ANNUAL REPORT
COMPREHENSIVE RESEARCH ON RICE
January 1, 2003 - December 31, 2003

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) Continue to determine 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 liposomes-impregnated pellets on the inhibition of tadpole shrimp reproduction in the rice field.

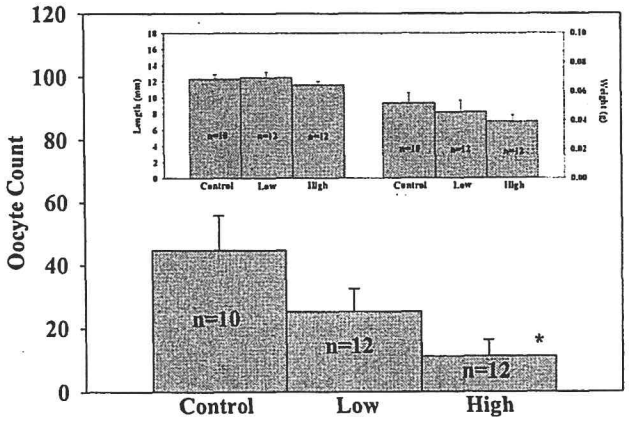
SUMMARY OF 2003 RESEARCH (MAJOR ACCOMPLISHMENTS) BY OBJECTIVE:

1) Ground application of Bluestone

Random block design was used to assign treatments to 16 rings placed around the checks. Ground application of copper sulfate was performed using appropriate protective gear after the rings were in place. At an application level equivalent of 15# per acre, no tadpole shrimp were found in the enclosure rings. 40 to more than 100 tadpole shrimp were found in control exclosure rings. Observations were stopped after the rice stalks reached 10 cm above the water level. Our observations were made at water temperatures between 24° - 27°C. These data strongly support our contention that ground applications of copper sulfate will eliminate tadpole shrimp from the rice fields. We hope to determine if lower concentrations will be equally as effective in lowering tadpole shrimp damage. Lower concentrations of copper sulfate will decrease the cost per acre in tadpole shrimp control. Our plan to test lower concentrations of copper sulfate concentrations was thwarted by miscommunications with water managers and times of field flooding. We are planning to complete the copper sulfate application at lower concentrations to determine the minimum levels necessary to eliminate tadpole shrimp.

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 possible oxidation of MF, the 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 lecithin, wheat gluten and casein. This feed mixture was consumed in previous and current assays and attracts the carnivorous tadpole shrimp. 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).

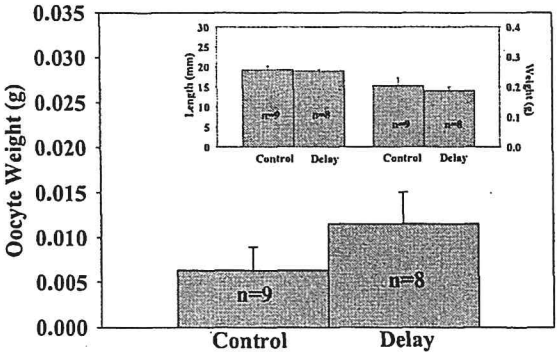


[Figure 1. MF-Liposome Pellets Reduce Oocytes in Tadpole Shrimp at Day 5 – In the laboratory, preliminary data indicates that MF-liposome pellets effectively decrease the number of oocytes in tadpole shrimp ovaries. "Low" concentration refers to 0.0001% and "High" refers to 0.001% MF in pellets. * indicates significant difference from control. **Inset**: MF-laden pellets had no effect on somatic indices, length or weight.]

In an effort to develop a MF-pellet that can be used as a single application, we constructed pellets in which MF was incorporated into liposomes, which were then incorporated

into pellets. Using MF-coated pellets, MF concentrations (0.0001% and 0.001%) were shown to be effective in previous trials. Our most recent efforts did not show any effect. We believe that this is due to the incomplete incorporation of MF into liposomes, and the rapid oxidation of MF during the pellet production process. Our earlier data (Fig 1) indicates that when properly protected by liposomes, the MF is stable and will likely provide an effective means of tadpole reproductive control. Because of the labile and oxidation properties, these data also indicate that MF is a good candidate for organic control of tadpole shrimp.

To determine whether the MF was indeed affecting the development of the reproductive system, we fed the MF-liposome pellets (from the initial study) to sexually maturing tadpole shrimp (5 days old). We found that the MF had no effect on the total oocyte weights when compared to control (Fig 2). These data suggest that the MF is acting as a juvenilizing factor that inhibits the development of reproductive organs in tadpole shrimp.



[Figure 2. MF Pellets Do Not Affect Ovary Weight of *Triops longicaudatus* when administered to adults. In laboratory trials, MF-pellets fed to day 5 tadpole shrimp had no effect on ovary weight at Day 10. No differences were detected in somatic factors (length and weight) of tadpole shrimp. **Inset**: MF- pellets had no effect on somatic indices, length or weight.]

The efficacy of the pellets utilizes relatively minimal amounts of MF (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 air. The breakdown products of MF are water and farnesoic acid, a harmless fatty acid. During the off-season, we hope to demonstrate that the liposomes protect the MF from oxidation, and therefore allow us to use smaller concentrations of MF. 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%) and ~$87/acre (0.0001%). We hope to be able to use lower concentrations (0.00001%), which would lead to study costs of~$8.70/acre. 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 a peripheral study, we determined that none of the major organs (gut, maxillary gland, ovary, and hepatopancreas, using muscle & saline as controls) of the tadpole shrimp were unable to degrade MF. This suggests that the tadpole shrimp will be less likely to develop a resistance to MF treatment.

During the off-season, we are continuing to develop the MF-liposomes for use in MF-pellets. These efforts are currently being funded by our California Agricultural Technology Institute – Agricultural Research Initiative grant.

PUBLICATIONS OR REPORTS:

Linder, C.J., W.K. Nelson and B. Tsukimura. Methyl farnesoate inhibits ovarian development in the tadpole shrimp, *Triops longicaudatus*. (in prep)

Presentations

Nelson, W.K. and B. Tsukimura. 2003. Reproductive inhibition by methyl farnesoate in the tadpole shrimp *Triops longicaudatus*. Society for Integrative and Comparative Biology, Toronto, Canada. Integr. Comp. Biol. 42:1284

Nelson, W.K. and B. Tsukimura. 2003. Potential organic control of the invasive Riceland tadpole shrimp, *Triops longicaudatus*, using methyl farnesoate. 24th Annual Central Calif. Research Symposium (CCRS)

CONCISE GENERAL SUMMARY OF CURRENT YEAR' S RESULTS:

Ground application of copper sulfate (15#/acre) eliminated tadpole shrimp from enclosure rings (1.2m diameter) while control rings contained from 40 – 100+ tadpole shrimp. Observations for the presence of tadpole shrimp continued until rice stalks were 10 cm above the water surface. These studies were conducted at relatively warm water temperatures (24° - 27° C). We plan to test the efficacy of ground application of copper sulfate at lower temperatures and concentrations next season. Application of copper sulfate typically occurs after the adult tadpole shrimp have released 100's of eggs that will develop and grow in successive years. To reduce or eliminate the reproduction of tadpole shrimp, we have developed MF- liposome pellets. These studies initially suggest that MF levels will significantly reduce tadpole shrimp reproduction at concentrations of 0.001% and 0.0001% MF (by weight). This year's efforts to generate liposome pellets were unsuccessful. We believe that this is a problem in production and not of the MF treatment. We will continue to develop MF-liposomes in the off season, as well as create an effective pellet for single applications. We believe that copper sulfate applications in combination with MF-treatment will both reduce the number of tadpole shrimp that damage crops and the number of eggs that will impact future plantings. 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.