Imidacloprid, potatoes, and honey bees in Atlantic Canada: is there a connection?

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Abstract

In 2000, some beekeepers in Prince Edward Island (PEI), Canada, experienced substantial, unexplainable honey bee colony losses. Prince Edward Island is an Atlantic Canadian province of approximately 5,684 km² with about 261,482 hectares (46%) of its land base involved in agriculture, and about 135,600 hectares of this agricultural land is in rotation with potato crops (extrapolated from PEI Agricultural Statistics for 1999). About 90% of the potato crop is treated with imidacloprid in-furrow. Some beekeepers in France felt that imidacloprid use on sunflower crops was negatively affecting honey bee health in that country. Beekeepers who experienced the high colony mortality on Prince Edward Island wanted this possible connection investigated as well. Their concerns were heard, and as a consequence, a residue study was done in 2001. The results did not show detectable residues of imidacloprid or two of its metabolites in bee forage plants or hive products at the limit of quantification of 2 ppb. Unexplained and substantial honey bee colony losses continue to plaque and mystify some beekeepers around the world. With increasing demand for honey bees for pollination of fruit crops in Atlantic Canada, this problem is a very real concern. Therefore, a comprehensive, mulitractor investigation was initiated in the spring of 2002. Project planning input was sought from beekeepers, and partnerships with beekeeper associations, producer organizations, individuals, corporations, institutions and governments were developed. The final version of the project includes seven major components which encompass many of the factors that beekeepers agree can negatively impact honey bee health. Also, these factors are in agreement with those listed in a Canadian Association of Professional Apiculturists (CAPA)/Canadian Honey Council (CHC) joint committee recommendation regarding the need for broad, factor based studies. The results of the 2001 investigation, and the methodologies of the 2002/2003 multi-factor study are presented.

Key words: *Apis mellifera*, honey bee, imidacloprid, bee health, Prince Edward Island, New Brunswick, Nova Scotia, Atlantic Canada, multi-factor study, residue study, potatoes, wildflowers, soil, canola, pesticides, bee diseases.

Introduction

Admire® is a Bayer CropScience plant protection product that contains the active ingredient imidacloprid. Imidacloprid is a synthetic systemic chloronicotinyl insecticide, which is registered in Canada for the management of Colorado potato beetles, aphids, flea beetles, and leafhoppers on potato crops, as well as other crops such as apples. It has an agonist mode of action at nicotinic acetylcholine receptors, and it demonstrates selective toxicity for insects over vertebrates. Since the initial registration of an imidacloprid product in France in the early 1990's, the Pest Management Regulatory Agency (PMRA) in Canada has received many applications requesting the registration of imidacloprid. In April 1995. Admire® 240F was granted temporary registration under section 17 of the Pest Control Products Act for the management of Colorado potato beetles on potatoes in Eastern Canada. In April 1999, it was approved for use on potatoes across Canada. Imidacloprid products are presently registered for use in 100 countries for use on over 65 crops, as well as for veterinarian applications.

Admire® has high molecular mobility in the xylem of treated plants as a result of its water solubility which is 510 mg/L (Elbert *et al.*, 1998). The molecular ability of imidacloprid, and its toxicity to sucking insects, makes it an ideal candidate for use on potatoes and numerous other crops (e.g. apples, lettuce, tomatoes, mustard, canola, cucumber, corn). This chloronicotinoid has

long-term persistence in soil and therefore, it has been used extensively as an in-furrow treatment for Colorado potato beetle on potato crops. The recommended infurrow rate of application is 850 ml to $1.3\ L$ / ha. Imidacloprid's residual activity has contributed to making it the most popular active ingredient for management of Colorado potato beetle.

Despite worldwide recognition, the use of Admire® has been in question following reports by French beekeepers of "disoriented" honey bees that had been foraging in Gaucho® (a.i. imidacloprid) treated sunflower fields. The beekeepers in France also reported that the honey bees had high rates of mortality, and low honey production because of reduced colony strength. In Canada, the PMRA's initial review of imidacloprid concluded that although pollinators (honey bees) could be at risk because of its high toxicity to bees directly exposed, the risk could be mitigated by a label statement contraindicating application of the product to blooming crops when bees are visiting the treatment area. Since that time, the question of whether systemic residues of imidacloprid may occur in nectar and pollen of flowering crops at concentrations harmful to honey bees has been the focus of many studies. For example, Schmidt and Schmuck (2000) examined the effects of sunflowers, grown from Gaucho® treated seeds, on honey bees and found no observable evidence of adverse effects. In an investigation on the foraging behaviour and orientation ability of honey bees by Kirchner (1999), changes concentrations from 20-100 ppb. Although the effects on the behaviour of bees were observed to start at imidacloprid concentrations of 20 ppb, no damage to the test populations were observed for the range of concentrations tested up to 100 ppb.

With the release of the information from France, some beekeepers in Prince Edward Island, and New Brunswick, expressed similar concerns in relation to placement of colonies near clover fields that had been previously treated with Admire®. They requested a moratorium on the use of Admire® in Prince Edward Island. In response, a study was initiated in the spring of 2001 to determine if imidacloprid residues could be found in potato field soil, clover leaves and flowers, bee collected pollen and nectar, uncapped honey, and wildflowers one and two years following application of Admire®. In 2002, a follow-up study aims to investigate a comprehensive range of factors that could be part of the overall bee health issue. The following paper is a report on the results of the 2001 study, and a summary of the factors and methods of the 2002/2003 investigation.

Materials and Methods

2001

Study sites

The collections were conducted at eighteen sites between Charlottetown and Summerside in Prince Edward Island, and at five sites between Woodstock and Florenceville in New Brunswick. Duplicate samples were collected for reserves.

The three field classifications used in this study were based on year of crop rotation, 1) potato (Year 1 field), 2) underseeded grain (Year 2 field), 3) clover (Year 3 field). Runoff areas of some Year 1 and Year 2 fields were subcategories for soil and wildflower sampling.

The fields used in this study had been planted in potatoes and treated with an in-furrow application of Admire® at the rate of 850 ml per hectare at the time of planting, except for the following fields: 1) field numbers 15 and 37 (Control fields, no treatment), 2) field 03 (about half of the field treated at the rate of 850 ml/hectare, and the other half was treated at the rate of 1300 ml/hectare), 3) field 110 (foliar application). Underseeded grain fields were planted in either oats or barley and underseeded with a mixture of red clover (*Trifolium pratense*), alsike clover (*Trifolium hybridum*), and timothy (latin name needed here) in the second year of the crop rotation. Clover was the dominant follow-up plant in the third year of rotation. First flowering clover fields were fields that were sampled

prior to the first cut, and second flowering clover fields were fields that were sampled during the second bloom of clover following the first cut.

Soil

A composite sample of 160 soil cores (18 cm length x 13mm diameter) per field were collected from eleven fields. A 2 hectare (5 acre) plot was measured and staked out on each field, and divided into twenty collection points. Eight pairs of soil cores were collected at each point. Each core pair was spaced one foot apart along crossed yard sticks to ensure that a treated furrow would be sampled.

A composite sample of twenty soil cores (18 cm length x 13 mm diameter) per field were collected from seven runoff areas. At runoff locations, the lowest field edge was divided into 20 sampling points along a transect parallel to the field edge and about adjacent to a border of wildflowers. At each sample point, one pair of soil cores were collected near the base of a wildflower plant.

Plants

Clover leaves and flowers

Clover leaves and flowers were collected based on the same grid system used for the soil collection (i.e. 2 hectare plots with a 4 X 5 grid creating 20 collection points). The leaves collected were the upper-most, fully expanded leaves, and the flowers chosen (i.e. inflorescences) were fresh, and fully opened.

A composite sample of 80+ clover flowers were collected from each of eight fields in Prince Edward Island and each of five fields in New Brunswick. A minimum of four flowers were collected per sample point. Depending upon the type of plant present (red versus white clover, or a mix), for every one red clover flower collected, two white clover flowers were collected to compensate for the reduced size and weight of the white clover flowers. The flowers were carefully removed from each plant using sterile dissection scissors and latex gloved hands. Each freshly cut flower was immediately placed in a 2 kg plastic bag and, after completing the flower collection in a field, the bag was sealed with a twist tie, double bagged, sealed again, and then stored in a cooler containing dry ice.

A composite sample of 400 clover leaves (20 leaves/point) were collected from each of eight fields in Prince Edward Island. In New Brunswick, 160 clover leaves (8 leaves/point) were collected from each of five fields. The leaves were carefully removed from each plant using sterile dissection scissors and latex gloved hands. Each freshly cut leaf was immediately placed in a 2 kg plastic bag and sealed and stored the same way as the flowers were treated.

Table 1. Field year designation as determined by year of in-furrow application of Admire.

Rotation year	Year of in-furrow admire application	Crop planted
Year 1 field	Spring 2001	Potato field
Year 2 field	Spring 2000	Grain field
Year 3 field	Spring 1999	Clover field
Year 1 runoff	During season 2001	Potato field edge

Wildflowers

A composite sample of forty grams per species of goldenrod (*Solidago canadensis*) inflorescences, fireweed (*Epilobium angustifolium*) flowers, and aster (*Aster novi-belgii*) flowers were collected when present from each of seven runoff fields. The twenty collection points used to collect runoff soil were also used as collection points for the wildflowers. Wildflowers were collected and treated as per clover flowers and leaves.

Bees and hive products

Bees

Hives of honey bee colonies were placed on five selected sites to supply the foraging bees from which the pollen and nectar would be collected. The hives and colonies of honey bees were supplied by the Prince Edward Island division of Jasper Wyman & Son. They also supplied additional supers when needed for colony management. The bees were New Zealand stock imported in the spring of 2001 and the equipment was previously used (i.e. imported from western Canada). Eight hives of honey bees, on two pallets of four, were moved to the edge of each of four second flowering clover fields, and one second flowering control field, on July 18 (n=40 hives). The hives were positioned in such a way as to optimize foraging activity on the study fields.

On July 25-27, all colonies at each site were equalized (i.e. "adjusted for strength – similar quantities of food stores (pollen and nectar), brood in all stages of development and adults covering at least 10 frames"), qualitatively assessed for general colony health, and managed for swarm prevention. Colonies were again assessed on September 14-15. Apistan strips and sticky boards were installed at the time of the second assessment to survey for varroa mite. The strips and boards were removed and inspected for varroa on September 17. Jasper Wyman & Son removed the colonies from the study fields on September 18.

Using a portable bug vac and a serpentine collecting pattern, pollen and nectar collecting honey bees were collected from each second flowering treatment field, and second flowering control field, during the period late July to early September. Each bee was individually collected onto dry ice. After several specimens were gathered they were placed in a cooler of dry ice until they could be transported, at the end of each day, to the laboratory at the University of Prince Edward Island (UPEI). Here they were placed in a freezer at -20°C ± 5°C for long-term storage. In October, the collected honey bees were transported, on dry ice, to an Agriculture and Agri-food Canada (AAFC) laboratory in Kentville, Nova Scotia, and then stored in an upright freezer.

Nectar and pollen

The bees were sorted into pollen and nectar carrying bees at the AAFC lab. The sorting was done in a coldroom to control condensation on the specimens. Pollen and nectar recovery was done in the lab at room temperature, with the aid of microscopes and sterile implements

Nectar was extracted by inserting a mechanical stage

mounted 12.7 mm ultra-fine hypodermic needle, attached to a 0.3 cc syringe, into the honey stomach of the subject honey bee. This was done on a diagonal at the anterior edge of the first abdominal terga. The stomach contents were drawn off from each of the nectar bees in a sample, and then ejected into a sterile vial. Each finished vial was sealed and then placed in a Ziploc® plastic bag and frozen immediately. Minimum required nectar sample weight was 1 g and the target weight was 2-4 g.

For pollen recovery, at least 200 bees were processed for the main sample, and another 200+ bees for the reserve sample. Before recovery, the bees were dried to prevent moisture uptake by condensation on the pollen loads. The drying process involved placing a single layer of bees in a sterile petrie dish, covering the dish with an offset cover, and then placing the unit in a drying oven at 24° C for 45 minutes. After drying, the pollen was removed using sterile forceps and probes. All pollen loads from a sample were placed in a sterile vial, bagged, and stored in a freezer. Minimum required pollen sample weight was 2 g and the target weight was 4-5 g.

On October 30 all samples were transported back to UPEI on dry ice. On October 31, the samples were shipped to Enviro-Test for analysis.

Unripe Honey

Unripe honey was collected from the hives on August 22 and September 14, 2001. The equivalent of 1-2 frames of unripe honey was collected from each apiary location, either on drawn comb, or comb freshly drawn in an empty frame space. The unripe honey was extracted by cutting the comb into chunks and placing them into a strainer over a plastic bowl. After crushing the comb, the honey was allowed to drip through the strainer for several hours. A 45 g sample of honey was pored into a sterile sample vial, labeled, and frozen. A reserve sample was also collected. All samples were shipped on dry ice to Enviro-Test on October 30.

Residue analysis

Residue analysis was performed by Enviro-Test Laboratories, Edmonton, Alberta. As reported in the analytical report from Enviro-Test, data was generated in compliance with PMRA DiR 98-01 which outlines the requirements of OECD GPL principals and in compliance with Good Laboratory Practices according to EPA-FIFRA section 4- CFR part 160 (Oct 16, 1989). The quality assurance unit of Enviro-Test Laboratories inspected and/or audited the analytical phase of the study and the report, and reported its findings to the Study Director, and to ETL Management.

The analytical report also stated that the objectives of this part of the study were: 1) To determine LOD/LOQ (Limit of Detection/Limit of Quantification) and validate the modified analytical methods: Method No. 00554, Method No. 00537, Method 00537/E001 and Method 106428 (Soil Method dated Aug. 24/94), 2) To analyze soil, pollen, nectar, honey, and plant samples for imidacloprid, NTN 35884 (Olefin metabolite), and WAK 4103 (Hydroxy metabolite). The methods used

for reference were Bayer method no. 00537 (report no. MR-551/98), method no. 00537/E001 (report no. MR-568/99), and no. 00554 (report no. MR-812/98). The Limit of Quantification (LOQ) was established to be 2.0 ppb.

Samples that had been stored in a freezer at $-20 \pm 5^{\circ}$ C at the University of Prince Edward Island, were shipped to Enviro-Test Laboratories in coolers containing dry ice. Samples were received in good condition and were immediately stored in a freezer at $-25 \pm 5^{\circ}$ C. The nectar, honey, pollen and most of the flower samples were received processed and did not require further processing. Flower and leaf samples were prepared in a food processor in the presence of dry ice. Soil samples were sieved into a homogenous mixture. The % moisture was determined for all soil samples. The samples were then analyzed by High Performance Liquid Chromatography-Electrospray Ionization Mass Spectrometry (HPLC-MS/MS). Quantification was accomplished by using weighted (1/x) linear regression from an eight to nine point calibration curve.

The method of validation for pollen, nectar, and honey consisted of 6 spiked samples: 1 control, 3 at LOQ (Level of Quantification), and 2 at 5 times LOQ. For soil, leaf, and flowers, the method of validation consisted of 8 spiked samples: 1 control, 5 at LOQ, and 2 at 5 times LOQ. The average verification and in-phase recoveries were good for all analyses.

2002

Study sites

Apiaries were chosen to include four regimes: 1) apiaries with no problems that are in areas with few problems, 2) apiaries with no problems that are in areas with problems, 3) apiaries with problems that are in areas with few problems, and 4) apiaries with problems that are in areas with problems. The geographic distribution of apiaries includes all of Prince Edward Island, a U shaped route along the major highways of western, eastern and southern New Brunswick, and the area between Berwick, Windsor, and Truro in central Nova Scotia. Buffer zones of 1.5 kilometer radius were determined for each apiary as areas for vegetation surveys. Apiaries included winter, summer, temporary, permanent, and pollination locations for bee hives.

Bee management (interviews)

Personal and telephone interviews with 40-50 beekeepers will be completed during the course of this study. The questions concern bee stock, seasonal management plans, overwintering preparation and success, winter loss history, and disease identification and management skills. All management practices will be compared to "Best Management Practices".

Pesticides residues (bees, wax, honey, pollen, canola flowers)

Samples of adult honey bees, wax, pollen, and honey will be collected from all inspected colonies 1-4 times over the duration of the study. In addition, 40 hives in Nova Scotia will be included in this portion of the

study. This will include colonies that suffer unexplained decline and mortality and colonies that appear healthy and normal. Another smaller investigation in New Brunswick involved sampling canola flowers from plants being grown for seed production. These flower samples will be analyzed for the presence of imidacloprid and thiamethoxam residues. A subset of all of the other samples will be analyzed by Enviro-Test Laboratories for residues of crop protection products used in the buffer zones and bee protection products used in the hives.

Pesticide use survey

Pesticide use records are being requested for agricultural crop fields within the 1.5 km buffer zones around apiaries.

Diseases and pests

Colony inspections for brood diseases, parasitic mites, atypical symptoms, chilled brood, skunks, viruses, spiroplasmas, honey and pollen stores, and colony strength were performed in study colonies in Prince Edward Island and New Brunswick.

Forage (vegetation survey and plant phenology)

A list of Prince Edward Island and New Brunswick plants important to honey bees was prepared. Dates of first flowering of each species will be determined from plant community surveys in buffer zones around study apiaries. This will provide sequence of bloom, species distribution, and abundance data.

Apiary suitability

Map coordinates for each site were recorded using global positioning system (GPS). Summer, winter, permanent, temporary, and pollination apiary sites were included. Sites were evaluated for suitability factors including proximity to bee pasture and crops, pesticide use in area, adequate shelter, source of water, proximity to other apiaries, timing of forage availability, and other factors. Beekeepers will be asked to provide honey production figures.

Varroa and AFB resistance testing

Some fluvalinate resistance testing will be performed on *Varroa destructor*. Also, samples of American foulbrood, *Paenibacillus larvae*, will be cultured and tested for susceptibility to oxytetracycline.

Viruses and spiroplasmas

A separate project is establishing protocols and techniques for investigating the distribution of certain honey bee viruses, and to establish the presence or absence of honey bee spiroplasmas.

Analyses

The following analytical methods will be used, as appropriate, to determine factor relationships and predictors

• Spatial analysis will be performed on certain aspects of the data to determine spatial relationships among vector map features through the use of map math, and

integrated vector-raster analysis and modeling.

- Multivariate analysis will be used to estimate the coefficient of the linear equation. This will help determine
 one or more independent variables that can be used to
 best predict the value of the dependent variable.
- Factor, or principal component, analysis will be used to identify underlying factors that explain the pattern of correlations within a set of observed variables. This method can be used as a data reduction technique "to identify a small number of factors that explain most of the variance observed in a much larger set of manifest variables".

Results

2001

Soil residues

Residue levels of imidacloprid in Prince Edward Island field soil samples ranged from <2.0 ppb to 38 ppb. Levels of hydroxy and olefin metabolites were not tested. Samples from underseeded grain fields ranged from 27 ppb to 38 ppb (average = 32 ppb). Samples from first flowering clover fields ranged from 16 ppb to 38 ppb (average = 24.6 ppb). Samples from second flowering clover fields ranged from 14 ppb to 25 ppb (average = 20 ppb). The control field had no quantifiable residues of imidacloprid.

Residue of imidacloprid in Prince Edward Island field runoff soil samples was detected in only one sample. Levels of hydroxy and olefin metabolites were not tested. Samples from potato field runoff areas were all below detection limit. One sample from underseeded grain fields had a level of 3.7 ppb, all other underseeded grain field samples were below LOQ. The control runoff field had no quantifiable residues of imidacloprid.

Plant residues

Clover Flowers

Residue levels of imidacloprid and the hydroxyl and olefin metabolites in Prince Edward Island and New Brunswick clover flower samples from first and second flowering clover fields were all below level of quantification (<2.0 ppb). The control field clover flowers were also <2.0 ppb.

Clover Leaves

Residue levels of imidacloprid in Prince Edward Is-

land and New Brunswick clover leaf samples ranged from <2.0 ppb to 4.4 ppb. Levels of hydroxy and olefin metabolites were below detection limit (<2.0 ppb) for all fields tested . Samples from underseeded grain fields ranged from <2.0 ppb to 4.4 ppb. Samples from second flowering clover fields in Prince Edward Island ranged from <2.0 ppb to 2.5 ppb. Samples from second flowering clover fields in New Brunswick were all below detection limit (<2.0 ppb). The second flowering control field in Prince Edward Island had no quantifiable residues of imidacloprid or metabolites.

Wildflowers

Residue levels of imidacloprid and the hydroxyl and olefin metabolites in Prince Edward Island wildflower samples (goldenrod, fireweed, and asters) from runoff areas of potato fields and underseeded grain fields were all below LOQ (i.e. <2.0 ppb). The control field had no quantifiable residues of imidacloprid or metabolites.

Nectar

Residue levels of imidacloprid and the hydroxy and olefin metabolites in Prince Edward Island nectar samples collected from honey bees in second flowering clover fields were all below level of detection. The control field had no quantifiable residues of imidacloprid or metabolites.

Pollen

Residue levels of imidacloprid and the hydroxy and olefin metabolites in Prince Edward Island pollen samples collected from honey bees in second flowering clover fields were all below level of detection (<2.0 ppb). The control field had no quantifiable residues of imidacloprid or metabolites.

Hive residues

Unripe Honey

Residue levels of imidacloprid and the hydroxy and olefin metabolites in Prince Edward Island unripe honey samples collected from hives placed in second flowering clover fields were all below level of quantification (<2.0 ppb). Unripe honey from the control field also had no quantifiable residues of imidacloprid or metabolites.

2002

The 2002 study extends into the spring of 2003. Therefore, the results will not be available until mid 2003.

Table 2. Summary of the presence of imidacloprid and two metabolite (olefin and hydroxy) residues in soil, plant and bee product samples from Prince Edward Island and New Brunswick, Canada, 2002.

Material	Imidacloprid	Metabolites
Soil	Yes (100% of fields in potato rotation; 16.7% of runoff locations)	Not analyzed
Clover Leaves	Yes (barely detectable levels in 27.3% of fields)	No
Clover flowers	No	No
Wildflowers	No	No
Pollen	No	No
Nectar	No	No
Unripe honey	No	No

Discussion

As any beekeeper or bee researcher knows, there are many factors that can contribute to failing honey bee colony health. Sometimes even the most experienced apiculturist can only surmise what might be involved in losses of colonies. However, monitoring of colony health over a longer period of time, and investigation and analysis of other possible factors contributes to a systematic approach to determining cause and effect. In the three Atlantic provinces of Canada involved in this study, honey bee losses are not consistent among the provinces. In fact, Nova Scotia reported an above average honey production year in 2002, while some PEI beekeepers suffered serious honey bee colony losses. Even though the current study will not be completed until mid 2003, some patterns are beginning to emerge. Once the residue results are available, and the overall analysis of the large data set is complete, a much clearer story can then be presented.

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