Interactions in Entomology: Utilization and Management of New Genetic Techniques for Insect Control in Southern Field Crops[1]

John All, H. Roger Boerma,[2] Wayne Parrott,[2] C. Neal Stewart, Jr.,[3] Paul Raymer,[2] Brian Rector, Suresh Ramachandran, David Walker[2] and Mike Treacy[4]

Department of Entomology, College of Agricultural and Environmental Sciences, University of Georgia, Athens, GA 30602 USA

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Recently, new genetic technologies have been introduced for management of insect pests of field crops that may be competitive with conventional control methods including the use of synthetic insecticides. The two methods currently being utilized by farmers in the United States are the use of insect-resistant transgenic crops and the application of genetically-engineered microbial insecticides. For the most part, these new genetic technologies have low negative toxicity to non-target organisms and are environmentally benign. There is probably more social and political controversy over deployment of genetically-modified organisms than other insect control strategies. Additionally, new contract policies that seed companies have introduced for protecting genetic technologies require more awareness by farmers of the inherent capabilities and limitations of transgenic crops. The purpose of this report is to evaluate the practical potential of certain of the new genetic methods in the context of the existing insect management methods that are being used in the southeastern U.S.

New crop improvement technologies for developing insect resistant cultivars. Developing insect resistant crop cultivars using conventional breeding methods is time consuming and laborious and is often frustrated by difficulty of combining resistance characters with high yield and other desirable agronomic properties for the crop. These difficulties are compounded in a program directed at multiple pest resistance such as pursued in the Center for Soybean Improvement at the University of Georgia (Boerma et al. 1988). Screening of high-yielding soybean breeding lines for resistance to insects, plant-feeding nematodes, and frogeye leaf spot, *Cercospora sojina* Hara, is conducted with greenhouse and field-testing methods designed to evaluate hundreds of plant lines each season (Boerma et al. 1993). In the insect program, a greenhouse assay using the corn earworm, *Helicoverpa zea* Boddie, is

[1]Received 13 August 1998; accepted for publication 15 September 1998.
[2]Department of Crop and Soil Sciences, University of Georgia, Athens.
[3]Department of Biology, University of North Carolina, Greensboro.
[4]American Cyanamid Corporation, Princeton, NJ.

used to screen hundreds of segregating F_2-F_4 lines for antixenosis resistance (All et al. 1989). Promising genotypes are retested in the greenhouse with the soybean looper, *Pseudoplusia includens* (Walker), and velvetbean caterpillar, *Anticarsia gemmatalis* Hübner, to verify interspecies resistance (Rowan et al. 1991). During the field season, large field cages covered with a light-emitting mesh fabric are used to test hill plantings of the hundred or more best greenhouse lines identified for season-long resistance to weekly artificial infestations of corn earworm larvae. Additionally, 40 to 50 soybean cultivars recommended in Georgia by the Cooperative Extension Service are screened and compared with the insect-resistant genotypes in the greenhouse and field cage tests (All et al. 1993).

A project has been initiated within the University of Georgia Crops Genomic Laboratory to identify the soybean gene(s) responsible for resistance to defoliating insects using marker-assisted selection. The project is utilizing restriction fragment length polymorphisms (RFLP) as molecular markers. Currently, considerable genetic mapping of soybean has been conducted using RFLPs, and we are using this information in cooperative endeavors with colleagues at Iowa State University (USDA Public Soybean Genetic Map) to identify quantitative trait loci (QTL) associated with antixenosis and antibiosis. The donor parents for insect resistance were PI 171451, PI 227687, and PI 229358 which are the same breeding lines that are used by most soybean insect resistance programs in the U.S. These three PIs were crossed with 'Cobb', a cultivar highly susceptible to defoliating insects. F_2 derived lines were assayed with corn earworm for antixenosis and antibiosis. Three QTLs associated with antixenosis have been identified, one shared by all three genotypes, one unique for PI 227687, and one shared by PI 229358 and PI 171451. One QTL associated with antibiosis was shared among the three PIs. Currently, other less expensive and time consuming molecular marker technologies, i.e., amplified fragment length polymorphisms (AFLP) and simple sequence repeats (SSR) are being investigated as genetic marker tools for soybean insect resistance research. Use of marker-assisted selection in soybean improvement programs affords tremendous opportunities for understanding genetic mechanisms involved in plant resistance to insects and has practical advantages for introgression of insect resistance genes from soybean plant introductions into elite breeding lines with minimal linkage drag (Rector et al. 1994).

Recent advances in several areas of biotechnology have made it possible to genetically engineer crops for improved insect resistance. The successful application of this technology was first tested in the field in 1987 with the introduction of delta-endotoxin genes from *Bacillus thuringiensis* (*Bt*) into tobacco and tomato (Barton et al. 1987, Fischoff et al. 1987). In 1997, approximately 1.05 million ha of *Bt* corn, 1.0 million ha of *Bt* cotton, and 10,000 ha of *Bt* potatoes were planted in the U.S. Crop seed was produced by several companies with gene technology licensing agreements with Monsanto Corporation (St. Louis, MO). Approximately 60,000 ha of *Bt* cotton were used in Australia and 4,000 ha of *Bt* corn were produced in Canada in 1997 in similar biotechnology licensing agreements between Monsanto and the respective countries. In 1997, *Bt* crop seed was produced for 1998 sale in Argentina, Australia, Brazil, China, Europe, Mexico, and Zimbabwe; whereas, *Bt* cotton adapted for India is proposed for 1999 (G. Barton, Monsanto Corp., pers. comm.).

Approximately 220,000 to 240,000 ha of *Bt* cotton were used in Georgia in 1997 and 1998, respectively, which was 37 to 45% of total cotton production. No commercial production of *Bt* corn or potatoes occurred in 1997, but a small acreage of *Bt* corn was used in Georgia in 1998. There is great concern about *Bt* corn contributing to

development of *Bt* resistance by corn earworm and tobacco budworm in cotton. Research over the past five years with various varieties of *Bt* cotton verifies the high toxicity to tobacco budworm and moderate effectiveness for control of corn earworm and other cotton pests in the families Noctuidae and Pyralidae. It was found that certain *Bt* corn cultivars containing the YieldGard® (Monsanto Corporation, St. Louis, MO) gene construct are resistant to the lesser cornstalk borer, *Elasmopalpus lignosellus* (Zeller) (J. N. All, unpubl. data). Lesser cornstalk borer is not a major pest in the midwestern corn growing areas of the U.S. and most of the interest for *Bt* corn is for control of the European corn borer, *Ostrinia nubilalis* (Hübner), and southwestern corn borer, *Diatraea grandiosella* Dyar. These borers are not serious pests of corn in Georgia.

Generation of soybean and canola plants containing the Cry1A(c) *Bt* gene construct was accomplished at the University of Georgia in 1994 and 1995, respectively, and each crop was tested in the field the year following development. The *Bt* soybean plants were derived from the high yielding cultivar 'Jack' and the canola from cultivars 'Oscar' and 'Westar' and the breeding line UGA188-20B. Laboratory, greenhouse, and field tests have shown that the UGA *Bt* soybean lines have strong resistance to corn earworm, tobacco budworm, velvetbean caterpillar, and soybean looper; whereas, the *Bt* canola is highly resistant to the diamondback moth, *Plutella xylostella* L., and the cabbage looper, *Trichoplusia ni* Hübner (Parrott et al. 1994, Stewart et al. 1994, 1996a, b).

Entomologists and others are very concerned about insect resistance development to *Bt* crops. A *Bt*-resistant laboratory colony of diamondback moth produced substantial feeding damage and successfully survived on *Bt* canola (Ramachandran 1998). One approach to resistance management in *Bt* crops is to combine (pyramid) additional insect resistance genes into plants that possess different modes of action as compared to the *Bt* toxins (McGaughey and Whalon 1992). Research is being conducted in soybean to combine *Bt* transformation and genetic marker-assisted selection technologies to produce *Bt*-engineered plants with the insect resistance genes of PI 229358 (RFLP tagged) backcrossed into them. The goal is to determine if the presence of RFLP-tagged genes can reduce the advantage of *Bt*-resistant tobacco budworm feeding on soybean transgenic with *Bt* (Boerma et al. 1995).

Use of genetically-manipulated microorganisms as improved microbial insecticides. Sprayable *Bt* products are used only to a limited extent in field crops in the southeastern U.S., primarily due to low efficacy and limited residual activity a few days after application. Attempts to genetically improve *Bt* strains to enhance insecticidal toxicity against specific target pests have been successful, but the level of improvement in efficacy has been limited in field crops. Products such as Condor®, Cutlass®, and Foil® developed by Ecogen Corporation (Langhorne, PA) utilized natural processes for transfer of plasmids with genes encoding for production of specified insecticidal crystal proteins; whereas, Mycogen Corporation (San Diego, CA) introduced the CellCap® technology utilizing *Pseudomonus flourescens* as a protective carrier of *Bt* toxins. *Bt* strains developed by recombinant DNA technology have been registered for specialized uses, for example, the bioinsecticide Lepinox® contains a recombinant variant strain of *Bt* developed from EG2348 (the active ingredient in Condor®). Lepinox® has substantially improved efficacy of beet armyworm, *Spodoptera exigua* (Hübner), and fall armyworm, *Spodoptera frugiperda* (J. E. Smith), as compared to other *Bt*-based products (All et al. 1994, 1996).

Baculoviruses (i.e., species of Baculoviridae, the most common of the eight fami-

lies of insect viruses) have unique advantages over other insecticides for management of insects in agricultural crops. Probably no other control agent can be as selectively deployed against specific pests without harming nontarget insects and other animals. One species of baculovirus is the nuclear polyhedrosis virus (NPV) of the alfalfa looper, *Autographa californica* (Speyer) (AcNPV). This pathogen is highly infective to larvae of a few serious noctuid pests and is moderately pathogenic to a small number of other lepidopteran species. AcNPV does not infect insect natural enemies or other beneficial insects (Possee et al. 1993, Treacy et al. 1997a). Entomologists have recognized the advantages of baculoviruses such as AcNPV for many years, but have not had much success in deploying the pathogens as a practical pest management tool. Erratic field performance of naturally-derived (feral) baculovirus-based insecticides in cotton has been attributed to the long disease incubation of 5 to 10 d prior to causing insect death. In field environments feral baculoviruses are ingested as polyhedral inclusion bodies (PIB) which dissolve in the alimentary canal to release the virions for cell infection. Disease development revolves around virion replication within susceptible cells. Insect feeding is often undiminished during much of the disease incubation period (Treacy et al. 1997b).

Considerable progress on genetic characterization of AcNPV has occurred and recombinant strains with enhanced capabilities for controlling pests have been developed. Of the recombinant strains developed so far, probably the most promising are those that express an insect specific toxin gene (i.e., AaHIT [or AaIT] gene) from the scorpion *Androctonus australis* Hector (AcNPV-AaIT strains). Upon infection by AcNPV-AaIT, insect cells produce a toxin that acts as a sodium channel antagonist. The main difference in the disease syndrome of AcNPV-AaIT as compared to AcNPV is that insect feeding is rapidly terminated and the death rate of infected larvae is increased to within 48 h. The accelerated killing characteristics of AcNPV-AaIT greatly improve its prospects for use by pest managers because it is expeditious enough to prevent damage by insects such as tobacco budworm and cabbage worm in cotton and cabbage looper in cabbage (Treacy and All 1996, Treacy et al. 1997a, All and Treacy 1997).

One of the first field tests conducted with AcNPV-AaIT occurred in Georgia in 1995 in cotton with additional field testing in 1996-1998. Also, tests were conducted using AcNPV-AaIT in conjunction with *Bt* cotton for control of tobacco budworm and corn earworm. It was found that AcNPV-AaIT had a positive interaction with *Bt* cotton, and corn earworm damage was reduced in an interactive manner (AcNPV-AaHIT + *Bt* toxin) on the transgenic plants. Because the mode of action of *Bt* is different from AcNPV and AcNPV-AaHIT, the use of these biological control agents could be compatible with the insect resistance management strategies being utilized by farmers growing *Bt* cotton (All and Treacy 1997).

In conclusion, genetic technologies will be utilized extensively in 21st century pest management programs for southern field crops. Genes that protect crops from insects and diseases or allow plant tolerance of selected herbicides will be a value-added attribute that will be available along with desirable agronomic characteristics when buying seed. A grower will be able to select one or many pest resistance attributes when considering an individual cultivar for purchase of seed. Combining insect resistance traits in field crops via transgenes and/or inherent genes will probably be utilized both for management of insect resistance to single dominant genes and for control of several different species of insects. Genetically enhanced biological control agents such as baculoviruses could be used to help manage insect adaptation to

plant protective genes and as a control method for pests that are not targeted by crop resistance.

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