FHP TECHNOLOGY DEVELOPMENT PROGRESS REPORT

PROJECT STATUS: Complete. Work continuing under no-cost extension.

PROJECT NUMBER: R8-99-03

PROJECT TITLE: Development of a mass rearing scheme for the bark beetle natural enemy *Roptrocerus xylophagorum.*

SUBJECT: Biological control, bark beetles, *Dendroctonus*, *Ips, Roptrocerus xylophagorum.*

PROJECT OBJECTIVES: The core objective of this project is the development of a cost-efficient mass rearing procedure for the bark beetle larval/pupal parasitoid, *Roptrocerus xylophagorum*, capable of producing adequate numbers of insects for possible use in augmentative release programs aimed at controlling bark beetle infestations. No methods currently exist to rear *R. xylophagorum* in a cost-effective manner on its natural hosts (*Ips* or *Dendroctonus* beetles), because large quantities of tree tissue are required to rear the beetles themselves. We propose to develop methods for rearing *R. xylophagorum* on an artificial diet devoid of insect components. To meet this goal the project must address the following two component objectives:

(1) *Identification and synthesis of an oviposition stimulant for* R xylophagorum. This parasitoid normally oviposits only on their natural hosts when these are enveloped in tree tissue. Collection and transfer of eggs from such "natural" oviposition sites to artificial media would be prohibitively labor intensive and too costly for large-scale implementation. To overcome this problem, we must identify the natural cues (chemical and physical) involved in oviposition, and then develop a synthetic oviposition stimulant. This stimulant would be used to induce the parasitoids to deposit eggs directly on the diet or onto surfaces where the eggs might be easily recovered and manipulated.

(2) *Optimization of an artificial diet for rearing* R. xylophagorum. Previous work with Dr. M. Guadalupe Rojas (USDA-ARS) has resulted in a functional artificial diet capable of rearing *R. xylophagorum* from egg to adult, one that has no insect-derived components. However, further tests are to needed to measure the quality of the individuals produced on the artificial diet. We will compare the fecundity, longevity, size, etc. of adult parasitoids reared on the diet with those reared on natural hosts, and if necessary enrich or modify the diet to obtain similar performance.

BRIEF DESCRIPTION OF PROJECT: Studies will be conducted to meet component objectives 1 and 2 above.

(1) *Studies will be undertaken to characterize and then artificially reproduce the chemistry of* R. xylophagorum's *natural oviposition stimulant(s).* Volatile and non-volatile compounds associated with *R. xylophagorum* oviposition sites in host-infested bark will be isolated by a variety of techniques and identified using coupled gas chromatography-mass spectrometry. Compounds distinguishing the oviposition sites from other locations will be obtained in pure

form, and synthetic blends of candidate oviposition stimulants will be bioassayed to check for oviposition-stimulating activity. Methods for presenting the stimulants to ovipositing parasitoids will be refined in order to maximize the facility with which eggs may be collected and transferred to media. Optimally, it will be possible to present the stimulants in such a way that oviposition occurs directly on media cells (see below), hence eliminating the need for manual or automated transfer.

(2) *Studies will be conducted to fully evaluate the fitness of* R. xylophagorum *reared on artificial diet.* Trays with multiple cells will be partially filled with the diet for *R. xylophagorum* and then eggs will be placed within each cell near the diet, as in our preliminary studies. Survivorship from egg to emerging adult will be recorded, and then measurements made of the fecundity, longevity, and size of the resulting adult wasps. Performance of *R. xylophagorum* on the diet will then be compared with those reared on natural hosts, and if necessary the diet will be modified/enriched until similar performance is obtained.

FHP PEOPLE WHO WILL LEAD PROJECT: Steve Clarke & Don Duerr, R8

CO-PRINCIPAL INVESTIGATORS: Dr. Brian T. Sullivan and Dr. C. Wayne Berisford, Department of Entomology, University of Georgia, Athens, Georgia 30602; Dr. John D. Reeve, Southern Research Station, Pineville, Louisiana 71360; Dr. M. Guadalupe Rojas, USDA ARS, New Orleans, LA 71360

Contributions: Dr. Sullivan will work on developing a synthetic oviposition stimulant for *R. xylophagorum* (see Methods, part 1), while Dr. Berisford will provide the necessary analytical equipment, oversight, and salary for Dr. Sullivan's work.. Dr. Reeve will work on optimizing the diet for *R. xylophagorum* (see Methods, part 2), in collaboration with Dr. Rojas. All rearing and quality testing will be done at the Southern Research Station, with diet preparation and modification conducted at an ARS facility in New Orleans.

ACCOMPLISHMENTS AND RESULTS:

We have succeeded in obtaining a crude extract capable of stimulating ovipositor probing/drilling by female *Roptrocerus xylophagorum*. The stimulant is a component present in a water extract of the larval frass of the bark beetle *Ips grandicollis*, and is likely to be nitrogenous waste product of the larvae. The extract is only capable of stimulating probing/drilling in an environment saturated with the odors of *Ips*-infested pine bark. The next step will be to chemically identify both this probing/drilling stimulant and the necessary environmental stimulants. In addition, egg deposition appears to require contact between the ovipositor and a host larva or larva surrogate. We have succeeded in obtaining egg deposition using a host larva surrogate consisting of homogenized insect larvae sandwiched between layers of Parafilm. However, we have been unable to obtain this egg deposition consistently, in part because we are still in the process of developing a reliable ovipositor probing/drilling stimulant. We intend to test modifications of the larva surrogate until we can reliable and quickly obtain egg deposition.

We have encountered and potentially solved several problems in developing an artificial diet for *R. xylophagorum*, but final success has yet to be achieved. In this part of the study, *R. xylophagorum* eggs were implanted in cells partially filled with artificial diet, and the subsequent behavior of the hatched larvae observed. Difficulties were first encountered in retaining the highly active larvae within the cells, and with bacterial and fungal contamination of the diet during implantation. These problems were overcome by better sealing of the cells and improved sterile technique. We then encountered a problem with excess moisture in the cells, which was overcome by inserting a drying period during dispensing of the diet. It was then discovered that the pH of the diet was too acidic, causing high mortality of the larvae. This problem can be easily fixed by adjusting the pH with a KOH solution. The diet does appear to be palatable to *R. xylophagorum*, however, because in all cases the larvae were observed feeding on the diet. We plan additional work to correct these problems, beyond the end of the STDP.

DOCUMENTATION:

Sullivan, B.T., K.C. Seltmann and Berisford, C.W. 2000**.** Response of the bark beetle parasitoid *Roptrocerus xylophagorum* (Ratzeburg) in a Y-tube olfactometer to odors associated with the host-tree complex. In review.

Sullivan. B.T., K.C. Seltmann and Berisford, C.W. 1999. A simple continuous rearing technique for the bark beetle parasitoid, *Roptrocerus xylophagorum* (Ratzeburg). J. Entomol. Sci.: 34:260-264.

FIRST YEAR FUNDED: FY 1999
YEAR SCHEDULED TO END: FY 2000
ACTUAL YEAR TO END: FY 2000

PRODUCTS AND DUE DATES IDENTIFIED IN THE ORIGINAL PROPOSAL: A cost-efficient process for mass rearing the parasitoid *R. xylophagorum* for use in inundative releases against bark beetle pests. We expect this process to be developed by the year 2000.

STATUS OF PRODUCTS: A probing/drilling stimulant has been identified, and work continues to identify an oviposition stimulant. A palatable diet has been developed, and it is being refined to increase larval survival. The mass rearing system is nearing completion, and work will continue beyond the end of the STDP.

FUNDS OBLIGATED FROM BEGINNING OF PROJECT THROUGH END: $53,477.

POST PROJECT TECHNOLOGY SUPPORT: The transfer of this technology will involve all cooperators on this proposal (University of Georgia, Southern Research Station, and FHP personnel). Once a mass rearing procedure for *R. xylophagorum* has been developed, we plan to conduct field trials of augmentation in *D. frontalis* or *Ips* infestations, and funding for this work

is needed. If efficacy is demonstrated at this level, the technology would then be made available to various private companies that rear beneficial insects, who would then provide the parasitoids to federal, state, and private landowners. We envision that the companies that are interested in this technique would provide the support to produce and market it. Landowners would be trained on the use of this method by FHP personnel with the help and support of the company.

LOOK TO THE FUTURE: We expect augmentation of *R. xylophagorum* to become one part of an IPM program for SPB. To accomplish this integration, other questions should be investigated.

1. Is this technique is applicable to other SPB parasitoids?
2. How does augmentation meld with other new methods of SPB suppression such as supplemental parasitoid feeding and the use of semiochemicals?
3. Recommendations as to when and how to augment parasitoid populations must be developed. For example, does augmentation work best at the onset of SPB epidemics, or toward the end when natural enemy populations are increasing? How many parasitoids should be released based on infestation size, host type, average tree DBH, etc.?
4. Can augmentation, alone or in concert with other techniques, suppress individual spots, and/or is the effect on a larger scale, a reduction in the duration and severity of outbreaks?