Final Project Report to the NYS IPM Program, Agricultural IPM 2002-2003

1. Title:

Development of Bt Collard as a Trap Crop for Cabbage

2. Project Leader:

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3. Cooperators:

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4. Type of Grant:

Pest-resistant crops

5. Project location:

Wherever cabbage is grown. The general principle being tested (GM-trap crops) is relevant nationally.

6. Abstract

This work seeks to protect crops from insect pests by combining approaches from biological control and biotechnology. More specifically, it aims to protect cabbage from diamondback moths (DBM) through use of a collard trap crop expressing insect-resistance genes from *Bacillus thuringiensis* (Bt). DBM lay more eggs on collard plants than on cabbage but the larvae that hatch from these eggs survive. Bt-transgenic collard plants would attract DBM egg-laying and would also kill the hatched larvae, providing more effective insect control in a mixed field of cabbage and collard. During this grant period, we introduced two different Bt genes into collard varieties "Champion" and "McCormack's Green Glaze" and confirmed that DBM larvae die on leaves of these plants. Plants with high production of the insecticidal Bt protein were identified. Some of these plants have been self-pollinated or crossed with cytoplasmic male sterile (CMS) cabbage. The crosses with cabbage are a first step toward production of CMS Bt-collard, which would eliminate any problems of transgenic pollen flow. Seeds are being recovered from the crosses. These seeds and other Bt- collard plants available will soon be used in comparisons of DBM egg-laying and insect damage on standard collard and cabbage. Field tests of various ways to deploy the Bt-trap crop and cabbage are planned for next summer.

7. Background and justification

Cabbage is a major New York State and U.S. vegetable crop with serious insect pest problems. Insecticides are the primary method of control. Managing insect pests via trap crops is an attractive biological control concept but it is often not very effective in actual implementation (Hokkanen 1991). Published reports suggest that collard has potential as a trap crop for cabbage (Mitchell et al. 2000); however, collards do not kill the larvae of insects attracted to them. Collards could be a more effective trap crop if they killed Lepidopteran insects by virtue of expression of a suitable Bt-transgene. The Earle lab has produced many types of Bt crucifers (broccoli, cauliflower, cabbage, Chinese cabbage, rapeseed) and so was in a good position to create Bt-collard as well. The cooperators are well qualified to conduct greenhouse and field tests comparing a Bt-collard trap crop with other insect management systems for cabbage and then to take the project to an implementation stage, if appropriate.

The system proposed avoids several concerns often raised about current Bt-transgenic crops:

1) Public acceptance: the cabbage crop would not be transgenic.
2) Gene flow to other plants: male-sterile Bt-collards that produce no pollen would largely eliminate this problem. Moreover, collards are biennial and would not flower during the growing season.
3) Development of resistant insects: the cabbage crop would serve as a large refuge on which some susceptible insects could survive. Use of collard plants simultaneously expressing two different Bt genes would slow development of resistance on the collards plants, as demonstrated in recent studies in Shelton's program (Zhao et al. unpublished data).

This project provides an excellent test of the concept that there is no inherent conflict between GMO and IPM approaches, i.e., that transgenic plants can be part of an effective IPM system. If results are positive, several further outcomes are likely. One is deployment of Bt-collard by growers of cabbage (or other crucifer crops) with reduction of insecticide use. Research on transgenic trap crops suitable for other horticultural crops will also be stimulated. Such additional transgenic crops might incorporate either Bt genes or other types of insect control genes, as they become available. Furthermore, success in this project might help individuals or groups currently hostile to GMO reevaluate their positions on the basis of a more environmentally friendly and less risky application of GMO technology.

8. Objectives

The overall aim is to determine whether transgenic approaches can be effectively combined with other biological control methods via production of a useful transgenic trap crop. Specific objectives are as follows:

1) Produce collard lines with high expression of a *cry1C* and/or a *Cry1Ac* gene from *Bacillus thuringiensis*. These genes both encode proteins that kill Lepidopteran insects.
2) Compare oviposition, larval mortality, and insect damage on cabbage plants grown alone or together with non-transformed or Bt-transgenic collard plants.
3) Produce cytoplasmic male-sterile Bt-transgenic collard lines.
4) Conduct field trials in Ithaca, Geneva, and Charleston, SC to determine the ratios, arrangements, and timings of cabbage and Bt-collard plantings that give best control of Lepidopteran pests.
5) Conduct field trials comparing insect control using Bt-collard as a trap crop with other insect control methods used for cabbage.
6) Evaluate the efficacy of this approach and, if appropriate, develop plans for larger scale testing and implementation of the transgenic trap crop strategy.

Note: These objectives will clearly require more than one year of work. The full list of objectives is presented to indicate the scope of the whole project.

9. Procedures

The procedures are listed for objectives 1) and 3), which are the ones addressed in the work to date.

1) Two collard (*Brassica oleracea* var. *acephala*) lines were used: Champion (non-glossy leaves) and McCormack's Green Glaze (glossy leaves). *Agrobacterium tumefaciens*-mediated transformation of seedling explants was used to introduce a *cry1C* or a *cry1Ac* Bt gene into these lines. Putative transformants were identified via their resistance to hygromycin or kanamycin, associated with the *cry1C* or *cry1Ac* gene, respectively. Integration of the genes

was confirmed by polymerase chain reaction assays, using primers specific for each Bt gene. Bt protein production was measured by ELISA assays. Resistance to second instar diamondback moth larvae was assayed by scoring leaf damage and larval mortality on detached leaves after 5days. Standard susceptible DBM larvae and larvae resistant to Cry1A or Cry1C Bt proteins were used. The details of the procedures used are presented in Cao et al. (2002).

3) Several Champion plants with high expression of Cry1C protein were vernalized for 10 weeks at 4° C to induce flowering. Vernalized non-transgenic cytoplasmic male sterile (CMS) cabbage plants were shipped to Ithaca from South Carolina by collaborator Farnham. The Bt-collard plants were self-pollinated and also used to pollinate the CMS cabbage plants.

10. Results and discussion

PRODUCTION OF COLLARD PLANTS CARRYING BT GENES

A total of 28 hygromycin-resistant collard plants were obtained from two transformation experiments using the *cry1C* + hygromycin construct (16 Champion and 12 McCormack's Green Glaze [MGG]). A total of 10 kanamycin-resistant plants was obtained from two transformation experiments using the cry1Ac + kanamycin construct (6 Champion and 4 MGG). Transformation efficiency (number of independent transgenic plant / explants used) ranged from 0.8-3.6% in different treatments.

ANALYSIS OF PLANTS OBTAINED IN THE TRANSFORMATION EXPERIMENTS

Presence of Bt gene in the antibiotic-resistant plants

Polymerase chain reaction assays of 11 hygromycin Champion plants and 5 MGG plants showed that they contained the *cry1C* Bt gene (as expected). The kanamycin-resistant plants will be assayed for presence of the *cry1Ac* Bt gene when they are larger. Based on our previous studies, they should contain the *cry1Ac* gene.

Production of Bt proteins

Fifteen Champion plants were assayed for their levels of Cry1C protein. Five showed high level, 7 had moderate-low levels, and the other had low levels of the protein. Both of the MGG plants assayed had high levels of Cry1C protein. To date, two of the plants transformed with the *cry1Ac* gene have been tested to date, and both were positive for the Cry1Ac protein.

Insect Resistance

Fourteen Champion plants expressing the cry1C gene were used in bioassays for control of DBM larvae. The transgenic plants with high expression of Cry1C protein suffered no leaf damage from the larvae. Leaf damage on plants with moderate or low Cry1C protein levels was slightly higher, ranging from 0 to <5%. Nevertheless, all Bt- transgenic plants caused 100% mortality of susceptible DBM larvae. Non-transgenic controls caused no larval mortality and suffered 95% leaf damage from the susceptible DBM larvae. The Bt-transgenic plants also suffered little or no damage from Cry1A-resistant DBM larvae, in contrast to the non-transgenic control, which was severely damaged. As expected, the *cry1C*-transgenic plants did not control DBM larvae with high resistance to Cry1C protein.

RECOVERY OF PROGENY

Five *cry1C* Champion plants have been vernalized and grown to flowering. They are setting seeds after self-pollination. Two CMS cabbage plants were pollinated with pollen from Champion plants producing high levels of Cry1C protein. The CMS plants are also setting seeds.

CURRENT STATUS OF PLAN T MATERIALS

We have 44 *cry1C* plants growing in soil (37 Champion, 7 MGG). Some of these are clones from the original transformed plants produced to increase the population of plants for further testing. The 10 *cry1Ac* plants are still in tissue culture.

DISCUSSION AND FURTHER WORK PLANNED

Initiation of this project required production of the needed plant materials. We now have a substantial population of Bt-collard plants from two different varieties, one with non-glossy leaves and one with glossy leaves. Two different Bt genes are represented, at different levels of expression. Seed progeny will soon be ready for harvest. As a result we are well positioned to address objective 2) of the project: comparisons of DBM oviposition and insect damage on the various transgenic collard plants and on standard collard and cabbage. We expect that DBM will lay more eggs on transgenic collard than on cabbage (as is the case with non-transgenic collard), but the larvae produced will fail to survive. If this is the case, we will be able to move on to the next steps in the project, starting with a small field test examining insect damage in plots with different arrangements of the cabbage and Bt-collards.

11. References

Cao J, Zhao JZ, Tang JD, Shelton AM, Earle ED. 2002. Broccoli plants with pyramided *cry1C* and *cry1Ac* Bt genes control diamondback moths resistant to Cry1A and Cry1C proteins. Theor. Appl. Genet. 105:258-264

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Mitchell ER, Hu G, Johanowicz D. 2000. Management of diamondback moth (Lepidoptera: Plutellidae) in cabbage using collard as a trap crop. HortScience 35:875-879