PROJECT NO. RP-12

ANNUAL REPORT
COMPREHENSIVE RESEARCH ON RICE
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PROJECT TITLE: Effective control of tadpole shrimp damage to rice yield: Methyl farnesoate inhibition of reproduction using liposome carriers

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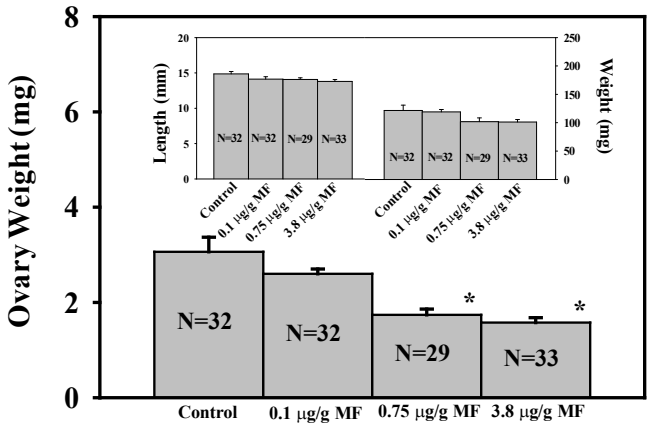
OBJECTIVES AND EXPERIMENTS CONDUCTED BY LOCATION TO ACCOMPLISH OBJECTIVES:

1) Complete the determination of the efficient order of ingredients for MF-liposome pellets;
2) To lower future damage by tadpole shrimp, we propose to determine the efficacy of methyl farnesoate (MF) liposomes pellets on the inhibition of tadpole shrimp reproduction in the rice field;
3) Determination of the capacity of tadpole shrimp to synthesize MF

SUMMARY OF 2005 RESEARCH (MAJOR ACCOMPLISHMENTS) BY OBJECTIVE:

1) Complete the determination of the efficient order of ingredients for MF-liposome pellets
In previous experiments, we demonstrated inhibitory reproductive impacts from MF-coated pellets; however, directly incorporating MF into pellets was no better than controls. We attributed this ineffectiveness to MF oxidation during the processing of the pellets. To prevent the oxidation of MF, this terpenoid was incorporated into liposomes (Sigma). These liposomes, at concentrations of 0.001% and 0.0001% MF (by weight), were blended into a protein mixture similar to that used above. These pellets contain a standard crustacean feed mixture of casein, lecithin, wheat gluten and albumin. As the tadpole shrimp devour the pellets, they also consume the liposomes laden with MF. The liposomes are lipid-bilayers that will protect the MF from chemical degradation. Previous attempts to use MF liposome pellets yielded poor results. This year, we found that the order in which ingredients are added to the pellet mixture greatly influences the efficacy of the tadpole shrimp treatment. Basically, we determined the order of ingredients to best preserve the liposomes during the production of pellets. Having tried several combinations, we found that adding the salt-mix to the distilled water, then the MF-liposomes, dry ingredients (corn starch, non-nutritive bulk, gluten, lecithin, vitamin mix, albumin, casein), followed by the lipids (cholesterol, Vit. E, Vit. A, cod liver oil, and corn oil), has given us pellets that still contain MF.

Lab trials, using these pellets, conducted for 5 days using the newly formulated pellets containing MF liposomes showed no significant differences in either somatic index or brood wt. was found between MF-treated tadpole shrimp controls. However, all the MF-treated groups differed significantly from controls in GSI (Fig. 1).



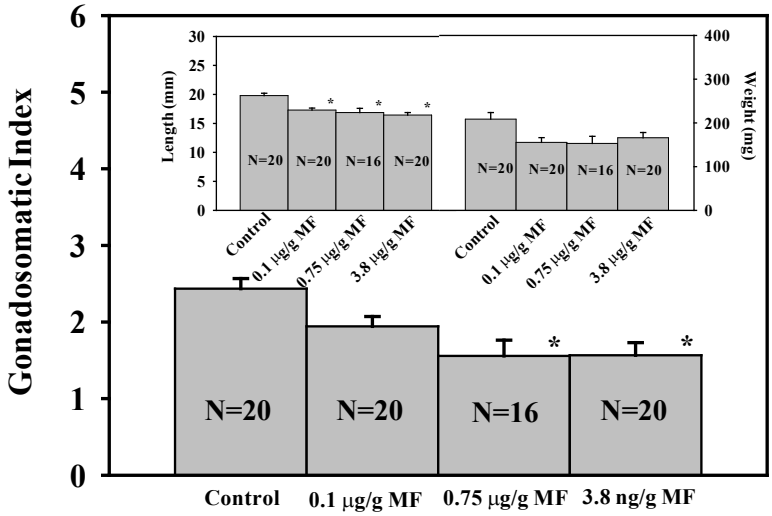
[Figure 1. Day 5 Analysis of MF-Liposome Pellets on ovary weight in *T. longicaudatus*. Decreases in ovary weight were seen in the 0.75 μg/g (Dunn's, $p<0.05$) and 3.8 μg/g MF treatment (Dunn's, $p<0.05$). Inset: Day 5 Somatic Growth Indices of *T. longicaudatus* fed with MF-Liposome Pellets. Neither body weight nor length was affected by either MF treatment.]

2) Methyl Farnesoate Liposomes-Pellets Effect on Tadpole Shrimp Reproduction in Field
After laboratory testing of the pellets has shown that our pellets had some effect, we made HPLC measurements of the pellets to determine pellet MF content. We found the pellets to contain 3.8 μg/g, 0.75 μg/g and <0.1 μg/g MF pellets. In the fields, tadpole shrimp were isolated using 16 plastic exclosure rings (1.22 m diameter), which were set up in the field prior to inundation. In a rice check, treatment groups were assigned to the 16 rings in a Latin-square

design: controls, and the three MF-pellets treatment groups. The efficacy of the 0.75 μg/g MF pellets indicated that lower MF concentrations may be effective in reducing gonad growth in tadpole shrimp. Thus, we created a liposome pellet with lower MF concentrations, <0.1 μg MF /g pellet.

Field studies were also carried out using the MF-liposome pellets. Because of a non-normal distribution in ovary weights within experimental groups, gonadosomatic indices (GSI, ovary weight as a percentage of total body weight) were calculated as a measure of oocyte production, to normalize the data. Differences in mean GSI were found among the groups (ANOVA, $p<0.001$) (Fig. 10). The 0.75 μg/g MF ($\bar{x}$ =15.575, n=16, Tukey's, $p<0.002$) and 3.8 μg/g MF ($\bar{x}$ =15.656, n=20, Tukey's, $p<0.001$) treatments caused decreases in GSI from controls ($\bar{x}$ =24.348, n=20) (Fig. 2). No decrease was seen in the group treated with 0.1 μg/g MF pellets ($\bar{x}$ =19.428, n=20, Tukey's, $p< 0.111$). Dunn's pairwise comparisons test showed no differences between groups for body weights. Lengths for the three MF-treatment groups were found to be different from controls ($\bar{x}$ =19.8, n=20): 0.1 μg/g MF, $\bar{x}$ = 17.3, n=20, Dunn's, $p<0.05$; 0.75 μg/g MF, $\bar{x}$ =16.875, n=16, Dunn's, $p<0.05$; 3.8 μg/g MF, $\bar{x}$ =16.45, n=20, Dunn's, $p<0.05$.

Fig. 2 Day 5 Gonadosomatic index of *T. longicaudatus* fed with MF-liposome pellets in a field experiment.



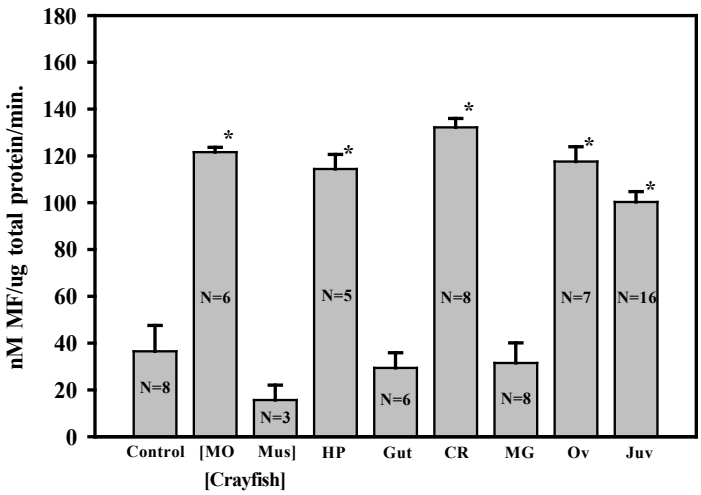
GSI was reduced in the groups fed 0.75 μg/g MF- (Tukey's, $p<0.002$) and 3.8 μg/g MF-pellets (Tukey's, $p<0.001$), as compared to controls. No difference was found between controls and the group fed 0.1 μg/g MF-pellets. Inset: Day 5 Somatic Growth Indices of *T. longicaudatus* fed with MF-Liposome Pellets in field experiments. No differences body weights were found between control and experimental groups in Dunn's pairwise comparisons test. All MF treatment groups were found to have reduced body lengths compared to controls in Dunn's pairwise comparisons test: 0.1 μg/g MF, $p<0.05$; 0.75 μg/g MF, $p<0.05$; 3.8 μg/g MF, $p<0.05$.

In the coming year, we will continue to test the hypothesis that pellets laden with MF-filled liposomes will inhibit reproductive processes in tadpole shrimp. These experiments will examine the efficacy of single treatments and the timing, relative to field inundation, of those treatments.

In additional studies, we plan to determine which, if any of the major organs (gut, maxillary gland, ovary, and hepatopancreas, using muscle & saline as controls) of the tadpole shrimp are capable to degrading MF. This will test whether the tadpole shrimp will be able to develop a resistance to MF treatment. Past support from the Rice Research Board has helped in acquiring a new California Agricultural Technology Institute – Agricultural Research Initiative grant (CATI-ARI). Rice Research Board funding is used as a match for this grant (CATI-ARI) on tadpole shrimp control.

3) **Determination of the capacity of tadpole shrimp to synthesize MF**

We have investigated the capacity of the tadpole shrimp to determine the capacity to synthesize MF. To determine synthetic capacity, a radiochemical synthesis assay for Farnesoic Acid O-Methyl Transferase (FAOMeT) activity. Crayfish (*P. clarkii*) mandibular organ tissue was used as a positive control. It has been reported to have high levels of FAOMeT activity. FAOMeT activity [mM MF/protein (mg/mL) /min.] was found in the crayfish mandibular Organ (MO) and the hepatopancreas (HP). In the tadpole shrimp FAOMeT activity was high in the cephalic region (CR), Ovary (Ov), and whole juvenile homogenates of the tadpole shrimp (Fig 3). No activity was found in the gut or maxillary gland (MG) of *Triops*.



[Figure 3. Radiochemical Synthesis Assay for Farnesoic Acid O-Methyl Transferase (FAOMeT) Activity in *T. longicaudatus*. Gut, hepatopancreas (HP), maxillary gland (MG), cephalic region (CR), and ovarian (Ov) tissue homogenates, as well as whole body homogenates of juveniles were incubated in farnesoic acid and [3H-methyl] SAM.

These data indicate that MF is indeed a native compound within the T. longicaudatus body. In addition, these data confirm previous studies that demonstrated MF as a component of tadpole shrimp hemolymph (Tsukimura et al, 2006, Comp. Biochem. Physiol., *In press*).

PUBLICATIONS OR REPORTS:

Tsukimura, B., C.J. Linder, and W.K. Nelson. (2006, *in press*) Inhibition of ovarian development in by methyl farnesoate in the tadpole shrimp, *Triops longicaudatus*. Comp. Biochem. Physiol.

Presentations

Tsukimura, B., and W.K. Nelson. 2005. Ovarian development inhibition by methyl farnesoate in the tadpole shrimp, *Triops longicaudatus*. 15th International Congress of Comparative Endocrinology, Boston, MA. pp. 128

Completed Graduate Degrees:

W.K. Nelson. 2005. Juvenilizing Effects of Methyl Farnesoate on Reproduction and Development in the Riceland Tadpole Shrimp, *Triops longicaudatus*. Department of Biology, California State University, Fresno.

CONCISE GENERAL SUMMARY OF CURRENT YEAR'S RESULTS:

As the potential for increased copper sulfate regulation looms, we are developing a method of treating tadpole shrimp using an organic hormone, methyl farnesoate. This terpenoid compound has been successfully incorporated into liposomes, which protects MF from oxidation. The blending of these MF-liposome pellets has been problematic. During the past year, we have developed a methodology that protects the liposomes during the production of the MF-liposome pellets. These pellets have been shown to be effective inhibitors of gonad development in juveniles, both in the laboratory and in the field. In addition, we have MF synthetic capabilities in the cephalic region, ovary, and whole juvenile homogenates of the tadpole shrimp. This indicates that this terpenoid compound is naturally synthesized in the animal.