HARC

HAWAII AGRICULTURE RESEARCH CENTER

FORMERLY HAWAIIAN SUGAR PLANTERS' ASSOCIATION
99-193 AIEA HEIGHTS DRIVE, SUITE 300, AIEA, HAWAII 96701-3911
TELEPHONE: (808) 487-5561 FAX: (808) 486-5020
http://www.hawaiiag.org/harc

COPY FOR YOUR INFORMATION LK

Final Report

Hawaii DOA Contract Number 53519

Ferric Phosphate for the Control of Golden Apple Snail in Wetland Taro: Efficacy Testing

P.I. Mel C. Jackson, Ph.D.

September 2006

Problem Addressed

Hawaii taro production in 2003 was estimated at 5.0 million pounds; an 18% decrease from 2002 (Hawaii Agricultural Statistics Service, Feb 9, 2004). This is the lowest ever recorded production and was in large part due to the effects of the Golden Apple snail (*Pomacea canaliculata*). The value of sales in 2003 was about $2.7 million, compared with $3.294 million the previous year. Golden apple snail was introduced into Hawaii, Japan, and many other countries in South-east Asia from South America as a source of food in the early 1980s. However, after its commercial markets failed, discarded and escaped snails invaded taro and rice ecosystems and have been causing significant economic damage. In Hawaii, these snails were also purposely introduced into taro paddies (lo`i), the reasoning being that they could be harvested for food (Tanji, 1990). However, the consequences of this action were not fully understood at the time. The snails are voracious, fast growing and have a huge reproductive potential. A single female can produce as many as 15,000 offspring per year, and can thrive in water at a density of 1,000 snails per square meter (Anderson, 1993). They mature within 60 – 85 days and spawn at weekly intervals and have been described as the most damaging pest ever to hit neotropical areas (Halwart, 1994). The snails very quickly spread throughout taro lo`i, via the extensive and interconnected irrigation system (Tanji 1990, Ashizawa, 1992). In 1996, the Hawaii State Department of Agriculture statistical services recorded that in 1992 approximately 60,000 lb of fresh taro was marketed from the islands of Oahu, Molokai, and Maui, yet in 1996, only approximately 10,000 lb was marketed; an 84% decline, largely due to apple snails. This picture of rapid and overwhelming infestation is reflected elsewhere in the world. For example, $1 million has been spent annually to control the snails in rice paddies in Taiwan since 1982 (Cheng, 1989). In addition, an estimated 20% of farm income was spent on apple snail control in the Philippines in 1993 (Anderson, 1993). Since taro grows in water where there are aquatic animals sharing the same ecosystems, chemicals are not allowed for pest control.

Biological control agents such as predatory animals and parasites are not effective for control of this snail. Similarly, conventional pesticides have been found to be non-specific and can pose significant ecological problems. In 1994, a formulation of copper sulfate was registered for use in taro lo'i (EPA registration number 1109-21), but was soon discontinued due to its non-specific and highly toxic nature. Extracts from natural products have been screened for potential molluscicide activity throughout Asia. For instance, in the Philippines, Starflower (*Calotropis gigantea*), was tested and showed anti-molluscicide activity (Lobo, et al., 1991); however, the effective rate of application was very high (200Kg/ha.). Morallo-Rejesus and Punzalan, (1997) examined a total of 138 extracts from Philippine plants as potential molluscicides, including extracts from neem leaf. While some were found to be effective, none were subsequently followed up. In addition, mechanical control has also been studied (Awadhal and Quick, 1994); however, this is an extremely labor intensive practice.

Ferric phosphate is registered for use on terrestrial snails, but has never been tested on aquatic snails such as the Apple snail. The aim of this project was therefore to test the efficacy of this material for the control of apple snails in wetland taro lo'i.

The original objectives of this project were as follows

1. To determine the efficacy of NEU1165M Slug and Snail Bait™ (1% ferric phosphate) on Apple snail populations in commercial lo'i at the currently registered label rate and at a 5X exaggerated rate.

2. To determine the effects of NEU1165M Slug and Snail Bait™ (1% ferric phosphate) on non-target species residing within the lo'i.

3. To determine the best means to integrate the application of the test substance with the taro growers' current cultural practices and to determine the frequency of application needed for adequate control.

4. To initiate the EPA process (through IR-4 and the manufacturer) of amending a current label so that that this material is registered for use in wetland taro lo'i.

In November 2005, a request was made to change the objectives of the project to include two plant extracts provided by Dr. Meepagala from USDA-ARS, Mississippi. These were an extract from the Pacific mugwort (*Artemesia douglasiana Besser*) which in preliminary studies Dr. Meepegala has shown to be effective in controlling Apple snails in a laboratory setting (Joshi, R.C., et al., 2005). In addition, Dr. Meepagala has partially purified a compound called vulgarone B from this plant (Meepagala K.M., et al., (2003). Vulgarone B appears to be the active ingredient responsible for snail mortality in laboratory tests. This material was provided to HARC for use in this project. In addition, Dr. Meepagala included an extract from the Yucca plant (*Yucca schidigera*) which in preliminary studies at her laboratory had shown activity against unrelated aquatic snails. It was decided to include these two extracts in the current project so that their activities against the Golden Apple snail in taro lo'i in Hawaii could be determined.

Laboratory and Field Test Methods and Results

Laboratory Tests

Ferric Phosphate: Ferric Phosphate formulated as NEU1165M Slug and Snail Bait was provided by Neudorff North America, VA (EPA Registration #67702-3). This material contains 0.76% by weight ferric phosphate and is formulated with an extruded starch based carrier. A laboratory experiment was undertaken to determine whether this formulation was physically stable in water. A few extruded granules were placed in a glass beaker and 200 mls of distilled water added. The mixture was left without stirring at room temperature for seven days. After seven days the physical characteristics of the material were observed. It was noted that the test material was intact after seven days, indicating that it should not break down in the taro lo'i before the snails had a chance to ingest it. It was also noted that the test material was heavier than water and sunk. This was a pre-requisite for any test material to be used in this project in taro lo'i. The lo'i are ecologically sensitive environments. Had the test material floated, it would have been available to non-target avian species.

Vulgarone B and Yucca extract: The vulgarone B and the Yucca extract were received from the ARS Louisiana in May 2005. The vulgarone B extract was a clear crystalline solid. Meepagala et al, (2005) had shown that in laboratory studies an LD50 of approximately 30uM was observed. Therefore, it was not considered necessary to undertake any preliminary laboratory test of this material. A 1X concentration of 75uM was considered sufficient to see significant mortality in subsequent field tests.

The Yucca extract had not been tested on snails before. It was a thick black viscous liquid and appeared to represent a very crude extract. This was therefore tested in the laboratory prior to testing in the field. Two grams of the extract were dissolved in 10mls of distilled water to provide a 0.2g/ml stock solution. Five mls of the stock was diluted to 1 L to give a 1000ppm solution. This was diluted sequentially to give 500ml aliquots of solutions containing 500ppm, 400ppm, 300ppm, 200ppm, and 100ppm, respectively. 500 mls of each of these solutions was added to beakers containing four live snails. A positive control, with 500mls of distilled water in place of the treatment solution was also run. A known number of lettuce leaf pieces were added so that feeding behavior over time could also be monitored. After 24 hours all of the snails in the 500ppm and 400ppm treatments were dead. The 300ppm treatment showed three dead snails and one alive but not feeding. The 200ppm, 100ppm and positive control all had zero mortality and feeding snails after 36 hours. It was therefore decided that the 300ppm concentration should be the 1X concentration in the subsequent field test.

Field Tests

Test Plot Setup: Test plots were 4-ft diameter circular plots, isolated from the rest of the loi by mesh and plastic barriers. A length of 4-ft high 1" wire mesh was cut so that it had a diameter of 4 ft. The ends of the length were strapped together so that the mesh formed a circle. The circle was placed in the lo'i and pushed into the mud to a depth of about 3". A length of 2-ft wide linoleum floor covering was cut and taped so that it formed a circle wide enough that it slid over the wire mesh circle and formed a solid barrier on the outside of it. Test plots were placed approximately 4 ft away from each other. The rings were placed in such a way that four taro plants were within the boundaries of each ring. Taro lo'i were selected that contained taro from 3 to 6 months of age. Prior to initiating a field test, the lo'i was drained to a depth above the mud of 1 inch. This was done for a number of reasons. Primarily, having a known depth across the lo'i and a comparable depth between lo'i allowed for a known concentration of test substance to be determined within the test plot. This allowed for a lowering of the absolute amount of test material that was needed to obtain the desired concentration. Another effect was to drive non-target species out of the lo'i, as they could move a lot faster than the target species. This served to limit any effects on non-target species. Consequently, no frogs, toads or fish were seen in lo'i that had been drained to 1 inch. Each test plot was cleared of any Apple snails that could be found. Apple snails were collected from a nearby field according to their size and sex. Snails were only collected with shell widths of greater than 1 inch, but less than 2 inches. This resulted in the collection of adult snails that were typical of the population as a whole. Snail sex was determined by inspection of the calcified hinged flap, called an operculum, used to seal the snail shell. Males posses a slightly concave rim to the operculum, whereas females possess a completely concave operculum. Test plots were seeded with collected snails prior to the initiation of field tests.

Ferric Phosphate: For the ferric phosphate field tests, Mr. Rodney Haraguchi's family farm and Dr. Adam Asquith's family farm, both in Hanalei, Kauai, were used as test locations for the ferric phosphate tests. The label rate of 1 lb of NEU1165M per 1,000 square feet of area, for terrestrial use was used as the 1X rate of the test application. Within a single lo'i, ½X, 1X and 5X test rates were used. This translated to 2.85 grams, 5.7 grams and 28.5 grams respectively of NEU1165M used for each test rate. A total of 15 snails were placed within each test plot, consisting of five males and ten females. Each treatment was run in triplicate within a lo'i. After placing the snails in the test plots, the applications were made and in addition to triplicate test applications at the three rates described above, a single control was also installed, consisting of 15 snails and no test material. Test plots were randomized within the lo'i. Observations of the number of dead snails, number of remaining plants, and number of egg clusters laid were made three, seven and 21 days after the test application was made. The results at each location are given in the following Tables 1 and 2:

Table 1. Ferric Phosphate Data from Haraguchi Farm

Time (days after start)	*Plot #*	*Application Rate*	*# Dead Snails*	*# Plants Remaining*	*# of Egg Clusters*	*Phyto*
3	1A	½ X	0	3	0	N
7	1A	½ X	1	0	2	N
21	1A	½ X	1	0	3	N
Totals	1A	½ X	2	0	5	N
3	1B	½ X	0	4	0	N
7	1B	½ X	0	2	0	N
21	1B	½ X	0	0	4	N
Totals	1B	½ X	0	0	4	N
3	1C	½ X	0	2	0	N
7	1C	½ X	2	1	0	N
21	1C	½ X	2	0	5	N
Totals	1C	½ X	4	0	5	N
3	2A	1 X	0	4	1	N
7	2A	1 X	1	1	3	N
21	2A	1 X	2	0	5	N
Totals	2A	1 X	3	0	9	N
3	2B	1 X	0	4	2	N
7	2B	1 X	1	1	2	N
21	2B	1 X	1	0	2	N
Totals	2B	1 X	2	0	6	N
3	2C	1 X	0	3	1	N
7	2C	1 X	0	2	2	N
21	2C	1 X	0	0	4	N
Totals	2C	1 X	0	0	7	N
3	3A	5 X	0	2	4	N
7	3A	5 X	0	0	1	N
21	3A	5 X	4	0	0	N

Time (days after start)	*Plot #*	*Application Rate*	*# Dead Snails*	*# Plants Remaining*	*# of Egg Clusters*	*Phyto*
Totals	3A	5 X	4	0	5	N
3	3B	5 X	1	2	4	N
7	3B	5 X	2	1	0	N
21	3B	5 X	1	0	2	N
Totals	3B	5 X	4	0	6	N
3	3C	5 X	0	4	0	N
7	3C	5 X	2	2	2	N
21	3C	5 X	3	0	3	N
Totals	3C	5 X	5	0	5	N
3	Control	0	2	0	3	N
7	Control	0	0	1	3	N
21	Control	0	0	1	1	N
Totals	Control	0	2	1	7	N

Table 2. Ferric Phosphate Data from Asquith Farm

Time (days after start)	*Plot #*	*Application Rate*	*# Dead Snails*	*# Plants Remaining*	*# of Egg Clusters*	*Phyto*
3	1A	½ X	0	4	2	N
7	1A	½ X	0	2	2	N
21	1A	½ X	1	0	6	N
Totals	1A	½ X	1	0	10	N
3	1B	½ X	0	4	0	N
7	1B	½ X	0	2	0	N
21	1B	½ X	0	2	0	N
Totals	1B	½ X	0	2	0	N
3	1C	½ X	0	4	0	N
7	1C	½ X	0	2	1	N
21	1C	½ X	0	0	4	N
Totals	1C	½ X	4	0	5	N

Time (days after start)	***Plot #***	***Applicatio n Rate***	***# Dead Snails***	***# Plants Remaining***	***# of Egg Clusters***	***Phyto***
3	2A	1 X	0	4	0	N
7	2A	1 X	3	1	0	N
21	2A	1 X	0	0	5	N
Totals	2A	1 X	3	0	5	N
3	2B	1 X	0	4	2	N
7	2B	1 X	1	1	1	N
21	2B	1 X	2	0	0	N
Totals	2B	1 X	3	0	3	N
3	2C	1 X	0	0	0	N
7	2C	1 X	0	0	0	N
21	2C	1 X	2	2	7	N
Totals	2C	1 X	2	2	7	N
3	3A	5 X	0	4	1	N
7	3A	5 X	3	3	1	N
21	3A	5 X	2	1	3	N
Totals	3A	5 X	5	1	5	N
3	3B	5 X	1	4	0	N
7	3B	5 X	2	4	0	N
21	3B	5 X	1	3	2	N
Totals	3B	5 X	4	3	2	N
3	3C	5 X	0	4	0	N
7	3C	5 X	2	2	2	N
21	3C	5 X	3	0	3	N
Totals	3C	5 X	5	0	5	N
3	Control	0	0	4	1	N
7	Control	0	0	0	4	N
21	Control	0	3	0	4	N
Totals	Control	0	3	0	8	N

It can be seen from the tables above that the ½X rates and the 1X rates at both locations were not significantly different from the controls in either number of dead snails, or number of plants remaining, or the number of egg clusters laid. However, at the exaggerated 5X rate, the number of dead snails doubled on average to a mortality rate over 21 days of 45%, versus 25% in the control plots. Analysis of variance showed this to be a highly significant difference (Asquith Farm $p = 0.0421$, Haraguchi Farm $p = 0.0322$). This represents an 80% increase in kill rate versus the control over 21 days. The test was halted after 21 days, because most of the taro plants had been consumed by the snails at this time.

Vulgarone B and Yucca Extract: Mr. Hobie Beck's taro farm in Hanalei, Kauai was the site for the testing of vulgarone B and the Yucca extract. All tests were conducted at the same time and in a single lo'i. Test plots were set up as described for the ferric phosphate study, with some minor modifications. As the taro in the test lo'i was older than that used for the ferric phosphate study and thus larger, 20 snails (15 female, 5 male) were used rather than ten. This increased pressure on the plants and therefore increased the probability that crop damage could be observed and compared between treatments. In addition, the snails were placed in a single pile within each test plot, prior to application of test material. This allowed observations to be made on the relative speed at which the extracts affected the snails, by noting how far snails had dispersed from the initial placement.

The 1X rate for vulgarone B was 75uM, as discussed earlier. A 5X rate was also used (375uM). For each 1X test, 1 gram of vulgarone B was dissolved in 20 mls of 75% (v/v) ethanol. Ten mls of this stock solution was added to 250mls of water. For the 5X rate, 50 mls of the stock solution was made up to 250mls with water. The control application was made with 50 mls of 75% ethanol, made up to 250mls with water. All calculations were based upon a 1" water depth in the lo'i, which translates to a 30 liter volume in each test plot.

For the Yucca extract, the 1X rate was based upon the preliminary laboratory experiment that indicated that 300ppm of the extract would be sufficient to cause significant mortality. A $3^1/_3$X rate (1,000ppm) was also tested. The control used for the vulgarone B test was also used for the yucca extract test. Applications of both test substances were made using a watering can with a sprinkler.

All tests were conducted in triplicate in a random block design. Photograph of the setup is shown below:


Observations were made four days after the application and again seven days after the application. The results for the vulgarone B and the yucca extract are shown below:

Vulgarone B

Time (days after start)	*Plot I.D.*	*Application Rate*	*# Dead Snails*	*# Plants Remaining*	*# of Egg Clusters*	*Phyto*
4	5	1X	0	4	3	N
7	5	1X	0	4	1	N
Totals		1X	0	4	4	N
4	8	1X	1	4	0	N
7	8	1X	0	4	1	N
Totals		1X	1	4	1	N
4	15	1X	2	4	2	N
7	15	1X	0	4	2	N
Totals		1X	2	4	4	N
4	4	5X	20	4	0	N
7	4	5X	0	4	0	N
Totals		5X	20	4	0	N
4	6	5X	20	4	0	N
7	6	5X	0	4	0	N
Totals		5X	20	4	0	N
4	12	5X	20	4	0	N
7	12	5X	0	4	0	Y*
Totals		5X	20	4	0	Y*
4	Control 3	0X	1	4	3	N
7	Control 3	0X	0	4	4	N
Totals		0X	1	4	7	N
4	Control 10	0X	0	4	2	N
7	Control 10	0X	0	4	2	N
Totals		0X	0	4	4	N
4	Control 14	0X	0	4	2	N
7	Control 14	0X	1	4	1	N
Totals		0X	1	4	3	N

* In plot #12, after seven days some phytotoxicity was seen in the weed mat, floating in the water surrounding the four taro plants. However, no phytotoxicity was seen in the taro in this plot or any of the other test plots.

The data above shows that at the 1X rate, the vulgarone B's effects did not appear to be significantly different from the untreated control, with similar snail mortality and egg cluster counts. However, at the exaggerated rate, all of the snails were dead within the four-day intervening period between application and the first observations. It is highly likely that in the plots exposed to the exaggerated rate, the vulgarone B acted very quickly, because the snails were still in the original pile that they were placed in prior to the application, whereas the surviving snails in the 1X treatments and the controls had dispersed in a random fashion throughout each of the test plots. In all of the test plots, it was observed that there was abundant non-target insect species, including mosquito larvae, pond skippers and dragonfly larvae. The lack of effect of the 1X vulgarone B rate was not expected, given the data from the laboratory experiments conducted by Dr. Meepagala. A reason was therefore sought in the experimental design that might explain this discrepancy. On further observation of the lo'i, it was realized that the lo'i depth, rather than the prescribed 1" upon which all concentration calculations were made, was in fact 2½" deep. This meant that the actual vulgarone B concentration in the 1X treatment was 30uM, not the expected 75uM, and the 5X treatment was 150uM, rather than the 375uM calculated. This explained the lack of effect in the 1X treatment, as this concentration was only around the LD50 concentration seen in the laboratory by Dr. Meepagala. The vulgarone B test was therefore repeated at a later date with 1" water depth, and will be reported upon later in this report.

The following yucca extract results were interpreted with the knowledge that the actual extract concentration in the 1X treatment was 120ppm rather than the calculated 300ppm, and the 5X rate was 400ppm rather than the 1,000ppm originally calculated.

Yucca extract

Time (days after start)	*Plot I.D.*	*Application Rate*	*# Dead Snails*	*# Plants Remaining*	*# of Egg Clusters*	*Phyto*
4	1	1X	14	4	0	N
7	1	1X	0	4	0	N
Totals		1X	14	4	0	N
4	9	1X	18	4	0	N
7	9	1X	0	4	0	N
Totals		1X	18	4	0	N
4	13	1X	8	4	0	N
7	13	1X	4	4	0	N
Totals		1X	12	4	0	N
4	2	3.3X	20	4	3	N
7	2	3.3X	0	4	0	N
Totals		3.3X	20	4	3	N
4	7	3.3X	20	4	0	N
7	7	3.3X	0	4	0	N
Totals		3.3X	20	4	0	N
4	11	3.3X	20	4	0	N
7	11	3.3X	0	4	0	N
Totals		3.3X	20	4	0	N
4	Control 3	0X	1	4	3	N
7	Control 3	0X	0	4	4	N
Totals		0X	1	4	7	N
4	Control 10	0X	0	4	2	N
7	Control 10	0X	0	4	2	N
Totals		0X	0	4	4	N
4	Control 14	0X	0	4	2	N
7	Control 14	0X	1	4	1	N
Totals		0X	1	4	3	N

It can be seen from the table above that the yucca extract, even at the lower application rates of 120ppm and 400ppm were highly effective in causing significant snail death. At the 1X rate

(120ppm), an average mortality rate of 73.3% was achieved within four days after the application. Compared with the control this was highly statistically significant ($p = 0.0028$). As with the vulgarone B results the speed at which the snails were affected was apparent from the observation that all of the dead snails were in the original pile that they had been placed in prior to the application. This suggests that the yucca extract was almost immediately effective in at least incapacitating the snails. Abundant insect life, including mosquito larvae, pond skippers and dragonfly larvae were observed in all test plots on both observation days. There was no phytotoxicity evident in any of the tests. It is also interesting to note that there were only three egg clusters found in the combined low and high rate test plots, even though some snails survived the test applications at the lower rate. This compares with a combined total of 14 egg clusters laid during the test period in the control plots.

At this point, given the results from the two test substances studied, the field trial was terminated. However, to further elucidate the effect of vulgarone B on snails, given that the first trial showed no significant effect at the 1X rate, a small scale test was installed to determine the effect of vulgarone B at the 75uM concentration that was originally intended. Four test plots were set up in a different part of the same lo'i that had been used for the previous tests. The water level was drained so that the depth was 1". Twenty snails (15 female, 5 male) were placed in a pile within each test plot. Vulgarone B was added to three of the test plots, to a final concentration of 75uM. The fourth test plot was treated as the control in the same manner as previously described. After four days, observations were made on the parameters previously described. The results are shown in the table below.

Effect of 75uM vulgarone B on snails four days after application.

Plot I.D.	***Application Rate***	***# Dead Snails***	***# Plants Remaining***	***# of Egg Clusters***	***Phyto***
1	1X	14	4	1	N
2	1X	20	4	0	N
3	1X	17	4	1	N
4	Control	0	4	10	N

It can be seen from the table above that at the 75uM final concentration, vulgarone B was highly effective at killing snails, with an average of 85% mortality within the four-day observation period. Analysis of variance showed this to be highly significant ($p = 0.0004$) when compared with the data from the combined control plots from both vulgarone B experiments. It was evident that the vulgarone B acted very quickly, as the dead snails in all of the tests remained in the pile that they were originally placed. Due to the high mortality rate, egg laying was severely reduced compared with the untreated control. There was no observable phytotoxicity. As with other tests described earlier, non-target insect species were evident in all of the test plots.

Conclusions

From the studies and the results described above, it is evident that all three of the test substances; ferric phosphate, vulgarone B and the Yucca extract, were effective to varying degrees in the control of Apple snails in taro lo'i. Ferric phosphate was marginally effective and took a relatively long time before its effect was evident. Although no phytotoxic effects were seen, a mortality rate of 45% is not considered effective enough to have any real long-term effect on snail numbers, considering the high reproduction rate of this species. In addition, an application rate of 5lb/1,000 sq ft would require 218 lbs per acre. To be effective, multiple applications would be required through the year, resulting in as much as thousands of pounds per acre needed for adequate control. This would be very cost-prohibitive. Vulgarone B looked very promising, and much more effective than ferric phosphate, with around 85% mortality observed within hours after one application at a rate of 75uM. However, at the higher rate of 150uM, some small signs of phytotoxicity were observed in the pond weed that co-exists in the taro lo'i. This weed provides food, shade and camouflage for a number of animals that inhabit the lo'i. It also serves as an alternate food source for the apple snails, indirectly providing some measure of protection to the taro from damage by the snails. The potential toxicity arising from higher rates of application of vulgarone B needs to be studied further. The yucca extract was also very effective in controlling apple snails. At a 120ppm application rate, about a 75% mortality occurred within hours after one application. No phytotoxic effects were observed. In both the yucca extract and the vulgarone B tests it is interesting to note that very few (four total) snails died after the first observation time (four days after the application). This suggests that both extracts are acutely toxic and have a very short residual activity. This is highly desirable in an ecologically sensitive environment such as a taro lo'i.

Vulgarone B and yucca extract have both been shown in this study to be highly effective means of Golden apple snail control. Further studies are now needed to determine the effects of these extracts on non-target species, such as fish, toads and crustaceans. It is recommended that the Hawaii State Department of Agriculture fund further studies so that these extracts can be moved towards registration with the EPA.