

Toxicity, Persistence, and Potency of Sabadilla Alkaloid Formulations to Citrus Thrips (Thysanoptera: Thripidae)

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ABSTRACT Toxicity of the major alkaloids present in commercial formulations of sabadilla, Veratran D, was determined in laboratory bioassays with adult female citrus thrips, *Scirtothrips citri* (Moulton). Both cevadine and veratridine, the 2 major components of the insecticidal fraction of sabadilla, were highly toxic to citrus thrips. LC_{50} s of cevadine and veratridine were 18.25 and 29.91 ng/cm², respectively, whereas veracevine, the parent alkanolamine, was much less toxic (LC_{50} of 17,314 ng/cm²). A field trial with Veratran D showed that alkaloid levels on leaves declined to 60% of the initial deposit within 20 h of application and to undetectable levels within 7 d. Analyses of stored commercial samples of Veratran D from each year 1990-1994 indicated that levels of each of the 2 major alkaloids, as well as the "total" alkaloid content determined gravimetrically were similar. In contrast, >3-fold variation in the level of veratridine was noted in 4 samples of Veratran D taken in 1995 despite similar levels of total alkaloids among samples. Future formulations of Veratran D might be improved by standardizing the levels of cevadine and veratridine rather than the level of total alkaloids.

KEY WORDS *Scirtothrips citri*, sabadilla alkaloids, Veratran, cevadine, veratridine, citrus pest management

INTEGRATED PEST MANAGEMENT implies that techniques used to manage one pest species should not disrupt techniques used to manage other pests of the same crop. In particular, the choice of pesticides for integrated pest management is governed not only by consideration of the efficacy against the target pest, but also by considerations of the effect on biological control agents of both target and non-target pests. In citrus pest management in California, this situation is well illustrated in the choice of pesticides for the management of one major pest, citrus thrips, *Scirtothrips citri* (Moulton), without disruption of several effective biological control agents of the other major pest, California red scale, *Aonidiella aurantii* (Maskell).

Currently, native natural enemies provide some control of citrus thrips. However, with very susceptible varieties and in environmentally conducive regions (e.g., San Joaquin Valley navel oranges, coastal lemons, desert citrus), citrus thrips populations often exceed economic thresholds (Haney et al. 1992, Morse 1995) and relatively few insecticides are available and effective (Grafton-Cardwell et al. 1994, Morse 1994). In most situations, California red scale can be managed adequately without pesticides by using a series of augmentative releases of the introduced parasitoid, *Aphytis melinus* DeBach (Haney et al. 1992). One insecticide that has almost no toxicity to *A. melinus* is a formulation of sabadilla alkaloids, Veratran D

(Dunhill, Rosemead, CA.) (Bellows et al. 1985, Morse and Bellows 1986). Unfortunately, the efficacy of sabadilla appears to be more variable in commercial application than synthetic chemical insecticides, and such variation has hindered its widespread inclusion in integrated pest management for citrus.

Crude mixtures of alkaloids from the seeds of sabadilla, *Schoenocaulon officinale* Grey (Liliaceae), have been used as an insecticide since pre-historic times and were widely used as a commercial insecticide until they were replaced by synthetic insecticides after World War II (for a review see Crosby 1971). The major components of the insecticidal fraction of sabadilla are cevadine and veratridine, each of which is an ester of the steroidal alkanolamine, veracevine. A number of related alkaloids occur in sabadilla, but at far lower concentrations (Holan et al. 1984). Previous evaluations of the toxicity of cevadine and veratridine to insects showed that although both were more toxic than veracevine, their relative toxicities were species-specific. Veratridine was more toxic than cevadine to houseflies, *Musca domestica* L. (Ikawa et al. 1945, Bergmann et al. 1958), but cevadine was more toxic than veratridine both to the large milkweed bug, *Oncopeltus fasciatus* (Dallas), and the redlegged grasshopper, *Melanoplus femurrubrum* (De Geer) (Allen et al. 1945).

The standard method to quantify sabadilla alkaloids in pesticide formulations involves solvent extraction followed by solvent removal and weighing of the residue (Helrich 1990); individual components are neither separated nor quantified. Consequently, although the total quantity of alkaloids in a commercial formulation may be standardized, the potency of the formulation to different pests may vary because of variation in the relative concentration of individual components having varying toxicities.

To better use sabadilla in citrus pest management and to understand the causes for the apparent variation in the effectiveness of sabadilla for citrus thrips control in commercial citrus, we determined the toxicity of veratridine and cevadine individually to citrus thrips, determined the persistence of each alkaloid on treated citrus foliage in the field, and determined the extent of variation in the relative abundance of both total and individual biologically active alkaloids in different commercial preparations.

Materials and Methods

Thrips Population. *S. citri* used in this study were collected from wild laurel sumac, *Rhus laurina* (Nuttal), in a semidesert region near Jesus Maria, Baja, Mexico, July 1987 (same as Baja87 [Immaraju et al. 1989]). Thrips were reared since that time in a greenhouse room on laurel sumac by using methods described by Tanigoshi and Nishio-Wong (1981).

Chemical Procedures. Veratridine was obtained commercially (Sigma, St. Louis, MO). Cevadine was purified either from commercial veratrine (Sigma) or Veratran D (Lot # 30405, Dunhill) by using preparative high performance liquid chromatography (HPLC) as described previously (Hare 1996). Veracevine was produced by hydrolysis of veratridine as described by Pelletier and Jacobs (1953).

Solutions for bioassaying each alkaloid were as follows. First, a solvent solution was prepared from HPLC-grade water, sucrose (15.6 g/liter) and a surfactant, Tween 80 (Sigma) (1.0 ml/liter). Sucrose is a feeding stimulant that is added to Veratran D at approximately this level for field applications. The surfactant was added to ensure even distribution over treated leaves.

Stock solutions of veratridine or cevadine were made by dissolving or suspending 2.0 mg of either cevadine or veratridine in 100 ml of the carrier solution to yield a concentration of 20 mg/liter. Test solutions/suspensions at lower concentrations were made from the stock solutions by dilution with the carrier solution. Initial studies on both alkaloids were done at concentrations of 20, 10, 5, 2.5, and 1.0 mg of alkaloid per liter. To estimate the toxicity of cevadine, additional concentrations of 4.0, 0.64, and 0.4 mg/liter also were included. For toxicity tests with of veratridine, additional

concentrations of 5.5, 3.5, 1.8, 0.55, and 0.35 mg/liter were included.

Solutions of veracevine were prepared initially at concentrations of 1,000, 100, 10, and 1.0 mg/liter but statistical analyses were done with data from bioassays at concentrations of 5,000, 2,000, 1,000, 500, 200, 100, and 50 mg/liter. Because these alkaloids are sparingly soluble in water, care was taken that all insoluble material was uniformly dispersed through the solution by vigorous stirring during serial dilution and subsequent transfer to the surface of test leaves.

Laboratory Bioassays. Bioassays were done with leaves placed in Munger cells (Munger 1942, Morse et al. 1986), which provide a closed test arena 3.2 cm in diameter. Leaves were collected from untreated lemon, *Citrus limon* L. Burm., trees at the University of California, Riverside, Citrus Experiment Station. Full-sized, newly expanded leaves were selected at random from the outer canopy between 1 and 2 m from the ground. This leaf age class was chosen on the basis of previous studies that showed their attractiveness to citrus thrips.

The center 8 cm² portion of the adaxial surface of each leaf exposed to thrips in each Munger cell was treated with 200 μ l of one of the alkaloid solutions. Control leaves were treated with 200 μ l of the carrier solution. The 200- μ l droplet was evenly spread over the exposed 8-cm² leaf area with a glass stirring rod and allowed to dry for 1–2 h. Five replicate cells were prepared for each alkaloid at each concentration per trial. Alkaloid concentrations are reported as the quantity of each applied per square centimeter of leaf. (Two hundred microliters of a 1 mg/liter solution contains 200 ng; when this is spread evenly over the 8 cm² of leaf area exposed in the Munger cells, the resultant quantity applied is 25 ng/cm².)

Between 10 and 15 adult female citrus thrips of unknown age were collected from the greenhouse colony into a clear plastic straw with a vacuum aspirator. Thrips within the straw were anesthetized with CO₂ for \approx 45 s, then introduced into the Munger cell. Healthy adult females in each cell were counted when thrips recovered from anesthesia. To reduce the possible effects of pesticide fumigation, air flow through the test arena was provided through air holes (0.3 cm diameter) covered with fine mesh screening and connected to a forced air supply. Mortality was assessed after 48 h. Two to 4 trials were performed on each alkaloid at each concentration. A total of 1,684 thrips (including 244 controls) was used to determine the toxicity of veratridine, 1,573 (including 311 controls) for cevadine, and 979 (including 153 controls) for veracevine. Data were analyzed assuming the probit model (LeOra Software 1987).

Field Bioassays and Chemical Analyses. Seven mature 'Marsh Seedless' grapefruit trees, *Citrus paradisi* MacFayden, were selected from a mixed planting of citrus varieties at the University of Cal-

ifornia, Riverside, Citrus Experiment Station. One hundred leaves in the north quadrant of each tree were tagged on 8 June 1995. Their upper surfaces were gently cleaned with distilled water and a cotton cloth to remove field dust and any other surface contaminants. A mixture of 12 g of Veratran D and 6 g of sucrose was brought up in one liter of water, approximating the recommended concentration of 10 lb of Veratran D + 5 lb of sugar per 100 gal of water per acre used for commercial field application.

Each tagged leaf was sprayed to runoff with the Veratran D solution with a hand-pump sprayer, and the leaves were allowed to dry. The volume of solution applied to each tree was monitored, and the mean \pm SEM volume applied was 95.0 ± 3.4 ml/100 leaves. Fifteen treated leaves from each of the 6 trees were collected at random after the leaves had dried (≈ 4 h). These leaves were then assigned randomly to 1 of 2 groups of 40 leaves each for chemical analysis. The remaining 10 leaves were discarded. Fifteen treated leaves per tree from each of the 6 trees also were collected as above 1, 4, 7, 11, and 15 d after treatment. Eighty leaves were assigned randomly to 1 of 2 groups of 40 for chemical analysis; the remaining 10 leaves were used for bioassays. In addition, 10 cleaned but untreated leaves were collected from a 7th grapefruit tree and used as untreated controls in each bioassay. Bioassays of alkaloid residues were done as described above, except that 10 replicates were set up each day.

Surface Alkaloid Extraction. Each leaf was dipped in 80 ml 5% (vol:vol) acetic acid in water, then wiped clean with a cotton ball moistened with 5% acetic acid. After all traces of surface material were removed, the top and bottom of the leaf were dried with a dry cotton ball, and the leaf was set aside for measurement. The cotton ball used to dry leaves was replaced as it became saturated. After all leaves per treatment had been wiped clean and dried, the used cotton balls were accumulated and placed in the 5% acetic acid bath used for dipping the leaves initially and stirred for 30 min. The cotton balls were then removed, squeezed to remove as much liquid as possible, then discarded. (Preliminary experiments showed that no detectable alkaloids remained on the leaves after treatment, nor did any detectable alkaloids remain in the cotton.)

The acetic acid solution was brought to an exact volume of 100 ml, and 150 μ l of a 0.01 mg/ml solution of papaverine in 5% acetic acid was added to the leaf surface extract. Papaverine has been shown to be a useful internal standard for the quantification of veratridine and cevadine (Hare 1996). The leaf surface extract was vacuum filtered through Whatman No. 1 filter paper to remove insoluble debris, then brought to pH ≥ 10.5 with 4N NaOH in water. The leaf surface extract was extracted 3 times with 150 ml of methylene chloride. The methylene chloride fractions were combined

and concentrated to ≈ 20 ml by rotary evaporation at 45°C.

Alkaloids were further purified by solid-phase extraction. A silica solid-phase extraction column (Fisher PrepSep, 300 mg, Pittsburgh, PA) was conditioned by passing 5 ml methylene chloride through the column with vacuum. The 20 ml of methylene chloride extract (above) was then loaded onto the extraction column with vacuum. The alkaloids were eluted with two 1-ml portions of a 1:1 mixture of methanol:methylene chloride. The eluate portions were combined and evaporated to dryness under a stream of N₂, then redissolved in 150 μ l of methanol.

Veratridine and cevadine were quantified by HPLC as described previously by Hare (1996), except that a Hewlett-Packard Model 1050 UV-vis diode array detector was used for chemical detection and measurement (Hewlett-Packard, Avondale, PA). The total area of all 40 leaves from which alkaloids were surface-extracted was then determined with a leaf area meter (Li-Cor 3000, Lincoln, NE), and the quantity of alkaloids extracted from each batch of 40 leaves was divided by the total leaf area to yield the quantity of each alkaloid per square centimeter of leaf surface.

Quantitative Variation in Alkaloid Concentrations in Formulated Sabadilla. One sample (≈ 100 g) of Veratran D was provided from each of 4 manufacturing dates in 1995 (1 March, 6 and 9 June, and 12 July). In addition, samples pooled over all manufacturing dates each year were provided for 1990 through 1994 from the manufacturer's reference collection. The quantities of "total alkaloids" were determined from duplicate 10-g samples using the official Association of Official Analytical Chemists (AOAC) gravimetric method (Helrich 1990, p. 173). The quantities of veratridine and cevadine also were determined as described above in additional duplicate 250-mg portions of each formulation, as well as the standard lot used in all laboratory and field bioassays.

Although the AOAC method uses 100°C temperatures for drying in the final step, these temperatures caused some breakdown of veratridine (J.D.H., unpublished data). Therefore, we did not attempt to determine the quantities of veratridine and cevadine in the total alkaloid extracts from the AOAC procedure. Variation in the quantities of veratridine, cevadine, their sum, and total alkaloids was determined among years, and among batches in 1995, by analysis of variance (SAS Institute 1988).

The expected quantities of veratridine and cevadine in these samples when applied in the field were calculated on the basis of the concentration of these 2 alkaloids relative to those in the standard laboratory lot, and the quantity of veratridine and cevadine recovered after applying this standard lot to leaves in the field (see *Results and Discussion*). The potency of these samples was then estimated by calculating the expected toxicity of each alkaloid

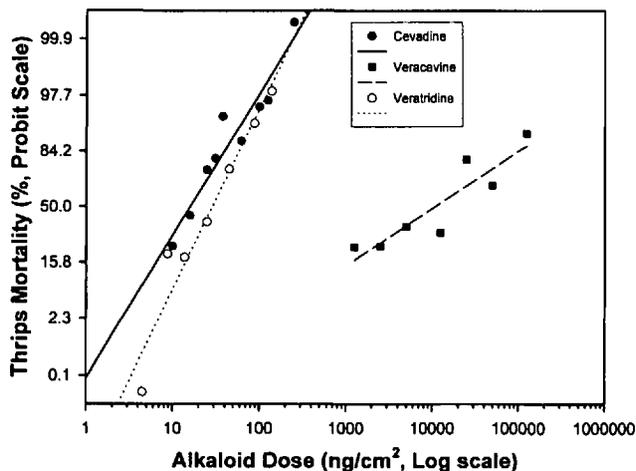


Fig. 1. Concentration–response relationships between alkaloid concentration and thrips mortality. Results of probit analyses: Slope \pm SEM = 3.064 \pm 0.206 for veratridine; 2.433 \pm 0.143 for cevadine; and 1.291 \pm 0.130 for veracevine. Values plotted are mean mortalities for each dose.

mixture by using the model of independent, joint action (Bliss 1939).

Results and Discussion

Toxicity of Veratridine and Cevadine to Citrus Thrips. Both veratridine and cevadine were highly toxic to citrus thrips (Fig. 1). The LC₅₀ (\pm 95% FL) for veratridine was 29.91 ng/cm² (23.13–35.99), whereas that for cevadine was 18.25 ng/cm² (13.68–22.62). The LC₉₀s were 78.36 ng/cm² (65.60–99.55) and 61.37 ng/cm² (50.21–79.42) for veratridine and cevadine, respectively. Veracevine,

in contrast, had similar toxicities only at concentrations 500–3,000 times higher (LC₅₀ = 17,314 ng/cm² (5,026–37,760); LC₉₀ = 170,260 ng/cm² (66,925–3,381,800)). The slopes of concentration–response lines of veratridine and cevadine differed significantly (chi-square from POLO-PC = 8.84, df = 1, P = 0.003). The LC₅₀ of cevadine was significantly different from that of veratridine as indicated by their lethal concentration ratio and 95% CL (ratio = 0.610, 95% CL = 0.516 and 0.722) and ascertaining that the confidence limits did not include the value 1.0 (Robertson and Preisler 1992, p. 42).

Persistence of Toxicity of Sabadilla Residues in the Field. Our application procedures resulted in 30.9 \pm 4.9 ng/cm² of veratridine and 35.3 \pm 6.1 ng/cm² (mean \pm SEM) of cevadine applied to grapefruit foliage (Fig. 2). By the next morning (\approx 20 h later) when the 1st thrips bioassay began, the quantities of veratridine and cevadine had declined to 59.5 and 59.8% of the initial levels (18.4 \pm 1.3 and 21.1 \pm 2.1 ng/cm², respectively). The concentration of veratridine continued to decline moderately through day 7, whereas that of cevadine declined rapidly to 0 between days 4 and 7. Neither alkaloid was recovered from foliage after day 7. We attribute this effect not only to breakdown of the esters, but also to the likelihood that any remaining alkaloids could have been washed from the leaves by (unseasonable) rainfall of 1.8 cm on days 8 and 9 (i.e., 16 and 17 June 1995).

Thrips mortality was 73.6 \pm 8.0% on day 1, then declined to 54.6 \pm 12.7% on day 4, and to 49.5 \pm 12.7% on day 7. No significant mortality was apparent on treated leaves after the leaves had been exposed to rainfall. We predicted expected thrips mortalities on days 1, 4, and 7 based on the observed concentration–mortality relationships ob-

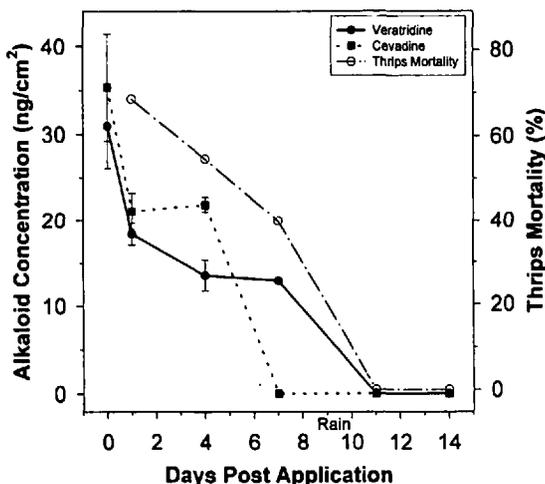


Fig. 2. Persistence of veratridine and cevadine, and thrips mortality for leaves treated with Veratran D. Values are means and standard errors of the mean for alkaloid concentration and mean corrected thrips mortality. Rain (1.8 cm) fell on days 8 and 9.

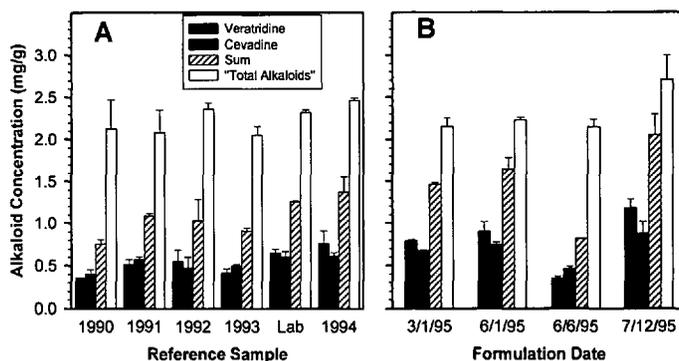


Fig. 3. Mean \pm SEM concentrations of veratridine, cevadine, the sum of veratridine and cevadine, and total alkaloids determined by gravimetry. Plot A: 1990–1994 reference samples, and lot #30405A, which was used in all field and laboratory bioassays. Plot B: Samples formulated on the indicated dates in 1995.

served in the laboratory and the quantities of each alkaloid extracted from the leaf surface assuming no antagonism or synergism between the 2 alkaloids. Our observed thrips mortality was similar, although somewhat lower than predicted (day 1, 73.6% observed versus 89% predicted; day 4, 54.6% observed versus 78% predicted; day 7, 49.5% observed versus only 17% predicted). The greatest deviation occurred on day 7, when cevadine was unexpectedly undetectable.

Quantitative Variation in Alkaloid Concentrations in Formulated Sabadilla. The yearly reference samples of pooled Veratran D production did not differ in quantities of veratridine, cevadine, their sum, or total alkaloids ($F = 2.80$, $P = 0.14$ for veratridine; $F = 1.65$, $P = 0.30$ for cevadine; $F = 2.48$, $P = 0.17$ for their sum; and $F = 1.37$, $P = 0.36$ for total alkaloids; $df = 4, 5$ in all cases, Fig. 3A). In contrast, we found significant, > 3 -fold variation in the concentration of veratridine from the 4 individual batches formulated in 1995 ($F = 19.76$; $df = 3, 4$; $P = 0.007$, Fig. 3B). The formulation of 6 June contained the least amount of veratridine (0.352 mg/g), whereas that formulated on 7 July had the most (1.18 mg/g). However, variation in the concentration of

cevadine ($F = 5.45$; $df = 3, 4$; $P = 0.07$), was not significant. The sum of veratridine and cevadine also differed among these 4 lots ($F = 13.03$; $df = 3, 4$; $P = 0.016$), and the pattern of variation followed that of veratridine. Concentration of total alkaloids did not vary significantly ($F = 2.64$; $df = 3, 4$; $P = 0.19$). This result suggests that there might have been significant variation in the proportions of active (e.g., veratridine and cevadine) and inactive alkaloids (e.g., veracevine) in the seed lots used to formulate Veratran D on these 4 dates. (Note that veracevine is not detectable by HPLC using UV detection [Hare 1996]). Thus, whereas the quantities of seeds used in the 1995 formulations were correctly adjusted to provide nearly equivalent quantities of alkaloids in all 4 formulations, and at least the advertised quantity of 0.2% total alkaloids (equivalent to 2.0 mg/g), this did not result in formulations with equivalent levels of bioactive alkaloids.

The significance of this quantitative variation in alkaloid concentration in different formulations is not clear. For example, all 1995 samples contained sufficient alkaloids to expect at least 83% thrips mortality shortly after application (Table 1). (Note that expected toxicity in Table 1 was calculated using the model of independent joint action [Bliss 1939] because the slopes of the dose mortality lines of each alkaloid were not parallel and the limited data from the field trial did not provide evidence for synergism between veratridine and cevadine [Robertson and Preisler 1992, p. 90].) However, the rapid loss of alkaloids within 24 h of application suggests that the samples with relatively low concentrations of veratridine and cevadine probably would have shorter residual activity against citrus thrips. The significance of the variation in potency of the yearly reference samples is even more difficult to evaluate because the variation could be the result not only of variation in the potency of seeds used in different years, but also the result of breakdown during storage over the past 1–5 yr.

Table 1. Expected quantities of veratridine and cevadine and expected thrips mortality, if reference samples were to be applied to leaves at the same rate as lot #30405A, as in Fig. 2, and independent, joint action is assumed

Sample	Veratridine, ng/cm ²	Cevadine, ng/cm ²	% expected mortality
1990	17.2	19.8	67.0
1991	28.4	31.6	86.8
1992	26.2	27.8	82.3
1993	19.5	29.1	84.0
1994	36.1	35.9	90.4
3/ 1/95	37.6	39.4	92.6
6/ 6/95	42.8	43.2	94.3
6/ 9/95	15.8	28.5	82.3
7/12/95	56.1	51.0	96.7

In conclusion, sabadilla is toxic to citrus thrips at rates conventionally applied, although toxicity is lost rapidly after application. The rapid loss of activity of sabadilla formulations when exposed to air and sunlight after application was noted in some of the earliest investigations on its potential role as an insecticide (Anderson 1945, Walton 1947, reviewed by Crosby 1971). With regard to integrated pest management, the rapid breakdown of materials usually is advantageous in minimizing reductions to natural enemy populations; however, the lack of residual toxicity also puts a premium on optimal timing of the pesticide application against the target pest.

Veratran D apparently is being formulated consistently to provide at least 2 mg/g of total alkaloids, although formulating on the basis of total alkaloid concentration may be insufficient to produce a formulation with consistent quantities of veratridine and cevadine. A more consistent product might be developed if the quantities of seeds incorporated into commercial sabadilla formulations were adjusted on the basis of veratridine and cevadine content, and not simply on the basis of total alkaloid content. Nevertheless, with few exceptions, most batches should contain sufficient bioactive alkaloids to provide significant initial thrips mortality.

Our results may provide little explanation for the apparent variability in efficacy of sabadilla in the field. Such variability was present even in our laboratory bioassays where we carefully controlled the quantity of alkaloids applied to leaves used in our bioassays. This suggests that the processes and time course in which individual citrus thrips contact and ingest these alkaloids may require more detailed investigation before mortality to thrips from sabadilla can be predicted more accurately.

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