Special Research Report #213: Insect Management

Development of a Novel Control Strategy for Thrips and Tospoviruses

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BACKGROUND

Thrips and *Tospoviruses (no ital)* present significant problems to the ornamental industry. The insects are particularly difficult to control because of their enormous fecundity and proclivity to develop resistance to insecticides. To make matters worse, they are the vectors of viruses that cause major damage to many important ornamental crops. Our main research objective is to develop practical, bio-rational systems to control these problems. When thrips feed on infected plants, viruses are ingested and they cross the gut wall by means of a receptor mediated event. After this initial prerequisite, they infect the insect which then becomes a vector for life. This is a very specific process. We have identified the molecules on the surface of *Tospoviruses (no ital)* that specifically target them to the thrips gut tissues. Also, we have produced small soluble versions of them *in vitro* which we can purify and use in experiments. We call these GnS because they represent a soluble truncated form of the wild type viral molecule. We are attempting to co-opt this attachment specificity by exchanging the molecular domain on the *Bacillus thuringiensis* (Bt) toxin (which in a similar manner targets this insecticidal toxin to other insects such a Lepidoptera or coleopteran) for the viral domain (GnS) that targets the virus to the thrips gut. In this way, we hope to derive a method to specifically kill thrips to mitigate feeding damage and prevent the spread of *Tospoviruses*, particularly in greenhouse settings. Once we have developed such a fusion and tested its efficacy in the laboratory, we will work with extension entomologists towards developing ways to deploy it in an applied setting. Some ideas for this include using it in a bait situation such as pollen (which thrips feed on voraciously) laced with this material, transgenic plants expressing the fusion protein (following on many examples of natural Bt being used in transgenic plants effectively), and using transgenic sentinel plants to provide strategic exposure to the material. We believe that these approaches would be particularly useful in the ornamental industry.

MATERIALS AND METHODS

This research requires supplies for raising thrips colonies, standard cloning procedures to produce fusion proteins comprised of Bt toxins and the viral ligand, and for purification and expression of these fusion proteins so they can be test fed to target and non-target insects.

RESULTS

We have succeeded in producing several fusions of the viral targeting protein (GnS) with the specific Bt toxin Cry3A which is normally used to control Colorado potato beetle. These include fusions to the N-terminus of the whole molecule (full length fusion) and construct in which we have removed the wild type binding domain of the toxin

and replaced it with the viral targeting sequence. We have expressed these proteins *in vitro* using a wheat germ system, in plants using standard Agrobacterium transient expression systems and have created viral expression vectors to test these fusions in systemic infection format. Significant studies have been done to verify that the fusions have been correctly expressed. We have tested some of them against the normal target of Cry3A and found that they are still active. This is important because it demonstrates that the fusions are still biologically active in their original setting. We have done limited feeding experiments with Thrips and have not observed any effects. However, these are very preliminary observations.

CONCLUSIONS

We have demonstrated the general feasibility of the approach. We feel that the retention of toxicity to the original target organism suggests that fusions proteins could be used to re-direct the toxin to thrips. We are encouraged by the expression of the fusion proteins in plants and in vitro because this will provides a mechanism for wide scale production of many different versions of these molecules for efficacy testing.

IMPACT TO THE INDUSTRY

While this research remains in the preliminary stage of development, we believe that, should our approach will be successful and that it would have enormous importance to the industry. The potential is that we will develop a specific, safe, and effective means to control thrips in both greenhouse and field settings to defray the major losses to the industry created by both thrips feeding and transmission of *Tospovirus* (no ital) diseases.

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