

Final Report to the California Department of Pesticide Regulation

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Contract Manager: Lyndon Hawkins

Title: Development of a Reduced-Risk Pest Control Program in Ornamental Horticulture

Principal Investigators:

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Executive Summary:

The goal of this program was to explore and develop reduced-risk methods for controlling pests in ornamental greenhouse systems. To this end, we evaluated the potential for the combined use of three reduced-risk control agents, *Beauveria bassiana*, an entomopathogenic fungus (Botanigard, Mycotech Corp., Butte MT), Cinnamic aldehyde (Cinnacure, ProGuard Inc., Suisun CA) and two natural enemy species for control of the Melon aphid on greenhouse fresh cut lilies.

Our project has successfully evaluated the combined use of *B. bassiana* and parasitoids for aphid control (objective 1). We also assessed the compatibility between the reduced-risk pesticide cinnamic aldehyde, *B. bassiana*, and aphid parasitoids (objective 2). However, completion of the greenhouse demonstration trials (objective 3) was confounded by several factors. First communication errors between growers and applicators resulted in the first two evaluations being 'over-sprayed' with conventional pesticides. More importantly, changes in pest management practices have eliminated the interest in reduced-risk controls. Bulb producers in the Netherlands have begun dipping lily bulbs in dilutions of imidacloprid. This treatment has effectively eliminated aphids during much of the cropping cycle reducing or eliminating the need for further control measures.

Our results though, have produced valuable information on the integrated use of biological and reduced-risk materials for pest control. Together these results demonstrate that a multi-tactic pest management program could be developed that relies exclusively on biological or reduced-risk materials. As a result of this work, two scientific publications have been generated as described below. The first publication is attached as a supplement to the final report.

Publications

Murphy B. C., D. von Damm-Katari & M. P. Parella. (1999). Interaction between fungal pathogens and natural enemies: Implications for combined biocontrol of greenhouse pests. Submitted to the International Organization for Biological control (IOBC), Brest, France.

Murphy B. C., D. von Damm-Katari, E. K. Fogg, C. L. Alexander & M. P. Parella. (in prep). Coexistence between fungal pathogens and natural enemies: Implications for combined biological control of greenhouse pests. *Environmental Entomology*.

Objective 1. Determine the compatibility and interaction of microbial fungi with parasitoids and predators against *Aphis gossypii* in ornamental lilies.

The experiment was evaluated within a randomized complete block design with seven treatments (4 replications/treatment) in a 25,000 sq. ft. commercial greenhouse. Lilies are produced in 2 by 1 foot boxes. Each replicate in the experiment consisted of 1 box enclosed in a cage with a netted cover to prevent movement of aphids and natural enemies. A total of 28 cages were constructed for the experiment. Treatments included an untreated control, a Botanigard only treatment, a parasitoid (*Aphidius colemani*) and Botanigard treatment, a Botanigard and predator (*Aphidoletes aphidimyza*) treatment and a predator, parasitoid and Botanigard treatment. Treatments were applied 2 weeks after infesting each cage with at least 50 *A. gossypii* nymphs and adults. Two fungal spore applications were made 14 days apart, and a single release of predators and parasitoids were made prior to the first fungal application.

Four cage trials were completed between August 1997 and September 1998. Two of these trials were interrupted by applications of conventional pesticides by the grower and data were not collected. The other two cage trials were successfully completed.

Aphid and parasitoid numbers were monitored to estimate mean aphid densities and fungal infection rates at the conclusion of each trial (4-5 weeks). Fungal infection rates were determined by disinfecting and plating aphids and parasitoids on a selective agar medium.

A supplemental laboratory trial was conducted to provide a more precise estimate of the compatibility of using Fungi with natural enemies and determine the influence of timing on aphid control. The trials were performed against melon aphid on individually caged chrysanthemum leaves. This experiment was done within a randomized design with six treatments. Aphids only, parasitoids (*Lysiphelbus testaceipes*) and aphids only, fungi and aphids only, and 3 combinations of parasitoids and Fungi. In the first combination, the parasitoids were released 3 days prior to fungi application; in the second, parasitoids and fungi were released at the same time; and in the third parasitoids were released 3 days after the fungi spray. At the conclusion of the study (3 weeks) aphids and parasitoid number were recorded.

Results

In all of the caged trials the *Aphidoletes aphidimyza* releases did not result in good establishment, thus, effects from this predator were not readily seen. Data on fungal infection for aphids indicated high infection rates in both the adult and juvenile aphids in the treated cages and very little was seen in the control cages.

Comparison of the mean number of melon aphids for the 2 successful cage trials combined are depicted in Figure 1. The combined mean aphid densities for both cage trials showed significantly lower aphid densities in the *Beauveria* treated cages relative to the controls ($P < 0.05$). However, no significant differences were detected between cages treated with *Beauveria* alone, parasitoids alone or combinations of natural enemies and *Beauveria*. Mean trends suggest that the combined treatments tended to have lower densities. The laboratory trials were conducted to provide better resolution in determining if combined natural enemies improved control.

Similarly, results of the laboratory trials showed the *Beauveria* alone treatments and the parasitoid alone treatment had similar levels of control and both were significantly lower than the controls (Fig. 2). Comparison of the three parasitoid plus *Beauveria* treatments did show enhanced levels of control when parasitoids and fungi were applied simultaneously. When parasitoids were released 3 days before or after the *Beauveria*

sprays levels of control were not significantly greater than *Beauveria* alone or parasitoids alone. These results suggested that the timing in release of parasitoids relative to the fungi applications were important in determining the level of control achieved. Simultaneous releases of the parasitoid and fungal applications resulted in the largest aphid reduction.

We also conducted a multiple regression analysis on both the field cage and laboratory trials to determine which factors, release of parasitoids or fungal applications were most important in determining aphid densities. Results are depicted in figures 3 & 4 and show both factors to be significant in determining aphid numbers. This analysis indicated that both factors contribute to aphid control and when used in combination can result in additive mortality effects. Thus our study demonstrates that parasitoids and fungi are compatible within a pest management program, and used together can achieve greater control than either separately.

2) Determine the compatibility of the biorational pesticide Cinnacure with fungi and parasitoids.

a) Phytotoxicity evaluation of Cinnacure applications to lilies.

Commercial stands of 'Stargazer' lilies were spray with 0.3 and 0.5% ai/v applications of Cinnacure using a backpack sprayer to determine plant safety for use of this product. Applications were made weekly for 4 weeks to approximately 400 lilies. At the end of 4 weeks all plants were inspected and any phytotoxic symptoms recorded.

Results determine that at these concentrations of Cinnacure no detrimental effects to plant growth or flowering were noted. Thus, we concluded Cinnacure appears to be safe for use on this crop.

b) Effect of direct Cinnacure applications to *Aphidius* sp. adults.

Objective: Evaluate Cinnacure for compatibility with *Aphidius colemani* adults. These materials will be compared with a water only spray and a growers' standard spray.

Treatments: Positive control (Orthene), Negative control (Water only), 0.3% active CNMA

Replications: 5

of *Aphidius colemani* per replication: 15 adults

Amount of material sprayed: 1 ml

Inspection interval: 6 hours, 24 hours, 48 hours, 72 hours

Cage and food: Experimental Arena (Murphy et al, 1997). Honey water thinly streaked on a small piece of parafilm.

Spray application: Spray applied to insects at a constant distance of 12-16 cm.

Procedure:

Hour 0: Collect 15 insects per replication and place them in the experimental arenas. Shake material thoroughly, then spray treatments with 1 ml of material. Add food material to cage.

Hour 6: Count number of insects dead and alive.

Hour 24: Count number of insects dead and alive.

Hour 48: Count number of insects dead and alive.

Hour 72: Count number of insects dead.

Results:

Direct applications of Cinnicure resulted in severe mortality comparable to Orthene (Figure 5). Results suggest that exposed adults will be susceptible to Cinnicure applications.

c) Effect of residual Cinnicure on leaves to *Aphidius* sp. adults.

Objective: Evaluate Cinnicure for compatibility with *Aphidius colemani* adults. This material will be compared with a water only spray and a growers' standard spray.

Treatments: Positive control (Orthene), Negative control (Water only), 0.3% active CNMA

Replications: 5

of *Aphidius colemani* per replication: 15 adults

Amount of material sprayed: 1 ml

Inspection interval: 6 hours, 24 hours, 48 hours, 72 hours

Cage and food: Experimental Arena (Murphy et al, 1997). Honey water thinly streaked on a small piece of parafilm.

Spray application: Spray applied experimental arena at a constant distance of 12-16 cm.

Procedure:

Hour 0: . Shake material thoroughly, then spray treatments with 1 ml of material. Spray experimental arenas and allow material to dry.

Hour 2: Collect 15 insects per replication and place them in the experimental arenas. Add food material to cage.

Hour 8: Count and record the number of insects dead and alive.

Hour 26: Count and record the number of insects dead and alive.

Hour 50: Count and record the number of insects dead and alive.

Hour 74: Count number of insects dead. Place experimental arenas in the freezer for ten minutes and count the total number of insects in the container by removing each insect as it is counted. Thus the difference between the total and number dead gives the number living at the time of the final count.

Results:

Residual Cinnicure material resulted in minor increases in mortality that were not significantly different than water only and significantly lower than Orthene (Figure 6).

d) Effect of Cinnicure to fungal spores.

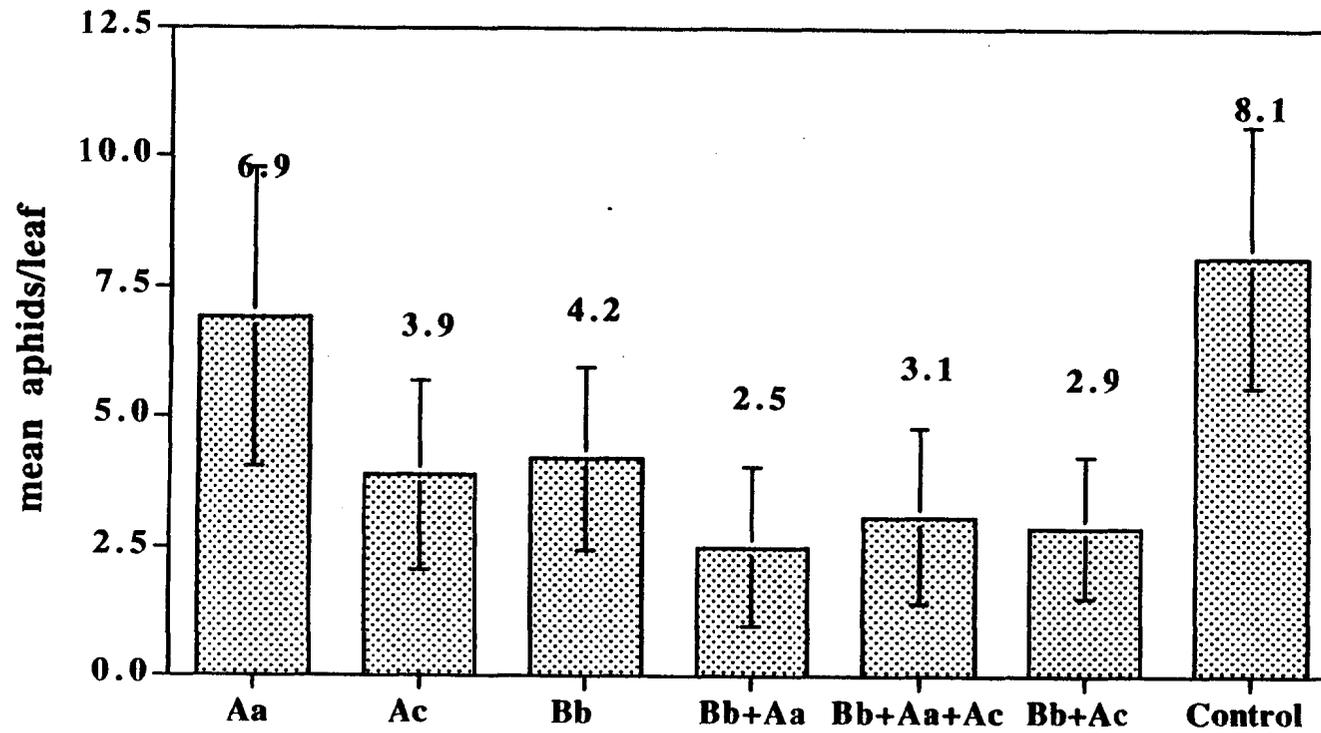
The mycotech Corp. (producers of the fungal pathogen) evaluated the effect of 0.3% ai/v Cinnicure to the fungal spores. Result determined that virtually 100% of the spores were killed by this application. Result indicates reapplication of spores will be necessary after Cinnicure applications.

3) Conduct full scale greenhouse demonstration trials integrating the three tactics under commercial production practices.

Two full scale replicated trials were attempted comparing the alternative aphid control program with the growers conventional pest control practices. In both attempts miss communication between the greenhouse manager and greenhouse workers resulted in conventional sprays being applied to the experiment. This prevented us from being able to evaluate the alternative control tactics.

Future attempts were also confounded by changes in cultural practices. Bulb producers in the Netherlands have begun dipping lily bulbs in dilution's of imidacloprid. This treatment apparently protects lilies from aphids for much of the cropping cycle eliminating or greatly reducing the need for additional control sprays. Thus under current practices the need for aphid control had been greatly reduced. No further attempts to conduct commercial seem warranted at this time.

Figure 1

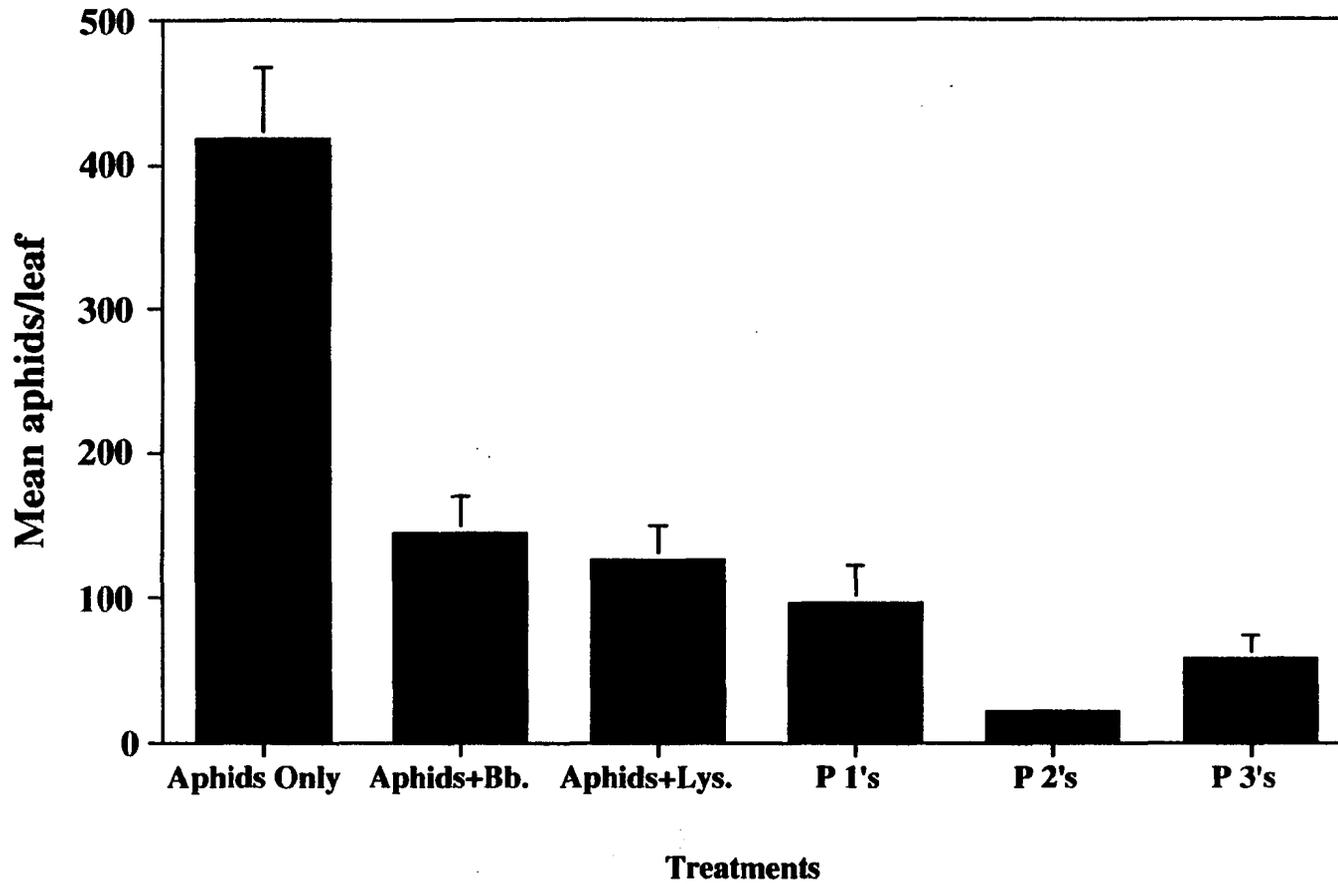


Bb = *Beauveria bassiana* (fungal pathogen)

Aa = *Aphidoletes aphidimyza* (predator)

Ac = *Aphidius colemani* (parasitoid)

Figure 2



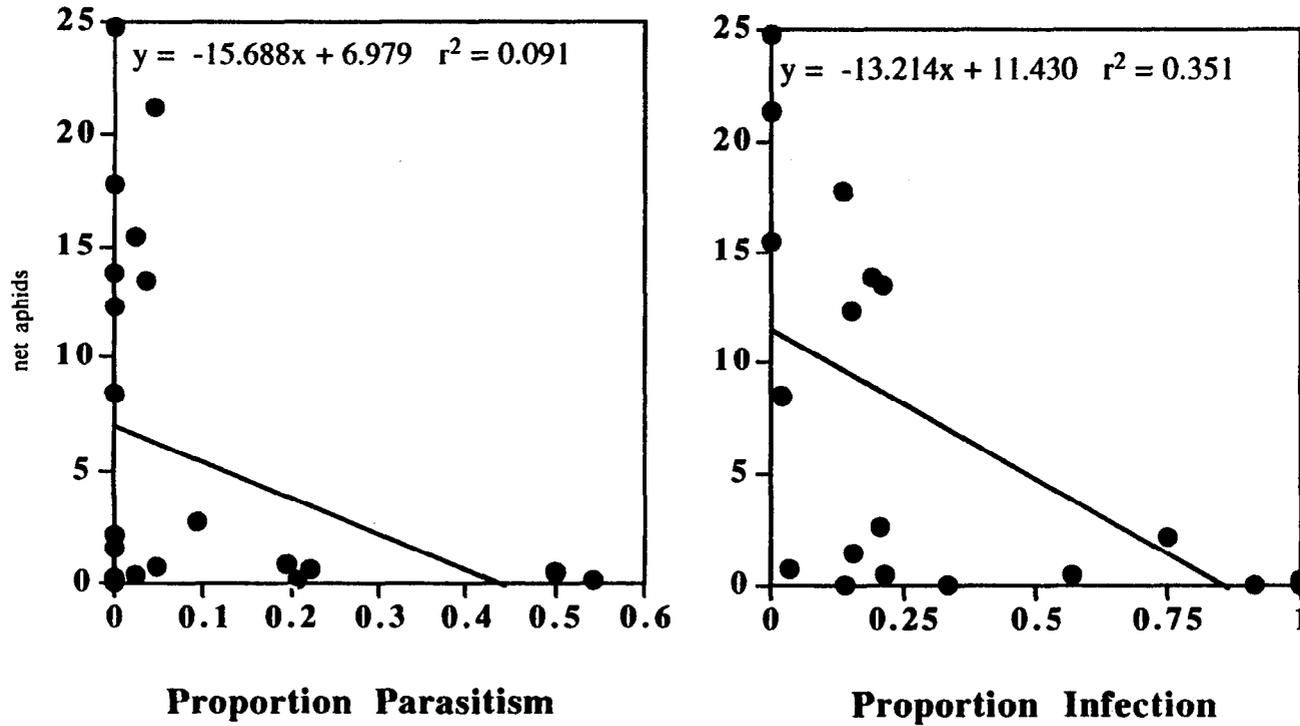
P1, parasitoids released 3 days before fungi, P2, released at the same time; P3, parasitoids released 3 days after fungi.

Bb = *Beauveria bassiana* (fungal pathogen)

Lys = *Lysiphlebus testaceipes* (parasitoid)

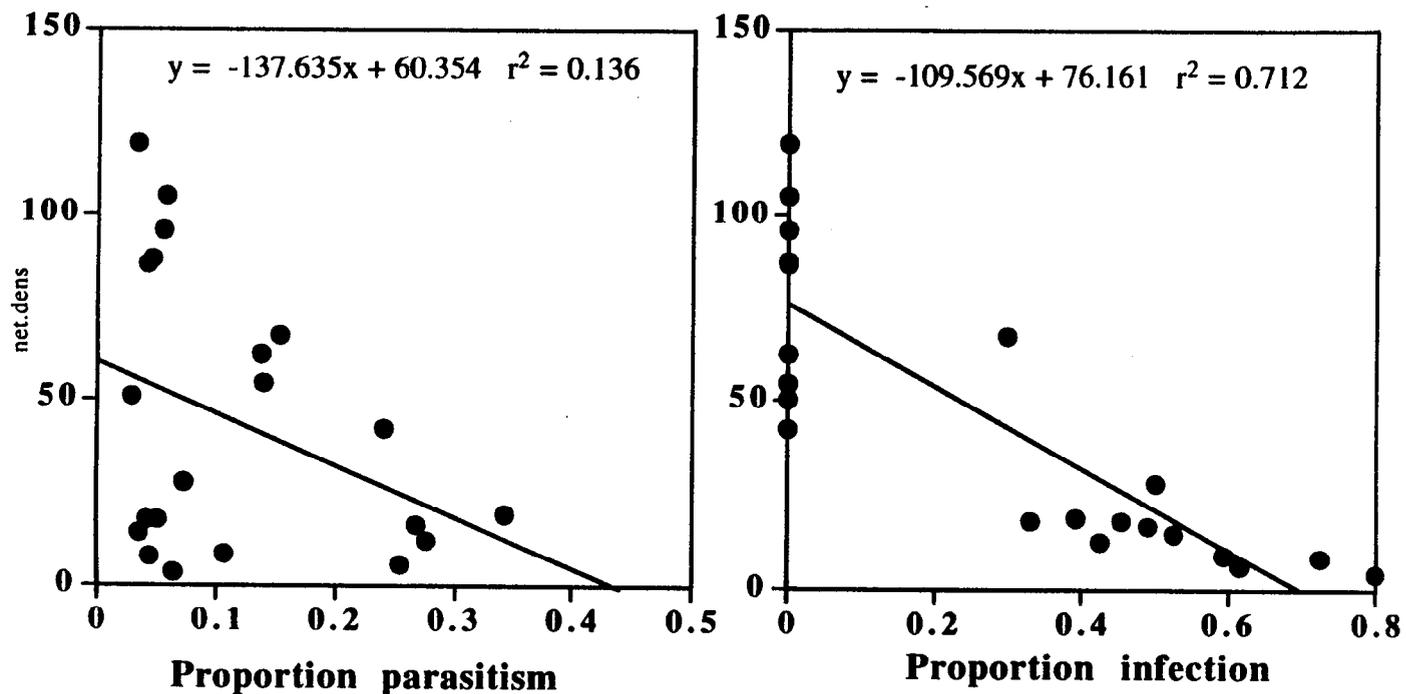
Figure 3

Melon Aphid on Fresh cut Lilies Caged Greenhouse Trial



<u>Multiple Regression</u>	<u>Df</u>	<u>SS</u>	<u>F ratio</u>	<u>Prob.</u>
Parasitism rate	1	1152.8	16.2	0.0002*
Infection rate	1	531.3	67.4	0.0084*
Interaction	1	3.5	6.5	0.8257

Figure 4
Melon Aphid on Chrysanthemum
Laboratory Trial



<u>Multiple Regression</u>	<u>Df</u>	<u>SS</u>	<u>F ratio</u>	<u>Prob.</u>
Parasitism rate	1	156448.5	10.5	0.0022*
Infection rate	1	191414.6	12.9	0.0008*
Interaction	1	14649.6	1.0	0.3261

Figure 5

Aphidius colemani Direct Bioassay

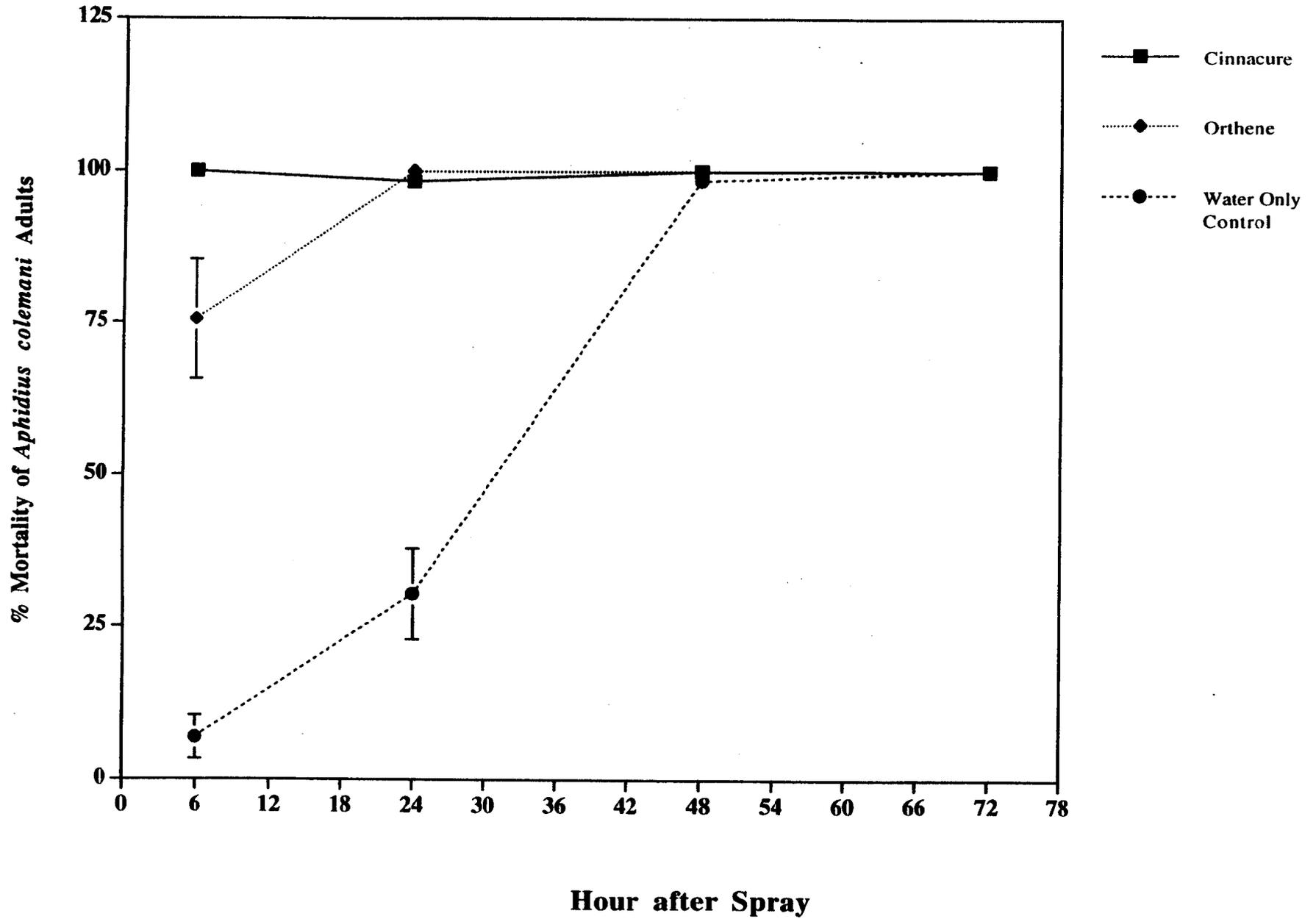
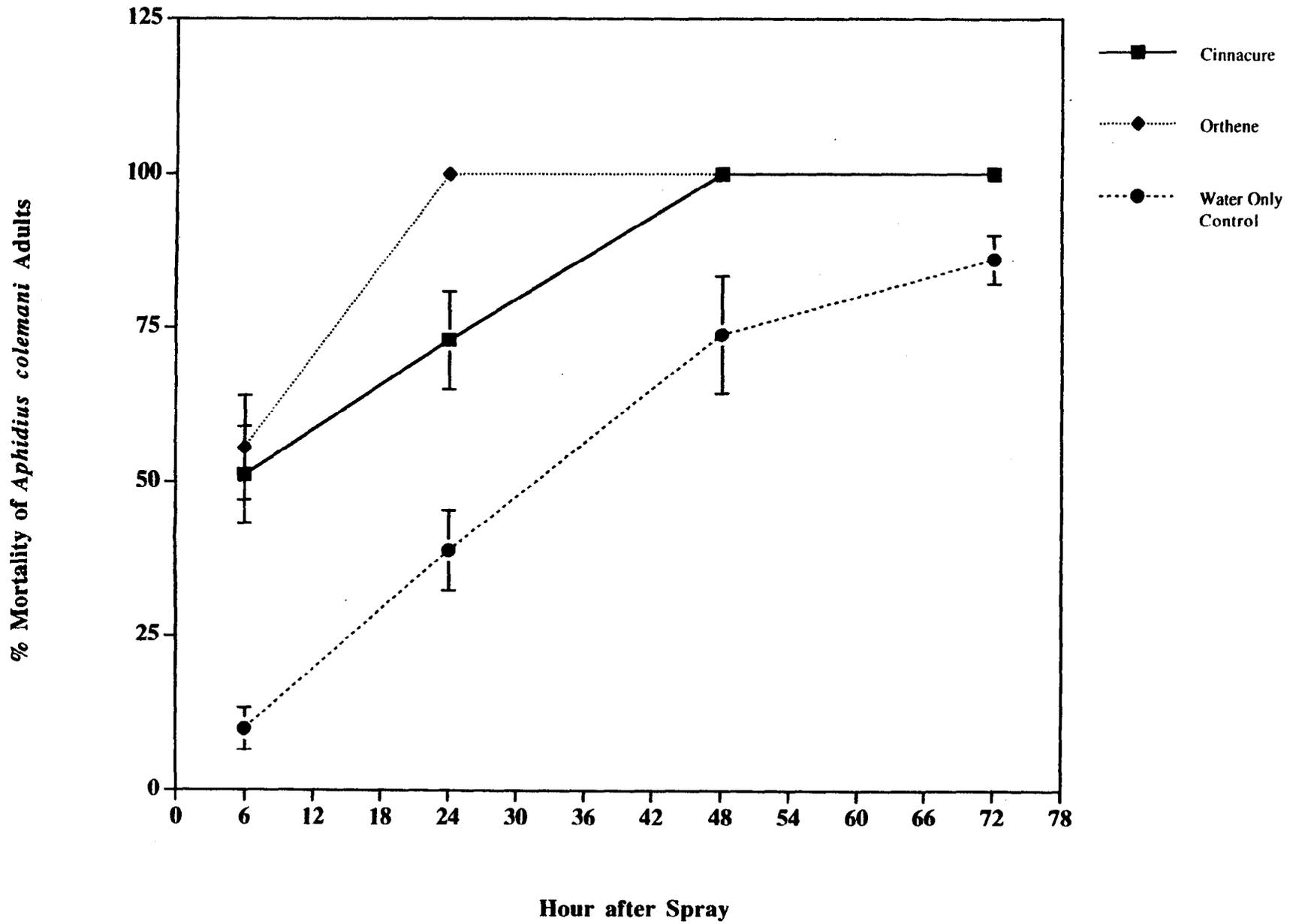


Figure 6

Aphidius colemani Residual Bioassay



Interaction between fungal pathogens and natural enemies: Implication for combined biocontrol of greenhouse pests

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Abstract: Advancements in fermentation and formulation technologies have led, in recent years, to the widespread commercial availability of effective entomopathogenic fungi for pest control programs in greenhouses. Natural enemies have been observed to persist in the presence of one fungal pathogen suggesting both natural enemies and fungi could be employed within pest control programs. Experimental trials were conducted to evaluate the degree of compatibility between the fungus, *Beauveria bassiana* and two economically important natural enemies. Results indicated *B. bassiana* did not significantly impact populations of the parasitoids *Lysiphlebus testaceipes* or *Aphidius colmani* attacking the melon aphid, *Aphis gossypii*. Furthermore, evidence suggests that when used together, both parasitoids and the fungus contributed toward aphid suppression.

Key words: ornamentals, *Lysiphlebus testaceipes*, *Aphidius colmani*, *Aphis gossypii*, *Beauveria bassiana*, biological control

Introduction

Effective integrated pest management (IPM) programs for ornamental crops have yet to be fully implemented. Over reliance on repeated applications of traditional pesticides have presented a number of problems for growers including the development of pesticide resistance, threats to worker safety and the environment, and interference with IPM tactics such as biological control.

A major obstacle to implementing IPM in ornamental crops has been the lack of effective alternatives to conventional pesticides. In recent years, advances in fermentation technologies and formulation have made effective microbial pesticides commercially available for greenhouse and field use. One such product is the entomopathogenic fungi, *Beauveria bassiana* (Mycotech corp., Butte MT, USA). *Beauveria bassiana* has been shown an effective alternative control agent for many important ornamental pests including the western flower thrips, the melon aphid, rose and green peach aphid and whiteflies.

Preliminary studies and observations have also shown some natural enemies of key ornamental pests can persist under *B. bassiana* applications suggesting both fungi and natural enemies could be used together in pest control programs.

Here, we present the results of experimental trials designed to test the impact of *B. bassiana* applications in the presence of two important parasitoid species, *Lysiphlebus testaceipes* and *Aphidius colmani* attacking the melon aphid, *Aphis gossypii*. The trials were undertaken to examine the interaction between *B. bassiana* and parasitoid populations when used simultaneously for pest control.

Materials and methods

Caged field trial. This study was established to test the effect of fungi and natural enemies alone and in combination against *A. gossypii* populations infesting asiatic lilies. Field cages were established in a commercial fresh cut lily greenhouse in Arcata, California, USA. Twenty eight cages measuring 2 x 1 x 3 ft were placed over asiatic lilies (17 to 25 lilies per cage). Each cage was randomly assigned to one of four treatment groups; *A. colmani* only, *B. bassiana* only, *A. colmani* plus *B. bassiana* (8 replicate cages each) and an untreated control (4 replicate cages). Fifty mixed age melon aphids (*Aphis gossypii*) were introduced to the cages and allowed to reproduce for 2 weeks uninhibited. *Beauveria bassiana* was applied at the label rate of 1.2 gm/liter to the appropriate cages. Within twenty four hours approximately 10 newly emerged

3 leaves from each of six plants within each cage replicate were sampled and the number of aphids, parasitized aphids and fungus infected aphids were recorded. For both the caged field trial and laboratory trial, parasitism rates were determined by holding a subsample of aphids for 7 days and examining them for evidence of parasitism. **Fungal infection was determined by disinfecting the aphid cuticle of ungerminated spores with a 0.5 percent solution of hypochlorite and plated on a selective agar medium and examined for evidence of *B. bassiana* infection after 7 days.**

Laboratory trial. A laboratory trial was conducted on potted chrysanthemums infested with *A. gossypii* and used to determine how the timing of parasitoid release, with respect to *B. bassiana* applications, effected aphid control. The experiment was conducted using single rooted chrysanthemum cuttings ('Icecap') planted in 4 inch square pots. Chrysanthemums were grown in the greenhouse for 3 weeks and then pinched to four fully formed leaves. Each plant was then caged individually using a 2.5 inch by 6 inch clear plastic cylinder with the top end screened to prevent movement of aphids or natural enemies. Each plant replicate contained a starting population of 25 *A. gossypii*. Aphids were allowed to reproduce uninhibited for 3 days before treatment applications. The experiment consisted of 6 treatments using 8 potted plant replicates per treatment (48 replicates total). The treatments were, 1. aphids only, 2. *L. testaceipes* only, 3. *B. bassiana* only, 4. aphids plus *B. bassiana* and *L. testaceipes* (parasitoids released 3 days prior to *B. bassiana* spray) 5. aphids plus *B. bassiana* and *L. testaceipes* (parasitoids released immediately after *B. bassiana* application) 6. aphids plus *B. bassiana* and *L. testaceipes* (parasitoids released 3 days after *B. bassiana* application). Parasitoids were released at a rate of 15 per replicate and *B. bassiana* was applied at the label rate of 1.2 gm spores / Liter.

At six days post treatment, one leaf (second leaf from the top) was sampled from four of the plant replicates for determining aphid infection by *B. bassiana*. Fungal infection was determined by disinfecting the aphid cuticle of ungerminated spores with a 0.5 percent solution of hypochlorite and plated on a selective agar medium. Seven days after plating the samples were counted for evidence of *B. bassiana* infection. Fourteen days after all treatment applications, the remaining leaves of the plant replicates were removed and the number of live aphids, dead aphids and number of parasitized aphids were recorded.

Statistical analyses. Results were subjected to analysis of variance using the treatment factor as the independent variable and either aphid density, parasitism or fungal infection rate as the response variable. Mean comparisons were accomplished using Tukey-Kramer HSD tests to detect differences among treatment means. Multiple regression analysis using fungal infection and parasitism rates as the independent variables and aphid densities as the dependent variable were used to determine which of these factors explained aphid density.

Results and discussion

Caged field trial. The ANOVA tests showed that at the conclusion of the trial there were significantly different aphids densities among treatments ($F = 8.76$; $df = 4, 27$; $P = 0.0002$), and infection ($F = 3.46$; $df = 3, 16$; $P = 0.0481$), but not for the parasitism rate ($F = 1.6$; $df = 3, 19$; $P = 0.2264$). Mean comparisons tests determined that all treatments resulted in significant reductions in aphid numbers relative to the control cages (Table 1). No differences in aphid densities were seen among the individual *B. bassiana* and *A. colmani* treatments. For the fungal infection rate, the *B. bassiana* plus *A. colmani* treatment resulted in greater aphid infection relative to all other treatments including the *B. bassiana* alone treatment. In addition, a sub-sample of mummified aphids were removed from the *A. colmani* only and *A. colmani* plus *B. bassiana* treatments and held for 10 days and the number of emerged parasitoids recorded. Parasitoid emergence for the *A. colmani* only treatment was 100%, and 70% emergence was recorded for the *A. colmani* plus *B. bassiana* treatment.

For the caged greenhouse trial, our results had shown that parasitoids and fungi caused significant reductions in aphids whether used singly or combined. The lack of difference in parasitism rate between the *A. colmani* and *B. bassiana* plus *A. colmani* treated cages did

persistence when using the fungus for pest control. However, observations on parasitoid emergence did suggest some enhanced mortality may be suffered by *A. colmani* in the presence of the fungus. The enhanced *B. bassiana* infection seen in the *B. bassiana* plus *A. colmani* treatment may indicate a positive effect when both species are used together. However, the greater infection rates did not translate into greater aphid reductions. The reason may have been that aphid densities were too low to test for treatment effects.

Table 1. Replicated caged greenhouse trial comparing *A. colmani*, *B. bassiana*, and *B. bassiana* plus *A. colmani* treatments on *A. gossypii* densities, *A. gossypii* parasitism and *B. bassiana* infection.

Treatment	Aphid density ^a	% parasitism	% infection
Aphids only	7.0 ± 2.3 a	0.2 ± 0.2 a	2.0 ± 2.0 a
<i>A. colmani</i>	0.58 ± 0.4 b	35.4 ± 14.3 a	20.0 ± 20.0 a
<i>B. bassiana</i>	0.75 ± 0.4 b	0.0 ± 0.0 a	42.3 ± 16.1 a
<i>B. bassiana</i> + <i>A. colmani</i>	1.33 ± 1.1 b	20.0 ± 20.0 a	73.3 ± 18.6 b

^a Mean separation using Tukey - Kramer HSD test.

Laboratory trial. Results of the ANOVA showed significant differences among treatment means for aphid density ($F = 25.7$; $df = 5,47$; $P < 0.0001$), parasitism ($F = 6.88$; $df = 5,47$; $P < 0.0001$), and fungal infection ($F = 5.21$; $df = 5,47$; $P = 0.0008$). Mean comparison tests showed that aphid densities were significantly lower among all treatments relative to the aphids only (control) treatment (Table 2). Furthermore, the *B. bassiana* plus *L.testaceipes* simultaneous release (zero day) treatment had significantly lower aphid numbers among all treatments. For parasitism rate, the *L.testaceipes* alone treatment had the highest mean parasitism rate but not significantly greater than the *B. bassiana* plus *L.testaceipes* treatments. Fungal infection was also found to be significantly greater in the *bassiana* plus *L.testaceipes* simultaneous release (zero day) than all other treatments.

The laboratory trial also showed that parasitoids and fungi caused significant reductions in aphids whether treated singly or combined. Within the combined treatments, application of both fungi and parasitoids on the same day resulted in the lowest aphid numbers thus supporting the notion that parasitoids and fungi could be used effectively for pest control. Again, no significant differences in parasitism was detected among the *L.testaceipes* and the combined treatments. Similar to the caged trial, significant increases in *B. bassiana* infection was seen, but only for the combined treatment when released on the same day. The reason for this is uncertain. One possible explanation may be that when *L.testaceipes* and *B. bassiana* are released on the same day, oviposition punctures in the host by parasitoids provide a more effective route for infection by germinating spores than when parasitoids are absent. When *L.testaceipes* is released 3 days before *B. bassiana*, most oviposition activity may have already taken place before the spores arrive. When *L.testaceipes* is released 3 days after the fungi, most fungi may have germinated and not benefited from oviposition activities.

Table 2. Replicated laboratory trial comparing *L.testaceipes*, *B. bassiana*, and *B. bassiana* plus *L.testaceipes* treatments at 3 release times on *A. gossypii* densities, *A. gossypii* parasitism and *B. bassiana* infection.

Treatment	Aphid density	% parasitism	% infection
Aphids only	417.6 ± 49.2 a	1.6 ± 1.4 a	4.9 ± 1.9 a
<i>L.testaceipes</i>	125.8 ± 24.8 b	16.6 ± 2.8 b	8.0 ± 3.8 a
<i>B. bassiana</i>	144.0 ± 26.5 b	2.0 ± 0.8 a	11.1 ± 3.7 a
<i>L.testaceipes</i> + <i>B. bassiana</i> -3d ^b	92.0 ± 25.2 b	7.9 ± 1.7 b	20.0 ± 4.4 a
<i>L.testaceipes</i> + <i>B. bassiana</i> 0d ^c	22.9 ± 4.7 c	9.1 ± 2.4 b	31.9 ± 6.6 b

^a Mean separation using Tukey - Kramer HSD test. ^b*L.testaceipes* released 3 days before *B. bassiana*. ^c*L.testaceipes* released on the same day as *B. bassiana*. ^d*L.testaceipes* released 3 days after *B. bassiana*.

The multiple regression analysis for both the laboratory and caged field trials revealed that percentage parasitism and percentage fungal infection of aphids were both significant factors explaining aphid density for both trials (Tables 3 & 4). As parasitism and/or infection rates increased, there was a corresponding decrease in aphid densities. The lack of significance for the interaction term suggested both factors operated in an independent manner.

Table 3. Multiple regression analysis from the caged greenhouse trial using parasitism and *B. bassiana* infection as the independent variables and *A. gossypii* as the dependent variable.

Treatment	DF	SS	F ratio	Probability
Parasitism rate	1	1152.8	16.2	0.0002*
Fungal infection rate	1	531.3	67.4	0.0084*
Parasitism x infection	1	3.5	6.5	0.8257

Table 4. Multiple regression analysis from the laboratory trial using parasitism and *B. bassiana* infection as the independent variables and *A. gossypii* as the dependent variable.

Treatment	DF	SS	F ratio	Probability
Parasitism rate	1	191414.3	12.90	0.0008*
Fungal infection rate	1	156448.5	10.53	0.0022*
Parasitism x infection	1	14649.6	0.99	0.3261

Overall, our results have demonstrated that for at least some species *B. bassiana* can conserve natural enemies within pest control programs. Results of the manipulative experiments and regression analyses further indicate that both could be used in combined biological control programs with each factor contributing an independent source of mortality and thereby provide greater control of pests than either factor acting independently.