Mating Disruption/SIR

Genetic Engineering of the Codling Moth: Sterility Without Irradiation

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Alternative control strategies are needed for the codling moth in order to reduce our reliance on chemicals. We are working on an alternative non-chemical method to establish pest-free zones which is similar to the Sterile Insect Release Program in British Columbia but does not involve irradiation. It involves the genetic engineering of a lethal trait into the codling moth.

Researchers at the University of California—Riverside have been working on such an alternative genetic control strategy for the suppression of pink bollworm. The lethal trait they examined in *Drosophila* is a mutation of the *Notch* gene which is required for normal development of the insect embryo. The mutation is expressed at temperatures below 20°C (68°F). Thus, moths which possess this mutation could be reared in the laboratory above 20°C. However, once released in the field, matings with wild moths would produce eggs that would die at temperatures below 20°C. During the growing season, temperatures drop below 20°C nearly every night and often during spring and fall daytime. Laboratory trials of this mutation with the *Drosophila* fruit fly led to the extinction of the population within three generations. This alternative genetic control strategy has been named Autocidal Biological Control by Karl Fryxell and Tom Miller at UC Riverside.

We have been developing the technology to genetically transform codling moth. During the past two years we have made progress toward a stably transformed codling moth. A microinjection system used to deliver DNA into the newly laid eggs has been optimized. We have injected several DNA vectors which are pieces of DNA used as a vehicle to transport the gene of interest into the egg. These vectors contain "jumping genes" such as *piggyBac* and *hobo,* which are capable of inserting themselves into the chromosome at specific sites. Once the gene is integrated into the chromosome, it is passed down to the offspring. We are using both *piggyBac* and *hobo* vectors. Preliminary assays with *piggyBac* have proven that the gene is capable of functioning as a jumping gene in the codling moth embryonic environment.

Stable genetic transformation is proven by determining if the foreign gene is heritable. With our initial injections with a *piggyBac* vector, we now have evidence of inheritance of the injected DNA in generations 1-5 (using polymerase chain reaction [PCR] analyses of DNA). When a *hobo/Notch* vector (with the lethal *Notch* mutation) was microinjected, codling moths grown at lower non-lethal temperatures (21°C) showed eye mutations, which was expected. This indicates that the *Notch* DNA may be affecting eye color or eye development genes. For these experiments, further molecular analyses will pinpoint the exact location of the inserted DNA in the chromosomes.

A fluorescent microscope, specifically designed to detect signal from green

fluorescent protein (GFP), was recently purchased for screening transformants. In preliminary experiments, a low level of gene expression of the GFP has been found in codling moth injected with *piggyBac/GFP*. Future injections will involve higher concentrations of DNA and using a vector containing *piggyBac,* GFP, and the lethal *Notch* mutation. Both the transformed "green" moths and the created eye mutant lines have great potential for future codling moth basic biology and genetic research.