SUMMARY SHEET OF MOSQUITO LARVAL TRAP TESTING CONDUCTED AT THE UNIVERSITY OF FLORIDA

Background: Experiments were conducted during the months of August, September, and November, 2003, utilizing the original patented mosquito larva traps (U.S. Patent No. 6,886,293). Subsequent to these and other tests, revisions were made that further enhanced the trapping abilities of the product (U.S. Patent No. 7,134,238).

Objectives: After determining the proper test procedures, the objectives of the testing were to:

1) Ascertain trap effectiveness.
2) Identify potential stimulants to further attract gravid mosquitoes.

Methodology: Each test consisted of the deployment of twenty traps in various locations around the Entomology building on campus. Although this deployment in a small area diminished the attractant abilities of any single trap due to competition from nearby units, the overall results of the tests in relation to total kills and best stimulants verses the control units containing water only remain valid.

The definitive test results are found in the second round of testing; the first round being utilized to refine the methods used in the second round. The results of this second testing are portrayed in graph form on page 15 of the attached report. The combined capture rate of the traps over a thirty-eight day period was estimated to be 17,400 mosquitoes or approximately 458 mosquitoes per day.

(116 egg rafts (page 15) x 150 eggs per raft (page 3) = 17,400 mosquitoes)

Summary: Gravid (egg bearing) mosquitoes generally live their entire lives, (approximately 15 days as adults), in an area about the size of a football field (less than ½ acre) and lay eggs 3 – 5 times during their adult life. The ***Bite Back!*** traps collect and destroy the offspring of the adults, thus breaking the exponential cycle of mosquito infestation in a given area during the breeding months.

Field deployment of traps has proven the product to be highly effective in mosquito eradication while being safe to use around children and pets. With the stimulant being rice based, the ***Bite Back!*** trap uses no poisons or harmful chemicals. Rather than electrocution or death by poison, the ***Bite Back!*** trapping action relies on the natural tendencies of the mosquito larva to dive for protection and rest.

Conclusion: This product is the only mosquito control system available today that is environmentally friendly, truly safe to use around children and pets, has been tested and proven effective in eliminating mosquito problems, and is inexpensive to deploy and maintain.

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December 2, 2003

James D. Forehand
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Dear Jimmy

The Forehand Mosquito Larval Trap report is enclosed.

Sincerely,

J. F. Butler, Professor Med. Vet. Entomology

SUBJECT:

Test Forehand Mosquito Larval Traps.

PEST: Mosquitoes.

TITLE:

Forehand Mosquito Larval Trap Evaluations Trial 1 and 2.

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OBJECTIVES:

The objectives were: (1.) To develop testing procedures to evaluate mosquito traps. (2.) To determine the effectiveness of the Forehand inverted cone mosquito larval trap. (3.) To add potential egg laying stimulants to the trap to improve egg and larval capture rate.

MATERIALS and METHODS:

Mosquito Larval Trap Trial 1: Forehand Mosquito Larval/Egg Traps were evaluated with different types of media as an attractant for natural egg laying. Five treatments with four replications were used (20 traps). Traps were placed in four different areas near the Entomology and Nematology Building 970. Trap treatments were randomized with 4 replications per treatment. Traps were labeled with date, treatment number, and location with white tape placed on the trap side. A one by six cm strip of filter paper was placed in the cone for egg attachment.

Medias evaluated in Trap 1 are as follows:

Media 1:
- 2 Liters of water
- 2 grams of powdered Pot Bellied Pig Chow
- 0.5 gram of brewers yeast

Media 2:
- 2 Liters of water
- 2 grams of powered Timothy Hay
- 0.5 gram of brewers yeast

Media 3:
- 2 Liters of water
- 2 grams of powered Pure Rice Bran
- 0.5 gram of brewers yeast

Media 4:
- 2 Liters of water
- 2 grams of powered Alfalfa Pellets
- 0.5 gram of brewers yeast

Media 5:
- 2 Liters of water

The traps with media were placed outside but protected from rain by a roof overhang. The windowsills of the inner courtyard and out side of building 970 were used. Observations were made after the first 7 days to determine if mosquitoes had begun laying eggs. The number of egg rafts present on the water surface as well as individual eggs placed on small blotter paper strips at the side of the cone leading into the water was monitored. After 5 weeks the residue in the traps was evaluated for mosquito larvae and other arthropods present. Adult mosquitoes in the upper trap area were monitored on a weekly basis.

Mosquito Larval Trap Trial 2:

Forehand Mosquito Larval Traps were set up as in Trial 1. Five treatments with four replications were used. The traps with media were placed outside of the windowsills in four different areas of building 970 in the inner courtyard. Evaluation of egg rafts and egg deposition were the only data monitored for Trial 2 as these criteria were found to be the most reliable data from Trial 1. The one by six cm strip of filter paper was placed in the cone for egg attachment and attractant placement. The strip was changed every 4 days. The following media were evaluated in Trial 2.

Media 1:

1 gram of powered Pure Rice Bran
1 gram of powered Alfalfa Pellets
2 Liters of water
0.5 gram of brewers yeast

Media 2:

2 Liters of water
2 grams of powered Pure Rice Bran
0.5 gram of brewers yeast in

Media 3:

2 Liters of water
2 grams of powered Alfalfa Pellets
0.5 gram of brewers yeast

Media 4:

1 gram of powered Pure Rice Bran
1 grams of powered Alfalfa Pellets
0.005 gram of attractant on the top of the attached filter paper weekly
2 Liters of water
0.5 gram of brewers yeast

Media 5:

2 Liters of water

Observations were made as noted in the tables for the number of mosquito egg rafts present on the surface of the water and the individual eggs attached to the filter paper inserts. Previous counts of mosquito larvae and other insects present in Trial 1 were limited in value as the number of mosquitoes that died in the 1st and 2nd instar stages could not be evaluated. The most valuable counts in Trial 1 were the number of egg rafts in the surface of the water and the number of individual eggs present on the attached paper strip. These gave a better evaluation of the potential mosquitoes exposed in the trap. A mosquitoe normally produces 2-3 egg rafts in her lifetime due to natural mortality rates. Therefore one egg raft or egg deposition represents about one third of their lifetime expected reproductive potential. A mosquito egg raft contains about 150 eggs. The observations of egg raft counts were timed to record the numbers prior to hatching and dispersal of 1st instar larvae into the trap.

Observations as egg and egg rafts were made daily for 2 weeks to determine the number of mosquito eggs laid. Adult mosquitoes surviving in the upper trap area were monitored on a weekly basis.

RESULTS and DISCISSION:

There were three objectives: (1.) To develop testing procedures to evaluate mosquito traps. (2.) To determine the effectiveness of the Forehand inverted cone mosquito larval trap. (3.) To evaluate an egg laying stimulants added to the trap.

Trial 1 was a developmental step to determine how data could be collected for this new type of trap and to measure potential collection rates. As mosquitoes visiting and laying eggs in the trap could not be counted the egg rafts or individual eggs were counted within a time interval and summed over time for evaluation. As the media matured with fermentation the mosquito egg laying attraction generally increased (Figure 5). When the eggs hatched the small larvae generally migrated beneath the black funnel to the lighter area beneath and were trapped and drown. A few first instar larvae were able to migrate through the small holes in the retention ring where they continued development to adults.

As the media became older or about 2 weeks there was a debris buildup allowing some of the larvae to stay in the black funnel. We were not able to assess the numbers of larvae that were trapped and drown. The total number of mosquitoes present was estimated by the number of egg rafts, which were on the surface. An egg raft would result in 150 larvae for *Culex quinquefasciatus*. *Aedes albopictus* females lay eggs singularly at or above the water line. These were assessed by placing 1 cm strips of blotter paper from the top of the trap into the water at the center. Success considered for *Aedes sp.* was limited, as the number of eggs collected seemed low. I think that the surface of the shiny black funnel should be rough or non-glossy allowing for some moistening of the plastic/water interface.

Data collected for Trial 1 are presented in Tables 1-5. Results of Trial 1 are best shown in Figures 1-3. There were no significant differences in Trial 1 (Table 4, 5) due to the placement of the replications on the outer windowsills of building 970. The mosquito populations on the outside of the building were not comparable with the inner courtyard. We were able to develop assay methods for the trapping system however, with egg rafts on the surface of the water and eggs placed upon the filter paper used to determine the number of potential mosquitoes developing. The presence and number of trapped insects were varied (Table 1). It was difficult to evaluate surviving/dead insect numbers from those remaining and decaying in the trap so the number of eggs and egg rafts were adopted as a measure of potential mosquitoes exposed. Some adult mortality was seen in those insects trapped above the retention ring of the trap.

The suitability of media in the traps was also observed. Table 1 gives the general observations on trap conditions and catches rates for traps during August 2003. In Trial 1 the condition and odor produced by the media was noted with the powered Pot Bellied Pig Chow and brewers yeast being particularly rank. This media would not be acceptable to the general public. This media is also not on the GRAS list. The other media were more acceptable and the ingredients are on the list.

The media collecting the most *Culex quinquefasciatus* was the Powered Pure Rice Bran and brewers yeast (Figure 2). This material could easily be packaged with the traps. Aedes eggs were highest for Powered Timothy hay and brewers yeast (Figure 1). Powered Timothy hay is not on the GRAS list. The fermenting pig chow also attracted many other small Diptera maggots as demonstrated in Figure 3 and Table 1. Statistical differences could not be demonstrated in Trial 1 due to high variance in the replications presented between to outer windowsill and inner courtyard placement of traps.

Trial 2 was more statistically reliable with replication placement allowing data collected to be analyzed. It also gave a measure of suitable media and augmentation of trap catch rates with potential attractants. Table 6-8 and Figures 4-6 presents these results. In this Trial powdered Rice Hulls and brewers yeast media were compared with powered Alfalfa Pellets and brewers yeast as well as their mixtures (Figure 5). The powdered Rice Hulls was significantly more attractive than Rice + Alfalfa + Dibutyl succinate which was significantly better than Alfalfa alone (Tables 6-8). The mixture of powdered Rice Hulls + powered Alfalfa Pellets were not significantly different than the water control. The mixtures of Rice Hulls + powered Alfalfa Pellets and Rice Hulls + powered Alfalfa Pellets + Dibutyl Succinate cannot be directly compared to the other treatments as the amount of each in the mixture was less than the other treatments. This was to maintain the same total 2 g amount of dry media at one gram of each in the mixture tested. The significant difference seen between the Rice Hulls + Alfalfa Pellets and Rice Hulls + Alfalfa Pellets + Dibutyl Succinate demonstrated that Dibutyl Succinate increased the catch rates for the traps. When Dibutyl Succinate was added to the filter paper on the top of the trap (0.005 g) the number of *Culex quinquefasciatus* egg rafts were doubled indicating that this material is a good attractant for stimulation of egg laying (Figure 5).

Media conditioning and longevity is noted in Figure 5. A delay of 12 days was noted before mosquitoes were attracted to the traps. By day 29 the media had lost its major attractiveness. Traps will need to be serviced about once per month, cleaning out the debris and adding new water and media.

SUMMARY:

The powdered rice hulls at 2 grams + 0.5 g brewers yeast, in 2 Liters of water was the best both in numbers of eggs laid and suitability of product odor. One loading of a trap is good for about 30 days and then productivity goes to near 0. Our best data collection was a count of mosquito egg rafts (150 eggs/raft), as larval mortality could not be reliably estimated. We had some survival of mosquito larvae to adults during the test. The traps may need to be modified and or disturbed to assure trapping of all the larvae. I think that 6 small 2 cm no return cones with 3mm holes placed in the positioning ring (the ring just under the black funnel) would allow larvae to move into this area to breath air but be unable to return to the center exit of the trap. Larva in this region of the trap would survive to adult and then die allowing a precise trapping measure of numbers and species. This would also be a visible demonstration of adult mosquitoes being trapped and

killed for the consumer. The water levels in the traps will also need to be maintained to keep traps effective.

The trap that included Dibutyl Succinate as an attractant demonstrated double the catch rate of one gram each of Rice Hulls and Alfalfa. I assume that it would also work for rice hulls alone. The mosquitoes, which are the vectors of West Nile Virus (*Culex quinquefasciatus*), were the majority those trapped. Aedes mosquitoes were not attracted to laying eggs on the slick black surface and only laid a few eggs on the filter paper installed for the purpose. The shiny black surface should be converted to a mat or brushed surface to one centimeter above the water line, which may increase the egg laying for these mosquitoes.

Table 1: Forehand Mosquito Larval general observations of Insects Collected and Media Conditions in Traps.

Site 1 - inner courtyard, west
Site 2 - inner courtyard, east
Site 3 - outer courtyard, west
Site 4 - outer courtyard, east

a - alfalfa
p – Pot Bellied Pig Chow
r – powered Pure Rice Bran
t - Timothy hay
W- water (control)

1a (translucent green, neutral smell) 1 egg raft
9-10 each 2-3rd instars larvae (dead)
1 each 4th instar mosquito Larva (dead)

1p (near putrid)
1 egg raft
1 adult *Culex quinquefasciatus* adult female
1 pupa
25 dead adult *Cx. quinquefasciatus*.
4 each 4th instar *Cx. quinquefasciatus* larvae
2 each 3rd instar larvae
Estimated 65 2nd instar larvae

1r (putrid, brown milky color)
40 each 2-3rd instar mosq larvae
1 sewer fly, Psychodidae
1 fruit fly, Drosophilidae
2 adult *Cx. quinquefasciatus* females, 1 male

1t (not putrid, translucent brown)
1 egg raft
9 Chironomid larvae
1 Psychodidae adult (dead)

1w (clear water)
3 Culicid larvae
4 Chironomidae larvae
2 each 4th instar *Cx. quinquefasciatus*

2a (brownish-green, slight musky smell)
80 each 2-3rd instar mosquito larvae

2p (brown putrid)
1 pupal cast skin mosq
84 each Chironomid cases on clear plastic part of trap
9 each Chironomid cases on black lip

2r (brown putrid)
2 adult female *Cx. quinquefasciatus*, 1 male
1 egg raft
2 each 4th instar mosquito larvae
10 each 2-3rd instar mosquito larvae
2 Chironomid larvae (bloodworms)

2t (brown, slight non-putrid odor)
Collembola; about 10 each dead on water
1 Psychodidae adult dead
1 Psychodidae larva

2w nothing

3a (heavily green, lots of algae)
1 each 1st instar mosquito larva
1 adult female *Cx. quinquefasciatus*

3p (green water, nasty putrid)
1 each 2nd instar mosquito larva (dead)

3r (green water, slight odor)
98 each 2-4th instar mosquito larvae (dead)

3t (green, slight odor)
nothing

3w (some algae, very slight)
nothing

4a (brown, moderate stink)
1 each adult Culex (escaped)
3 each 4th instar larvae
15 pupae

4p (putrid brown) 75 each 4th instar *Cx. quinquefasciatus*
1 mosquito pupa
Approximately 100 Chironomid larval cases

4r (putrid brown, orange film on bottom of trap)
26 each 3-4th instar mosquito larvae *Cx. quinquefasciatus*
Several dead Collembola

4t (brown, some odor)
105 each mosquito larvae,
2-4th instar *Cx. quinquefasciatus*
50 immature cyclorraphids

4w 15 mosq larvae, 2-4th instar, *Cx. Quinquefasciatus*

Table 2: Forehand Mosquito Larval Traps Trial 1

Mosquito Larvae Present in Traps

Media tested	S1	S2	S3	S4	Sum
Alfalfa	11	80	2	19	112
Pig	98	1	3	76	178
Rice	42	14	98	26	180
Timothy	0	0	0	105	105
Water	5	0	0	15	20

Table 3: Forehand Mosquito Larval Trap Culex Egg Rafts Trial 1

Culex rafts	S1	S2	S3	S4	Raft Total
Alfalfa	10	3	0	0	13
Pig	7	3	0	0	10
Rice	28	5	0	2	35
Timothy	2	1	0	0	3
Water	0	0	0	0	0
					61

Table 4 stat. Forehand Mosquito Larval Trap Culex Egg Rafts Trial 1
ANOVA: Single Factor

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
Alfalfa	4	13	3.25	22.25
Pig	4	10	2.5	11
Rice	4	35	8.75	168.9167
Timothy	4	3	0.75	0.916667
Water	4	0	0	0

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	189.7	4	47.425	1.167624	0.364112	3.055568
Within Groups	609.25	15	40.61667			
Total	798.95	19				

Table 5 stat. Forehand Mosquito Larval Trap Aedes Egg Counts Trial 1
ANOVA: Single Factor

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
Alfalfa	4	0	0	0
Pig	4	43	10.75	358.25
Rice	4	35	8.75	106.25
Timothy	4	93	23.25	956.9167

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	1103.188	3	367.7292	1.034824	0.412102	3.4903
Within Groups	4264.25	12	355.3542			
Total	5367.438	15				

Table 6: Forehand Mosquito Larval Trap Aedes Egg Counts Trial 1

	S1	S2	S3	S4	Raft Total	
Alfalfa	0	0	0	0		0
Pig	4	0	0	39		43
Rice	20	0	15	0		35
Timothy	26	1	0	66		93
Water	1	0	1	11		13
						184

Table 7: Forehand Cone Trap Trial 2
Culex quinquefasciatus egg raft(s)

Treatment	Material Used	Site 1/rep	Site 2/rep	Site 3/rep	Site 4/rep	Trt total	
1	Rice + Alfalfa		0	2	9	3	14
2	Rice		9	18	9	4	40
3	Alfalfa		2	7	7	7	23
4	Rice + Alfalfa + Dibutyl succinate		7	12	7	6	32
5	Water-Control		0	2	4	1	7
							116

Table 8 stat. Forehand Cone Trap Trial 2
Culex quinquefasciatus egg raft(s)
ANOVA: Single Factor

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
Rice + Alfalfa	4	14	3.5	15
Rice	4	40	10	34
Alfalfa	4	23	5.75	6.25
Rice + Alfalfa + Dibutyl succinate	4	32	8	7.333333
Water-Control	4	7	1.75	2.916667

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	176.7	4	44.175	3.372137	0.037048	3.055568
Within Groups	196.5	15	13.1			
Total	373.2	19				

ANOVA: Single Factor

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
Rice + Alfalfa + Dibutyl succinate	4	32	8	7.333333
Water-Control	4	7	1.75	2.916667

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	78.125	1	78.125	15.2439	0.007944	5.987374
Within Groups	30.75	6	5.125			
Total	108.87 5	7				

ANOVA: Single Factor

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
Water-Control	4	7	1.75	2.916667
Rice + Alfalfa	4	14	3.5	15

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	6.125	1	6.125	0.683721	0.439946	5.987374
Within Groups	53.75	6	8.958333			
Total	59.875	7				
Total		373.2	19			

Figure 1: Forehand Mosquito Larval Trap
Culex Egg Rafts and Aedes eggs Trial 1

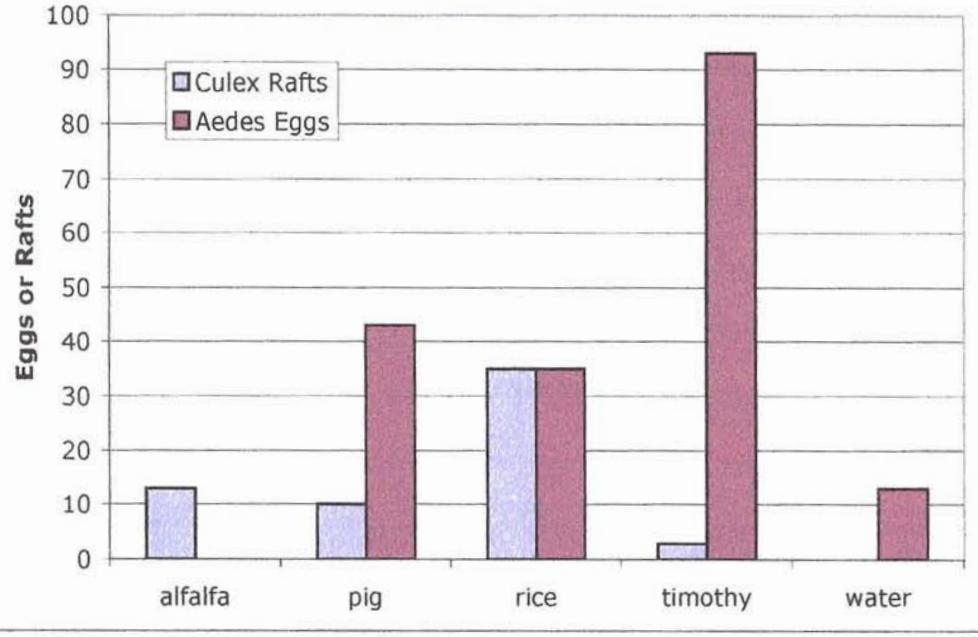


Figure 2: Forehand Mosquito Larval Traps
Sum of Culex Larvae Present

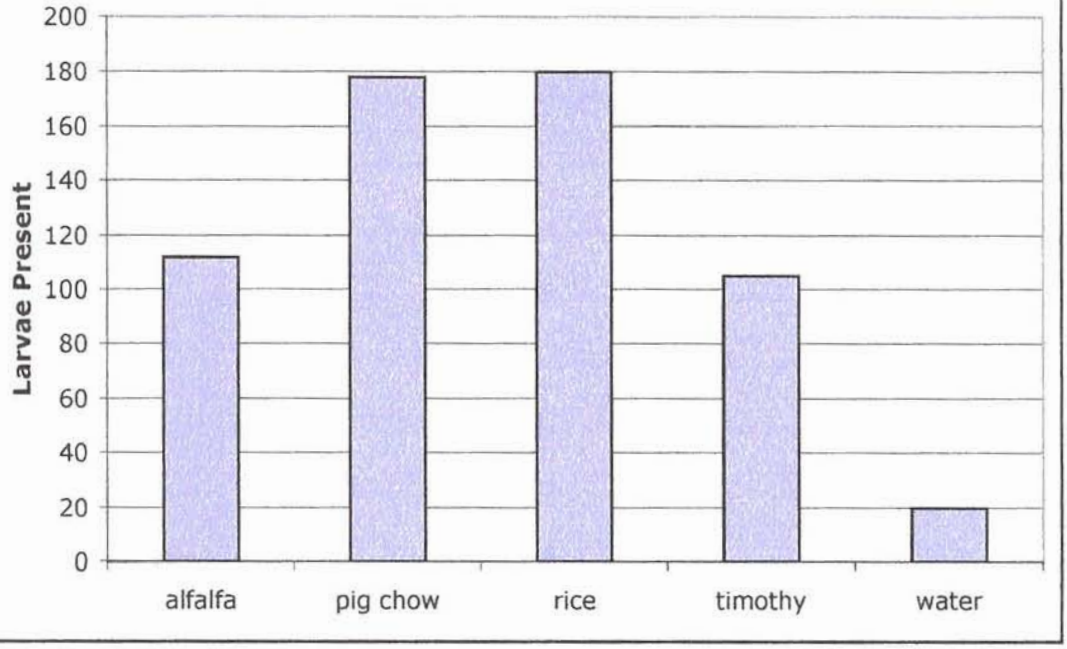


Figure 3: Forehand Mosquito Larval Traps
Sum of Other Insect Larvae Trial 1

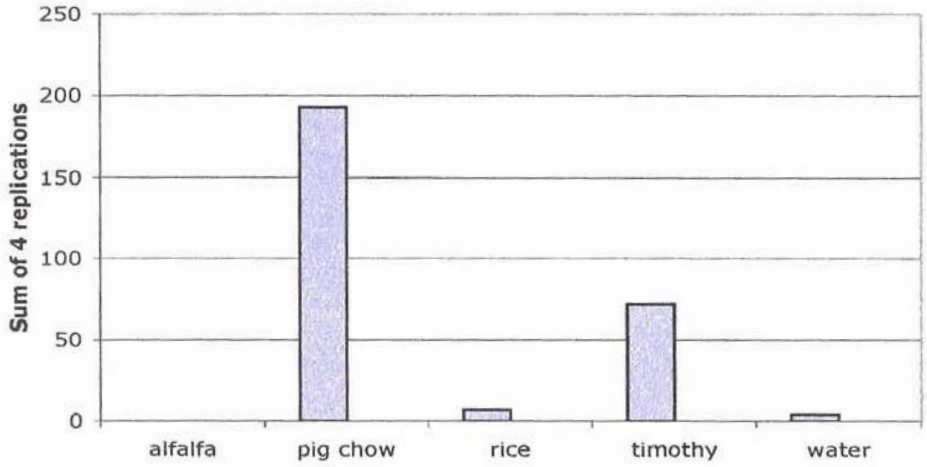
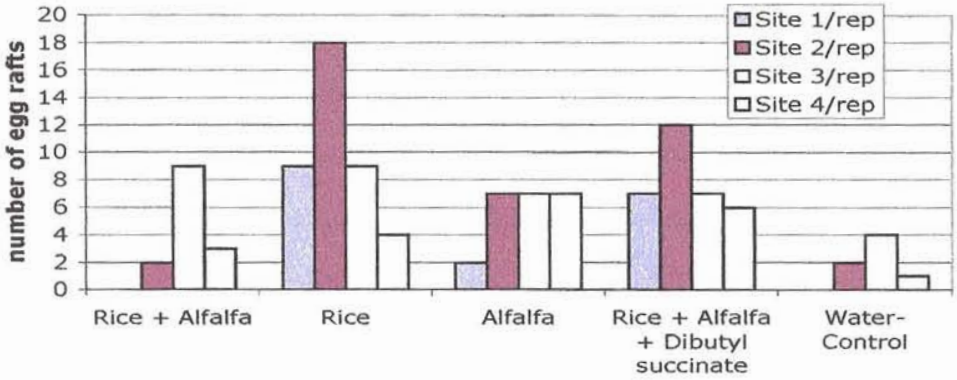


Figure 4:
Forehand Cone Trap Trial 2:
as Replication/Treatment



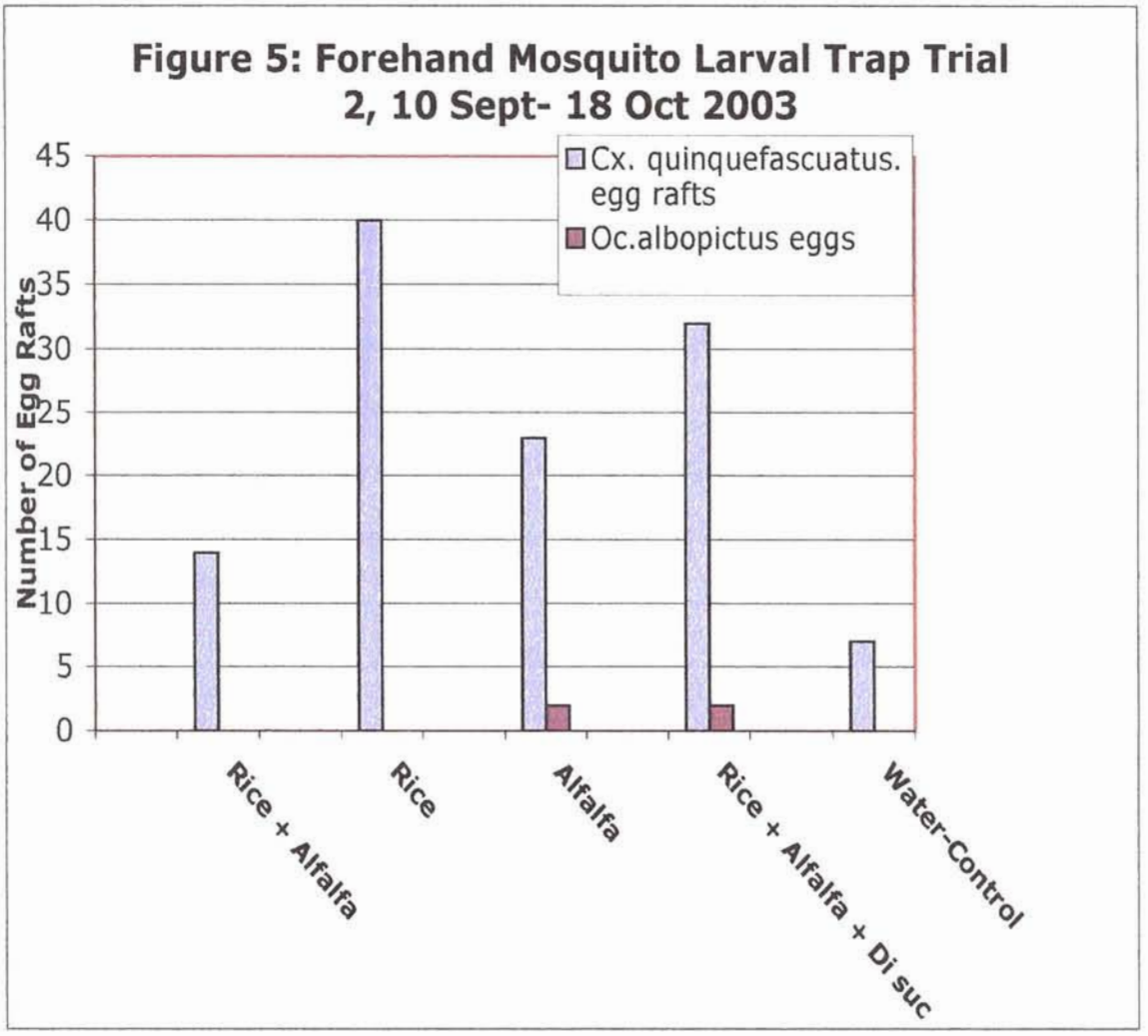


Figure 5: Forehand Mosquito Larval Trap Trial 2, 10 Sept- 18 Oct 2003
Number of Egg Rafts
45
40
35
30
25
20
15
10
5
0
Cx. quinquefascuatus. egg rafts
Oc.albopictus eggs
Rice + Alfalfa
Rice
Alfalfa
Rice + Alfalfa + Di suc
Water-Control

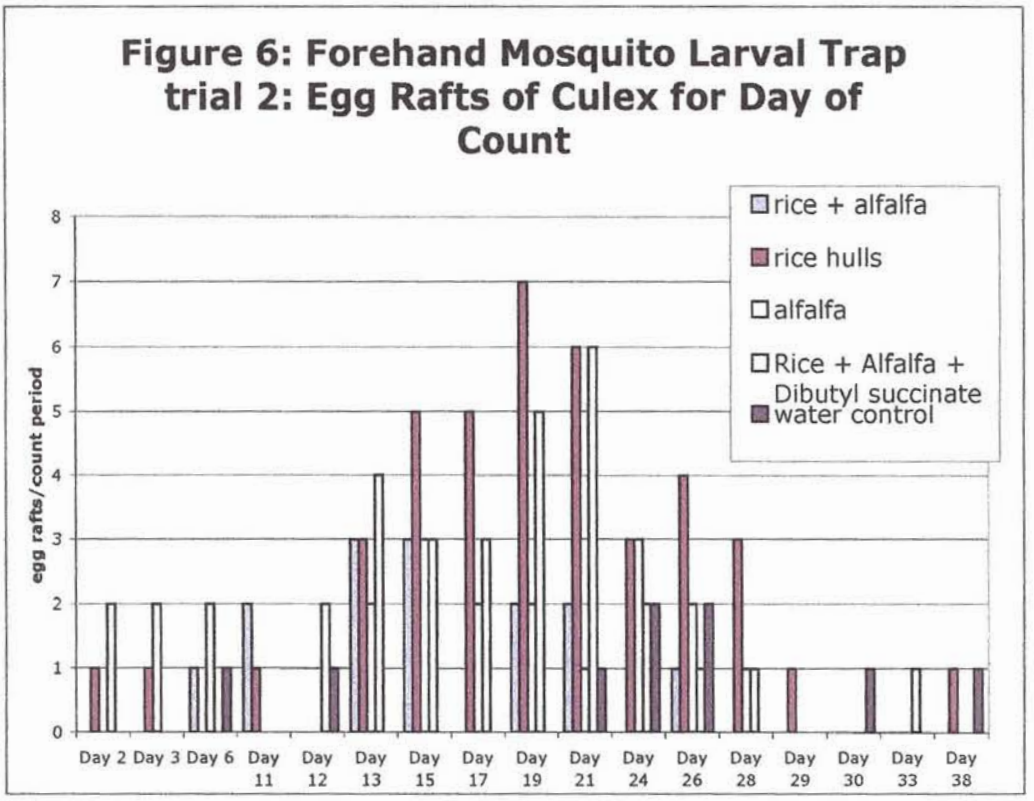


Figure 6: Forehand Mosquito Larval Trap trial 2: Egg Rafts of Culex for Day of Count
egg rafts/count period
0
1
2
3
4
5
6
7
8
Day 2
Day 3
Day 6
Day 11
Day 12
Day 13
Day 15
Day 17
Day 19
Day 21
Day 24
Day 26
Day 28
Day 29
Day 30
Day 33
Day 38
rice + alfalfa
rice hulls
alfalfa
Rice + Alfalfa + Dibutyl succinate
water control