Potential Use of a Conditional Lethal Transgenic Pink Bollworm *Pectinophora gossypiella* in Area-Wide Eradication or Suppression Programmes

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ABSTRACT The sterile insect technique (SIT) has been used successfully for over 30 years to keep a large cotton growing area in the Central Valley of California, USA free of the pink bollworm *Pectinophora gossypiella* (Saunders). Releases of sterile pink bollworm as part of an integrated pest management programme were recently carried out on 36 400 hectares of cotton in Texas, New Mexico and northern Mexico for the eradication of this pest. The sterile releases will soon be expanded into Arizona and California. To achieve eradication and continue to effectively operate the Central Valley containment programme, a more effective and lower cost programme is needed. Irradiation of pink bollworm with a sterilizing dose greatly reduces mating competitiveness and the development of a conditionally lethal strain of pink bollworm as an alternative or supplement to sterilization by irradiation may allow a more effective and less costly programme. A pink bollworm strain carrying a conditionally lethal gene was recently developed using the "release of insects with a dominant lethal mutation" (RIDL) technology, and larval mortality levels ranging from 60 to 92% have been obtained in laboratory tests. With further development, a strain of pink bollworm carrying a conditionally lethal gene may serve as a complete replacement for radiation-based sterility, or as a supplement to the traditional SIT by providing a genetic sexing mechanism, a safeguard against accidental release, or as a means for lowering the radiation dose to produce a more competitive insect.

KEY WORDS autocidal biological control, RIDL, Lepidoptera, effects of radiation, pink bollworm, eradication

1. Introduction

Pink bollworm *Pectinophora gossypiella* (Saunders) infestations cost US cotton producers USD 47 million per year in direct losses and control measures (National Cotton Council, March 2005, unpublished brief). The United States Department of Agriculture-Animal Plant Health Inspection Service-Plant Protection and Quarantine (USDA-APHIS-PPQ) along with several states, and grower cooperative organizations are involved in two area-wide control programmes for pink bollworm that integrate the release of sterile moths. These are (1) an area-wide containment programme in the Central Valley of California, and (2) an eradication programme in Texas, New Mexico and northern Mexico combining the release of sterile insects with the use of *Bacillus thuriengensis* (Berliner) (*Bt*) cotton, mating disruption with pheromones, and pesticides. The use of the SIT was expanded to 36 400 hectares in 2005 when sterile releases were added to the eradication programme areas in Texas and New Mexico in the USA and the Juarez Valley in northern Mexico.

The SIT containment programme has been effective in keeping the Central Valley of California free of pink bollworm for 30 years (Bloem et al. 2005). However, increased cotton production costs, worldwide competition and the increasing demands of the expanded pink bollworm eradication programme require a more effective and lower cost programme, and one way to achieve this is by increasing the competitiveness of the insects in the field. The final competitiveness of the insect in the field is affected by a combination of stresses caused by many factors, e.g. long-term mass-rearing, sterilization, handling, transport and release. The relative effects of each of these factors on the final competitiveness in the field have not been determined for many insects. However, in Lepidoptera, where high doses of radiation are needed for full sterility, an alternative strategy is the use of inherited sterility (Carpenter et al. 2005). In addition, completely replacing radiation could be done provided the alternative strategy does not itself compromise other components of the biology of the insect. In this paper some of the negative effects of radiation on released pink bollworms in SIT programmes and how development of an autocidal biological control system (Fryxell and Miller 1995) to create a conditionally lethal pink bollworm could be useful to pink bollworm genetic control programmes, are summarized. Also, preliminary data are provided on several transgenic pink bollworms strains that carry a conditional lethal gene based on a system called ”release of insects with a dominant lethal mutation” (RIDL) (Thomas et al. 2000).

2. Radiation Effects on Pink Bollworm and Lepidoptera in General

Numerous studies have shown that the very high doses of radiation needed to sterilize male Lepidoptera are associated with decreases in quality, field performance, sperm transfer and dispersal ability in many species (North 1975, LaChance 1985, Carpenter et al. 1997, Bloem et al. 1999). Decreasing the radiation dose is associated with increased mating ability and superior sperm competitiveness (Carpenter et al. 1997) and this has led to the use of lower doses in field programmes. In pink bollworm, very early data showed that high radiation doses reduced longevity, decreased sperm transfer by males, decreased sperm receptivity by irradiated females, decreased female attractiveness and decreased control efficacy (Graham et al. 1972, Flint et al. 1973, Flint et al. 1974, Flint et al. 1977, Bartlett 1978, Miller et al. 1994). Because of the negative effects of radiation along with the negative effects of mass-production, handling, transport and release mentioned above, effective programme operation requires release ratios of 60 sterile males to one wild male (based on males caught in pheromone monitoring traps) and high frequencies of release (4-7 days per week). A more robust and competitive moth could reduce the release ratio and frequency required for effective control.

3. Development of a Strain of Pink Bollworm Carrying a Conditional Lethal Gene

The use of a conditional lethal or autocidal strain of pink bollworm created with transgenic technology as a possible supplement to, or replacement of, radiation-based sterilization may offer advantages that could improve the effectiveness of the SIT for pink bollworm.

A strain carrying a conditional lethal gene could also be used in combination with a conventional radiation strategy, as a safeguard or precaution to guard against an accidental release of fertile moths. This may become more important for operation of the current rearing facility as the eradication programme, when it expands westward toward Arizona and the area around the rearing facility therefore becomes free of pink bollworm. A moth carrying a conditional lethal gene used in this way would also allow the use of a lower radiation dose, helping increase the competitiveness of the released moths. Mortality associated with conditional lethality could not be used in an inherited sterility strategy (Carpenter et al. 2005) as F_1 progeny would die before passing on increased sterility to the next generation.

Lastly, while many researchers consider mixed-sex release desirable for effective SIT against lepidopteran pests, there is theoretical and empirical evidence that, when one sex of the sterile insects is more competitive than the other, single-sex release would be advantageous, either female only (Knipling 1979, Van Steenwyk et al. 1979, Henneberry and Keaveny 1985) or male only (Knipling 1979, Marec et al. 2005). The use of a sex-limited or sex-linked conditionally lethal system would make large-scale sex separation feasible in order to test these principles (Heinrich and Scott 2000, Thomas et al. 2000, Alphey and Andreasen 2002, Marec et al. 2005).

These last two examples of transgenic conditionally lethal technology for use in a pink bollworm genetic control programme would result in a hybrid programme where both the new technology and a standard SIT approach are mixed. While work continues to develop a conditionally lethal system that could potentially serve as a complete replacement for radiation-based genetic control of pink bollworm, these other approaches would be compatible with the existing programme. They may also have advantages where resistance due to public concerns about the use of genetically modified insects are a factor, and for refinements to the standard SIT strategy such as the addition of a sexing strain of pink bollworm

4. Development of Pink Bollworm Strains Carrying RIDL Constructs

Recently, twenty independent transformed lines of pink bollworm with RIDL constructs have been produced and tested. Of these, five lines with construct LA1124 express lethal phenotypes when reared on chlortetracycline-free diets. LA1124 is a lethal construct controlled by a tetracycline repressible transactivator protein (tTA). Lethality is produced by a positive feedback cycle, in which binding of tTA to its specific target sequence tetO drives production of more tTA. In the absence of tetracycline, this leads to lethality by high expression of tTA. When tetracycline is present, tTA does not bind tetO, and so the positive feedback cycle is not established and tTA remains at a low, non-lethal level (Gong et al. 2005 provide more details on the tetO-tTA system). Tetracycline (in the form of chlortetracycline) is a normal part of the pink bollworm artificial diet, so these strains of pink bollworm could readily be incorporated into the current mass-rearing system.

In the laboratory, pink bollworms, heterozygous for the LA1124 construct, were crossed to wild types to produce an F_1 generation with a 1:1 ratio of progeny of LA1124 heterozygotes and wild type. These were reared with and without chlortetracycline and mortality was scored at larval, pupal, and adult stages. This experiment was designed to simulate the mortality of progeny that would occur from the mating of a moth, homozygous for a RIDL construct with a wild-type pink bollworm after release of moths carrying RIDL constructs in a cotton field, while also including an internal wild-type control (the wild-type siblings of the heterozygous F_1 transgenics).

To date, over 37 000 individuals have been tested and significant levels of mortality were observed for progeny heterozygous for a RIDL construct (genotype = LA1124/+)

reared on a chlortetracycline-free diet. Most of the mortality occurred in the prepupal-pupal stage with mean levels of mortality of 60-92%. Rates of mortality in the control treatments (LA1124/+ progeny reared on the chlortetracycline TC diet, and +/+ progeny reared on chlortetracycline-free diet) were low at 2-15%. A more thorough description of the construction and testing of these transgenic pink bollworm lines with the LA1124 construct will be reported elsewhere.

5. Conclusions

Conditionally lethal strains of pink bollworm expressing partial mortality have recently been developed with RIDL technology. Although further improvements and testing of the strains are needed to determine if a fully functionally pink bollworm strain carrying RIDL constructs can be developed, the technology shows promise and may eventually serve as a replacement or supplement to the current technology using radiation sterilization. Work currently underway and planned for the future includes: (1) creating new pink bollworm strains doubly homozygous for the LA1124 constructs by crossing together individual lines with independent insertions of the LA1124 construct, (2) testing LA1124 doubly homozygous lines on a small scale on cotton plants in quarantine field cages to estimate mortality rates and control efficacy under the more realistic conditions of the actual plant host under field conditions, (3) testing adult longevity of LA1124 lines (important for both mass-rearing and field efficacy estimates), and (4) testing the mortality rates of constructs with autocidal effector genes other than tetO-tTA. The strategy is to combine the lethal effects of two separate effector genes into a single pink bollworm strain to assess if mortality rates can be increased or if lethality will occur at earlier larval stages when compared with the LA1124 strain construct alone. The results of these experiments will lead to a greater understanding of the function of RIDL constructs in pink bollworm and the potential for the incorporation of conditionally lethal pink bollworm strains into the existing control programme.

6. Acknowledgements

We thank Mickey Sledge and Guolei Tang for technical assistance in this project, and Patrick Shiel, Jim Dargie and two anonymous reviewers for their valuable comments that improved this manuscript.

7. References

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