# Response of the Predatory Mite Phytoseiulus macropilis (Acari: Phytoseiidae) To Pesticides and Kairomones of Three Spider Mite Species (Acari: Tetranychidae), and Non-Prey Food

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Source: Florida Entomologist, 92(4) : 554-562

Published By: Florida Entomological Society

URL: https://doi.org/10.1653/024.092.0404

# RESPONSE OF THE PREDATORY MITE *PHYTOSEIULUS MACROPILIS* (ACARI: PHYTOSEIIDAE) TO PESTICIDES AND KAIROMONES OF THREE SPIDER MITE SPECIES (ACARI: TETRANYCHIDAE), AND NON-PREY FOOD

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ABSTRACT

The predatory mite *Phytoseiulus macropilis* Banks (Acari: Phytoseiidae) is native to Florida. Some biology and ecology of this phytoseiid have been documented, but its potential as a biological control agent of phytophagous mites (Acari: Tetranychidae) has received less attention. The response of *P. macropilis* to 12 acaricides, 3 tetranychid mite species and 5 potential alternate foods was evaluated in laboratory bioassays. Pesticide residual effects on *P. macropilis* were evaluated by a double-disk leaf residue method. The synthetic pyrethroids Tame (fenpropathrin), Cymbush (cypermethrin) and Mavrik (fluvalinate) were highly toxic. Tolerance was observed to the acaricides, Omite (propargite), and Avid (abamectin), while Vendex (hexakis), Pentac (dienochlor), and Kelthane (dicofol) were highly toxic. The insecticides Orthene (acephate) and Diazinon and the fungicides, Domain (thiophanate-methyl) and Cleary (thiophanate) were not toxic to *P. macropilis*. Field efficacy tests of fenpropathrin and dicofol indicated that these chemicals lose toxicity to *P. macropilis* 21 and 7 d after application, respectively. In olfactometer bioassays, female predators were attracted to kairomones produced by their rearing host *Tetranychus urticae* Koch on bean leaves but not to kairomones of the tetranychids *Oligonychus ununguis* (Jacobi) and *T. evansi* Baker and Pitchard on their respective host plants. Predators did not respond significantly to selected alternate foods: pollen from the hybrid daylily *Hemerocallis* spp., *Phylloxera* spp. larvae, eggs of false oleander scale *Pseudaulacaspis cockerelli* (Cooley), a sugar-water solution and water. This study identified several pesticides that could be integrated with use of *P. macropilis* as a biological control. Results also indicate that the predator may have a narrow prey range and require specific species of mite prey for survival and oviposition.

Key Words: predatory mite, insecticide, attractant, spider mite

RESUMEN

El ácaro depredador *Phytoseiulus macropilis* Banks (Acari: Phytoseiidae) es nativo de la Florida. Algunos aspectos de su biología y ecología han sido documentados, pero su potencial como un agente de control biológico de ácaros fitófagos (Acari: Tetranychidae) ha recibido menor atención. La respuesta de *P. macropilis* a 2 acaricidas, 3 especies de ácaros tetraniquidos y 5 clases de comida alternativa potencial fue evaluada en bioensayos en el laboratorio. Los efectos de los residuos de pesticidas sobre *P. macropilis* fueron evaluados utilizando el método de residuos sobre doble-discos de hojas. Los piretroides sintéticos Tame (fenpropatrin), Cymbush (cypermetrin) y Mavrik (fluvalinate) fueron altamente tóxicos. Tolerancia fue observada hacia los acaricidas Omite (propargite) y Avid (abamectin), mientras Vendex (hexakis), Pentac (dienochlor) y Kelthane (dicofol) fueron altamente tóxicos. Los insecticidas Orthene (acefate) y Diazinon y los fungicidas, Domain (thiofanate-metil) y Cleary (thiofanate) no fueron tóxicos a *P. macropilis*. Las pruebas de eficacia en el campo para feopropatrin y dicofol indicaron una perdida de toxicidad a *P. macropilis* de los 21 y 7 días después de aplicación, respectivamente. En los bioensayos utilizando el olfactometro, depredadores hembras fueron atraídas a las kairomonas producidas por su hospedero *Tetranychus urticae* Koch sobre hojas de frijol sobre lo cual fueron criados pero no fueron atraídas a kairomonas de los tetraníquidos *Oligonychus ununguis* (Jacobi) y *Tetranychus evansi* Baker y Pritchard sobre sus plantas hospederas respectivas. Los depredadores no respondieron significativamente de las clases de comida alternativa seleccionadas incluyendo polen de lirio híbrido *Hemerocallis* spp., larvas de *Phylloxera* spp., huevos de la escama diaspidido *Pseudaulacaspis cockerelli* (Cooley), una solución de agua-azúcar, y solo agua. Este estudio identifico varios pesticidas que pueden ser integrados con el uso de *P. macropilis* como un agente de control biológico. Los resultados indican que el depredador posiblemente tiene un rango de presa estrecho y requiere especies de presa especificas para sobrevivir y ovipositar.

The economic losses caused by phytophagous mites (Acari: Tetranychidae) in various agricultural crops in most areas of the world is well documented (Huffaker et al. 1970). The genera *Tetranychus*, *Oligonychus*, *Eotetranychus* and *Panonychus* are some of the world's most important pests of agricultural crops (Jeppson et al. 1975). Tetranychid mites, including the twospotted spider mite *Tetranychus urticae* Koch, the southern red spider mite *Oligonychus ilicis* (McGreger), and the spruce spider mite *O. ununguis* (Jacobi), are important pests of ornamental nursery crops in the southern United States, with *T. urticae* being the predominant pest species. Nursery and landscape plants have an "aesthetic" economic threshold with low tolerance for pest damage. Therefore, nurseries frequently use pesticides to combat pest outbreaks (Mizell & Schiffhauer 1991). Nursery production requires intensive labor and high volumes of water, both subject to high risk of pesticide exposure. Rapidly increasing pesticide costs, social clamor against hazardous effects of chemicals, legal regulations regarding worker safety, and the risk of pesticide resistance development among pest species indicate a need to develop alternate integrated pest management strategies that reduce or eliminate pesticide use and its side effects in nurseries. Integration of natural enemies with selective pesticides could be a viable strategy for some pest situations in nurseries.

Although natural enemies of phytophagous mites have been reported from several acarine families (Lord 1949; Knavel & Salheime 1967), the majority of the well known predatory mites belong to the family Phytoseiidae. Phytoseiids as biological control agents of phytophagous mites are effective in many agricultural systems (Flaherty & Huffaker 1970; Pickett & Gilstrap 1986; Hamlen & Lindquist 1981; Mizell & Schiffhauer 1991). Phytoseiids locate their prey through chemical cues known as "herbivore induced plant volatiles" (van Wijk et al. 2008) emitted from host plants as a result of spider mite feeding activity (Dicke et al. 1990; Takabayashi & Dicke 1992; Takabayashi et al. 1994; van Wijk et al. 2008). The perception of kairomones increases the probability of prey finding by phytoseiids (Hislop & Prokopy 1981; Dicke et al. 1990; Takabayashi et al. 1994; van Wijk et al. 2008). Rosen & Huffaker (1982) regard searching ability as the most important attribute of an effective predator. Some phytoseiids also feed on non-prey foods, such as pollen, honeydew, and plant juices (McMurtry 1982), which may help to sustain the predators through periods when prey are at low densities (Huffaker & Flaherty 1966).

Despite many documented successes against tetranychid mites in diverse agricultural systems, the family Phytoseiidae has only a few well-known species. Thus there is a need to research and develop new, particularly native, predators to suppress phytophagous mite outbreaks. The predatory mite, *Phytoseiulus macropilis* Banks, indigenous to Florida (Muma & Denmark 1970), has shown its efficacy against phytophagous mites in cotton (Saba 1971) and glasshouse crops (Hamlen 1978; Hamlen & Lindquist 1981) and may have excellent potential as a biological cont1rol agent of tetranychid mites of nursery ornamentals. Aspects of the biology and ecology of this phytoseiid are well documented (Smith & Summers 1949; Prasad 1967; Shih et al. 1979; Ball 1980; Hislop & Prokopy 1981; McMurtry & Badii 1989; Mesa et al. 1990), but *P. macropilis* response to common pesticides and volatiles of tetranychid mites-host plant species (except *T. urticae*) is still unknown. In addition, potential use of alternate food by *P. macropilis* has not yet been determined. This study evaluated the response of *P. macropilis* to (1) pesticides commonly used in nurseries, (2) volatiles from potential tetranychid prey-host plant species, and (3) selected alternate food to determine the predator's potential use in an integrated pest management program against tetranychid mites in nurseries.

## Materials and Methods

### Predator Rearing

*Phytoseiulus macropilis* (Banks) were sub-cultured from a laboratory colony established in 1987 by R. F. Mizell, III from a wild population feeding on twospotted spider mites at Monticello, Florida. Mite identification was performed by H. A. Denmark and vouchers specimens are housed in the Florida Collection of Arthropods, Gainesville, Florida. Predators were reared in the laboratory during 1993 and 1994 at 27-34°C, 60 ± 5% relative humidity (R.H.) and a photoperiod of 16:8 light:dark (L:D). Rearing units consisted of a rectangular sponge (length = 23.5 mm, width = 16.5, thickness = 2 mm) placed in a metal tray (length = 27 mm, width = 17.5, height = 3.5 mm). The tray was filled with tap water until the sponge was saturated. A black plastic plate (length = 22 mm, width = 12.3 mm, thickness = 0.2 mm) was seated on the sponge as the rearing arena.

Lima beans, *Phaseolus vulgaris* L., 'Henderson', were grown in a greenhouse (25°C and 60% ± 5% R.H. under fluorescent lights) at the North Florida Research and Education Center in Monticello, Florida. Twospotted spider mites were reared on 1-2-week-old lima bean seedlings at 27-31°C, 60 ± 5% R.H. under fluorescent lights in the laboratory. Predators were fed on the rearing units every 24 h by adding 2-4 lima bean leaves infested with all stages of *T. urticae*. The old dried leaves from rearing units were discarded after removing predator eggs, nymphs, and adults. Adult female predators 3-5 d old were used in all bioassays.

Residue Bioassays

Residue bioassays were used to determine the toxic effects of selected registered pesticides on *P. macropilis* by a modified double-leaf-disk method. The double-leaf-disk method described by Schiffhauer & Mizell (1988) was modified so that the ring was closed with a treated leaf instead of cloth. Rings cut from standard PVC pipe (diameter = 20 mm; thickness = 2.5 mm) were attached to a 7-d-old cotyledon leaf of lima bean with contact cement (DAP Inc., Dayton, Ohio). The seedlings with the PVC rings were dipped in the test concentrations for 5 s. After drying seedlings in a hood, eggs and other life stages of *T. urticae* were placed inside the ring arena with a fine brush. Ten to 15 female predators from the colony were aspirated into a small straw with a vacuum device. The straw was sealed with parafilm and held in ice for about 5 min to slow down predator movement to prevent their escape. Predators were transferred into the ring arena, and the latter was closed on top by the second cotyledon leaf with contact cement. Treated and untreated control seedlings were held in beakers with their roots immersed in water. The number of concentrations tested varied by chemical, but most were tested at 5 serial dilutions of the label rates (Table 1). Six replicates of 10 predators were tested for each concentration of 12 acaricides. Untreated control groups (6 replicates of 10 predators), were included with each test. After 72 h, the top leaf from each ring was removed and mortality was assessed under a steromicroscope by a gentle touch to the predators with a fine probe. Predators that could not walk one body length when probed were counted as dead. These bioassays were carried out at 27-34°C and 50± 15% R.H. under fluorescent lighting. Toxicity was rated as in IOBC/WPRS (Hassan et al. 1987). Dose-response lines, $LC_{50}$ values and slopes were calculated for 5 acaricides by the Probit Procedure in SAS (SAS 1994).

Field Efficacy of Fenpropathrin and Dicofol Residues

The pesticides, fenpropathrin and dicofol, were applied to *Euonymus japonica Thunb* to runoff at the 1× rate (Table 1) with a backpack sprayer. Seven to 10 cm long shoots were cut from treated plants and leaf replicates and numbers of predators tested were as described in the lima bean bioassays at 0, 3, 7, 14, and 21 d in order to determine the mortality of the residues to *P. macropilis*. The final mortality was adjusted for untreated control mortality by Abbott's formula (Abbott 1925).

Olfactometer and Response to Prey-host Emitted Volatiles

A glass Y-tube olfactometer of diameter 1.1 cm with arms 8.5 cm long (Mizell & Schiffhauer 1991) was used to observe the behavioral response of gravid female *P. macropilis* to plant- and prey-emitted volatiles. The central arm of the Y-tube was connected to an air outlet and a Nalgene water vacuum pump (11.5 L/minute capacity, NO. 6140-0010) on a water spigot via a plastic tube. Each end of the Y-tube was connected to adjustable plastic containers that contained the test treatments. Air flowed through a canister of activated charcoal into the adjustable plastic containers and through the Y-tube. Air flow was adjusted with flowmeters at 100 mL/min in both arms of the Y-tube. Treatments were placed upwind in the plastic containers. The Y-tube was placed horizontally on a wooden block over white paper to improve observation of predator movement. Adult female predators were placed in the main arm of the olfactometer with a fine probe.

Predatory Mites

Adult female *P. macropilis* reared on *T. urticae* Koch on 'Henderson' lima bean were starved for 24 h before the tests. Thirty-six predators (used only once) were tested individually for each treatment. Each predator was observed for a maximum of 10 min. After 10 predators were tested, the treatments in the olfactometer arms were rotated to remove possible positional effect(s) and the olfactometer was washed with acetone. Predators that did not choose one of the arms of the olfactometer after 10 min were removed and scored as no response. Predators were scored '+' or '–' when they reached the upper end of one of the Y-arms of the olfactometer. The tests were carried out at 27-34°C and 60-80% R.H., under fluorescent light from 1130-1800 EDT. The following treatments were tested to observe the response of *P. macropilis* to kairomonal cues from the prey and host plant: (1) blank versus blank (to test the validity and potential positional bias of the olfactometer); (2) blank *vs* uninfested lima bean leaves; (3) *T. urticae* infested lima bean leaves *vs* blank; (4) *T. urticae*-infested bean leaves *vs* uninfested lima bean leaves; (5) lima bean leaves previously infested by *T. urticae* but with all traces of mites removed with a light brushing *vs* blank; (6) blank *vs* uninfested juniper leaves; (7) *O. ununguis* infested juniper leaves *vs* uninfested juniper leaves; (8) *T. urticae* on lima bean leaves *vs O. ununguis* on juniper leaves; (9) *T. evansi* infested tomato leaves *vs* blank; (10) *T. evansi* on filter paper *vs* blank; and (11) *T. urticae* on lima bean leaves *vs T. evansi* on tomato leaves. The results were statistically analyzed by the Sign-test at $P = 0.05$ (Conover 1971).

Alternative Food Bioassays

Selected non-prey food: water, water and sugar solution (16:1), daylily pollen *Hemerocallis sp.*,

TABLE 1. PESTICIDES TESTED FOR THEIR EFFECT ON ADULT *PHYTOSEIULUS MACROPILIS*.

| Pesticide trade name (active ingredient) | Manufacturer | Label[1] rate (1X) mg a.i./L | Serial dilution[2] | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | 3X | 2X | 0.1X | 0.01X | 0.001X | 0.0001X | 0.00001X |
| 1. Abamectin (Avid) | Merck Sharps Dhome[3] | 5.8 | | | + | + | + | + | + |
| 2. Vendex (Hexakis) | E.I. Du Pont De Ne Mours & Co. (Inc.) Wilm. | 450.6 | + | | + | + | + | + | + |
| 3. Kelthane (Dicofol) | Rohm & Haas | 478.4 | | | + | + | + | + | + |
| 4. Pentac (Dienochlor) | Sandoz | 480.0 | | | + | + | + | + | + |
| 5. Mavrik (Fluvalinate) | Zoecon Corp. | 139.2 | | | | | | + | |
| 6. Cymbush Cypermethrin) | ICI Americas, Inc. | 208.5[4] | | | | | | | |
| 7. Tame (Fenpropathrin) | Chevron Chemical Co. | 138.0 | | | | | | | |
| 8. Omite (Propargite) | Uniroyal Chemical, Inc. | 360.0 | + | + | + | + | + | + | + |
| 9. Orthene (Acephate) | Chevron Chemical Co. | 1132.5[3] | | | | | | | |
| 10. Domain (Thiophanate- methyl) | Grace Sierra Corp. | 626.0[3] | | | | | | | |
| 11. Cleary (Thiophanate) | Cleary Chemical | 1198.4[3] | | | | | | | |
| 12. Diazinon | Ciba-Geigy Corp. | 1764.0[3] | | | | | | | |

[1]Rates recommended by manufacturers for spider mite control on nursery plants.
[2]Concentration was tested in addition to the label rate.
[3]Manufacturers for some compounds have changed since the data collection, but these were the origin of the compounds in the experiments.
[4]Only label rate was tested.

eggs of false oleander scale *Pseudaulacaspis cockerelli* (Cooley), and *Phylloxera sp*. nymphs were presented to adult female predators to study their effect on survival and fecundity. The experimental units consisted of an inverted plastic Petri dish (5.3 mm dia.) closed with silicone (Alex Plus, DAP Inc. Dayton, Ohio 45401). A moistened filter paper placed at the bottom retained humidity and also served as the arena for the subjects. The inverted dish had 2 holes at the base; one was used for predator and food placement (fitted with a cork) and the other was fitted with a stub of filter paper to supply moisture. Food was provided every 24 h. The number of eggs and live predators were recorded per 24 h. Five replicates of 6 satiated, gravid female *P. macropilis* were used for each treatment. The treatments were continued until the tested predators died. A control group of 5 replicates of 6 predators with all life stages of *T. urticae* as food was included with each treatment. Bioassays were conducted at 27-34°C and 60-80% R.H. Results were analyzed by the randomization test at $P = 0.05$ (Conover 1971).

## Results and Discussion

### Residue Bioassays

Synthetic pyrethroids were highly toxic as permethrin, fluvalinate, and fenpropathrin at1X label rates caused 100% mortality (Table 2). However, in the field efficacy test, fenpropathrin toxicity decreased to 30% at 21 d after application (Table 3). Other studies of synthetic pyrethroid toxicity to phytoseiids reported similar results (Croft et al. 1983; Riedl & Hoying 1983; Mizell & Schiffhauer 1991). Fenpropathrin under field conditions showed high toxicity during the 2 weeks after application, but its toxicity declined to 30% after 21 d (Table 3). Thus, *P. macropilis* might be released into nurseries 3-4 weeks following treatment with fenpropathrin.

Dienochlor at the 1X concentration caused high mortality, but at lower concentrations mortality declined sharply (Table 3). These results agree withreports of Mizell & Schiffhauer (1991) for *N. collegae* De Leon, and Malezieux et al. (1992) for *N. fallacis* (Garman). The $LC_{50}$ value was 335.54 mg a.i./L, which is more than half of the registration field rate (480 mg a.i./L). *Phytoseiulus macropilis* is relatively unharmed by exposure to dienochlor, which is an organochlorine compound no longer available for use (Table 4).

The insecticide Diazinon surprisingly caused only 18% mortality whereas acephate caused only 5% mortality at 1× rate and was the safest of all pesticides tested (Table 2). Nevertheless, Hassan et al. (1987) reported acephate as highly toxic to *Phytoseiulus* spp. Similarly, Diazinon has been observed as highly toxic to *A. hibisci* (Bartlett 1964) and *A. fallacis* (Croft & Nelson 1972). However, results similar to ours were reported by Babcock & Tanigoshi (1988) on *T. occidentalis* and Zacharda & Hluchy (1991) on *T. pyri* Scheuten. Apparently both insecticides could be used in nurseries targeted to pest species other than mites and cause little harm to *P. macropilis*.

The acaricide dicofol at the 1× rate caused 100% mortality and the $LC_{50}$ was very low 0.083 mg a.i./L in comparison to the label rate. Under field conditions, however, residual toxicity declined 7 to 14 d after application to 12% and 1%, respectively (Table 3). Heretofore, this chemical has been reported as nontoxic (Rock & Yeargan 1971; Theiling & Croft 1988), slightly toxic (MacPhee & Sanford 1961), moderately toxic (Bartlett 1964; Zacharda & Hluchi 1991) and highly toxic to phytoseiids (Van de Vrie 1962; Hassan et al. 1987; Mizell & Schiffhauer 1991). Based on our observation of dicofol in the field, it appears that *P. macropilis* could be safely integrated with this chemical for tetranychid suppression in nurseries 7-14 d after its application.

The acaricides abamectin and hexakis at the 1X rate caused high *P. macropilis* mortality (Table 4). The $LC_{50}$ value for hexakis was 74.54 mg a.i./L and for abamectin 0.7513 mg a.i./L. Propargite at the 1X rate caused low mortality (Table 4). The acaricide propargite was harmless, both at the label rate and at concentrations 2, 3,

Table 2. Response of *Phytoseiulus macropilis* females after 72 h to commercial pesticides at label rates in a laboratory bioassay.

| Pesticide | Label rate[1] (1X) mg ai. $L^{-1}$ | Mean % mortality ± SE | Classification[2] |
|---|---|---|---|
| Fluvalinate | 139.2 | 100 ± 0 | 4 |
| Fenpropathrin | 138.0 | 100 ± 0 | 4 |
| Cypermethrin | 208.0 | 100 ± 0 | 4 |
| Acephate | 1132.5 | 5.0 ± 0.84 | 1 |
| Thiophanate-methyl | 626.0 | 35.0 ± 0.26 | 1 |
| Thiophanate | 1198.4 | 48.0 ± 0.84 | 1 |
| Diazinon | 1764.0 | 18.0 ± 0.76 | 1 |

[1]Rates recommended by manufacturers for spider mite control on nursery plants.
[2]Toxicity rating after the convention of IOBC/WPRS, 1 = harmless (<50%), 4 = harmful (99%).

TABLE 3. RESIDUE TOXICITY OF 5 ACARICIDES TO *PHYTOSEIULUS MACROPILIS*.

| Acaricide | Total no. of predators tested | Label rate (1x)[1] mg a.i./L | $LC_{50}$ (95% CI mg a.i./L) | Slope ± SE |
|---|---|---|---|---|
| Dienochlor | 420 | 480.0 | 335.54 (37.86 - 451.36) | 1.46 ± 6.80 |
| Hexakis | 540 | 450.6 | 74.54 (10.21 - 303.85) | 0.87 ± 2.47 |
| Abamectin | 420 | 5.8 | 0.7513 (0.444 - 588.71) | 0.77 ± 1.69 |
| Dicofol | 480 | 478.4 | 0.083 (0.000248 - 1.561) | 2.10 ± 4.39 |
| Propargite | 420 | 360.0 | 8917.0 (423.6 – 8.30413 x $10^{16}$) | 12.20 ± 40.30 |

[1]Rates (middle of range) recommended by manufacturers for spider mite control on nursery plants.

TABLE 4. RESPONSE OF P. MACROPILIS TO RESIDUES OF FENPROPATHRIN AND DICOFOL (AT LABEL RATES) UNDER COMMERCIAL NURSERY CONDITIONS.

| | Days after treatment | | | | |
|---|---|---|---|---|---|
| Pesticide | 0 | 3 | 7 | 14 | 21 |
| | Mean % mortality[1] | | | | |
| Fenpropathrin | 100 | 73 | 100 | 100 | 30 |
| Dicofol | 100 | 100 | 12 | 1 | |

[1]Mortality corrected by Abbott's formula.

and 6-fold of the label rate. In contrast, propargite was reported slightly toxic to *A. hibisci* (Bartlett 1964) and *A. fallacis* (Croft & Nelson 1972). However, these results are similar to those reported by Rock & Yeargan (1971) on *T. occidentalis*, Babcock & Tanigoshi (1988) on *N. fallacies*, and Mizell & Schiffhauer (1991) on *N. collegae*. The $LC_{50}$ was recorded as 8917 mg a.i/L, more than 10 times the label rate.

The fungicides thiophanate and thiophanate-methyl at the 1X rates caused 58% and 35% mortality, respectively (Table 2). The predator's tolerance to tested fungicides is important relative to its use in nurseries, as fungicides are frequently applied for disease control.

In this study a double-leaf-disk method was used to avoid 'runoff' mortality. The method used was similar to Schiffhauer & Mizell (1988), with the difference that the ring was closed with a treated leaf instead of a cloth. This modification provided more exposure of the treated surface to the predators. *Phytoseiulus macropilis*, like many other phytoseiids, has a wandering tendency, and is difficult to confine in a treated arena during experiment set up, often trying to escape the treated arena and is killed in the contact glue. This prob-

TABLE 5. RESPONSE OF ADULT FEMALE *PHYTOSEIULUS MACROPILIS* TO ODORS IN AN OLFACTOMETER PRODUCED BY TETRANYCHID MITE SPECIES. STATISTICAL SIGNIFICANCE IS SCORED WITH RESPECT TO PREFERENCE FOR THE FIRST TREATMENT IN THE COUPLETS. ZERO AND N.S. APPLIES TO NO SIGNIFICANT DIFFERENCE BETWEEN THE TREATMENTS, ++ EXPLAINED AS COMMENTS.

| Treatment | Outcome | | | Probability | Comment |
|---|---|---|---|---|---|
| | No. | (+) | (-) | | |
| 1. Blank vs Blank | 36 | 0 | 0 | n.s.[1] | Bioassay is valid |
| 2. *Tetranychus urticae* on lima bean leaves vs uninfested lima beans. | 36 | 22 | 0 | <0.05 | attraction |
| 3. *T. urticae* infested lima bean leaves vs blank. | 36 | 25 | 2 | <0.05 | attraction |
| 4. Uninfested lima bean leaves vs blank. | 36 | 0 | 0 | n.s. | no attraction |
| 5. Previously *T. urticae* infested lima bean leaves vs blank. | 36 | 20 | 3 | <0.05 | attraction |
| 6. *Oligoncyhus ununguis* on juniper leaves vs blank. | 36 | 3 | 1 | n.s. | no attraction |
| 7. Uninfested juniper leaves vs blank. | 36 | 2 | 3 | n.s. | no attraction |
| 8. *T. evansi* on tomato leaves vs. blank. | 36 | 0 | 1 | n.s. | no attraction |
| 9. *T. evansi* on filter paper vs blank. | 36 | 0 | 0 | n.s. | no attraction |
| 10. *T. urticae* on lima bean leaves vs *O. ununguis* on juniper leaves. | 36 | 19 | 4 | <0.05 | attraction to T. urticae |
| 11. *T. urticae* on lima bean leaves vs *T. evansi* on tomato leaves. | 36 | 20 | 0 | <0.05 | attraction to *T. urticae* |

[1]Determined using Sign test, $P = 0.5$.

lem was countered by drawing predators into straws, and putting the latter in ice for about 5 min. This cooling technique (Mizell & Schiffhauer 1991) facilitated predator transfer into the treated arena. However, the double-leaf-disk method, like any other residue testing technique, has some tradeoffs. For example, behavioral response of subjects to pesticide residue cannot be observed. The possible fumigation effect inside the arena may cause some mortality among confined subjects that may not occur on an open treated surface. However, possible fumigation effects in this study were deemed negligible in comparison to the problems with predator escapes from other arenas.

Olfactometer Bioassays

Of the 38 *P. macropilis* tested, 22 responded significantly ($P < 0.05$) to lima bean leaves infested with *T. urticae*, whereas none of the *P. macropilis* responded to uninfested lima bean leaves ($P < 0.05$). The response was also significantly different ($P < 0.05$) when 1 treatment was lima bean leaves infested with *T. urticae* and the other blank. The *P. macropilis* response to *T. urticae*-infested lima bean versus a blank (25:2) and to lima bean leaves that were previously infested by *T. urticae* (20:0) to a blank were both significantly different ($P < 0.05$) (Table 5). The *P. macropilis* did not respond significantly to *O. ununguis* on juniper leaves both versus uninfested juniper leaves and versus the blank (Table 5). *Phytoseiulus macropilis* were not attracted significantly to *T. evansi* either on tomato leaves or on filter paper (Table 5).

The significant response of adult *P. macropilis* to lima bean leaves infested with *T. urticae* Koch, both versus blank and uninfested lima bean leaves, was similar to the results reported by Hislop & Prokopy (1981). Predators did not respond significantly to uninfested lima bean leaves. Sabelis et al. (1984) also reported that *P. persimilis* did not respond to uninfested lima bean leaves. However, *P. macropilis* responded significantly to infested lima bean leaves from which *T. urticae* were removed.

Takabayashi & Dicke (1992) observed that phytoseiids reared on twospotted spider mite on lima bean leaves respond to volatiles from lima bean leaves but the predators reared on the twospotted spider mites from cucumber leaves did not respond to lima bean volatiles. *Phytoseiulus macropilis* used in this study were reared on 'Henderson' lima beans for about 7 years. Thus, the significant response of *P. macropilis* to the kairomones of *T. urticae* on lima bean leaves versus the negative response to *O. ununguis* and *T. evansi* on juniper and tomato leaves, respectively, may possibly be explained by the rearing history. The response of *P. macropilis* to *T. evansi* was not significant, even when the latter were offered on filter paper, which removed the effect of the tomato leaves. Thus, it appears odors produced by *T. evansi* on tomato do not attract *P. macropilis*, or perhaps volatile(s) carried over by *T. evansi* from feeding on tomato leaves made them repellent. Results regarding the response of *P. macropilis* to these 2 tetranychid species warrant further investigation (van Wijk et al. 2008). The significant response of *P. macropilis* to one of the predominant phytophagous pest of nursery ornamentals *T. urticae* indicates that *P. macropilis* has potential as a biological control agent for twospotted spider mite and warrants further study.

Alternate Food Bioassays

There was no significant difference between the survivorship and fecundity ($P < 0.05$) of predators fed on alternate food and the controls after 24 h (Table 6). However, after 48 hr, mortality increased and fecundity decreased sharply for *P. macropilis* in the alternate food treatments, whereas the *P. macropilis* controls fed spider mites remained alive and continued ovipositing (Table 6). These results suggest that *P. macropilis* cannot survive or oviposit when fed only on nonprey food. The rate of mortality, however, varied among predators fed on different alternate foods (Table 6). Interestingly, the highest observed survivorship for predators after 48-72 h was for those fed on the solution of sugar and water. The predators provided with sugar-water solution survived up to 120 h, which was the maximum survival period in any of the alternate food treatments. Similarly, egg production decreased rapidly for treatments after 24 h except for the predators that were fed on the eggs of false oleander scale *P. cockerelli* and on sugar-water solution where they oviposited for up to 72 h. Apparently, the decreased egg production among treated predators indicates either that the predators' food reserves were exhausted after 24 h or foods other than *T. urticae* are nutritionally deficient for egg production. These results are similar to Kennett & Homai (1980), who reported that *P. persimilis* and *T. occidentalis* provided with food other than tetranychid prey did not oviposit.

## Conclusions

The results from the 3 study types suggest that *P. macropilis* may have potential for use in an integrated biological and chemical control program for tetranychid mites in nurseries. The discrepancies involved in double-leaf-disk residue method such as prevention of behavioral response by predators and fumigation effect inside the treated arena could have affected the residual efficacy of tested pesticides. Thus, there is a need to field test these and other chemicals for their possible inte-

TABLE 6. EFFECTS OF NONPREY DIET ON THE SURVIVORSHIP AND EGG PRODUCTION OF ADULT GRAVID FEMALE *PHYTOSEIULUS MACROPILIS* ($N$ = 30), OVER 4-5 D.

| Diet | Mean No. Alive Hours after treatment | | | | | Mean egg production ± SE Hours after treatment | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | 24 | 48 | 72 | 96 | 120 | 24 | 48 | 72 | 96 | 120 |
| Control, *T. urticae* (all stages) | 5.4 ± 0.5 A[1] | 4.8 ± 0.8 A | 4.6 ± 1.3 A | 4.7 ± 0.2 A | 4.2 ± 0.2 A | 4.1 ± 0.5 A | 3.7 ± 0.6 A | 3.4 ± 0.9 A | 3.0 ± 0.5A | 3.0 ± 0.3 A |
| *Hemerocallis spp.*, pollen | 5.0 ± 0.3 A | 2.8 ± 0.4 B | 2.2 ± 1.9 B | 1.0 ± 0.0 B | 0B | 3.4 ± 0.3 A | 1.04 ± 0.0B | 0 B | 0 B | 0 B |
| *Phylloxera* spp. larvae | 5.3 ± .04 A | 4.3 ± 0.4 A | 2.2 ± 0.6 B | 2.0 ± 0.1 B | 0B | 3.4 ± 0.9 A | 0 B | 0 B | 0 B | 0 B |
| *P. cockerelli* eggs | 4.5 ± 0.3 A | 4.4 ± 0.3 A | 3.4 ± 0.2 B | 2.0 ± 0.5 B | 0B | 3.5 ± 0.2 A | 2.5 ± 0.4 A | 1.2 ± 2.0 B | 0 B | 0 B |
| Sugar-water solution | 5.4 ± 0.2 A | 4.4 ± 0.2 A | 3.2 ± 0.7 B | 2.6 ± 0.2 B | 2.8 ± 0.4 B | 3.4 ± 1.3 A | 2.6 ± 0.2 A | 2.0 ± 0.5 B | 0 B | 0 B |
| Water | 4.5 ± 0.2 A | 3.4 ± 0.2 A | 0.4 ± 0.2 B | 0B | 0B | 3.4 ± 0.3 A | 1.7 ± 0.4 B | 0 B | 0 B | 0 B |

[1]Means not followed by same letter are significantly different from the control, $P = 0.05$, as determined by the randomization test (Conover 1971).

gration with *P. macropilis* to control spider mites in nurseries. Nurseries use overhead irrigation that over time may reduce residues on plants. This was evident in the field test of dicofol, where the chemical lost considerable toxicity to *P. macropilis* 7-14 d after application. For fenpropathrin, mortality in the field decreased from 100% to 30% three weeks after application. Because *P. macropilis* apparently does not use alternate food, chemicals potentially integrated with the predator must not kill all the prey, thereby starving the predator. The response by *P. macropilis* to *T. urticae* on lima beans suggests that the predator uses host kairomones to find its prey. Therefore, further olfactometer studies with other species of ornamental plants that are *T. urticae* hosts are warranted.

## ACKNOWLEDGMENTS

Thanks to Dr. Michael D. Hubbard, Peter C. Andersen, and Brent V. Brodbeck for improvements to a previous draft of the manuscript. Special thanks to Ms. Cheryl Manasa for technical support.

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