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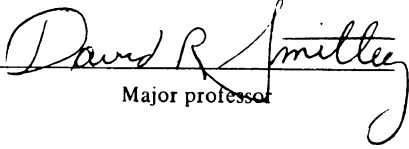
Biological Control of Tetranychus Urticae Koch with
Phytoseiulus Persimilis Athias-Henriot in a Rose
Production System

presented by

Anthony J. D'Angelo

has been accepted towards fulfillment
of the requirements for

Master's degree in Entomology


Major professor

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BIOLOGICAL CONTROL OF TETRANYCHUS URTICAE KOCH WITH
PHYTOSEIULUS PERSIMILIS ATHIAS-HENRIOT IN A ROSE
PRODUCTION SYSTEM

By

Anthony John D'Angelo

A THESIS

Submitted to
Michigan State University
in partial fulfillment of the requirements
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ABSTRACT

BIOLOGICAL CONTROL OF TETRANYCHUS URTICAE KOCH WITH
PHYTOSEIULUS PERSIMILIS ATHIAS-HENRIOT IN A ROSE
PRODUCTION SYSTEM

By

Anthony John D'Angelo

Twospotted spider mites have been a major pest of greenhouse grown crops for at least 40 years with heavy infestations resulting in serious economic losses. Fluctuations in the intensity of spider mite infestations can be attributed to the development of mite resistance to previously effective miticides. Standard slide-dip and 36 h residue tests were used to evaluate selectivity of commonly used miticides, fungicides and insecticides against twospotted spider mite and the predator mite, Phytoseiulus persimilis. Longer term efficacy studies and predator feeding studies were found to be useful in determining indirect toxicity of miticides to the predator. Absolute population densities and distributions of spider mites on 14 rose plants were determined. For research purposes, random samples of 20 leaflets per plant will provide an acceptable estimate of population density. Sampling schemes designed to detect mites at low density should be aimed at the base of rose plants where the oldest leaves are found.

To my family

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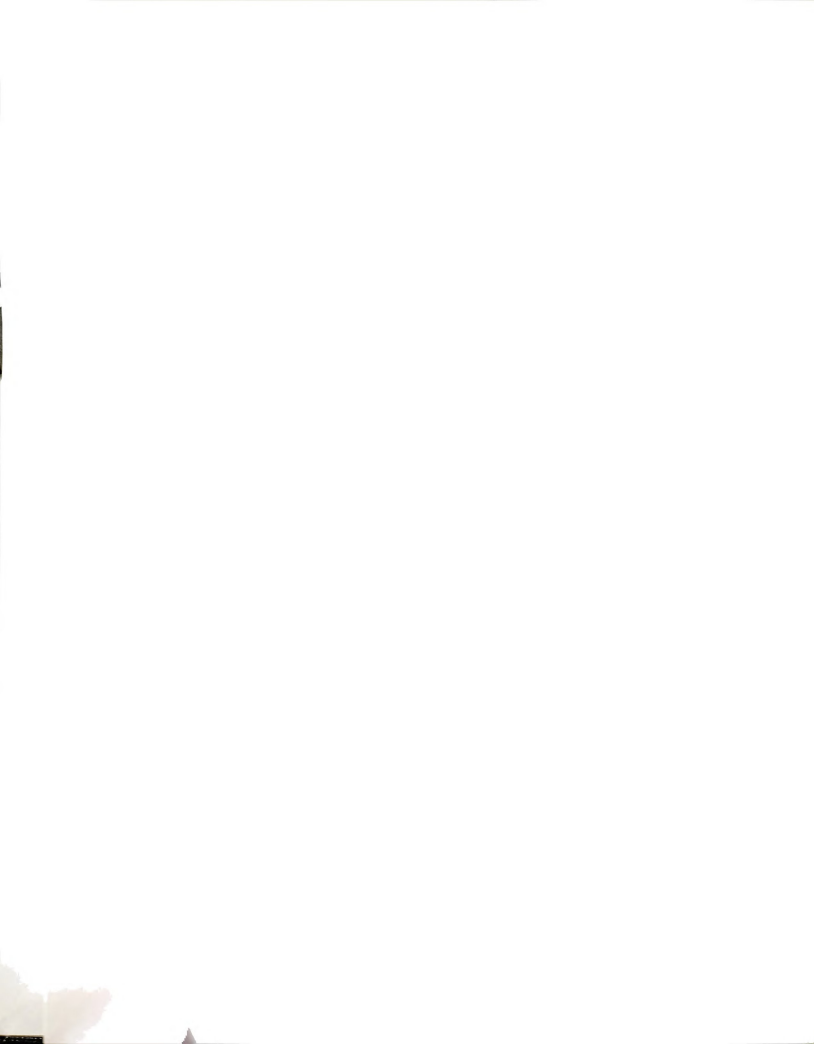


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INTRODUCTION

Predaceous Phytoseiid mites are probably the most important biological control agents of spider mites in deciduous orchards, vineyards, and greenhouses around the world (Hoyt, 1982). Control programs utilizing natural occurring Phytoseiids have been successful on a number of orchard and field crops. Typhlodromus occidentalis Nesbitt was first used successfully on apples where mite control costs have been significantly reduced (Hoyt & Caltagirone 1971). Important factors contributing to the success of this program were; the predator developed resistance to azinphosmethyl used for codling moth, Lasperyresia pomonella L., and an alternate food source, the apple rust mite, Aculus schlechtendali (Nalepa), was present and could maintain T. occidentalis when Tetranychus macdanieli McGregor was scarce (McMurtry 1982). By applying the same principles used on apples, similar programs utilizing T. occidentalis have been successful on peaches (Hoyt & Caltagirone 1971), pears (Westigard 1971), grapes (Flaherty & Huffaker 1970, Kinn & Dout 1972), and walnuts (McMurtry & Flaherty 1977).

Amblyseius fallacis (Garman) has been used successfully to control European red mite, Panonychus ulmi (Koch) in apples when ground cover maintained for overwintering sites provides an alternate Tetranychus host. A. fallacis moves into the trees in spring where it is effective in controlling P. ulmi as well as Tetranychus species (Croft & McGroarty 1973, Meyer 1974). As with T. occidentalis an important factor in its success is its resistance to certain pesticides (McMurtry 1982).

Typhlodromus pyri Scheuten is another predator which occurs on apple trees in various parts of the world (Schuster & Pritchard 1963,

Specht 1968). Although T. pyri is recognized as an effective predator of P. ulmi in some situations (Downing & Moilliet 1972), its use in integrated mite control programs began only after developement of resistance to various pesticides (Croft 1982). A number of other naturally occurring Phytoseiid mites have shown potential for spider mite control on a variety of crops (Oatman 1973).

Over the last two decades interest in biological and integrated pest management programs utilizing mass production and release of Phytoseiid mites has increased significantly. (van Lenteren & Woets 1988). Twenty years ago there were few commercial producers of natural enemies. As of 1988 there were about 15 large producers supplying beneficial arthropods (van Lenteren & Woets 1988). Large scale production and transportation techniques have been described for a variety of Phytoseiid mites (Kamborov 1966, Scriven & McMurtry 1971, Hoy et al. 1982, Hussey & Scopes 1985).

Biological control of spider mites in field crops by mass release of predators has been largely developed on strawberries (McMurtry 1982). Good control has been achieved in several studies using predator mites. On strawberries, Phytoseiulus persimilis Athias-Henriot provided good control of spider mites at a rate of 5 to 10 mites per plant (Oatman & McMurtry 1966, Oatman et al. 1968, 1976, 1977). Phytoseiulus persimilis has also shown good control of spider mites on field grown ivy and dahlias (Gould & Light 1971).

Interest in biological control in greenhouses did not begin until 1926 when a tomato grower noticed blackened pupae among the normal white pupae of the greenhouse whitefly, Trialeurodes vaporariorum Westwood. The parasites that emerged were identified as Encarsia formosa (Speyer

1927). Within a few years a research station in England was supplying 1.5 million of these parasites annually to 800 nurseries in Britain. Use of E. formosa spread to other countries until distribution was discontinued when more convenient and efficient insecticides were introduced after World War II (van Lenteren & Woets 1988). However, the first signs of pesticide resistance in spider mites brought about renewed interest in biocontrol. Since then considerable advances have been made using Phytoseiid mites to control spider mites on greenhouse grown crops (van Lenteren & Woets 1988).

Phytoseiulus persimilis is the most widely used natural enemy for spider mite control in greenhouses (van Lenteren & Woets 1988). Interest in the potential of P. persimilis as a biocontrol began when Dosse (1958) reported on its high reproductive rate and efficiency in reducing spider mite populations (McMurtry 1982, van Lenteren & Woets 1988). This potential was again demonstrated by a number of experiments using P. persimilis to control spider mites on greenhouse grown crops (Chant 1961, Bravenboer & Dosse 1962, Bravenboer 1963). Small-scale application of spider mite biological control in commercial greenhouses started in 1968. (van Lenteren & Woets, 1988). By 1980 use of P. persimilis in Europe on greenhouse grown cucumbers had increased to over 60% of the acreage in the Netherlands, 75% in England and 75% in Sweden, Denmark (McMurtry 1982, van Lenteren & Woets 1988) and Finland (Markkula & Tiittanen 1980). Programs have also developed in British Columbia, Canada (Tonks & Everson 1977, Costello et al. 1984) and the USSR (Beglyarov & Smetnik 1977). Commercially P. persimilis has also been used on greenhouse grown peppers (van Lenteren & Woets, 1988), and tomatoes (French et al. 1976, Gould 1977, Stenseth 1980). In research

tests P. persimilis has been effective on Strawberries (Simmonds 1971, Gould & Vernon 1978, Port & Scopes 1981) and chrysanthemums (Hamlen & Lindquist 1981).

In the United States, grower interest in greenhouse biocontrol has increased substantially in the last 10 years (Osborne et al., 1985), although very few commercial greenhouses have actually tried biocontrol as a pest control approach. In greenhouse rose production the major pests include the twospotted spider mite, Tetranychus urticae Koch, rose aphids, Macrosiphum rosae (L.), western flower thrips, Frankliniella occidentalis Pergande, and powdery mildew, Sphaerotheca pannosa. Twospotted spider mites have been a major pest of greenhouse grown crops for many years, with heavy infestations resulting in serious economic loss (Anonymous 1961, Hamlen & Lindquist 1981, Field & Hoy 1984). On greenhouse grown roses, as many as 19 miticide applications per year are applied by some growers (Field & Hoy 1984). The development of mite resistance to previously effective miticides has caused fluctuations in the intensity of spider mite problems on roses and other crops (McMurtry et al. 1970, Brador 1977, Georghiou & Saito 1983). Increased interest in biocontrol has resulted not only from miticide control failures, but also from pesticide toxicity to workers, phytotoxicity to plants, and increased government regulations.

In order to implement a successful integrated pest management program in a greenhouse a grower must have a detailed plan. He must know what predators to use, when to release them, and how many to release. Previous studies have determined the predator/prey ratio necessary for successful biocontrol (Oatman et al. 1976). A grower must know what selective pesticides can be used and when they should be used. The primary

focus of this study was to evaluate pesticides commonly used in commercial rose production greenhouses for their relative toxicity to P. persimilis and T. urticae.

Secondly, biocontrol of spider mites requires more intensive monitoring of mite populations than chemical control. Accurate sampling methods are needed for researchers studying biocontrol of mites on roses, and for greenhouse growers trying to detect spider mites at low densities to properly time release of predator mites. A quantitative approach to biological control of spider mites using predaceous mites requires estimating population densities (Nachman 1984). To be useful, a sampling program should provide estimates having the highest accuracy commensurate with the amount of work expended (Southwood 1978). Intelligent decisions concerning pest control strategies are directly dependent upon knowing the status of all important species (Beardsley et al. 1979).

In order to determine if a successful integrated pest management program for T. urticae can be developed it is necessary to know if the release of predators is compatible with natural controls and pesticides used for the other major pest problems. Boys and Burbutis (1972) suggested that with the use of a short-lived selective miticide P. persimilis may have potential value in an integrated pest management program on commercially grown greenhouse roses. The probability of successful initiation of a spider mite biocontrol program could be increased by the use of a selective miticide to suppress spider mite populations. In conclusion, potential problems in initiating predator mite biocontrol programs in rose production greenhouses are: (1) adequate monitoring of spider mite

populations; (2) use of fungicides; (3) pesticide residues on foliage; and (4) outbreaks of aphids and thrips.

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**Determining the selectivity of the miticides abamectin,
dienochlor and propargite to Tetranychus urticae
or Phytoseiulus persimilis**

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Key Words: Acari, Twospotted spider mite, Tetranychus urticae, Phytoseiulus persimilis, miticides, selectivity.

ABSTRACT

Standard slide-dip and 36 h residue tests were used to evaluate activity of abamectin, propargite and dienochlor against twospotted spider mites, Tetranychus urticae Koch and the predaceous mite, Phytoseiulus persimilis Athias-Henriot. Results showed that abamectin was more toxic to T. urticae than P. persimilis, while dienochlor and propargite had little or no effect on either mite. Dienochlor was shown to be toxic to spider mites in a long term experiment. A predator feeding study, where spider mites from populations initially recovering from a miticide application were fed to predator mites, was found to be useful in determining indirect toxicity of miticides to P. persimilis.

INTRODUCTION

The twospotted spider mite, Tetranychus urticae, has been a major pest of greenhouse crops for at least 40 years. Heavy infestations frequently result in serious economic losses (Anonymous, 1961; Field and Hoy, 1984; Hamlen and Lindquist, 1981). As many as 19 miticide applications per year are applied by some rose growers to control spider mites (Field and Hoy, 1984). Fluctuations in the intensity of spider mite problems on roses and other crops in the last 40 years can be attributed to the development of mite resistance to previously effective miticides (Brador, 1977; Georgiou and Mellon, 1983; McMurtry et al., 1970). In some cases resistance is present in greenhouse and field populations of mites several generations removed from those originally treated (Overmeer et al. 1975, van Zon & Overmeer 1975). Studies have shown that T. urticae populations in a rose production greenhouse were still resistant to tetradifon and dicofol up to 7 years after the miticides had last been used (Overmeer et al. 1975, Helle and van de Vrie, 1975). In addition to control failure, the issues of labor costs, pesticide toxicity to workers, phytotoxicity to rose plants, and increased government regulations have resulted in an increased interest in biocontrol.

The most widely adopted greenhouse predator release strategy is the use of Phytoseiulus persimilis for control of T. urticae. In the United States, grower interest in greenhouse biocontrol has increased substantially in the last 10 years (Osborne et al. 1985), although very few commercial greenhouses have actually relied on biocontrol as their major pest control approach.

Investigators have reported successful control of T. urticae on roses in research greenhouses by release of Metaseiulus occidentalis (Nesbitt) (Field & Hoy 1984, 1986) or P. persimilis (Simmonds 1972, Hamlen & Lindquist 1981, Burgess 1984, Rasmy & Ellaithy 1988). However, successful biocontrol of spider mites becomes less certain when release programs are scaled up for use in commercial rose greenhouses. Some projects have successfully held spider mite damage to a tolerable level (Burgess, 1984; Martin, 1987), while others have failed (Boys & Burbutis, 1972; Lindquist et al., 1980). Potential problems in initiating predator mite biocontrol programs in rose production greenhouses are: (1) adequate monitoring of spider mite populations; (2) use of fungicides; (3) insecticide residues on foliage; and (4) outbreaks of aphids and thrips. The probability of successful initiation of a spider mite biocontrol program could be increased by the use of a selective miticide to suppress spider mite populations until undesirable pesticide residues are no longer toxic to P. persimilis.

The objective of this study was to evaluate three miticides for relative toxicity to T. urticae and P. persimilis.

MATERIALS AND METHODS

The toxicity of abamectin, dienochlor and propargite to T. urticae and P. persimilis was evaluated in three different tests: (1) a standard slide-dip; (2) a 36 h residual activity study on bean leaves; and (3) a predator feeding study.

Tetranychus urticae cultures were established from mites collected from the pesticide research center greenhouses at Michigan State

University in 1986. Twice each year T. urticae were collected at the same place and added to the culture. Tetranychus urticae were maintained in the green-house and laboratory on 'Henderson Bush' and 'Eastland Bush' lima beans (Phaseolus vulgaris).

Phytoseiulus persimilis was originally obtained from 'Nature Control', Medford, Oregon. The population was maintained under laboratory conditions on excised bean leaves infested with spider mites. The relative humidity (RH) was kept at 95% and the temperature at 27°C. Spider mites were tapped from infested bean leaves to provide a daily food source.

Slide-dip test. In slide-dip tests with spider mites, a 2 cm piece of double-sided Scotch® tape (666 07340-3 3M Commercial Office Supply Division, St. Paul, MN 55133-3053) was placed across a standard microscope slide. Scotch® nylon ribbed packaging tape (34-7023-2006-9, 3M Home products Division, St. Paul, MN 55133-3053) was placed on top of the double-sided tape with its sticky side facing upwards. Adult female mites on bean leaves were placed in a CO₂ filled sealable plastic bag. After 15 minutes the mites were tapped off the leaves onto a Tyler equivalent 32 mesh screen (500µm pore size). Shaking the screen allowed immature and male mites to fall through, leaving only adult females. Adult females were removed with a fine paint brush and placed on slides so that their legs could move freely. After each slide was fitted with twenty mites it was placed in a covered plastic holding chamber at 95% relative humidity (RH) and 27°C. Miticide solutions were prepared at concentrations of 1x, 1/5x, 1/25x, 1/650x and 0x, where x is the recommended concentration on the U.S.A. federal product label for greenhouse application. The 1x rates for abamectin, dienochlor and propargite were 5.6, 150, and 360 ppm active

ingredient, respectively. Three hundred ml of each solution was prepared in a 400ml beaker. Triton AG-98 was added to each solution at a concentration of 0.12%. All treatments were replicated twice and randomized with respect to time of mite placement on slides. Slides with mites were agitated in test solutions for 5 seconds, and placed on edge in a drying rack lined with absorbent paper towel to remove excess moisture from the slides. After 15 minutes the slides were placed back into the holding chamber. After 36 h the mites were examined to determine survival. Each mite was touched with a fine paint brush. If it responded with movement it was considered alive.

A similar procedure was followed for the P. persimilis slide-dip tests. However, the nylon ribbed packaging tape did not hold the predators in place. They were able to turn themselves over and crawl off the tape. Duct tape (Nashua 357, Watervleit, N.Y. 12189) has a stronger adhesive than packaging tape and holds the predator mites in place better. This tape was used for all predator mite slide dip tests. Also, 15 P. persimilis were used per slide compared with 20 T. urticae. Treatment rates and all other details were the same as discussed for the T. urticae dip tests.

36 hour residue test. Residual activity of abamectin, dienochlor, and propargite were tested by spraying bean leaves at 5 P.M., allowing them to dry overnight, and placing mites on the leaves between 9 and 11 A.M. the next day. In each of these tests miticides were applied at 1x, .5x, .25x, .125x, .0625x and 0x, where 1x is the recommended concentration on the U.S.A. federal product label. As in the slide dip tests, the 1x rates for abamectin, dienochlor and propargite were 5.6, 150, and 360 ppm active ingredient, respectively. The treatments were replicated four times. Treatments were applied in a spray tower equipped with a conveyor belt

that moves at a constant rate of 9.1cm/sec. Miticide solutions were applied through a TeeJet 8001-E even flat spray nozzle. Excised bean leaves in petri dishes layered with absorbent cotton were sprayed uniformly by running the petri dish through the spray tower. Miticides were applied beginning with the lowest concentration first. Petri dishes were arranged in numerical order on a large cafeteria tray after treatments were applied. After the spray had dried on the leaves, one rubber hose washer was glued to each leaf using Elmer's Glue-all® (Borden's Inc., Dept. CP, Columbus, Ohio 43215). The washers served as arenas to hold the mites in place. Twenty adult female T. urticae and six P. persimilis were placed in each arena. A small circle of nylon mesh screening was glued over the arenas after the mites were in place. The cotton base was kept moist to prevent desiccation of the leaves.

Phytoseiulus persimilis adults were transferred to arenas by working in a 5°C room with a fine paint brush. In all tests the mites were kept in arenas on treated bean leaves for 36 h. After this time the arena covers were removed and mortality data taken.

Predator feeding study. Predaceous mites were allowed to feed on spider mites surviving spray treatments to determine if the predators received a toxic dose of miticide from ingesting mite prey. Four greenhouse grown potted rose plants were sprayed to run-off with the miticide to be tested. After three days each plant was inoculated with four spider mite infested bean leaves. The introduced spider mite population rapidly declined at first due to the miticide residue. A small proportion of the introduced mites survived on the treated plants and the mite population began to increase some 4-6 weeks after the initial miticide application. At that time spider mites that had been feeding on the miticide treated plants

were fed to the predator mites over a five day period. Small (60ml) clear plastic diet cups were used as arenas. Six P. persimilis were placed in each arena along with enough spider mite food to last five days. The diet cups were then sealed with plastic covers. To allow for oxygen exchange a hole had been made in the center of each cover. Fine mesh screening was placed over the hole to keep the mites from escaping. All treatments were replicated six times. Spider mites from untreated plants were added to another set of diet cups for a control treatment.

Long-term population studies. Long-term studies of the effect of miticides on T. urticae and P. persimilis were conducted because the results of slide-dip tests and 36 h residue tests suggested that dienochlor did not have any effect on spider mites. This miticide is known to be slow acting. Therefore, efficacy of dienochlor was evaluated over a longer (15 day) test period. Abamectin was also tested in a long-term mixed population study to see how results of slide-dip and 36 h residue tests compared with survival of T. urticae and P. persimilis on rose plants treated with abamectin. In the dienochlor study, each of eight greenhouse grown potted rose plants were inoculated with four spider mite infested bean leaves. Spider mites were allowed to establish and increase for two weeks. After this time a fifteen leaflet precount sample was taken from each plant. Four plants were then sprayed to run-off with a 150 ppm concentration of dienochlor. Four were left untreated as controls. Fifteen leaflets were sampled from each plant every five days and the number of live T. urticae recorded.

The long term residual effect of abamectin on roses was studied by spraying 20 potted rose plants previously infested with T. urticae. Three weeks later 1000 P. persimilis were released. Tetranychus urticae and P.

persimilis were sampled approximately every seven days for a 45 day period starting one day before application of abamectin on 23 July. Each sample consisted of 60 leaflets from five rose plants (12 per plant). Each five plant unit was replicated four times. The temperature was maintained at $25 \pm 2^{\circ}\text{C}$.

Statistical analysis. An arcsin square root transformation was performed on all percent mortality data before analysis. Dose effect of abamectin, dienochlor and propargite on T. urticae and P. persimilis was analyzed by regressing transformed percent mortality of mites against the concentration of the test solution (Wilkinson 1987). If the slope of this regression was greater than zero ($p < 0.05$), the miticide was determined to have a dosage effect. In 36 h residue tests, mortality of T. urticae was compared with that of P. persimilis at each miticide concentration with the Student's T-test (Steel and Torrie, 1980). Mite mortality was also regressed against miticide concentration as described for the slide-dip tests. In the predator feeding study the effect of each miticide on P. persimilis was evaluated by testing for differences (Student's T-test) in survival of P. persimilis fed spider mites from miticide treated plants with survival of P. persimilis fed spider mites from untreated plants. In the long term population studies with dienochlor, the number of spider mites per sample from miticide treated plants was compared with that on control plants at each sample date with an analysis of variance (Steel and Torrie, 1980).

RESULTS

Slide-dip tests. When mites were dipped in test solutions, abamectin was more toxic to T. urticae than P. persimilis. At a

concentration of 0.224 ppm abamectin killed 100% of T. urticae and only 28% of P. persimilis tested (Figure 1a). When T. urticae mortality was regressed against concentrations of abamectin, the slope of the regression line was greater than zero (Figure 1A, $F = 115.94$, $p < 0.01$). Regression of P. persimilis percent mortality against concentration of abamectin gave a slope that was not different from zero (Figure 1, $F = 3.92$, $p = 0.076$). Therefore, a dosage effect of abamectin on mite mortality was observed for T. urticae but not for P. persimilis.

Dienochlor as evaluated in our slide dip tests had no 36 hour contact activity against T. urticae or P. persimilis. Regression of T. urticae or P. persimilis against test concentrations of dienochlor yielded slopes that were not different from zero (Figure 1B, $F = 0.146$, $p = 0.710$ and $F = 0.276$, $p = 0.611$ respectively).

Propargite also had little effect on T. urticae or P. persimilis at any concentration in the slide-dip tests. Regression of T. urticae or P. persimilis mortality against concentration of propargite gave slopes that were not different from zero (Figure 1C, $F = 0.297$, $p = 0.598$ and $F = 1.338$, $p = 0.274$ respectively).

36 hour residue tests. Abamectin was more toxic to T. urticae than to P. persimilis at all concentrations in 36 hour residue tests on bean leaves. When T. urticae mortality was regressed against concentration of abamectin, the slope of the regression line was greater than zero (Figure 2A, $F = 30.750$, $p < 0.001$). Regression of P. persimilis mortality against abamectin concentration gave slopes that were greater than zero at $p = 0.10$ but not at $p = 0.05$ (Figure 4, $F = 4.227$, $p = 0.052$). Although 50% mortality of P. persimilis was observed at 5.6 ppm abamectin, this was not different from the control treatment ($T(.05) = 1.445$, d.f. = 6). Mortality

of T. urticae was significantly higher than that of P. persimilis at a concentration of 5.6 ppm ($T(.05) = 7.659$, d.f. = 6).

In 36 h residue tests dienochlor had little effect on T. urticae or P. persimilis, and no selectivity for either species. Regression of T. urticae mortality against concentrations of dienochlor was not significant. Phytoseiulus persimilis mortality increased slightly as the concentration of dienochlor increased in test solutions (Figure 2B). Although the maximum mortality to P. persimilis was only 20%, regression analysis indicates a positive relationship to concentrations of dienochlor ($F = 5.5$, $p = 0.029$).

Propargite also had no effect on T. urticae or P. persimilis mortality at any concentration in 36 hour residue tests. Regression of T. urticae or P. persimilis mortality against concentrations of propargite gave slopes that were not different from 0 (Figure 2C, $F = 3.714$, $p = 0.067$ and $F = 0.368$, $p = 0.550$, respectively). Tetranychus urticae mortality was not different from that of P. persimilis at the standard concentration of 360 ppm propargite ($T(.05) = 0.659$, d.f. = 6).

Predator feeding study. When P. persimilis were fed T. urticae from populations initially recovering from a miticide application, significant mortality to the predaceous mites was observed for two of three miticides tested (Figure 3). Tetranychus urticae were collected from rose plants 6 weeks after application of 5.6 ppm abamectin, 150 ppm dienochlor, 360 ppm propargite, or water. When P. persimilis were fed T. urticae from abamectin and dienochlor treated plants, P. persimilis mortality was greater than when they were fed T. urticae from untreated plants ($T(.05) = 4.27$, and $T(.05) = 4.38$, respectively). When P. persimilis were fed T. urticae from propargite treated plants, P. persimilis survival

was the same as when they were fed T. urticae from untreated plants (Figure 3).

Long term population study. When dienochlor was applied to potted rose plants it effectively suppressed T. urticae populations for the 15 days of the study (Figure 4). Tetranychus urticae populations at 5, 10 and 15 days after treatment were reduced by 95, 80 and 85%, respectively, compared with populations on control plants (Figure 4).

Abamectin applied to 20 potted rose plants infested with T. urticae steadily reduced T. urticae populations from 189 per sample prior to application to 1.8 per sample 4 weeks after application (Table 1). Tetranychus urticae populations slowly increased during the following three weeks to 9.3 per sample at 7 weeks after application. Phytoseiulus persimilis adults released onto the same rose plants at 3 weeks after application of abamectin disappeared to undetectable levels within 2 weeks following release (Table 1).

DISCUSSION

The results of standard slide-dip and 36 h residue tests showed that dienochlor and propargite had little or no effect on T. urticae or P. persimilis (Figs. 1,2). Dienochlor was shown to be toxic to spider mites in a long term experiment (Figure 4). Efficacy testing of dienochlor and propargite in previous studies demonstrate that they are highly effective against T. urticae (Shultz & Coffelt 1987, Horsburgh & Cobb 1988). Apparently, propargite and dienochlor work slowly and require more than 48 hours before mortality is observed in spider mite populations. Abamectin was much more toxic to T. urticae than to P. persimilis in slide-

dip and 36 h residue tests (Figs. 1,2). Zhang and Sanderson (1990) had similar results when T. urticae and P. persimilis were placed on abamectin treated leaf discs. Our tests with abamectin verified its effectiveness against spider mites but raised the question of a food-chain toxicity problem. Predator mites disappeared two weeks after they were released on abamectin treated plants. In further tests, where spider mites collected from populations recovering from miticide treatments were fed to predator mites, abamectin and dienochlor were toxic to predator mites feeding on spider mites from treated plants, while propargite still showed no effect on P. persimilis (Figure 3). Previous studies have demonstrated that organophosphate compounds remain in resistant spider mites for several weeks in sufficient amounts to kill predators feeding on them (Binns 1971, Hussey & Parr 1965, Hussey 1968). In similar studies Lindquist and Wolgamott (1980) found that acephate at concentrations of 75, 150 and 300 ppm applied as a soil drench were toxic to P. persimilis. In that study nearly all P. persimilis feeding on T. urticae on treated plants died 21 days after acephate application. In this study food chain effects of miticides were critical in evaluating their probable impact on mixed populations of spider mites and predaceous mites. The likely impact of a dienochlor or abamectin application is a 4-8 week suppression of T. urticae followed by an outbreak period where the miticide residues are too small to effect T. urticae but are still lethal to P. persimilis feeding on the spider mites.

In reviewing the responses of arthropod natural enemies to insecticides, Croft and Brown (1975) cited 13 studies where insecticides were determined to be relatively non-toxic to Amblyseius fallacis. The methods used to determine selectivity were divided approximately equally among slide-dip, residue, and field tests. Little attention was paid to food-

chain toxicity; and most of the food-chain studies that were reviewed were for systemic insecticides (Croft & Brown 1975). Some systemic insecticides and acaracides, initially proclaimed as non-toxic to predators, were found to have a food-chain toxicity effect on predators as early as 1967 (McClanahan 1967). Zhang and Sanderson (1990) found only a slight decrease in survival of P. persimilis when predators were fed spider mites "intoxicated"(immobilized) from feeding on abamectin treated leaves. However, In the same study Zhang and Sanderson (1990) found that the reproductive rate of P. persimilis was significantly reduced. Grafton-Cardwell and Hoy (1983) showed a similar decrease in fecundity in M. occidentalis when the predators were exposed to leaves treated with 4, 8 and 16 ppm abamectin. The survival of adult M. occidentalis was also greatly reduced at these rates.

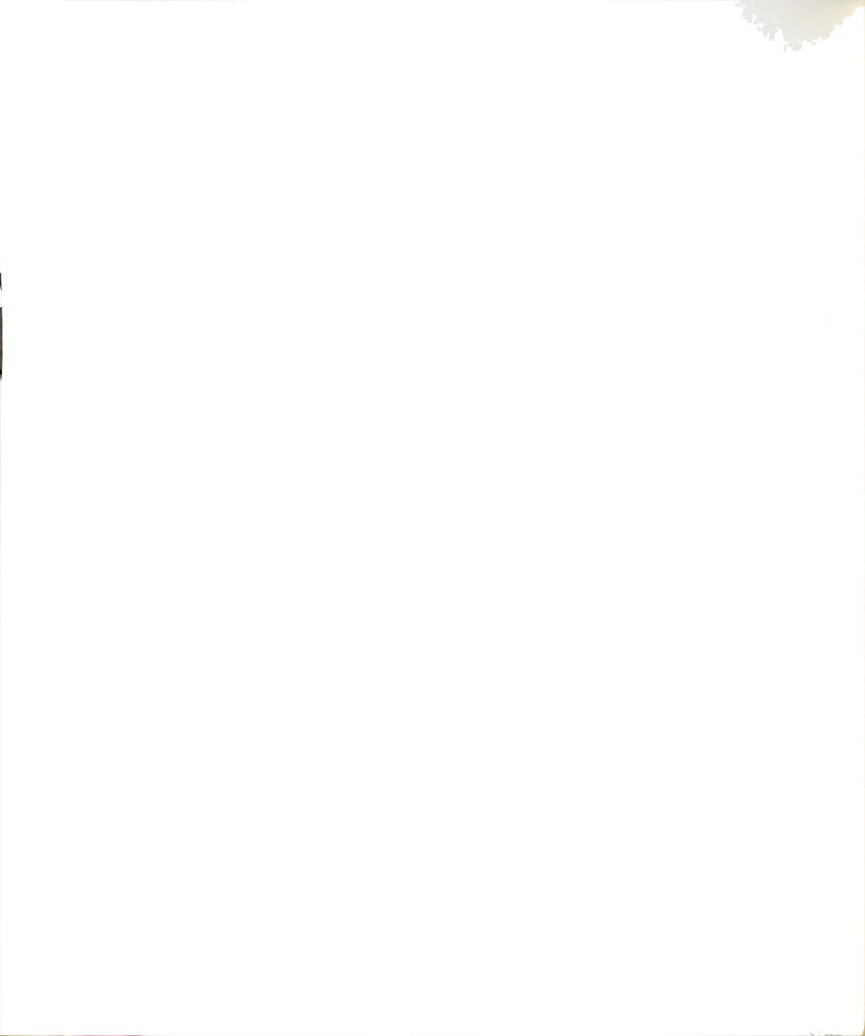
One difference between our study and that of Zhang and Sanderson (1990) is that in our study the mites fed to predators were those that survived miticide application and were active at the time of our test. These mites were from an actively reproducing population on abamectin treated plants. In the Zhang and Sanderson (1990) study immobile spider mites were collected 24 hours after treatment with abamectin. These differences in test methods may account for the greater mortality of predator mites in our tests compared with Sanderson and Zhang (1990). In non-replicated greenhouse tests, abamectin applied to cucumbers at 7 and 14 day intervals reduced the number of predator mites after the first week, and eliminated them completely after 2 weeks (Heyler & Ledieu 1986). Predator mites may have been eliminated because of a direct effect of abamectin on survival and reproduction of predator mites or a lack of prey as a result of

quick elimination of spider mites, or both (Zhang & Sanderson 1990). Further testing is needed before any definite conclusions can be made.

A selective miticide is needed for adjusting predator/prey ratios in integrated mite programs (Grafton-Cardwell & Hoy 1983, Hoy & Cave 1985, Zhang & Sanderson 1990). Of the three miticides tested, propargite appears to be the most compatible with *P. persimilis*. Further testing is needed to determine the impact of avermectin on predator mites.

Résumé

On a utilisé trois sortes d'essai biologique pour déterminer la sélectivité des miticides contre Tetranychus urticae et Phytoseiulus persimilis: 1) le comportement d'alimentation des prédateurs sur la proie, 2) un essai dont des acariens à deux points étaient adhérents à une lame de microscope et immergés dans du miticide et 3) un essai dont on a étudié l'effet du miticide résiduel, sur des acariens à deux points, à 24h après l'arrosage des feuilles. Utilisant ces deux derniers essais normalisés on a évalué l'efficacité d'abamectin, de proparjite et de dienochlor contre T. urticae et P. persimilis. Les résultats de ces deux essais-ci étaient fallacieux et ne correspondaient pas aux études à long terme de la population. Pour déterminer la toxicité indirecte des miticides contre P. persimilis on a exposé aux prédateurs des acariens à deux points qui venaient d'une population déjà traitée par une dose de miticide. Des études du comportement d'alimentation des prédateurs sur la proie et des études à long terme d'une population qui consiste de proie et de prédateur sont les plus utiles pour prévoir l'activité sélective des miticides. A partir des résultats de cette expérience il serait possible de créer un essai normalisé dont les acariens à deux points, traités par une dose sous-létale de pesticide, seraient exposés aux acariens prédateurs afin d'évaluer l'effet, dans la chaîne alimentaire, du miticide sur P. persimilis.



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Table 1. Number of T. urticae and P. persimilis per sample on potted rose plants. Samples were collected approximately every seven days for a 45 day period starting one day before application of abamectin on 23 July. Each sample consisted of 60 leaflets from five rose plants (12 leaflets per plant). Each five plant unit was replicated four times. One thousand Phytoseiulus persimilis were released evenly throughout the 20 plants on 8 August.

Sampling date	<u>T. urticae</u> per sample	<u>P. persimilis</u> per sample
22 July	189.1 $\pm$ 130.0	0
29 July	27.5 $\pm$ 17.9	0
5 August	2.6 $\pm$ 9.9	0
10 August	6.4 $\pm$ 8.0	1.0 $\pm$ 0.8
15 August	1.8 $\pm$ 2.6	0
19 August	3.2 $\pm$ 5.0	0
25 August	7.3 $\pm$ 21.1	0
6 September	9.1 $\pm$ 14.2	0

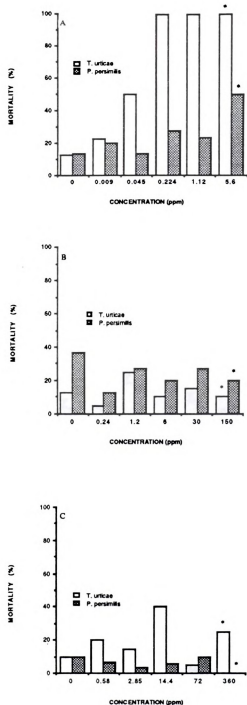
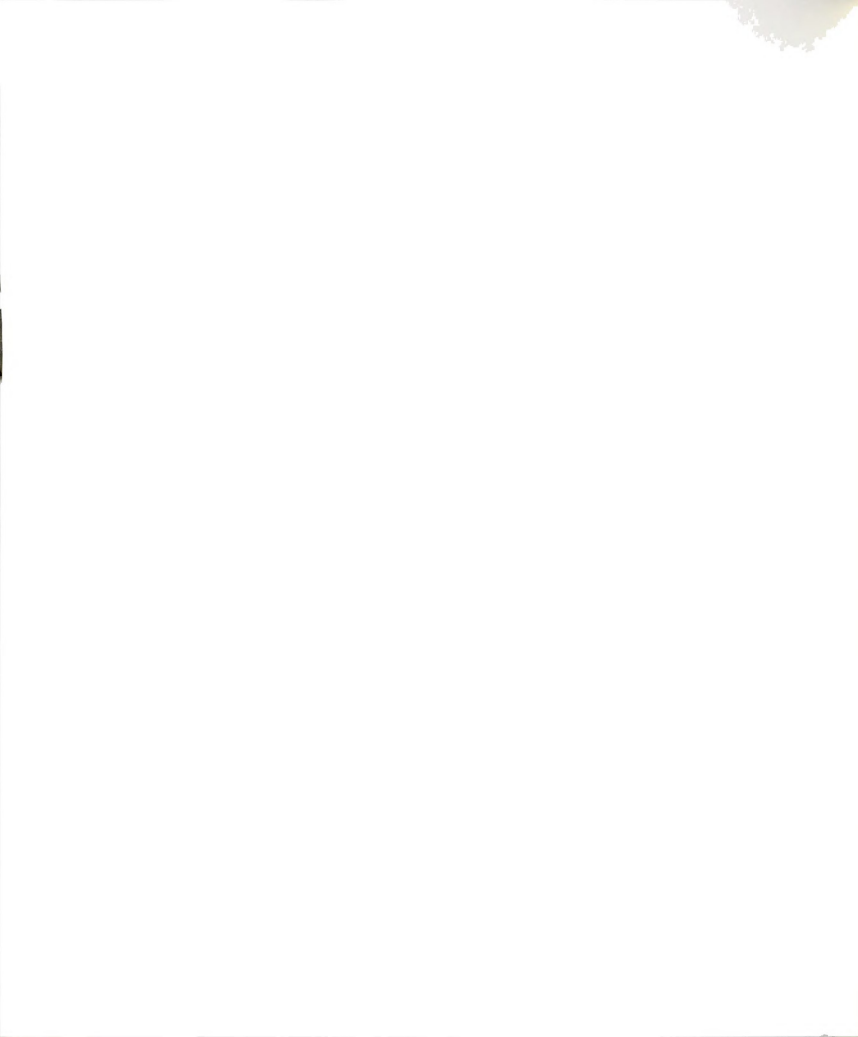


Figure 1. Slide-dip evaluation of three miticides commonly used in greenhouse rose production to *Tetranychus urticae* and *Phytoseiulus persimilis* (A = Abamectin, B = dienochlor, C = propargite.) The concentration listed on the U.S.A. federal product label for greenhouse application is denoted by an asterisk (*) above those bars on the graph.



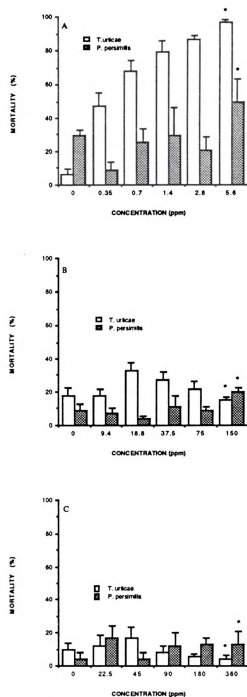


Figure 2. Mortality of *T. urticae* and *P. persimilis* placed on bean leaves treated with abamectin (A), dienochlor (B), or propargite (C) in a 36 h residue test. The concentration listed on the U.S.A. federal product label for greenhouse application is denoted by an asterisk (*) above those bars on the graph. The standard error of each treatment mean is indicated by the brackets on each bar.

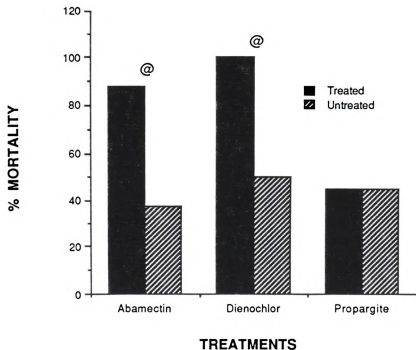
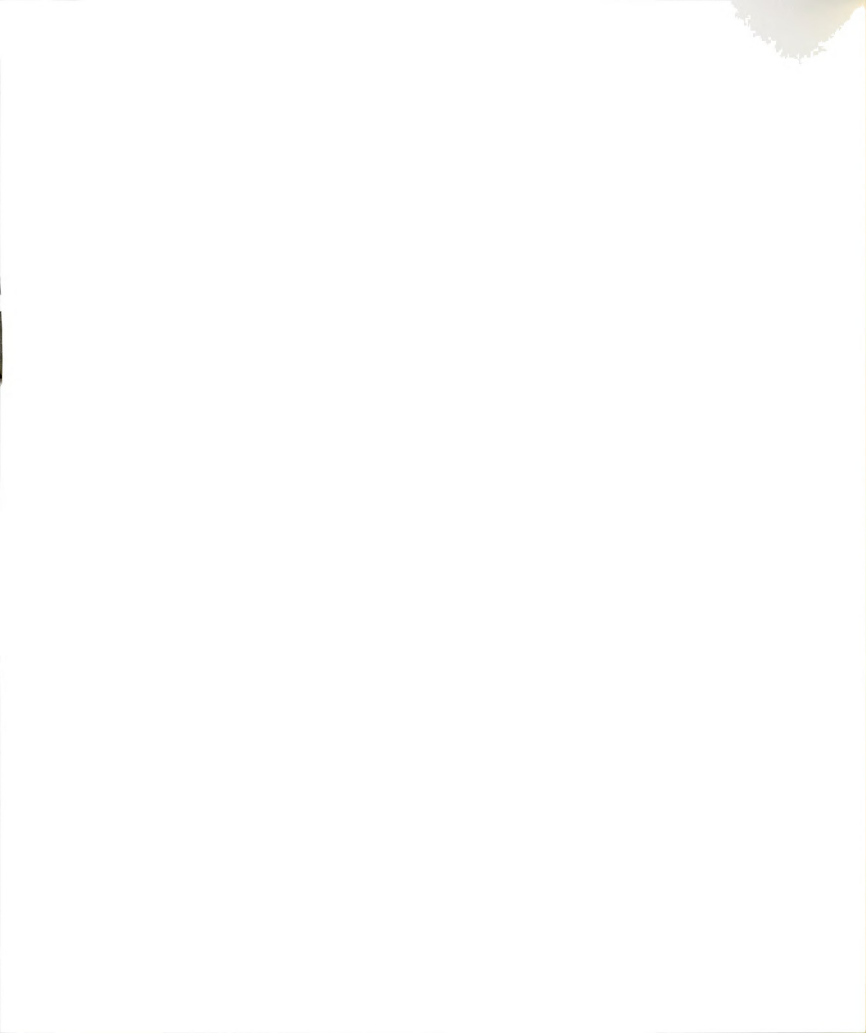


Figure 3. Mortality of P. persimilis fed spider mites surviving applications of abamectin, dienochlor or propargite compared to the mortality of P. persimilis fed spider mites from untreated plants. (@ indicates that treated and untreated means are different, ANOVA $P < .05$).



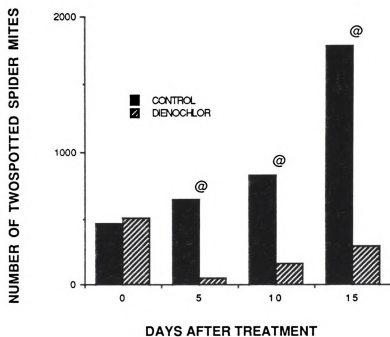


Figure 4. Efficacy of a single treatment of 150 ppm dienochlor in suppressing populations of *T. urticae* 5, 10 and 15 days after treatment as compared with an untreated population. (@ indicates that treated and untreated means are different, ANOVA $P < .05$).

**Selectivity of Benomyl, Dodemorph Acetate, Piperalin,
Triadimefon, Triforine, Acephate and Endosulfan for
Phytoseiulus persimilis or Tetranychus urticae**

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Key words: Acari, Twospotted spider mite, Tetranychus urticae, Phytoseiulus persimilis, fungicides, insecticides, selectivity, testing

ABSTRACT

Standard slide-dip and short residue tests were used to evaluate activity of the fungicides benomyl, dodemorph acetate, piperalin, triadimefon, and triforine; and the insecticides acephate and endosulfan against twospotted spider mites, Tetranychus urticae Koch and the predaceous mite Phytoseiulus persimilis Athias-Henriot. None of the five fungicides tested were toxic to T. urticae or P. persimilis. Both insecticides were more toxic to P. persimilis than to T. urticae. Therefore, benomyl, dodemorph acetate, piperalin, and triadimefon have potential for use in an integrated mite management system for greenhouse grown roses, while acephate and endosulfan should be avoided because of their toxicity to P. persimilis.

INTRODUCTION

Release of the predator mite, Phytoseiulus persimilis Athias-Henriot, has been effective for control of Tetranychus urticae Koch on a number of greenhouse grown vegetable and ornamental plants (Scopes 1970, Gould 1971, Parr & Scopes 1971, Simmonds 1971, Simmonds 1972). Pesticides used for control of other pests may be toxic to predators used in biological control programs (Croft & Brown 1975, Schulten et al. 1976, Ledieu & Stacey 1978). Predators may be affected by pesticides through direct contact with sprays, contact with spray residues, or via the food chain (Lindquist & Wolgamott 1980).

In greenhouse rose production the major pest problems are twospotted spider mites, Tetranychus urticae, rose aphid, Macrosiphum rosae (L.) western flower thrips, Frankliniella occidentalis Pergande, and powdery mildew, Sphaerotheca pannosa. In order to determine if a successful biological control system for T. urticae using P. persimilis can be developed it is necessary to know if the release of predators is compatible with pesticides used for the other major pest problems.

This study was initiated to evaluate five fungicides used to control powdery mildew and two insecticides frequently used in aphid control, for compatibility with the use of P. persimilis in integrated control of T. urticae in rose production greenhouses.

MATERIALS AND METHODS

Toxicity of the fungicides benomyl, dodemorph acetate, piperalin, triadimefon, and triforine; and the insecticides acephate and endosulfan to Tetranychus urticae and Phytoseiulus persimilis was evaluated using a slide-dip test and a 36 hour residual activity test with bean leaves. Spider mites used for these experiments were maintained in the greenhouse and laboratory on 'Henderson' and 'Eastland Bush' lima beans (Phaseolus vulgaris). The culture was initiated in 1986 from spider mites collected at pesticide research center greenhouses, at Michigan State University. Twice every year, additional mites are collected from the same greenhouse range and added to the culture. Phytoseiulus persimilis was originally obtained from "Nature's Control" in Medford, Oregon. The population was maintained under laboratory conditions with periodic additions to the population supplied from Nature's Control. The predators were fed spider mites from our lima bean culture.

Slide-dip test. In slide-dip tests with T. urticae, a 2.5 cm-long piece of double sided Scotch® tape (666 07340-3 3M Commercial Office Supply Division, St. Paul, MN 55133-3053) was placed across a standard microscope slide. A piece of Scotch® nylon packaging tape (34-7023-2006-9, 3M Home products Division, St. Paul, MN 55133-3053) was placed on top of the double-sided tape with its sticky side facing upwards. Twenty T. urticae were placed on each slide. Spider mites on each slide were dipped into test solutions for 5 seconds. Fungicide solutions were prepared at concentrations of 1x, and solutions of insecticides at

concentrations of 1x, 1/5x, 1/25x, 1/125x, and 1/625x, where x is the recommended concentration on the U.S.A. federal product label for greenhouse application. For the fungicides benomyl, dodemorph acetate, piperalin, triadimefon, and triforine the 1x rates were 600, 900, 150, 150, and 269 ppm, respectively. The 1x rates for the insecticides acephate and endosulfan were 600 and 412 ppm respectively. The treatments for the fungicide tests were replicated four times in a randomized complete block design with respect to time of mite placement on slides and treatment. The treatments for the insecticide tests were replicated twice in the same manner. Adult female mites were collected from spider mite infested bean leaves which were placed for 15 minutes in a CO₂ filled sealable plastic bag. The mites were tapped off the leaves onto a Tyler equivalent 32 mesh screen (500µm pore size). Unwanted mite stages fell through the screen leaving only adult females. The collected adults were then observed under a microscope where the needed numbers were removed with a fine paint brush and placed on slides. The mites were placed on the tape so that their legs could move freely. After each slide was fitted with mites it was placed in a holding chamber at approximately 95% relative humidity and 27°C. Three hundred ml of each solution was prepared in a 400ml beaker. Triton AG-98 was added to each solution at a concentration of .12%. Slides with mites were agitated in test solutions for 5 seconds, and placed on edge in a drying rack lined with absorbent paper towel to remove excess moisture from the slides. After 15 minutes the slides were placed back into the holding chamber and allowed to sit for 24 hours. Each mite was touched with a fine paint brush. If a mite showed any movement it was considered alive.

A similar procedure was followed for the P. persimilis slide-dip tests. However, the nylon ribbed packaging tape did not hold the predators in place. Duct tape (Nashua 357, Watervleit, N.Y. 12189) was stickier and proved to be a suitable replacement. Also, 15 mites were used per slide instead of 20. Treatment rates and all other details were kept the same.

36 hour residue test. Residual activity of benomyl, dodemorph acetate, piperalin, triadimefon, triforine, acephate, and endosulfan were tested by spraying bean leaves at 5 P.M., allowing them to dry overnight, and placing mites on leaves between 9 and 11 A.M. the next day. In each of these tests the fungicides were applied at concentrations of 4x, 2x, 1x, 1/2x, 1/4x and 0x. The insecticides were applied at concentrations of 1x, 1/2x, 1/4x, 1/8x, 1/16x and 0x, where x is the recommended concentration on the federal product label for greenhouse application. The 1x rates for all pesticides remained the same as in the slide-dip tests. The treatments were replicated four times in a complete randomized block design. Treatments were applied in a spray tower equipped with a conveyor belt that moves at a constant rate of 9.1 cm/second. Miticide solutions were applied through a TeeJet 8001-E even flat spray nozzle. Excised bean leaves in petri dishes layered with absorbent cotton were sprayed uniformly by running the petri dish through the spray tower. Miticides were applied beginning with the lowest concentration first. Petri dishes were arranged in numerical order on a large cafeteria tray after treatments were applied. After the spray had dried on the leaves, one rubber hose washer was glued to each leaf using " Elmer's® Glue-all" (Borden's Inc., Dept. CP, Colombus, Ohio 43215). The washers served as arenas to hold the mites in place. Twenty adult female T. urticae and six P. persimilis were used in each arena for their respective tests. Adult female spider

mites were collected by the CO₂ method described in the previous section. A fine paint brush was used to place mites in arenas. A small circle of nylon mesh screening was glued over the arenas after the mites were in place. The cotton base in the petri dishes was kept moist to prevent desiccation of the leaves.

In residue tests with P. persimilis, the mites were added to leaves in a 5°C cold room to prevent them from leaving before the screening was glued in place. In all tests the mites were kept in arenas on treated bean leaves for 24 hours. After this time the arena covers were removed and mortality data taken.

Statistical analysis. An arcsin square root transformation was performed on all percent mortality data before analysis. Dose effect of the fungicides benomyl, dodemorph acetate, piperalin, triadimefon, and triforine; and the insecticides acephate and endosulfan was analyzed by regressing transformed percent mortality of mites against the concentration of test solution (Wilkinson 1987). If the slope of this regression was greater than zero ($p < 0.05$), the pesticide was determined to have a dosage effect. In 36 h residue tests, mortality of T. urticae was compared with that of P. persimilis at each of pesticide concentraion with the Student's T-test (Steel & Torrie 1980). Mite mortality was also regressed against pesticide concentration as described for the slide-dip tests.

Results

Slide-dip tests. The powdery mildew fungicides benomyl, piperalin, and triadimefon had no contact activity against T. urticae or P. persimilis in slide-dip tests (Table 1). Dodemorph acetate was toxic to spider mites but not to predator mites, while triforine was toxic to spider mites and predator mites.

Dodemorph acetate treatment caused 29% mortality of T. urticae adults compared with only 9% mortality of mites treated with a water control ($T_{(.05)} = 3.312$, $df = 6$). Dodemorph acetate did not appear to have any effect on P. persimilis adults ($T_{(.05)} = 0.873$, $df = 6$). Triforine had the greatest effect on T. urticae and P. persimilis. Spider mites dipped in triforine suffered 47% mortality compared with 24% in the control, while 46% of the predator mites died compared with 22% in the control ($T_{(.05)} = 3.243$, $df = 6$ and $T_{(.05)} = 2.561$, $df = 6$, respectively, Table 1). Regression of T. urticae or P. persimilis mortality against concentrations of benomyl, piperalin and triadimefon solutions indicate no relationship between variables (Table 3). The concentration of dodemorph acetate was related to spider mite mortality but not to P. persimilis mortality ($r^2 = 0.67$, $F = 12.4$; $r^2 = 0.11$, $F = 0.7$, respectively). Also, T. urticae and P. persimilis mortality increased as the concentration of triforine increased ($r^2 = 0.53$, $F = 6.9$; $r^2 = 0.57$, $F = 8.0$, respectively, Table 3).

The concentrations of acephate and endosulfan solutions were not related to mortality of T. urticae or P. persimilis mortality (Table 4).

36 hour residue test. The powdery mildew fungicides benomyl, piperalin, triadimefon, and triforine had no activity on T. urticae or P. persimilis in the residue tests (Table 2). Dodemorph acetate was toxic to

spider mites at rates above the 1x rate of 900 ppm (22% mortality compared with 11% mortality in control), but had no effect on spider mites at 1x (11% mortality). Dodemorph acetate was not toxic to P. persimilis.

Regression of P. persimilis mortality against concentrations of benomyl and dodemorph acetate indicates no relationship between variables. The same regression analysis for T. urticae indicated a decrease in mortality with an increase in concentration of benomyl (Table 3).

The insecticides acetate and endosulfan had greater activity against predator mites than spider mites. Predator mite mortality increased with an increase in the concentration of acephate or endosulfan applied to bean leaves (Table 4). The same regression of spider mite mortality against concentrations of acephate and endosulfan indicates no relationship between variables (Table 4).

DISCUSSION

None of the five powdery mildew fungicides tested had a major impact on spider mite or predator mite survival in our slide-dip or short residue studies. Triforine and dodemorph acetate caused mortality in slide dip tests but not in residue tests. In the residue tests the activity of dodemorph acetate only affected spider mites when applied at rates above the 1x rate of 900 ppm and therefore cannot be considered useful in helping to control spider mites in an integrated control program. Although these tests suggest that the fungicides tested are not harmful to P. persimilis, a food-chain toxicity test is needed to determine if spider mites feeding on fungicide treated plants have any effect on predators feeding on them (Lindquist & Wolgamott 1980). Field and Hoy (1986) also saw no

impact of benomyl, piperlin, and triforine on female twospotted spider mites in residue tests. However, other studies have shown that use of benomyl may decrease the fecundity of twospotted spider mites (Spadafora & Lindquist 1972, Field & Hoy 1986). In one study benomyl applied as a soil drench controlled powdery mildew with no adverse effects to P. persimilis (Parr and Binns 1971).

The insecticides acephate and endosulfan were more toxic to P. persimilis than T. urticae in short term residue tests. Therefore, application of acephate or endosulfan may induce outbreaks of spider mites by removing predator mites. Other studies have also shown that acephate (Lindquist et al. 1979, Lindquist & Wolgamott 1980) and endosulfan (Smith et al. 1963) are toxic to P. persimilis.

In conclusion, the fungicides tested seem to be compatible with use of P. persimilis. Benomyl may have an effect on mite fecundity and should be investigated further in population studies before determining its role in integrated control programs.

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Table 1: Contact toxicity of commonly used fungicides to *I. urticae* and *P. persimilis*. Data are expressed as the mean percent mortality.

	n	Fungicide Treatment				
		benomyl (600 ppm)	dodemorph acetate (900 ppm)	piperalin (150 ppm)	triadimefon (150 ppm)	triforine (269 ppm)
<u>I. urticae</u>						
treated	4	.21	.29*	.12	.14	.46*
control	4	.28	.09	.12	.09	.12
<u>P. persimilis</u>						
treated	4	.29	.12	.19	.09	.44*
control	4	.30	.08	.08	.08	.21

* An asterik indicates that mortality of mites dipped in fungicide solution was greater than that in the control treatment (Student t-test, $P = 0.05$).

Table 2: Residual activity of commonly used fungicides to I. urticae and P. persimilis at selected rates. Data are expressed as mean percent mortality.

Fungicide Treatment						
	n	benomyl (ppm)	dodemorph acetate (ppm)	piperalin (ppm)	triadimefon (ppm)	triforine (ppm)
<u>I. urticae</u>						
treatment						
Control	4	.16	.11	.21	.16	.16
0.25x	4	.11	.11	.06	.13	.20
0.50x	4	.05	.05	.11	.25	.14
1.00x	4	.04	.11	.15	.16	.14
2.00x	4	.12	.11	.11	.25	.07
4.00x	4	.01	.22	.16	.13	.16
<u>P. persimilis</u>						
treatment						
Control	4	.13	.08	.17	.50	.25
0.25x	4	.10	.08	.34	.35	.04
0.50x	4	.29	.12	.21	.24	.13
1.00x	4	.17	.04	.25	.42	.17
2.00x	4	.05	.17	.13	.39	.13
4.00x	4	.25	.12	.18	.40	.23

Table 3: Regression statistics for contact and residual activity of five fungicides to *I. urticae* and *P. persimilis*.

Fungicide	Mite	Test	Regression Statistics			
			Model†	F	P	r ²
benomyl(ppm)	<i>I. urticae</i>	slide-dip	y = 558 - 0.146x	1.300	0.298	0.178
	<i>P. persimilis</i>	slide-dip	y = 580 - 0.042x	0.131	0.729	0.021
	<i>I. urticae</i>	residue	y = 308 - 0.091x	4.682	0.042*	0.175
	<i>P. persimilis</i>	residue	y = 283 + 0.060x	0.763	0.392	0.034
dodemorph acetate(ppm)	<i>I. urticae</i>	slide-dip	y = 295 + 0.292x	12.420	0.012*	0.674
	<i>P. persimilis</i>	slide-dip	y = 245 + 0.103x	0.762	0.416	0.113
	<i>I. urticae</i>	residue	y = 262 + 0.055x	5.132	0.034*	0.189
	<i>P. persimilis</i>	residue	y = 213 + 0.027x	0.471	0.500	0.021
piperalin(ppm)	<i>I. urticae</i>	slide-dip	y = 315 - 0.083x	0.006	0.941	0.001
	<i>P. persimilis</i>	slide-dip	y = 245 + 1.250x	2.886	0.140	0.325
	<i>I. urticae</i>	residue	y = 315 + 0.093x	0.236	0.632	0.011
	<i>P. persimilis</i>	residue	y = 461 - 0.321x	1.089	0.308	0.047
triadimefon(ppm)	<i>I. urticae</i>	slide-dip	y = 295 + 0.483x	1.081	0.339	0.153
	<i>P. persimilis</i>	slide-dip	y = 245 + 0.100x	0.013	0.913	0.002
	<i>I. urticae</i>	residue	y = 414 - 0.012x	0.004	0.950	0.000
	<i>P. persimilis</i>	residue	y = 654 - 0.142x	0.231	0.636	0.010
triforine(ppm)	<i>I. urticae</i>	slide-dip	y = 315 + 1.217x	6.876	0.039*	0.534
	<i>P. persimilis</i>	slide-dip	y = 482 + 0.957x	7.967	0.030*	0.570
	<i>I. urticae</i>	residue	y = 372 - 0.083x	0.486	0.493	0.022
	<i>P. persimilis</i>	residue	y = 281 + 0.176x	1.775	0.196	0.075

† For all regression equations percent mortality was converted using arcsin-square root transformation and then multiplied by 1000.

* The regression model is significant at P = 0.05.

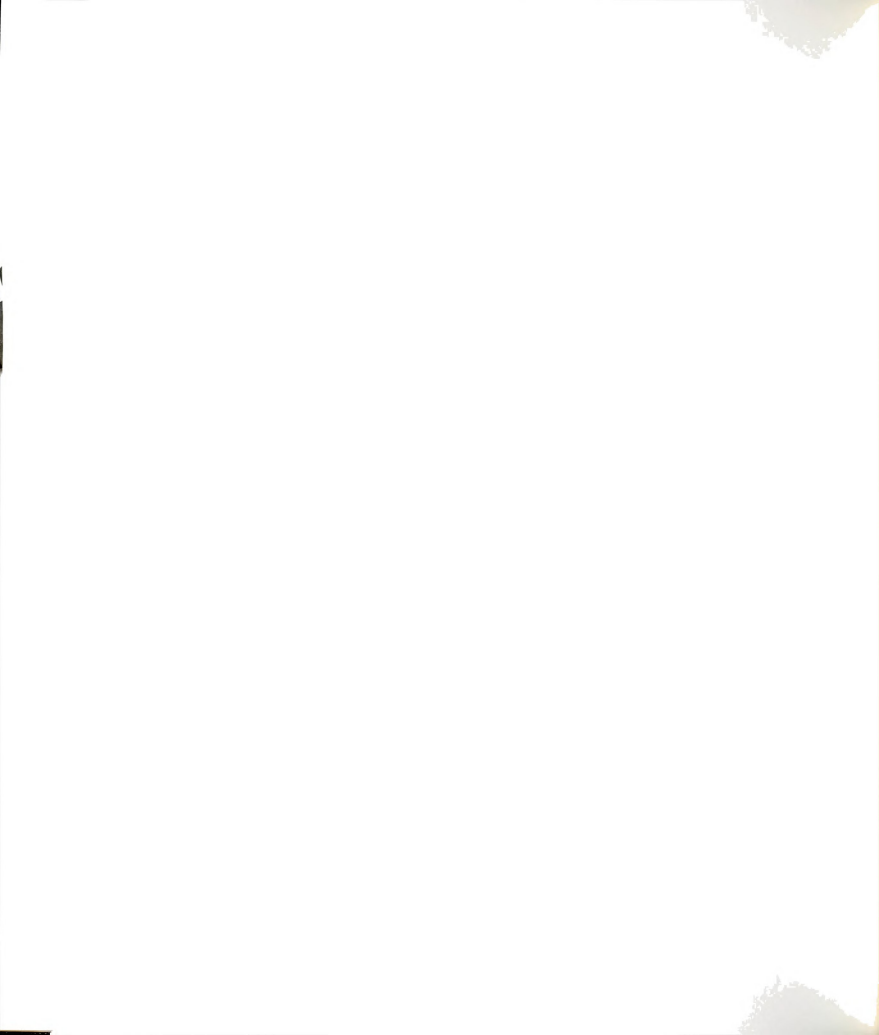


Table 4: Regression statistics for contact and residual activity of acephate and endosulfan to *I. urticae* and *P. persimilis*.

Insecticide	Mite	Test	Regression Statistics		
			Model†	F	r ²
Acephate(ppm)	<i>I. urticae</i>	slide-dip	y = 399 + 0.333x	0.696	0.424
	<i>P. persimilis</i>	slide-dip	N/A	N/A	N/A
	<i>I. urticae</i>	residue	y = 506 - 0.074x	0.133	0.719
	<i>P. persimilis</i>	residue	y = 614 + 3.080x	21.292	0.000*
Endosulfan(ppm)	<i>I. urticae</i>	slide-dip	y = 271 + 0.149x	0.145	0.711
	<i>P. persimilis</i>	slide-dip	y = 334 + 0.393x	0.641	0.442
	<i>I. urticae</i>	residue	y = 430 + 0.348x	3.464	0.076
	<i>P. persimilis</i>	residue	y = 168 + 1.876x	16.555	0.001*

† For all regression equations percent mortality was converted using arcsin-square root transformation and then multiplied by 1000.

* The regression model is significant at P = 0.05.

**Estimating Population Densities of Twospotted Spider Mites,
Tetranychus urticae, on Greenhouse Grown Roses.**

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Key Words: Acarina, Twospotted spider mite, Tetranychus urticae, sampling, bias, accuracy, precision, roses

ABSTRACT

Absolute population densities and distributions of spider mites on 14 rose plants were determined by counting the number of mites on every leaflet (450 -900 leaflets per plant) on each plant. Leaf, leaflet and stem positions were also noted and entered into a data file. The number of mites found on leaves 1 and 2, the oldest leaves, was 1-8 fold greater than the number of mites found on randomly selected leaves. The actual mean number of mites per leaflet was most closely estimated by the means of leaflets 3 and 4. Eight replications of simulated samples of 10, 20, and 30 randomly selected leaflets were taken from each plant. This process was repeated using only leaflets 3 and 4 from leaves 1 and 2. Because samples from leaves 1 and 2 are consistently higher than random samples from the entire plant, the bias was determined and used for a sample adjustment computation that more accurately reflected the actual mite density. The precision and accuracy of all sample methods were determined. When mite populations are low (< 2.5 mites per leaflet) samples of leaflets 3 and 4 from leaves 1 and 2, adjusted for known bias (multiplied by conversion factor 0.548), and random samples from the entire plant are equally accurate and precise. When mite populations exceed 2.5 mites per leaflet random samples from the entire plant are more accurate but not more precise than leaflet samples from leaves 1 and 2 only. For research purposes, random samples of 20 leaflets per plant will provide an acceptable estimate of spider mite population density (average $s^2/\bar{x} = 0.79$). Because spider mites are most heavily distributed among the oldest leaves, sampling schemes designed to detect mites at low density should be aimed at the base of rose plants where the oldest leaves are found.

INTRODUCTION

Frequent outbreaks of twospotted spider mite, Tetranychus urticae Koch, on roses and strict guidelines proposed by the United States Environmental Protection Agency for re-entry of greenhouses after pesticide applications has led to increased interest in developing an integrated pest management (IPM) program using the predator mite Phytoseiulus persimilis Athias-Henriot. Biocontrol of spider mites requires more intensive monitoring of mite populations than chemical control. A quantitative approach to biological control of spider mites using predaceous mites requires estimating population densities (Nachman 1984). To be useful, a sampling program should provide estimates having the highest accuracy commensurate with the amount of work expended (Southwood 1978). Accurate sampling methods are needed for researchers studying biocontrol of mites on roses, and for greenhouse growers trying to detect spider mites at low densities to properly time release of predator mites. Intelligent decisions concerning pest control strategies are directly dependent upon knowing the status of all important species (Beardsley et al. 1979).

The purpose of this project was to determine the distribution of spider mites on greenhouse grown rose plants, and to use this information to develop sampling procedures with known precision and accuracy limitations that can be adapted by other researchers and rose growers.

MATERIALS AND METHODS

Rose plants of Livia and Darling varieties were received in good condition from Devor Nurseries on 26 June, 1987. Two hundred roses were planted in 8 liter plastic nursery pots filled with Baccto® Professional Plant Mix potting soil. Rose plants were maintained with biweekly irrigation and weekly fertilization. An average of 300 g of 20-10-20 of Peter's® professional fertilizer (W. R. Grace and co., Fogelsville, PA 18061) applied as a soil drench using a 10-1 proportioner and a 5 gallon stock tank.

Mite cultures were established in 1986 from mites collected from the Pesticide Research Center greenhouses at Michigan State University. Once per year additional twospotted spider mites are collected at the same place and added to the culture. Twospotted spider mites were maintained in the laboratory and in the greenhouse on 'Henderson Bush' and 'Eastland Bush' lima beans (Phaseolus vulgaris). Mites were allowed to infest the rose plants naturally by movement from other parts of the greenhouse. This prevented any bias in mite positioning on the plants that might occur from hand infesting of plants.

In order to develop and determine the most efficient sampling method for twospotted spider mites on greenhouse grown roses it was decided to determine the actual population and distribution of spider mites on selected rose plants, enter the data into a file and simulate sampling procedures with a computer. When the potted rose plants reached a size of approximately 75 to 95 cm, individual plants were chosen for sampling by

visual inspection for mite injury. Selected plants had a range of 450 to 900 leaflets, supporting from 50 to 24,000 mites. Spider mite numbers on every leaflet were recorded for fourteen rose plants. Each plant was counted within a 3-day time period to avoid significant shifts in mite distribution.

Numbering System: A five digit identification number was assigned to each leaflet based on its position on the plant. Digits 1 to 5 of the identification number represent the stem, stemlet, branch, leaf or leaflet, respectively (Figure 1). Leaves can arise from either the stem, stemlet or branches. Any plant part not present was assigned a 0. When leaflets were missing the lowest leaflet present was labeled as #1.

Spider Mite Counts. Counts were made starting with the lowest leaf on stem #1. This pattern was repeated for all stems. Each leaf was taken from the plant for counting. Leaflets were assigned the appropriate identification number and all motile mites were counted using a binocular dissecting scope. Data were recorded as the number of motile mites per leaflet. Eggs were not counted. A data file for all sampled plants was created in Systat® (Wilkinson 1987) to allow computer sampling.

Sampling. Data collected were used to determine: (1) total number of mites/plant; (2) mean number of mites/leaf; (3) mean number of mites/leaflet; and (4) distribution of mites on the plant. For each plant, eight simulated random samples of 10, 20, and 30 leaflets were taken from the entire plant and from leaves 1 and 2, leaflets 3 and 4 only. Using Systat®, random numbers were generated and assigned to each leaflet. For each plant the random numbers were sorted and arranged from lowest to highest numerical value. Groups of 10, 20 and 30 random leaflets were pulled from the data file for statistical analysis in Systat® (Wilkinson

1987). The means of all samples were calculated and compared to the actual plant means for their accuracy and precision.

Data Analysis. Accuracy of a sample estimate is the difference between the sample estimate and the true population parameter being estimated (Fowler & Witter 1982). Therefore, in our study accuracy can be defined as a measure of the deviation of the sample mean ($\bar{x}$) from the true population mean (μ). Precision of a sample is the difference between the sample estimate and the mean of the estimates of all possible samples that can be taken from the population (Fowler & Witter 1982). Therefore it can be said that precision is a measure of the variance among the sample means. In order to determine the accuracy and precision of our sampling method the variance of sample means (precision) and the accuracy of random leaflet samples were calculated for all fourteen plants. The mean accuracy was calculated using the formula:

$$\text{Accuracy} = \frac{\sum(x - \mu)}{n} \quad \text{where; } n = 8$$

Accuracy was calculated in this manner for all sampling variations. Because the sample means were consistently higher for leaves 1 and 2 than the actual mean, a conversion factor was calculated to adjust samples from leave 1 and 2 to more accurately reflect actual mite density. First, the percent accuracy for each plant was obtained by the formula:

$$\% \text{ Accuracy} = \frac{\text{accuracy}}{\mu}$$

The mean percent accuracy for all fourteen plants was used to calculate the conversion factor by the formula:

$$\text{Conversion factor} = 1.0 / (1.0 + \text{mean } \% \text{ Accuracy})$$

By this method an average conversion factor of 0.548 was determined for all 14 plants. Sample means were multiplied by this value for each plant and the accuracy, variance and sample means were calculated with the adjusted values. For research purposes, random samples of 20 leaflets per plant will provide an acceptable estimate of spider mite population density (average $s^2/\bar{x} = 0.79$, Figure 6).

All other data were analyzed with Systat® and Mystat® statistical programs from Macintosh® (Wilkinson 1987). Regression of twospotted spider mites distribution against leaf position was performed for high, medium and low populations on greenhouse grown roses.

RESULTS AND DISCUSSION

When the mean number of mites per leaf for high (5.0 -36.1), medium (1.5 - 5.0), and low (0 - 1.5 mites per leaflet) populations were regressed against leaf position, the slopes of the regression lines were $0.045x$, $-0.52x$ and $-1.34x$, respectively (Figure 2). Leaf positions 1 and 2, the oldest on any given stem, contained the highest density of mites (Figure 1B).

Mite density decreased as leaf position increased. On a potted rose plant leaf positions 1 and 2 are mostly located in the center of the plant closest to the ground. The actual mean number of mites per leaflet were compared with the means for various leaflet sample patterns. In most cases, the actual mean was most closely estimated by the mean of leaflets 3 and 4 (Table 1). Therefore, it was theorized that the most sensitive estimate of mite populations on experimental rose plants could be obtained by sampling only leaves 1 and 2, and counting the live mites on leaflets 3 and 4. In

order to test this assumption randomly selected samples of 10, 20, and 30 leaflets were taken from each rose plant using the data file created from the mite population counts. Two sets of samples were taken. One set from leaves 1 and 2 with only mites on leaflets 3 and 4 being counted. The other set of samples were taken randomly from the entire plant.

Samples from leaves 1 and 2, leaflets 3 and 4 only, were more precise and less accurate than random samples taken from the entire plant (Table 2; Figure 3). Samples from only leaves 1 and 2 generally gave estimates that were too high (Table 3; Figure 5). When the data were adjusted by multiplying the sample means by the conversion factor 0.548, the accuracy of the estimates were increased (Table 4; Figure 4). When mite populations were below 2.5 mites per leaflet random samples from all leaflets and samples from leaflets 3 and 4 on leaves 1 and 2 were equally accurate and precise. When mite populations exceed 2.5 mites per leaflet random samples taken from the entire plant were found to be more accurate and less precise than the adjusted leaflet samples from leaves 1 and 2.

In summary, the most accurate but not the most precise estimate of mite populations are determined from samples taken from the entire plant. Samples taken from areas of highest mite concentrations only consistently give estimates that are too high. When this data is adjusted using a known accuracy conversion factor the accuracy and precision of the estimate is increased. The adjusted data were the most precise of the three samples.

An important step in biological control programs is the early detection of pest populations followed by release of the control organism (French et al 1976, Hamlen & Lindquist 1981). Selective sampling of leaflets 3 and 4 from leaves 1 and 2 is a good method for detecting low

populations of spider mites on potted rose plants. Further research is needed to confirm that spider mite populations on the larger rose plants grown in production greenhouses have the same spatial distribution as we found for mites on potted rose plants.

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Table 1. Spider mites per leaflet for computer generated samples of selected leaflet groups compared to spider mites per leaflet for the entire plant.

Rose Plant	Spider mites per leaflet			
	All Leaflets	Leaflets 1 & 2	Leaflets 3 & 4	Leaflets 5 - 9
A	0.102	0.046	0.067*	0.241
B	0.244	0.167	0.321	0.254*
C	0.341	0.188	0.301*	0.795
D	2.012	1.780	2.328	1.936*
E	2.638	1.489	2.503*	4.093
F	2.677	2.131	2.613*	3.580
G	2.725	2.455	2.969	2.905*
H	3.162	3.176*	3.830	2.439
I	6.265	3.558	6.480*	9.523
J	6.368	5.051	8.174	5.946*
K	8.395	5.442	8.732*	11.868
L	11.513	8.138	13.141*	15.608
M	14.822	8.724	15.594*	24.008
N	36.105	29.428	40.955*	42.200

* Selected leaflet samples with means closest to the mean number of spider mites per leaflet for entire plant are indicated by an asterisk.

Table 2. The accuracy and variance of mean estimates for random leaflet samples. Accuracy ($\sum(\bar{x}-\mu)/n$ where n = eight sample replications.) and variance of mean estimate were determined for samples of 10, 20, and 30 leaflets per plant from roses with various mite densities. The actual mean (μ) was determined from absolute samples. Mean estimates ($\bar{x}$) is the number of mites per leaflet determined from samples of 10, 20, or 30 leaflets.

Rose Plant	Mites per leaflet (μ)	10 Leaflet Samples				20 Leaflet Samples				30 Leaflet Samples			
		Mites per leaflet ($\bar{x}$)	Acc.†	Var.†		Mites per leaflet ($\bar{x}$)	Acc.†	Var.†		Mites per leaflet ($\bar{x}$)	Acc.†	Var.†	
A	0.102	0.050	-0.052	0.006		0.106	0.004	0.016		0.120	0.018	0.035	
B	0.244	0.263	0.019	0.128		0.350	0.106	0.152		0.217	-0.027	0.075	
C	0.341	0.401	0.060	0.150		0.510	0.170	0.600		0.240	-0.100	0.050	
D	2.01	1.26	-0.75	3.03		1.54	-0.47	1.87		2.25	0.24	1.16	
E	2.64	2.03	-0.61	2.62		3.46	0.82	4.42		2.42	-0.22	1.09	
F	2.68	2.86	0.18	4.15		2.80	0.12	1.68		2.38	-0.30	0.89	
G	2.73	1.96	-0.77	1.86		2.86	0.13	1.78		2.75	0.02	1.53	
H	3.16	3.46	0.30	4.52		3.08	-0.08	1.41		3.31	0.15	1.06	
I	6.27	8.04	1.77	20.80		6.36	0.09	10.65		6.78	0.51	3.78	
J	6.37	6.28	-0.09	5.78		6.63	0.26	2.39		6.03	-0.34	10.49	
K	8.40	10.30	1.90	20.80		8.48	0.08	5.40		8.29	-0.11	0.20	
L	11.51	14.28	2.77	65.97		10.23	-1.28	2.08		10.63	-0.88	14.56	
M	14.82	16.59	1.77	23.66		14.90	0.08	12.50		14.40	-0.42	7.25	
N	36.11	30.90	-5.20	47.10		35.80	-0.30	49.80		38.20	2.10	51.20	

† Accuracy and variance of eight mean estimates.

Table 3. The accuracy and variance of mean estimates from samples of leaflets 3 and 4 from leaves 1 and 2. Accuracy ($\sum(\bar{x}-\mu)/n$ where n = eight sample replications.) and variance of mean estimate were determined for samples of 10, 20, and 30 leaflets per plant from roses with various mite densities. The actual mean (μ) was determined from absolute samples. Mean estimates ($\bar{x}$) is the number of mites per leaflet determined from samples of 10, 20, or 30 leaflets.

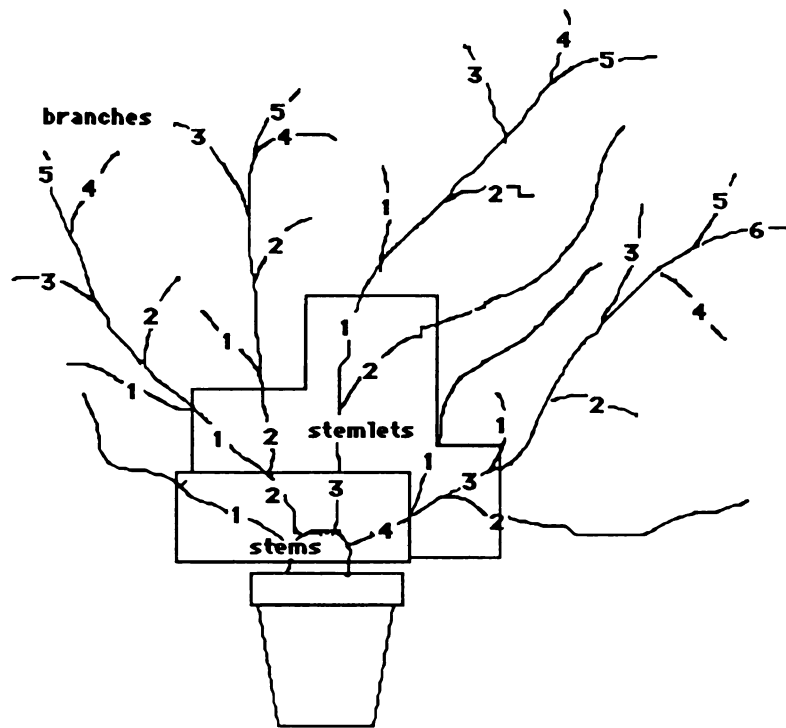
Rose Plant	Mites per leaflet (μ)	10 Leaflet Samples				20 Leaflet Samples				30 Leaflet Samples			
		Mites per leaflet ($\bar{x}$)	Acc.†	Var.†		Mites per leaflet ($\bar{x}$)	Acc.†	Var.†		Mites per leaflet ($\bar{x}$)	Acc.†	Var.†	
A	0.102	0.075	-0.027	0.01		0.069	-0.033	0.002		0.081	-0.021	0.003	
B	0.244	0.588	0.344	0.66		0.681	0.497	0.06		0.916	0.672	0.34	
C	0.341	0.625	0.269	0.36		0.825	0.484	0.18		0.716	0.375	0.38	
D	2.01	2.64	0.63	1.91		3.26	0.94	1.37		3.57	1.56	2.09	
E	2.64	2.99	0.28	4.82		3.12	0.48	1.13		3.39	0.75	1.51	
F	2.68	3.26	0.59	7.97		2.93	0.25	0.80		2.67	-0.01	1.04	
G	2.73	9.64	6.91	15.46		8.98	6.25	6.49		9.06	6.33	2.73	
H	3.16	6.95	3.79	7.07		6.98	3.82	2.36		7.52	4.36	2.61	
I	6.27	9.16	2.89	12.49		8.13	1.86	5.86		7.92	1.65	3.94	
J	6.37	10.85	4.48	19.08		10.72	1.67	1.03		11.09	4.72	11.79	
K	8.40	10.04	1.64	4.45		10.07	4.35	14.91		9.85	1.45	1.78	
L	11.51	20.23	8.72	47.83		20.93	9.41	3.76		20.66	9.15	3.24	
M	14.82	23.04	8.22	37.42		23.52	8.70	18.13		23.53	8.71	8.91	
N	36.11	54.30	18.19	83.91		54.73	18.62	58.92		56.52	20.41	50.41	

† Accuracy and variance of eight mean estimates.

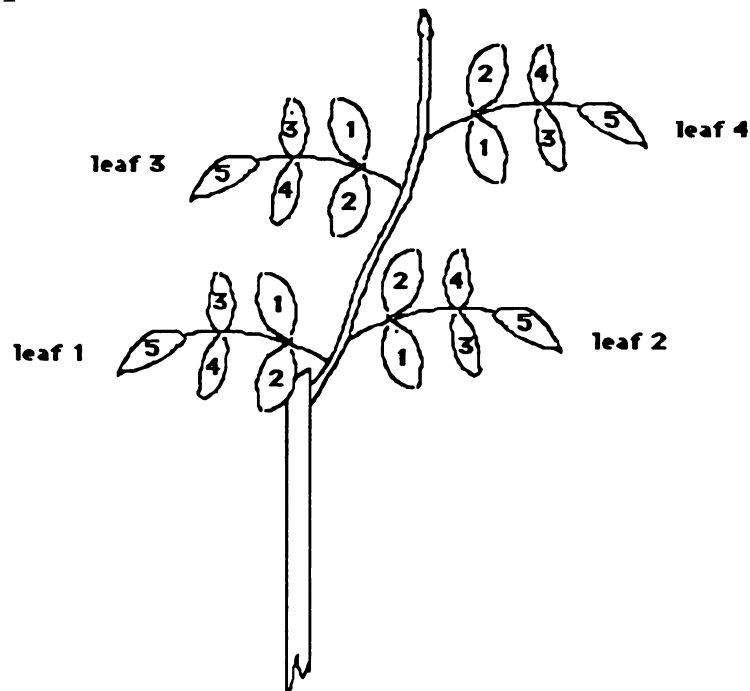
Table 4. The accuracy and variance of mean estimates from samples of leaflets 3 and 4 from leaves 1 and 2 multiplied by the conversion factor 0.548 before statistics calculations. Accuracy ($\sum(\bar{x}-\mu)/n$ where n = eight sample replications.) and variance of mean estimate were determined for samples of 10, 20, and 30 leaflets per plant from roses with various mite densities. The actual mean (μ) was determined from absolute samples. Mean estimates ($\bar{x}$) is the number of mites per leaflet determined from samples of 10, 20, or 30 leaflets.

Rose Plant	Mites per leaflet (μ)	10 Leaflet Samples				20 Leaflet Samples				30 Leaflet Samples			
		Mites per leaflet ($\bar{x}$)	Acc.†	Var.†		Mites per leaflet ($\bar{x}$)	Acc.†	Var.†		Mites per leaflet ($\bar{x}$)	Acc.†	Var.†	
A	0.102	0.041	-0.061	0.002		0.038	-0.064	0.001		0.045	-0.057	0.001	
B	0.244	0.322	0.078	0.199		0.373	0.129	0.019		0.502	0.258	0.101	
C	0.341	0.343	0.002	0.107		0.452	0.111	0.054		0.393	0.052	0.114	
D	2.01	1.45	-0.56	0.57		1.78	-0.23	0.41		1.95	-0.06	0.63	
E	2.64	1.64	-1.00	1.45		1.72	-0.92	0.34		1.86	-0.78	0.45	
F	2.68	1.79	-0.89	2.39		1.61	-1.07	0.24		1.46	-1.22	0.31	
G	2.73	5.28	2.55	4.64		4.92	2.19	1.95		4.96	2.23	0.82	
H	3.16	3.81	0.65	2.12		3.82	0.66	0.71		4.12	0.96	0.78	
I	6.27	5.02	-1.25	3.75		4.45	-1.82	1.76		4.34	-1.93	1.18	
J	6.37	5.95	-0.42	5.73		5.87	-0.50	4.48		6.08	-0.29	3.54	
K	8.40	5.50	-2.90	1.34		5.52	-2.88	0.31		5.40	-3.00	0.53	
L	11.51	11.08	-0.43	14.36		11.48	-0.03	1.13		11.32	-0.19	0.97	
M	14.82	12.63	-2.19	11.24		12.89	-1.93	5.45		12.90	-1.92	2.67	
N	36.11	29.76	-6.35	25.20		30.01	-6.10	17.68		30.98	-5.13	15.13	

† = Accuracy and variance of the eight mean estimates.



A



B

Figure 1. Rose plant numbering system. (A) Generalized plant showing the numbers assigned to stems and branches. (B) The numbering system used to identify leaf and leaflet positions.

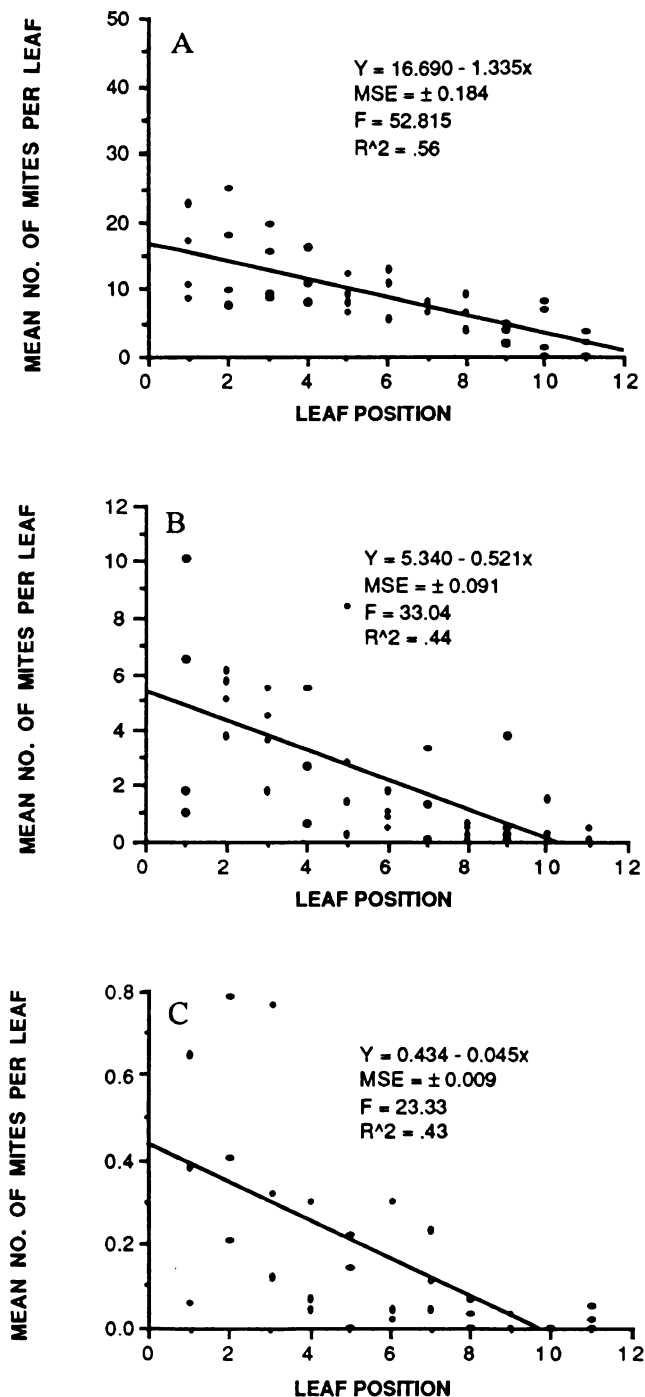


Figure 2. Mean number of spider mites per leaf in relation to leaf position. (A) High populations (plants with 4000 - 7000 mites per plant); (B) medium populations (plants with 1000 - 3000 mites per plant); (C) low populations (plants with less than 1000 mites per plant).

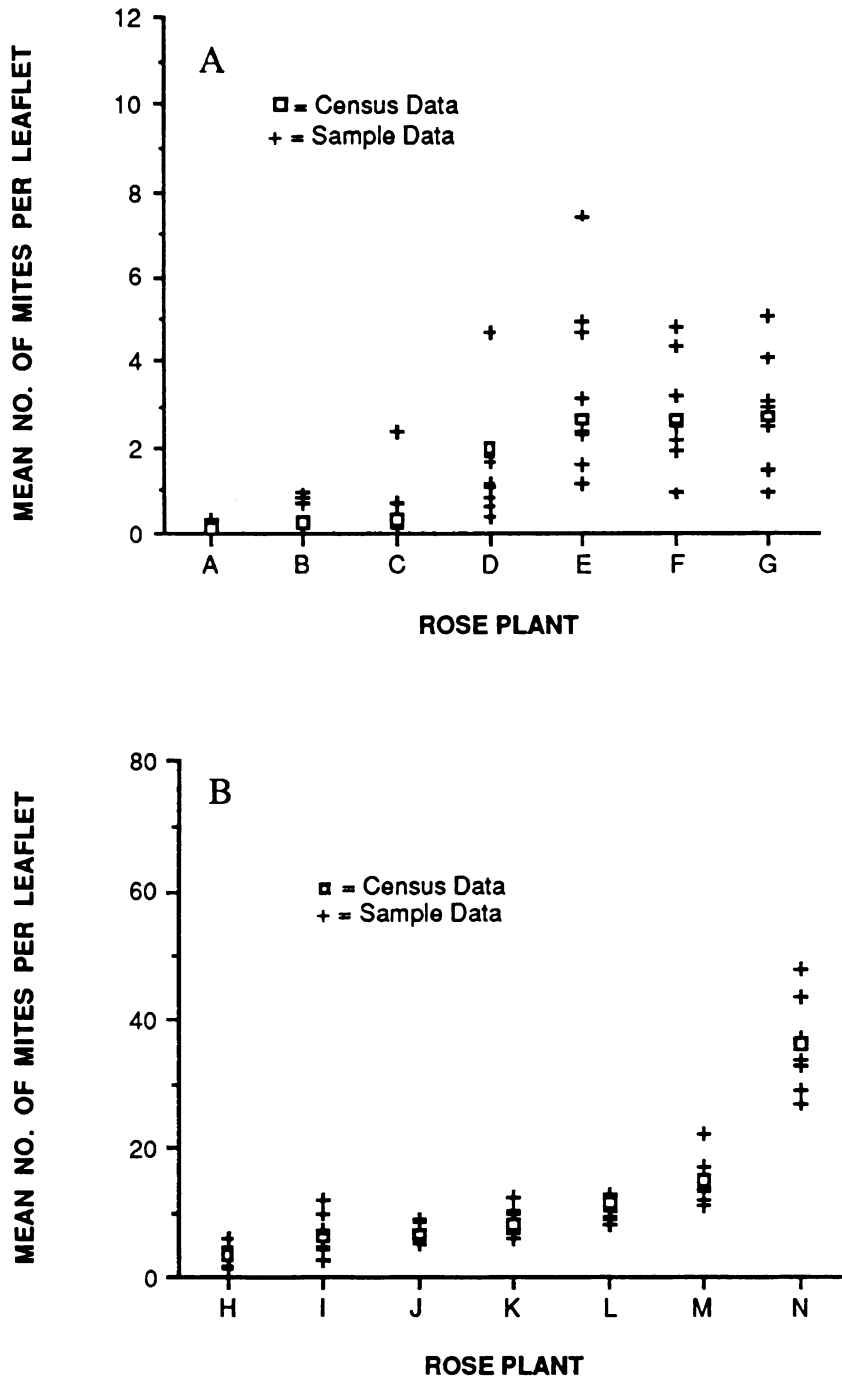


Figure 3. Actual (from absolute samples) and sample estimates (eight random samples of twenty leaflets/plant) of mean mites per leaflet for rose plants with different population densities. (A) Plant with lowest mean number of mites per leaflet; (B) plants with highest mean number of mites per leaflet.

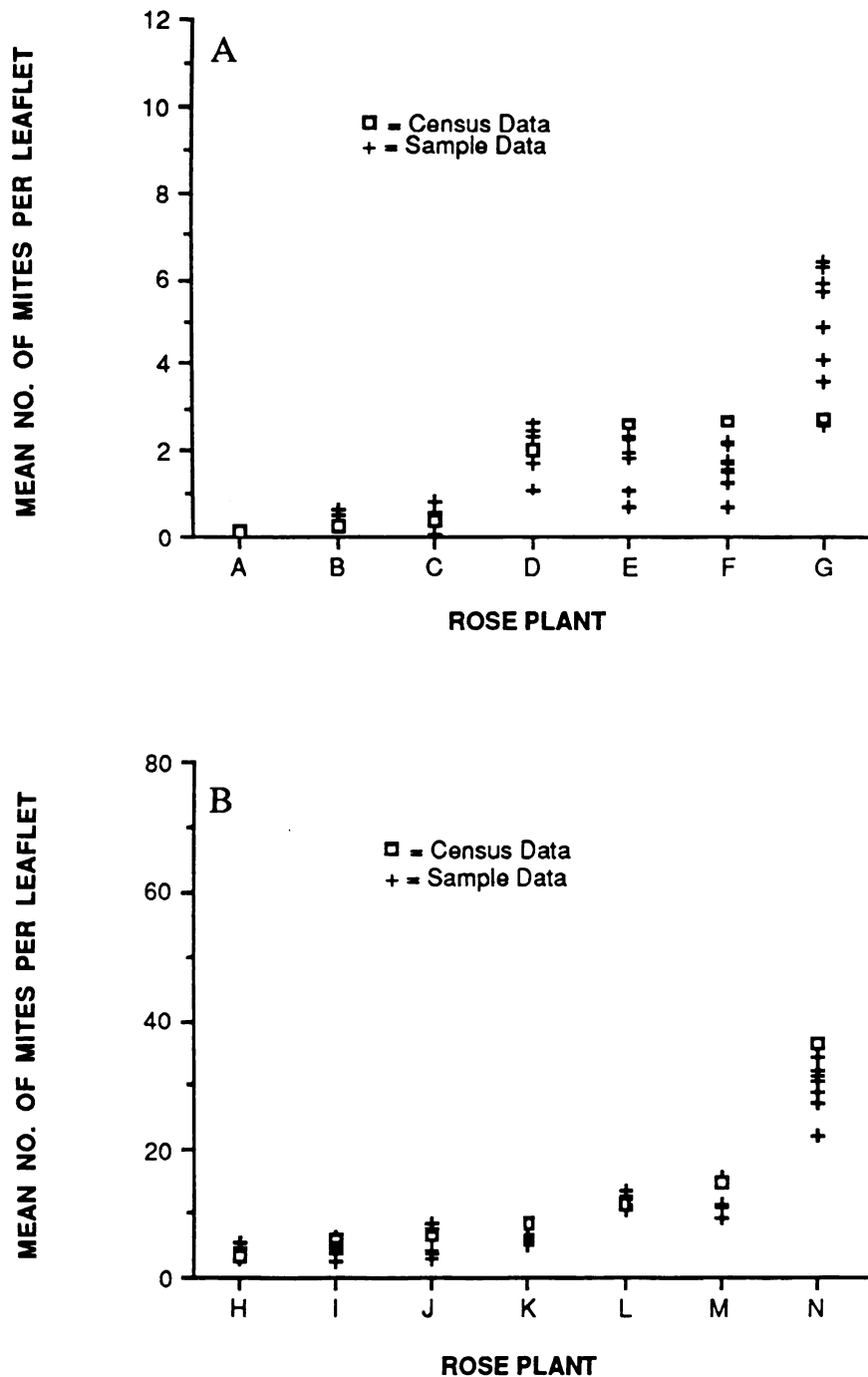


Figure 4. Actual (from absolute samples) and sample estimates (eight random samples of twenty leaflets/plant) of mean mites per leaflet rose plants. Samples of leaflets 3 and 4 from leaves 1 and 2. Sample means multiplied by conversion factor. (A) Plant with lowest mean number of mites per leaflet; (B) plants with highest mean number of mites per leaflet.

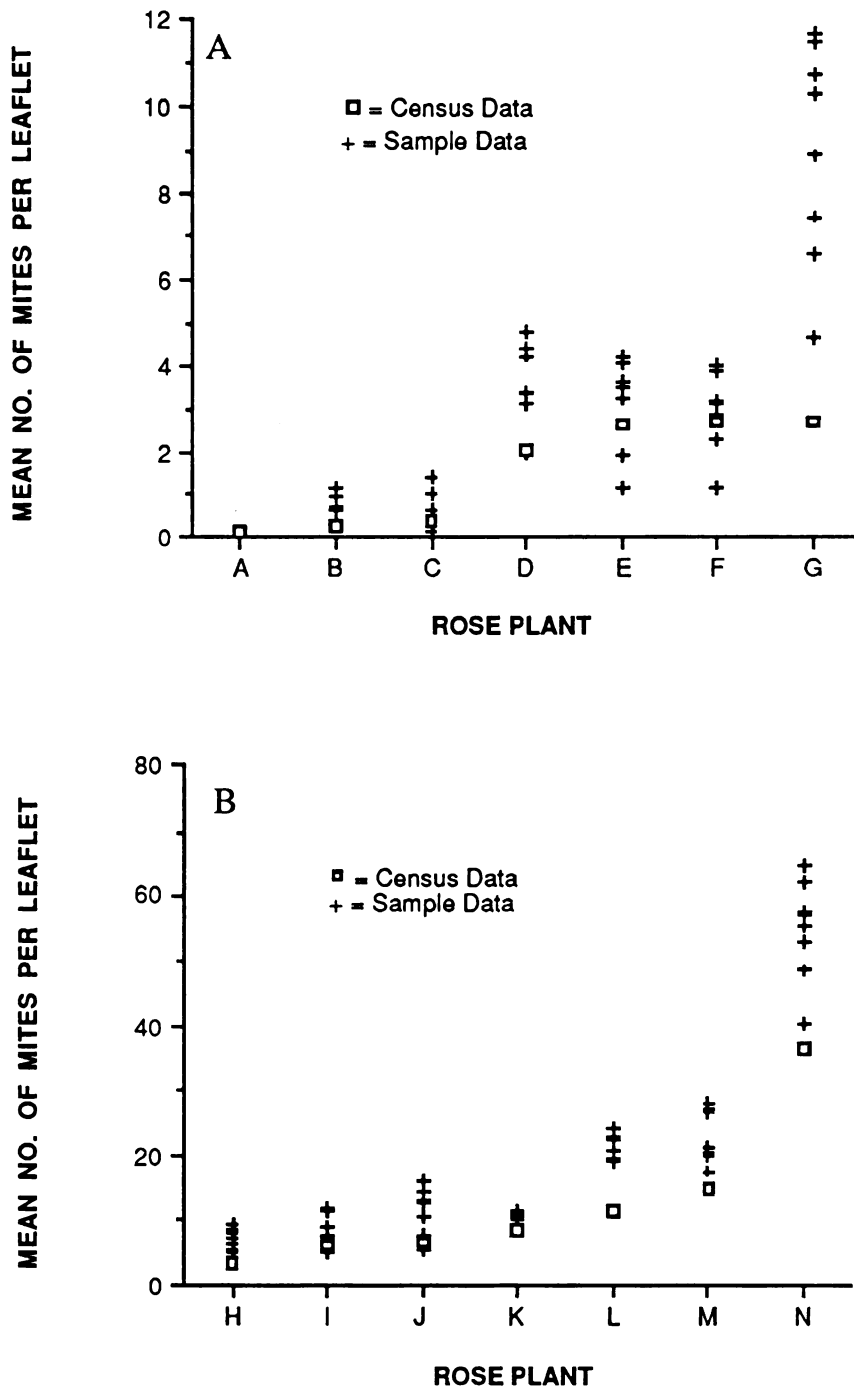


Figure 5. Actual (from absolute samples) and sample estimates (eight random samples of twenty leaflets/plant) of mean mites per leaflet for rose plants with different population densities. Samples of leaflets 3 and 4 from leaves 1 and 2. (A) Plant with lowest mean number of mites per leaflet; (B) plants with highest mean number of mites per leaflet.

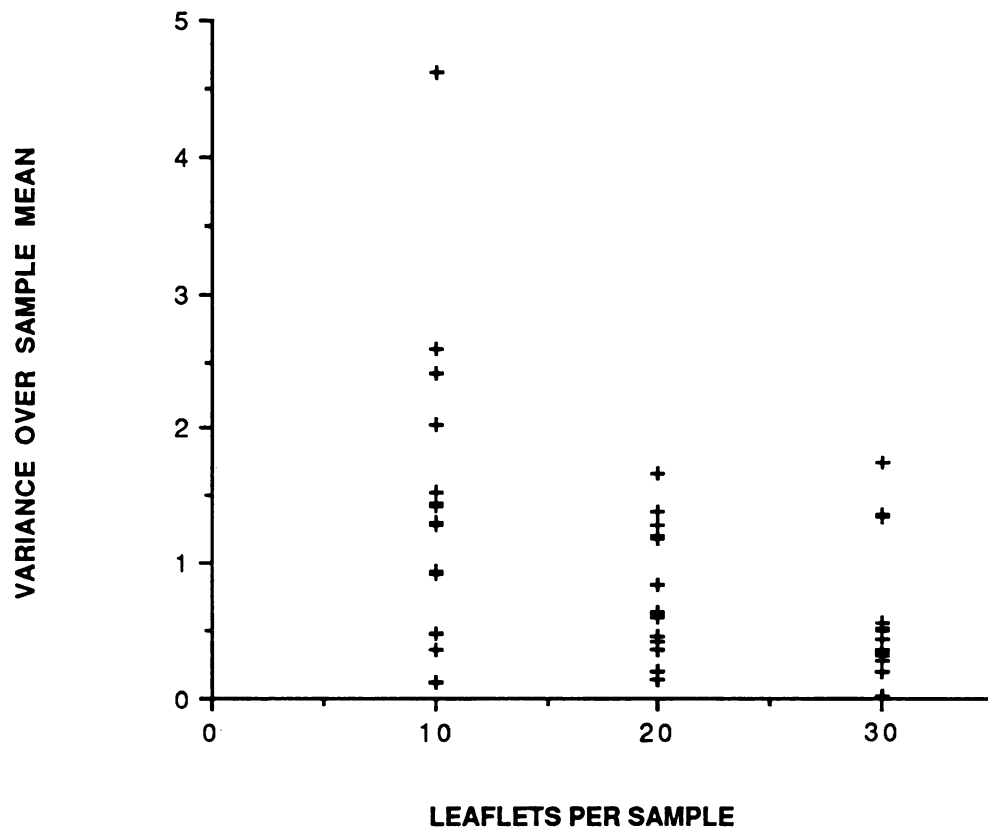


Figure 6. Average variance/sample mean for rose plants with different population densities. Eight random samples of 10, 20 and 30 leaflets per plant.

SUMMARY AND CONCLUSION

It has been suggested that with the use of a short-lived selective miticide P. persimilis may have potential value in an integrated pest management program on commercially grown greenhouse roses. The probability of successful initiation of a spider mite biocontrol program could be increased by the use of selective pesticides to suppress spider mites and other pest populations.

Standard slide-dip and 36 h residue tests were used to evaluate activity of the miticides abamectin, propargite, and dienochlor; the fungicides benomyl, dodemorph acetate, piperalin, triadimefon, and triforine; and the insecticides acephate and endosulfan against twospotted spider mites, Tetranychus urticae and the predaceous mite Phytoseiulus persimilis. Results showed that abamectin was more toxic to T. urticae than P. persimilis, while dienochlor and propargite had little or no effect on either mite. Dienochlor was shown to be toxic to spider mites in a long term experiment. In the predator feeding study, spider mites from populations initially recovering from miticide applications were fed to predator mites. This test was found to be useful in determining indirect toxicity of miticides to P. persimilis.

None of the five fungicides tested were toxic to T. urticae or P. persimilis. Both insecticides were more toxic to P. persimilis than to T. urticae. Therefore, benomyl, dodemorph acetate, piperalin, and triadimefon have potential for use in an integrated mite management system for greenhouse grown roses. Predator feeding studies need to be performed before recommendations for use can be made. Acephate and endosulfan

should be avoided because of their toxicity to P. persimilis. A number of biological control and cultural control methods need to be looked at as control of aphids and thrips.

Biocontrol of spider mites requires more intensive monitoring of mite populations than chemical control. A quantitative approach to biological control of spider mites using predaceous mites requires estimating population densities. Our findings show that spider mites are most heavily distributed among the oldest leaves therefore, sampling schemes designed to detect mites at low density should be aimed at the base of rose plants where the oldest leaves are found. For research purposes, random samples of 20 leaflets per plant will provide an acceptable estimate of spider mite population density.



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