

# Oviposition Response of *Tetranychus urticae* (Acari: Tetranychidae) to Direct Treatment and Residue of Pyrethroids on Soybean<sup>1</sup>

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**ABSTRACT** The oviposition response of the twospotted spider mite (TSSM), *Tetranychus urticae* Koch, to direct treatment and residues of fenvalerate and permethrin on soybean was examined in the laboratory. Contact with pyrethroid residues (120 ppm) reduced eggs per mite-day for 72 h, then oviposition rates returned to normal. Most eggs (99%) on the control leaflets were oviposited directly on the leaf surface, while 83% and 78% of the eggs in the fenvalerate and permethrin treatments were suspended off the leaf surface onto trichomes, cage walls or in the silk webbing. Oviposition rate on untreated soybean foliage following five days on pyrethroid residues was essentially the same for all TSSM; however, the mites previously exposed to fenvalerate or permethrin residue still oviposited 85% and 37% of their eggs off the untreated leaf surface, respectively. A 120 ppm concentration of fenvalerate directly applied to TSSM resulted in 83% and 100% mortality after 24 h and 48 h and no oviposition. A similar direct exposure to permethrin (120 ppm) resulted in 54% mortality after 48 h, and a 58.5% reduction in eggs per mite-day. Direct exposure to a lower dosage rate (12 ppm) caused little mortality but significantly reduced the number of eggs produced in both fenvalerate and permethrin treatments for 48 h. Oviposition returned to normal after 72 h.

**KEY WORDS** *Tetranychus urticae*, oviposition, fenvalerate, permethrin, soybean.

Concern regarding spider mite outbreaks following pyrethroid insecticide applications has risen in recent years due to the widespread use of these products in a wide array of crops. Pyrethroid insecticides have been reported to influence the dispersal behavior of the twospotted spider mite, *Tetranychus urticae* (Koch) (TSSM) on soybean (Donahue et al. 1988), lima beans (Iftner and Hall 1983) and French dwarf beans (Penman and Chapman 1983). Spider mites escape pyrethroid residues by either walking off the residue surface or by descending from a silken thread (Penman and Chapman, 1983), or by dispersing aerially (Margolies and Kennedy 1988).

Earlier studies (Luckey 1968) reported that pest reproductive stimulation by sublethal dosages of an insecticide fit into the general pharmacological hypothesis of hormoligosis. McKee and Knowles (1984) proposed that hormoligosis could

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potentially contribute to spider mite outbreaks following pyrethroid applications. Penman et al. (1981) reported that field collected TSSM (from apples) oviposition was reduced for a short time following direct treatment and exposure to residues of fenvalerate on French beans and broad beans.

Specific effects of pyrethroid insecticides on TSSM oviposition in soybeans are not fully understood. Therefore, this study was undertaken to evaluate TSSM oviposition response and egg distribution under three conditions of exposure to fenvalerate and permethrin insecticides applied to soybeans. The controlled conditions included direct exposure to pyrethroid residues, direct exposure to residues followed by a period on untreated foliage, and direct treatment of TSSM with pyrethroids followed by a period on untreated leaf surface.

### Materials and Methods

TSSM's were taken from a laboratory colony maintained on soybeans, cv. Essex, held at 20-30°C, 30-60% RH, and 16:8 (L:D). Plants were grown under greenhouse conditions in Metro-Mix 500® potting medium. Three plants were grown in each plot, then thinned to two at the V1 or unifoliate stage (Fehr and Caviness 1977). Only plants in the early vegetative stages (V<sub>3</sub>-V<sub>4</sub>) were utilized.

Small leaf cages (Hughes et al. 1966) of 4.0 cm<sup>2</sup> plastic tubing were used to confine the TSSM to the leaves. The weight of the leaf cages was supported by brackets. A perpendicular crossbrace 21 cm long was attached approximately midway on a 42 cm × 2 cm × 0.5 cm wooden upright. A small wire brad was inserted at each end of the crossbrace, facing downward. One support was placed in each pot, each brad gripping the cork portion of a leaf cage, thus securing the cage in an upright position.

**Exposure to pyrethroid residues.** Treatments were prepared as water emulsions of commercially formulated fenvalerate (Pydrin 2.4 EC [emulsifiable concentrate]; E. I. DuPont De Nemours and Company, Wilmington, DE) and permethrin (Pounce 3.2EC; FMC Corp., Philadelphia, PA) (120 ppm). These solutions approximated a field use rate of 0.11 kg AI/ha. Insecticides were applied to runoff on the first trifoliate or V2 stage soybeans (Fehr and Caviness 1977), using a hand-held pump sprayer. Distilled water was applied to the untreated controls. Six plants were randomly assigned to each of the three treatments. Cages were attached after all plants were allowed to dry for about 1 h. A leaf cage was centered over the midrib, of the middle leaflet of each treated trifoliate, and attached to the abaxial surface. Six cages served as replicates for each treatment.

Three freshly molted and fertilized TSSM adult females (1-72 hours old) were transferred to each cage using a number 3 artist brush, for a total of 18 TSSM per treatment. Experimental units were held in a plant growth chamber at 25°C/19°C ± 0.5°C and 16:8 (L:D). Cages were examined every 12 hours for the numbers of mites present. Egg counts were made every 24 h. The distribution of eggs oviposited directly on the leaf surface vs. suspended on trichomes, cage walls, or in the silk webbing was recorded daily. Eggs were removed daily from all cages using a number 3 artist brush.

After five days, new cages were affixed to the next newest trifoliate on each test plant as previously described. The new trifoliate leaves had not expanded at the time of the insecticide application, and therefore were not exposed to residues. Surviving TSSM were transferred from the original cage to the new leaf site on

each plant, where they were not directly exposed to any pyrethroid residues. TSSM presence and oviposition were monitored for an additional six days. From the raw data, a value of eggs per mite-day oviposition was calculated. Analysis of variance was used to detect differences between the treatment means within a particular time and the cumulative egg production per mite for the 0-5 day (on residues) and 6-11 days (removed from residues) time brackets. When statistical significance was found using ANOVA, treatment means were separated using the Least Significant Difference procedure (Steel and Torrie 1960). Egg distribution data were analyzed by arcsine square root probability transformation of the surface oviposition frequencies, with statistical significance determined using the Kruskal-Wallis non-parametric procedure (Siegel 1956). Means were separated using the non-parametric means separation procedure suggested by Zar (1974).

**Direct treatment of TSSM.** Treatments were prepared as water emulsions of commercially formulated fenvalerate (Pydrin 2.4 EC, 120 ppm) and permethrin (Pounce 3.2 EC, 120 ppm). The experimental units were identical in design to those used in the residue experiment, but all plants were left untreated. Six plants were assigned to each of the insecticide treatments and control.

Forty freshly molted and fertilized female TSSM (1-48 hours old) were selected for each treatment and the control. Each of these cohorts was transferred (with a 3/0 artist brush) to an excised soybean leaflet maintained in a petri dish. Each leaflet was sprayed to runoffs, using a hand-help pump sprayer. Treated leaflets/mites were placed in a plant growth chamber at  $25^{\circ}\text{C} \pm 0.5^{\circ}\text{C}$  and continuous light until dry (approx. one hour). Four treated TSSM females then were transferred to each leaf cage; 24 mites per treatment. Cages were monitored for mite survival and egg production as previously described. TSSM mortality was analyzed using chi-square. Egg production between the control and insecticide treatments was analyzed using one way analysis of variance.

This experiment was repeated with the pyrethroid concentration decreased one magnitude to 12 ppm. For this experiment, leaf cages were moved up each plant to the middle leaflet of the second trifoliolate, of the plants used in the 120 ppm direct application experiment. TSSM presence, egg production, and egg distribution was recorded and analyzed as previously mentioned; however, egg production and distribution were evaluated every 12 h instead of every 24 h. The monitoring was continued for a total of seven days after treatment.

## Results

**Exposure to residues.** Contact with pyrethroid residues significantly reduced the numbers of eggs oviposited in the first 72 hours (Table 1). Oviposition observed after one day in the permethrin and fenvalerate treatments was comparable but significantly less than the control. The relationship between treatments observed after two days was similar, with permethrin comparable to fenvalerate, but significantly less than the control. The number of eggs oviposited in the permethrin treatment after three days was more moderate, significantly more than the fenvalerate treatment, but still significantly less than the control ( $9.6 \pm 0.4$ ). No significant differences between treatments and controls occurred at the four and five day ratings. Oviposition dropped off sharply in the control after the third day, falling to  $6.0 \pm 0.5$  by the fifth day. Cumulative egg production calculated on per mite basis (Table 2) showed the pyrethroids to be comparable in significantly

**Table 1. Mean number of eggs per mite-day of *T. urticae* exposed to permethrin or fenvalerate residues (120 ppm) on soybean foliage.**

Treatment	n	Eggs per mite-day on each day				
		1	2	3	4	5
Control	6	7.3a*	10.2a	9.6a	8.2a	6.0a
Permethrin	6	2.1b	4.4b	5.8b	6.3a	5.9a
Fenvalerate	6	2.0b	3.6b	4.8b	5.9a	4.9a

\* Column means followed by the same letter are not significantly different, ANOVA and LSD, P=0.05.

**Table 2. Cumulative number of eggs oviposited by *T. urticae* over the duration of each experiment, and cumulative exposure to pesticides, expressed as eggs per mite for the exposure time specified.**

Treatment	Experimental exposures to pesticides		
	5 days on residues*	6 days on untreated†	7 days after directed spray‡
Control	41.3a	37.8a	38.0a
Permethrin	24.5b	39.2a	31.2b
Fenvalerate	21.2b	28.2a	26.0b

\* Column means followed by the same letter are not significantly different, ANOVA and LSD,  $P=0.05$ .

† TSSM for the "5 days on residues" experiment, transferred to untreated soybean foliage for an additional 6 days.

‡ TSSM treated directly with 12 ppm concentration, and transferred to untreated soybean foliage for 7 days.

reducing the numbers of eggs oviposited (permethrin  $24.5 \pm 3.7$ , fenvalerate  $21.2 \pm 2.4$ ) compared to oviposition in the controls ( $41.3 \pm 0.9$ ). Fenvalerate and permethrin residues altered the distribution of eggs in the leaf cages (Table 3). Only 17% (fenvalerate) and 23% (permethrin) of the total eggs were oviposited directly on the leaf surface containing pyrethroid residues over the five days. Egg distribution in the control cages was significantly different, with  $> 99\%$  of the eggs oviposited directly on the leaf surface.

No significant differences were detected in numbers of eggs oviposited one day after being transferred to fresh untreated foliage from the fenvalerate ( $3.3 \pm 0.5$ ), permethrin ( $4.9 \pm 0.8$ ) and control ( $5.2 \pm 0.7$ ) mites. On day two, oviposition in the control ( $7.6 \pm 0.4$ ) and permethrin ( $6.0 \pm 0.6$ ) mites increased and remained comparable, while oviposition of the fenvalerate mites was significantly reduced ( $4.1 \pm 1.1$ ). A similar relationship was observed on day three. No significant differences between the control, permethrin, and fenvalerate treatments were observed on day four (9 days after treatment) and beyond. Cumulative egg production calculated on a per mite basis (Table 2) showed the mean number of eggs produced by a female in control, permethrin, and fenvalerate treatments were not significantly different, although there was a consistent trend toward lower oviposition in the fenvalerate treatment ( $P = 0.07$ ).

Mites from the pyrethroid treatments continued to oviposit significant proportions of their eggs suspended above the leaf surface, even though pyrethroid residues were not present on the leaf surface (Table 3). Fenvalerate induced the most dramatic response, with only 15% of the eggs oviposited on the leaf surface. The response to permethrin was less severe (63%), but still significantly different from the control where  $> 97\%$  of the eggs were oviposited directly on the leaf surface.

**Direct treatment.** The 120 ppm concentration of permethrin was found to be moderately lethal to females, resulting in 54% mortality after 24 hours (Table 4). The same concentration of fenvalerate was found to be highly lethal, resulting in 83% mortality after 24 hours, and 100% mortality after 48 hours. No eggs were oviposited by the fenvalerate treated mites. Oviposition by the surviving permethrin treated mites at 24 h after treatment was significantly less than the control. Oviposition continued at significantly reduced levels in the permethrin treatment after 48 hours, compared to the control.

The lower pyrethroid dosage rates (12 ppm) significantly reduced the number of eggs oviposited in both the fenvalerate and permethrin treatments (Table 5). Pyrethroid treated mites initially displayed symptoms of pyrethroid poisoning, such as spasmodic movements of appendages and difficulty in remaining secured to the leaf surface. Fenvalerate showed the more severe effect in reducing oviposition at the 24 and 48 h ratings. However, oviposition in the permethrin treatment at 24 and 48 h also was significantly reduced below the untreated controls. The permethrin treatment values at both times were significantly greater than the corresponding fenvalerate values. No significant differences between the fenvalerate, permethrin, and control treatments were observed at the 3- day through 7- day ratings.

Cumulative numbers of eggs oviposited per mite over the seven day duration of the 12 ppm direct exposure was comparable in the fenvalerate and permethrin treatments (Table 2), but significantly less than oviposition in the control. Direct treatment of females did not result in a significant alternation of egg distribution in the leaf cages (Table 3). Greater than 97% of the total number of control eggs

**Table 3. Distribution of eggs of *T. urticae* exposed to three different experimental exposures to pesticides, expressed as the percentage of egg totals oviposited directly on the soybean leaf surface.**

Treatment	Experiment		
	5 days on on residues	6 days on untreated †	7 days after direct ‡
Control	99.5a*	97.4a	97.5a
Permethrin	22.5b	63.0b	96.0a
Fenvalerate	17.2b	15.2c	84.3a

\* Column means followed by the same letter are not significantly different, Kruskal-Wallis non-parametric procedure, followed by non-parametric means separation procedure described by ZAR (1974).

† TSSM for the "5 days on residues" experiment, transferred to untreated soybean foliage for an additional 6 days.

‡ TSSM treated directly with 12 ppm concentration, and transferred to untreated soybean foliage for 7 days.

Table 4. Percent mortality and eggs per mite-day for *T. urticae* treated directly with permethrin and fenvalerate (120 ppm).

Treatment	n	Hours Post Treatment			
		24		48	
		Percent mortality*	Egg production †	Percent mortality ‡	Egg production §
Control	24	0	3.87a	4	6.17a
Permethrin	24	54	0.80b	54	2.56b
Fenvalerate	24	83	0.00	100	—

\* Chi square on 3 \* 1 contingency Table = 34.7, df = 2, P &lt; 0.005.

† Analysis of variance (fenvalerate treatment excluded), F=26.1, df=10, P &lt; 0.01.

‡ Chi-square on 3 \* 1 contingency table = 44.3, df=2, P &lt; 0.005.

§ Analysis of variance (fenvalerate treatment excluded), F=30.8, df=10, P &lt; 0.01.



**Table 5. Mean number of eggs per mite day of *T. urticae* on untreated soybean foliage following direct treatment with permethrin or fenvalerate (12 ppm).**

Direct Treatment (12 ppm)	n	Eggs per mite-day on each day following treatment						
		1	2	3	4	5	6	7
Control	24	6.1a*	6.4a	6.2a	5.4a	4.8a	5.4a	3.7a
Permethrin	24	4.3b	5.2b	5.4a	4.9a	4.4a	3.7a	3.3a
Fenvalerate	24	1.2c	2.5c	4.2a	5.3a	4.6a	4.7a	3.5a

\* Column means followed by the same letter are not significantly different, ANOVA and LSD, P=0.05.

oviposited, were found directly contacting the leaf surface, compared to 96% in the permethrin treatment and 84% in the fenvalerate treatment.

### Discussion

Numbers of eggs oviposited daily by TSSM held on pyrethroid residues gradually increased over time. Dittrich et al. (1974) noted the possible influence of DDT residue age on the ability of the chemical to elicit a response in the spider mite, with fresher residues having greater activity. Southwick et al. (1983) in studies of permethrin compartmentalization in cotton leaves, noted a rapid decline in permethrin surface residues over the 5 days following application. This decline was attributed to volatilization losses, although pyrethroids as a class are considered to be only slightly volatile (Elliott et al. 1978). Either effect alone or both combined, might explain the gradual restoration of oviposition in the pyrethroid treatments. Leaf expansion during the test also could have been a factor. Overall, the suppressive effect of fenvalerate on the eggs per mite-day continued for three days following removal from the residues, while only slight reductions were noted in the mites previously exposed to permethrin. These results indicate fenvalerate to be more active in depressing TSSM egg production than permethrin.

Data from the direct exposure study suggest that the TSSM used in this study were susceptible to both pyrethroid insecticides, more so than colonies used in published slide dip toxicological studies (Roush and Hoy 1978, Rock 1979, Wong and Chapman 1979, Iftner and Hall 1983). The parent stock colony in this study had been in culture (USDA Floriculture Laboratory, Beltsville, MD, U.S.A.) for about 17 years on bush lima bean, while the subcolony had been maintained on soybeans for about one year.

Pyrethroid residues at 120 ppm were essentially nonlethal to even these highly susceptible TSSM. This agrees with the observations of Dennehy et al. (1982) of the potential discrepancy in susceptibility of mites to residues versus direct contact.

Direct applications of a 12 ppm concentration of permethrin to TSSM females moderately reduced oviposition for at least 48 hours after treatment. Fenvalerate produced a greater reduction in oviposition. TSSM eventually recovered from the effects of both insecticides, and egg production was essentially the same for all three treatments on days 3-7.

Slide dip toxicological tests have shown fenvalerate to be more toxic to TSSM than permethrin (Iftner and Hall 1983). This relationship appears true for the degree and duration of the oviposition reduction observed in this study on soybeans. TSSM afflicted with acute pyrethroid poisoning show symptoms of neural disfunction, such as uncontrolled appendage movements, and lack of motor coordination. TSSM with these symptoms have difficulty remaining attached to the leaf surface. Suppression of oviposition remains even after the visible symptoms of the poisoning have disappeared, and feeding has resumed. The temporary continuation of the oviposition reduction effect following removal of the TSSM from actual contact with pyrethroid residues might be due to chronic poisoning of TSSM while in contact with residues. Over time, the toxins could be enzymatically metabolized gradually eliminating the toxins accumulated during the exposure period.

Results presented here add to an understanding of the factors that contribute to pyrethroid induced outbreaks of spider mites in soybeans, and possibly other crops. These results indicate that hormoligosis is not a likely contributor to TSSM outbreaks following pyrethroid applications, at least at the rates that were examined. Further investigation into the complex pyrethroid avoidance behavior displayed by the TSSM, and the contribution of this factor to TSSM outbreaks in many field crops is needed.

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