

Mating Disruption/SIR

Functionally Sterile Codling Moths Through Genetic Engineering: An Alternative Control Program

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Alternative control strategies are needed for the codling moth in order to reduce our reliance on chemicals. Continued use of organophosphates during the growing season kills the beneficial organisms that help to keep pest populations down, puts orchard workers at risk for insecticide exposure, and increases the likelihood of insecticide resistance developing in the codling moth. In addition, the only chemical used for the postharvest disinfestation of fruit, methyl bromide, is scheduled for removal from use in the U.S. by the year 2001 because it is an ozone depleter. We are working on an alternative nonchemical 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 a gene which is required for normal development of the insect embryo (Fryxell and Miller 1995). 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 (ABC) (Fryxell and Miller 1995).

We have been developing the technology to genetically transform codling moth. A pressurized microinjection system is used to deliver DNA into the newly laid eggs. We use a DNA vector which contains a "jumping gene," which is capable of inserting itself into the chromosome at a specific site. Once the gene is integrated into the chromosome, it is passed down to the offspring. The vector we are using, called *piggyBac*, has been developed for and has been proven in transforming moth species. As soon as it has been determined that *piggyBac* works to transform the codling moth, the lethal gene will be inserted into the vector and injected into eggs to produce a mutant, functionally sterile strain of codling moth.

Laboratory assays with *piggyBac* have proven that the gene is capable of functioning as a jumping gene in the codling moth embryonic environment. This indicates that the vector will most probably be successful in transforming the codling moth which may be proven by determining if the foreign gene is heritable. To date, a total of 1320 codling moth eggs have been

injected with *piggyBac* (Table 1). The resultant moths are mated to wild type moths, and their offspring are screened, using a sophisticated selection procedure, for transformants. From the 48 moths mated in the first set, a total of 1719 larvae was screened, but only one resulted from the selection. Further analysis is needed to confirm if the foreign gene is present in this individual. Also, future injections will involve lower DNA concentrations to decrease the chances that potentially transgenic embryos die due to lethal effects of the jumping gene.

Table 1. Codling moth egg microinjections with *piggyBac* DNA.

Date of injection	No. injected	No. hatched	No. mated	No. offspring screened	No. potentially transgenic
10/11	425	94	48	1719	1
10/31	459	47	still pupal		
11/1	436	70	still pupal		

Fryxell, K.J. and T.A Miller. 1995. Autocidal biological control: A general strategy for insect control based on genetic transformation with a highly conserved gene. *J. Econ. Entomol.* 88: 1221-1232.