-treated red kidney bean leaves were prepared as described above for tests with adult thrips up to the point of completed air drying. At that point, whole leaves were fixed to a disc of white paper cut to fit inside a plastic 15 x 1.5 cm petri dish (Falcon, Becton-Dickinson, Franklin Lakes, NJ). The edges of the leaves were taped with scotch tape to the disc and a thin barrier of tangletrap sticky material (Tanglefoot Co., Grand Rapids, MI) was applied around the whole edge of the leaf to produce a thrips-proof border. This ensured that test animals were continuously confined on the treated surface, which was not possible for winged adults.

With a fine paint brush, 10 immature (first or second instar larvae) thrips from the UMASS colony were placed near the center of the treated bean leaf in each of 24 pesticide-treated and 25 water-treated petri dishes. Petri dishes then were placed on trays and incubated for 24 h in a growth chamber at 22°C on a light cycle of 16:8 (L:D), and about 30% RH. After 24 h, petri dishes were closely examined and the number of dead thrips larvae counted.

#### Experiment #3: Toxicity to Both Mite Species

Bean leaves were treated with spinosad following the same procedures as described for adults thrips and allowed to dry in a greenhouse for 2, 24, or 48 h to simulate the natural degradation of the material expected under conditions of green-

house use. Spinosad was applied at full label rate (0.1198 g a.i./ml solution) for all tests. Leaves with aged residues were taped into petri dishes and a tangletrap barrier was created around the leaf as in the experiment with larval thrips. Unlike conditions in the thrips tests, in the mite tests a dusting of red apple pollen and a 6-mm disk of moistened filter paper were placed on the bean leaf to supply mites with food and water. In tests with *N. cucumeris* and *I. degenerans*, 10 mites (any motile stage) were added per petri dish. Petri dishes were stacked on trays and placed in a growth chamber and held at 22°C, with a photoperiod of 16:8 (L:D) and about 30% R.H. Numbers of petri dishes per treatment varied from 6 to 27, as indicated in Table 2. Water disks were remoistened twice during the 24 h incubation period. After 24 h, the arenas were inspected and the numbers of live, dead, or missing mites recorded.

#### Experiment #4: Repellency to Both Mite Species

This test was run with 2-h-aged residues of spinosad, applied at the full label rate (0.1198 g a.i./ml solution) to the leaves of red kidney bean, as described above. Freshly sprayed leaves were allowed to dry indoors for 2 h. A test arena was constructed by cutting a spinosad-treated leaf in half along one side of the mid-vein and then taping it to half of a water-treated control leaf cut down the opposite side. The two leaf halves were overlapped slightly and white glue used to join them seamlessly. On the surface formed by these joined leaf halves, tanglefoot was applied as a barrier to create a square arena (10 cm on a side) centered on the dividing line so that exactly half of the enclosed surface was treated and half untreated. To initiate the test, 10 mites (the same procedure for both mite species) were placed on the line between the treated and untreated leaf halves. The arena was then continually observed for 15 min and mites on the treated and untreated areas were counted every 3 min. The five resulting counts were then summed for an individual replicate, such that a complete lack of repellency would be indicated by a 25:25 division of mite-observations between the two parts of the arena. For *N. cucumeris*, the responses of 300 mites (30 replicates) were examined and for *I. degenerans*, 240 mites (24 replicates) were observed.

#### Experiment #5: Effect of Spinosad on Mite Oviposition

Spinosad-treated red kidney bean leaves were prepared as in the toxicity experiments described above, and then leaf squares (2 x 1.5 cm) were cut from the treated leaves after 2 h drying time and placed individually in 1-dram glass shell vials, as above. Vial caps were cut for ventilation and

screened with Nitex screening (03-95/33) (Sefar America, Inc., Briarcliff Manor, NY). A 6-mm dia piece of moistened filter paper was added to each vial to provide humidity, along with a sprinkle of red delicious apple pollen (Antles Pollen Supplies, Wentachee, WA). For replicates testing oviposition of *I. degenerans*, 10 first instar thrips from the UMASS colony also were added to each vial as an additional food source for this species only. For replicates with *N. cucumeris*, only pollen was provided, as this is a sufficient food source for reproduction in this species. With a moist paintbrush, one mite of the test species was placed in each vial.

Vials were then placed on trays and incubated for 24 h in a growth chamber at 20 °C, with a 16:8 (L:D) photoperiod and 50-70% RH. After 24 h, the surface of the leaf square and the inside of the vial were examined for mite eggs. Each surviving mite was then transferred into a fresh vial with a new leaf square cut from a plant that had been treated at the start of the experiment. The new vial also contained a fresh water disc, a sprinkle of apple pollen and, for *I. degenerans*, 10 new first instar thrips. This process was repeated so that oviposition was measured for three consecutive 24 h periods after set up.

Statistical Analyses

For experiments 1, 2, and 3 (toxicity to adult thrips, larval thrips, and mites, respectively), we used 2-sample *t*-tests to compare rates of mortality for treated and control groups. For experiments 4 (repellency) and 5 (effect on oviposition), we used the 2-sample *t*-tests with the Proc Mixed procedure of SAS to assess effects on repellency or mite oviposition.

RESULTS

Experiments #1 and #2: Toxicity to Adult and Larval Thrips

Western flower thrips confined on 2-h-aged spinosad residues at the labeled rate (0.1198 g a.i./ml solution) for 24 h showed low survival: 3% vs. 99% in controls for adults ( $t = 84.7, P < 0.0001, df = 78$ ) and 12% vs. 96% for larvae ( $t = 34.9, P < 0.0001, df = 40$ ).

Experiment #3: Toxicity to Mites

In contrast to thrips, which suffered low survival when exposed to spinosad, *N. cucumeris* survival was high (74%) on even the least aged residue (2 h) and not significantly different from survival of mites on the water-treated control (78%) ( $t = 0.59, P < 0.56, df = 35$ ). Most of the mortality suffered by *N. cucumeris* in either treatment was due to mites becoming stuck on the tanglefoot barrier around the arena or not being found at the end of the test (Table 1).

Survival of *I. degenerans* on fresh residues (2 h) was 56.3%, compared with 72.9% for controls ( $t = 4.25, P < 0.0001, df = 46$ ). For 24 h-residues, survival rates were not different (65% on spinosad-treated leaves and 68% on water-treated controls). As with *N. cucumeris*, most mites that did not survive died by becoming stuck in the tanglefoot barrier.

Experiment #4: Repellency of Spinosad to Mites (Both Species)

Neither mite species showed any avoidance of leaf surfaces with freshly dried spinosad (full la-

TABLE 1. TOXICITY OF SPINOSAD RESIDUES (0.1198 G A.I./ML SOLUTION) AGED 2, 24, OR 48 H ON BEAN LEAVES, FOR TWO PREDATORY MITES (*NEOSEIULUS CUCUMERIS* AND *IPHISEIUS DEGENERANS*) CONFINED IN GLASS VIALS WITH TREATED LEAF DISKS.

Average (SE) no. of mites in category (of 10 per vial) after 24 h						
Mite	Treatment	Residue age (replicates)	Live	Dead in tanglefoot	Dead on leaf	Missing
<i>N. cucumeris</i>	spinosad	2 h (22)	7.4 ± 0.4	1.0 ± 0.3	0.3 ± 0.1	1.3 ± 0.4
		24 h (22)	7.3 ± 0.3	1.3 ± 0.2	0.4 ± 0.1	1.1 ± 0.2
		48 h (21)	7.7 ± 0.3	1.3 ± 0.2	0.3 ± 0.1	0.7 ± 0.1
	water	2 h (15)	7.8 ± 0.4	1.1 ± 0.3	0.5 ± 0.2	0.6 ± 0.3
		24 h (11)	8.5 ± 0.5	0.9 ± 0.4	0.2 ± 0.1	0.5 ± 0.2
		48 h (12)	8.4 ± 0.4	0.8 ± 0.3	0.1 ± 0.1	0.7 ± 0.3
<i>I. degenerans</i>	spinosad	2 h (27)	5.6 ± 0.6	2.9 ± 0.3	1.3 ± 0.2	0.2 ± 0.1
		24 h (6)	6.5 ± 0.6	2.2 ± 0.4	1.3 ± 0.6	0.0 ± 0.0
	water	2 h (21)	7.3 ± 0.2	1.7 ± 0.3	0.9 ± 0.2	0.1 ± 0.1
		24 h (6)	6.8 ± 0.5	2.3 ± 0.3	0.7 ± 0.4	0.2 ± 0.2

bel rate, 2-h-aged residue): *N. cucumeris* (mean  $25.2 \pm 4.6$  mites per treated zone vs.  $23.3 \pm 4.3$  on the water control zone,  $n = 30$ ) ( $F = 1.06$ ,  $P < 0.38$ ,  $df = 4$ , 116) and *I. degenerans* ( $24.0 \pm 4.9$  mites per treated zone vs.  $24.9 \pm 5.1$  on the water control zone,  $n = 24$ ) ( $F = 1.28$ ,  $P < 0.29$ ,  $df = 4$ , 92).

#### Experiment #5: Effect of Spinosad on Mite Oviposition (Both Species)

Exposure to freshly dried (2 h) spinosad residues reduced oviposition of *N. cucumeris* on days 2 and 3 after confinement on pesticide-treated foliage by 48 and 76%, from 1.0 and 0.7 eggs per day on d 2 and 3 for controls to 0.5 and 0.2 on spinosad-treated leaves (Table 2), with  $n = 22$ -25 females for the spinosad-treated and 32 for the water-treated leaves (treatment:  $F = 13.55$ ,  $P < 0.0004$ ,  $df = 1$ , 105; time:  $F = 5.83$ ,  $P < 0.004$ ,  $df = 2$ , 105; interaction:  $F = 3.50$ ,  $P < 0.034$ ,  $df = 2$ , 105).

Oviposition of *I. degenerans* was reduced on days 2 and 3 by 41 and 70%, from 1.0 and 0.8 eggs per day for controls to 0.6 and 0.3 for mites on spinosad-treated leaves (Table 2), with  $n = 44$  females for the spinosad-treated and 54 for the water-treated leaves (treatment:  $F = 18.52$ ,  $P < 0.0001$ ,  $df = 1$ , 186; time:  $F = 3.13$ ,  $P < 0.046$ ,  $df = 2$ , 186; interaction:  $F = 3.91$ ,  $P < 0.022$ ,  $df = 2$ , 186).

#### DISCUSSION

As anticipated, spinosad residues caused high mortality to immature and adult western flower thrips, but caused much less mortality to either *N. cucumeris* or *I. degenerans*. However, a significant reduction in mite oviposition was observed in both mite species when confined for three d on leaves

freshly treated with spinosad. This decline in oviposition was not evident in the first day, but was progressively greater over d 2 and 3 of the test. These findings suggest that reproduction of either mite would be suppressed following spinosad application, inhibiting mite population growth. Because natural increase of mite numbers through reproduction in the greenhouse after mite releases is essential for these mites to have an impact on thrips, we predict that spinosad applications and mite releases may not be compatible. This is contrary to initial expectations and a previous report indicating spinosad did not depress *N. cucumeris* oviposition (Kim et al. 2005).

A subsequent field trial comparing events in spring bedding plant crops in greenhouses receiving spinosad alone, *N. cucumeris* releases alone, or mites + spinosad (Van Driesche et al. 2006) supported this prediction for *N. cucumeris*. In greenhouses with mites + spinosad, *N. cucumeris* population densities showed no evidence of population increase over the life of an impatiens crop. In contrast, in greenhouses receiving only *N. cucumeris* (at the same rate), regression lines fitted to mite counts over time had significantly positive slopes, indicating that in the absence of a single mid crop spinosad application, mite populations increased over time.

It is possible that reproduction depression requires exposure to fresh residues (as in the tests presented here) and mites and spinosad might be compatible in longer term crops if the depression of reproduction after pesticide use is only temporary. Greenhouse studies are needed to assess this possibility.

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TABLE 2. OVIPOSITION RATES (EGGS PER FEMALE MITE PER DAY) FOR TWO PREDATORY MITES (*NEOSEIULUS CUCUMERIS* AND *IPHISEIUS DEGENERANS*) WHEN CONFINED ON RED KIDNEY BEAN LEAVES, TREATED WITH SPINOSAD ON DAY ZERO, ALLOWED TO DRY FOR 2 H AND THEN PLACED IN GLASS VIALS, VERSUS UNTREATED LEAVES IN VIALS.

	Eggs laid per female mite (mean, SE, n)	
	Spinosad-treated	Water control
<i>N. cucumeris</i>		
day 1	0.8 ± 0.1 (25)	0.8 ± 0.1 (32)
day 2	0.5 ± 0.1 (25)	1.0 ± 0.1 (32)
day 3	0.2 ± 0.1 (22)	0.7 ± 0.1 (32)
<i>I. degenerans</i>		
day 1	0.7 ± 0.1 (44)	0.8 ± 0.1 (54)
day 2	0.6 ± 0.1 (44)	1.0 ± 0.1 (54)
day 3	0.3 ± 0.1 (44)	0.8 ± 0.1 (54)

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