

MPIC-11-10

Proposal Title: Physiology of resistance to imidacloprid in Michigan overwintered resistant beetles

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Synopsis of Proposal:

The physiology of overwintered Michigan Colorado potato beetles (CPB) resistance to imidacloprid will be studied. Oral LD₅₀ will be determined. Excretion, metabolism, and inhibition of metabolism to imidacloprid will also be evaluated in CPB adults.

Potential Impact on Michigan Plant Agriculture/Industries:

Development of resistance of CPB to neonicotinoids is occurring in Michigan. The experience of the cost in resistance for the in the past resistance cost Michigan potato industry about \$1.4 million/year. There are few alternatives to control CPB neonicotinoid-resistant populations. Resistance management is therefore a critical aspect of CPB control. This project will provide critical insights into the physiology of resistance of Michigan overwintered CPB and will improve how resistance is managed. Lower resistance in overwintered beetles is desirable because potato fields treated with imidacloprid still provide some control of overwintered beetles.

Will this project involve human subjects, genetic material, or animals requiring university approval? (Note: if project is approved, funds will not be released until university approval is received)

Yes _____ No X

Signatures:

David Mota-Sanchez

Ernest S. Delfosse

Principal Investigator _____
Date 12/09/09

Lead Unit Administrator _____
Date 12/9/09

Physiology of resistance to imidacloprid in Michigan overwintered resistant beetles

David Mota-Sanchez, Zsafia Szendrei, and Mark Whalon. MSU Entomology

1. PROBLEM STATEMENT

The Colorado potato beetle (CPB), *Leptinotarsa decemlineata* (Say), is the principal insect pest of potatoes in North America and Europe (Weber and Ferro 1994). If this pest is not controlled it can cause the total defoliation of the crop and severe economic losses. Many methods have been used to control CPB, and insecticides are the most effective method. However, CPB has developed resistance to 52 compounds and is ranked the fourth most resistant species to pesticides in the world (Whalon et al. 2007). The cost of CPB insecticide resistance to the Michigan potato industry has been estimated at \$1.4 million per year (Grafius 1997). Imidacloprid, a neonicotinoid compound that targets the nicotinic acetylcholine receptors (nAChRs) in the insect central nervous system (Bai et al. 1991), was registered for use on potatoes in 1995 and soon became the primary means to control CPB resistant to organophosphates, pyrethroids, and carbamates in Michigan and other areas in the US (Grafius 1997).

Despite excellent initial efficacy of imidacloprid in 1997, a 100-fold level of resistance to imidacloprid was detected in a CPB population collected from an imidacloprid-treated commercial potato field in Long Island, NY (Zhao et al. 2000). In 1998 resistance of CPB to imidacloprid was widespread in Long Island (Mota-Sanchez et al. 2000), and more recently resistance has been detected in several other potato-growing regions in the northeast USA (Dively 2006, Alyokhin et al. 2007). Resistance to imidacloprid in a population from Long Island, NY also resulted in cross-resistance to several neonicotinoids never used on the field including thiamethoxam, dinotefuran, chlothianidin, thiacloprid, and acetamiprid (Mota-Sanchez et al. 2006). Resistance to imidacloprid and cross-resistance to thiamethoxam and chlothianidin has been detected in other areas of the eastern USA and Canada (Alyokhin et al. 2007, Alyokhin et al. 2008). In Michigan, resistance of CPB to imidacloprid and low levels of resistance to thiamethoxam were detected in 2004 and more resistance cases are appearing close to the original site of detection (Grafius 2006). Insecticide resistance management efforts including crop rotation and trap crop have been implemented (Grafius 2006). Despite some resistance cases, many CPB populations are still susceptible to imidacloprid and other neonicotinoids, including thiamethoxam. Taking a proactive approach at this point in time will help to preserve the efficiency of this class of chemicals.

Resistance to imidacloprid in other insects including white flies, *Drosophila* and German roaches suggest that microsomal oxidases are responsible for resistance (Rauch and Nauen 2003, Nauen and Denholm 2005). Also a mutation in the nicotinic acetylcholine receptor was responsible for resistance in the Brown plant hopper (BPH) (Liu et al. 2006). However, the BPH resistance case occurred in laboratory selected populations and may not be representative of field resistance. In a CPB population from Long Island, NY, cloning of the alpha subunits where imidacloprid may bind the nicotinic acetylcholine receptors have been conducted in the past two years by Dr. Ke Dong at

MSU. No mutation has been identified in the target site responsible for resistance (Ke Dong, personal communication). In addition, no differences were found in binding receptors to ³H-imidacloprid in resistant and susceptible beetles (Nauen and Denholm 2005). However, nerve recording suggested that insensitivity at the target site may be another mechanism of resistance from populations from Long Island, NY (Tan et al. 2005). The only resistance mechanism that has been identified in these populations and Michigan populations, to date, has been metabolism of imidacloprid as indicated by synergized resistance observed in resistant beetles treated with the mooxigenasee inhibitor, piperonyl butoxide (Zhao et al. 2000, Mota-Sanchez et al. 2006, Mota-Sanchez et al. unpublished). In combination with metabolism, fast excretion removes imidacloprid from the insect body in populations from Michigan, Maine, and Long Island, NY (Mota-Sanchez 2003, Mota-Sanchez et al. unpublished).

Resistance to imidacloprid is lower in overwintered beetles (also called spring-emergent adults) in comparison with the resistance of late summer adults in Massachussets (Baker and Porter 2008), and Michigan (Mark Otto, personal communication). Lower levels of resistance may be due to age, fitness cost of the resistance, or diapause. We have performed research in summer generation beetles to determine the mechanism of resistance, and enhanced metabolism is strong evidence of resistance of CPB to imidacloprid (Fig 1). However, there is lack of data on the physiology of resistance in overwintered CPB because research has focused on beetles with the highest levels of resistance. Lower resistance in overwintered beetles can be a desirable condition because fields treated with imidacloprid still can provide some control of overwintered beetles. Therefore, the objective of this proposal is to study the physiology of resistance in Michigan resistant overwintered CPB in comparison with summer beetles.

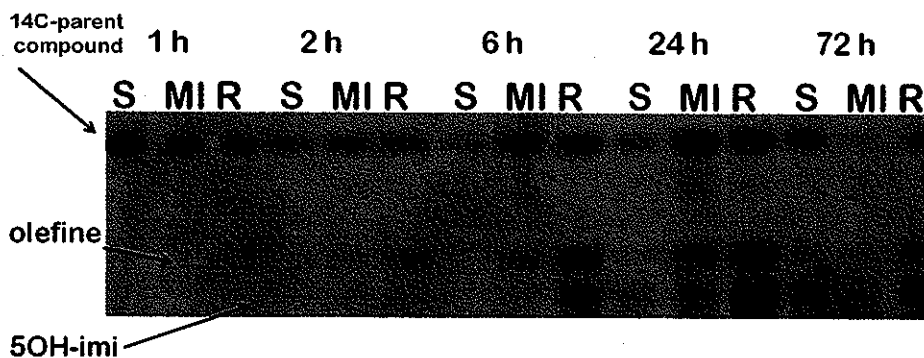


Fig. 1. Autoradiogram of external fractions after ¹⁴C-imidacloprid application

III.

SPECIFIC OBJECTIVES AND HYPOTHESES

Specific objectives:

The mechanism of resistance has been investigated by topical application of imidacloprid, but the main route of uptake of imidacloprid in adult and larvae of CPB is by ingestion of potato leaves. Imidacloprid (Admire®) is translocated by the xylem to the leaves after in furrow application at planting. Fast excretion of ¹⁴C-imidacloprid from the insect body has been observed when imidacloprid was applied orally (Mota-Sanchez et al. unpublished). Therefore, *in vivo* study by oral application of imidacloprid will provide more accurate insights on the resistance levels, excretion, metabolism and mechanism of resistance of CPB in measuring the physiology of the resistance in overwintered and summer Michigan beetles.

- 1) Determine the oral LD₅₀ of overwintered and summer Michigan beetles to imidacloprid and calculate the resistance levels among these generations.
- 2) Determine the physiology of resistance in overwintered and summer generation beetles to imidacloprid via rates of excretion, and metabolism of labeled imidacloprid in Michigan CPB treated with this compound.
- 3) Evaluate the rate of inhibition of excretion and metabolism when beetles are treated with piperonyl butoxide, an inhibitor of monooxygenases (a mechanism of resistance of beetles to imidacloprid), before insecticide treatment.

Hypothesis:

Diapause will cause a reduction in levels of resistance, excretion and metabolism of imidacloprid in overwintered Michigan resistant beetles in comparison with summer beetles. Understanding the physiology of resistant to imidacloprid in overwintered and summer generations will also provide insights about resistance to imidacloprid and other neonicotinoids.

IV. SPECIFIC METHODS AND PROCEDURES

Oral Bioassays.

Compounds. Imidacloprid technical grade will be used for the bioassays (imidacloprid 98.7%, Bayer Corporation, Kansas City, MO). Piperonyl butoxide (90%) will be obtained from Aldrich Chemical Co., Milwaukee, WI.

Insects. Overwintered resistant Michigan beetles will be collected at the woodlot at the Sackett farm Lot-22 as soon as they start emerging. Summer beetles will be collected also in areas adjacent to Lot-22 of the Sackett farm. They will be brought to the laboratory for bioassays. Also overwinter and summer beetles from Long Island, NY and a laboratory susceptible population (New Jersey) will be used to compare results.

Bioassays. Oral bioassays will be used to assess adult resistance. Imidacloprid will be dissolved in DMSO, at least five concentrations that result in more than 0% and less than 100% mortality based on preliminary assays will be used. A 2-ul pipette will be used to administer 0.4 ul of insecticide solution in the middle on the mandibles. The

control beetles will be treated with 1 μ l of water. Three to five replications per concentration will be performed, each replication will consist of 10 beetles. After treatment, beetles will be placed in Petri dishes containing potato leaves and kept at 28 °C, 50 % relative humidity, and photoperiod 16:8 (L:D). Mortality will be assessed 1, 3, 5, 7 and 10 d after treatment. Beetles unable to right themselves or walk a distance equal to their own body length when disturbed will be counted as dead. To determine the suppression of resistance an hour before insecticide treatment 10 μ g of PBO will be applied in a similar way via oral application and then imidacloprid will be applied.

Physiology of the resistance in overwintered beetles.

The rate of excretion and metabolism of imidacloprid in Michigan resistant and susceptible populations will be identified and compared with populations from Long Island, NY and a susceptible strain. This will include measuring imidacloprid-induced mortality with and without metabolic synergists such as piperonyl butoxide, an inhibitor of oxidases, PBO. In addition, 14 C-imidacloprid will be applied via oral application. Fractions of excreta, and internal body will be extracted with solvents methanol and acetonitrile. Samples will be dried with nitrogen and condensed samples will be spotted and thin layer chromatography will be used to determine the amount of imidacloprid metabolism. Standards of imidacloprid metabolites will be used to identify the metabolites.

Insects. Overwintered and summer beetles of CPB resistant to imidacloprid from Michigan (Sackett farm Lot22) will be used in this study. Those populations will be compared with a highly resistant field population from Long Island, NY and a susceptible population reared in the laboratory (New Jersey).

Dosing. 14 C-imidacloprid will be applied via oral application: a low dose (2200 dpm/beetle) in all strains. After feeding, beetles will be transferred to a 20 ml glass scintillation vial. Up to four beetles will be held in a vial. Two time intervals will be used (6 and 24 h). Three replications will be performed per exposure time. At the end of each time interval, beetles will be killed and then they will be dried at 70 °C for 48h. The dried beetles will be oxidized in a biological oxidizer and the resulting 14 CO₂ will be trapped in a scintillation vial with 15 ml of cocktail fluid. Radioactivity of each exposure time will be measured in a liquid scintillation counter (LSC).

Excretion. To calculate the amount of excreted 14 C from imidacloprid, holding vials for each time period will be rinsed with 3 ml of methanol. An aliquot of 400 μ l of acetone will be put in a scintillation vial with 15 ml of cocktail fluid and will be counted. To get the total amount of 14 C excreted, the results of all fractions will be combined.

Metabolism. Metabolism will be determined in excreta and internal body at 6, and 24 h. Beetles will be homogenized in a tube with 10 ml of acetonitrile using a high speed mechanical homogenizer (VirTishear). Following homogenization, tubes will be centrifuged for 5 min at 7000 rpm. The supernatant will be decanted into a scintillation vial. An aliquot will be taken from each extract to count the radioactivity. Aliquots from the excreted and internal samples will be put in scintillation vials and dried with a gentle stream of nitrogen in a bath of water at 40° C to a volume of 100 μ l. Samples will be spotted on TLC plates (250 mm thick silica gel plates Whatman LK5F, Clifton, NJ). The

parent imidacloprid and standard metabolites will run on one strip as a marker. To confirm the identity of the parent compounds and metabolites the plates will be developed by using a mobile phase of single dimension system or double dimension systems. After drying, the plates will be covered by a thin mylar film (0.001 mm) and put in a phosphor screen. A day after exposure, the screen will be scanned in a phosphorimager analyzer (BioRad). In addition, the TLC plates will be scraped in bands according to the pattern of metabolites and the silica gel will be transferred to a scintillation vials for reading in the LSC. The non-labeled metabolites on the plates will be identified after developing using a UV chamber. The area of the metabolite will be marked with a pencil, and then the plates will be put with phosphor screens and scanned. The image will be printed as a transparency, and matched with the original plate to determine the co-location of the ¹⁴C-unknown metabolite and the non-labeled metabolites.

V. IMPACTS OF PROPOSAL:

Field efficacy and development of resistance of CPB to neonicotinoids is occurring in Michigan. The cost of resistance for the Michigan potato industry in the past was about \$1.4 million/year. There are few alternatives to control CPB neonicotinoid-resistant populations. However, these alternatives should be used judiciously. This project will provide critical insights of the physiology of the resistance in Michigan overwintered potato beetles and the way to manage resistance for Michigan potato growers.

Successful potato production in large part depends on grower ability to control CPB. Imidacloprid and serve as a foundation of beetle control on commercial farms. These insecticides combine relatively low mammalian toxicity, high efficiency against target pests, and affordable price. Loss of their availability for controlling the CPB is likely to be a serious problem even when alternative chemistries still suppress resistant populations. We will also expect that this research will generate important data for an AFRI proposal about the gene expression and identification of CPB to detoxify neonicotinoids in the near future.

IX. LITERATURE CITED

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Project Budget Form

Project Title:
Physiology of resistance to imidacloprid in Michigan overwinter resistant beetles
Project Principal Investigator: David Mota-Sanchez

Budget Item	FY-10	FY-11	FY-12	Non-Project funds*
A. Personnel Wages				
A1. Research associates & post-docs				
A2. Other professionals				
A3. Secretarial & clerical				
A4. Technical, shop & other				
B. Fringe Benefits (Must be charged as direct costs.) See below**				
A5. Undergraduate students ***	10,000			
A6. Graduate students – including associated fringes**				
C. Total Personnel Costs (A+B=C)	10,000			
D. Nonexpendable equipment (Attach explanation)				
E. Materials & Supplies	2,000			40,000 ^a
F. Travel				
G. Publication				
H. Other Direct Costs (Attach explanation, list of items and individual costs.)				
TOTAL	12,000			40,000

^a Including cost of labeled materials 14C-imidacloprid (a generous gift of the company), metabolites of imidacloprid, TLC plates, solvents, maintenance of LSC counter and oxidizer equipment, and liquid scintillations cocktails. The industry and recently an AFR1 proposal make possible to request a low budget to the MPIC.

Justification:

A1. No salary is requested for any of the PIs

A5. Total (\$10,000). Salary of an undergraduate student for about 5 months to perform many activities related to the research (rearing and feeding insects, assisting with the labor-intensive experiments). \$10,000 is budgeted (787 hours @ \$12 per hour). Fringe is budgeted at 7.65% of hours worked during the summer semester (\$ 542).

G.

E. Scintillation vials, fertilizer for potatoes, pots and rearing insect materials.