

PEST MANAGEMENT GRANTS-APPLIED RESEARCH FINAL REPORT
TITLE PAGE

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Contract Title: Evaluation of efficacy of green lacewings, *Chrysoperla rufilabris* (Burmeister), delivered using a liquid release technique, for management of lettuce aphid, *Nasonovia ribis-nigri* (Mosley), in organic and reduced-risk (IPM) leaf lettuce.

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Disclaimer

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Executive Summary

The lettuce aphid, *Nasonovia ribis-nigri*, has emerged as an extremely important new lettuce pest to California. To control lettuce aphid, growers have increased their use of highly toxic pesticides, including organophosphates and carbamates. Some organic growers have been driven to reduce their lettuce acreage due to this serious new pest. This project sought to utilize knowledge and experience gained during our first year of funding in 1999 to improve a liquid delivery system for green lacewing eggs in lettuce and evaluate the hatch of the released eggs and efficacy of subsequent lacewing larvae in decreasing lettuce aphid populations. Specific objectives of the project were: 1.) to improve the mechanical liquid distributor originally designed for grapes and modified for lettuce (row crop) production systems; 2.) to evaluate the effect of potential liquid sticker carriers on egg hatch in laboratory bioassays; 3.) to evaluate lacewing egg hatch in organic, unsprayed and Admire-treated lettuce fields distributed using the modified liquid distributor; and 4.) to evaluate the efficacy of lacewings in reducing lettuce aphid populations in the field.

The mechanical liquid distributor was modified to improve efficient delivery of lacewing eggs to lettuce. The vessel and tubing connections were made air and liquid-tight to prevent leaking, the number of emitter valves was increased from four valves to ten valves to enable coverage of more seed lines with one tractor pass, manifolds were constructed to enable flexibility in positioning the valves over the lettuce seed lines, and the electrical system and control box were made more robust to enable greater capacity.

Six liquid carriers, including two food-grade starches, three organically approved products and a dilute agar solution, were compared to water and an untreated control in laboratory bioassays for effects on egg hatch and adhesion. The carriers were evaluated after the eggs were submersed in the carriers for 15 minutes while gently stirring. Hatch of the treated eggs was compared relative to an untreated control. Although several of the carriers did not negatively effect egg hatch relative to the untreated controls, two carriers, a 0.05% solution of Nu-Film P™ and a 1% C DrySet™, were observed to give both good adhesion and good hatch, 86% and 95% mean relative egg hatch respectively, and were chosen for further field testing.

Low spring populations of the target pest, *Nasonovia ribis-nigri*, prevented early lacewing egg releases in Admire™-treated fields. Releases were conducted in two organic fields in late summer. At the first site, the releases failed due to problems with conditioning of the eggs, the grower's irrigation schedule, and equipment failure. In the second site, three releases using the modified distributor were conducted with four treatments: eggs distributed using water as the carrier, eggs distributed using Nu-Film P™ as the carrier, eggs distributed using C-DrySet™ as the carrier and an untreated control. Monitoring results showed the mean number of lacewing larvae was highest in the plants that received lacewing eggs using water as the carrier (0.5 lacewing larvae/plant on the last monitoring date), significantly higher, at $p=0.05$, than either the plants that received eggs in C-DrySet™ (0.2 lacewing larvae/plant) or the untreated (0 lacewing larvae/plant) but not significantly different from those plants which received eggs in Nu-Film-P™ (0.27 lacewing larvae/plant). The mean number of apterous *Nasonovia* was highest in the untreated plants (44 *Nasonovia*/plant on the last monitoring date) and plants which received eggs in C-DrySet™ (43 *Nasonovia*/plant), significantly higher, at $p=0.1$, than either the plants that

received eggs in NuFilm-P™ (32 *Nasonovia*/plant) or the plants that received eggs in water (31 *Nasonovia*/plant). However, syrphid larvae were also observed to be good predators of *Nasonovia* and were highest in the plants that received lacewing eggs in water (2.1 syrphid/plant).

We conclude that lacewing egg releases using the modified liquid distributor can increase the number of subsequent lacewing larvae in a field and reduce the number of apterous *Nasonovia* in those plants that receive eggs over untreated plants, especially when water is used as the egg carrier. However, in our releases, we found that syrphid larvae were also higher in our release plots and therefore we cannot attribute the reduction in *Nasonovia* in those plots to the lacewing alone. The use of augmentative biological control will continue to be an important component of IPM and organic systems, as demonstrated by grower's interest, and there continues to be a need to improve the efficiency and effectiveness of this potential tool.

Report.

a. Introduction

Lettuce aphid, *Nasonovia ribis-nigri*, is a new pest to California that colonizes the center of the lettuce head where it is difficult to reach with pesticide sprays. Lettuce infested with lettuce aphid is unmarketable. To control lettuce aphid, growers have increased their use of highly toxic pesticides, including organophosphates and carbamates. Organic lettuce growers have few, if any, acceptable control options and many have reduced their lettuce acreage because of losses due to *Nasonovia* infestations. The overall goal of this project was to evaluate the efficacy of augmentative biological control, specifically green lacewing eggs, for lettuce aphid, *Nasonovia ribis-nigri*, in both organic and reduced-risk (IPM) lettuce production systems. The project addressed the following priority areas: alternatives to highly toxic pesticides, reduction of worker exposure to pesticides, and protection of surface and ground water quality.

This project sought to utilize knowledge and experience gained in our first year of funding in 1999 to improve a liquid delivery system for lacewing eggs in lettuce and evaluate the hatch of the released eggs and efficacy of subsequent lacewing larvae in decreasing lettuce aphid populations. During the 1999 season, green lacewing egg releases were conducted in the Salinas Valley using water as the liquid carrier. Results showed good insectary quality and showed that the delivery system did not harm the eggs. However, only one release resulted in lacewing larvae in the field as evaluated by both clip cage and in-field monitoring. Dislodging of eggs from the plant surface after distribution but prior to hatching appeared to be one key factor in poor recovery of lacewing larvae after our egg releases. We concluded that a need exists to identify liquid carriers other than water for distributing the eggs, carriers that improve adhesion of the eggs to the plant without decreasing egg hatch.

The specific objectives and associated tasks for this project were as follows:

Objective 1: Improve the mechanical liquid distributor system originally designed for grapes and modified for lettuce (row crop) production systems.

Tasks: 1.) modify the suspension reservoir and replace tubing and fittings to reduce leaks and improve reliability; 2.) resizing emitter valves so as to reduce clogging with egg suspension; 3.) design adjustable distributor valve tips (nozzles) and/or adjustable valve mountings to help direct eggs to the center of lettuce heads which are growing over time; 4.) increase the number of distributor valves from 4 valves to 8-10 valves in order to cover 4-40" lettuce beds each with 2 seed lines or 2-80" lettuce beds each with 5 seed lines in one pass; 5.) improve the electrical system and control box electronics to enable capacity of the 8-10 valves.

Objective 2: Evaluate the effect of potential liquid sticker carriers on lacewing egg hatch in laboratory bioassays.

Tasks: 1.) identify potential liquid sticker carriers appropriate for use in organic systems; 2.) mix eggs with the carriers in laboratory bioassays; 3.) plate eggs after submersion into individual cells on cell plates; 4.) evaluate the effect of carriers by observing adhesion of eggs to plates and counting hatched larvae.

Objective 3: Evaluate lacewing egg hatch in organic, unsprayed and Admire-treated lettuce fields for eggs distributed using the modified liquid distributor.

and

Objective 4: Evaluate the efficacy of released lacewings in reducing lettuce aphid populations in the field.

Tasks: 1.) communicate with growers and PCAs to identify field sites and arrange for equipment use and tractor driver assistance; 2.) flag fields for trial set-up, 3.) monitor for *Nasonovia* presence in lettuce; 4.) condition eggs and prepare carriers for releases; 5.) conduct releases; 6.) collect plate and clip cage data for released lacewings; 7.) collect field monitoring data for *Nasonovia*, lacewings and native natural enemies; 8.) enter data and conduct data analysis; 9.) supervise a field assistant.

b. Materials and methods.

Note: for all experiments reported here, laboratory and field, green lacewing eggs, *Chrysoperla rufilabris* (Burmeister), were obtained from a commercial insectary (Beneficial Insectary, Oak Run, CA.). Eggs were packed without carrier and shipped either overnight standard or overnight priority. Upon receipt, three “control plates” of eggs were prepared to determine egg viability in the absence of any experimental effects, as in Wunderlich and Giles, 1998. Since green lacewing larvae are generalist predators and cannibalistic, the control eggs were separated by placement into individual cells on cell plates and incubated until hatch. Each plate held a maximum of sixty eggs, with a total sample size of approximately 180 eggs/date.

Objective 1: Improve the mechanical liquid distributor system originally designed for grapes and modified for lettuce (row crop) production systems.

The mechanical liquid distributor that was used during the 1999 project season was delivered to Ken Giles, UC-Davis Biological and Agricultural Engineering Dept., for performance assessment and improvements. To reduce air leaks in the original suspension reservoirs, the top-ends of the reservoirs were replaced by machined grooves for a tighter o-ring fit. The four nylon-threaded rods of each vessel were replaced with cadmium-coated steel to enable a tighter screw down. The original tubing from vessel to each valve was replaced with smaller diameter tubing and all of the compression fittings for the tubing were replaced to provide leak-proof flow. The entire electrical system was improved for increased valve capacity and for ease of field use.

A ten-liter Nalgene™ liquid carboy was modified and tested as replacement suspension reservoir. The bottom of the carboy was drilled with an inlet hole for air, so the vessel could be pressurized, and an exit hole for the egg suspension. The cap of the carboy was drilled and a 24-volt motor installed and attached to turn an inserted paint stirrer at 60 RPM to provide agitation to the egg suspension. A pressure gauge was inserted in the cap to measure vessel pressure.

The prototype carboy vessel was evaluated in the laboratory for mortality of green lacewing eggs after submersion over time in the vessel agitated by the stirrer and for uniformity of the egg suspension after such agitation and discharge from the system. The carboy vessel was filled with

8 liters of water and 7.2 g. of green lacewing eggs were added to bring the suspension to a concentration of 10 eggs/ml based on our observations of approx. 11,155 eggs/g. The carboy vessel was pressurized to approx. 1-3 psi. Four distributor valves were attached to the outlet of the vessel and the valves were set for output at 4 Hz., 20% duty cycle. The egg suspension was allowed to stir for approx. 20 minutes and then the valves were turned on and egg suspensions were collected from the valves, counted for uniformity, and plated for egg mortality. The experiment was conducted on two dates: 6/1 and 6/22. Five ml. of eggs were collected every 5 min for 50 min. on 6/1; while 10 ml. of eggs were collected every 5 min. for 20 min. on 6/22.

New emitter valves were constructed to increase the size of the distributed droplets and to improve the flexibility in directing the droplets towards the center of the lettuce plants where the target pest, *Nasonovia ribis-nigri*, colonizes. The number of emitter valves was increased from 4 to 10 to allow for efficiency in covering a typical "pass" (4-40" lettuce beds each with 2 seed lines or 2-80" lettuce beds each with 5 seed lines). The steel manifold "tees" for mounting the valves on a standard tractor tool bar were also modified for flexibility: a slot was cut into the tee and the valves were fitted with mounting screws which could be slid along the slot and tightened at the appropriate position.

Objective 2: Evaluate the effect of potential liquid sticker carriers on lacewing egg hatch in laboratory bioassays.

We considered both biological and practical implications to narrow the list of candidate carriers for testing, including: ease of mixing, availability to growers, reported previous success (either anecdotal or scientific), and propensity to attract unwanted interfering predators (i.e. sugar solutions attracting ants). Later, during field-testing, the issue of acceptable certified organic materials became an extremely important factor.

Researchers from Colorado State University, (Mannix, et al.,1999), used a 1% solution of a modified food starch to deliver green lacewing eggs into trees with reported success. We contacted the source of this starch, Cerestar Inc. (Hammond, Indiana), and included two food-grade starch products (C-DrySet™ and C Gel Instant™) in our testing. Corn calcium (Peaceful Valley Farm Supply, Grass Valley, Ca.) was included because it was recommended by a local PCA (Israel Morales, pers. comm.). A 0.05% solution of agar was recommended by a colleague working in biological control (Pete Gothro, pers. comm.). We also included the following organically-accepted adjuvants, based on EPA List 4 and the approved OMRI (Organic Materials Review Institute) list of organic materials: Nu-Film P™ (Miller Chemical and Fertilizer Corp., Hanover, Penn.), and Therm-X70™ (Cellu-con, Inc., Strathmore, Ca.).

For each date, the eggs were received from Beneficial Insectary (Oak Run, Ca.) and were placed into control plates as described above. The control plates containing the untreated eggs and the remaining cup of eggs (containing the eggs to be submerged in the carriers) were placed into a plastic box with a lid and a cup of water for humidity. The box, containing the eggs, was then placed in a growth chamber at 28°C (+/- 2°C) for 24-48 hours, until eggs turned from bright green to grayish-brown in color, indicating the lacewing were close to hatch.

When eggs appeared gray-brown in color the submersion experiments were conducted. Since there was little information on the carriers tested, several dilutions of each carrier were screened. Except for the C-DrySet™ solutions, each carrier was prepared the day of the submersion test: 100 to 200ml. of carrier solution was prepared and placed into a glass beaker with a stir bar. The C-DrySet™ solution was usually prepared the day before by heating in a hot water bath for 15 minutes at 90°C to make the solution stickier by hydrolyzing the starch as recommended by the manufacturer. The solution was allowed to cool completely before submersion of eggs.

A mass of eggs equivalent to 10 eggs per ml. of carrier solution, calculated using the estimated average density of 11,155 eggs/gram, was weighed and added to the carrier solution in the beaker. The eggs in solution were then stirred slowly on a stir plate for 15 minutes. A water control in which the eggs were submerged into water alone and stirred for 15 minutes to assess any effects on the eggs due to stirring was also included for each date. After 15 minutes, the solution containing the eggs was poured onto three plates and the eggs were immediately singulated into the plate cells using a fine tipped paintbrush, as was done with the control eggs. For each date, three plates (with 60 cells containing an egg per plate or a maximum of 180 eggs/treatment) were prepared for each carrier tested, for the untreated control eggs, and for the water control. All of the plates were placed inside of plastic boxes with lids and a cup of water for humidity (one plate from each treatment per box) and placed into the growth chamber at 28°C (+/- 2°C) until hatch of the eggs. The number of eggs hatched was counted for each plate and normalized to the untreated control hatch for that date.

Observations were made on each carrier solution as it was tested: the ease of mixing and relative solubility of the carrier, the texture (“stickiness”) of the solution by feel between the fingertips, and the apparent adhesion of the eggs was observed. The adhesion of each carrier was assessed by turning the plates containing the eggs upside down and observing the stick of the eggs to the organza layer of the plates.

Objective 3: Evaluate lacewing egg hatch in organic, unsprayed and Admire-treated lettuce fields for eggs distributed using the modified liquid distributor.

Objective 4: Evaluate the efficacy of released lacewings in reducing lettuce aphid populations in the field.

Spring commercial fields: Hazienda 502 and 610.

We met with several challenges in conducting our field releases in the 2000 season. The first was the unexpected low populations of the target pest, *Nasonovia ribis-nigri*, in spring and early summer lettuce fields. Two commercial romaine fields (neither organic) located in the southern Salinas Valley near King City were targeted for releases and monitored. The first field, Hacienda Ranch Lot 502, (approx. 10 acres) did not receive Admire™ under any part of the field and was divided into two sides: one side to receive lacewing eggs once *Nasonovia* were detected and the other to receive Standard conventional sprays for *Nasonovia* in leaf lettuce (Provado™, and/or Dimethoate™ and/or Diazinon™). Thirty plants were monitored weekly in each part of the field (60 plants total) for seven weeks, April 5 through May 23.

The second field, Hacienda 610, was treated pre-plant with Admire™ under most of the field. Forty-eight beds along the edge were left untreated (no Admire™) so that lacewings could be applied to that section of the field. We also planned on releasing lacewings onto the treated portion of the field to see if the Admire™-treated plants would have any effect on the lacewing hatch. We monitored 20 plants weekly on each part of the field (40 plants total) for six weeks, April 25 through June 5.

Summer organic field trial: Coke Ranch.

Since *Nasonovia* pressure was low in spring conventional lettuce fields, we contacted several organic growers who presumably would have a greater likelihood of having an aphid-infested field. We identified the Coke Ranch as an organic site with *Nasonovia* present and the grower agreed to provide a tractor and driver for the releases.

We originally planned on applying two treatments: lacewing eggs using water as the carrier and lacewing eggs using C-DrySet™ as the carrier. When we contacted the grower and his PCA regarding our planned use of the C-DrySet™, we were notified of the need to get approval from the organic certifier (California Certified Organic Farmers) for the use of the C-DrySet™ material. Although C-DrySet™ is a food grade product, it is not currently on the OMRI-approved list of organically acceptable materials. We contacted CCOF and discussed the possibility of a CCOF approved Experimental Use Policy. This, however, would have jeopardized the grower's organic certification of the crop, and possibly, the organic certification of the land. We therefore decided to withdraw the use of the C-DrySet™ in this field trial and instead focused on field-testing our improved distributor using water as the carrier for the eggs.

A 3.2 acre block of Romaine was divided and flagged into 4 replicated blocks of untreated plots and plots to receive lacewing eggs. Plots were 0.4 acres each, consisting of 4, 80-inch beds wide. Each 80-inch wide bed had five seed lines planted on it. Two releases were made to the field (on July 19 and July 27) using the newly modified equipment and water as the carrier. We planned to release approx. 125,000 eggs/acre or 2-3 eggs/plant at the estimated planting density of 49,000 plants/acre. For the first release, however, we applied a higher rate (approx. 300,000 lacewing eggs/acre), since we did not have the C-DrySet™ treatment and had extra eggs. For the second release on July 27, we attempted to apply approx. 125,000 eggs/acre.

For all of our releases, the electronically controlled liquid delivery system was transported to the field site and mounted on the tractor tool bar the morning of the release. The cooperating growers provided the tractor and the tractor driver for each release, which took up to five hours including mounting, troubleshooting and actual application time.

For all of the releases a mass of 2.7g of eggs was measured into container cups, based on 11,155 eggs/gram and a release concentration of 10 eggs/ml in three liters of water. Tap water was used for the carrier and transported to the site. Eggs were mixed into the water immediately before loading and distribution in the field. One cup, or approx. 30,000 eggs, was mixed with three liters of water in each of the two vessels.

To assess handling and application effects, eggs mixed in water were collected from the distributor valves after travel through the apparatus during the field release and plated for

comparison with the control plates. Three plates, or a maximum of 180 eggs, were evaluated. Evaluation of environmental effects, temperature and wind, on egg hatch in the field after distribution was measured by covering the eggs with clip cages immediately after release. Fifteen eggs in each plot (60 eggs total) were caged after the first release. Only 25 eggs total were caged during the second release due to problems with the equipment (see results). The eggs were evaluated for hatch the following day by unclipping the cage and noting whether the egg was present, missing, crushed, or if the egg had hatched, a lacewing larva was present.

To evaluate lacewing larvae survivorship, and efficacy against the aphid *Nasonovia*, each field was monitored before and after each release for the presence of *Nasonovia* and lacewing larvae. The day before the release and weekly thereafter, (July 14 to August 10) ten plants were monitored in each of the four replicated plots receiving eggs and in the untreated plots. Lettuce plants were inspected and the number of aphids as well as other insect pests and any natural enemies (including lacewings) present were counted.

Late Summer/Early Fall : Tanimura & Antle Experimental Field.

In order to evaluate the C-DrySet™ carrier and compare it to water and Nu-Film P™ in a randomized block design, we moved our last release site to the Tanimura & Antle experimental field located in Spreckels, Ca. This enabled us to do field releases on organically managed ground without jeopardizing the grower's organic certification.

A 0.4-acre block of Romaine was divided into a randomized complete block design with 4 replicates of 3 treatments and an untreated control. Plots were approximately .02 acres in size: 4, 40-inch beds wide and 67 feet long. Treatments consisted of lacewing eggs released in water as the carrier, lacewing eggs released in Nu-Film P™ as the carrier, and lacewing eggs released in C-DrySet™ as the carrier. Three releases were made: on August 30, September 7, and September 13. A flagger directed the tractor driver to each of the appropriate blocks for application of the treatments. Due to a lack of time, no clip cages were placed over the released eggs nor were eggs collected from the valves.

The field was monitored weekly from August 23 to September 20. Ten plants from each replicate, or forty plants per treatment, were monitored. Only plants from the middle two beds of each plot were inspected.

c. Results.

Objective 1. : Improve the mechanical liquid distributor system originally designed for grapes and modified for lettuce (row crop) production systems.

Improvements made to the vessel and fittings succeeded in making the distributor air and water tight, no leaking was observed during field releases.

Egg hatch from eggs which were suspended in water in the Nalgene carboy prototype vessel, stirred for 10, 20 or 40 minutes and then plated for hatch showed no difference from untreated controls. Therefore, the stirring agitation did not appear to effect egg viability. Uniformity of distribution was affected, however. On June 1, the eggs/ml. distributed varied from 0 eggs at

T=10 min. to 27 eggs at T=30 min. Likewise, during the second experiment on June 22, the eggs/ml. varied from 3 eggs at T=15 min. to 11 eggs at T=20 min. Therefore, the stirring method of agitation employed in the carboy prototype did not adequately keep the eggs uniformly suspended and effected the distribution of the discharged eggs. Because of these problems, we decided not to use the carboy vessel prototype, and instead used the improved original suspension reservoirs for our field experiments. The original reservoirs have demonstrated very uniform discharge of the eggs, with only a 0.4% decrease in egg concentration per minute of agitation (Wunderlich, 1997).

Several problems were noted while laboratory testing of the new emitter valves, including electrical problems with firing of the valves and apparent clogging of eggs in the newly reconfigured valves. The electrical problems were solved by inspection of the control box and repair. Because of the intermittent clogging of eggs in the new valves, the original valves were re-employed in the system. Later, during the first field release at Coke Ranch (see objective 3), we encountered egg clogging in the smaller diameter tubing, which led us to replace it with the original sized tubing.

Objective 2: Evaluate the effect of potential liquid sticker carriers on lacewing egg hatch in laboratory bioassays.

Six carrier solutions, in addition to water, were screened in laboratory assays. Table 1 in Appendix 1 lists the carriers, manufacturers, rate and observations from the carriers we screened. Figure 1 shows the percent mean egg hatch, relative to the control eggs, for each of the tested carriers. The (n) or number of plates replicated differs for each carrier since those carriers which did not show some promise in both adhesive qualities and egg viability in early tests were eliminated from further testing to facilitate the timeline of the experiment.

Some of the carriers were difficult to mix up and prepare, including the agar solution (which had to be autoclaved to dissolve the agar), and the corn calcium, which was a very viscous material and was difficult to accurately measure.

C-Gel™ at a 1% and 5% concentration decreased hatch of the eggs to a mean of 56-65% hatch, which was considered unacceptable, therefore it was not considered further. Likewise, Therm-X70™ (10x) and NuFilm-P™ (2x and 10x) also decreased hatch as compared to the untreated controls and were therefore omitted from further testing.

Although corn calcium (1x), Therm-X70 (1x), and agar solution (.05%) showed no detrimental effect on egg hatch as compared to the untreated control, none of these three carriers appeared to stick the eggs to the surface of the plate in our observations. Therefore, these carriers were tested on only one or two dates before they were discarded from the pool of candidates.

Of the six carriers tested, only C-DrySet™ (1% and 3%) appeared to give the best adhesion without effecting hatch of the lacewings. Since there was no significant difference in hatch of eggs after immersion in the 3% solution compared to the 1%, we dropped the 3% solution and continued testing with the 1% C-DrySet™ concentration. We repeated testing on 7 dates, for a total of 21 replicated plates. The mean relative hatch from eggs submerged in the 1% C-DrySet

solution across all dates was 95% (standard error of 3.6%). Although the mean hatch from 9 replicates of NuFilm-P™ (1x) was only 86%, we still considered it a good candidate since it appeared to give good adhesion of the eggs, was available to growers and relatively easy to mix, and was an organically approved material. At the conclusion of our laboratory testing of carriers, we prepared to go to the field with two candidate carriers: NuFilm-P™, and C-DrySet™.

Objective 3: Evaluate lacewing egg hatch in organic, unsprayed and Admire-treated lettuce fields for eggs distributed using the modified liquid distributor.

Objective 4: Evaluate the efficacy of released lacewings in reducing lettuce aphid populations in the field.

Spring commercial fields: Hazienda 502 and 610.

In Hacienda Ranch Lot 502, only 3 plants were found to have *Nasonovia*: one plant had eight apterous *Nasonovia* aphids on May 5, and two plants had one alate *Nasonovia* each on May 23. In Hacienda 610, no *Nasonovia* on either the Admire-treated or the untreated side; therefore, no lacewing releases were made into either of these fields.

Coke Ranch.

Several problems were encountered during the releases at Coke Ranch. For the first release, we received the eggs from the insectary on July 18, the day before the scheduled release, and placed them into the growth chamber @ 25°C as per our protocol. However, on the morning of the release the eggs were still greenish in color, indicating they were still not close to hatch. Because of the scheduling of both the tractor driver and the grower's irrigation schedule, we conducted the release with the eggs even though they were not as close to hatch as we would have preferred.

On July 20, the day after the first release, there was no hatch in either the control plates or the eggs collected from the distributor and plated. On July 21, two days after the release, the mean hatch from the control plates was 94.4%, and the relative mean hatch of the eggs collected from the distributor valves after travel through the apparatus during the field release was only 69.7%, much lower than observed in previous testing.

Unsurprisingly, there was no hatch from the eggs we had clip caged when we checked them at 3:20 p.m. on July 20, the day after the release. At that time, of the 60 eggs caged, 46 of them were not yet hatched, 11 eggs were missing, and 3 eggs had been crushed. The grower then irrigated the field using sprinklers the evening of July 20.

During our second release, on July 27, we encountered problems with plugging of the tubing during the release. After repeated clogging, we stopped the release and did not put eggs onto the fourth replicate. For the plating procedure, we only collected eggs from the two valves that were not clogging. Because of the time spent trying to unclog the tubing, we only were able to clip a total of 25 eggs in the first three replicates. After this release, we replaced the tubing with the larger-diameter tubing and encountered no further problems with clogging.

The mean control plate hatch from the second release was 92.7%, and the mean hatch from eggs collected after immersion in the water and travel through the distributor and the two valves that were not clogging was 95.4%. On July 28, the day after the second release, 5 of the 25 clip caged eggs had hatched and contained a lacewing larva, 2 of the 25 eggs were missing, and the remaining 18 eggs were unhatched.

In Appendix 2, Figure 2.1 and Figure 2.2 show the results from our field monitoring at Coke Ranch. Figure 2.1 shows the mean number of *Nasonovia*/plant, which reached a peak on August 2 with a mean of 53 aphids/plant in the untreated plots and 44 aphids/plant in the plots that received lacewing eggs. We do not attribute this difference to our releases, however.

Figure 2.2 shows the mean number of either lacewing or syrphid larvae in the field on the same dates. We did not find any lacewing larvae in the field the week following our first release. We believe this release failed to result in any larvae, due to the young age of the eggs and the grower's irrigation, even though we put out a rate of eggs equivalent to 300,000 per acre. On August 2, the week following our second release, we found a total of three lacewing larvae on three of the 40 plants we monitored. Given the problems with the clogging of the equipment, we were not surprised by this low number. Syrphids appeared to be the dominant natural enemy of the aphids in the field and reached a mean of 4.5 syrphid larvae per plant in the plants that received lacewing eggs and 2.75 syrphid larvae per plant in the untreated plants. We are unsure why there were greater numbers of syrphids in our release plots and we have no reason to believe our releases encouraged the presence of syrphids.

Tanimura and Antle Field.

In Appendix 3, Figures 3.1 and 3.2 show the results from our field monitoring after the three releases at the Tanimura and Antle field site. Figure 3.1 shows the mean number of apterous *Nasonovia*/plant found in each treatment compared to the untreated control. We had good aphid pressure in this field: the mean number of *Nasonovia*/plant grew steadily in all blocks, including our release plots, throughout the month. On Sept. 20, the last date we monitored the field, the mean number of *Nasonovia*/plant was highest in the untreated block (44 *Nasonovia*/plant) and also in the block which received lacewing eggs in the C-DrySet™ carrier (43 *Nasonovia*/plant) both significantly higher (at $p=0.1$) than the blocks which received lacewing eggs in either Nu Film P™ (32 *Nasonovia*/plant) or in water (31.3 *Nasonovia*/plant).

Figure 3.2 shows the mean number of lacewing larvae found in each treated block as compared to the untreated. We found the highest number of lacewing larvae in the block that received eggs using water as the carrier (mean of 0.5 lacewing larvae/plant on Sept. 20), significantly higher than either the plants that received eggs in C-DrySet™ (0.2 lacewing larvae/plant) or the untreated plants (0 lacewing larvae/plant) but not significantly different from those plants which received eggs in Nu-Film-P™ (0.27 lacewing larvae/plant). We found no lacewing larvae in the untreated plots at any time.

We also found native syrphid larvae in the plots. On Sept. 20 there were a significantly higher mean number of syrphid larvae in the plots which received lacewing eggs in water (2.1 syrphid larvae/plant) than either the C-DrySet™ (0.8 syrphid larvae plant) or the untreated (0.3 syrphid

larvae/plant). The Nu-Film P™ treatment had a mean of 1.5 syrphid larvae/plant on the same date.

d. Discussion

Results from our work demonstrate the importance of field-testing results from laboratory experiments designed for field application. Changes made in the laboratory to our distributor, new emitter valves and narrower tubing, did not bear out in our field tests and we had to replace the emitters and tubing with the originals. Likewise, the carriers C-DrySet™ and Nu-Film P™ demonstrated good egg adhesion and hatch after egg submersion in the carriers in beaker experiments in the laboratory. We expected the adhesion lent to the eggs by the carriers to result in better stick of the eggs to the plants and subsequently more lacewing larvae in the field. But when we conducted field releases using the mechanical distributor to dispense the lacewing eggs immersed in the C-DrySet™ or Nu-Film P™ compared to water as the carrier, neither of the carriers significantly improved the number of lacewing larvae found in the field over water. We are unsure why this was the case. It could be that the carriers somehow made the eggs more susceptible to damage during delivery or more susceptible to desiccation once distributed. Therefore, we were unable to identify a carrier for lacewing egg distribution that is better than water, and in fact, found the greatest number of lacewing larvae in the plots which distributed eggs using water as the carrier.

Furthermore, we cannot conclude that the decrease in the mean number of *Nasonovia* in the plots that received lacewing eggs was a result of the preying of the lacewing larvae on the *Nasonovia* alone. This is because other natural enemies, especially syrphid larvae, were present in the release plots (which were organic and did not receive any other sprays, not even organically-approved materials), and the syrphid larvae were also observed to prey on the *Nasonovia*. Interestingly, in both of our field trials we found more syrphid larvae in the plots that received lacewing eggs than in the untreated plots. We are unsure why this was the case.

Feedback from several growers and PCAs indicated a great interest in the mechanical release technology, but with a clear need to demonstrate lacewing survivorship in the field once egg distribution was made. For example, several of our cooperators were already purchasing lacewing eggs in large quantities and distributing them using their own equipment, but most PCAs complained they could not find lacewing larvae after their egg distribution. In our field test at Tanimura and Antle, we were able to show cooperating PCAs lacewing larvae in plants which received the egg treatments using our experimental equipment, which had been carefully tested not to harm the eggs during delivery (Wunderlich, 1997). This increased the PCAs confidence in the lacewing release strategy. Organic growers especially, some of whom made repeated applications for lettuce aphid with organically approved pesticides such as Neem without good results, have a need for continuing work on improving biological control methods. We should note, however, that it is extremely difficult to test new potential organic materials in commercial organic fields without risking grower's loss of certification.

e. Summary and Conclusions.

The overall goal of this project was to evaluate the efficacy of augmentative biological control, specifically green lacewing eggs, for lettuce aphid, *Nasonovia ribis-nigri*, in both organic and reduced-risk (IPM) lettuce production systems. We conclude that lacewing egg releases using

the modified liquid distributor can increase the number of subsequent lacewing larvae in a field and reduce the number of apterous *Nasonovia* in those plants that receive eggs over untreated plants, especially when water is used as the egg carrier. However, in our releases, we found that syrphid larvae were also higher in our release plots and therefore we cannot attribute the reduction in *Nasonovia* in those plots to the lacewing alone.

This work made the following accomplishments:

We improved the mechanical liquid delivery system originally designed for green lacewing egg releases in grapes and modified it for efficient lacewing egg releases in row-crops.

We tested six liquid carriers for effects on green lacewing egg adhesion and hatch in laboratory bioassays and identified two carriers, C-DrySet™ (Cerestar USA, Inc., Hammond, Indiana) and Nu-Film P™ (Miller Chemical and Fertilizer Corp., Hanover, Penn.) for further field testing.

We conducted five green lacewing egg releases in two field sites using the mechanical delivery system and compared egg hatch and survivorship after release in water, C-DrySet™ and Nu-Film P™ as carriers to an untreated control.

We concluded from the results of our monitoring after field releases that the carriers C-DrySet™ and Nu-Film P™ did not improve lacewing survivorship in the field over water as the egg carrier.

We found that, relative to an untreated control, *Nasonovia* populations decreased in plots that received lacewing eggs using water as the carrier and that lacewing larvae and syrphid larvae increased in those plots which received lacewing eggs. The syrphid larvae, with the lacewing larvae, most likely contributed to the *Nasonovia* population decline.

Appendix 1. Laboratory Results.

Table 1. Carriers screened in laboratory bioassays.

Carrier Name	Manufacturer	1x rate	Observed adhesion of eggs to plate.	Remarks
C DrySet	Cerestar USA, Inc. Hammond, Indiana.	1%	Good.	Converted corn dextrin. Derived by dry-heating unmodified starch in the presence of acid (HCl). GMO-free corn will be available in future. Need to heat while mixing for better stickiness.
C DryGel	Cerestar USA, Inc. Hammond, Indiana.	1%	Fair.	Dried glucose syrup obtained by acid conversion of common corn starch.
Corn calcium	Peaceful Valley Farm Supply Grass Valley, Ca.	0.2%	None.	According to Peaceful Valley, ingredients are: corn syrup, sugar cane molasses and barley malt extract. Corn syrup is obtained through a hydrolysis method where corn starch and water are put with HCl and enzymes and then neutralized with NaOH. No "calcium" per se. Difficult to mix.
Nu-film P	Miller Chemical and Fertilizer Corp. Hanover, Penn.	0.05%	Fair-good.	OMRI approved.
Therm X70	Cellu-con, Inc. Strathmore, Ca.	0.05%	None.	20% saponin from Yucca, 80% plant extract.
Agar solution		0.05%	None.	Needs to be autoclaved to dissolve.

Appendix 1. Laboratory Results.

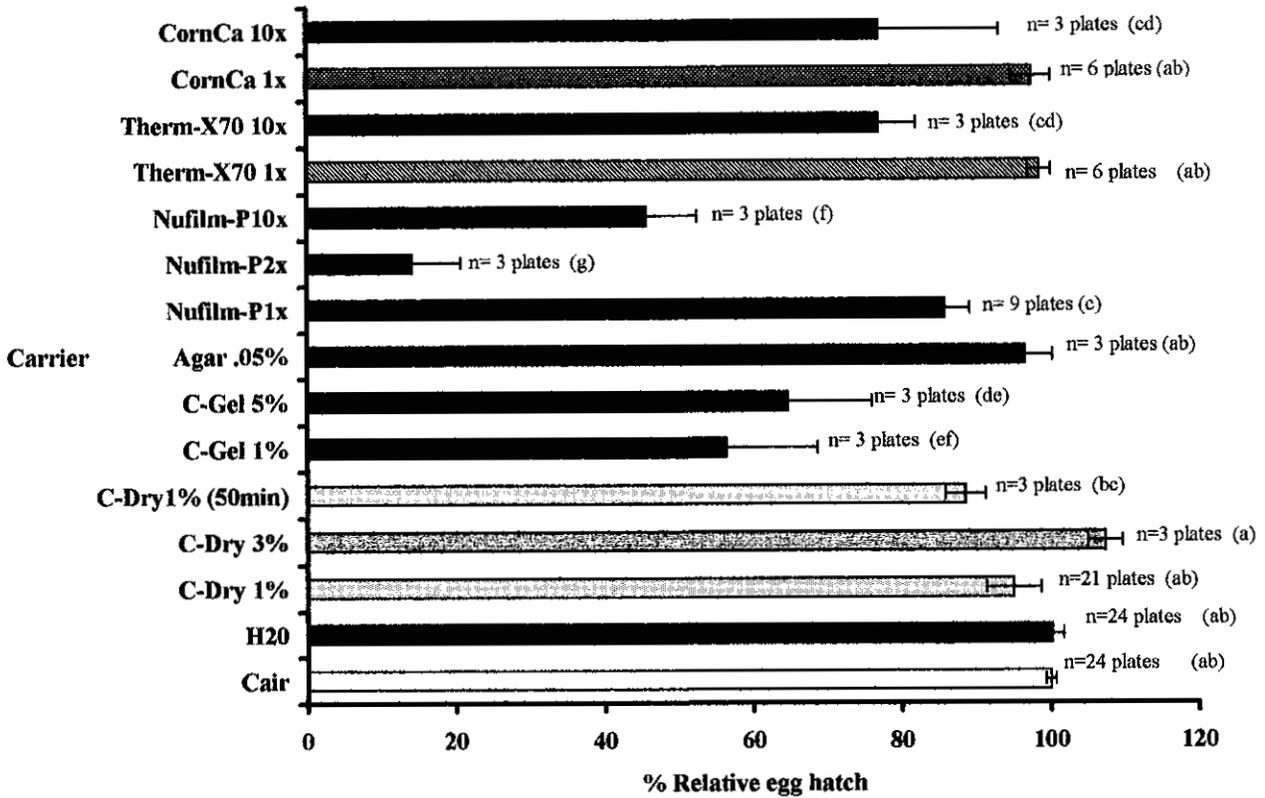


Figure 1. Percent relative lacewing egg hatch after immersion in various liquid carriers during laboratory bioassays. Bars indicate standard errors. The number of replicated plates is indicated by n. Different letters indicate significant differences in treatments based on Fisher's LSD at $p = 0.05$.

Appendix 2. Field results from Coke Ranch releases.

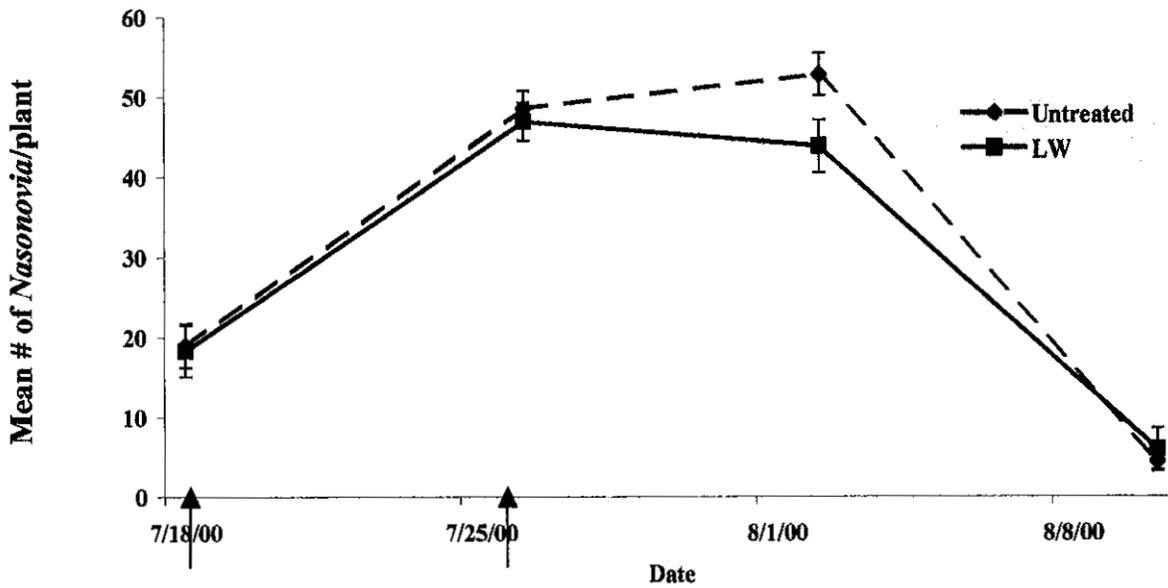


Figure 2.1 Mean number of *Nasonovia*/plant in untreated and lacewing egg release plots at Coke Ranch. Bars indicate standard errors. Arrows indicate release dates.

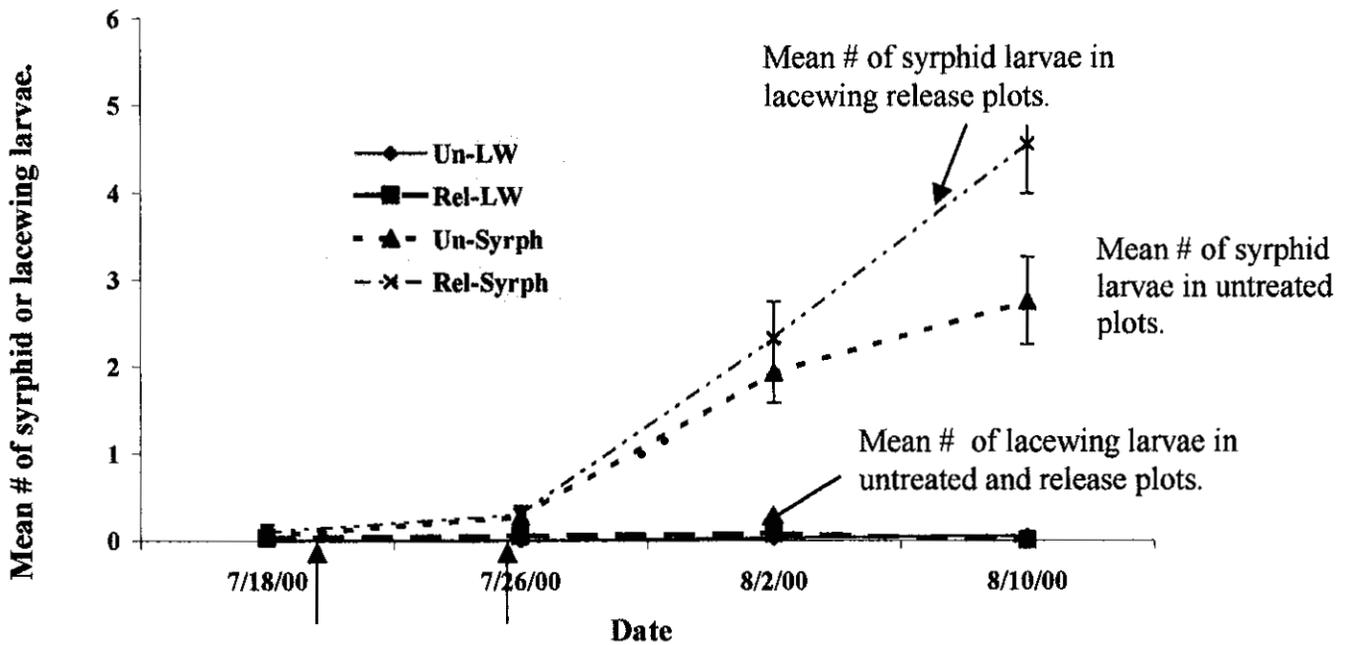


Figure 2.2 Mean number of syrphid and lacewing larvae in untreated and lacewing release plots at Coke Ranch. Bars indicate standard errors. Arrows indicate release dates.

Appendix 3. Field results from T&A releases.

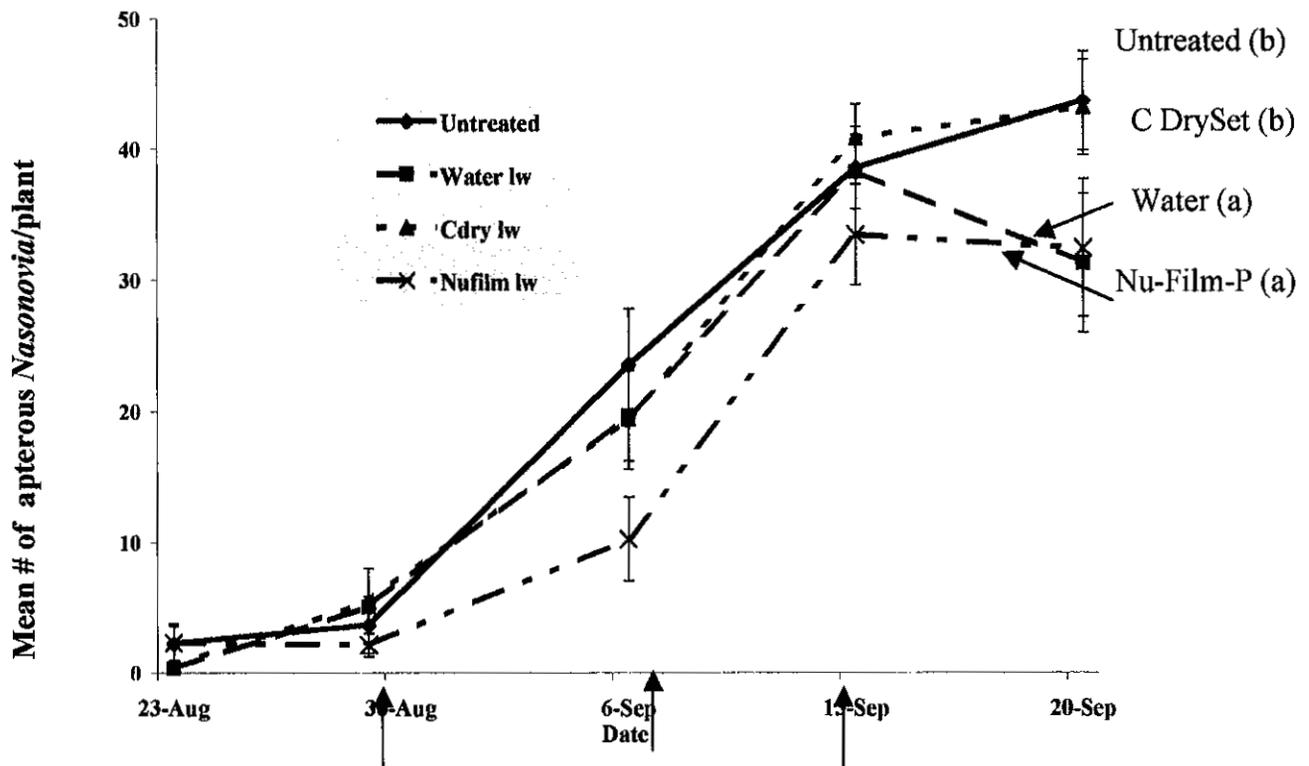


Figure 3.1 Mean number of apterous *Nasonovia* in untreated plots and plots that received lacewing eggs in various carriers. Bars indicate standard errors. Arrows indicate release dates. Different letters indicate significant differences in treatments based on Fisher's LSD at $p = 0.1$.

Appendix 3. Field results from T&A releases.

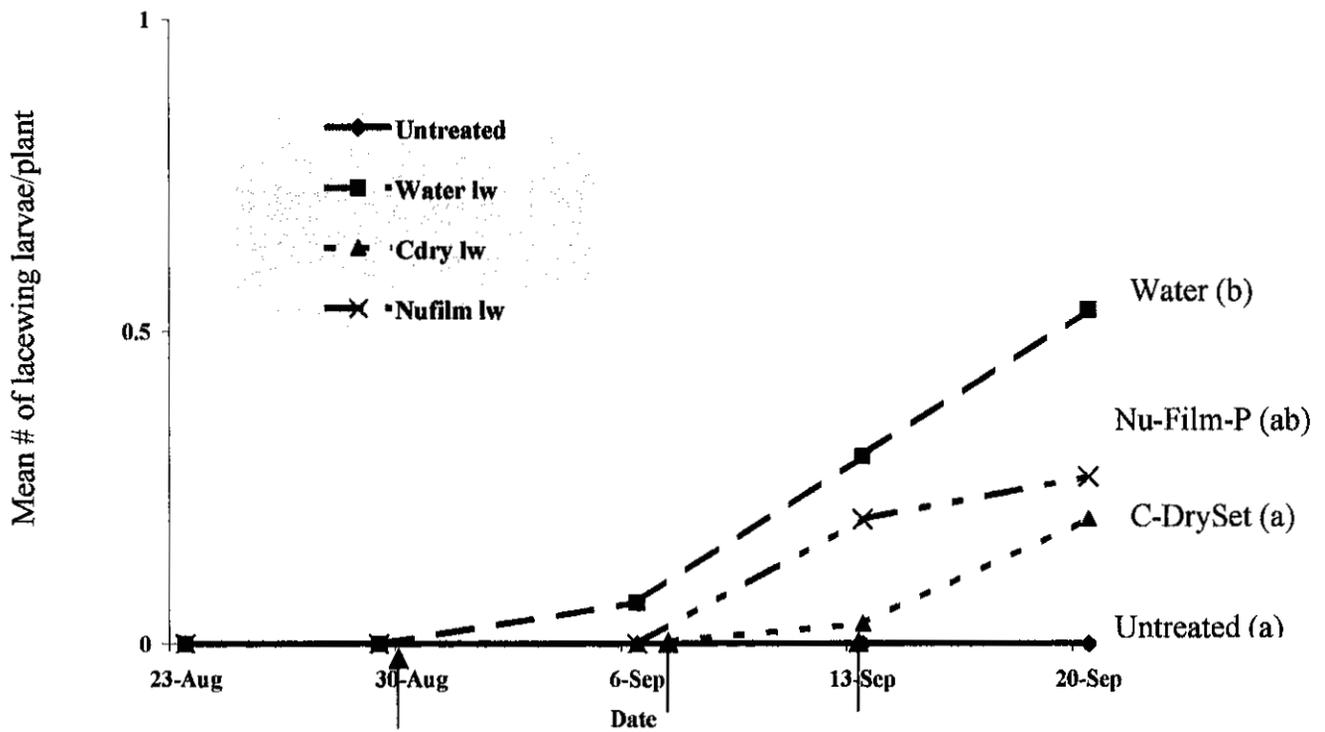


Figure 3.2 Mean number of lacewing larvae in untreated plots and plots which received lacewing eggs in various carriers. Bars indicate standard errors. Arrows indicate release dates. Different letters indicate significant differences in treatments based on Fisher's LSD at $p = 0.05$.

Appendix 4: References Cited.

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