PROJECT NO. RP-12

ANNUAL REPORT
COMPREHENSIVE RESEARCH ON RICE
January 1, 2004 - December 31, 2004

PROJECT TITLE: Effective control of tadpole shrimp damage to rice yield: Ground application of copper sulfate and methyl farnesoate-liposome pellets

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OBJECTIVES AND EXPERIMENTS CONDUCTED BY LOCATION TO ACCOMPLISH OBJECTIVES:

1) Complete the assessment of the efficacy of ground application of copper sulfate on lowering the damage caused by tadpole shrimp in rice yields. Determine the potential cost savings of ground application.
2) To lower future damage by tadpole shrimp, we propose to determine the efficacy of methyl farnesoate (MF) liposomes-impregnated pellets on the inhibition of tadpole shrimp reproduction in the rice field.

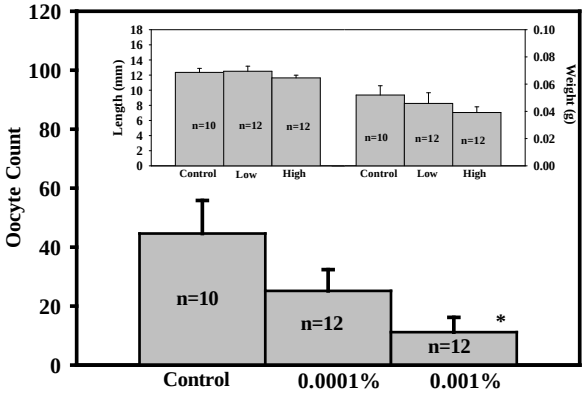
SUMMARY OF 2004 RESEARCH (MAJOR ACCOMPLISHMENTS) BY OBJECTIVE:

1) Ground application of Bluestone

At an application level equivalent of 15# per acre, no differences in tadpole shrimp populations were found between treated and control checks. A second aerial application was required to control tadpole shrimp during the second week of inundation. Observations were stopped after the rice stalks reached 10 cm above the water level. Our observations were made at water temperatures between 20° - 24°C. These data did not support our hypothesis that ground applications of copper sulfate would eliminate or lower tadpole shrimp in the rice fields.

2) **Methyl Farnesoate Liposomes-Pellets Effect on Tadpole Shrimp Reproduction**

In previous experiments, we demonstrated inhibitory reproductive impacts from MF-coated pellets, however, directly incorporating MF into pellets was no better than controls. 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. Preliminary data in laboratory tests suggest that this feed effectively reduced the number of oocytes found in developing tadpole shrimp (Fig 1).

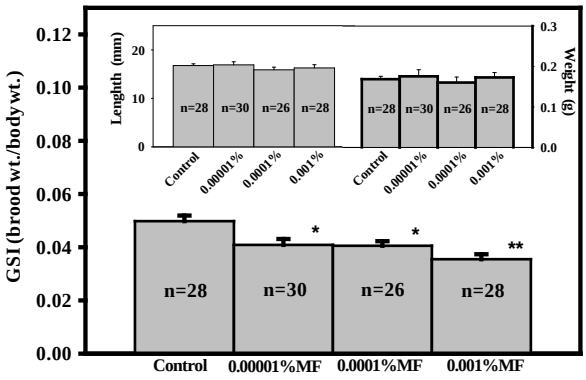


[Figure 1. MF-Liposome Pellets Reduce Oocytes in Tadpole Shrimp at Day 5 – In the laboratory, preliminary data indicates that MF-liposome pellets effectively decrease the number of oocytes in tadpole shrimp ovaries. *= significant difference from control. **Inset**: MF-laden pellets had no effect on somatic indices, length or weight.**]**

Successive attempts in synthesis of MF-liposome pellets yielded poor results. We have since found that the order in which ingredients are added to the pellet mixture greatly influences the efficacy of the tadpole shrimp treatment. Lab trials conducted for 5 days using pellets containing MF liposomes showed no significant differences in either somatic index or brood wt. was found between MF-treated tadpole shrimp controls. Recent experiments have involved the development of the pellets. We have spent the last year adjusting the order of ingredients to best preserve the liposomes during the production of pellets. Initially dry ingredients were mixed (corn starch, non-nutritive bulk, gluten, lecithin, vitamin mix, albumin, casein and salt-mix) then the lipid based ingredients (cholesterol, Vit. E, Vit. A, cod liver oil, corn oil, and liposomes) were added, followed by distilled water. This ingredient order yielded no MF in the resultant

pellet. Having tried several combinations, we found that added the salt-mix to the water, then the liposomes, dry ingredients, followed by the lipids, has given us pellets that still contain MF. Recent studies ultimately became experiments showing the importance of maintaining the structure of the liposome during pellet production.

The efficacy of the 0.0001% 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, 0.00001% MF. Laboratory experiments using the MF-liposome pellets (new recipe) significantly lowered the gonadosomatic index (GSI) of the treated tadpole shrimps relative to controls (Fig. 2).



[Figure 2. Day 5 Analysis of MF-Liposome Pellets on the gonadosomatic index in *T. longicaudatus*. MF-liposome pellets caused a significant reduction in GSI in the 0.00001% MF, mean=0.0409 (Tukey, $p<0.01$); 0.0001% MF, mean=0.0405 (Tukey, $p<0.01$); and 0.001% MF, mean=0.0355 (Tukey, $p\leq0.001$) treatments, as compared to controls, mean=0.0498. Inset: Neither body weight nor length was affected by any of the MF treatments.]

The efficacy of the pellets utilizes relatively minimal amounts of MF (0.00001%, 0.0001%, and 0.001%). An additional benefit is that MF is an "organic" compound (extracted from plants) that is rapidly oxidized when exposed to oxygen. The breakdown products of MF are water and farnesoic acid, a harmless fatty acid. During the off-season, we hope to refine the MF-liposome pellet. MF currently costs 1$/mg from Echelon Research Laboratories, UT. This cost is high because there is no common use for MF and production is minimal. Extraction costs will decrease significantly, if there were a higher demand for MF and a market developed. At these current costs, we calculated the cost for initial field testing to be ~$870/acre (0.001%), ~$87/acre (0.0001%) and ~$8.70/acre (0.00001%). In the coming year, we plan to continue to test the hypothesis that pellets laden with MF-filled liposomes will inhibit reproductive processes in tadpole shrimp. 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. These efforts are currently being funded by our California Agricultural Technology Institute – Agricultural Research Initiative grant. Rice Research Board funding is used as a match for a campus grant (CATI-ARI) on tadpole shrimp control.

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PUBLICATIONS OR REPORTS:

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

Presentations

Nelson, W.K. and B. Tsukimura. 2004. Reproductive Inhibition By Methyl Farnesoate in the Riceland Tadpole Shrimp *Triops longicaudatus*. 85th Annual Pacific Division meeting of the American Association for the Advancement of Science, Logan, UT.

Nelson, W.K. and B. Tsukimura. 2004. Methyl farnesoate: Possible Endocrine alternative for population control of the invasive Riceland tadpole shrimp, *Triops longicaudatus*. 25th Annual Central Calif. Research Symposium (CCRS). pp.62.

CONCISE GENERAL SUMMARY OF CURRENT YEAR'S RESULTS:

As the potential for increased copper sulfate regulations 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. Briefly, this methodology requires precision in the order in which ingredients are added. In laboratory studies, this new method significantly decreased tadpole shrimp gonad development. Though the gonad decreases were moderate, we plan to further analyze the order of ingredients to increase the efficacy of gonad inhibition. During the off-season we are further refining the pellet recipe for field experiments during the 2005 growing season. Ground application of copper sulfate (15#/acre) did not significantly reduce tadpole shrimp populations in rice checks. Additional tadpole shrimp control was required in these fields to control tadpole shrimp damage.

As farm equipment is the most likely vector for spread of the tadpole shrimp between fields, we recommend that farm equipment that has been operated in infected field be rinsed prior to use in clean fields.