Research Project Report

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Titles: Developing sustainable methods for controlling invasive pests pre- and post-invasion on ornamental cuttings and plants; Developing sustainable methods for controlling invasive pests on ornamental plant cuttings
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Abstract

The project entitled "Developing sustainable methods for controlling invasive pests on ornamental plant cuttings" had 6 objectives (Table 1) with two primary goals of studying the newly introduced *Duponchelia fovealis* and to examine methodologies to reduce interstate and international shipment of invasive species using model crop-pest systems. An unstated objective was to disseminate the newly gathered information to stakeholders via technical literature and presentations to growers. Our research team determined the minimal and maximum temperature thresholds for *D. fovealis* development with the optimal temperature being 32.2ºC. The current *D. fovealis* population within San Diego county was surveyed over time demonstrating multiple generations per year. In examining hot water immersion treatments as a potential means of disinfestation, rooted chrysanthemum cuttings exhibited higher heat tolerance than unrooted cuttings and subsequent growing conditions can impact growth and market readiness. Poinsettia cuttings were not able to tolerate the same temperatures as chrysanthemums. For pre-shipment efficacy on cuttings, aphid, mite and thrips populations were reduced by dips into natural products but not consistently to the level required to eliminate interstate or international shipping of these pests. For actual shipping, citrus mealybug populations were also reduced with dip treatments generally providing better control than spray treatments. BotaniGard and Safari reduced mealybug populations to virtually zero by approximately 2 weeks after application. With silverleaf whitefly, none of the natural products sufficiently reduced populations after shipping to warrant their use as regulatory pre-shipment treatments.

Table 1. Project Objectives and Milestones

Objectives	Milestones
Duponchelia fovealis	
Obj. 1: Describe *D. fovealis* life cycle	Development of *D. fovealis* life stages under different temperature regimes
Obj. 2: Study seasonal abundance of *D. fovealis*	Conduct an area-wide monitoring program using pheromones with focus on key pepper and strawberry producing areas of CA
Obj. 3: Determine impact of entomopathogens, heat treatments and conventional materials on *D. fovealis*	Comparative efficacy levels determined for various treatments under actual shipping
Mitigation of arthropods during shipping	
Obj. 4: Study performance and crop safety of entomopathogens, heat treatments and conventional materials prior to shipment	Comparison of biological tools with conventional chemical standard(s) during preliminary experiments Determine whether products cause injury to test crops.
Obj. 5: Study performance of biological tools during simulated shipping	Comparison of biological tools with conventional chemical standard(s) during simulated shipping of boxed cuttings
Obj. 6: Study performance of tools during shipping among CA, FL and HI	Comparison of biological tools with conventional chemical standard(s) during actual shipping of boxed cuttings efficacy for model arthropods such as *B. tabaci*, *T. urticae, P. phenacoccus citri*, and *C. hesperidum*.

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European Pepper Moth

Developing methodology to rear Duponchelia fovealis

An efficient method of rearing the European Pepper Moth (EPM) has been developed. At least two of each sex (at least 4 total/vial) were placed in a 40 dram plastic vial with a hole drilled in the bottom, and covered by mesh for ventilation. A 3-inch square of paper towel was folded and pushed through a pin attached to the lid of the vial. The paper towel was moistened with water and brushed with honey to provide a food and water source. Eggs were laid on the inner surface of the vial and hatch in approximately 5-7 days.

Once neonates were observed, 5 – 10 larvae were brushed into a 3/4 X 5/8 inch round container supplied by Durphy Packaging (47 Richard Road, Ivyland, Pennsylvania). The food source was unprepared Tobacco Hornworm Diet (Bio Serv, One 8th Street, Suite One, Frenchtown, New Jersey). A 1 cm^3 cube of diet was added to the containers, with each dish numbered and dated. The neonates were given a couple days to establish and then counted. Dishes were stored on a countertop at room temperature (72.4° F± 3.0°, RH 34% ± 10%). At these temperatures, it takes approximately 35.8 days for *Duponchelia* to develop from neonate to adult. Larvae were sometimes transferred to other/new dishes to keep a low population in each dish. More than ten larvae cause poor pupal development/dish. Each dish was counted every 4-5 days to monitor the progress of the larvae. Larvae were fed a 1 cm^3 cube of diet once a week. Pupae were formed using the frass available in the dishes. Adults emerged 7-14 days later. Adults were collected, the sex recorded, and added back into a 40 dram vial to reproduce.

In 2012, Dr. Bethke's laboratory produced an average of 250 eggs, 112.53 larvae and 35.65 adults per week. Males were produced slightly more than females (51% vs. 49%). Approximately eight vials were used per week to house the adults.

Objective 1: Describe D. fovealis life cycle

Studies on the life history of EPM began on 1 Dec 2011 and were completed on 20 Jan 2012. Observations were made on 68 individual females and accompanying males. Some individuals were removed from the analysis due to handling mortality. Occasionally, a female was moved to another vial and a newly hatched male was added if the original male died prior to the end of female life (n=19). Longevity, fertility and fecundity were recorded. Observations of longevity were observed on 28 males and 37 females. Not all individuals were observed for mortality and some were removed from analysis because the mortality was missed. Fifty percent of the females laid eggs but were infertile and some females laid no eggs. Since an individual male was paired with a single female, there was potential for lack of mating due to female choice. Females that laid no eggs were excluded from the analysis (Table 8).

Table 2. Longevity (±STD) of male and female European pepper moth adults, and female European pepper moth fecundity and fertility under selected environmental conditions.

GENDER	N	Longevity (days)	N	Mean no. Eggs	Range	Mean No. Hatched	Range	Mean % Hatch
Females	37	9.6 ± 3.1	58	266.8 ± 166.8	23-584	121.4 ± 127.1	0-450	65.0 ± 79.1
Males	28	11.8 ± 3.8						
Combined	65	10.6 ± 3.5						

Dr. Jim Bethke studied the development of *D. fovealis* from 50° to 55° F. Newly hatched larvae from the maintained colony were added to a Durphy friction sealed container. One larva was added per dish and assigned a number. Thirty larvae were used per temperature. Each set was placed in a Percival environmental chamber where the humidity was kept at 50% and light cycle at 10/14 light/dark. Larvae were observed every day, and their head capsules measured to document instar changes. Head capsules were measured in micrometers using a slide under a stereoscope. Temperatures ranging from 50 to 100° F were tested at 5° intervals.

One hundred percent mortality (no development to pupae) occurred at 50 and 95° F. At lower temperatures, EPM sometimes had five instars but at four at higher temperatures (Table 3).

Table 3. Average days for total *D. fovealis* development at different temperatures.

Temperature °F	Temperature °C	Average days from egg hatch to adult emergence
60	15.5	105.4
70	21.1	40.0
80	26.6	25.2
90	23.5	23.5

To develop data for a degree day model, the length of time was assessed for *D. fovealis* to develop from one instar to the next at various temperatures under controlled conditions. Initial studies demonstrated that *D. fovealis* did not develop at 10ºC and 35ºC so temperatures were selected between these extremes. Neonate larvae fed on an artificial diet were introduced to environmental chambers at one of 7 constant temperatures (10ºC, 12.8ºC, 15.5ºC, 21.1ºC, 26.6ºC, and 32.2ºC, and 35ºC) and photoperiod of 16:8 (L:D). Individuals were monitored daily for molting to the next stage or death.

Mean time to adult was fastest (20.7 d) for *D. fovealis* maintained at 32.2ºC (Table 4). The mean development times (days) for each instar at 26.6ºC, first through fifth instar, respectively, was 3.0, 2.2, 4.2, 6.3, and 5.8 days. When data for larvae which died were excluded, similar mean development times occurred (Table 5). Male and female moths completed life cycles at consistent times with the exception that female larvae did not develop at 32.2ºC (Table 6, Table 7).

Using nonlinear regression, we estimated that the lower developmental threshold was 10.04ºC, with a maximum threshold of 35.0ºC and the optimum developmental rate at 32.2ºC (Figure 1, Figure 2). Adjusting for a lower minimum threshold calculated by linear regression, we determined that an adjusted lower developmental threshold was approximately 10.25ºC, with 454.5 degree-days required to complete development from newly hatched larva to adult. Temperature also significantly affected the overall survival of larvae and the overall time from larvae to adult as estimated by survival time analysis. Utilizing the estimated minimum and maximum developmental temperatures has implications for modeling where *D. fovealis* might invade with changing climate.

Table 4. Length of time *Duponchelia* moths completed their development within each stadium but not necessarily their entire development at each of seven temperatures (°C).

	Mean Time (days)						
Temperature (°C) [*n*]	First	Second	Third	Fourth	Fifth	Pupae	Total
10.0	-	-	-	-	-	-	-
12.8 [14]	13.7 (0.4)	14 (0.8)	17.9 (1.1)	32.5 (2.6)	47.1 (2.5)	39.4 (0.8)	164.7 (4.0)
15.5 [19]	11.8 (0.2)	7.6 (0.6)	13.2 (0.7)	13.6 (0.6)	30.4 (2.2)	28.9 (0.4)	105.5 (2.6)
21.1 [20]	3.9 (0.2)	5.1 (0.3)	7.2 (0.4)	4.5 (0.3)	9.0 (0.4)	10.1 (0.2)	39.7 (0.7)
26.6 [9]	3.0 (0.0)	2.2 (0.2)	4.2 (0.3)	6.3 (0.7)	5.8 (0.2)	7.9 (0.2)	29.4 (0.8)
32.2 [3]	2.3 (0.3)	3.7 (0.3)	2.7 (0.3)	5.7 (0.3)	-	6.3 (0.3)	20.7 (0.3)
35.0	-	-	-	-	-	-	-

*No fifth instar at 32.2°C.

A '-' denotes that development did not occur for that stadium at that temperature.

Table 5. Length of time *Duponchelia* moths completed their development from egg hatch to adult at each of seven temperatures (°C) [*n*].

Temperature (°C)	First	Second	Third	Fourth	Fifth	Pupae	Total
10.0	-	-	-	-	-	-	-
12.8	13.6 (0.3) [30]	13.4 (0.4) [30]	18.0 (0.7) [30]	33.4 (2.2) [30]	47.1 (2.5) [14]	39.4 (0.8) [14]	164.7 (4.0) [14]
15.5	11.7 (0.2) [30]	7.8 (0.4) [30]	12.7 (0.6) [30]	13.8 (0.6) [29]	30.8 (2.1) [19]	28.9 (0.4) [19]	105.5 (2.6) [19]
21.1	4.0 (0.2) [27]	4.9 (0.2) [27]	7.1 (0.3) [27]	4.5 (0.2) [27]	9.0 (0.4) [20]	10.1 (0.2) [20]	39.7 (0.7) [20]
26.6	3.0 (0.1) [22]	3.4 (0.7) [22]	7.3 (1.4) [21]	7.2 (1.3) [17]	5.8 (0.3) [9]	7.9 (0.3) [9]	29.4 (1.1) [9]
32.2	2.3 (0.1) [29]	3.9 (0.1) [29]	2.6 (0.1) [29]	5.7 (0.3) [3]	-*	6.3 (0.3) [3]	20.7 (0.3) [3]
35.0	5.4 (0.5) [14]	-	-	-	-	-	-

*No fifth instar at 32.2°C.
A '-' denotes that development did not occur for that stadium at that temperature.

Table 6. Length of time female *Duponchelia* moths completed their development from egg hatch to adult at each of seven temperatures (°C) [*n*].

Temperature (°C) [*n*]	First	Second	Third	Fourth	Fifth	Pupae	Total
10 [0]	-	-	-	-	-	-	-
12.8 [10]	13.4 (0.4)	14.2 (1.1)	18.7 (1.3)	33.1 (2.7)	46.5 (3.3)	38.9 (0.9)	164.8 (4.9)
15.5 [11]	11.6 (0.3)	7.1 (0.4)	12.8 (0.8)	14.9 (0.8)	31.5 (2.8)	28.9 (0.6)	106.8 (3.7)
21.1 [8]	4.6 (0.3)	5.5 (0.5)	6.6 (0.5)	5.0 (0.5)	8.0 (0.6)	10.0 (0.4)	39.1 (0.9)
26.6 [5]	3.0 (0.0)	2.0 (0.3)	4.0 (0.4)	6.4 (1.1)	6.0 (0.3)	7.6 (0.2)	29.0 (1.4)
32.2 [0]	-	-	-	-	-	-	-
35	-	-	-	-	-	-	-

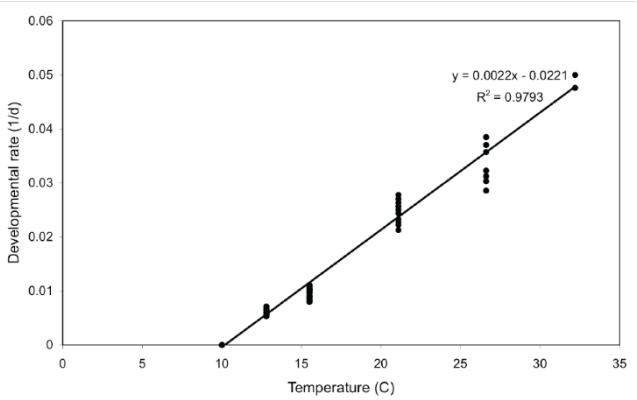
*No fifth instar at 32.2°C.
A '-' denotes that development did not occur for that stadium at that temperature.

Table 7. Length of time male *Duponchelia* moths completed their development from egg hatch to adult at each of seven temperatures (°C) [*n*].

Temperature (°C) [*n*]	First	Second	Third	Fourth	Fifth	Pupae	Total
10 [0]	-	-	-	-	-	-	-
12.8 [4]	14.5 (1.2)	13.5 (1.0)	16.0 (1.4)	31.0 (6.8)	48.8 (4.0)	40.8 (1.6)	164.5 (8.2)
15.5 [8]	12.1 (0.2)	8.4 (1.2)	13.6 (1.4)	11.8 (0.5)	29.0 (3.7)	28.9 (0.6)	103.8 (3.5)
21.1 [8]	3.7 (0.1)	5.0 (0.5)	7.3 (0.5)	4.3 (0.4)	9.5 (0.5)	10.3 (0.3)	40.1 (1.0)
26.6 [5]	3.0 (0.0)	2.5 (0.6)	4.5 (1.0)	6.3 (1.8)	5.5 (0.6)	8.3 (0.5)	30.0 (2.0)
32.2 [3]	2.3 (0.3)	3.7 (0.3)	2.7 (0.3)	5.7 (0.3)	-*	6.3 (0.3)	20.7 (0.3)
35	-	-	-	-	-	-	-

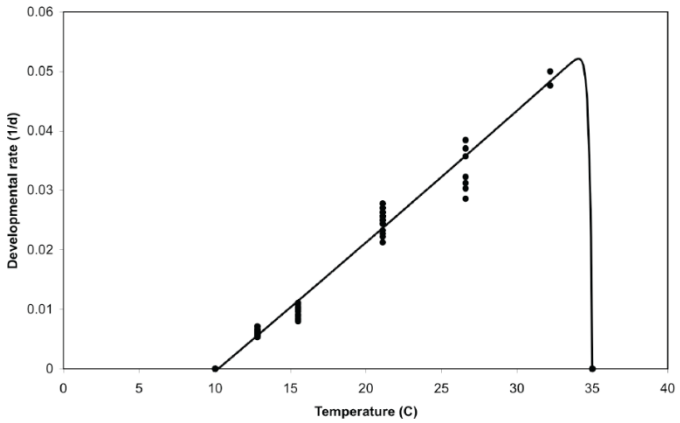
*No fifth instar at 32.2°C.
A '-' denotes that development did not occur for that stadium at that temperature.

Figure 1. A developmental growth rate curve for European pepper moth.



This curve is for the overall (=total) development rate of moths. Simulation estimates show that the developmental threshold (=Tmin) of this insect is 10.25°C, with the fastest rate of development estimated to be 34.1°C (=highest point on this curve). Simulated maximum temperature (=Tmax) is 35°C. This curve was created with predicted development rates in Proc NLIN of SAS (nonlinear regression, Pseudo $R^2 = 0.98$).

Figure 2. The regression line presented this line estimates Tmin at 10.04°C.



The cumulative degree days is 454.5. If this is compared against the simulated Tmin of overall development from Fig. 1 (=10.25°C), this linear regression is only 0.2°C different.

Objective 2: Study seasonal abundance of D. fovealis

This research shifted to focus on trapping in ornamental horticulture production nurseries rather than the initially planned strawberry fields. Traps were placed and data collected from ornamental horticulture nurseries in Encinitas and San Luis Rey.

During 2014, we collected *D. fovealis* in six locations within San Diego County. Traps baited with pheromone were place in Oceanside, San Marcos, Del Mar, Pauma Valley, Kearney Mesa, and Chula Vista (Figure 3). Very high populations were detected in Oceanside; very few moths were collected Del Mar (Figure 4). None were detected in Kearny Mesa or Chula Vista.

During 2015, we monitored *D. fovealis* populations at a trap next to a portable weather station to compare temperature and counts over time on a monthly basis. Samples were collected from January through October. Fewer moths were collected from January through March, while higher populations were observed from April through October (Figure 4). Due to the traps proximity to a portable weather station, comparisons can be made to *D. fovealis* populations and temperature and moisture. When comparing the two data sets, populations seem to have a loose correlation to increased temperatures and dew points (Figure 5).

Figure 3. Pauma Valley, Oceanside, San Marcos, Kearny Mesa, Del Mar, and Chula Vista.

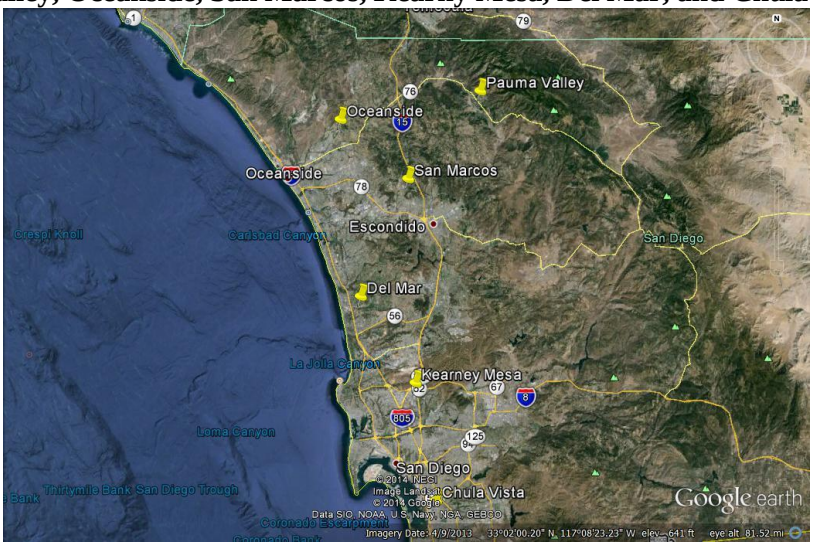


Figure 4. Population counts of *Duponchelia fovealis* during July and August 2014.

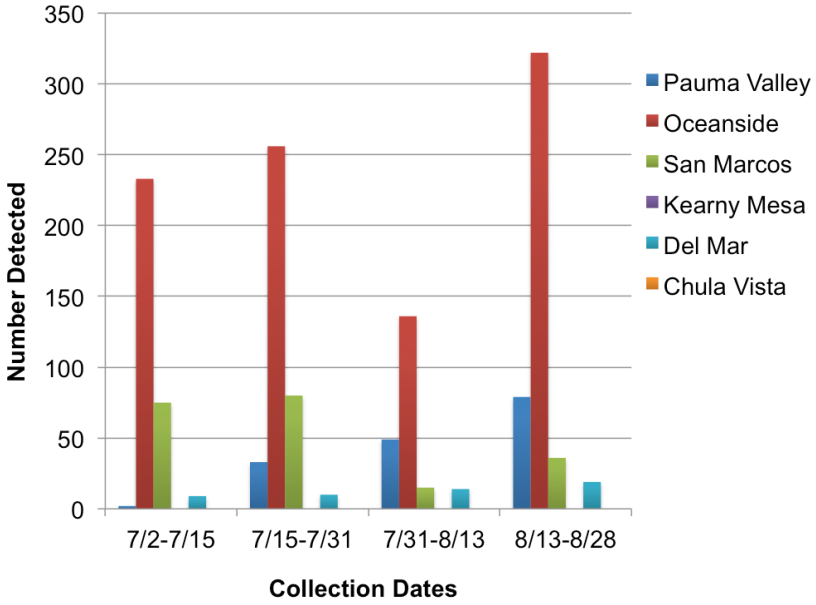


Figure 5. Population counts of *Duponchelia fovealis* during January through October 2015.

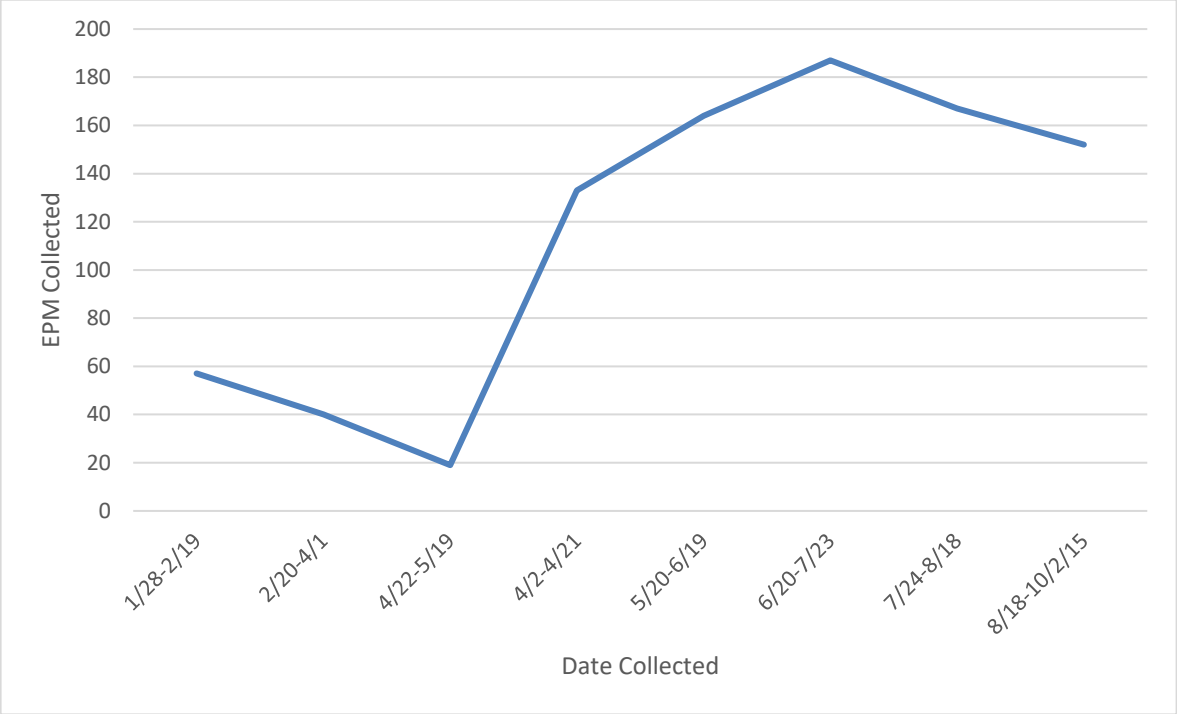
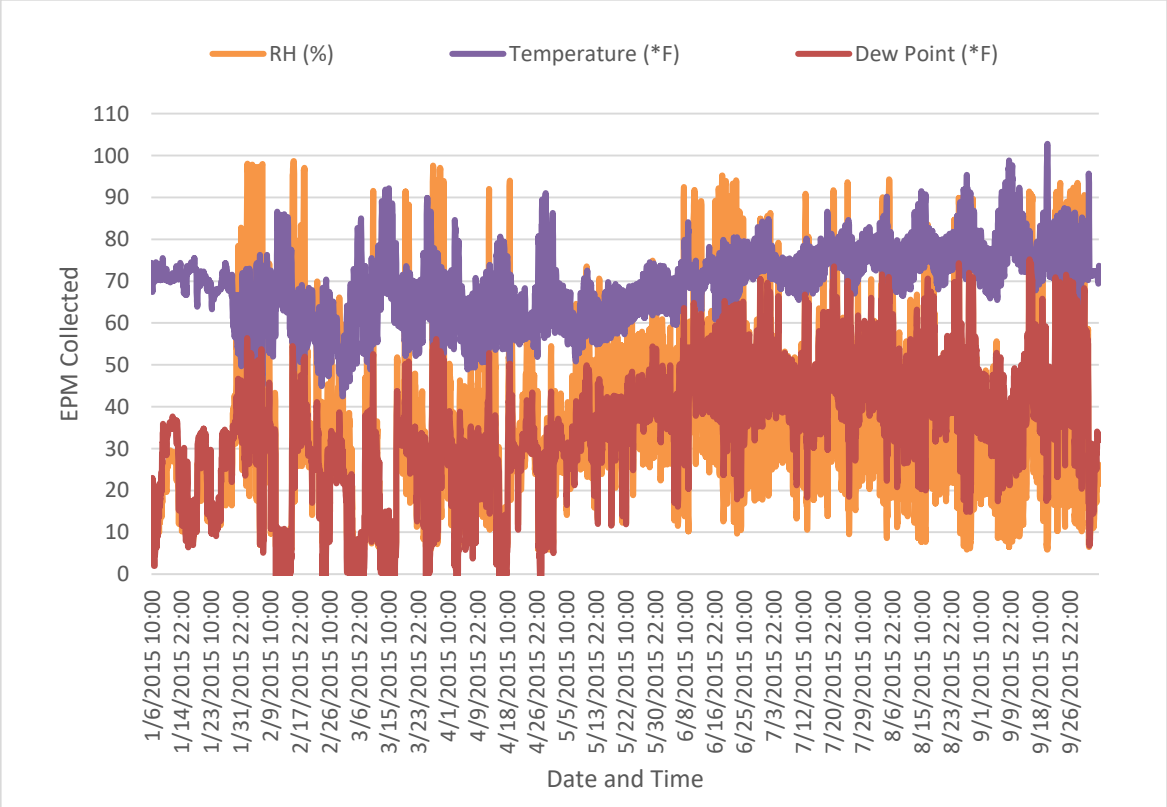


Figure 6. Weather data for *Duponchelia fovealis* trap sites during January through October 2015.



In addition, UCCE San Diego was able collect data in cooperation with the County of San Diego's Agriculture Weights and Measures' Light brown apple moth (LBAM) monitoring program. This monitoring was composed of 4419 trapping grids covering the entirety of San Diego County. We collected 614 individual European Pepper Moth (*D. fovealis*) samples from a total of 138 sites between 01-2015 and 09-2015. Each trap ranged from 0 to 310 individual EPM, with a mean of 8.6 EPM per trap and a sample standard deviation of 30 (Figure 7). Note the slope of the line of regression showing a steady increase in overall occurrences during the survey period. We see 6 distinct peaks in populations occurring in early January (57), early April (133), mid May (164), late June (310), late July (167), and mid August (189) which may represent distinct generations.

Geographic data was collected for each sample (Figure 8). With this data we are able to see where the highest concentrations of EPM were located and how they have moved over time. High-density populations occurred in a few locations, but further climate data is needed to determine if there are any correlations. Further correlation data will be presented at the 2015 Entomological Society of America's annual meeting in November.

Figure 7. 2015 survey results of *D. fovealis* sampling.

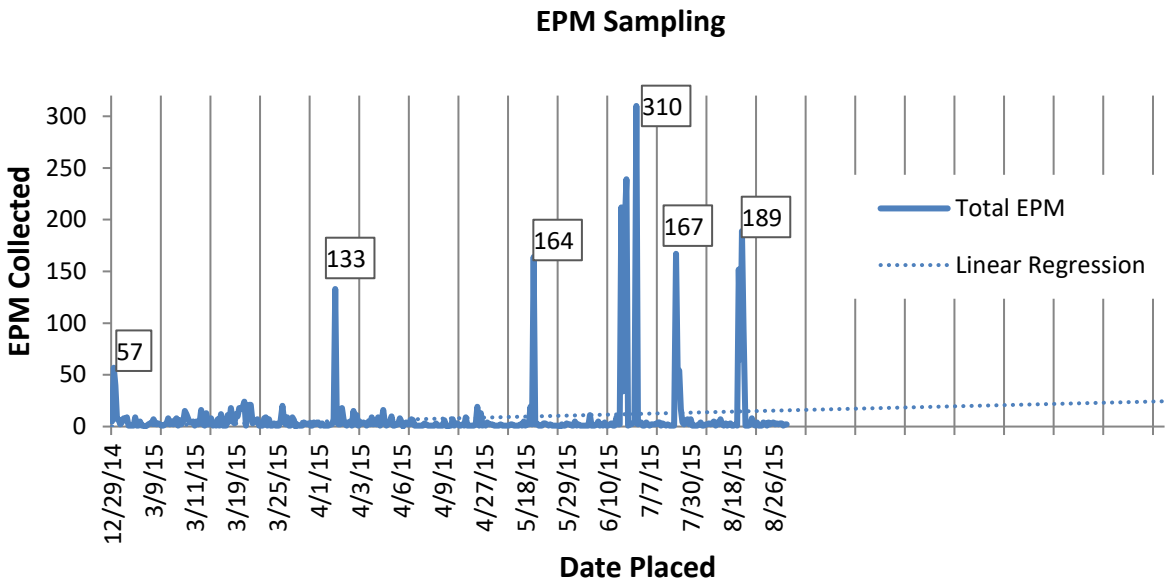
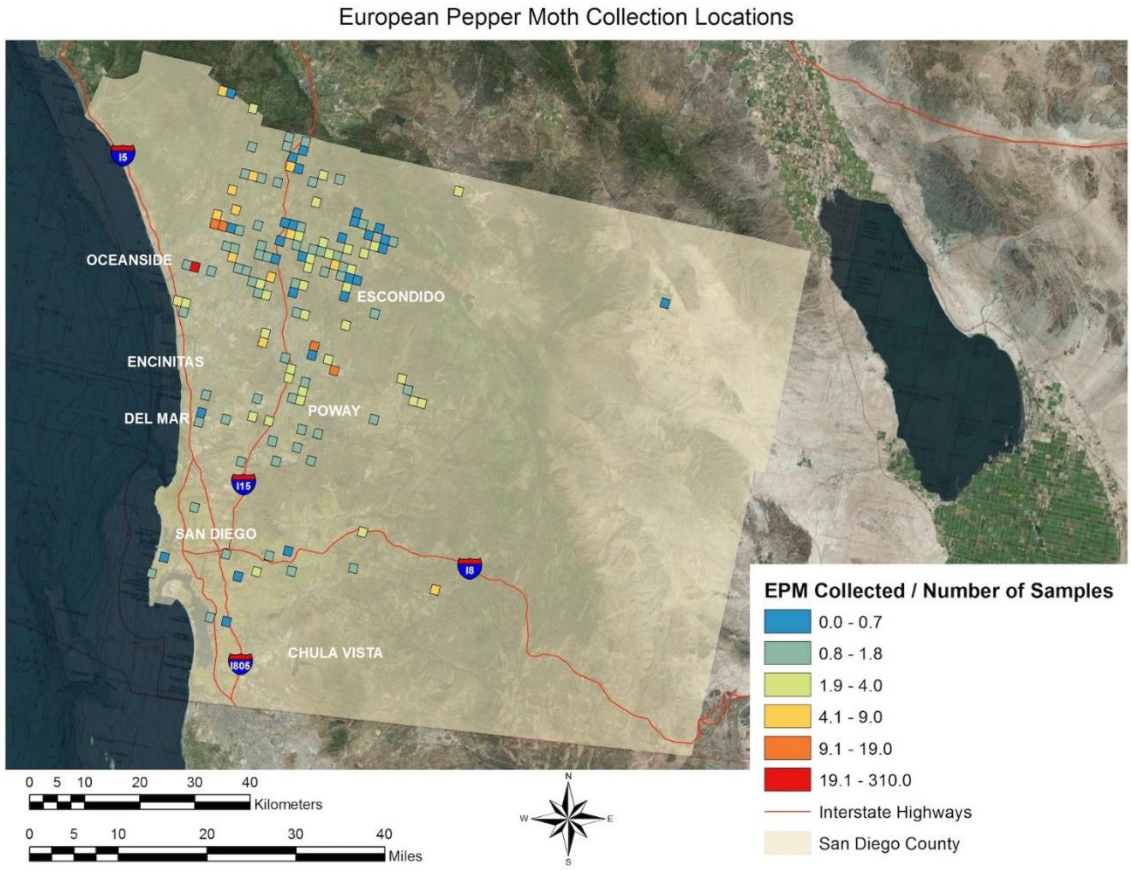


Figure 8. 2015 survey results of *D. fovealis* sampling.



Objective 3: Determine impact of entomopathogens, heat treatments and conventional materials on D. fovealis.

Dr. Bethke conducted two insecticide trials: a leaf dip assay against early instar larvae and a spray trial using infested plants.

For the leaf dip assay, second instar larvae were exposed to cowpea leaves dipped in the treatment solutions for one minute. After the leaves had air dried, one leaf was placed in a friction sealed plastic petri dish along with five larvae. The number of alive and dead larvae was counted at 1, 3 and 7 DAT.

For the plant fungicide experiment, *Vinca minor* plants were placed in an insect cage on an expanded metal bench in a temperature-controlled greenhouse. One week prior to the first fungicide application, five second instar larvae were added to each pot. After the second application, each pot was placed in a paper bag and the number of adult moths that developed was counted. Four post-application evaluations were completed at 7, 14, 21 and 28 days after the second application. Data were analyzed using ANOVA, and means were separated using Fisher's LSD ($\alpha = 0.05$). The data were arcsine square root transformed.

The percent survivorship for the leaf dip portion was recorded in Table 11. Proclaim with or without the addition of surfactant achieved excellent results 7 DAA. Scimitar and the check were statistically higher in survivorship than the rest of the treatments. Table 12 showed the average number of adults that developed per pot after treatments. Only the check and Scimitar recorded a significant number of developed adults as compared to the rest of the treatments.

Table 8. Efficacy of selected insecticides and surfactants on early instar larvae of European pepper moth in a leaf dip assay.

Treatment	Rate/100 gal	Surfactant v/v%	1 DAA	3 DAA	7 DAA
Check	---	---	90.0 a	90.0 a	90.0 a
Proclaim 5 SG +Dyne-amic	2.4 oz	0.5	68.8 abc	30.0 cb	5.0 c
Proclaim 5 SG +Dyne-amic	4.8 oz	0.5	73.8 ab	36.3 b	0.0 c
Proclaim 5 SG +Dyne-amic	9.6 oz	0.5	40.0 d	15.0 cb	0.0 c
Proclaim 5 SG +Ultra Pure Oil	4.8 oz	1.0	46.3 cb	0.0 d	0.0 c
Proclaim 5 SG	4.8 oz	---	95.0 a	31.3 b	0.0 c
A16901B WG +Dyne-amic	1.78 oz	0.5	46.3 cb	22.5 cb	0.0 c
Scimitar GC	4.8 oz	---	95.0 a	75.0 a	65.0 b
Coragen	5.0 oz	---	100.0 a	80.0 a	0.0 c
Belt SC	3.0 oz	---	95.0 a	70.0 a	5.0 c

Day	Df	F value	Pr>F
1	10	7.64	<.0001
3	10	8.81	<.0001
7	10	17.85	<.0001

Table 9. Efficacy of selected insecticides against early instar larvae of the European pepper moth infesting potted *Vinca minor*.

Treatment	Rate/100 gal	Surfactant v/v%	Avg. # adults per pot emerged
Check	---	---	1.34 a
Proclaim 5 SG +Dyne-amic	2.4 oz	0.5	0.12 b
Proclaim 5 SG +Dyne-amic	4.8 oz	0.5	0.00 b
Proclaim 5 SG +Dyne-amic	9.6 oz	0.5	0.00 b
Proclaim 5 SG +Ultra Pure Oil	4.8 oz	1.0	0.00 b
Proclaim 5 SG	4.8 oz	---	0.00 b
A16901B WG +Dyne-amic	1.78 oz	0.5	0.00 b
Scimitar GC	4.8 oz	---	1.01 a
Coragen	5.0 oz	---	0.00 b
Belt SC	3.0 oz	---	0.12 b

DF=10, F value= 14.24, Pr>F=<.0001

A commercially available source of rove beetle, *Atheta coriaria* was obtained and added to a container of Sunshine Mix potting soil. The container was stored at room temperature and the soil was kept moist. *Atheta* were aspirated and added to a friction sealed plastic petri dish. Various life stages of *Duponchelia* larvae were added and their interaction observed. As stated in other scientific literature (Messelink & VanWensveen, et. al. 2003), *Atheta coriaria* will feed upon first instar *Duponchelia* larvae (Figure 20). It was observed that once the *Duponchelia* molted to second instar, *Atheta* predatory feeding ceased due to the larger size of the larvae. It was reported that *Atheta* can decrease the population of *Duponchelia* larvae in a greenhouse potted plant system by approximately 60% (Messelink & VanWensveen, et. al. 2003).

UCANR, Bethke, 2012

To test the residual activity of three registered products against *D. fovealis* (Table 10), treatments were sprayed once to runoff using 100 gal/A. The Enfold treatments included Dyne-Amic spreader/sticker at 0.5% v/v, while Conserve and Scimitar did not have any additional surfactants. To measure the effectiveness, second instar larvae were contained in clip cages at 1, 5, 7, 14 & 21 days after the application. Two clip cages were placed on the upper surface of the leaves and two cages on the lower surface in order to document any change in mortality due to the amount of chemical reaching the underside of the leaves.

At 1 day after application (DAA) there was survivorship on all treatments except for Conserve where there was 0% survivorship on the surface and 100% on the lower surface (Table 10). By day seven, there was a reversal in

activity as the Enfold treatments had no survivors while Conserve had 100% survivors. In general, survivorship of larvae was greater on the undersides of leaves as shown in the last column. Based on these results, Enfold @ 2.4 proved most effective in reducing the number of *Duponchelia* larvae on both the upper and lower surfaces of leaves.

Figure 9. Rove Beetle attacking a first instar stage of *Duponchelia fovealis* (photo by Bryan Vander Mey)

Table 10. Percent survivability of larvae

Chemical	Cage Position	1 DAA	5 DAA	7 DAA	14 DAA	21 DAA	Mean
Enfold (emamectin benzoate) @ 2.4 oz per 100 gal	top	60	50	0	0	25	27
	bottom	100	50	0	33	100	56.6
Enfold @ 4.8 oz per 100 gal	top	50	50	0	100	60	52
	bottom	100	100	0	50	100	70
Scimitar @ 5 oz per 100 gal	top	100	33	0	50	60	48.6
	bottom	100	100	100	50	100	90
Conserve @ 22 oz per 100 gal	top	0	0	100	50	100	50
	bottom	100	50	100	75	83	81.6
UTC	top	100	100	100	50	100	90
	bottom	100	100	100	0	100	80

Duponchelia host range expansion to rosemary

A local grower of perennial evergreens contacted Dr. Bethke regarding a lepidopteron observed in his crops. The species turned out not to be *D. fovealis* but we took the opportunity to place larvae on rosemary and observe whether they survived or not. *D. fovealis* did indeed develop on rosemary and is documented in the Figure 10 showing a larva, feeding damage and webbing.

Figure 10. *Duponchelia* development on rosemary.

Mitigation of Arthropod During Shipping

Objective 4: Study performance and crop safety of entomopathogens, heat treatments and conventional materials prior to shipment

Literature Review.

For heat treatments, Dr. Arnold Hara reviewed the literature for previous heat treatment results for arthropods infesting nurseries in HI and on heat tolerance of model cropping systems. Between 43 °C and 54 °C provided optimal mortality with temperature and duration varying with each species (Table 11). In reviewing available data for impact of hot water treatments on ornamental horticulture crops, chrysanthemums appeared to tolerate high temperatures slightly better than poinsettias (Table 12). However, there did appear to be some variability in highest temperature and duration for survival without detrimental growth affects which might be due to different chrysanthemum species and cultivars.

Table 11. Literature review of heat treatments for pest species.

Pest	Life stage	Treatment Type	Temperature & Duration	Reference
Whitefly	*Bemisia tabaci* adult females	Hot Air	40 °C for 240 min for 100% mortality 40 °C for 300 for 100% moratlity with symbiotic bacterium (Rickettsia)	Brumin et al. 2011
	Bemisia argentifolii early nymphs	Hot Water	43 °C for 15 min for 95% mortality	Romero 2011
Mite	*Tetranychus urtica* diapausing	Hot Water	44 °C for 211 min for 99% mortality 47 °C for 67 min for 99% mortality 54 °C for 3.6 min for 99% mortality	Lester et al. 1997
	Tetranychus urtica non-diapausing	Hot Water	44 °C for 102 min for 99% mortality 47 °C for 34.9 min for 99% mortality 54 °C for 2.9 min for 99% mortality	
Mealybug	*Planococcus citri* nymphs	Hot Water	49 °C for 12 min for 100% mortality	Hara et al. 1997
	Planococcus citri adults	Hot Water	49 °C for 12 min for 99.2% mortality	
Soft Scale	*Coccus viridis* adults and crawlers	Hot Water	49 °C for 10 min for 99.9% mortality	Hara et al. 1994b
	Coccus viridis nymphs	Hot Water	49 °C for 10 min for 99.7% mortality	
Leafminers	*Liriomyza huidobrensis* eggs	Hot Water	45.5 °C for 4 min for 97.7% mortality	Cuthbertson et al. 2009
	Liriomyza huidobrensis pupae	Hot Water	45 °C for 18 min for 97.7% mortality	

Table 12. Literature review of hot water immersion treatments for pest species.

Crop	Treatment Type	Temperature & Duration	Impact	Reference
Chrysanthemum morifolium 'Iridon' tip cuttings	Hot Water	49 °C for up to 6 min	No negative effects on cuttings' survival, rooting, or vegetative growth	Hara et al. 1994a
Dendranthema grandiflorum 'Sunny Shasta' tip cuttings	Hot Water	41 °C for 5 min	No detrimental effects	Romero 2011
		41 °C for 15 min	84% survival	
		43 °C for 5 min	73% survival	
Potted chysanthemums (6-8 leaf stage)	Hot Water	Pretreated with incubation at 20 °C for 24 h followed by hot water immersion (43.5 °C for 10 min or 45 °C for 7 min)	No effect on stem height, shoot dry weight or root dry weight	Cuthbertson et al. 2009
Poinsettia (*Euphorbia pulcherrima*) 'Prestige' tip cuttings	Hot Water	41 °C for 5 min	91% survival and no detrimental effects to roots	Romero 2011
		43 °C for 5 min	28% survival	

Chrysanthemum Phytotoxicity and Growth response to Hot Water Immersion, Experiment 1
This study was conducted at the University of Hawaii at Manoa, Waiakea Agricultural Research Center and a cooperating nursery near Hilo, Hawaii, under greenhouse conditions (polyroof, 15% shade), to determine the tolerance of 'Shasta Improved' chrysanthemums to hot water dip treatments for insect disinfestation. Two stainless steel tanks (160L barrel type) were filled with tap water: one heated to 48.8°C using two immersion circulator/heaters (Isotemp model IC-2150, Fisher Scientific Inc., Pittsburgh, PA); the second tank held at ambient temperature (24°C). At the agricultural research center, 10 randomly selected rooted mum cuttings were placed in 30cm x 35cm organza bags, which were secured at the top with a drawstring, and then submerged in water for the allotted treatment. Treatments included: bags of plants held in the heated water for 2, 4, and 6 minutes, then cooled in ambient water for 1, 2 and 3 minutes, respectively; a treated check in which plants were dipped and held in ambient water for 9 minutes; and an untreated check. Each treatment was replicated three times on three separate dates. All plants were transferred to the cooperating nursery post treatment where they were transplanted into 5" plastic pots of peat:perlite (1:1.5) and topdressed with Apex (16-6-13 + micronutrients, 1 tbsp/pot), then spaced on benches 5" apart in a CRD. During the first two growing weeks all plants received additional 4 hours of light at night under incandescent lights. Once plants were 7-8cm tall, their tips were pinched, and they were moved to outer benches where they received additional nutrients in three minute drip irrigations (Excel: 15-5-15) twice daily until market ready (90% opened flowers, approximately 10-12 weeks old). A foliar growth regulator (B9) was applied at two and five weeks after planting. Pesticides, applied at 10-14 day intervals, included rotations of Pylon, Overture, Hachi Hachi and Avid.

During this trial the average temperature was 24.9°C (37.7°C high, 17.7°C low) and the average humidity was 81.6% (100% high, 37.9% low). Plants were observed for symptoms of heat damage at 3 day intervals for the first two weeks and then weekly up to 17 weeks after treatment (WAT). Changes in height, leaf, and flower development were recorded. Heights (pretreatment and market ready) and flower production (market ready) were compared using ANOVA (Tukey's Mean Separation, Minitab16).

There were no differences in pretreatment heights (Table 13). Plants submerged for 2 minutes in water heated to 48.8°C or for 9 min in ambient water showed little to no signs of heat damage with high rates of survival (96.7%, 100%) and were not different from the untreated check (Figure 11). However, less than 40% of the plants exposed to 48.8°C for 4 minutes (32.4%) and 6 minutes (36.1%) survived the hot water dip treatment ($P<0.05$), and when plants were market ready, heat treated mums were shorter than the treated check ($P<0.05$) (Table 13).

Plants in the untreated check, treated check and 2 minute exposure were market ready within 11 WAT (range 10-12 wk) (Figure 12), while the plants that survived the 4 and 6 minute treatments were market ready approximately 4-5 wk later. All heat treated plants had fewer flowers than the treated check ($P<0.05$) (Table 13, Figure 14).

Summary: Chrysanthemum cuttings (rooted, 4 cm) were able to tolerate 2 minute exposure to 48.8°C water with no difference in survival rate, height at market, and weeks to market as compared to the checks. Survival rate dropped considerably ($P<0.05$) to an average of 34.25% when exposure time increased to 4 and 6 minutes, and these cuttings required 4-5 wk longer to reach market-ready status. Overall, plants exposed to hot water had fewer flowers at market as compared to the untreated check. Exposure to lower temperatures for longer durations may be better tolerated by chrysanthemum cuttings.

Table 13. Maturity, height and number of chrysanthemum 'Shasta Improved' flowers produced after dipped hot water heat treatment

Treatment	% Survival	Height Pretrt (cm)	Number of Weeks until Market-ready	Mean Height at Market (cm)	Mean Number of Flowers at Market
No treatment	100.0 A	4.1	10.6 A	23.2 A	43.3 AB
48.8°C dip then 24°C for 9 minutes	100.0 A	4.5	10.6 A	22.2 AB	43.9 A
48.8°C for 2 min then 24°C for 1 minutes	96.7 A	4.4	10.7 A	21.1 B	35.9 C
48.8°C for 4 min then 24°C for 2 minutes	32.4 B	4.0	14.9 B	18.6 C	32.3 C
48.8°C for 6 min then 24°C for 3 minutes	36.1 B	4.0	15.1 B	20.9 BC	34.0 BC

Figure 11. Chrysanthemum 'Shasta Improved' tolerance to hot water immersion at 1 week after treatment

Untreated mums

Treatment 1: 9 minutes @ 24°C

Treatment 2: 2 minutes @ 48.8°C

Treatment 3: 4 minutes @ 48.8°C

Treatment 4: 6 minutes @ 48.8°C

Figure 12. Market-readiness of chrysanthemum 'Shasta Improved' at 11WAT.

Untreated Check	Treated Check	2 min	4 min	6 min

Figure 13. Height impact of hot water immersion treatments on chrysanthemum 'Shasta Improved'

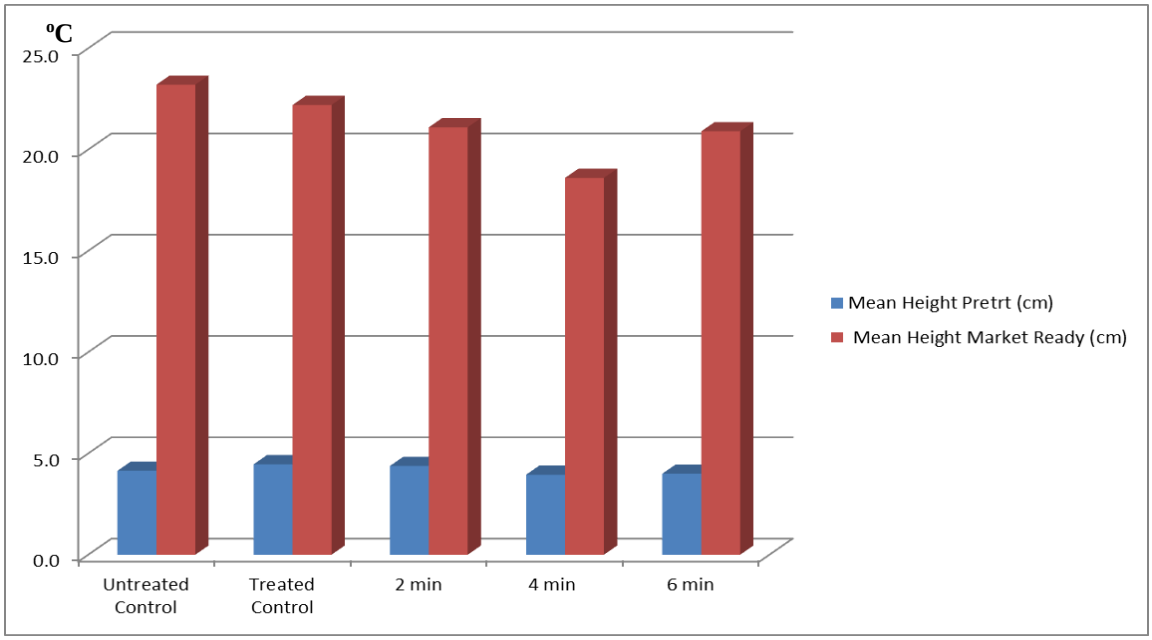


Figure 14. Mean flower number of chrysanthemum 'Shasta Improved' 10 to 12 weeks after treatment

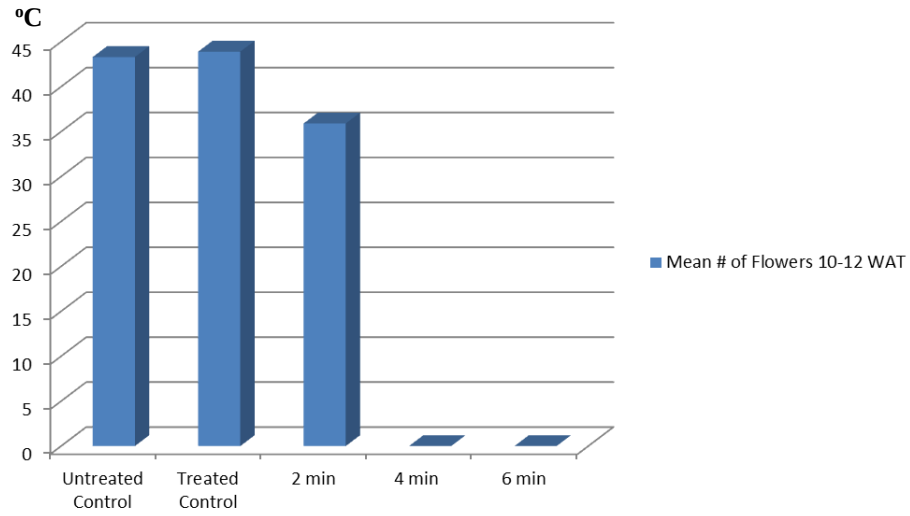
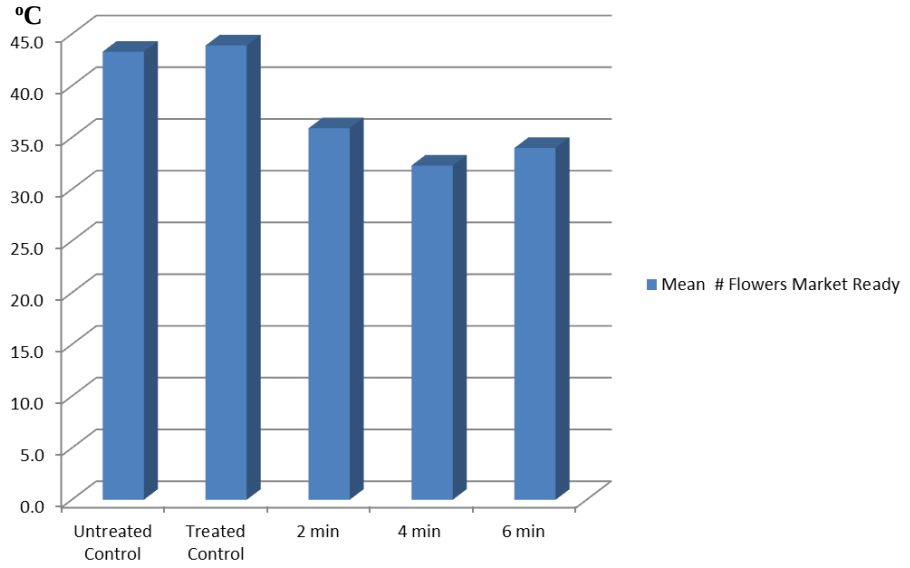


Figure 15. Impact of hot water immersion on bloom number of chrysanthemum 'Shasta Improved' when untreated controls were market ready



Chrysanthemum Phytotoxicity and Growth response to Hot Water Immersion, Experiment 2

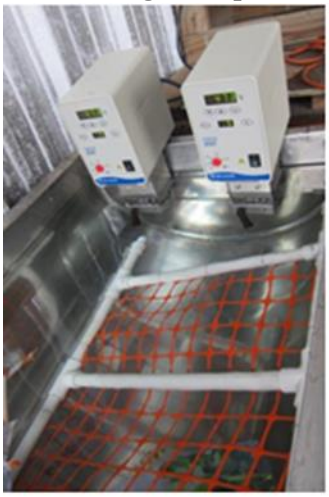
This study was to determine the tolerance of 'Bold New York' chrysanthemum unrooted cuttings to hot water dip treatments for insect disinfestation. Treatments were conducted at the University of Hawaii at Manoa, Waiakea Agricultural Research Center in Hilo, HI. Two stainless steel tanks (160L barrel type) were filled with tap water: one heated to three different target temperatures using two immersion circulator/heaters (Isotemp model IC-2150, Fisher Scientific Inc., Pittsburgh, PA); the second tank held at ambient temperature (32°C). Vegetative tips, cut to 5.0-7.5 cm (mean height of 6.2 cm) and leaves trimmed from the bottom two nodes, were wrapped in moistened paper towels for transport to the Waiakea Agricultural Research Center. Cuttings were randomly selected and assigned to a treatment, then placed in 30cm x 35cm organza bags, which were closed with a drawstring, and then submerged in water for the allotted treatment (Figure 16). Treatments were: 10 minutes at 46.1°C, 6 minutes at 47.2°C, and 2 minutes at 48.9°C; each treatment was followed by immersion in

cool water (ambient temperature, 32.0°C) for half the duration of the hot water treatment (5, 3 and 1 minutes, respectively), a treated check that consisted of submerging cuttings in ambient temperature water for 15 minutes, which was the equivalent of the longest hot water treatment and its respective cool water immersion time, and an untreated check.

The cuttings were dipped in 0.1% IBA (Hormex 1), planted into trays of vermiculite, then placed in a mist chamber for 12 days (Figure 2), at which time they were transplanted into 3" pots containing 3:1:1 peat, vermiculite, and perlite. The cuttings were observed for symptoms of heat sensitivity at 1, 2, 5, 7, and 12 days after treatment (DAT) and then at weekly intervals until 56 DAT when initial flowering was observed.

Figure 16. Chrysanthemum 'Bold New York' cuttings were trimmed, wrapped in moistened paper towels for transport, then assigned to treatments and placed in organza bags.

Figure 17. Two immersion heating/circulating pumps were attached to a stainless steel sink and used to achieve target temperatures.



Post treatment cuttings were dipped in 0.1% IBA, planted in trays of perlite and placed in a mist chamber.

Results

No damage was observed on untreated and treated control cuttings 1 day after treatment (DAT). However, signs of heat sensitivity were observed on cuttings exposed to 46.1°C, 47.2°C, and 48.9°C: 70% of cuttings exposed to 46.1°C had slight necrosis on the growing tips; 40% of the those exposed to 47.2°C also had slight necrosis on the terminal growth; 20% of the cuttings exposed to 48.9°C had slight necrosis while 40% of them had moderate to severe necrosis on the terminals (Figure 18). By 5 DAT, no further necrotic damage was observed. At 12 DAT all cuttings were transplanted. Cuttings exposed to 46.1°C were similar to controls. However, root development varied within 47.2°C and 48.9°C treatments from good to poor or none (Figure 19). At 21 DAT plant height of those with severe necrosis or poor root development were somewhat stunted compared to controls, and differences in plant height between treatments and controls continued through 28 DAT (Table 14, $P<0.05$). From 35 through 42 DAT all plants grew equally well, and there were no differences in plant heights ($P>0.05$). By 49 DAT plants exposed to hot water treatments that had had moderate to severe necrosis post treatment and/or poor root development had shorter internode lengths ($P < 0.05$).

Discussion

Of the three hot water dip treatments, 47.2°C produced the least heat damage to the cuttings and resulted in taller plants. Higher temperature exposure for less time and lower temperature exposure for a longer period of time caused more damage to cuttings and resulted in overall shorter plant heights compared to controls. Although the plants were shorter, they appeared fuller due to the shortened intermodal length. During this trial, all cuttings successfully rooted and produced flower buds by 56 DAT.

Figure 18. Symptoms of heat treatment damage 1 DAT for chrysanthemum 'Bold New York' cuttings

Top right and bottom left and right are examples of slight to severe heat damage on terminal growth observed within heat treatments.

Figure 19. Chrysanthemum 'Bold New York' cutting 12 DAT root development varied from good (top left) to adequate, poor and no root development (top center, right and bottom left and right, respectively).

Figure 20. All chrysanthemum 'Bold New York' cuttings successfully rooted and initiated flower buds by 56 DAT.

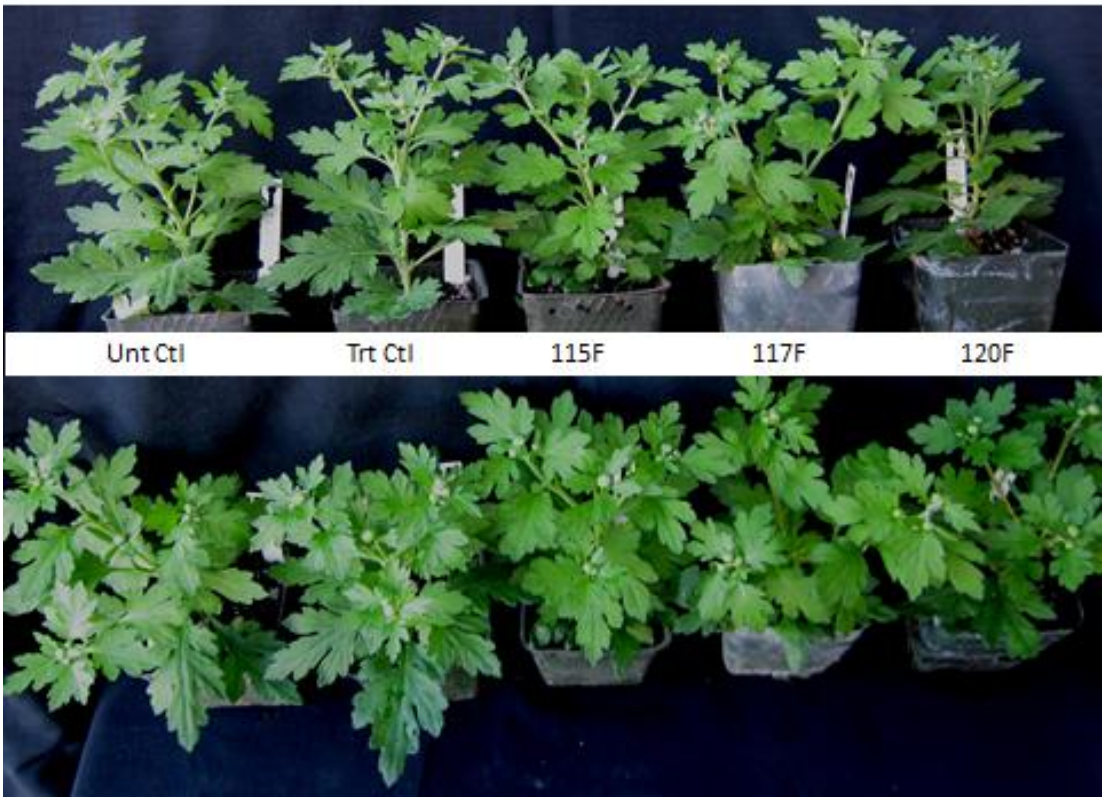


Table 14. Height of chrysanthemum 'Bold New York' heights through 56 days after heat treatment

	Mean Plant Height(cm)* Days after treatment					
	21	28	35	42	49	56
Untreated Control	4.9 ab	7.3 a	9.8	13.9	16.2 ab	18.0 ab
Treated Control	6.0 a	6.8 ab	9.6	14.2	16.5 a	18.6 a
46.1 °C, 10 min	3.7 b	4.9 b	7.7	11.4	13.9 ab	16.1 ab
47.2 °C, 6 min	4.3 b	4.8 b	7.9	11.3	14.2 ab	16.3 ab
48.9 °C, 2 min	4.4 ab	4.9 b	7.7	11.3	13.3 b	15.6 b

* Letters within columns followed by different letters are different (P<0.05 ANOVA and Tukey's Mean Separation).

Chrysanthemum Phytotoxicity and Growth response to Hot Water Immersion, Experiment 3

This study was a joint project between the University of Hawaii at Manoa and the Center for Applied Horticultural Research (CfHAR) in North San Diego County, California to determine the tolerance of 'Olympia' chrysanthemum rooted cuttings to hot water dip treatments for insect disinfestation and the effects of shipping conditions on the growth of heat-treated cuttings. Heat treatments were conducted at the University of Hawaii at Manoa, Waiakea Agricultural Research Center in Hilo, HI. Two stainless steel tanks (160L barrel type) were filled with tap water: one heated to three different target temperatures using two immersion circulator/heaters (Isotemp model IC-2150, Fisher Scientific Inc., Pittsburgh, PA); the second tank held at ambient temperature (32°C). Ten randomly selected rooted mum cuttings (mean height 6.47 cm, mean number of leaves 7, Aris Horticulture, Inc /Yoder Brothers, Inc, Alva FL), were placed in 30cm x 35cm organza bags, which were closed with a drawstring, and then submerged in water for the allotted treatment. Treatments were: 10 minutes at

46.1°C, 6 minutes at 47.2°C, and 2 minutes at 48.9°C; each treatment was followed by immersion in cool water (ambient temperature, 32.0°C) for half the duration of the hot water treatment (5, 3 and 1 minutes, respectively), a treated check that consisted of submerging cuttings in ambient temperature water for 15 minutes, which was the equivalent of the longest hot water treatment and its respective cool water immersion time, and an untreated check. There were two replicates per treatment; one replicate was transported to a nearby cooperating nursery in Hilo for post-treatment planting, and the other replicate was shipped to California by Fed Ex Next Day Air.

The elapsed time between hot water treatment and planting at a Hilo nursery was 2.5 hours. The rooted cuttings were transplanted into 5" plastic pots of peat: perlite (1:1.5) and top-dressed with Apex (16-6-13 + micronutrients, 1 tbsp./pot), then spaced on benches 5" apart in a CRD. During the first two growing weeks, these mums received additional 4 hours of light under incandescent lights at night in a polyroof greenhouse.

Once plants were 7-8cm tall (approximately 2 week after treatment), their tips were pinched to induce lateral stem formation, and the plants were moved to outer benches under 15% shade cloth where they received additional nutrients in three minute drip irrigations (Excel: 15-5-15) twice daily until market ready (50% partially and fully opened flowers, approximately 10-12 weeks old). Because the chrysanthemum cultivar 'Olympia' develops large, weighty flowers, the multiple flower buds initiated on each stem were disbudded to one per stem to avoid stem breakage. Disbudding occurred at bud initiation between 6 and 7 WAT.

The elapsed time between hot water treatment and planting in California was 7 days (2 days shipping, 5 days at California plant quarantine). One cutting from each treatment was retained at CDFA quarantine for nematode inspection, which reduced the number of plants per treatment from 10 to 9. The remaining cuttings were transplanted at CfHAR in San Diego into 4" pots, spaced 4" apart on benches. These cuttings received 2 additional days of darkness and refrigeration in shipping, no supplemental lighting and were housed in a climate controlled fully enclosed greenhouse for the duration of the trial. They were irrigated by spitter emitters for 3 minutes twice a day and received additional fertilizer via dosatron (Excel 15-5-15 at 100 ppm/n). Flower bud initiation began at 6 WAT, and axillary buds were not disbudded until 8 WAT, 2 weeks after bud initiation.

Plants were observed for symptoms of heat damage weekly 2 WAT (09-29-2014), 3 WAT (10-06-2014), 4 WAT (10-13-2014), 5 WAT (10-20-2014), 6 WAT (10-27-2014), 7 WAT (11-03-2014), 8 WAT (11-10-2014), and were market ready at 9 WAT (11-17-2014).

At both locations a foliar growth regulator (B9) was applied at two and five weeks after planting, and pesticides, applied at 10-14 day intervals, included rotations of Pylon, Overture, HachiHachi and Avid. During this trial the mean temperature in Hawaii was 24.8°C (39.5°C high, 18.4°C low) with a mean humidity of 86.9% (100% high, 39.1% low). In California the mean temperature was 24.2°C (54.4°C high, 12.0°C) with a mean humidity of 60% (96.6% high, 5.7% low).

Plants were observed for heat sensitivity symptoms at 3 day intervals for the first two weeks and then weekly up to 10 weeks after treatment (WAT). Changes in height and flower development were recorded. Heights (biweekly intervals) and flower production were compared using ANOVA, and a 2-Way ANOVA was conducted for location X treatment interactions (Tukey's Mean Separation, Minitab16). Although results at both sites showed similar trends, variability at each location (Table 15) impacted results sufficiently that outcomes were compared separately.

Table 15. Summary of mum growing conditions at two locations

Factor during grow-out period		Hawaii	California
Time elapsed between treatment and planting		2.5 hr	7 days
Greenhouse Conditions		Polyroof &15% shade	Enclosed greenhouse
Size of pot		5”	4”
Spacing between pots on bench		5”	4”
Lighting		+4 hours	-2 days
Irrigation		drip	spitter emitter
Ambient temperature (°C)	Mean	24.8	4.2
	Low	18.4	12.0
	High	39.5	54.4
Relative humidity	Mean	86.9	60.0
	Low	39.1	5.7
	High	100	96.6

Plant Height: Hawaii

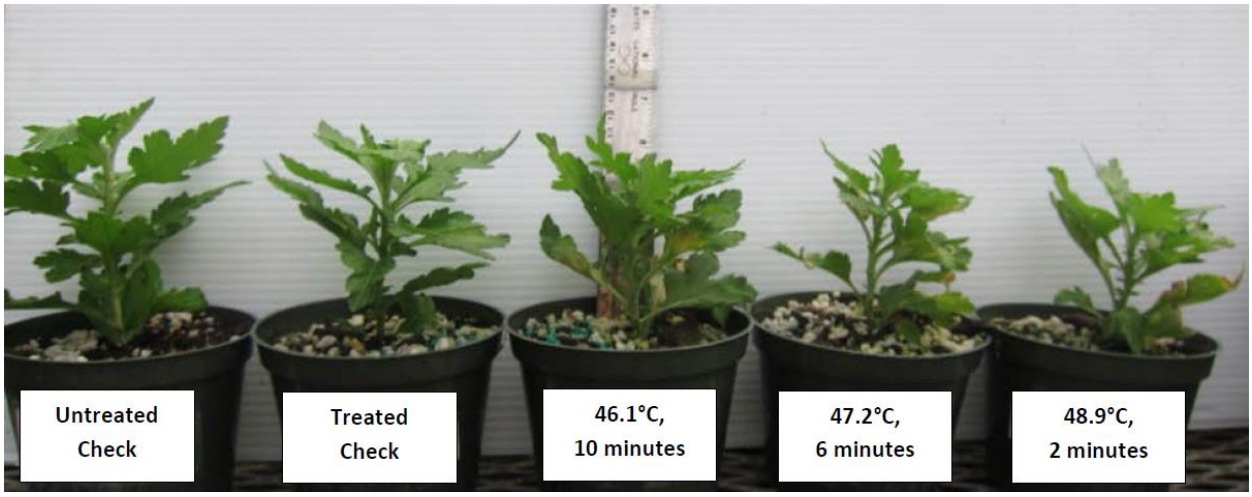
During the first four weeks of observations treatment heights were different. Although cuttings were randomly assigned, pre-treatment variations in height occurred, which resulted in one treatment group being shorter than the checks. At 2 weeks after treatment (WAT), signs of heat damage, including severe browning on lower leaves and growing tips, were visible on 10% of the heat treated plants (Figure 21), and plant growth was retarded within the three heat treatments as compared to the checks. The variations in the amount of stem pinched-back at 3 WAT also affected 4 WAT measurements ($P<0.05$) (Figure 22).

However, from 6 through 10 WAT there were no differences in mean height ($P > 0.05$) between treatments and checks. Rooted chrysanthemum cuttings tolerated exposure to 46.1°C for 10 minutes, 47.2°C for 6 minutes, and 48.9°C for 2 minutes with no differences in survival rate, height or the number of weeks until ‘Market Ready’ as compared to the checks at 10 WAT.

Flower Production: Hawaii

Initially, check treatments had more flower buds at 7 WAT than the three heat treated groups ($P < 0.05$) (Figure 23). By 8 WAT there were no differences in flower production between treatments. The hot water dip treatments did not adversely affect the amount, rate of maturation or quality of flowers, and there were no differences between treatments ($P >0.05$). At 10 WAT all plants were ‘Market Ready’ with 50% partial or fully opened flowers (Figure 24).

Figure 21. Heat-treated rooted chrysanthemum 'Olympia' cuttings: Hawaii.
a) At 2 WAT, 9 of 10 replicates tolerated exposure to 46.1°C for10 minutes, 47.2°C for 6 minutes, and 48.9°C 2 minutes with slight to no chlorosis on older leaves.



b) At 2 WAT, one plant in each heat treatment showed signs of severe burning onlower leaves and growing tip.

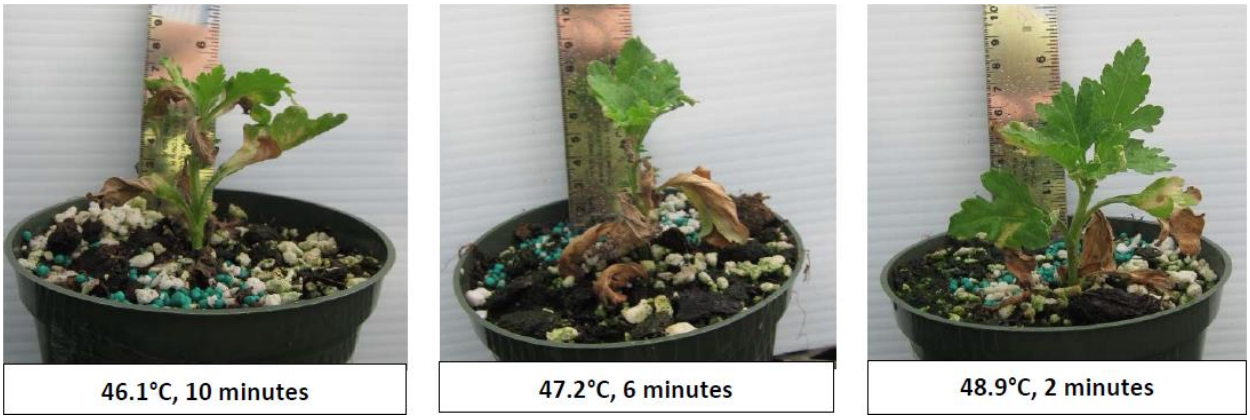


Figure 22. Height of rooted chrysanthemum 'Olympia' cuttings after hot water immersion: Hawaii.

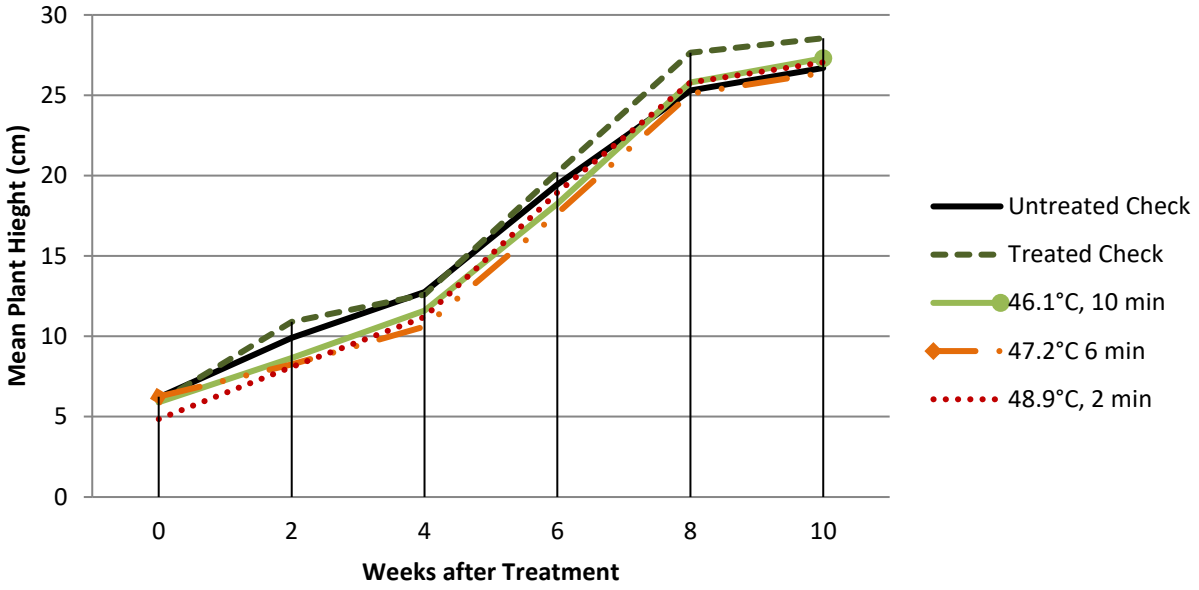


Table 16. Mean plant height (cm) of chrysanthemum 'Olympia' buds and flowers *

	Weeks after Treatment*					
	0	2	4	6	8	10
Untreated Check	6.2 a	9.9 ab	12.8 a	19.5	25.3	26.7
Treated Check	5.9 a	10.9 a	12.6 ab	20.2	27.7	28.6
46.1°C, 10 min	5.9 a	8.7 bc	11.6 abc	18.3	25.8	27.3
47.2°C 6 min	6.3 ab	8.3 c	10.6 c	17.7	25.1	26.4
48.9°C, 2 min	4.9 b	8.1 c	11.2 bc	19.0	25.8	27.1

*Means within columns followed by different letters are different ($P < 0.05$, Tukey's Mean Separation)

Figure 23. At 10 WAT all chrysanthemum 'Olympia' were 'Market Ready' with at least 50% partial and fully opened blooms: Hawaii.

Untreated Check

Treated Check

115°F, 10 minutes

117°F, 6 minutes

120°F, 2 minutes

Figure 24. Number of buds and flowers after hot water immersion on rooted chrysanthemum 'Olympia' cuttings: Hawaii.

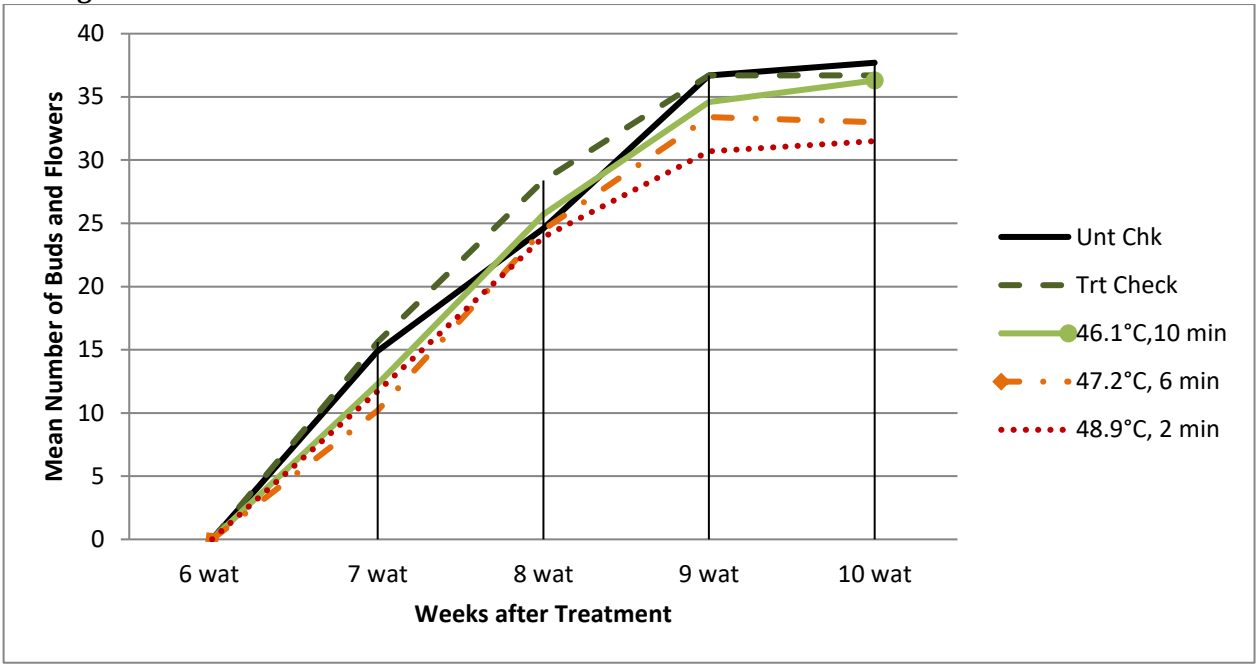


Table 17. Mean Number of chrysanthemum 'Olympia' Buds and Flowers: Hawaii

	Weeks after Treatment				
	6	7	8	9	10
Untreated Check	0	15 a	25	37	38
Treated Check	0	16 a	28	37	37
46.1°C,10 min	0	12 ab	26	35	36
47.2°C, 6 min	0	10 b	25	33	33
48.9°C, 2 min	0	12 ab	24	31	32

*Means within columns followed by different letters are different ($P < 0.05$, Tukey's Mean Separation)

A second set of treatments were shipped to Dr. Bethke in California. Upon arrival, a quarantine was imposed by the County of San Diego on the plants. One voucher specimen from each treatment was confiscated for nematode testing and plant were kept in shipping conditions for an additional day. After the quarantine was lifted, all plants were rooted to a depth just above the root zone in 4" pots using 70-80% Canadian Spagnum peat moss, dolomitic, limestone, Nutricote (13-13-13) Inorganic Fertilizer (1 plant/pot). Plants were kept in 75-80% sunlight for the first 2 weeks to stimulate vegetative growth. Plants were watered via one-minute drip irrigations 2 times daily. We measured each plant from the root callus to the growing tip. Plants were observed for signs of heat sensitivity/phytotoxicity at 4 days after treatment (DAT) recording the number of leaves produced and growth on 09-12-2014, 7 DAT (09-15-2014), 10 DAT (09-18-2014), 14 DAT (09-22-2014), and 17 DAT (09-25-2014). All changes were documented digitally (pictures) for burns, necrosis and/or plant death.

On the 7th day after planting top dress 17-3-6 (slow release) was applied and Excel (liquid feed 15-5-15 at a rate of 100 ppm nitrogen) 1 time a week twice daily until plants were market ready (90% opened flowers). Starting 14 DAT plants were observed in weekly intervals. Plant height from soil level to growing tip was measured, when flower buds appear, when buds are partially opened, and when the plants are market ready was noted. The number of buds and blossoms that were produced were noted to check for reduced flower production

and height to check for stunting. The growing tip was pinch back 12 days after planting, to induce branching and plants were placed into a growing area under black cloth to induce flowering (until 10-01-2014).

A foliar growth regulator (B9) was applied at 2 weeks after treatment (WAT) 09-29-2014 and 5 WAT (10-20-2014). Plants were maintained with drip irrigation, Excel liquid feed daily (twice daily under hot conditions) and pesticides were applied as needed for leafminers and thrips. Plants were observed for symptoms of heat damage weekly 2 WAT (09-29-2014), 3 WAT (10-06-2014), 4 WAT (10-13-2014), 5 WAT (10-20-2014), 6 WAT (10-27-2014), 7 WAT (11-03-2014), 8 WAT (11-10-2014), and were market ready at 9 WAT (11-17-2014).

Plant Height: California

Unlike mums grown out in Hawaii where there were differences in height the first 4 weeks of observations, there were no differences between treatments from 0 to 6 WAT ($P > 0.05$) (Fig. 5). The differences between mum growth in Hawaii and in California may be a result of the return shipment in which shipped cuttings were exposed to additional refrigeration and darkness for 2 days, delayed planting and late pruning.

Differences in plant height were due to adjustments made late in the trial ($P < 0.05$) (Figure 1). Lateral stems started to break prior to the 7 WAT observations; flower buds were removed 2 weeks after initiation which may have contributed stems breaking; and at 9 WAT stems were pruned back to reduce further damage, altering plant measurements.

Flower Production: California

Bud initiation occurred at 6 WAT, a week earlier than Hawaii, with check groups having more buds set than the heat treated groups ($P < 0.05$) (Figure 2). Higher temperatures in the greenhouse and smaller pot size may have contributed to the earlier bud set. From 7 through 9 WAT there were no differences in flower production between treatments. All plants were 'Market Ready' by 10 WAT with plants exposed to 46.1°C for 10 minutes producing more flowers than the other treatments ($P < 0.05$). The hot water dip treatments did not adversely affect the rate of maturation or quality of flowers.

Figure 25. Height of rooted chrysanthemum 'Olympia' cuttings after hot water immersion: shipment to and grow out in California

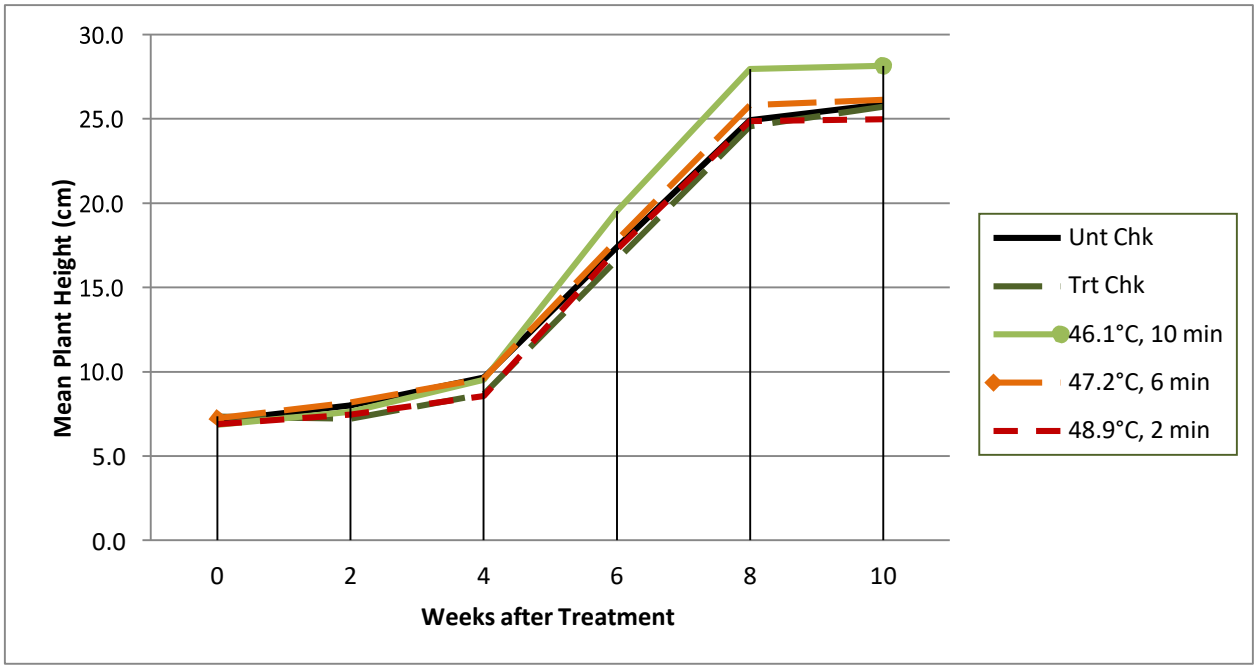


Table 18. Mean height of rooted chrysanthemum 'Olympia' cuttings after hot water immersion: shipment to and grow out in California*

	Weeks after Treatment					
	0	2	4	6	8	10
Untreated Check	7.1	8.0	9.7	17.4	24.9 b	25.9 ab
Treated Check	7.4	7.2	8.7	16.7	24.6 b	25.7 ab
46.1°C, 10 min	6.9	7.6	9.5	19.5	28.0 a	28.1 a
47.2°C, 6 min	7.3	8.2	9.6	17.8	25.8 ab	26.1 ab
48.9°C, 2 min	6.9	7.5	8.6	17.3	24.9 ab	25.0 b

*Means within columns followed by different letters are different (P < 0.05, Tukey's Mean Separation)

Figure 26. Number of buds and flowers of rooted chrysanthemum cuttings after hot water immersion: shipment to and grow out in California.

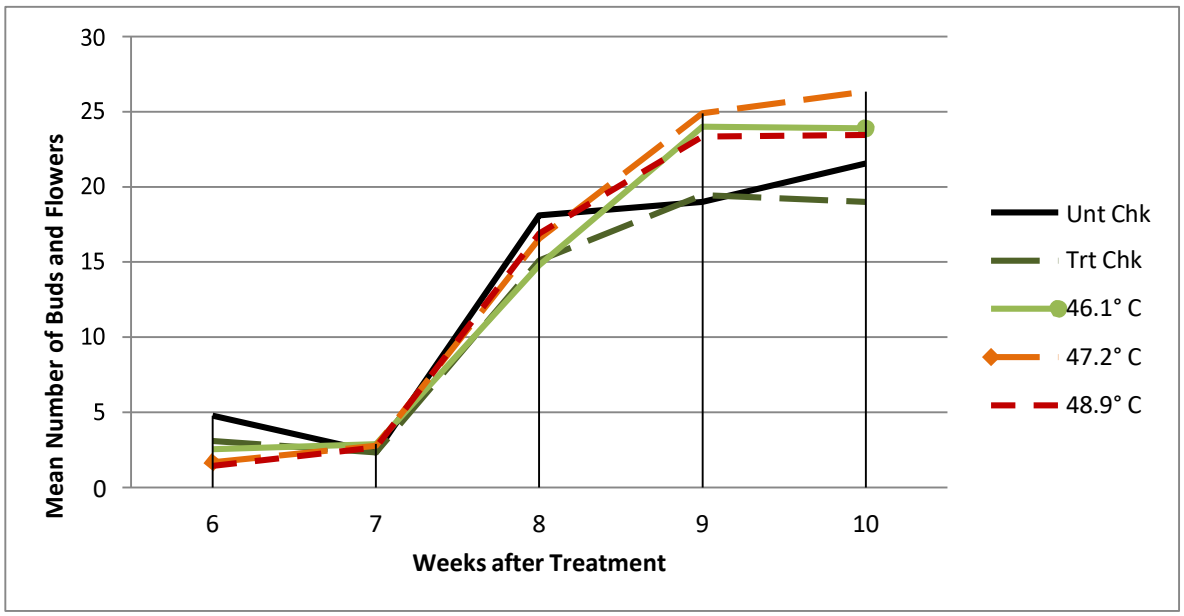


Table 19. Number of buds and flowers of rooted chrysanthemum cuttings after hot water immersion: shipment to and grow out in California

	Weeks after Treatment				
	6	7	8	9	10
Untreated Check	4.8 a	2.3	18.1	19.0	21.6 a
Treated Check	3.1 ab	2.3	15.1	19.4	19.0 b
115° F, 10 min	2.6 b	2.9	14.8	24.0	23.9 ab
117° F, 6 min	1.7 b	2.8	16.6	24.9	26.3 a
120°F, 2 min	1.4 b	2.7	16.9	23.3	23.4 ab

*Means within columns followed by different letters are different (P < 0.05, Tukey's Mean Separation)

Chrysanthemum Phytotoxicity to Entomopathogens, Conventionals, Soaps and Oils
Two phytotoxicity screens were performed using entomopathogens (BotaniGard, PreFeRal), synthetic chemistry (Safari) and natural chemistry (Publix Soap, Stoller Natur'l Oil, M-pede). Foliar sprays were applied to chrysanthemum nonrooted cuttings. Phytotoxicity was rated on a scale of 1 to 6 with 1 being no observed injury and 6 being plant death. The experiment in Florida was compromised by the mists system breaking during a weekend so it was terminated after two weeks (Figure 27). The experiment in California continued for 3 weeks after the first foliar application. No biologically significant injury occurred with any of the treatments (Figure 28).

Figure 27. Chrysanthemum phytotoxicity test, Florida.

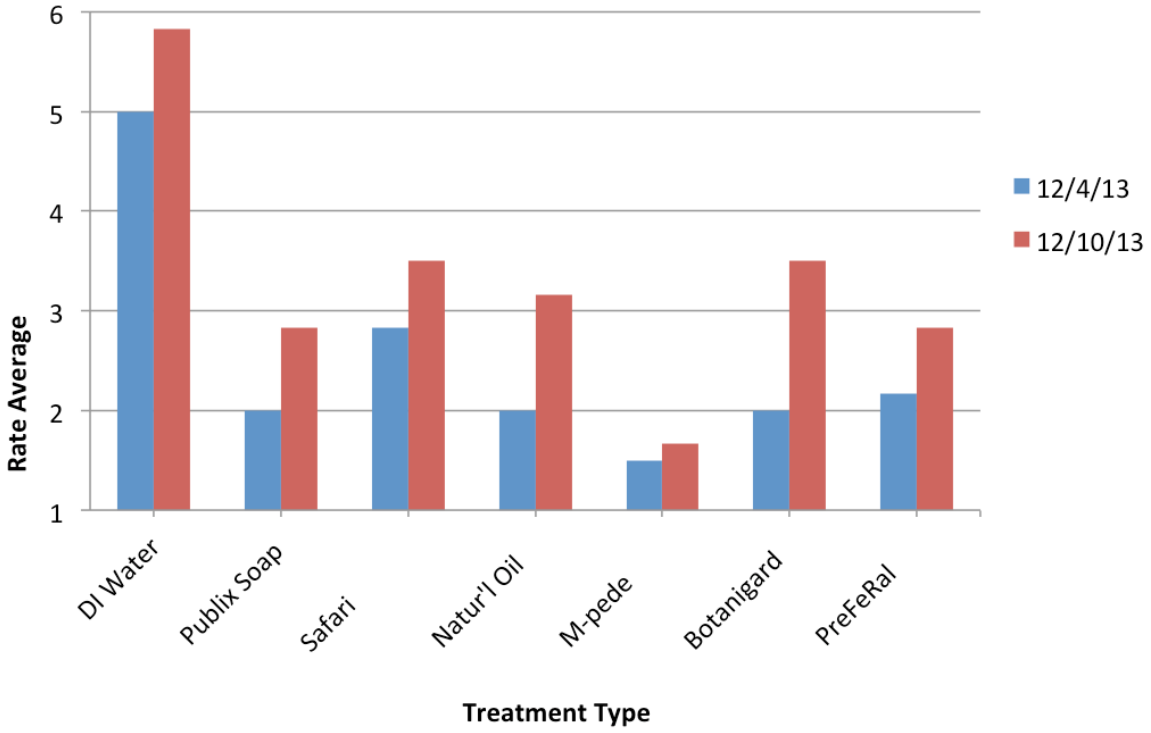
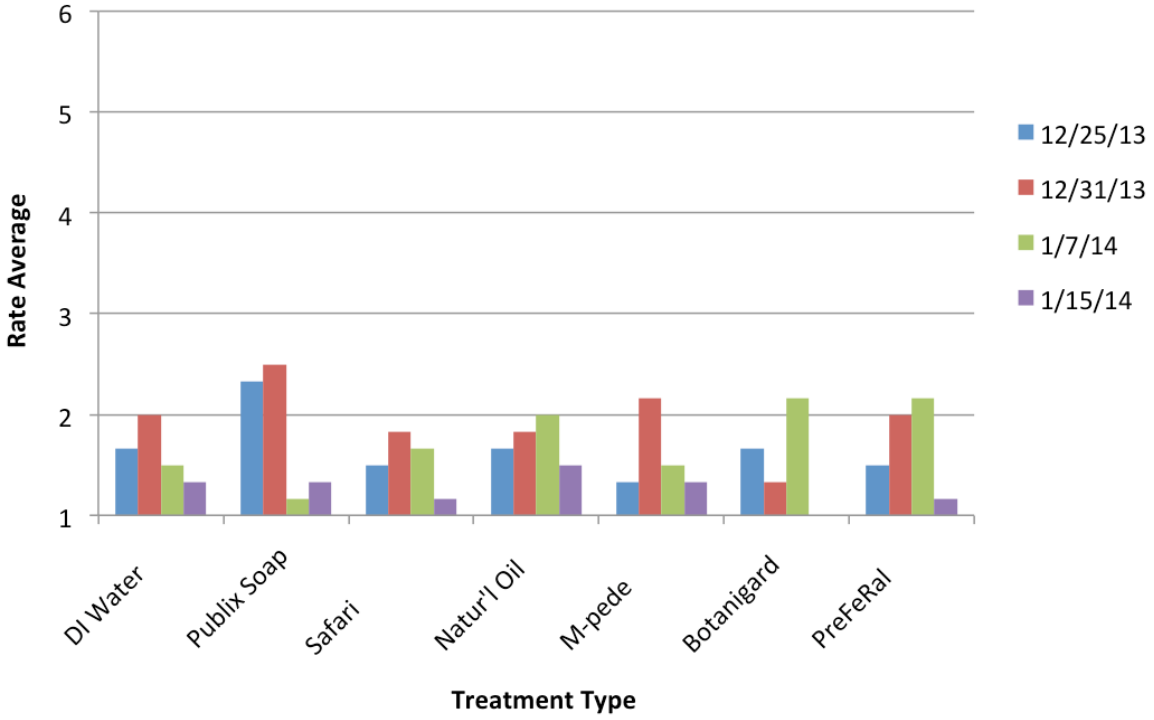


Figure 28. Chrysanthemum phytotoxicity test, California.



Poinsettia Phytotoxicity and Growth Response to Heat Treatment

The purpose of this experiment was to determine heat tolerance of poinsettia cuttings to hot water at exposure times and temperatures that target mealybugs, scale insects and whiteflies as an alternate pest management practice. All treatments were conducted at the University of Hawai‘i at Mānoa, Waiākea Research Station (Hilo, HI) in cooperation with a nursery near Hilo, Hawaii. Callused poinsettia cuttings imported by the nursery were randomly assigned by size and number of leaves to one of the following treatments: hot water dip at 120 °F (48.9 C) for 2, 4, or 6 min followed by 1, 2, or 3 min dip, respectively, in ambient temperature water at 77 ºF (25 C) as “cool down”; ambient temperature water dip for 9 min (longest duration of hot water dip with “cool down”) as a treated check, or no treatment (untreated check). Cuttings were approximately 2 cm in diameter at callused basal end, ranged in height from 2.5 to 4.0 cm (average 3.2 cm), and had an average of 7 leaves (range of 5 to 8 leaves) (Fig. 1a). All treatments were replicated ten times in a RCB design.

The hot water treatment system consisted of a 106 L stainless steel tank equipped with two Isotemp heating immersion circulators (model 730; Fisher Scientific, Pittsburgh, PA) to maintain a constant tank temperature within ±0.1 ºC of the target temperature. Water temperature was monitored throughout the treatment using a digital thermometer (Fluke model 52 Series II thermometer; 80PK-1 beaded probe thermocouple; Fluke Corp., Everett, WA). A second stainless steel sink was filled with ambient tap water for the cool down and treated check treatments. For each treatment, ten cuttings were placed in a 12” x 14” fine mesh organza bag and dipped into heated and/or ambient temperature water for the designated treatment duration (Figure 29). Within 30 minutes of treatment, the cuttings were potted in 4” diameter plastic pots approximately 0.5 cm deep in peat moss and perlite media (1:2 ratio) (Figure 29). All potted cuttings were placed on benches kept under mist. Plant height, leaf production, and symptoms of heat sensitivity were recorded 1, 2, 5, 8, and 16 days after treatment (DAT).

By 1 DAT all cuttings exposed to 120 °F for 2 to 6 min exhibited die-back, leaf browning, stem rot. By 16 DAT, no new growth was observed among any of the hot water-treated cuttings (Figure 30), whereas the treated and untreated check cuttings produced one to two new leaves during the same period and grew an additional 2 cm.

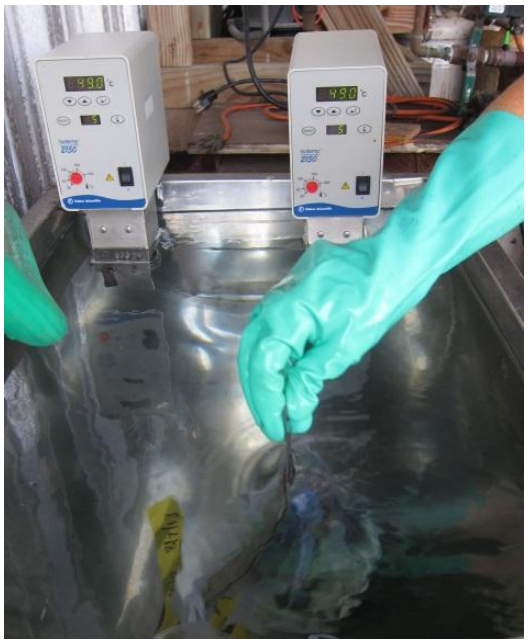
The highest temperature of hot water tolerated by poinsettia 'Prestige' tip cuttings was 106 ºF (41 °C) for 5 min without detrimental effects to survival (91%) and roots per plant but was not efficacious for *Bemisia tabaci* (Gennadius) biotype "B"; survival rate of cuttings fell to 28% when cuttings were treated at 109 °F (43 °C) for 5 min (Romero 2011). Romero (2011) concluded that dipping poinsettia cuttings in insecticidal soap at 20 ml/L for 1 min were highly efficacious against early and late nymphs of SPW, and dipping cuttings in horticultural oil at 5ml/L for 1 min were highly efficacious against eggs, early nymphs and late nymphs of SPW without any phytotoxicity to poinsettia cuttings. Therefore, horticultural oils could provide an effective dip treatment for poinsettia cuttings that are not tolerant to hot water treatment.

Figure 29. Preparation and hot water treatment of callused poinsettia cuttings.

a) Pretreatment measurements

b) Cuttings in organza bag

c) Hot water dipping of cuttings in organza bag



d) Post-treatment potting of cuttings

Figure 30. Callused poinsettia cuttings dipped in hot water (120 ºF, or 49 ºC) for 2, 4 or 6 min prior to planting, at 2 weeks after treatment(2 WAT) compared to treated and untreated checks.

2 min at 120 °F

Untreated check (no dipping)

4 min at 120 °F

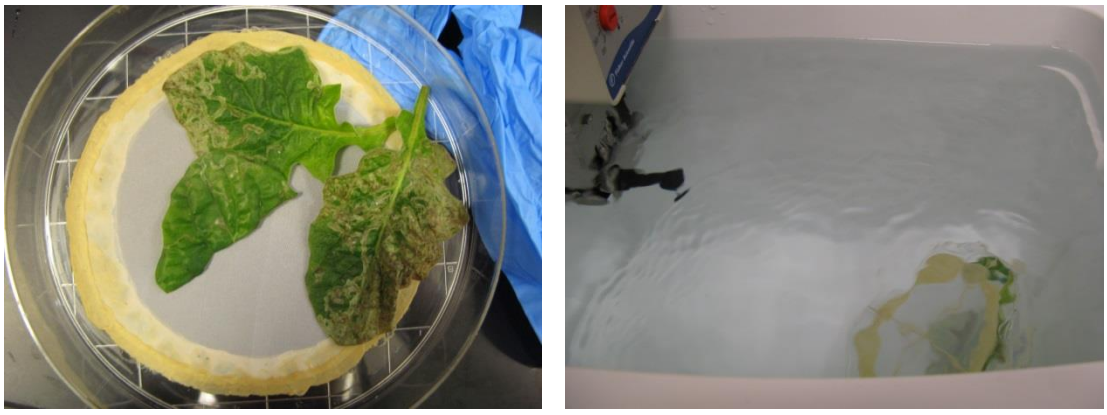
6 min at 120 °F

Treated check (dipped in ambient temperature water (77 ºF) for 9 min)

Gerbera – Leafminer – Heat Treatment Efficacy – Pre-shipment (HI)

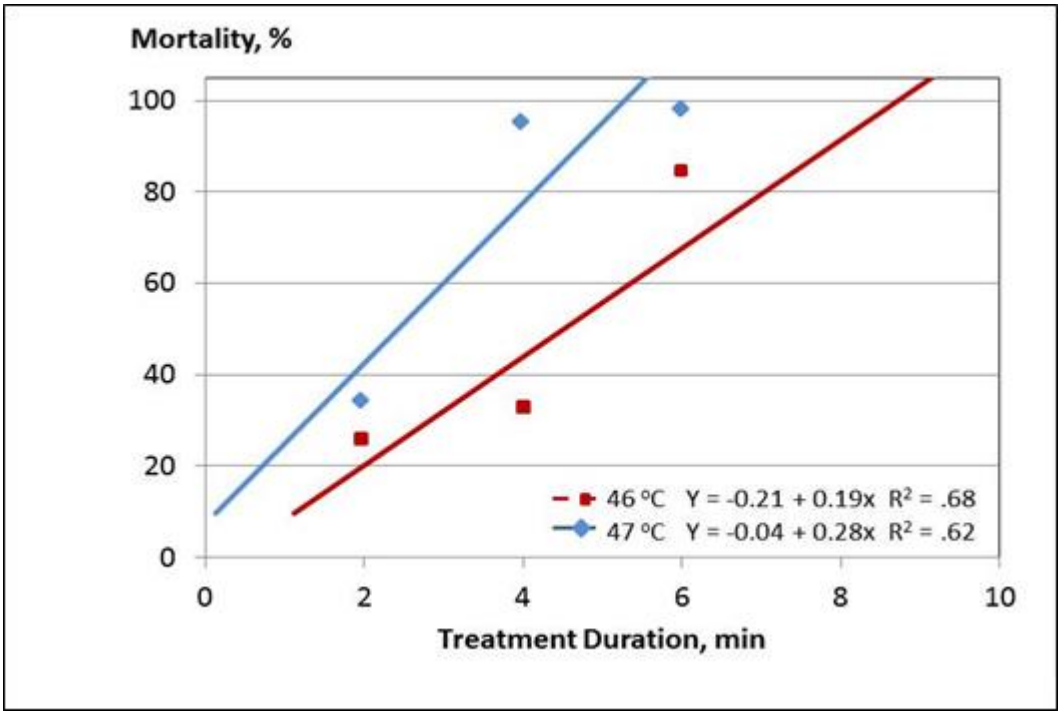
The efficacy of hot water on mining *Liriomyza trifolii* larvae was evaluated. Gerbera daisy plants (4" pots) were exposed to adult *L. trifolii* in a greenhouse for 30 days to allow oviposition. Leaves with actively mining larvae were excised and placed in plastic petri dishes (150 x 150 mm) modified with an 8.9 cm diameter hole on both cover and bottom screened with silk organza (74 µm pore size) secured in place with hot glue (Figure 31). Treatment tanks were set up in 26L insulated coolers (Igloo Products Corp., Model 00044559, Katy, TX) heated with immersion circulating heaters (Isotemp, Model IC-2150, Fisher Scientific, Inc., Pittsburg, PA). The petri dishes were held together with rubber bands and subjected to one of six treatments: submersion in hot water at 46.1 ºC for 2, 4 or 6 min, or at 47.2 ºC for 2, 4 or 6 min; each treatment was followed by immediate submersion in ambient temperature water (25.5 ºC) for half the duration of the hot water submersion. A treated check consisted of submersion in ambient temperature water (25.5 ºC) for 9 min. All treatments were replicated three times with 9 to 50 larvae per rep. All leaves were gently blotted with paper towels to remove excess water and held at room temperature for 24 h (21.5 ºC; 71.1 r.h.) (Figure 31). With the aid of a dissecting microscope, the larvae were probed and observed for movement. The numbers of live and dead larvae and live pupae were recorded at 1 and 2 days after treatment (DAT). Linear regression of data for hot water immersion at lower temperatures 46.1 °C and 47.2 °C predicted 100% mortality at 10 and 6 min, respectively (Figure 32).

Figure 31. Gerbera leaves with actively mining *Liriomyza trifolii* larvae were immersed in hot water to determine lethal temperature and duration as a quarantine treatment



Excised leaves in screened petri dish was covered and immersed in hot water.

Figure 32. Mortality of *Liriomyza* spp larvae in Gerbera daisy leaves immersed in hot water at 46.1°C or 47.2°C for 2, 4 or 6 min (regression equation and adjusted R^2).



Additional bioassay trials were conducted to confirm the efficacy of hot water immersion at temperatures tolerable to rooted chrysanthemum cuttings. Actively mining *Liriomyza* spp larvae were collected from tomato and Gerbera daisy leaves, excised and placed in plastic petri dishes (150 x 150 mm) modified with an 8.9 cm diameter hole on both cover and bottom that were screened with silk organza (74 µm pore size) secured in place with hot glue (Figure 33). Insulated treatment tanks (26 L, Igloo Products Corp., Model 00044559, Katy, TX) were filled with tap water heated with immersion circulating heaters (Isotemp, Model IC-2150, Fisher Scientific, Inc., Pittsburg, PA). The petri dishes were secured with rubber bands and subjected to one of 8 treatments: submersion in hot water at 46.1 °C for 2, 4, 6 or 10 min, at 47.2 °C for 2, 4, or 6 min, or at 48.9°C for 2 min. Following cool-down protocol for heat-treated plants and propagative material to expedite heat dissipation, each treatment was followed by immediate submersion in cool water (ambient temperature 25.5 °C) for half the duration of the hot water submersion. A treated check consisted of submerging excised *Liriomyza* spp larvae in cool water (25.5 °C) for 15 min, which was equal to the duration of the longest hot water treatment and its respective cool-down. All leaf sections were held at room temperature for 48 h for observation (21.5 °C; 71.1 % R.H.; Hobo Pro v2 data logger, Onsetcomp.com).

With the aid of a dissecting microscope, the larvae were probed and observed for movement at 1.5, 24 and 48 h after treatment (HAT) (Table 20). Larvae that appeared lifeless at 1.5 HAT often recovered from 'heat stupor' within 24 h; therefore, only rates of mortality (%) at 48 HAT were statistically analyzed for linear regression to predict treatment temperature and duration to achieve 100% mortality.

Discussion

Based on previous trials, linear regression models predicted hot water immersion at 46.1°C for 10 min should achieve 100% mortality, and responses of treated *Liriomyza* spp larvae observed at 1.5 and 24 HAT appeared to support the prediction; however, two larvae recovered from heat stupor by 48 HAT (Table 1). Hot water immersion for 4 minutes at 47.2°C or 2 minutes at 48.9°C achieved 100% mortality, while 6 min at 47.2°C did not. A high rate of parasitism occurred during the rearing of *Liriomyza* spp larvae throughout the spring and summer months, resulting in low replicate numbers for some treatments and use of possibly parasitized larvae in

others. Revised linear regression models predict 100% mortality with exposure of at least 11.7 min at 46.1°C, 6.1 min at 47.2°C (Figure 34), and 2 min at 49.2°C (Figure 35).

Figure 33. Excised leaves with a) viable *Liriomyza* spp larvae, b) placed in a petri dish, with organza inserts to allow flow of water, and c) secured prior to hot water immersion.

a) b) c)

Table 20. Efficacy of hot water treatments on *Liriomyza* spp. larvae in leaf tissue.

			% Mortality (Hrs after Treatment)		
Target Temperature	Duration	Reps	1.5	24	48
21.7° (71.1°F)	no dip, ambient room temp	11	0	0	0
24.7°C (76.4°F)	15 min, ambient water temp	10	0	0	0
46.1°C (115°F)	2 min w/ 1 min cool down	17	41	18	29
46.1°C (115°F)	4 min w/ 2 min cool down	17	65	35	24
46.1°C (115°F)	6 min w/ 3 min cool down	17	94	100	82
46.1°C (115°F)	10 min w/ 5 min cool down	10	100	100	80
47.2°C (117°F)	2 min w/ 1 min cool down	17	59	77	47
47.2°C (117°F)	4 min w/ 2 min cool down	18	94	100	100
47.2°C (117°F)	6 min w/ 3 min cool down	28	79	86	89
48.9°C (120°F)	2 min w/ 1 min cool down	10	100	100	100

Figure 34. Efficacy of hot water immersion of *Liriomyza* spp larvae at 46.1°C or 47.2°C for 2, 4, 6 or 10 min (regression equation and adjusted R^2).

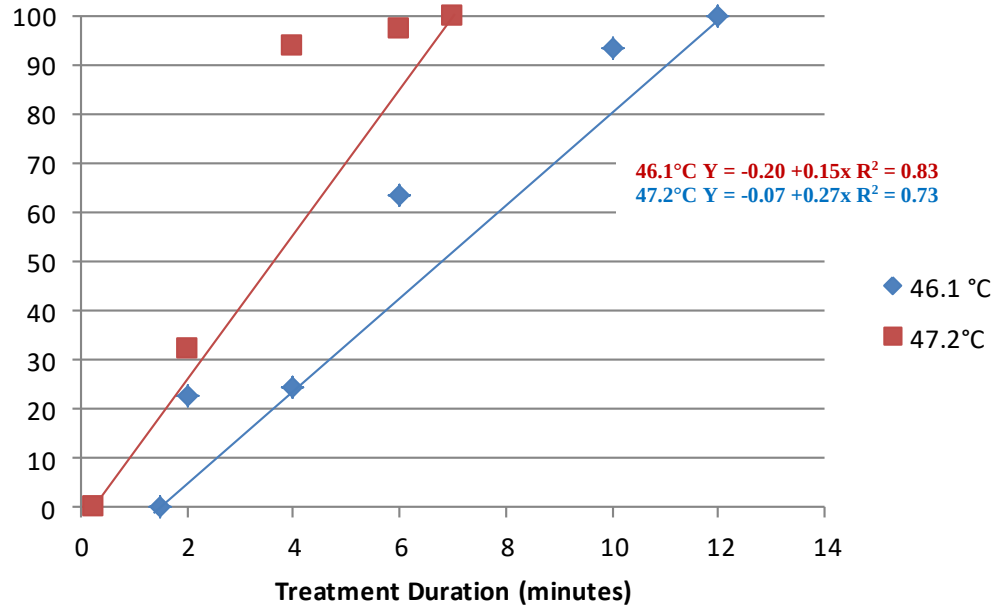
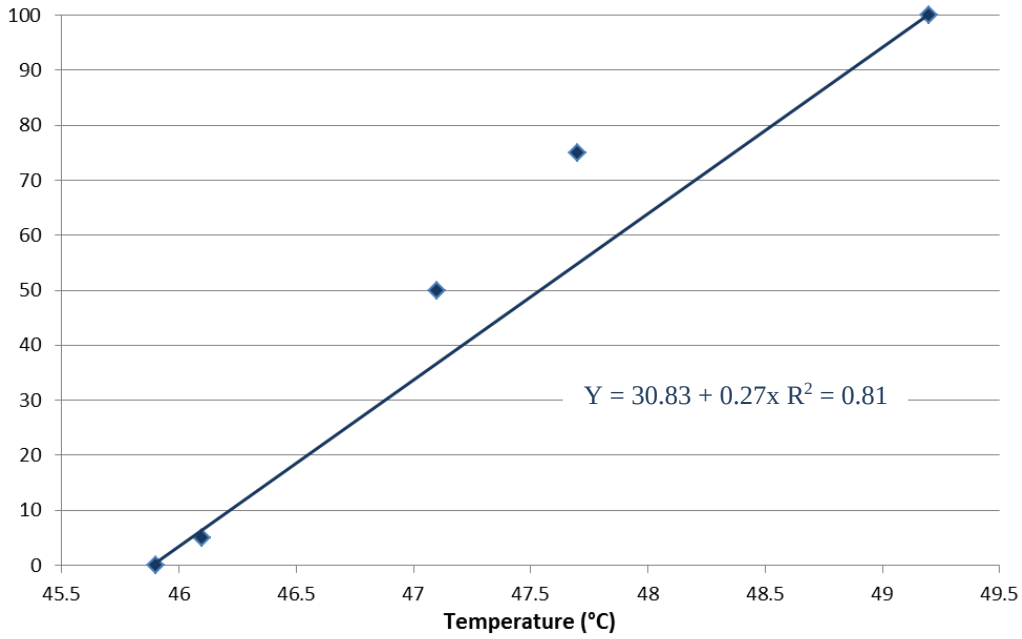


Figure 35. Efficacy of hot water immersion of *Liriomyza* spp larvae at 46.1°C, 47.2°C or 48.9°C for 2 min (regression equation and adjusted R^2).



Biopesticide compatibility with biorational products

PreFeRal

Dr. Osborne evaluated the potential to mix PreFeRal with various other biorational surfactant materials being evaluated: Publix Detergent, Natur'l Oil, Wetcit, VaporGard, hydrating wax (WashGard®) (Wax W), and Fire Blocking Gel (Barricade II) (Table 21, Table 22). In both experiments, the presence of the surfactants in media did not alter significantly germination of *I. fumosoreosea* spores. In the second experiment, water agar (Agar)

was compared to potato dextrose agar (PDA) to determine whether medium could impact germination. Under these test conditions, none of the surfactants appeared to inhibit PreFeRal germination (Table 22) or growth (Figure 36). For germination, PDA appeared to provide slightly better germination across surfactants, but this was not statistically significant.

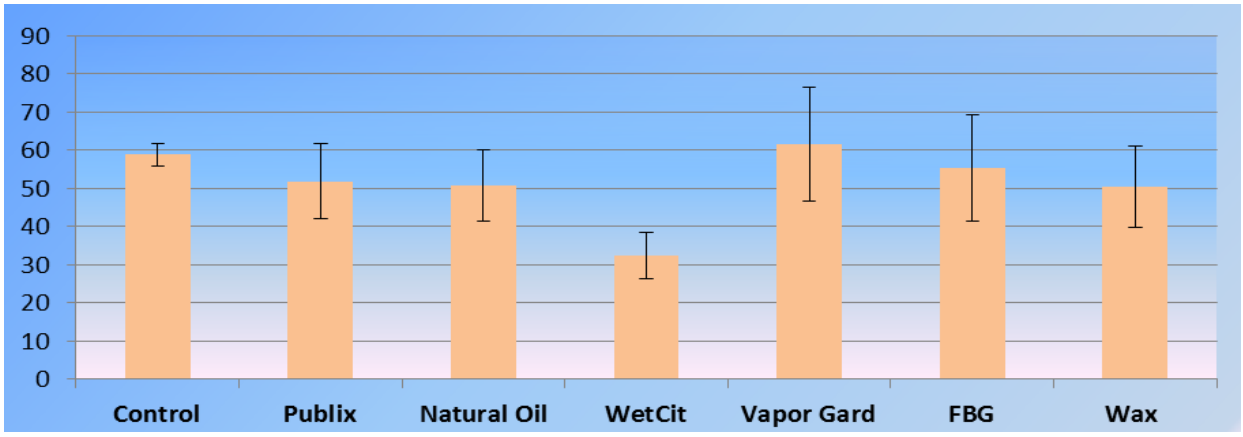
Table 21. Compatibility of PreFeRal (*Isaria fumosorosea*) with biorational products, A

Treatment	Germination %	CFU	Sta Dev	Growing 5 days	Sta Dev
Fire Blocking Gel (Barricade II)	60	87.5	14.63	10.5	0.7
Natur'l Oil	74	79.8	9.87	10.75	0.5
Publix Detergent	74	85.5	13.07	9.5	2.12
VaporGard	72	112.8	26.19	10.66	0.57
WashGard (hydrating wax)	52	80.6	15.41	0	0
WetCit	63	53.2	11.86	10.5	0.7
Non Treated Control	78	68.9	11.82	11	0

Table 22. Compatibility of PreFeRal (*Isaria fumosorosea*) with biorational products, B

	Germination %		STDEV	
Treatment	Agar	PDA	Agar	PDA
Fire Blocking Gel (Barricade II)	77.5	80	3.53	5.65
Natural Oil	73.5	78	12.02	1.41
Publix Detergent	67.5	82	3.53	3.53
VaporGard	83	76.5	1.41	3.53
WashGard (hydrating wax)	68	71	4.24	4.24
WetCit	74.5	78.5	6.36	1.41
Non Treated Control	70	76.5	1.41	1.41

Figure 36. Average Preferal colony forming units mixed with other biorational products.



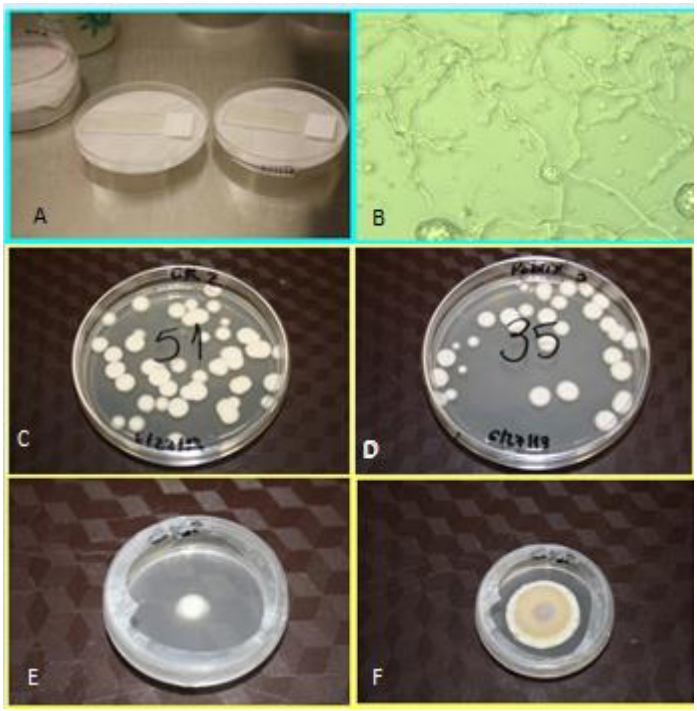
Beauveria bassiana

As a preliminary step for studying *Beauveria bassiana* in a similar fashion, material already present within Dr. Osborne's laboratory was tested for germination. The level of germination in the sample was 58% or less, so fresh samples were obtained.

Compatibility of *I. fumosorosea* and *B. bassiana* with surfactants: Under sterile laboratory conditions, compatibility of both entomopathogenic fungi was assessed with different surfactants (potential synergetic products for the fungi). All surfactants at 1% (concentration v/v) were mixed with both fungi in order to perform the compatibility test with three different evaluations (percentage of germination, number of colony forming units, and radial growing of the colony). In order to determinate the number of colony forming units (CFU), Petri dishes (9 cm diameter) with potato dextrose agar PDA (3.9 w/v, Difco Laboratories, Detroit, MI) were inoculated with suspensions of the surfactants mixed with the entomopathogenic fungi at 10^3 blastospores/mL of *I. fumosorosea* and 10^3 spores/mL of *B. bassiana* respectively. The number of CFU were assessed 4 days after the inoculation. Radial growth was also assessed 5 an d 10 days after inoculation into PDA in a separate experiment.

Washgard and Wetcit significantly reduced germination of and radial colony growth (5 days) of *I. fumosorosea* (Table 1). Publix, Natural Oil, VaporGard and FBC has no noticeable impact on germination or colony diameter 5 days after inoculation. For *B. bassiana*, all surfactants reduced germination (Table 2), but only WetCit, FBG, and WashGard reduced radial colony growth 5 days after inoculation. For both biopesticides, radial colony growth was not significantly different among treatments 10 days after inoculation.

Figure 37. Compatibility test design.



A- Slides glasses used for the evaluation of germination; B- Blastopores of *I. fumosorosea* 20 hours after the inoculation (400 X magnification) counting as percentage of germination of the fungi; C and D- Petri dishes used for the evaluate the number of colony forming units for the fungi *I. fumosorosea* 4 days after inoculation; E and F - Radial growing of the colony (*I. fumosorosea*) at 5 and 10 days after the inoculation respectively

Table 23. Compatibility of PreFeRal (*Isaria fumosorosea*) with biorational products, C

Treatments	Germination (%)	Number CFU	Diameter (mm) 5 Days Average ± STD	Diameter (mm0 10 Days Average ± STD
Preferal (Control)	72.1 ± 3.6 a	58.86 ± 12.7 a	10.10 ± 1 a	18.25 ± 2.2 a
Publix	68 ± 4.1 a	51.93 ± 26.4 a	9.08 ± 1.1 ab	17.4 ± 2.5 a
Natural Oil	70.7 ± 4.9 a	50.8 ± 22.2 a	9.3 ± 1.5 ab	17.07 ± 2.6 a
WetCit	57.9 ± 10.7 bc	32.36 ± 17.0 b	8.7 ± 1.8 b	16.3 ± 2.2 a
Vapor Gard	71.4 ± 7.3 a	61.46 ± 40.2 a	9.4 ± 1.1 ab	17.3 ± 2.7 a
FBG	67.2 ± 6.4 ab	55.40 ± 28.1 a	9.3 ± 1 ab	16.7 ± 2.7 a
WashGard	55.5 ± 7.5 c	50.4 ± 25.1 a	8.8 ± 1.1 b	16.7 ± 2.2 a
F	8.92	5.91	3.36	1.56
df	6	6	6	6
P	0.0001	0.0001	0.0035	0.1605

Percentage of germination, number of colony forming units (CFU), and radial growing of the colony (diameter in mm) at 5 and 10 days after inoculation at (25 °C ± 1, 70-80% relative humidity, and 12:12 h L:D photoperiod). Means within a column followed by the same letter are not significantly different at α = 0.05 (Tukey's HSD test)

Table 24. Compatibility of *B. bassiana* (Botanigard®) with different bio-pesticides.

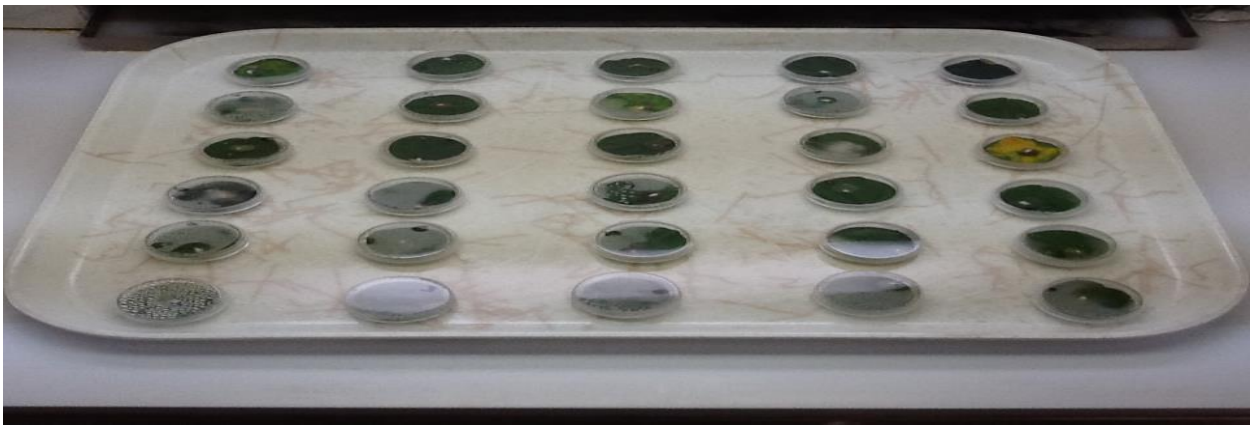
Treatments	Germination (%)	Number CFU	Diameter (mm) 5 Days Average ± STD	Diameter (mm) 10 Days Average ± STD
Botanigard (Control)	81 ± 4.1 a	96.4 ± 18.4 a	9.8 ± 0.87 a	21.9 ± 2.2 a
Publix	73.7 ± 3.1 b	89.5 ± 16.4 ab	9.2 ± 0.98 ab	21.1 ± 2.3 a
Natural Oil	72 ± 4.2 bc	80.2 ± 20.2 b	9.3 ± 1.14 ab	21.04 ± 2.1 a
WetCit	61.2 ± 4.2 d	98.5 ± 14.1 ab	8.8 ± 0.89 b	20.7 ± 2.02 a
VaporGard	67.6 ± 5.5 bcd	83.1 ± 11.1 ab	9.3 ± 0.86 ab	21.5 ± 1.8 a
FBG	65.7 ± 5.2 cd	53.02 ± 29.9 c	8.9 ± 1.06 b	21.2 ± 2.07 a
WashGard	69.8 ± 3.7 bc	83.1 ± 11.6 ab	9.1 ± 0.89 b	21.6 ± 1.8 a
F	16.53	35.01	4.43	1.18
df	6	6	6	6
P	0.0001	0.0001	0.0003	0.3204

Percentage of germination, number of colony forming units (CFU), and radial growing of the colony (diameter in mm) at 5 and 10 days after inoculation at (25 °C ± 1, 70-80% relative humidity, and 12:12 h L:D photoperiod). Means within a column followed by the same letter are not significantly different at α = 0.05 (Tukey's HSD test).

Hibiscus – Madeira Mealybug Efficacy with PFR – Initial Efficacy Screen (FL)

When applied to nymphs and adults, PFR 97® at 10^6 blastospores/mL has been known to infect and kill the 1st and 2nd instar nymphs. Adult males when in contact with the fungal contaminated ovisac were susceptible to PFR 97 infection. Dr. Osborne examined Madeira Mealybug as a model to determine if the entomopathogenic fungus PFR 97® 20% WDG can penetrate and infect the eggs inside the ovisac on leaf disks under optimum environmental conditions prior to shipping. The first step was to apply PFR 97 at 5 different concentrations (10^6, 10^5, 10^4 10^3, 10^2 blastospores/mL, plus water only) to ovisacs placed on Madeira hibiscus leaf discs (Figure 38). Percent egg hatch was assessed 14 days after treatment. The LD_{50} of PFR 97 for the trials was 10^2 blastospores / mL and the LD_{90} was 10^8 blastospores / mL when sprayed against the Madeira mealybug ovisacs.

Figure 38. Madeira hibiscus leaf disc in petri plates with Madeira Mealybug ovisacs



The efficacy of *Isaria fumosorosea* (= *PFR* 97®) on the leaf phylloplane over time for controlling Madeira mealybug, *Phenacoccus madeirensis* Green (Hemiptera: Pseudococcidae), nymphs before shipping plant products was assessed under laboratory conditions. Hibiscus leaves were dipped into beakers filled with 0, 1, 2, 4, 8, and 10 g of *PFR* 97® / L of water and tapped on brown paper towels to allow excess suspension to run off. Damp leaves were individually placed into empty plastic Petri dishes and a single 3rd - 4th-instar mealybug was exposed on the leaf. Each dish bioassay was sealed and placed in an environmentally controlled chamber at 25 °C under a 14 h L: 10 h D photoperiod. Percent mortality was determined after observing nymphs daily for 8 days post-exposure. The LT_{50} of nymphs after exposure to these dilutions varied from 6.9 – 11.1 days. Mortality of the mealybugs for concentrations 2, 4, 8, and 10 g / L was > 50% after 7 days post-exposure to PFR 97, but increased to 100% after final molting to the adult stage. The results suggest that molting may remove infective spores from penetrating the exoskeleton and subsequently decrease efficacy of the fungal treatments until the adult stage. There is potential for using *I. fumosorosea* as part of a strategy for mitigating and controlling mealybug populations on plants pre - and post – shipping.

Hibiscus – Madeira Mealybug Efficacy with Natural Products – Pre Shipment (FL)
Cuttings of coleus, copperleaf, hibiscus, and verbena were evaluated after dipping in various concentrations of Wetcit (a surfactant) and Natur'l Oil for phytotoxicity on a weekly basis. On week 4 post treatment (next week), the cuttings will be evaluated for the final time and the average root length (based on taking measurements of the three longest roots) and root volume for each cutting will be determined.

Thus far, Wetcit at a 2% concentration inflicted moderate to severe foliage phytotoxicity to coleus, copperleaf, hibsicus, and verbena after 2-3 weeks post-dipping. Most cuttings exposed to Wetcit at 2% concentration were considered unmarketable. Therefore, Wetcit at a 2% concentration was not used for subsequent dipping bioassays. Concentrations of Wetcit at 1%, 0.5%, and 0.1%, respectively, caused less foliage phytotoxicity than Wetcit at 2%. Cuttings exposed to lower Wetcit concentrations showed less severe phytotoxicity symptoms while cuttings exposed to higher Wetcit concentrations showed more severe phytotoxicity symptoms: dose response. Natur'l Oil showed less severe foliage phytotoxicity than Wetcit on all four host plants. However, Natur'l Oil showed similar phytotoxicity patterns as Wetcit in the following ways:

1) Natur'l Oil at a 2% concentration inflicted moderate foliage phytotoxicity to all cutting types after 2-3 weeks post-dipping. Most cuttings exposed to Natur'l Oil at 2% concentration were considered borderline marketable.
2) Concentrations of Natur'l Oil at 1%, 0.5%, and 0.1%, respectively, caused less foliage phytotoxicity than Natur'l Oil at 2%. Data collected showed an overall dose response: as we increased the % oil we increased the level or severity of phytotoxicity.

Surfactants have been known to cause plant injury after application under warm to hot temperature in the field. Coleus cuttings applied with Natur'l and Wetcit were exposed to 20C, 30C, 40C, and 50C. Natur'l Oil and

Wetcit treated coleus cuttings were extremely sensitive at 40C and 50C. Nearly all treated coleus cuttings exposed at 50C irrespective of Wetcit or Natur'l Oil treatment concentration were dead. Nearly all treated coleus cuttings exposed at 40C irrespective of treatment concentration were unmarketable. Currently all treated cuttings exposed to 20C (control) and 30C, respectively, show minimal phytotoxicity across all treatment concentrations tested for Wetcit or Natur'l Oil.

Additional research expanded the list of biorational products screened solely on coleus cuttings. This research was two-fold: phytotoxicity to coleus cuttings and efficacy for Madeira mealybug on cuttings using dips of the biorational products. Surfactants evaluated included Natur'l Oil, Vapor Gard, Wetcit, and Publix Mild Dish Detergent. Coleus (*Solenostemon scutellarioides*) cuttings were used for the phytotoxicity studies and coleus cuttings infested with the Madeira mealybug plant were used for the efficacy studies.

For the phytotoxicity concentration trials, a range of concentrations (0.1%, 0.5%, 1.0%, 1.5%) for each insecticidal dip was assessed. The phytotoxicity trials lasted for 3 weeks. Foliage was rated for phytotoxicity and the root volume and average root length determined. Through a process of elimination, the highest concentration of each "insecticidal" dip which caused the least phytotoxic symptoms, 0.1% Vapor Gard, 1.0% Publix Soap, 0.1% Wetcit, and 1.0% Natur'l Oil were selected for use in the efficacy studies.

Each efficacy study lasted two weeks and utilized 5 cuttings for each treatment (4 total) and control (1 total). Each cutting was infested with a mixture of 15 Madeira mealybug adults and nymphs. By process of elimination, the product and concentration which caused the highest mortality (preferably above 70%) was designated as the model treatment. Experiments will last for two weeks with mortality assessed on Day 1, 3, 7 and 14 post-treatment.

The data from the first two efficacy studies were presented below (Table 25, Table 26). Certain treatments greatly reduced the number of mealybugs on treated cuttings. Mortality was observed simply with the dipping process (non treated controls in both experiments). With the biorational treatments, dipping cuttings in the detergent and in Natur'l Oil seem to provide the most consistent and highest level of mortality with the least amount of phytotoxicity.

Table 25. Efficacy over time for Madeira Mealybug challenged with Biorational Products – Experiment 1.

	Number of reps (out of 5) that reach 70% mortality			
Product	Day 1	Day 3	Day 7	Day 14
Non Treated Control	0	0	0	1
Publix Detergent	0	1	3	5 *
Natur'l Oil	1	2	4	5 *
Wetcit	0	2	3	3
VaporGard	0	1	1	2

* These treatments reached 80% mortality

Table 26. Efficacy over time for Madeira Mealybug challenged with Biorational Products – Experiment 2.

	Number of reps (out of 5) that reach 70% mortality			
Product	Day 1	Day 3	Day 7	Day 14
Non Treated Control	0	0	2	2
Publix Detergent	0	3	3	5 *
Natur'l Oil	1	4	5	5 *
Wetcit	0	3	5	4 *
VaporGard	0	1	2	3

* These treatments reached 80% mortality

Bean – Mite Efficacy with PreFeRal – Pre Shipment (FL)
Several trials were conducted using Preferal alone and in combination with a surfactant as a dip of bean leaves infested with *T. urticae*. In general, the fungus was present but there was very little indication that there was any benefit from treating leaves with the fungal pathogen when it comes to controlling or even infecting *T. urticae*. The positive aspect of these studies is that the fungus did grow on the treated leaves and produce conidia. The surfactant caused a significant amount of phytotoxicity while having little impact on the pests.

Spearmint – Mite Efficacy with Natural Products – Pre Shipment (FL)
All dip-mist studies were conducted using Spearmint at the UF Apopka research center. Cuttings were taken from untreated stock plants and rooted commercial potting soil (Faford® Growing Mix 2/C-2 soil composed of Canadian sphagnum peat moss, perlite, vermiculite, dolomitic limestone, and wetting agent (2:1:1:1:1, vol:vol:vol:vol:vol)) planted in 4 inch plastic pots. The potted cuttings were then placed in mist that actuated every 10 minutes for 30 seconds from 0800 to 2000 hours. Once rooted and actively growing they were either infested naturally or by placing infested leaf pieces on them until a sufficient number of individuals were noted on plant. The mite studies were infested with *Tetranychus urticae.* Cuttings were then taken and the number of individual on marked leaves of each cutting were then counted, the infested cuttings were assigned to treatments, dipped it an appropriate treatment solution for 1 minute, allowed to air dry and then cuttings completely randomized under the mist system. We evaluated one rate of Stoller Natur'l Oil (10mL of Stoller Natur'l Oil per 990mL of de-ionized water), one rate of M-Pede (10mL of Stoller Natur'l Oil per 990mL of de-ionized water), and a control treatment of just de-ionized water. A second count of live mites or thrips was made 2 or 7 days post-application using a binocular microscope in the laboratory.

In the first two experiments, mites were reduced significantly with both Stoller Natur'l Oil and Mpede 2 days after treatment, but only Stoller Natur'l Oil exceeded greater than 90% control of motiles (Table 27, Table 28). Eggs were reduced significantly in Experiment 2, but this may not be to a sufficient level for interstate or international shipping. A similar trend was seen in the second two experiments where data were collected 7 days after treatment (Table 29, Table 30).

After talking to a number of growers, they feel that post-shipment treatments are most relevant to them. They don't think the cutting producers off-shore will dip cuttings. They feel their pest insurance will be to dip newly arriving cuttings themselves. These treatments didn't result in levels of control that would meet regulatory standards but the oil treatments achieved very high and acceptable levels of control without any noticeable phytotoxicity.

Table 27. Mean number of mites per leaf, Experiment 1

Treatment/formulation	Rate	DAT 0 (Pre-spray)	DAT 2
Stoller Natur'l Oil	1%	11.2a	0.0b (100)
Mpede	1%	10.8a	4.3b (62)
Untreated check	-	10.7a	11.3a
	F value	0.47	17.64
	Pr > F	0.6292	<.0001

Means in a column followed by the same letter are not significantly different ($P > 0.05$, Tukey test)
Henderson-Tilton's corrected percent mortality is presented in parentheses after each mean

Table 28. Mean number of mite eggs and motiles per leaf, Experiment 2

Treatment/ formulation	Rate product/ acre	Eggs		Motiles	
		DAT 0 (Pre-spray)	DAT 2	DAT 0 (Pre-spray)	DAT 2
Stoller Natur'l Oil	1%	18.1a	2.0b (80)	8.2a	0.9c (92)
Mpede	1%	16.6a	1.5b (84)	9.3a	6.6b (47)
Untreated check	-	17.3a	9.5a	9.2a	12.3a
F value		0.18	9.57	0.16	30.83
Pr > F		0.8378	0.0009	0.8561	<.0001

Means in a column followed by the same letter are not significantly different ($P > 0.05$, Tukey test)
Henderson-Tilton's corrected percent mortality is presented in parentheses after each mean.

Table 29. Mean number of mites per leaf, Experiment 3

Treatment/formulation	Rate	DAT 0 (Pre-spray)	DAT 7
Stoller Natur'l Oil	1%	11.8a	0.0b
Mpede	1%	10.8a	4.3b
Untreated check	-	10.7a	11.3a

Means in a column followed by the same letter are not significantly different ($P > 0.05$, Tukey test)

Table 30. Mean number of mites per leaf, Experiment 4

Treatment/formulation	Rate	DAT 0 (Pre-spray)	DAT 7
Stoller Natur'l Oil	1%	10.3a	5.5b
Mpede	1%	10.3a	41.7a
Untreated check	-	10.2a	50.5a

Means in a column followed by the same letter are not significantly different ($P > 0.05$, Tukey test)

Spearmint – Thrips Efficacy with Natural Products– Pre Shipment (FL)

All dip-mist studies were conducted using Spearmint at the UF Apopka research center. Cuttings were taken from untreated stock plants and rooted commercial potting soil (Faford® Growing Mix 2/C-2 soil composed of Canadian sphagnum peat moss, perlite, vermiculite, dolomitic limestone, and wetting agent (2:1:1:1:1, vol:vol:vol:vol:vol)) planted in 4 inch plastic pots. The potted cuttings were then placed in mist that actuated every 10 minutes for 30 seconds from 0800 to 2000 hours. Once rooted and actively growing they were either infested naturally or by placing infested leaf pieces on them until a sufficient number of individuals were noted on plant. The thrips studies were infested with *Echinothrips americanus*. Cuttings were then taken and the number of individual on marked leaves of each cutting were then counted, the infested cuttings were assigned to treatments, dipped in an appropriate treatment solution for 1 minute, allowed to air dry and then cuttings completely randomized under the mist system. We evaluated one rate of Stoller Natur'l Oil (10mL of Stoller Natur'l Oil per 990mL of de-ionized water), one rate of M-Pede (10mL of Stoller Natur'l Oil per 990mL of de-ionized water), and a control treatment of just de-ionized water. A second count of live mites or thrips was made 2 days post-application using a binocular microscope in the laboratory.

Table 31. Mean number of Echinothrips per spearmint leaf, Experiment 1

Treatment/ formulation	Rate	Larvae		Pupae		Adult	
		DAT 0 (Pre-spray)	DAT 2	DAT 0 (Pre-spray)	DAT 2	DAT 0 (Pre-spray)	DAT 2
Stoller Natur'l Oil	1%	10.9a	0.3b (91)	3.4a	0.6b (78)	2.6a	0.1ab (90)
Mpede	1%	9.5a	0.3b (90)	4.3a	2.3a (33)	2.8a	0b (100)
Untreated check	-	11.6a	3.7a	4a	3.2a	2a	0.8a
F value		0.37	14.46	0.76	8.85	0.86	5.22
Pr > F		0.7343	<.0001	0.6254	0.6999	0.4372	0.0462

Means in a column followed by the same letter are not significantly different ($P > 0.05$, Tukey test)
Henderson-Tilton's corrected percent mortality is presented in parentheses after each mean.

Table 32. Mean number of immature thrips (larvae, pupae) per spearmint leaf, Experiment 2

Treatment/ formulation	Rate	Larvae		Pupae		Adult	
		DAT 0 (Pre-spray)	DAT 2	DAT 0 (Pre-spray)	DAT 2	DAT 0 (Pre-spray)	DAT 2
Stoller Natur'l Oil	1%	1.9a	0.1b (91)	1.6a	0.2b (80)	1.5a	0b (100)
Mpede	1%	3.1a	0.6ab (68)	0.7a	0b (100)	1.6a	0.1b (95)
Untreated check	-	2.8a	1.7a	1.3a	0.8a	1.4a	1.9a
F value		0.37	0.70	5.25	1.30	0.11	22.33
Pr > F		0.7343	0.5060	0.0132	0.2915	0.8963	<.0001

Means in a column followed by the same letter are not significantly different ($P > 0.05$, Tukey test)
Henderson-Tilton's corrected percent mortality is presented in parentheses after each mean

Table 33. Mean number of immature thrips (larvae, pupae) per spearmint leaf, Experiment 3

Treatment/ formulation	Rate	Larvae		Pupae		Adult	
		DAT 0 (Pre-spray)	DAT 7	DAT 0 (Pre-spray)	DAT 7	DAT 0 (Pre-spray)	DAT 7
Stoller Natur'l Oil	1%	8.8a	8.6a	1.2a	0.4b	1.2a	1.2a
Mpede	1%	7.9a	8.9a	1.5a	0.1b	1.5a	1.8a
Untreated check	-	5.9a	8.5a	3.1a	3.4a	2.1a	2.5a

Means in a column followed by the same letter are not significantly different ($P > 0.05$, Tukey test)

Chrysanthemum – Mite Efficacy with Natural Products – Pre Shipment (FL)

All dip-mist studies were conducted using Chrysanthemum variety Chesapeake (*Dendranthema x grandiflora*). Cuttings were taken from untreated stock plants and rooted commercial potting soil (Faford® Growing Mix 2/C-2 soil composed of Canadian sphagnum peat moss, perlite, vermiculite, dolomitic limestone, and wetting agent (2:1:1:1:1, vol:vol:vol:vol:vol)) planted in 4 inch plastic pots. The potted cuttings were then placed in mist that actuated every 10 minutes for 30 seconds from 0800 to 2000 hours. Once rooted and actively growing they were either infested naturally or by placing infested leaf pieces on them until a sufficient number of individuals were noted on plant. The mite studies were conducted using *Tetranychus urticae*. Cuttings were then taken and the number of individual on marked leaves of each cutting were then counted, the infested cuttings were assigned to treatments, dipped it an appropriate treatment solution for 1 minute, allowed to air dry and then cuttings completely randomized under the mist system. We evaluated one rate a 1.0% rate of Stoller Natur'l Oil (10mL of Stoller Natur'l Oil per 990mL of de- ionized water), one rate of M-Pede (10mL of Stoller Natur'l Oil per 990mL

of de-ionized water), and a control treatment of just de-ionized water. A second count of live mites was made 7 days post-application using a binocular microscope in the laboratory.

Table 34. *Tetranychus urticae* on Chrysanthemum

	Live mites	
Treatment	Day 0	Day 7
Control	16.7a	7.8a
Stoller Natur'l Oil	16.7a	1.3b
M-Pede	16.5a	1.3b

Means in a column followed by the same letter are not significantly different ($P > 0.05$, Tukey test)

Chrysanthemum – Echinothrips Efficacy with Natural Products – Pre Shipment (FL)

All dip-mist studies were conducted using Chrysanthemum variety Chesapeake (*Dendranthema x grandiflora*). Cuttings were taken from untreated stock plants and rooted commercial potting soil (Faford® Growing Mix 2/C-2 soil composed of Canadian sphagnum peat moss, perlite, vermiculite, dolomitic limestone, and wetting agent (2:1:1:1:1, vol:vol:vol:vol:vol)) planted in 4 inch plastic pots. The potted cuttings were then placed in mist that actuated every 10 minutes for 30 seconds from 0800 to 2000 hours. Once rooted and actively growing they were either infested naturally or by placing infested leaf pieces on them until a sufficient number of individuals were noted on plant. Cuttings were infested with *Echinothrips americanus*. Cuttings were then taken and the number of individual on marked leaves of each cutting were then counted, the infested cuttings were assigned to treatments, dipped it an appropriate treatment solution for 1 minute, allowed to air dry and then cuttings completely randomized under the mist system. We evaluated one rate a 1.0% rate of Stoller Natur'l Oil (10mL of Stoller Natur'l Oil per 990mL of de-ionized water), one rate of M-Pede (10mL of Stoller Natur'l Oil per 990mL of de-ionized water), and a control treatment of just de-ionized water. A second count of live mites or thrips was made 7 days post-application using a binocular microscope in the laboratory. Thrips adults are very mobile and thus moved between treatments.

Table 35. *Echinothrips sp.* on Chrysanthemum, Experiment 1

	Live Echinothrips							
	Day 0				**Day 7**			
Treatment	**Adults**	**Larvae**	**Pupae**	**Total**	**Adults**	**Larvae**	**Pupae**	**Total**
Control	5.0a	20.2a	3.9a	29.1a	5.0a	27.3a	4.2a	36.5a
M-Pede	4.2a	20.3a	4.8a	29.3a	1.1b	20.9ab	0.4b	22.4ab
Stoller Natur'l Oil	4.6a	19.3a	4.6a	28.5a	1.2b	9.4b	0.0b	10.6b

Means in a column followed by the same letter are not significantly different ($P > 0.05$, Tukey test)

Table 36. *Echinothrips sp.* on Chrysanthemum, Experiment 2

	Live Echinothrips							
	Day 0				**Day 7**			
Treatment	**Adults**	**Larvae**	**Pupae**	**Total**	**Adults**	**Larvae**	**Pupae**	**Total**
Control	6.2a	20.4a	9.4a	36.0a	15.4a	28.5a	16.1a	60.0a
M-Pede	5.0a	19.5a	10.9a	35.4a	1.6b	11.7b	0.3b	13.6b
Stoller Natur'l Oil	5.2a	21.8a	8.7a	35.7a	3.6b	12.7b	0.1b	16.4b

Means in a column followed by the same letter are not significantly different ($P > 0.05$, Tukey test)

Table 37. *Echinothrips sp.* on Chrysanthemum, Experiment 3

Treatment	Live Echinothrips							
	Day 0				Day 7			
	Adults	Larvae	Pupae	Total	Adults	Larvae	Pupae	Total
Control	5.5a	7.9a	5.0a	18.4a	5.9a	8.8a	4.4a	19.1a
M-Pede	5.9a	9.3a	3.5a	18.7a	2.0b	11.4a	0.1b	13.5a
Stoller Natur'l Oil	5.0a	7.6a	5.3a	17.9a	2.8b	5.1a	0.1b	8.0a

Means in a column followed by the same letter are not significantly different ($P > 0.05$, Tukey test)

Basil – Aphid Efficacy with Natural Products – Pre Shipment (FL)

All dip-mist studies were conducted using Basil at the UF Apopka research center. Cuttings were taken from untreated stock plants and rooted commercial potting soil (Faford® Growing Mix 2/C-2 soil composed of Canadian sphagnum peat moss, perlite, vermiculite, dolomitic limestone, and wetting agent (2:1:1:1:1, vol:vol:vol:vol:vol)) planted in 4 inch plastic pots. The potted cuttings were then placed in mist that actuated every 10 minutes for 30 seconds from 0800 to 2000 hours. Once rooted and actively growing they were either infested naturally or by placing infested leaf pieces on them until a sufficient number of individuals were noted on plant. Basil cuttings were infested with melon aphid (*Aphis gossyppii*). Cuttings were then taken and the number of individual on marked leaves of each cutting were then counted, the infested cuttings were assigned to treatments, dipped it an appropriate treatment solution for 1 minute, allowed to air dry and then cuttings completely randomized under the mist system. We evaluated one rate of Stoller Natur'l Oil (10mL of Stoller Natur'l Oil per 990mL of de-ionized water), one rate of M-Pede (10mL of Stoller Natur'l Oil per 990mL of de-ionized water), and a control treatment of just de-ionized water. A second count was made 7 days post-application using a binocular microscope in the laboratory.

Table 38. Preliminary data for population reduction of melon aphid 7 days after dipping basil cutting in oil or Impede – Experiment 1

Treatment	7/14/2015	7/21/2015
Control	84	249
Stoller Natur'l Oil	88	3
Impede	82	0

Table 39. Preliminary data for population reduction of melon aphid 7 days after dipping basil cutting in oil or Impede – Experiment 2

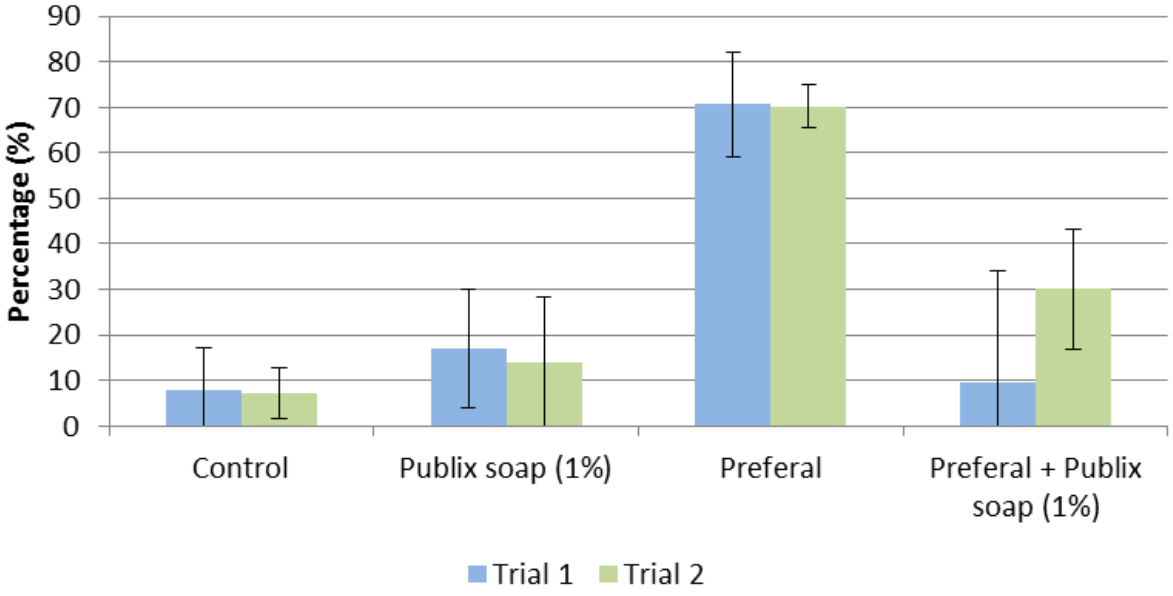
Treatment	7/21/2015	7/28/2015
Control	69	257
Stoller Natur'l Oil	69	6
Impede	70	1

Objective 5: Study performance of biological tools under simulated shipping

Dr. Obsorne's laboratory group studied the impact of Preferal, Publix detergent and the combination of these on *Bemisia tabaci* Biotype B on Mint (*Mentha* sp) as a test system. Plants were exposed 24 hours to adults, a length of time to allow oviposition on the leaves. Terminal cuttings were collected and then dipped for 60 seconds with the three treatments and placed in a mist bed. After 10 days under mist, the plants were subjected to simulated shipping by placing the cuttings in darkness at 70° F for 48 hours. Whitefly populations were evaluated before and after simulated shipping.

In both trials, the number of whiteflies in each treated population was not statistically different from the non-treated control plants. However there was a statistically significant decline in population with the Preferal dips. Population data converted with the Henderson-Tilton equation indicated approximately 70% efficacy (Figure 39). The addition of Publix detergent to Preferal and Publix detergent appeared to inhibit whitefly mortality with Preferal.

Figure 39. Reduction of whitefly population after the application of treatments and shipping simulation



Soon after completion of the initial screening studies, it was decided to ship between the USDA laboratory at Fort Pierce and the UF Experiment Station in Apopka, FL in addition to some preliminary simulated shipping studies. The results below are a combination of these experiments.

Drs. Bethke, McKenzie and Osborne developed a shipping protocol and shipped host plants and cuttings among the sites. Initially coleus was planned for Madeira mealybug, but this altered to chrysanthemum based on the number of mealybugs planned for shipping. Mint was selected as a model crop for *Tetranychus urticae* and *Bemisia tabaci*.

Mint – Silverleaf Whitefly – Actual Shipping Fresh Cuttings (FL > FL)
Several trials were conducted to evaluate the effectiveness of both entomopathogenic fungi *I. fumosorosea* (Preferal®) and *B. bassiana* (Botanigard®) mixed with two bio pesticides (the soap Publix® and the surfactant Natural Oil®) in order to reduce the population of whitefly *Bemisia tabaci* through pre-shipping treatments on ornamental plants. Each trial had 4 or 6 treatments with 6 repetitions per treatment. In 3 trials (Preferal® & Publix®), mint plants were exposed to adult whiteflies for 24 hours to ensure egg laying; afterwards, terminals were cut and treated with corresponding product by dipping the terminals in the solutions during 60 seconds. Then terminals were placed on a mist bed for 8 to 10 days. After rooting terminals were shipped to Fort Pierce FL or placed under simulated shipping (20°C ± 2, total darkness, and 60 - 80% RH) for 48 hours. Whitefly populations were assessed before the products were applied and 3, 7, and 14 days after shipping.

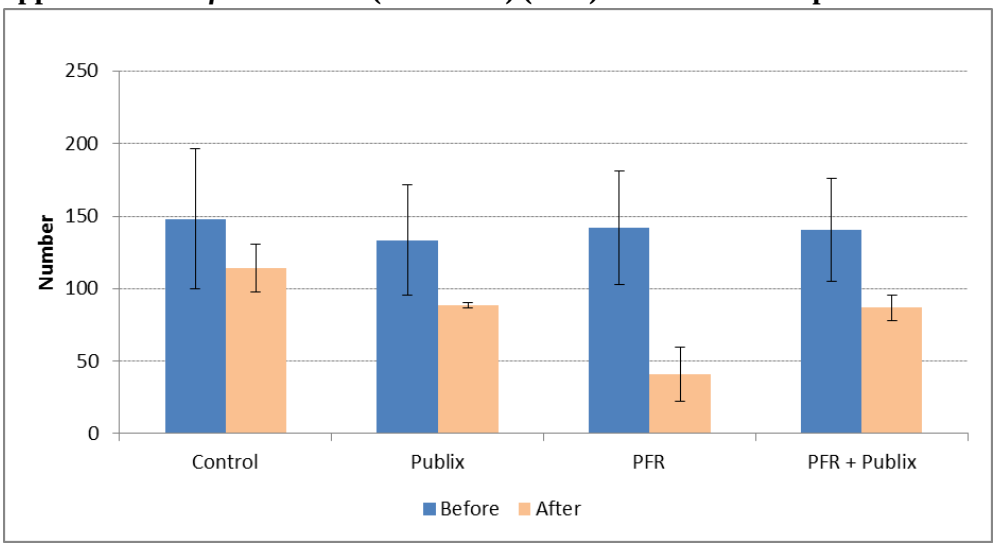
In the subsequent experiments, mint plants were exposed to whitefly adults for 6 days; this period of time guarantee the presence of both eggs and immature stages at the same time before treatments (Figure 40). Figure 41 and Figure 42 show the population of whitefly (eggs) before and after pre-shipping treatments (eggs, nymphs, and adults) and the mortality caused by *I. fumosorosea* (PFR), Publix (soap), and the combination of both of them (PFR + Publix). In this case, when the population of eggs was treated with *I. fumosorosea* before shipping, PFR significantly reduced the population of whitefly in contrast with the control and the other

treatments. The soap Publix applied alone or in combination with *I. fumosorosea* did not have a significant impact in the reduction of whitefly population. The highest impact in the reduction of the whitefly population was presented by the application of *I. fumosorosea (*Preferal®) alone which caused (62.4% ± 11.4) of mortality (Figure 42). The reduction of whitefly population by Publix applied alone or in combination with the fungi was low, less than 20% of mortality, (the mortality was corrected by Henderson-Tilton's Formula).

Figure 40. Shipping study of *Bemisia tabaci* reared on cabbage.

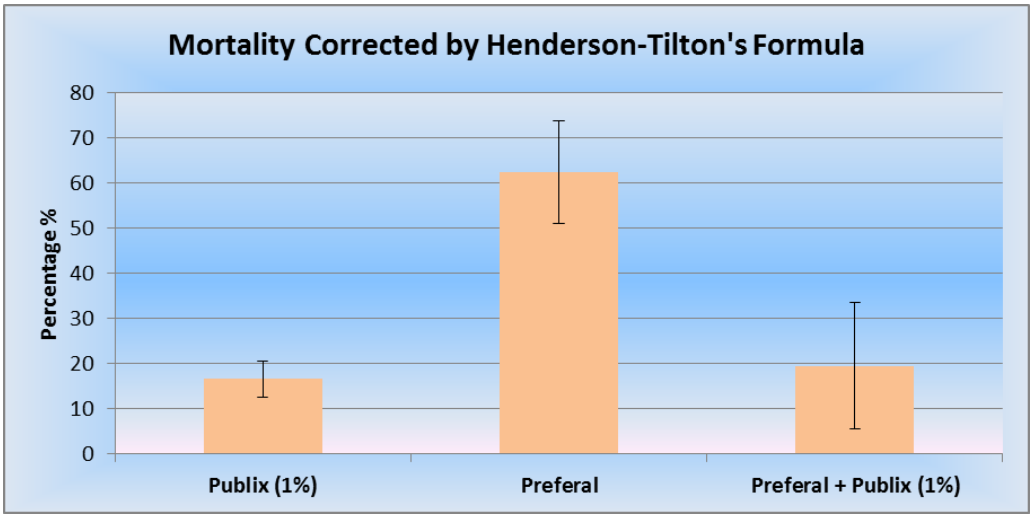
A- Colony of whitefly *Bemisia tabaci* reared on cabbage, *Brassica oleracea* (L); B- Mint plants, *Mentha* sp. (L) infected with whitefly (exposure 24 h or 6 days); C and D- Commercial carton boxes using for shipping the mint plants after pre-shipping treatments; E - Boxes with mint plants placed inside the simulation room for 24 – 48 hours (at 20 °C ± 2, 60 – 80 % Relative humidity and totally darkness); F – Mints plants placed in a greenhouse for evaluation at 3, 7, and 14 days after the shipping arrival.

Figure 41. Population of whitefly before and after pre-shipping treatments at 3, 7, and 14 days later; application of *I. fumosorosea* (Preferal®) (PFR) and Publix® soap.



Data correspond to the average from 3 trials.

Figure 42. Mortality of whitefly population corrected by Henderson-Tilton's formula. Pre-shipping treatments with *I. fumosorosea* (Preferal®) and Publix soap applied alone and when were applied mixed with the entomopathogenic fungi



Data correspond to the average from 3 trials.

Effect of *B. bassiana*, Publix and Natural Oil on immature stages of whitefly

The population of whitefly on mint plants infected with immature stages (eggs and nymphs) and treated with different bio-pesticides is presented in the figures 5 and 6. The reduction of whitefly population after pre-shipping treatments was significantly high with the application of *B. bassiana* (BB*)* alone and *B. bassiana* mixes with Natural Oil (BB + N. Oil), in contrast with the other treatments. The reduction caused by Natural Oil alone was significantly high in contrast with the control. However, its reduction was less than the application of *B. bassiana* alone or in the combination of *B. bassiana* with Natural Oil. The application of Publix alone or in combination with *B. bassiana* did not have a significant reduction of whitefly population. In Figure 43 and Figure 44, the sporulation of the entomopathogenic fungi *B. bassiana* (at 7 or 14 days after the shipping arrival) in the body of immature stages and adults is shown.

In addition, the reduction of whitefly population (mortality) was increasing through the time after the application (3, 7, and 14 days after pre-shipping treatments) for *B. bassiana* and *B. bassiana* mixed with Natural Oil (figure 5). In fact, the number of the immature stages of whitefly before the application of the bioinsecticides ranged between 130 and 170 individuals (eggs and nymphs) per leaf disc in all treatments. The population in controls 14 days after pre-shipping application was higher than 150 individual per left discs. In contrast, when *B. bassiana* was applied alone and *B. bassiana* was applied in combination with Natural Oil, the population of whitefly was reduced from 130 to 20 individuals per leaf disc and from 170 to 20 individuals per leaf disc respectively. This increasing in the reduction of population was showed because the effectiveness of the entomopathogenic fungus increased after the application and because the conditions were propitious to the fungus especially high humidity (> 80% under shipping simulation and > 70% under greenhouse).

Figure 43. The population of whitefly before and at 3, 7, and 14 days after pre-shipping treatments with biopesticides. Mint plants treated with *B. bassiana* (BB), soap (Publix), Natural Oil (N. Oil), and the combination of those products with the fungus (BB + N. Oil).

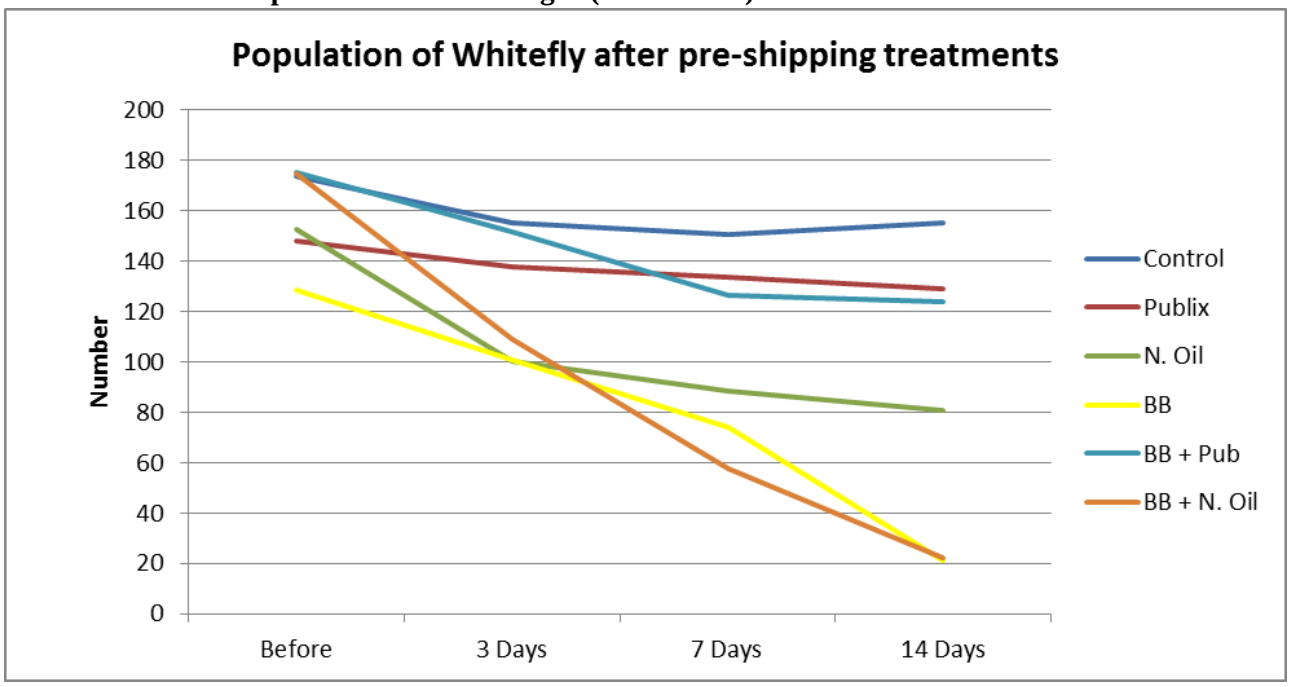
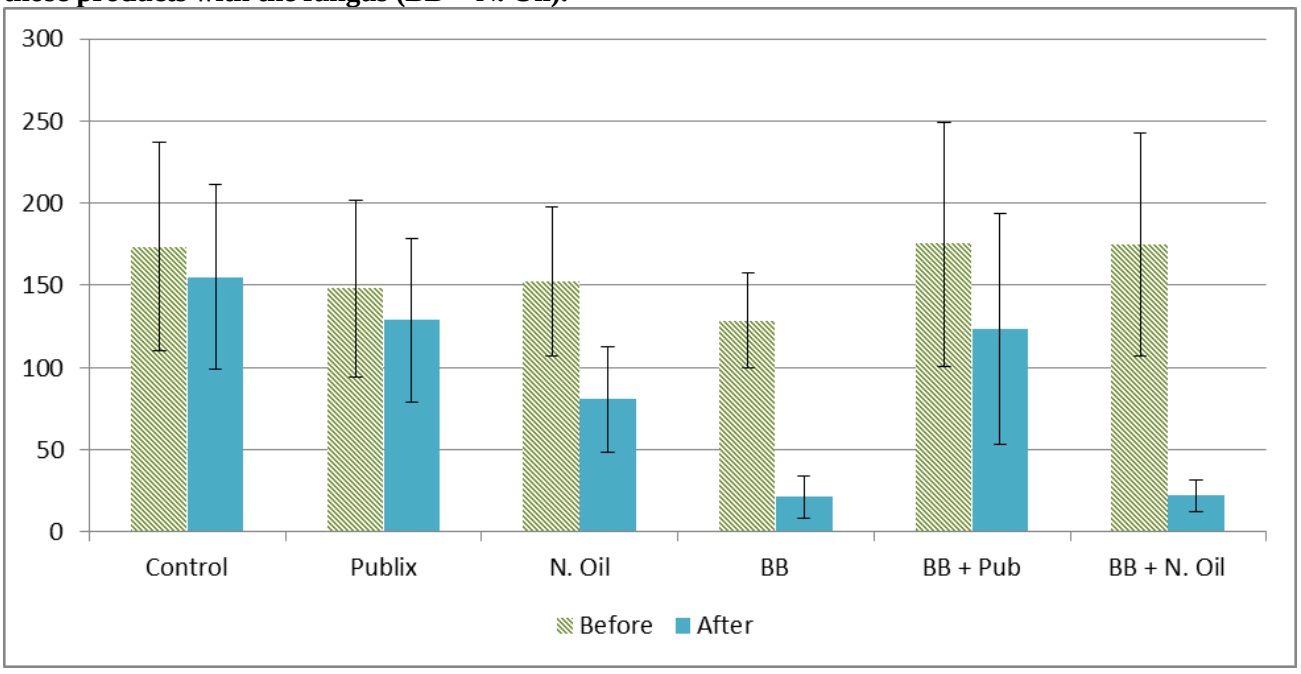


Figure 44. The population of whitefly before and 14 days after pre-shipping treatments with biopesticides. Mint plants treated with *B. bassiana* (BB), Publix (soap), Natural Oil (N. Oil), and the combination of those products with the fungus (BB + N. Oil).



Mortality of whitefly caused by two entomopathogenic fungi and two surfactants

The average of mortality (%) on whitefly population caused by all products through pre-shipping treatments is shown in Table 40 and Figure 45; mortality was corrected by Henderson-Tilton's Formula. The analysis of

variance (ANOVA) for the mortality showed significant differences between treatments. According with the Tukey's HSD test, the most efficient bio-pesticides to reduce whitefly populations on pre-shipping treatments were *B. bassiana* applied alone, *B. bassiana* in combination with Natural Oil, and *I. fumosorosea* in combination with Natural Oil. In contrast, when Publix, Natural Oil, and *I. fumosorosea* were applied alone the mortality on immature stages of whitefly was less than 50%.

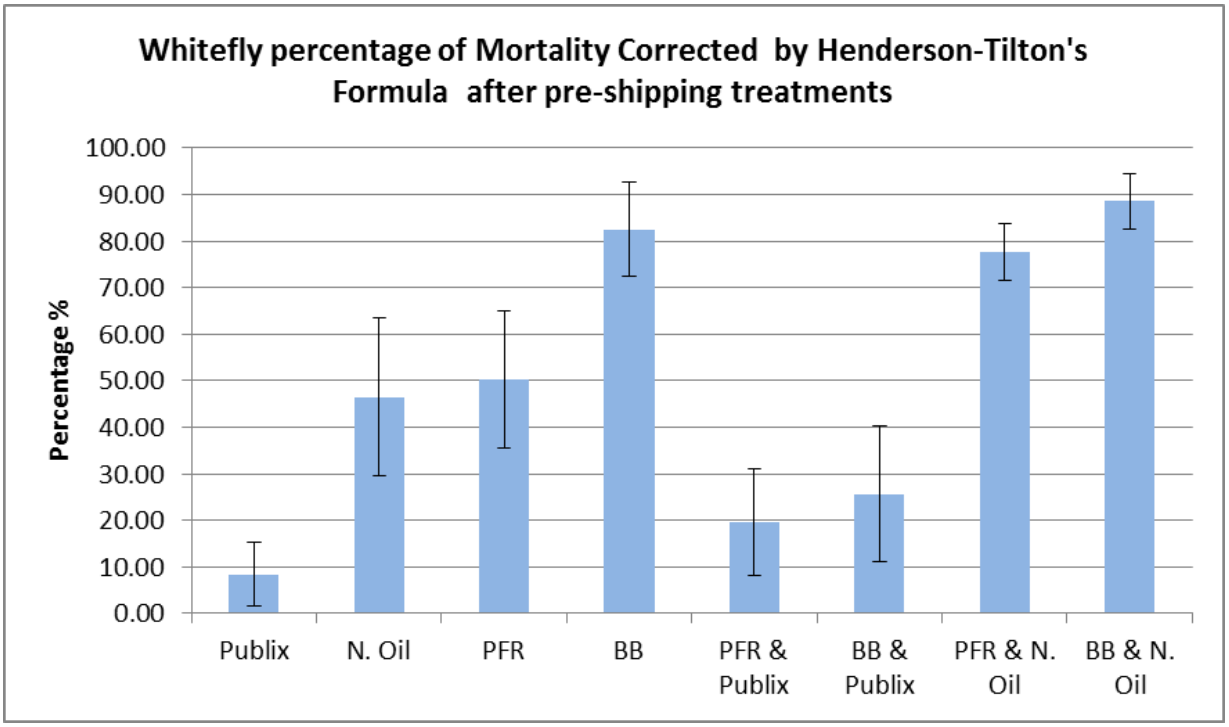
In some cases, the applications (dip during 60 seconds) of Natural Oil at (1%) alone or in combination with both entomopathogenic fungi caused phytotoxicity on mints plants. Not all plants presented the same symptom damages by phytotoxicity. Others plants were in good conditions in the greenhouse after the pre-shipping treatments with Natural Oil. Due that some plants (different ornamental species) could be more susceptible than others to different surfactant products; it is important conduct a test for phytotoxicity. Therefore, before use Natural Oil, is recommended to make a test for phytotoxicity on plants, with different concentrations (0.5 and 1%) and different periods of exposure (15, 30, and 60 seconds) in order to prevent damage of plant.

Table 40. Mortality of whitefly population after pre-shipping treatments.

Treatments	Mortality Average ± SDT
B. Bassiana & Natural Oil	88.53 ± 5.8 a
B. Bassiana	82.54 ± 10 a
I. fumosorosea & Natural Oil	77.68 ± 6.1 ab
I. fumosorosea	50.15 ± 14.7 bc
Natural Oil	46.43 ± 16.9 cd
B. Bassiana & Publix	25.67 ± 14.4 de
I. fumosorosea & Publix	19.55 ± 11.4 de
Publix	8.4 ± 6.8 e
F	23.24
df	7
P	0.0001

The mortality of whitefly was corrected by Henderson-Tilton's formula. Treatments with the same letter are not significantly different according with Tukey HSD test ($\alpha = 0.05$).

Figure 45. Mortality of whitefly population corrected by Henderson-Tilton's formula.



Pre-shipping treatments with *I. fumosorosea* (Preferal®), *B. bassiana* (Botanigard®), Publix® soap and Natural Oil® applied alone and when were applied mixed with the entomopathogenic fungi.

Figure 46. Adults and immature stages of whitefly *Bemisia tabaci* on mint plants at 7 and 14 days after the shipping arrival

A- Healthy immature stages (control); B- Healthy adult (control); C and D- Immature stage and adult infected by *I. fumosorosea* (Preferal®) respectively; E and F - Immature stage and adult infected by *B. bassiana* (Botanigard®) respectively.

Objective 5: Study performance of tools during shipping among CA, FL and HI

Mint – Silverleaf Whitefly – Actual Shipping Fresh Cuttings (FL > CA)

Unrooted mint cuttings treated with Publix Mild & Gentle Detergent or Natur'l Oil were shipped from Apopka, FL to Fort Pierce, FL and San Marcos, California. Upon arrival in CA, they were immediately potted in four inch pots so they could be grown and as stock for cuttings in future shipping studies. Since all were treated, phytotoxicity was assessed. At Fort Peirce, FL, the cuttings were placed in environments to promote rooting and evaluated for phytotoxicity. In all cases, there were no issues noted that resulted from the dip treatments. Small data recorders were obtained and tested in shipping containers prior to full use.

Permits necessary to receive shipments of infested plant material in Florida from California and vice versa were acquired.

Figure 47. Dr. Jim Bethke's whitefly colony being maintained on Mint (*Mentha sp.*)

Figure 48. Mint cuttings received by Dr. Jim Bethke and placed in to 4 inch pots for further evaluation.

The first three trials were run (dip-ship, spray-ship, and spray-dry-ship) with whitefly on cuttings. Each trial consisted of 6 treatments and a control with 6 replications each totaling 42 cuttings/plants per trial, an aggregate of 126 cuttings/plants for the first round.

The three leaves were then marked in sharpie with an “X” to denote those to be inoculated and to help with ease of relocation for future data collection (Figure 49). A team of seven researchers attached 378 clip traps to the indicated leaves on each designated cutting and aspirated 5 to 10 whitefly per clip trap for a total of 1890-3780 whitefly applied. These were left for the specified 48 hours in the greenhouse to reproduce. All clip traps were removed and left for an addition 24 hours to allow eggs to acclimate. After this 24-hour period, pretreatment evaluations were made prior to the insect shipping treatments (T1). The number of eggs was then counted on each of the designated leaves (Figure 49). After counting, the cuttings were removed from the rooted plants and immediately treated with the designated treatment type, packaged, and shipped to Florida next day air in a Styrofoam shipping container with ice packs.

Figure 49. Evaluating whiteflies on Mint (*Mentha sp.*)

Table 41. Average number of whitefly eggs prior to treatment.

Number of Cuttings/Plants	Total Leaves Counted	Treatment Type	Total # Eggs	Average # of Eggs Per Leaf Counted
42	126	Dip-Ship	701	16.69
42	126	Spray-Ship	1917	45.64
42	126	Spray-Dry-Ship	1839	43.79

There were a total of 12 trials and 3 shipments of mint cuttings infested with whitefly. In the initial shipment to the Florida lab as described above, four trials were conducted. However, mint cuttings did not standup to the shipping process and the 70% mortality rate was attributed to the high temperatures during treatment and shipping. On arrival, the plants displayed signs of disease and died over a two-day period. Data was collected upon arrival, but the cuttings died before additional counts were made, rendering the data unusable.

The following shipment was completed within more acceptable temperature conditions, and the cutting mortality rate dropped to 10%. However, due to additional infestations (predatory mites and thrips) the whitefly data was abysmal. While the plants were not dead, they were not healthy and the presence of whitefly was difficult to ascertain.

We treated the stock plants with Triact and Conserve, but the mint populations suffered from the treatments. We ran a mock trial without success. Due to the conditions in our greenhouse and the limited control we have over the prevention of stock plant exposure to other pathogens, it was decided that we might have more success if we went with a hardier and more resilient plant species to avoid further issues.

Plant Species Change

Chrysanthemum cuttings were ordered and the replacement process was started (11-14-2013). Background information on a "model" treatment for the mums was reviewed and a phytotoxicity test was started. These plants were shipped to the Florida lab (12-03-2013) and observed. While all of the treatments seemed to be fine, the control was apparently compromised by its proximity to the icepack during the shipment, causing nearly

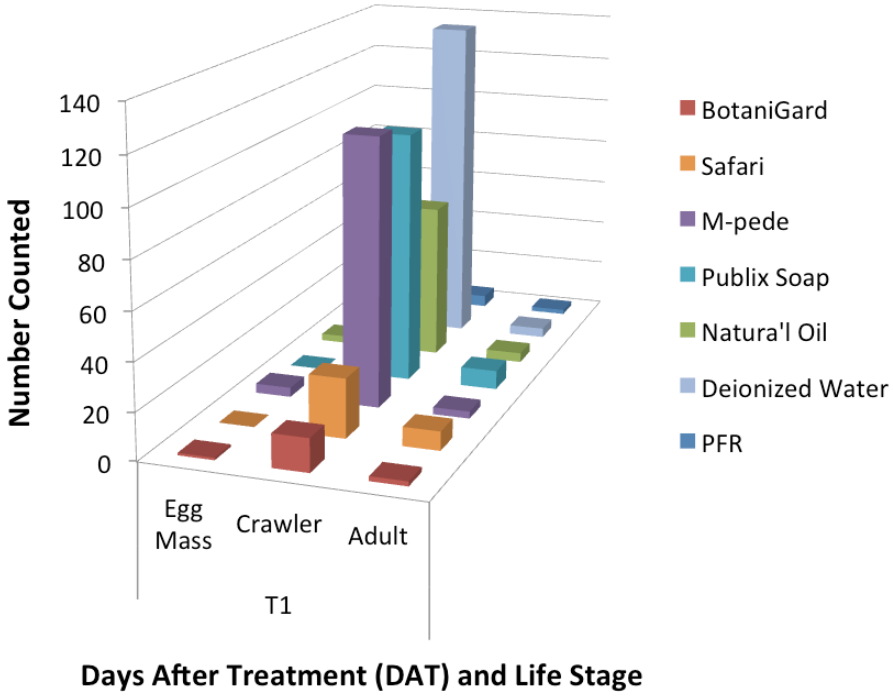
100% mortality. Due to this inconsistency, we performed a second phytotoxicity test at our lab and concluded with a mean phytotoxicity of 1.6 on a scale from 0 (no phyto) to 6 (dead).

Figure 50. Evaluating madeira mealybug on chrysanthemum

Chrysanthemum – Citrus Mealybug – Actual Shipping Fresh Cuttings (CA > FL), Experiment 1
Inoculations of Citrus mealybug on chrysanthemum have been very successful. The first trial for this combination commenced 02-25-2014 with inoculations. A total of 50 4" plants were artificially infested with Citrus mealybug by placing one egg mass and 5-7 adults per plant with 6 plants per treatment (42 plants). After inoculation, the plants were kept in insect cages placed on benches in a climate-controlled greenhouse. One week after inoculation, the plants were then treated with the selected products and held for an additional 24-hours after treatment.

Fresh cuttings were taken, dipped in rooting hormone and packaged to mimic the way we have been receiving them. They were separated into zip locked bags by treatment and shipped overnight via FedEx. The entirety of all six plants from each treatment were evaluated and counted in each subsequent evaluation. Evaluations were made prior to the insect shipping treatments, noted as T1 (Figure 51).

Figure 51. Citrus mealybug on chrysanthemum cuttings with a dip then ship treatment, pretreatment count, Experiment 1.

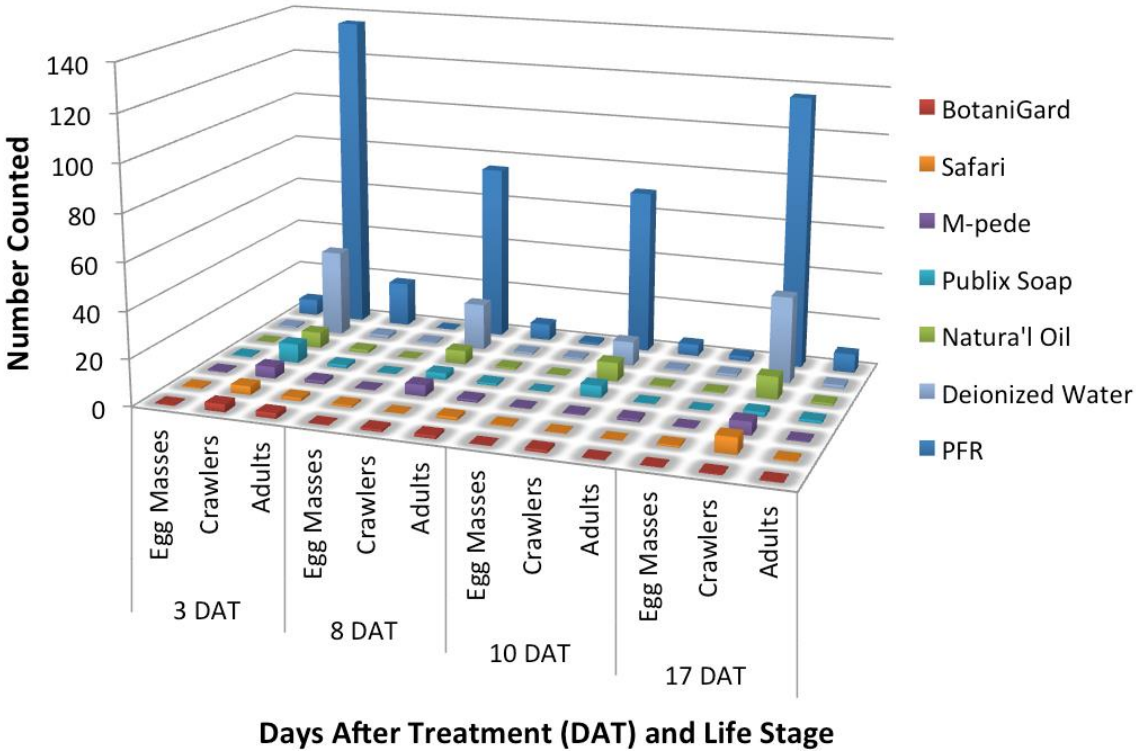


We started this portion doing destructive counts, since mealybugs tend to wedge themselves in hard to reach places (between the petiole, lateral bud, and the stem, inside the flowers, and in the terminal bud) and we were concern that the act of repeatedly counting each plant would cause too damaging and the accuracy of counts would suffer. However, once the cuttings were received the counts were not completed in the same manner.

After the shipment was received by the Florida lab, post shipment counts were completed on arrival (3 DAT); post shipment (8 DAT); post shipment (10 DAT); post shipment (17 DAT). All 6 plants for each treatment type were counted in the attempt to provide more data than what destructive counts would provide. In the future, pretreatment counts will be completed on all plants. It should also be noted that the 17 DAT evaluations were a destructive count (Figure 52).

Pictures of the plants were taken before the 17 DAT count and the cuttings showed little to no phytotoxicity.

Figure 52. Citrus mealybug on chrysanthemum cuttings with a dip then ship treatment, post shipment counts, Experiment 1.



Chrysanthemum – Citrus Mealybug – Actual Shipping Fresh Cuttings (CA > FL), Experiment 2
Fresh cuttings were collected from 4" pots and were utilized for three different application trials: Dip-Ship (DS) and Dip-Dry-Ship (DDS), Spray-Ship (SS). Inoculations for the DS and DDS trials occurred on 04-28-2014 and were treated the following week (05-05-2014). While, inoculations for the SDS trial occurred on 06-10-2014 and were treated 06-18-2014.

Cuttings were taken, treated, dipped in rooting hormone, and packaged to mimic the way we have been receiving them from the grower. They were separated into zip locked bags by treatment and shipped overnight via FedEx. The entirety of all six plants from each treatment were evaluated and counted in each subsequent evaluation. Evaluations were made prior to the insect shipping treatments (T1) and after the treatments were received by the Florida lab. Post shipment counts for DS and DDS were completed on arrival (1 DAT); post shipment (4 DAT); post shipment (7 DAT); post shipment (10 DAT); post shipment (15 DAT). All 6 plants for

each treatment type were counted and the 15 DAT evaluations were destructive counts (Figure 53 and Figure 54). Post shipment counts for SS were completed on arrival (1 DAT); post shipment (4 DAT); post shipment (7 DAT); post shipment (11 DAT); post shipment (14 DAT). All 6 plants for each treatment type were counted and the 14 DAT evaluations were destructive counts (Figure 55).

Both dipping treatments reduced populations through 10DAT (Figure 53, Figure 54); this reduction included the deionized water treatments. BotaniGard and Safari and Publix Soap continued to reduce populations to virtually nothing through 15DAT. The spray then ship treatments had mealybug populations throughout the experiment with the exception of Safari which had no mealybugs at 14DAT (Figure 55).

Figure 53. Citrus mealybug on chrysanthemum cuttings, Dip-Ship treatment counts.

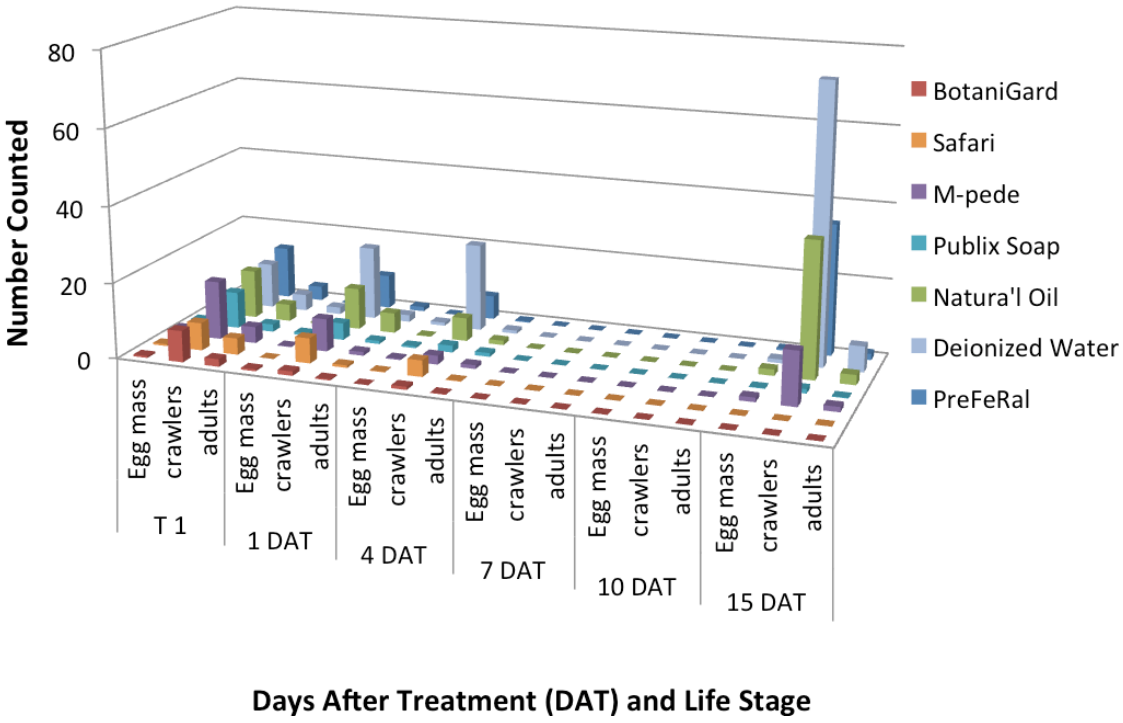


Figure 54. Citrus mealybug on chrysanthemum cuttings, Dip-Dry-Ship treatment counts.

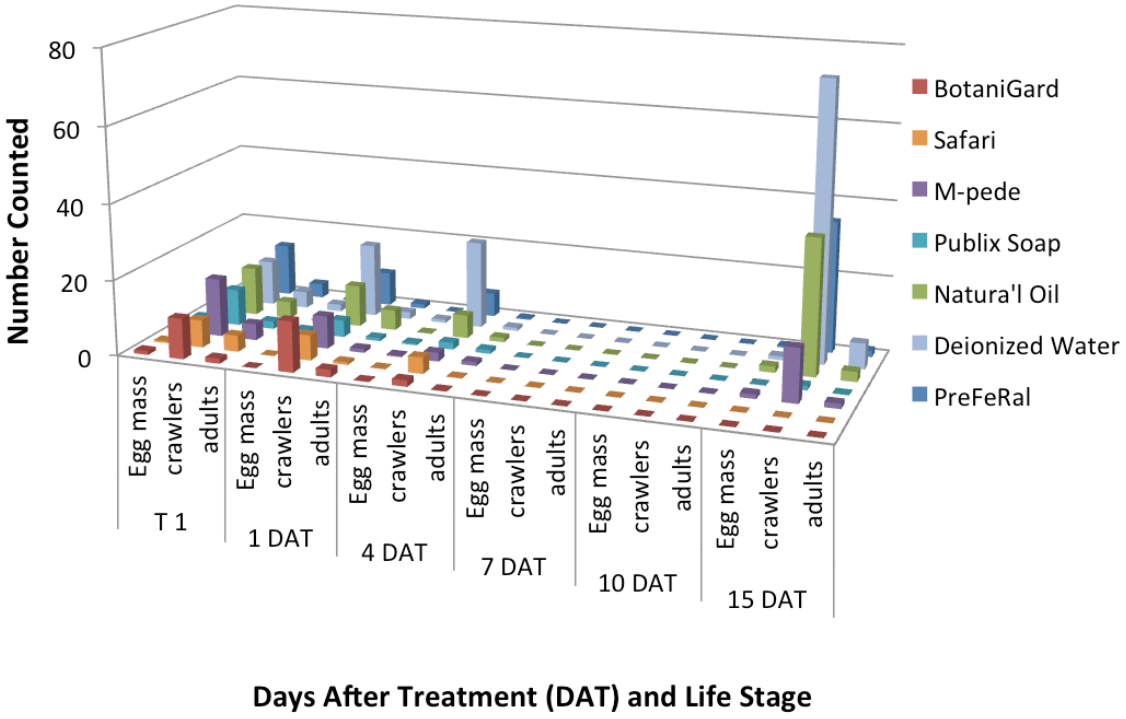
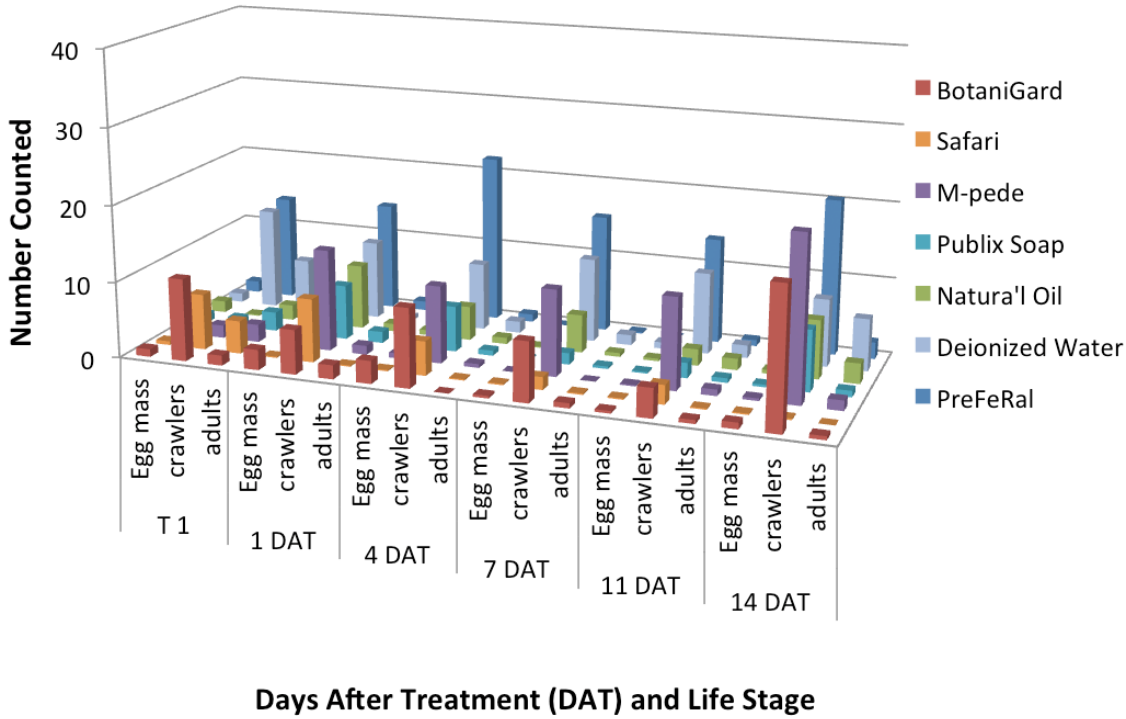
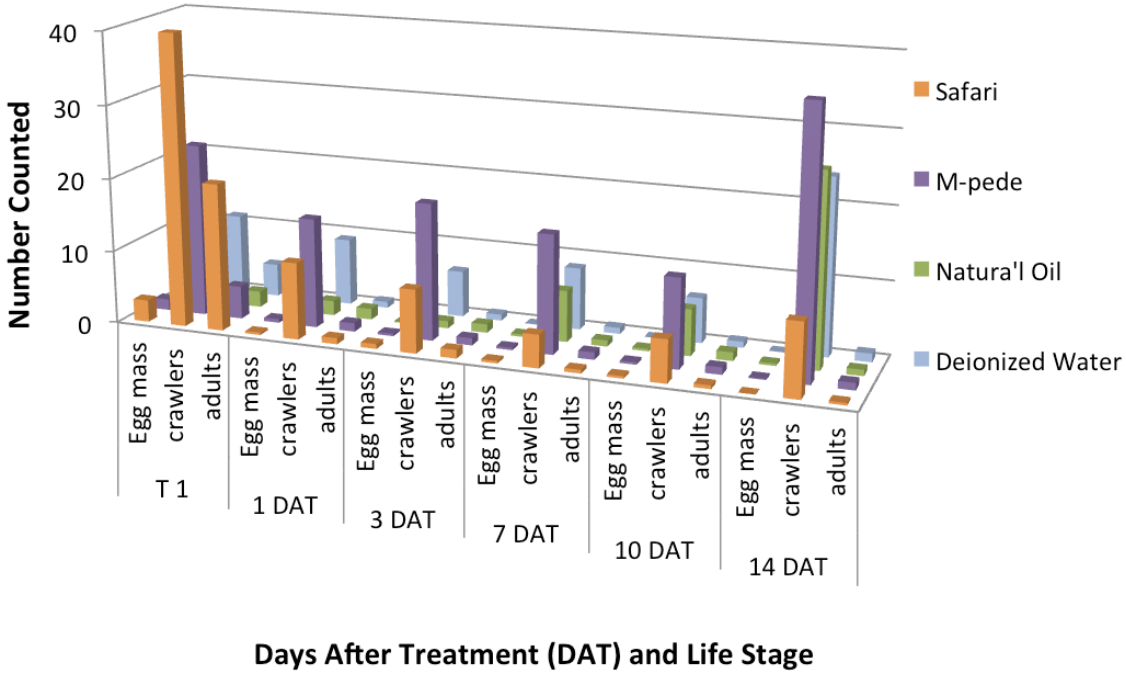


Figure 55. Citrus mealybug on chrysanthemum cuttings, Spray-Ship treatment counts.



Chrysanthemum – Citrus Mealybug – Actual Shipping Fresh Cuttings (CA > FL), Experiment 3
The last Citrus mealybug experiment limited the treatments to those that were likely to be used or adopted by the industry (Safari, M-pede, Natur'l Oil, and the control in the spray the trials and M-pede, Natur'l Oil, and the control in the dip trials for this quarter). Inoculations of Citrus mealybug on chrysanthemum had been very successful. Following procedures outlined above, we artificially infested a total of 40 4" chrysanthemums plants (for cuttings) by placing one egg mass and 5-7 adults per plant. From this we proceeded to use 24 of the infested plants to collect cuttings. Pretreatment counts were taken (07-15-2014) and the cuttings were shipped to Florida for further data collection, which was completed 07-30-2014.

Figure 56. Data from the final Citrus mealybug trial, Spray-Dry-Ship.



Chrysanthemum – Citrus Mealybug – Actual Shipping Rooted Cuttings (CA > FL), Experiment 1
Following the same procedure as previous experiments, we artificially infested a total of 100 rooted cutting in oasis and/or 4" plants (for cuttings) per batch shipped by placing one egg mass and 5-7 adults per plant. After inoculation, the plants were kept in insect cages placed on benches in a climate-controlled greenhouse. One week after inoculation, the plants were then treated with the selected products and held for an additional 24-hours after treatment.

Cuttings were rooted in oasis and were utilized in four application trials: Spray-Ship (SS) and Spray-Dry-Ship (SDS), Dip-Ship (DS) and Dip-Dry-Ship (DDS). Inoculations for the SS and SDS trials occurred on 04-07-2014 and were treated the following week (04-14-2014). While, inoculations for the DS and DDS trials occurred on 05-20-2014 and were treated 05-27-2014.

Cuttings were received from the grower and placed in oasis approximately two week prior to being inoculated to allow roots to establish. Once the mealybug populations were established the rooted cuttings were treated and packaged to mimic the way cuttings in oasis are shipped via growers. Each cutting was labeled (1-6), separated into individual boxes by treatment, and shipped overnight via FedEx. The entirety of all six plants from each treatment were evaluated and counted in each subsequent evaluation.

Evaluations were made prior to the insect shipping treatments (T1) and after the treatments were received by the Florida lab. Upon arrival in Florida, the initial batch of rooted cuttings had jettisoned out of the black plastic oasis trays. As a solution, all future shipments were pinned in place through the oasis to secure the rooted cuttings during shipment. Post shipment counts for DS and DDS were completed on arrival (1 DAT); post shipment (5 DAT); post shipment (13 DAT). All 6 plants for each treatment type were counted and the 13 DAT evaluations were destructive counts. Post shipment counts for SS and SDS were completed on arrival (1 DAT); post shipment (4 DAT); post shipment (7 DAT); post shipment (11 DAT); post shipment (14 DAT). All 6 plants for each treatment type were counted and the 13 DAT evaluations were destructive counts.

Figure 57. Citrus mealybug on chrysanthemum rooted cuttings, Dip-Ship treatment counts.

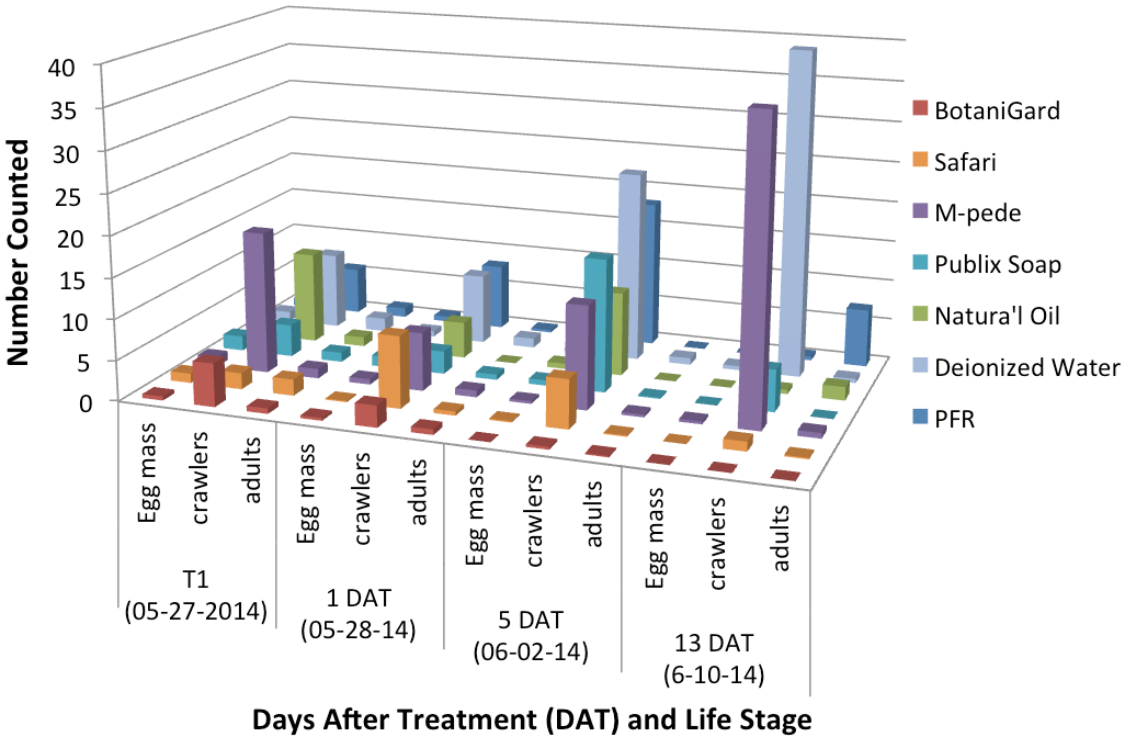


Figure 58. Citrus mealybug on chrysanthemum rooted cuttings, Dip-Dry-Ship treatment counts.

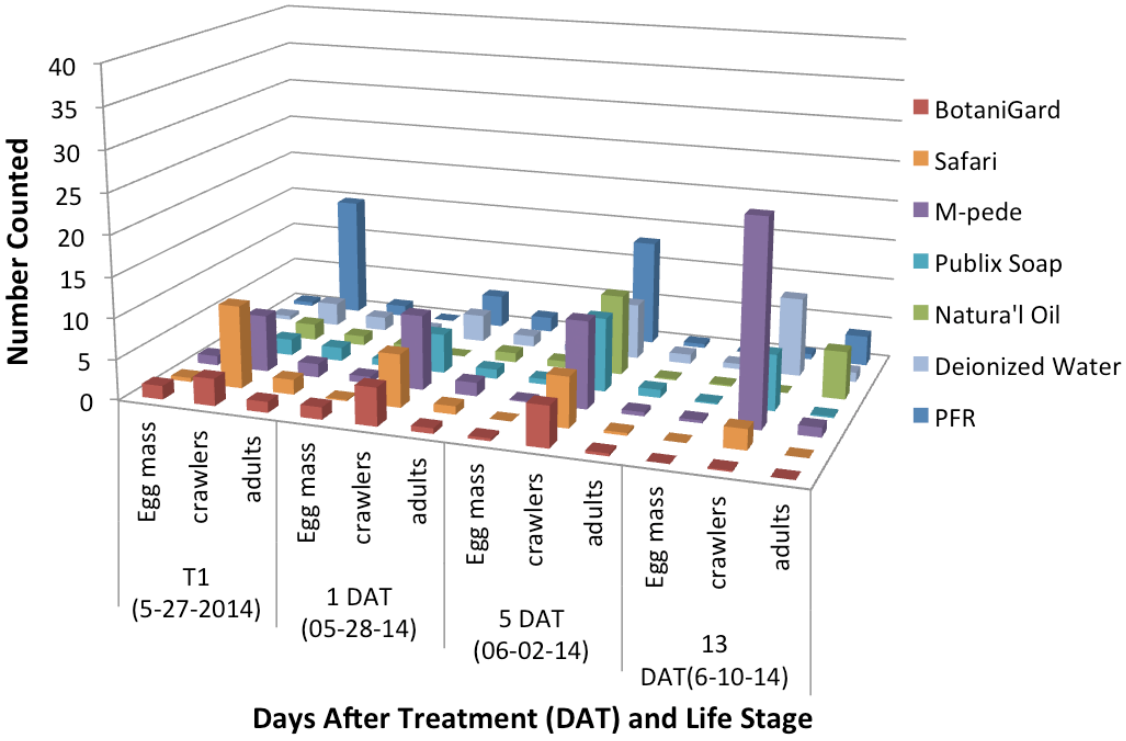


Figure 59. Citrus mealybug on chrysanthemum rooted cuttings, Spray-Ship treatment counts.

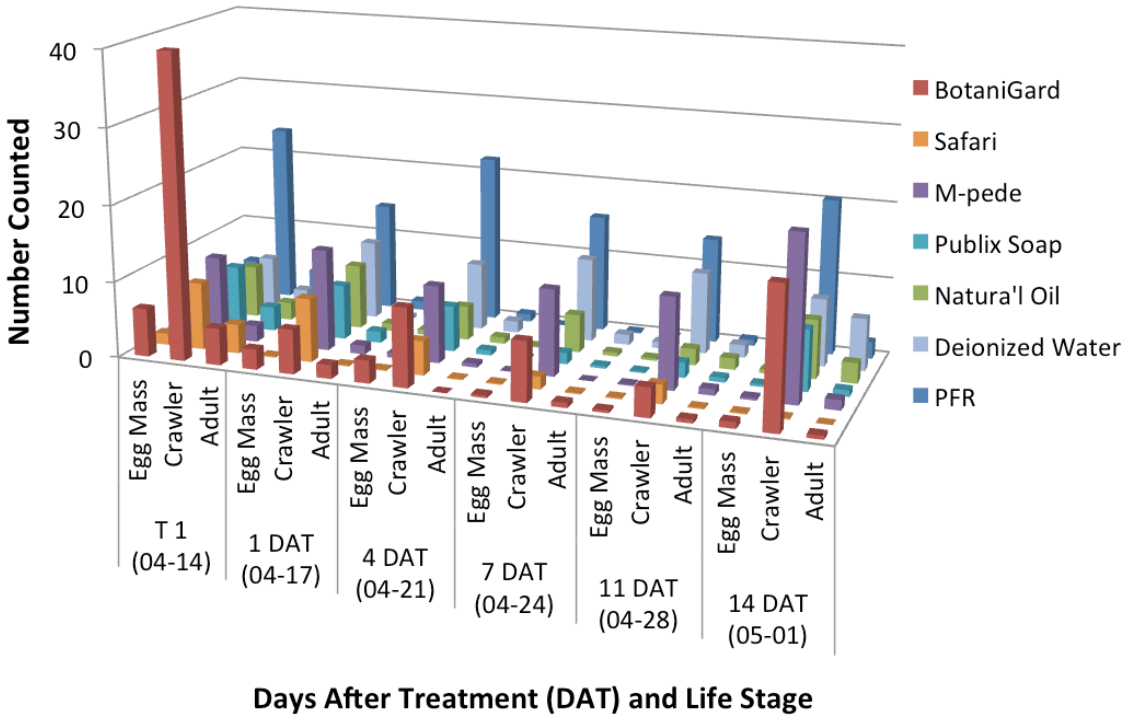
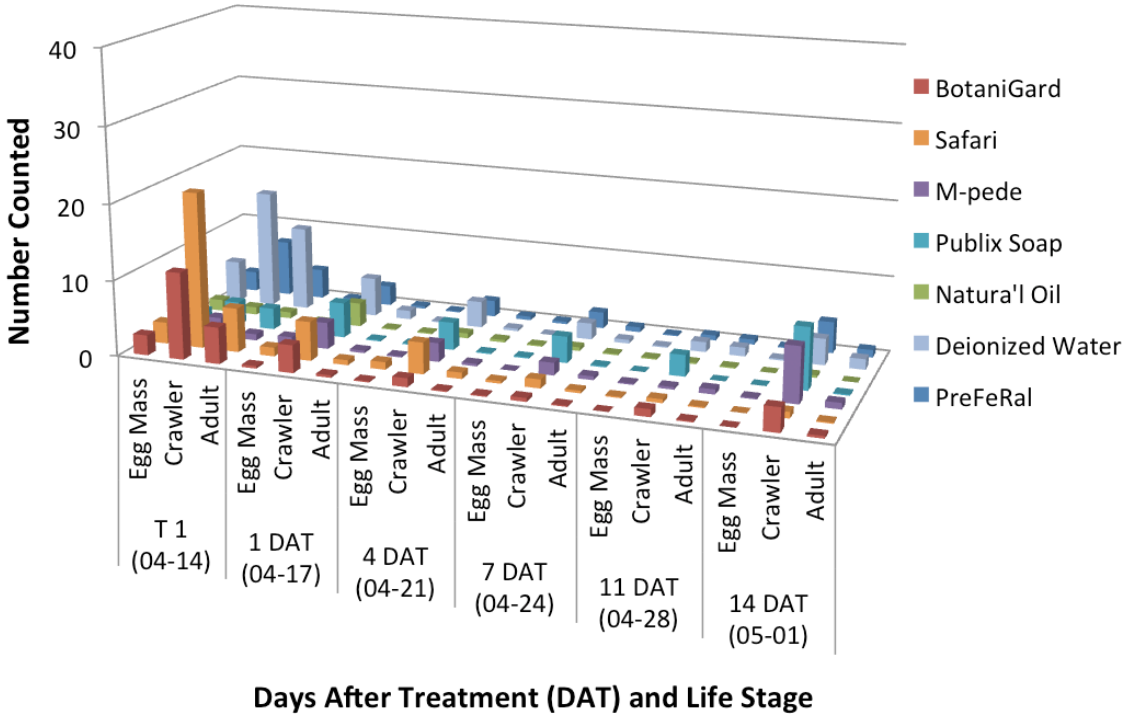


Figure 60. Citrus mealybug on chrysanthemum rooted cuttings, Spray-Dry-Ship treatment counts.



Chrysanthemum – Silverleaf Whitefly – Actual Shipping Fresh Cuttings (CA > FL)
The first, second, and fifth batches of plants were fresh cuttings utilized in four application trials: Spray-Ship (SS) and Spray-Dry-Ship (SDS), Dip-Ship (DS) and Dip-Dry-Ship (DDS). Pretreatment count for the SDS trial was completed 07-15-2014, the SS and DS 07-22-2014, and DDS counts were finished 09-09-2014.

Cuttings were received from the grower and inoculated with silverleaf whitefly (*Bemisia tabaci*). Once whiteflies were established, three leaves on each rooted cutting were selected, numbered, and whitefly eggs/nymphs counted. The cuttings were then treated and packaged to mirror previous shipments. Each cutting was labeled (1-6), separated into individual boxes by treatment, and shipped overnight via FedEx. The three indicated leaves on each of the six plants from each treatment were evaluated and counted in each subsequent evaluation.

Evaluations were made prior to the insect shipping treatments (T1) and after the treatments were received by the Florida lab. Post shipment counts collected on arrival (1 DAT); post shipment (7 DAT); post shipment (14 DAT).

M-pede completely eliminated all life stages when sprayed then shipped, but not when a period of drying was added (Figure 61, Figure 62). Sprays of Safari, M-pede or Stoller Natur'l Oil reduced whitefly populations but they were more effective without drying. Dips of M-pede or Stoller Natur'l Oil reduced but did not completely eliminate whiteflies whether or not cuttings were dried prior to shipping (Figure 63, Figure 64).

Figure 61. Silverleaf whitefly on chrysanthemum cuttings, Spray-Ship treatment counts

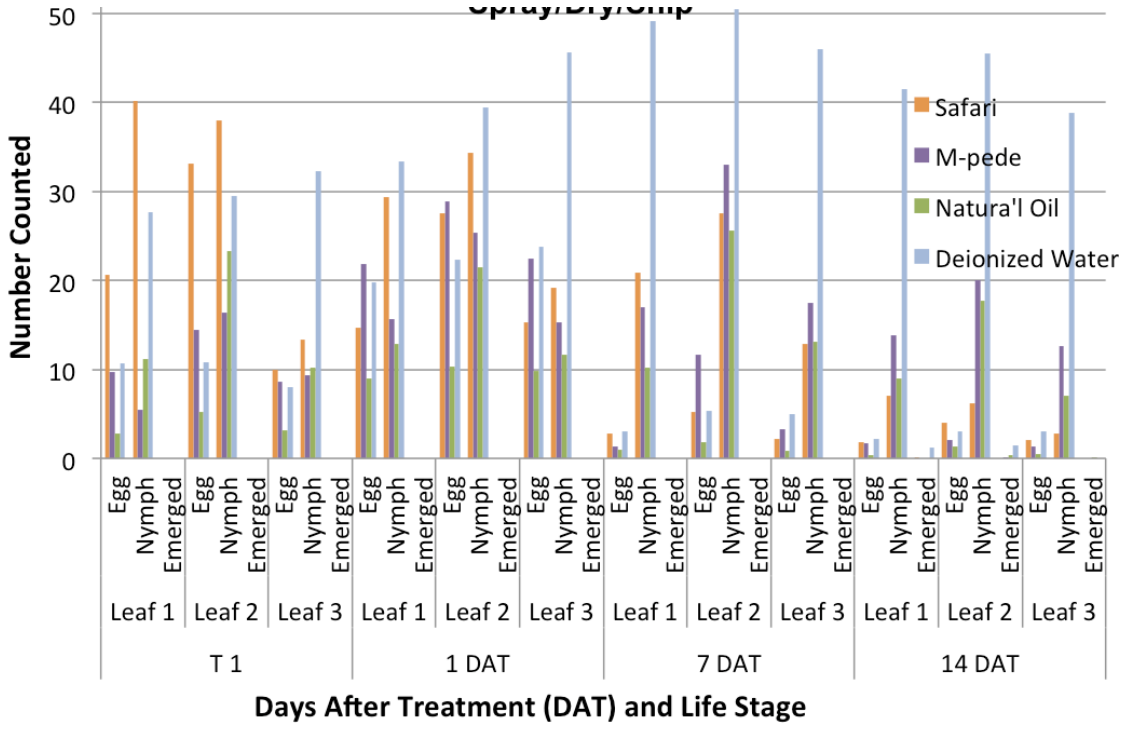


Figure 62. Silverleaf whitefly on chrysanthemum cuttings, Spray-Dry-Ship treatment counts

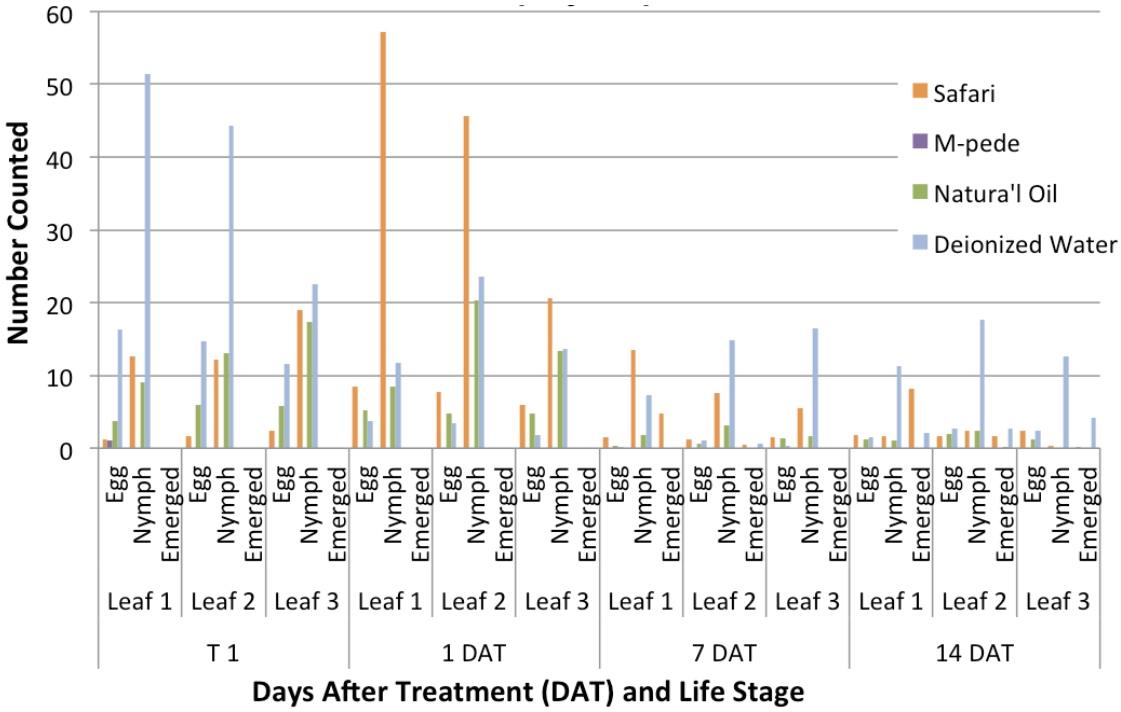


Figure 63. Silverleaf whitefly on chrysanthemum cuttings, Dip-Ship treatment counts

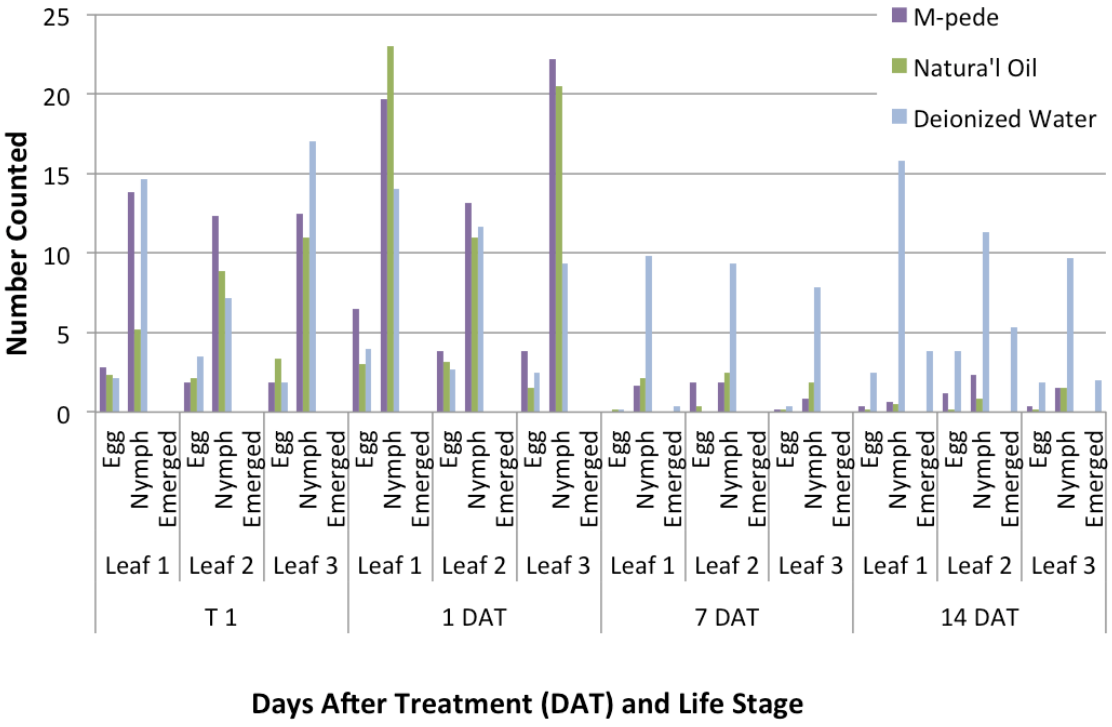
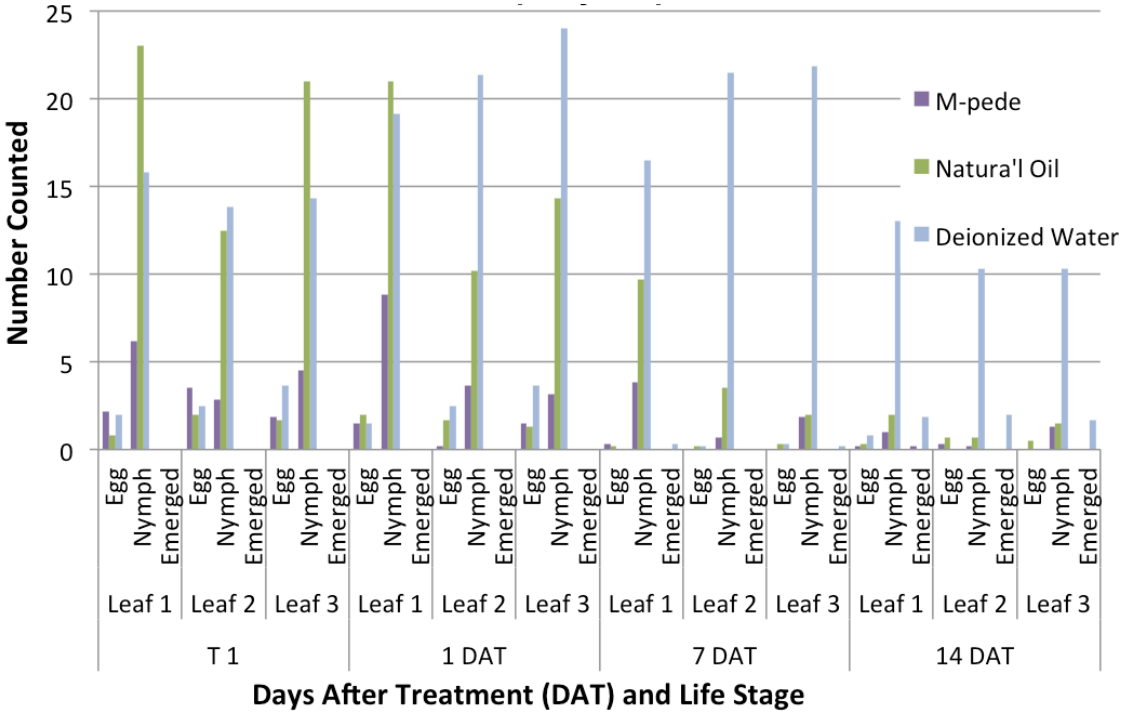


Figure 64. Silverleaf whitefly on chrysanthemum cuttings, Dip-Dry-Ship treatment counts



Chrysanthemum – Silverleaf Whitefly – Actual Shipping Rooted Cuttings (CA > FL)
The next series of experiments tested whether Silverleaf whitefly on rooted chrysanthemum cuttings could be managed with pre-shipment applications of M-pede or Stoller Natur'l Oil. Treatments consisted of Spray-Ship (SS), Spray-Dry-Ship (SDS), Dip-Ship (DS) and Dip-Dry-Ship (DDS).

Cuttings were received from the grower and placed in oasis approximately two week prior to being inoculated with silverleaf whitefly (*Bemisia tabaci*) to allow roots to establish. Once the whitefly exposure was accomplished, three leaves on each rooted cutting were selected, numbered, and whitefly eggs/nymphs counted, in California (T1). Pretreatment counts for the DS trial was completed 08-12-2014 and the SS, SDS, and DDS counts were finished 09-25-2014. The cuttings were then treated and packaged to mirror previous shipments. Each cutting was labeled (1-6), separated into individual boxes by treatment, and shipped overnight via FedEx to the Florida lab. The three indicated leaves on each of the six plants from each treatment were evaluated and counted in each subsequent evaluation. Post shipment counts were initially meant to be collected on arrival (1 DAT); post shipment (4 DAT); post shipment (7 DAT); post shipment (11 DAT); post shipment (14 DAT). The first trial (DS) was counted 2 DAT, 7 DAT, and 14 DAT. Due to the increased amount of time required to collect whitefly data, count intervals were altered to be less frequent (1 DAT, 7 DAT, and 14 DAT), making the evaluation process more practical.

For DS treatments without drying, Stoller Natur'l Oil reduced all whitefly stages at 2 and 7 DAT (Figure 65), but some eggs and nymphs started to appear on 14 DAT. With drying, dip treatments did not appear to be as effective (Figure 66). The spray treatments with and without drying did not appear to significantly impact whitefly populations (Figure 67, Figure 68).

Figure 65. Silverleaf whitefly on chrysanthemum rooted cuttings, Dip-Ship treatment counts.

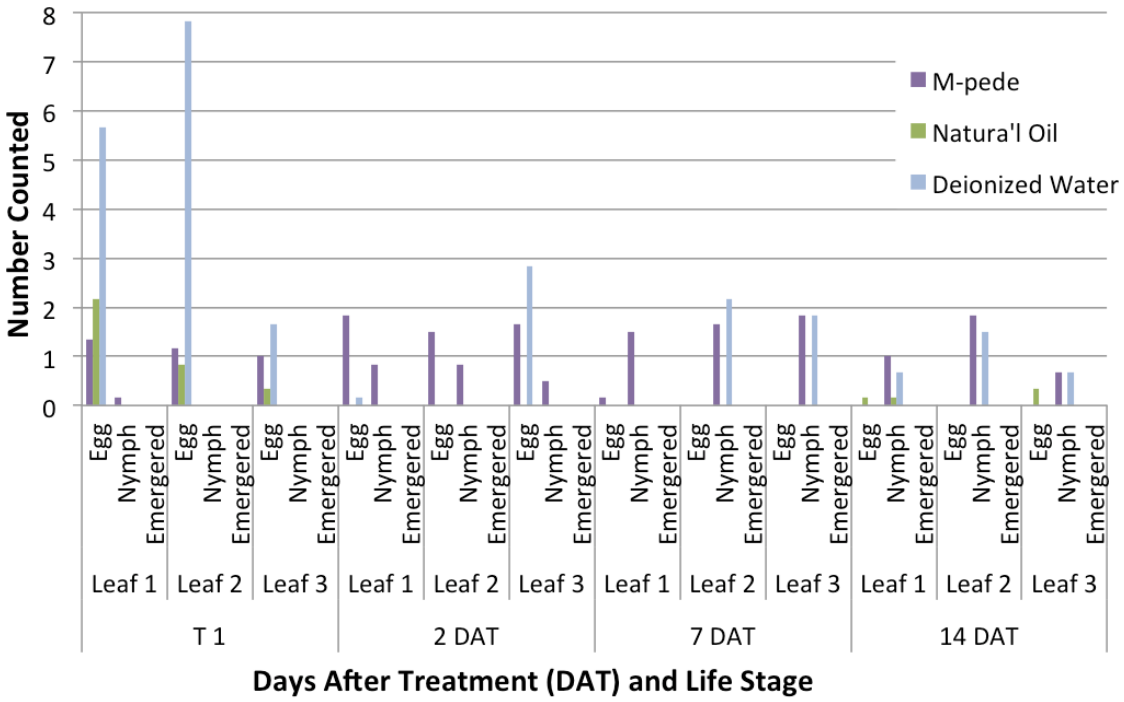


Figure 66. Silverleaf whitefly on chrysanthemum rooted cuttings, Dip-Dry-Ship treatment counts.

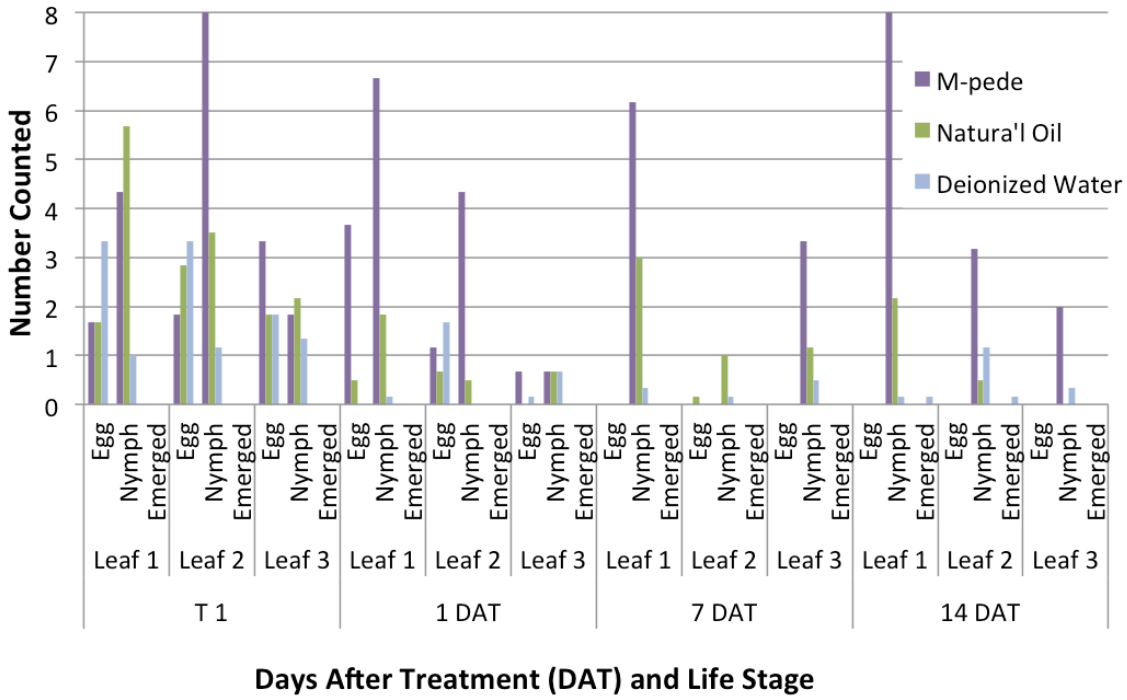


Figure 67. Silverleaf whitefly on chrysanthemum rooted cuttings, Spray-Ship treatment counts.

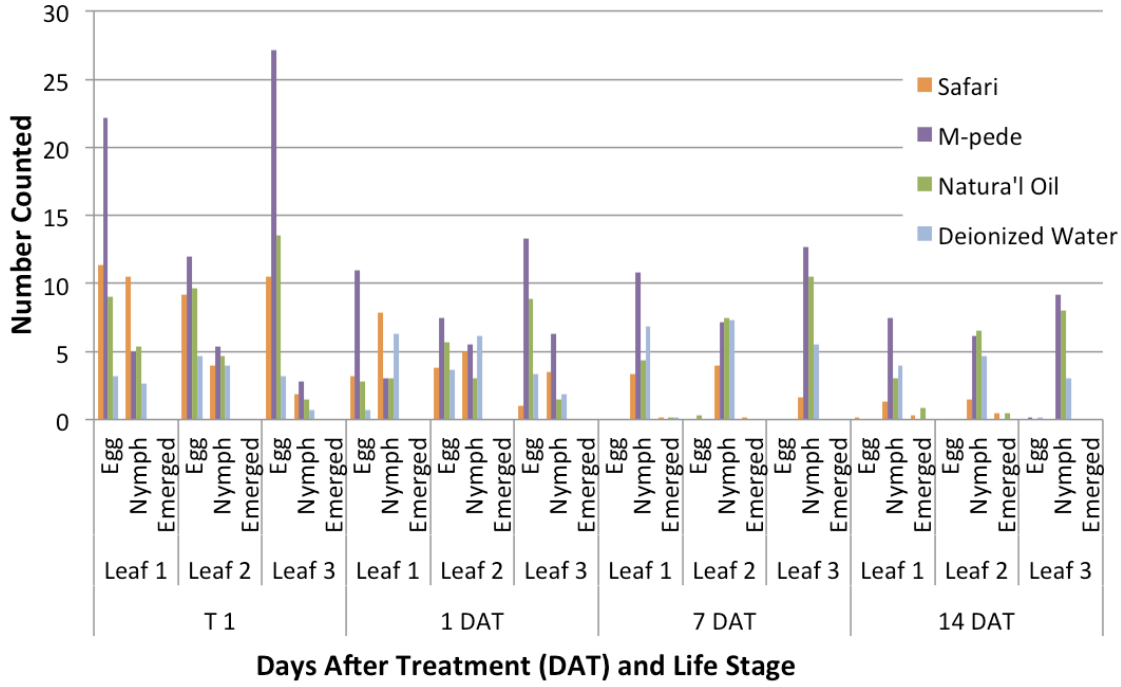
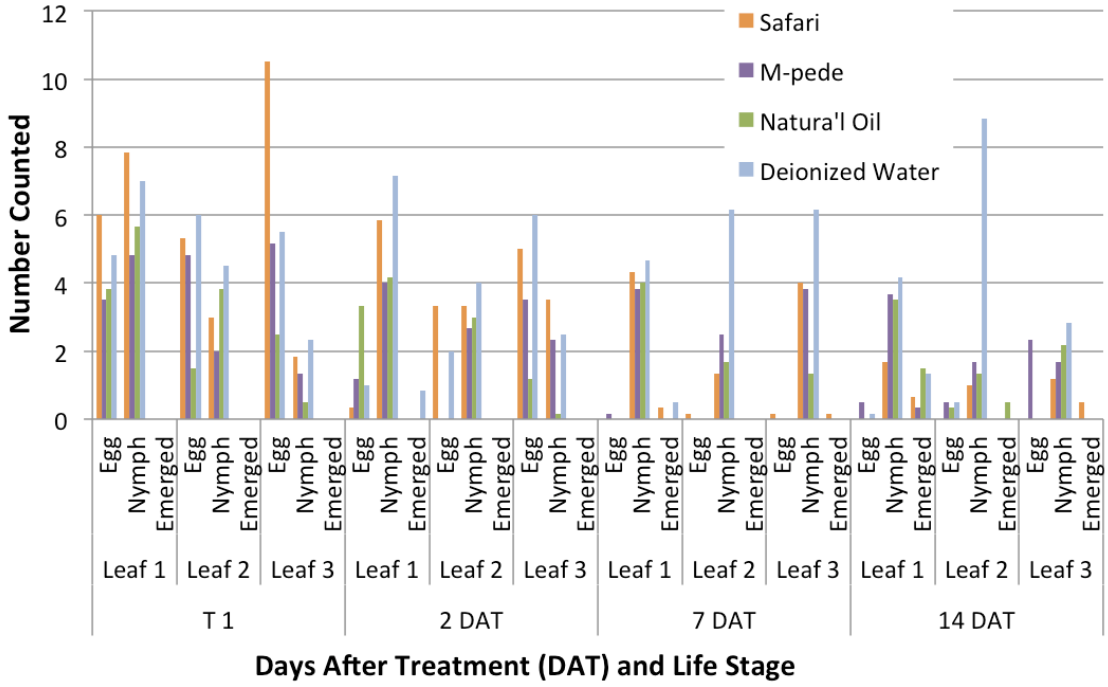


Figure 68. Silverleaf whitefly on chrysanthemum rooted cuttings, Spray-Dry-Ship treatment counts.



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Outreach Activities

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BMP for EPM. A draft BMP against EPM has been developed and presented to the Technical Working Group. The draft BMP can be located at the following web site: http://mrec.ifas.ufl.edu/lso/dupon/dupon.html. The BMP is a work in progress. New information will be added as research is completed. No information has been added during this reporting cycle.