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Project Title: Validation and Demonstration of Predacious Mite Releases for Management of Spider Mites in Cotton

Summary:

Predatory mite releases were evaluated for spider mite management in cotton. Factors that could influence the effectiveness of predatory mite releases were examined at UC experimental stations. These factors included: (1) predaceous mite release rates, (2) release technique, (3) compatibility of predaceous mites with naturally occurring insect predators, (4) commercial source of predatory mites, and (5) release time of day. Larger scale releases of predatory mites, primarily in grower fields, have been performed at five different sites that are located throughout California's Central Valley. Arthropod populations were monitored from the time immediately before the predaceous mite releases were performed to September using leaf and sweep sampling techniques. Preliminary analysis of the experiments conducted during 1997 indicates that predaceous mite releases were unable to increase predaceous mite numbers in open plot experiments during the early season. However, a cage experiment using higher release rates demonstrated that releases can greatly increase predatory mite populations, these predator populations then can further increase in abundance through recruitment, and they can suppress spider mite populations. Other open plot and cage experiments showed that hemipteran predators can have a negative impact on predatory mite persistence but can substantially improve spider mite suppression. Other tasks completed included, the evaluation of a mechanical release device, the evaluation of predatory mites sold by commercial insectaries, and extension efforts to increase the grower awareness of predaceous mite releases for spider mite control.

Results and Discussion:

1. To Evaluate Factors that Could Potentially Influence the Effectiveness of Predatory Mite Releases in Cotton.

The factors that we are evaluating that could potentially influence the efficacy of predatory mite releases include (A) release rates, (B) release technique, (C) compatibility of predaceous mites with naturally occurring insect predators, (D) commercial source of predatory mites, (E) and release time of day. Due to the large number of factors tested, experiments were performed in small replicated field plots (approx. 0.08 ac) and in replicated fine-mesh exclusion cages located at several UC experimental field stations. In the replicated plot experiments, arthropod populations (including spider mites, predatory mites, thrips, and predaceous hemipterans) were monitored from the time immediately before predaceous mite releases to September (when irrigation stopped) using leaf and sweep sampling techniques. Leaf samples were collected every two weeks and sweep samples were collected every four weeks. Leaf sampling involved randomly selecting 25 plants per plot and collecting the mainstem leaf located five nodes below the top of the plant or the most basal mainstem true leaf when plants had fewer than 6 nodes. Sweep samples were performed by taking 20 sweeps in each plot for June and early July and 10 sweeps in each plot for late July and August when predatory insects became more abundant. All arthropods were removed from the leaf material using a leaf washing method. All arthropods were quantified using a dissecting stereomicroscope. During October, cotton seed and lint were harvested from each plot to compare yields between treatments. The specific details of each of these experiments is discussed below.

A. Release Rate - Replicated Plot Experiment
In this experiment we compared the arthropod dynamics in low (2000 mites per acre), moderate (15,000 mites per acre), and high (100,000 mites per acre) release rate treatments with a no-release control. Releases were performed by hand from 7 AM to 9 AM on 6/5/97 at the UC Cotton Research Station (Shafter, CA). Western predatory mites (*Galendromus occidentalis*) were purchased from Biotactics Inc. (Riverside, CA). Plots were 90 ft. by 40 ft. (0.083 ac) and were assigned to treatments using a complete randomized design. Each treatment had seven replicates (n=7).

Preliminary results indicate that predaceous mite releases were unsuccessful in significantly increasing predatory mite numbers above naturally occurring background densities (Figure 1) in early season cotton (15 May through 30 June). Without augmentation of predatory mite numbers, we did not expect early season spider mite abundances to be influenced by the releases, and indeed they were not (Figure 2). However, spider mite and predatory mite samples taken during the mid and late season are still being processed, and will be described in future reports.

B. High Release Rate - Cage Experiment

To further examine how release rate affects spider mite suppression we conducted a cage experiment that was designed to quantify (1) the impact of *G. occidentalis* mite releases on *Tetranychus urticae* abundance under high predator and prey densities, and (2) the impact of *Frankliniella occidentalis* on spider mite and predatory mite abundance. The experiment was conducted from 31 May to 25 June, 1997 in a one acre experimental planting of *Gossypium hirsutum* cv. "Maxxa" at the UC Davis Plant Pathology Fieldhouse, Davis, CA. The experimental unit was a single plant. On 31 May, plants were randomly selected and thoroughly sprayed with an insecticidal soap (Safer® Inc.) at the labeled rate (20 mL soap/ L H_2O) to reduce resident populations of western flower thrips and other insects. Plants were then enclosed in cylindrical cages composed of a plastic PVC base and No-Thrips® mesh (Greentek® Inc.; pore size ca. 150 µm; cage dimensions: height 45 cm, diameter 30 cm).

On 1 June, plants were randomly assigned to one of four treatments, each replicated 14 times: (1) spider mites alone (*T. urticae*), (2) spider mites plus western predatory mites, (3) spider mites plus western flower thrips, and (4) spider mites, predatory mites, and western flower thrips. Spider mites were added to all replicates by placing two spider mite infested seedlings from a laboratory culture onto each plant; this delivered 471 ± 45 (mean ± 1 SE) spider mites to each replicate. Approximately 10 thrips adults were added to each replicate in the thrips treatments. Western predatory mites were purchased from Biotactics Inc. and were released within two days of receipt. On 1 June, approximately 10 adult predatory mites were added to each replicate of the predatory mite treatments. On 7 June, a second release of 68 ± 16 predator mites in a corn-cob grit carrier was added to each replicate in the predatory mite treatments to adjust the predator to prey ratio. This second release was performed because we originally underestimated the number of spider mites added to the plants. On 15 June, 9 out of the 14 replicates from each treatment were sampled destructively (census 1). The remaining five replicates of each treatment were collected on 25 June (census 2).

The outcome of this experiment differs from that observed in the replicated small plot release rate experiment. First, using high release rates (>60 predatory mites per plant), we were able to greatly increase the predatory mite population compared to the naturally occurring background population (Figure 3). Second, released predatory mite populations increased at least 60% above the initial release rates under conditions of high spider mite availability, low predation, and a sufficiently long duration (this result was only observed in census 2, 18 days following predatory mite release). This result is important in that it demonstrates that *G. occidentalis* populations can grow in cotton. Also, the predatory mite releases reduced spider mite abundance to 38% of the level reached in the controls (Figure 4). However, spider mite populations continued to build from day 8 to day 18 in the predatory mite release treatments, suggesting that predatory mites may be capable of suppressing but not controlling spider mite populations in cotton in the short term. Predatory mites may have been capable of adequately controlling spider mites had the predator to prey ratio been higher or had the experimental duration been longer. Western flower thrips manipulations were somewhat successful during the first portion of the experiment (census 1 - thrips treatment: 82 per plant, control 38 per plant) but had no detectable effect on predatory mite or spider mite abundance.

C. Release Technique - Mechanical vs. Hand Release.
In this experiment we compared the arthropod dynamics in treatments where predatory mites were released using (i) the Giles mechanical release device, (ii) the Carter release device (a new release device recently developed by the USDA engineers at UC Cotton Research Station including L. Carter, J. Chesson, and V. Piner), and (iii) the hand release technique. Release plots were also compared with no release controls. Releases were performed from 5:30 AM to 10:30 AM on 6/3/97 at the Kearney Agricultural Center (Parlier, CA). Western predatory mites were purchased from Biotactics Inc. Plots were 80 ft. by 40 ft. (0.074 ac) and were assigned to treatments using a complete randomized design. The Giles release device and the hand release treatments had seven replicates (n=7) while the Carter release device and the no-release treatments had six replicates (n=6). We included the new Carter release device to this year's research because it will provide a useful comparison to the Giles release device and the hand release technique. It is different than the Giles release device in several ways: the flow rate of the carrier-corn grit mixture varies with tractor speed, making the application rate constant; the application rates are also much lower than the Giles device (about one-sixth of Giles at normal tractor speeds). This eliminates the need to dilute the predatory mite-carrier mixture received from the insectary. Lastly, the Carter device keeps the predatory mite-carrier mixture cooler than the Giles device (about 40 vs. 55 °F). However, the Carter device needs further development before it can be attached to a tractor toolbar and withstand the harsh conditions encountered by farm equipment in cotton. Arthropod samples from this experiment are being processed and analyzed at this time.

D. Compatibility of Spider Mite Predators - Replicated Open Plot Experiment.
This experiment evaluated the compatibility of predaceous mites with naturally occurring insect predators. We used a complete factorial design to conduct this experiment: (i) pre-release application of acephate (Orthene®) and release of predatory mites, (ii) pre-release application of acephate and no release, (iii) release of predatory mites only, (iv) no manipulation control. Acephate was sprayed at 4.0 oz ai/ ac on 5/21/97 in an attempt to reduce naturally occurring populations of *Geocoris* spp., *Orius tristicolor*, *F. occidentalis*, and other arthropod predators. One week after the acephate application (5/30/97), a leaf disk bioassay using leaf material from sprayed and unsprayed plots was done to determine if residual levels of acephate would cause predatory mite mortality. Chi-square analysis of the bioassay results showed that mortality was the same for mites on leaf disks from sprayed and unsprayed plots ($P > 0.3$). Releases were performed by hand from 7 AM to 9 AM on 6/5/97 at the UC Cotton Research Station. Western predatory mites (*G. occidentalis*) were purchased from Biotactics Inc. Plots were 90 ft. by 40 ft. (0.083 ac) and

were assigned to treatments using a complete randomized design. Each treatment was replicated seven times.

Leaf and sweep samples showed that generalist insect predators (especially juveniles) were reduced in plots sprayed with acephate two weeks following application (Figure 5, 6C). The acephate application caused a large increase in spider mite abundance; spider mites in sprayed plots reached densities that were six times higher than unsprayed plots (Figure 6A). This increase in spider mite populations was most likely due to the decrease of generalist insect predation. Predatory mite numbers also increased in plots sprayed with acephate. The increase in predatory mites could have occurred for two reasons: (1) release from predation by generalist insect predators, (2) increase in prey availability of spider mites. We believe that the predatory mite increase was more influenced by the removal of generalist predators than the increase of spider mites because hemipteran predators can reduce predatory mite persistence (see below), and densities of spider mites in unsprayed plots were probably too high for predatory mites to be food limited (>10 spider mites per leaf, a predatory mite to spider mite ratio of 1:180).

E. Compatibility of Spider Mite Predators - Cage Experiment.

To further explore this question, we conducted a field cage experiment that was designed to quantify the individual impact of insect predators *Geocoris*, *O. tristicolor*, and *F. occidentalis* with the simultaneous presence of *G. occidentalis* on spider mite and predatory mite abundance. The experiment was conducted from 14 August to 30 August, 1997 in a 0.5 ac experimental planting of *Gossypium hirsutum* cv. "Maxxa" at the UC Davis Agronomy Field Plots, Davis, CA. The experimental unit was a single mainstem leaf located at the fifth node from the plant terminal. From 14 -15 August, plants were randomly selected and the fifth node mainstem leaf was thoroughly brushed to reduce resident populations of western flower thrips and other insects. Leaves were then enclosed in square cages composed of No-Thrips® mesh (Greentek® Inc).

From 21-22 August, cages were re-opened and brushed a second time to remove insects that hatched from egg stages embedded in the leaf tissue such as *F. occidentalis* or *O. tristicolor*. We waited 7 days before re-opening cages in anticipation that this would be sufficient time for all eggs to hatch. This removal technique was effective for *O. tristicolor* but only partially effective for *F. occidentalis*. Once brushed, caged leaves were randomly allocated to one of five treatments, each replicated eighteen times: (1) spider mites alone (*T. urticae*, 147 ± 15 per leaf), (2) spider mites plus predaceous mites (*G. occidentalis*, 10.6 ± 0.7 per leaf), (3) spider mites, predaceous mites, and *O. tristicolor* (4 first to third instar nymphs per leaf), (4) spider mites, predaceous mites, and *Geocoris* spp. (1 first to third instar nymph per leaf), and (5) spider mites, predaceous mites, and *F. occidentalis* (ca. 12 adults per leaf) (densities were chosen to reflect natural densities of predators in cotton when spider mite densities are high; Rosenheim, unpublished data). The duration of this experiment was 7 days (approximately the generation time for the spider mites, predaceous mites, and thrips). From 28-30 August, replicates were collected, cages were opened, and all herbivorous and predatory arthropods were quantified while still on the leaf in the laboratory with the aid of a dissecting stereomicroscope.

This experiment demonstrated that generalist hemipteran predators, such as *Geocoris* and *O. tristicolor*, can have negative impacts on *G. occidentalis* persistence. The addition of *Geocoris* and *O. tristicolor* reduced predatory mite densities to 23% and 0%, respectively, of the density observed in the predatory mites alone treatment (Figure 7A). Both of these hemipteran predators are very common in cotton and are important naturally occurring spider mite predators. Though simultaneous addition of predatory mites and hemipteran predators had negative effects on predatory mite persistence, it did not interfere with spider mite suppression (Figure 7B), at least over the short-term studied. Indeed, the best spider mite suppression in experiment 2 was in the *O. tristicolor* + predatory mite treatment.

F. Predatory Mite Source Experiment.
This experiment compared arthropods in release plots where the western predatory mites came from two commercial sources, Biotactics Inc. and Visalia Insectary Inc. (Visalia, CA). A third commercial insectary was excluded from the experiment because they mistakenly had reared and sold us the wrong species of phytoseiid (*Neoseiulus californicus*) and delivered lower that expected numbers of mobile predatory mites (Figure 8 - Insectary C). In contrast, the other two insectaries delivered numbers of mites that were close to or above expected numbers (Figure 8 - Insectary A & B). Release plots were also being compared with no release controls. Releases were performed by hand and were done from 5:30 AM to 10:30 AM on 6/3/97 at the Kearney Agricultural Center. Plots were 80 ft. by 40 ft. (0.074 ac) and were assigned to treatments using a complete randomized design. The Biotactics Inc. and Visalia Insectary Inc. treatments had seven replicates (n=7) while the no-release control had six replicates (n=6). Arthropod samples are being processed and analyzed for this experiment at this time.

G. Release Time of Day and Transfer Efficiency Experiment.
The release time of day experiment had two main objectives: (1) to determine if mortality of predatory mites was influenced by conducting the releases during the morning versus during the afternoon and (2) to estimate the absolute survivorship or transfer efficiency of predaceous mites (i.e. # mites found on plants / # mites released). The experiment had three treatments: early morning releases, late morning releases, and no release controls. This experiment was short-term and involved releasing predaceous mites at a very high rate (670,000 mites per acre) in small plots (52 ft. x 1 row); rigorously sampling the plots (20 whole plants per plot) on the day following the release; and rigorously processing the leaf material (machine and hand washing leaf samples). Morning releases were performed from 6 AM to 6:45 AM and afternoon releases were performed from 3 PM to 3:45 PMon 6/26/97 at the UC Davis Agronomy Field Plots. Soil temperatures around the cotton plants was much warmer during the afternoon compared to the morning (morning 15°C,

afternoon 51°C) indicating that predatory mite mortality could be different for the two release times. Western predatory mites were purchased from Biotactics Inc. Plots were assigned to treatments using a complete randomized design. Release treatments had ten replicates (n=10) and the control treatment had eight replicates (n=8). Arthropod samples are being processed and analyzed for this experiment at this time.

2. To Evaluate the Use of Inoculative Releases of Predaceous Mites for Management of Spider Mite Pests in Grower Cotton Fields.

Spider mite management with western predatory mites was examined in five grower cotton fields. Each field had a no-release control plot, and one or two release plots (5000 predatory mites per acre): (i) an early release plot (May 9), where predatory mites were released when the plants were between 1-6 nodes; and/or, (ii) a later release plot (May 30 - July 29), where predatory mites were released when the plants had greater than 7 nodes. We monitored a total of 4 conventional fields and a total of 6 organic fields from the beginning of the cotton field season (mid April) until the end of July. Predatory mites were only released into plots in fields that had reached a minimum 20% spider mite infestation level based on presence/absence sampling. Since not all of the fields we monitored met the minimum 20% spider mite density requirement, we completed a total of 1 early release and 5 late releases (see Table 1). The low number of early releases came about for two reasons; there were few spider mites during the first part of the early season, and warm spring conditions quickly moved cotton plants out of the early release developmental stages (2-6 nodes). We did not monitor as many organic fields as anticipated because we encountered difficulty in finding cotton farmers that were growing organic cotton. We contacted at least five cotton growers who grew organic cotton in the past but were now strictly growing cotton conventionally. To avoid having acaricides sprayed on release plots in conventionally managed fields, we asked conventional growers to withhold acaricide applications in experimental plots unless spider mite densities reached damaging levels. This approach generally kept plots from being sprayed; acaricides were applied to only one of the grower field release plots. To enable growers to treat our plots differently than the rest of the field and to reduce the amount of economic risk associated with not using acaricides, we reduced the size of the release and control plots (from 5 acres [1996] to 1-2 acres [depending on the field site]), and reduced the separation between plots (from 200 ft [1996] to 25 - 200 ft. [depending on the field site]). Arthropod samples are being processed and analyzed for grower field releases at this time.

3. To Develop and Demonstrate Quality Control Techniques for Mechanical Releases of Predaceous Mites in Cotton.

To evaluate the Giles mechanical release in a large scale release setting, half of the grower field releases were done using this device. Warren Sargent from Ag Attack® (Visalia, CA), a cooperator on this research project who is commercially producing the mechanical release device, made several modifications to the original Giles design. These modifications include: (1) the distributors can now hold 'blue ice' and are better insulated to keep predatory mites chilled and immobile, (2) the predatory mite - vermiculite mixture is contained in a movable plastic 3-gallon container that can be purchased directly from an insectary, (3) the 12 rpm 12 Vdc permanent magnet gearmotor was replaced with a 4.5 rpm 12 Vdc gearmotor in order to decrease the flow rate and make the distributor more appropriate for the large size of most cotton fields, (4) the air compressor was replaced with a blower which is able to withstand the large amounts of dust typically encountered in cotton fields, (5) wind guards were developed that attach to the sides of each release device to improve the placement accuracy of the mixture onto the cotton, and (6) sliding plates replaced rotating plates to decrease flow rates (reduce the frequency of refilling containers which is especially important in cotton where fields are large) and to reduce costs needed to construct mechanical release devices. These modifications resulted from observations made in the field and ongoing feedback between the cooperators and Ramy Colfer. These modifications make the release devices more suitable for the harsh conditions encountered in cotton. Furthermore, Warren Sargent has made a less expensive model of the mechanical release device (about 60% of the older model's cost) that has replaced the insulated sheet metal container (that holds the movable plastic 3-gallon container) with a container made of cardboard and styrofoam. However, we have not tested this new device in the field.

Quality control measures were implemented to verify that we received the western predatory mite in the correct concentration. As discussed above, we began the season by buying predatory mites from 3 different insectaries (Biotactics Inc., Visalia Insectary Inc., and a third insectary). The third insectary was not used because we repeatedly received the wrong species of phytoseiid mite (25 randomly selected predatory mites were identified to be *Neoseiulus californicus*) and the number of mobile predatory mites was on average 34% of the expected (Figure 8 - Insectary C). We contacted this insectary and informed them of our findings. This company subsequently moved out of the insectary business. A subsample of 25 predatory mites from the other two insectaries were slide mounted and identified as *G. occidentalis*. The number of mobile predatory mites from the other insectaries were on average 141% and 87% of the expected (See Figure 8 - Insectary A & B).

We also measured the number of mobile mites recovered from the predatory mite – vermiculite mixture that was collected from the mechanical release devices during the field releases. We found that a higher concentration of predatory mites was released by the distributors during the first half of the release in comparison to the predatory mite concentration found in the second half (Figure 9). Application rates of predatory mites were on average 10% above the expected application rate. These results are very similar to the results we received from the research we conducted in 1996.

Western predatory mites from insectaries are always sold with spider mites included as food (they generally use *Tetranychus pacificus* or *T. urticae*). Some growers were concerned with adding spider mites to their fields during the release of the predaceous mites. To eliminate this concern, we held the carrier + predatory mite mixture at room temperature for approximately 1 to 2 days until no spider mites were left in the carrier before these mites were released into grower cotton fields. To determine what impact holding predaceous mites and releasing them via the Giles release device had on predatory mite viability, we ran a bioassay. We randomly selected predaceous mites (n = 30) that had been held in the carrier at room temperature for 2 days and then mechanically released during a grower field release. These mites were taken back to the lab and placed on leaf disks with excess spider mite prey. Leaf disks were surrounded by water to prevent escape. After feeding on spider mites for 1 day, females began to lay eggs. On day 2, 93% of the adult females laid eggs (Figure 10), producing an average of 2.5 eggs per day which is within the range of previously published fecundities for *G. occidentalis*. The 26% of the mites missing from the leaf disks after two days is typical for leaf disk assays, even when using predatory mites that have not been exposed to these harsh treatments (R. G. Colfer, pers. observ.). These data indicate that the released mites were viable and capable of reproduction if sufficient food was available.

4. Promote Increased Awareness of Inoculative Releases of Predatory Mites for Mite Management in Cotton to Pest Control Advisors, Growers, and UC Cooperative Extension Personnel.

We have taken several approaches to promoting greater awareness of predatory mite releases to manage spider mites in cotton. First, we have discussed the purpose of our research with the growers that we are working with and have had them participate in releases. We have kept them updated on spider mite infestation levels in the fields where we did releases. Second, we contacted nearly every UC cooperative extension farm advisor in the San Joaquin Valley that was working in cotton and discussed with them the purpose of our research. We have worked closely with farm advisors Bill Weir, Dan Munk, and James Brazzle in locating growers interested in participating in our research. Ramy Colfer also attended two BIFS (Biologically Integrated Farming Systems; a group organized by Pete Goodell and Jeff Mitchell, West Side, CA) meetings and discussed our research with growers, farm advisors, and UC extension personnel involved in the group.

Lastly, Ramy Colfer presented the objectives and preliminary results of our research to a group of growers, farm advisors, UC extension personnel, journalists, and researchers at the June and September field days at the UC Cotton Research Station, at the 1998 Beltwide Cotton Conference in San Diego, and at the 1997 Pest Science Conference at UC Davis.

Table 1. Summary of predatory mite release sites, dates and initial percent of leaves infested with spider mites.

Site #	County	Early Release (ER) Date	Later Release (LR) Date	% plants infested (ER, LR)
Conventional 1	Merced	5/9/97	6/6/97	45% (ER), 55% (LR)
Conventional 2	Kern	none	5/30/97	20% (LR)
Conventional 3	Yolo	none	6/8/97	20% (LR)
Organic 1	Kern	none	7/29/97	30% (LR)
Organic 2	Kern	none	7/29/97	25% (LR)

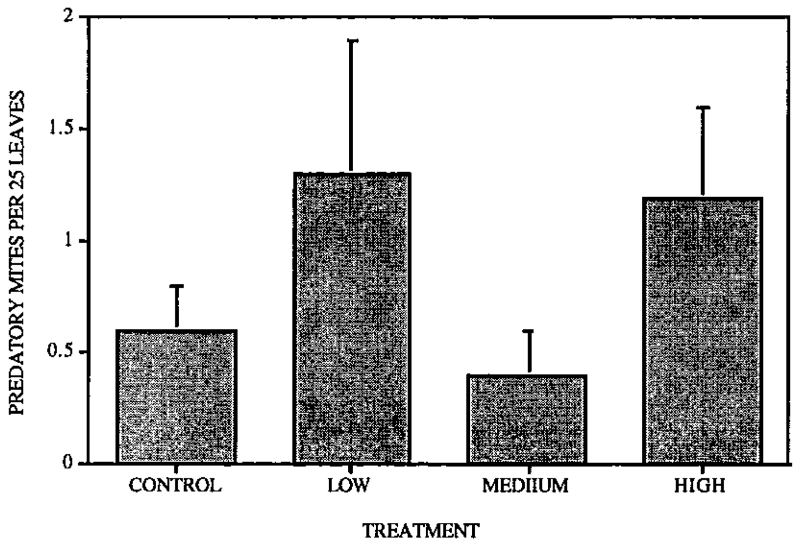

Figure 1 - Early season average predatory mite abundance in release rate treatments (low = 2000/ac; medium = 15,000/ac; high = 100,000/ac).

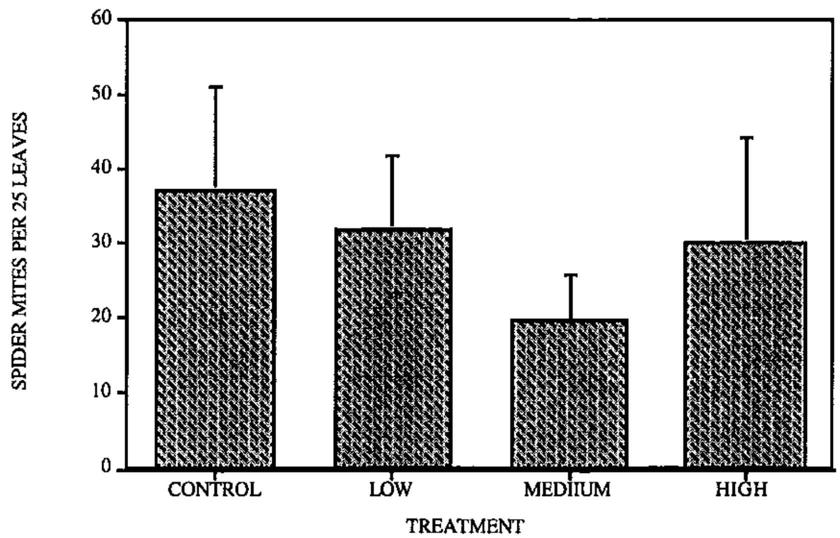

Figure 2- Early season average spider mite abundance in release rate treatments (low = 2000/ac; medium = 15,000/ac; high = 100,000/ac).

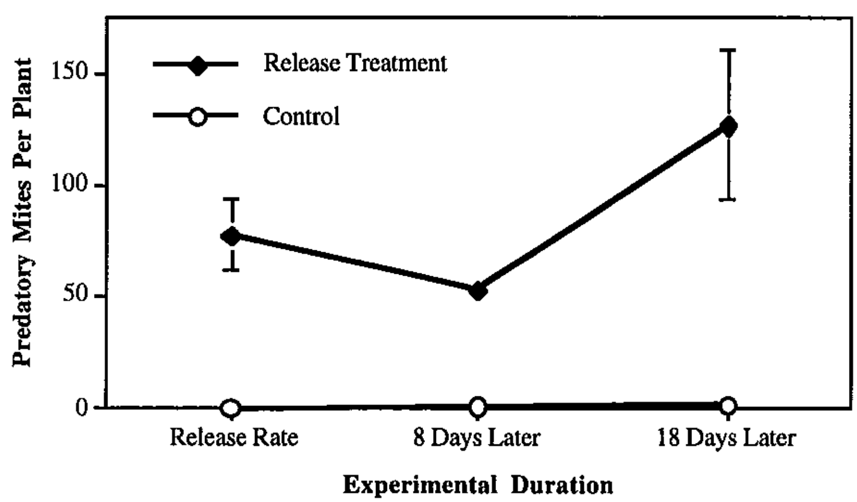

Figure 3 - Releases increased predatory mite numbers far above numbers occurring on control plants.

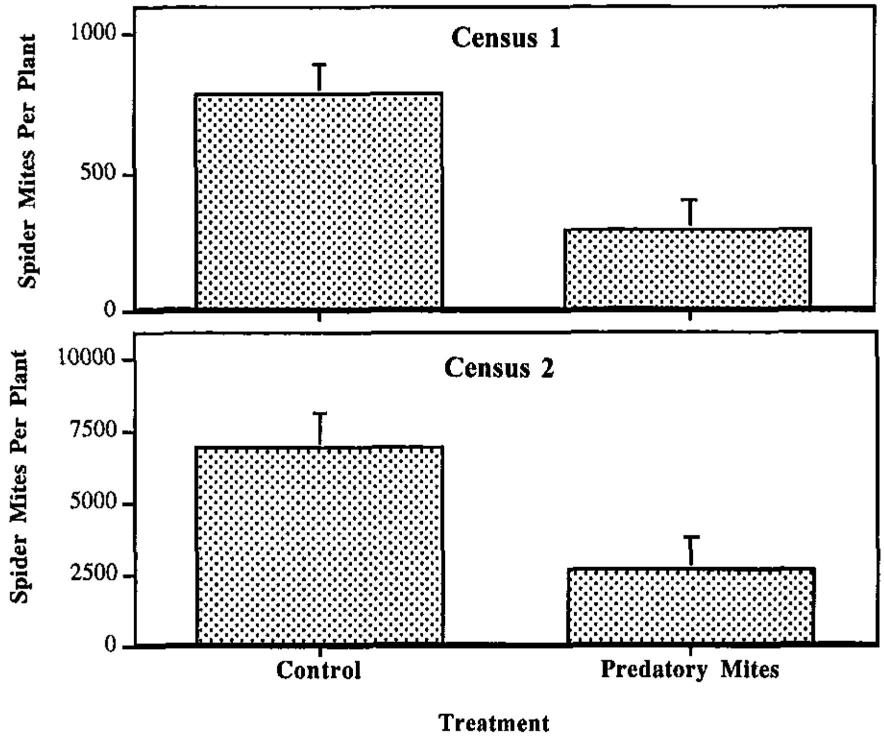

Figure 4 - Releases of *G. occidentalis* reduced spider mite densities to less than 40% of the densities in the control plants at both day 8 and day 18 of experiment 1. Spider mite density continued to increase in all treatments from day 8 to day 18 despite predatory mite releases.

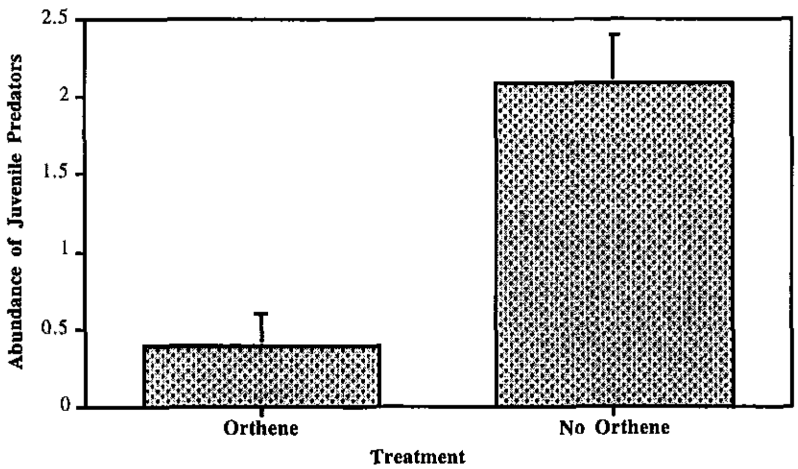

Figure 5 - Number of juvenile spider mite predators (excluding western flower thrips) in 20 sweep net samples per plot in sprayed and unsprayed treatments two weeks following the Orthene® application.

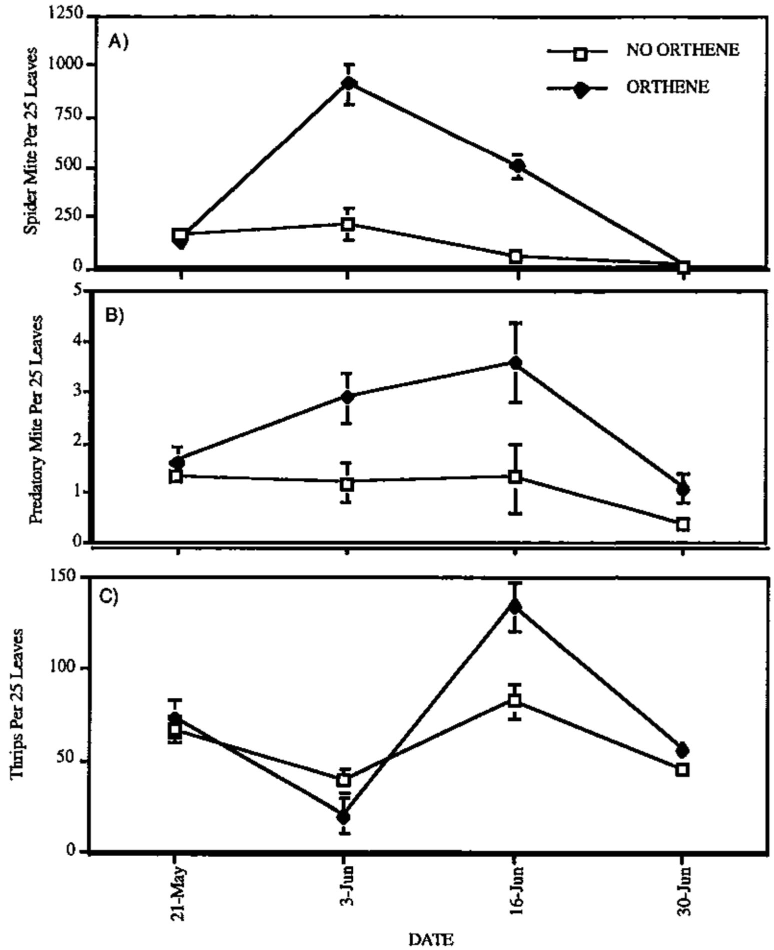

Figure 6 - The effect of Orthene® applications on (A) spider mite, (B) predatory mite, and (C) thrips populations dynamics during the early season.

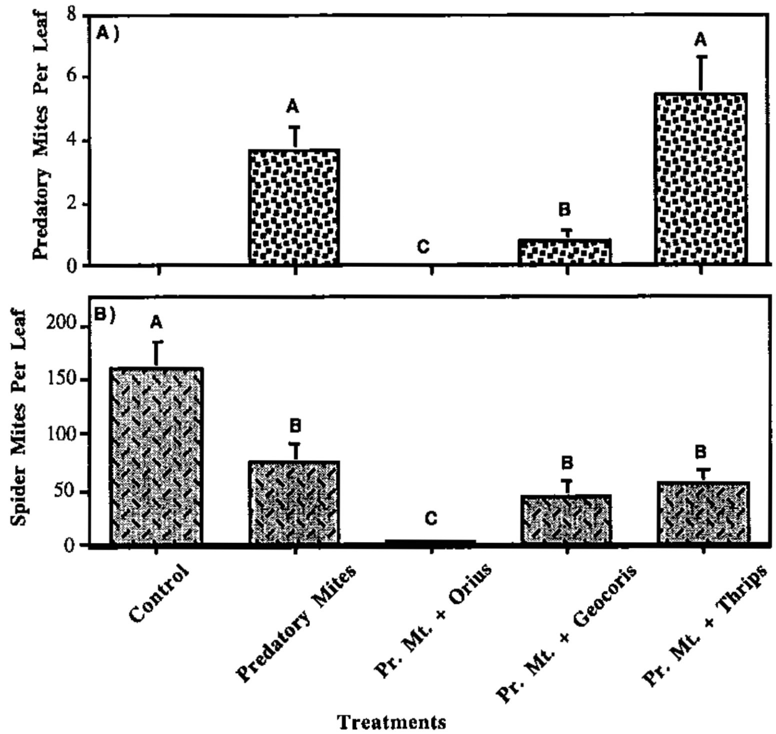

Figure 7 - Influence of predatory mite and generalist insect predator additions on (a) western predatory mite abundance per leaf and (b) twospotted spider mite *T. urticae* abundance per leaf. Differing letters above graph columns represent significant differences between treatments at $\alpha = 0.05$.

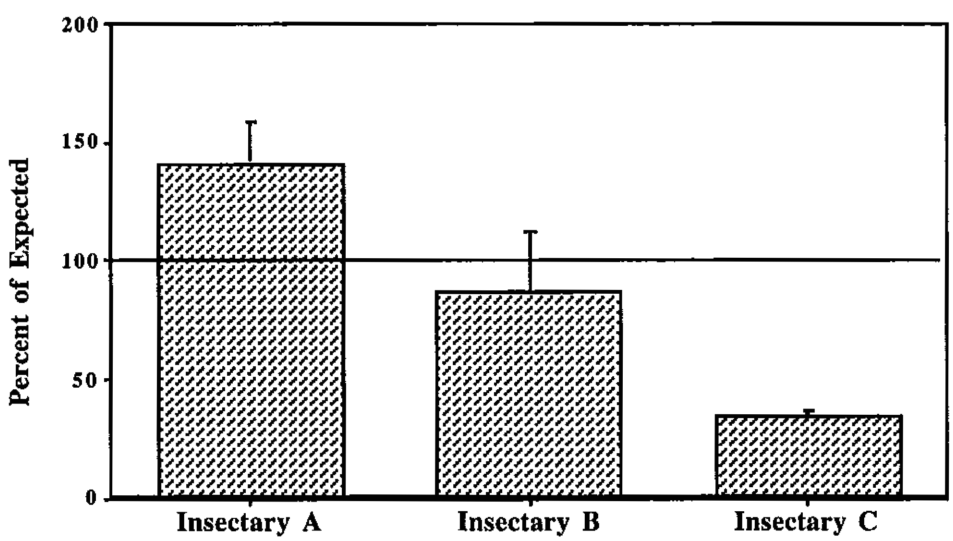

Figure 8 - Percent of the expected mobile predatory mites (means ± s.e.) from three different California insectaries.

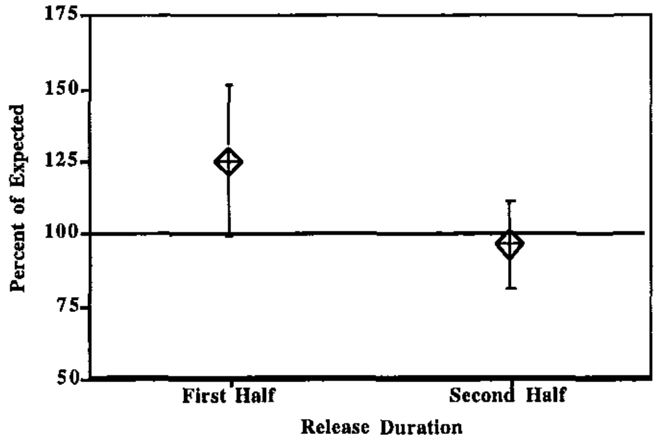

Figure 9 - Percent of mobile predatory mites collected during predatory mite releases from the Giles mechanical release device. Diamonds represent means and bars represent standard errors.

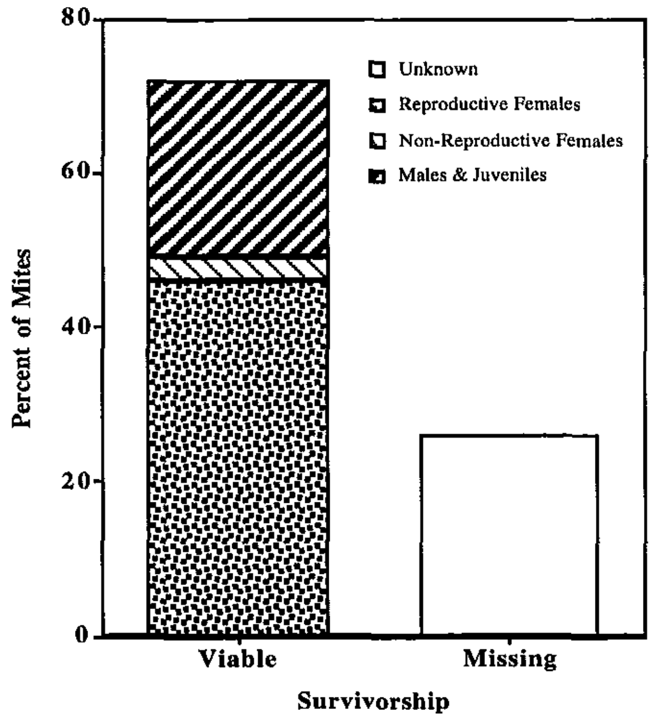

Figure 10 - Percent of predatory mites that survived and reproduced in a leaf disk bioassay. Previous to the assay, mites were held in corn grit carrier at room temperature until their spider mite food was gone and were then released via the Giles release device.