Cyhexatin Resistance and Enhancement with Calcium Chloride in Washington State Populations of Spider Mites (Acari: Tetranychidae) on Pome Fruit[1]

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ABSTRACT The enhancement of calcium salts, primarily calcium chloride, to the efficacy of cyhexatin in Washington populations of twospotted spider mite (TSSM), *Tetranychus urticae* Koch, McDaniel spider mite (MSM), *Tetranychus mcdanieli* McGregor and European red mite (ERM), *Panonychus ulmi* (Koch) was evaluated in the laboratory and in commercial apple orchards. Laboratory techniques used to test the 50WP, 5F and technical 85% cyhexatin were the slide-dip, leaf disk and leaf spray bioassays. Bioassay responses by various spider mite populations to cyhexatin and calcium salts indicated that the leaf spray method was most appropriate on apple foliage and that calcium chloride increased cyhexatin 50WP toxicity 6-fold for ERM and 31-fold for TSSM. The toxicity of formetanate 92SP, propargite 30WP, dicofol 1.6EC and fenbutatin-oxide 4L was not increased by combining with $CaCl_2$. Under field conditions, efficacy trials with ERM and MSM to cyhexatin and cyhexatin + $CaCl_2$ indicated that differences between treatment means were significant, but less effective than were predicted from laboratory bioassays. Inconsistent field control was apparently associated with respective adjuvant/cyhexatin concentrations, tetranychid population susceptibility and physical variables inherently associated with orchard management practices.

KEY WORDS Acari, cyhexatin, tetranychids, calcium chloride, resistance, tree fruit, *Tetranychus urticae, Tetranychus mcdanieli, Panonychus ulmi*..

Since the early 1970's, the registration of cyhexatin (Plictran 50 wettable powder (WP) Dow Chemical Company, Midland, MI) has provided Washington State orchardists with a selective organotin acaricide that effectively controls European red mite (ERM), *Panonychus ulmi* Koch, McDaniel spider mite (MSM), *Tetranychus mcdanieli* McGregor, and twospotted spider mite (TSSM), *Tetranychus urticae* Koch on pome fruit. Recent efforts to control these phytophages with physiologically selective acaricides have met with mixed results (Croft et al. 1987). Edge and James (1982, 1983, 1986) reported resistance development to cyhexatin in TSSM populations from apple and pear orchards in Australia. Widespread cyhexatin resistance in North America has since been reported for TSSM in strawberry, apple and pear (Croft et al. 1984; Hoyt et al. 1985, Miller et al. 1985) and for *T. pacificus* McGregor in almond (Keena and Granett 1987). Cyhexatin resistance has been reported for ERM on apple in New Jersey (Swift and Polk

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1984), New York (Welty et al. 1987), Washington (Croft et al. 1987) and Yugoslavia (Stemenkovic et al. 1985).

In 1981, first reports of control problems with ERM came from apple and pear orchardists in Washington who had used annual applications of cyhexatin for over 10 years. Washington's integrated mite program on apple has relied on the selectivity of cyhexatin to suppress economic levels of the apple tetranychid mite complex, while preserving the western predatory orchard mite, *Typhlodromus occidentalis* Nesbitt. The potential for conferring cross resistance to fenbutatin-oxide (Vendex 50 WP, Shell Chemical Company, Houston, TX) by cyhexatin resistant spider mite populations would leave Washington's mite program with few acaricides capable of correctively controlling spider mites. These organotin compounds provide minimal toxic impacts to phytoseiid predators. Because of cyhexatin's low toxicity to the OP-resistant phytoseiid, *T. occidentalis* (Babcock and Tanigoshi 1988, Croft et al. 1984, Hoyt et al. 1985), it and fenbutatin-oxide have been the standard acaricides used in Washington State. These organotins are selectively used when spider mite-predator ratios require field adjustment to promote continued integrated control in the orchard.

Calcium chloride and other calcium salts are commonly applied to some varieties of tree fruits to reduce various physiological disorders and to increase postharvest storage life of fruit (Bangarth 1979). Over the past five years, orchardists who tank mixed cyhexatin with $CaCl_2$ have noticed improved mite control.

The objectives of this study were: 1) To determine from bioassay techniques the relative toxicity of cyhexatin and synergistic effects of calcium salts and commercial formulations of calcium chloride to ERM, TSSM and MSM; 2) To correlate greenhouse and field trials of cyhexatin and $CaCl_2$ to lab bioassays; 3) To assess effects of other calcium chloride-acaricide combinations.

Materials and Methods

Bioassay Methods. A leaf-dip bioassay (Tanigoshi and Congdon 1983) was used. Leaf disks 15 mm diam were obtained from 'Henderson Bush' lima bean, (*Phaseolus limensis* Per.) and dipped for 5 s in appropriate concentrations of toxicant. Treated disks were airdried on wax paper to prevent the solution from wicking away. The leaf disks were then placed bottom side up on cotton sheets (Webril wipes, Kendall Company, Boston, MA) placed on water-soaked foam pads in stainless steel trays. Each concentration was replicated six times with 10-15 adult spider mite females per disk.

A standard slide-dip bioassay after Croft et al. (1976) was also used. Adult female mites were fixed on their dorsum to a standard glass microslide by a piece of Scotch double-sided sticky tape. Each slide was immersed in appropriate concentrations for 5 s and held in a slide box until evaluation. Each concentration was replicated six times with 15 adult spider mite females per slide.

A leaf-spray bioassay modified after Miller et al. (1985) was used for several bioassays. Ten to 15 female spider mites were placed on 15 mm bean leaf disks for 1 h before their topical treatment. Six replicates were used per concentration. The spray was applied to the undersurface of bean leaf disks placed on moist cotton in a disposable petri dish (87 mm inside diam) with no more than six disks per dish. Each disk was treated with a 1 s spray (0.2 ml) at 84 kPa from an air brush

(Badger Air-Brush Company, Franklin Park, IL). This application deposited beads of solution on the leaf disk and spider mites and simulated spray runoff.

Holding conditions were the same for all three bioassay techniques. Mites were held for 48 h (Carbonaro et al. 1986) in darkness at 25 ± 1°C at 60 - 70% RH in an incubator. Mortality was determined by prodding each 3-5 day-old female spider mite with a fine 000 camel hair brush; mites were scored dead if they were unable to repeatedly move their appendages. Water controls were included for each bioassay replication. The results were corrected for control mortality using Abbott's (1925) formula. Bioassay data were analyzed using PROC PROBIT (SAS Institute 1985, p. 639-645).

Spider Mite Populations. TSSM used for respective bioassays were obtained from a Washington State University, Pullman, WA, greenhouse population that had been maintained for 13 months on 'Henderson Bush' lima bean (Scriven and McMurty 1971). These spider mites originated from greenhouse populations that had been exposed for many years to commonly used broad-spectrum pesticides; however, they had never been exposed to cyhexatin.

The Wenatchee ERM and MSM populations were collected in September, 1986 from apple orchards having an extensive history of cyhexatin usage and a recent history for its commercial failure. The MSM colony was reared on detached bean leaves for over six months and was originally collected from a pear orchard population near Prosser, WA. The Wapato pear population and Tieton pear/apple interplant populations of MSM were reared in the lab from overwintering females. Neither populations from these two locations were controlled during the 1986 season with cyhexatin 50WP. Both spider mite populations were isolated and propagated on detached lima bean leaves.

Bioassay Evaluations. Cyhexatin formulations used were 50WP, XRM-4868 5 flowable (F) and 85% technical (Dow Chemical Company, Midland, MI). Calcium sources tested were: calcium chloride (99.8%); calcium nitrate (99.0%); calcium acetate (98.0%); Peladow (95% calcium chloride, Dow Chemical Company); This calcium (6% calcium chloride, Stroller Chemical, Houston, TX) and Link calcium (6% calcium chloride, Wilbur-Ellis, Fresno, CA). All calcium sources were used at or near field concentration rates of 1.5 g/liter (5.6 kg/ha) per 1514 liters of water. This and Link calcium are liquid formulations that were used at 2.5 ml/liter and 1.5 ml/liter, respectively. Other acaricides tested were formetanate (Carzol 92 soluble powder (SP) (Nor-Am Ag Products, Inc., Naperville, IL)), propargite (Omite 30 wettable powder (WP), (Uniroyal Chemical, Bethany, CT)), fenbutatin-oxide (Vendex 4 liquid (L), (Shell Chemical Company, Houston, TX)) and dicofol (Kethane 1.6 emulsifiable concentrate (EC), (Rohm and Haas Company, Philadelphia, PA)).

Greenhouse and Field Trials. Treatments of cyhexatin 50WP (1.13 kg [AI]/1514 liters) and cyhexatin 50WP (1.13 kg [AI]/1514 liters) + $CaCl_2$ (2.26 kg/1514 liters) were applied along with a water check to potted 'Delicious' seedlings in a WSU greenhouse and to commerical 'Delicious' and 'Golden Delicious' orchards located in Wapato and Selah. Applications were made with a CO_2-powered backpack sprayer fitted with a hand wand and an adjustable cone type nozzle. Trees in all tests were sprayed to the point of drip. Random samples were periodically taken by picking 10 leaves per tree for the greenhouse and orchard studies. A mite-brushing machine was used to remove mites from leaves; mite densities for each sample were counted under a stereoscopic microscope and counting grid. Data were

analyzed using the GLM procedure ($P < 0.05$; SAS Institute 1985, p. 483-506). Means were separated with Duncan's (1955) multiple range test.

'Delicious' seedlings were infested with a MSM population in late March, 1987 and arranged in a randomized complete block design consisting of four replicates and three treatments. Seedlings were sampled and treated on 15 April.

Wapato 1. The 'Delicious' trees in this orchard were about 3-4 m tall. All test trees were in a row directly adjacent to a pear block that had a history of MSM problems and poor control with cyhexatin 50WP. Samples were taken on 23 June (pretreatment), 29 June and 14 July 1987.

Wapato 2. This orchard consisted of 'Golden Delicious' trees about 2-3 m tall. It bordered a 'Delicious' orchard where cyhexatin 50WP had performed poorly in 1984 and was used again with mixed success in 1986. The plot was treated on 21 July and samples taken on 21 and 26 July 1987.

Selah. The test trees in this orchard consisted of 3 rows of 'Delicious' trees approximately 3-4 m tall. ERM was the major mite pest and had not been controlled well after the 1984 and 1985 seasons with cyhexatin 50WP. This trial was treated and sampled on the same dates as was the Wapato 2 orchard. All field trials consisted of three treatments and six replicates each.

Results

Bioassays. LC_{50} values were decreased for 50WP, 5F and technical 85% formulations of cyhexatin by the addition of various calcium formulations (Table 1). Combinations of $CaCl_2$ with cyhexatin 5F and technical 85% were 9 and 10-times more toxic than cyhexatin 50WP and $CaCl_2$. A 5-fold increase in toxicity for 50WP + $CaCl_2$ was obtained for the Wenatchee ERM population over that expressed by the 50WP formulation alone. Results of the leaf-dip bioassays for the Pullman TSSM population indicate technical cyhexatin to be 3 and 17-times more toxic than either 5F or 50WP, respectively.

The most enhancing form of calcium tested was reagent quality $CaCl_2$ (Table 1). It lowered the leaf-dip LC_{50} values for cyhexatin 50WP by 31-fold. Cyhexatin 5F and technical 85% LC_{50}'s were lowered by 51- and 18-fold by the addition of $CaCl_2$. Other commercial calcium formulations that produced an increase in toxicity when compared with cyhexatin 50WP were: This, 24-fold; Link, 9-fold; and Peladow, 18-fold.

Estimates for LC_{50}'s for calcium acetate and calcium nitrate were not obtained because of the data's lack of fit to the probit model. However, comparison of single concentration data will illustrate the different relative activities of these compounds (Table 2). At a cyhexatin and calcium acetate concentration of 1500 ppm, only 15% mortality was obtained from 130 TSSM tested; 43% mortality was observed from 240 TSSM tested at 1500 ppm cyhexatin and calcium nitrate. The same TSSM population exhibited 97% mortality from similar leaf-disk bioassays at 300 ppm cyhexatin in combination with 1500 ppm $CaCl_2$.

The regression slopes resulting from a $CaCl_2$ dilution series at a constant cyhexatin concentration of 300 ppm exhibited different results associated with the two populations tested (Fig. 1). The ERM population showed a higher mortality to cyhexatin at the zero $CaCl_2$ level and responded more slowly as $CaCl_2$ concentration was increased (lower slope). The more susceptible TSSM population responded quickly as $CaCl_2$ concentration was increased and a steeper slope was produced.

Table 1. Comparative toxicity of cyhexatin formulations tested independently and in combination with $CaCl_2$ to tetranychid mites.

Species	Population	Assay*	Treatment	N	LC50 (ppm Al)	(95% FL [ppm AL])	Slope	(± SEM)
P. ulmi	Wenatchee	LD	50WP	150	1430	(558-b)	0.74	(1.53)
P. ulmi	Wenatchee	LD	50WP + 2.4g $CaCl_2$	250	250	(59-469)	0.95	(1.05)
T. mcdanieli	Wenatchee	SD	50WP + 2.4g $CaCl_2$	550	530	(421-735)	1.54	(0.64)
T. mcdanieli	Wenatchee	SD	5F + 1.5g $CaCl_2$	400	50	(†-†)	1.96	(0.51)
T. mcdanieli	Prosser	LS	50WP	425	1400	(857-2260)	0.73	(1.37)
T. urticae	Pullman	LD	50WP	700	2190	(980-3540)	1.64	(0.59)
T. urticae	Pullman	LD	50WP + 1.5g $CaCl_2$	1085	77	(71-83)	2.57	(0.39)
T. urticae	Pullman	LD	5F	825	410	(356-483)	1.75	(0.57)
T. urticae	Pullman	LD	5F + 1.5g $CaCl_2$	800	8	(5-12)	2.08	(0.48)
T. urticae	Pullman	LD	85% Technical	520	128	(102-161)	1.38	(0.72)
T. urticae	Pullman	LD	85% + 1.5 g $CaCl_2$	560	7	(4-10)	1.60	(0.62)
T. urticae	Pullman	SD	50WP	650	150	(130-170)	3.30	(0.27)
T. urticae	Pullman	SD	50WP + 1.5g $CaCl_2$	900	110	(93-152)	4.37	(0.23)
T. urticae	Pullman	LS	50WP	330	120	(37-944)	1.51	(0.66)
T. urticae	Pullman	LS	50WP + 1.5g $CaCl_2$	550	23	(19-27)	2.26	(0.44)
T. urticae	Pullman	LD	50WP + THIS	650	90	(71-135)	2.57	(0.39)
T. urticae	Pullman	LD	50WP + PELADOW	925	120	(72-367)	2.12	(0.47)
T. urticae	Pullman	LD	50WP + 1.5 ml LINK	325	250	(208-337)	2.44	(0.41)

* LD = leaf disk, SD = slide dip, LS = leaf spray.

† Data not reported due to inadequate fit to the log-probit model.

Table 2. Leaf disk and spray responses of tetranychid populations to cyhexatin 50WP and calcium salt combinations.

Species	Population	n	Assay* Type	Calcium Source	Cyhexatin (ppm)	Calcium Salt (ppm)	% Mortality	(± SEM)
T. urticae	Pullman	240	LD	CaN_2O_6	1500	1500	43	19.8
T. urticae	Pullman	127	LD	$C_4H_6CaO_4$	1500	1500	15	4.1
T. urticae	Pullman	150	LD	$CaCl_2$	300	1500	98	2.4
T. urticae	Pullman	120	LD	O	300	0	8	4.0
T. urticae	Pullman	80	LS	$CaCl_2$	450	1500	97	2.7
T. urticae	Pullman	100	LS	O	450	0	58	4.6
T. mcdanieli	Wapato	125	LS	$CaCl_2$	450	1500	63	18.2
T. mcdanieli	Wapato	125	LS	O	450	0	30	16.0
T. mcdanieli	Prosser	130	LS	$CaCl_2$	450	1500	60	15.8
T. mcdanieli	Prosser	85	LS	O	450	0	7	9.1
T. mcdanieli	Tieton	117	LS	$CaCl_2$	450	1500	82	9.9
T. mcdanieli	Tieton	100	LS	O	450	0	64	15.6

* LD = leaf disk, LS = leaf spray

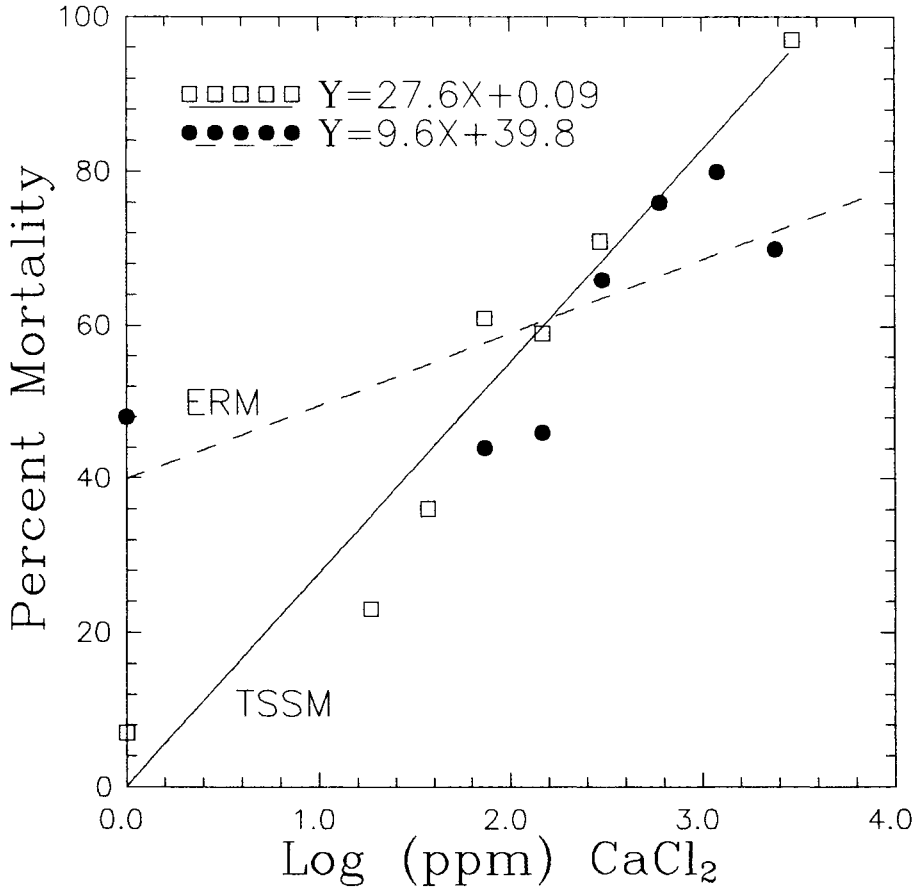

Fig. 1. Log $CaCl_2$ concentration/mortality responses of *P. ulmi* and *T. urticae* with a constant cyhexatin rate of 300 ppm.

At the high $CaCl_2$ concentration end of the response curve a greater level of mortality was obtained for the TSSM population. Both populations showed an increase in response with increasing $CaCl_2$ concentrations, however this response was not as pronounced for the ERM population. A cyhexatin resistant MSM strain from Tieton was also assayed with the same combination of cyhexatin and $CaCl_2$ as used above; however, for this population mortality at the highest $CaCl_2$ level tested was not increased over mortality obtained for cyhexatin alone. Upper levels of $CaCl_2$ are limited by the phytotoxic effect that may be produced at concentrations greater than 6 kg/ha.

Greenhouse and Field Trials. The combination of cyhexatin 50WP and $CaCl_2$ never provided a significantly greater level of population control for ERM and MSM when compared with cyhexatin alone (Tables 3 - 5). Mean values for the

Table 3. Effects of cyhexatin and its combination with $CaCl_2$ in *T. mcdanieli* populations, Pullman, WA.

	15 April*		22 April		29 April	
Treatment	e	m	e	m	e	m
Cyhexatin 50 WP (1.13 kg [AI]/ha)	391a	216a	867ab	152b	211a	284b
Cyhexatin 50WP (1.13 kg [AI]/ha) + $CaCl_2$ (2.26 kg/ha)	245a	177a	206b	43b	76a	38b
Untreated Control	344a	261a	1462a	597a	265a	1510a

Means within a column followed by the same letter are significantly different ($P < 0.05$; Duncan's (1955) multiple range test.

* e = egg/leaf, m = motile life stages/leaf.

Table 4. Effects of cyhexatin and its combination with $CaCl_2$ in *T. mcdanieli* populations, Wapato, WA.

	23 June*		29 June		14 July	
Treatment	e	m	e	m	e	m
Cyhexatin 50 WP (1.13 kg [AI]/ha)	67.7a	21.4a	43.8a	52.6ab	76.0b	34.0b
Cyhexatin 50WP (1.13 kg [AI]/ha) + $CaCl_2$ (2.26 kg/ha)	85.6a	19.0a	22.0a	18.8b	43.6b	18.0b
Untreated Control	58.4a	14.0a	56.8a	77.0a	307.0a	339.0a

Means within a column followed by the same letter are significantly different ($P < 0.05$; Duncan's (1955) multiple range test.

* e = egg/leaf, m = motile life stages/leaf.

combination were consistantly lower than those obtained with cyhexatin alone. This may indicate a subtle difference that the experiment was not statistically sensitive enough to substantiate. After 7 and 14 days postspray, motile life stages of the greenhouse MSM population on the untreated controls were 3.9- and 5.3-fold and 13.8- and 39.7-fold higher than the cyhexatin and cyhexatin + $CaCl_2$-treated seedling, respectively. While these reductions were significantly different for both sampling intervals, the response to the addition of $CaCl_2$ was sufficient to cause a difference of 2.8- and 7.5-fold for ovipositional rate and motile life stages. This numerical advantage corroborates empirical observations for cyhexatin resistance taken for a MSM in an orchard that had a history of cyhexatin failures at recommended rates. Generally, both treatments provided a significant level of control when compared with the water check; however, in both ERM field plots the treatments did not significantly lower their population levels commensurate with MSM.

Table 5. Effects of cyhexatin and its combination with $CaCl_2$ in *P. ulmi* populations, Wapato 2 and Selah, WA.

	Wapato 2				Selah			
	21 July*		26 July		21 July		26 July	
Treatment	e	m	e	m	e	m	e	m
Cyhexatin 50 WP (1.13 kg [AI]/ha)	100.4a	6.0a	73.6a	5.6a	70.5a	6.2a	44.3a	3.5a
Cyhexatin 50 WP (1.13 kg [AI]/ha) + $CaCl_2$ (2.26 kg/ha)	102.0a	6.0a	67.2a	4.8a	64.3a	5.0a	50.0a	3.0b
Untreated Control	98.8a	8.0a	88.8a	4.6a	50.2a	3.2a	57.3a	7.0a

Means within a column followed by the same letter are significantly different ($P < 0.05$; Duncan's (1955) multiple range test.

* e = egg/leaf, m = motile life stages/leaf.

Discussion

In late 1987, cyhexatin was withdrawn from the world market by Dow Chemical Company and its application since has consisted of the use of remaining inventories by orchardists. This change has limited the utility of study; however, the information presented here may be valuable to those interested in studying the behavior of miticides in combination with adjuvants, synergists and other materials. These data may also be useful for comparison of laboratory bioassay methods and of bioassay data with field results.

Comparisons of the three bioassay methods used in this study indicate that the most appropriate technique for spider mites on apple leaves is the leaf-spray. This method comprises residual and topical exposures that will closely approximate toxicant exposure of spider mites on apple foliage when applied by conventional ground orchard sprayers. The leaf-spray bioassay for the Pullman TSSM population produced an LC_{50} of 120 ppm, comparable to the slide-dip LC_{50} of 150 ppm. Slide-dip bioassays of the Pullman TSSM population provided reasonable estimates of cyhexatin susceptibility; however, slope values for the probit lines were much steeper than those obtained from the leaf-dip and leaf-spray bioassay methods (Table 1). These steep slopes may indicate greater homogeneity of response than is actually present under field conditions. The leaf-spray technique also deposits a more uniform toxicant distribution on the leaf surface than is obtainable through the leaf-dip or slide-dip method due to beading of the toxicant solution in the latter methods. From these empirical observations, we judged the leaf-spray method to be the most effective method for determining concentration-response lines for the spider mite species used for these studies.

This study demonstrated the potential for $CaCl_2$ to increase cyhexatin toxicity to spider mites. In the laboratory these increases in toxicity were often substantial; but may not be as apparent under orchard conditions because control failures have occurred even when cyhexatin was combined with $CaCl_2$. Welty et al. (1989)

showed that cyhexatin-ERM bioassay trends were generally apparent in field trials, however, the responses in the field were often more subdued. These results are similar to ours and as was pointed out by Welty et al. (1989) may indicate various extraneous factors that influence acaricide efficacy in the field such as environmental conditions, application techniques and coverage variations, varietal differences and host plant physiology. The degree to which $CaCl_2$ affects cyhexatin toxicity is related to the concentration of each compound, the inherent levels of population resistance and proportions of cyhexatin resistant genotypes. As was shown for the MSM population from Tieton, high concentrations of $CaCl_2$ may have little or no effect in increasing spider mite mortality even in laboratory assays when resistance has become widespread.

Unpublished data by the authors from a ^{14}C-cyhexatin radionuclide assay, indicated that the topical effect of $CaCl_2$ in combination with cyhexatin may be more physiological than physical in affecting penetration through the integument of female TSSM. Also, the hygroscopic nature of $CaCl_2$ may enhance the wetting activity of the toxicant-solution on the mite's integument and cyhexatin's availability in an aqueous medium on the target site. Though residual assays indicated a slightly higher external ^{14}C-cyhexatin concentration, this did not equate to higher internal concentrations. The rate of labeled cyhexatin accumulation was rapid through the first 6 h and then leveled-off between 6-20 hr when compared to the uncombined ^{14}C-cyhexatin solution. This leveling may be due to reduced mite feeding activity and movement relative to intoxication.

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