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Monitoring and Managing Colorado Potato Beetle Resistance

2010 Proposal to the Michigan Potato Industry Commission

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Background.

The registration of neonecotinoid insecticides in 1995 saved millions of dollars for the potato industry in control costs against Colorado potato beetle (CPB). Currently, imidacloprid or thiamethoxam are used on 72% of Michigan's potato acreage annually as in furrow or seed treatment and it would be very costly and difficult for Michigan growers to manage CPB without these insecticides. Colorado potato beetles resistant to imidacloprid were first found in Michigan in 2004 and by 2007, resistance had appeared in more than a dozen fields near the original location plus a number of sites 10 miles or more away. Low levels of resistance to thiamethoxam are present at many of the same locations. The high selection pressure provided by the repeated application of neonecotinoids is the cause for the gradual loss of the effectiveness of this insecticide class for CPB control. Without alternative solutions, the current situation could have serious impacts on the Michigan potato industry. CPB has developed resistance to all known insecticides, and so we must focus our efforts on learning the details of this broad resistance capability. Gaining an understanding of the molecular resistance mechanisms has enormous potential to shed light on how resistance evolves. This information is fundamental for designing informed management tactics that can break the vicious cycle of developing resistance to new control measures. The proposed research is designed to continue monitoring resistance and also to develop short and long term solutions to economically control CPB that are resistant to imidacloprid and thiamethoxam.

Specific objectives.

1. Continue to monitor Michigan populations of CPB for resistance to imidacloprid and thiamethoxam.
2. Evaluate alternative insecticides in appropriate field and laboratory tests for control of CPB.
3. Develop an understanding of the molecular genetic processes of resistance evolution in CPB.

Methods.

Objective 1.

Beetle collections: Colorado potato beetles will be collected in the spring from volunteer potatoes and trap crops following 2009 potato crops and in the summer from 2010 potato fields, both in locations that reported control problems and in areas where resistance has not yet been reported. Crop consultants, agribusiness representatives, and growers will be notified of the program and encouraged to contact us if they have concerns about resistance. We will continue to collect beetles from fields with known resistance problems in order to monitor any changes in resistance status that might be occurring over time. We will also collect beetles from areas that have not reported resistance in order to proactively track the first appearance of resistance.

Bioassay: Each beetle population will be screened to determine the relative susceptibility to imidacloprid and thiamethoxam. Adult beetles will be treated with 1 µl of an acetone/imidacloprid or acetone/thiamethoxam solution of known concentration applied to the underside of the abdomen. We will use 15 adults per concentration, five concentrations and two replications (150 beetles per insecticide). Treated beetles will be placed in petri dishes lined with

filter paper and fed fresh potato foliage and kept at 24°C (±1). Beetle mortality will be assessed 7 days after treatment. Doses lethal to 50% of the beetles (LD_{50}s) for imidacloprid and thiamethoxam will be determined by log dose/probit mortality analysis. LD_{50}s for field populations will be compared to LD_{50}s for susceptible beetles to determine if resistance to either chemical is increasing in the field. Resistant populations will be mapped to see if resistance appears to be spreading or occurring in new locations.

Objective 2. Insecticides that are alternatives to neonicotinoids are Radiant, AgriMek, Coragen, Belay, Alverde, Endigo, Voliam Xpress, and Rimon. Regent recently received a label for wireworm control in potatoes and we expect that it will also be effective against Colorado potato beetle. These products will be tested in standard field insecticide trials at the Montcalm Research Farm, in comparison to imidacloprid and thiamethoxam as at-planting applications. Other experimental products will also be tested, as they are made available by the agrichemical industry. We are scheduling meetings for early spring 2010 with industry representatives to find out about new products that will be included in our tests. Some active ingredients will be tested in the laboratory against beetles that have been selected for very high levels of resistance to imidacloprid and thiamethoxam to ensure that these products can be used in resistance management programs.

Objective 3. Genes induced by allelochemicals or xenobiotics, such as insecticides, encode enzymes involved in the metabolism of harmful compounds. Identification of these genes could be used to detect the enzymes with the capacity to metabolize the insecticides. The information on detoxification enzymes and target site gene expression is of importance not only to understand the ability of an insect to develop resistance, but also to monitor the development of resistance. The cloning of genes enables us to answer questions such as "do one or multiple genes control resistant phenotypes?" and "how do resistant mutations arise in a field population?". Microarray technology is a modern and powerful method that allows thousands of genes to be tested simultaneously so that a large-scale exploration of the fundamental genetic patterns of resistance can be carried out simultaneously.

We currently have five populations of CPB in culture originating from different geographic locations around the USA, representing susceptible and resistant populations (thiamethoxam, imidacloprid). We will use individuals from these five populations to construct a microarray library. RNA extraction from CPB will be done at MSU. RNA samples will be sent to Evrogen to develop and construct normalized full-length-enriched cDNA library. Sequencing of cDNA library will be done at the Research Technology Support Facility (RTSF) at MSU on a 454 GSFLX Titanium Sequencer. Once microarrays are available, we will use them to characterize and compare transcript abundances as resistance evolves in populations in the laboratory and in natural field populations to insecticides as well as to other sources of stresses, such as Bt toxins and allelochemicals. RNA from susceptible insects will serve as a reference. Populations with the following characteristics will be compared: (1) resistance can be created artificially in laboratory populations, by applying specific insecticides to select groups -this allows the observation of resistance development over time and to specific compounds; (2) field populations from MI, WI, NY, NJ, VA, and WA will be collected to compare geographic differences; and, (3) laboratory colonies reared on Bt-potatoes and different plant species will be compared.

Proposed Budget

Personnel	
Technical	$8,000
Fringes (45,02%)	3,602
Student Labor	4,500
Supplies	7,400
Travel	1,498
Total	$25,000

A proposal for 2010 CPB research funding will be submitted to Project GREEEN and the Rackham Foundation. A proposal has been submitted to the USDA National Potato Research Grants Program for funding to study resistance and cross-resistance to imidacloprid and thiamethoxam, to continue resistance monitoring on a national basis.

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