Research: Invasive Species

Imidacloprid contamination of maple sap following Asian longhorned beetle quarantine treatment

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The Asian longhorned beetle (ALB), threatens hardwood forest tree species in North America, especially maples. First detected in the U.S. in 2006, it has now been discovered in and quarantines have occurred in IL, MA, NJ, NY, and OH. In order to eradicate this foreign pest, USDA APHIS (Animal Plant Health Inspection Service) has a prescriptive treatment protocol: all infested trees are cut down, the stumps are ground, and portions of the trees are chipped to a maximum dimension of one inch, which is sufficiently small that no ALB larvae or pupae can survive. Nearby uninfested potential host trees are either: (1) removed and chipped, as though they were infested, or (2) treated in three successive years with imidacloprid, a systemic insecticide. Systemic insecticides are those products that, when applied to one part of the plant, are transported within xylem and/or phloem sap to other plant parts. Approved methods for applying imidacloprid to protect maple trees include trunk injection, as has been implemented in the Worcester, MA, quarantine area, or soil injection, which has been used along with limited trunk injection in all other quarantine areas (USDA 2011).

USDA considers basal soil injection of imidacloprid to be the most effective and cost-effective option (USDA 2005). In the eradication program, imidacloprid is applied to protect all host trees within a 0.5 mile radius of any infested tree. For soil injection, the maximum labeled dosage, or 1.42 g active ingredient of imidacloprid is applied per dbh (diameter at breast height) inch of trunk. For trunk injection, trees between 2 and 24 inches dbh are treated with one Mauget capsule per 2 inches dbh; for trees greater than 24 inches dbh, the dosage is increased to 2 capsules per 2 inches dbh (USDA 2011).

Both the Asian longhorned beetle and the use of imidacloprid to protect sugar maples from this pest pose a threat to maple syrup producers. Maple syrup is marketed as a pure, natural sweetener. Protection of maples from infestation by ALB or other pests could require trees to be treated with imidacloprid, and, if residues are found in the syrup from these trees, that syrup must not be marketed. We asked: (1) Do treatments following the quarantine protocol of sugar maples result in detectable imidacloprid in sap or syrup? (2) Do soil applications cause higher residues than trunk injection? (3) What concentrations may we expect in sap and processed syrup from trees treated

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to protect them from ALB? (4) For trunk injections, does tapping above or below the insecticide injection site influence detected contamination of the sap? and (5) Does boiling of sap to process it into syrup destroy imidacloprid residues?

Field Experiment

Maple trees were selected for this study at The Caroline A. Fox Research and Demonstration Forest, Hillsboro, New Hampshire, operated by NH Division of Forests and Soils. They had have never previously been exposed to imidacloprid. Trees were chosen based upon ease of access, diameter of 10 – 22 inches (large enough for tapping; 14.6 ± 0.4 dbh inches, mean ± se), and good overall health of the trunk. A randomized complete block design was established to allow the treatments (untreated trees, soil injected, trunk injected but tapped below the injection point, and trunk injected but tapped above the injection point) to have trees matched with respect to tree diameter. A minimum of 12 trees were used for each treatment group. A constraint on randomization was the requirement that untreated control trees physically matching the treated trees were located far enough from those being treated to prevent contamination via root uptake from soil.

Maple trees were treated on July 18, 2013. We used the imidacloprid soil injection dosages and trunk injection protocols for ALB quarantine specified by USDA APHIS (2011). For soil treatment, the root flare was exposed, and an imi-

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dacloprid suspension (a 1:10 dilution of Xytect 2F [Rainbow Tree Care]) sufficient to provide 1.42 g active ingredient per inch dbh was poured directly onto the exposed bark, and the soil was then returned to cover the root flare. For trunk injection, capsules (Mauget, Arcadia, CA) specifically designed for ALB quarantine treatment contained 4 ml per capsule of a 10% imidacloprid formulation. These were applied at 3 feet height on the bole of the tree at a dosage of one capsule for every 2 inches dbh. These injections are higher on the trunk than would be normal procedure, to allow tapping of trees in 2014 at positions above and below the injection sites. An 11/64" high helix drill bit was used to drill approximately 0.5 inches into the xylem, at a slight downward angle, and a feeder tube inserted into this hole. The injection capsule was then pressurized and tapped with a mallet onto the feeder tube to break the capsule's internal seal. After the capsule had emptied, the capsule was removed. The imidacloprid solution did not empty from a few capsules into trees: these were removed, and replacement capsules were inserted in freshly drilled holes on July 19 to ensure correct dosing of every tree.

Trees were tapped during the normal sap flow period in 2014, and four weekly samples (March 21, March 28, April 4, and April 11) of 15 ml from each tapped tree were frozen and shipped overnight to Villanova for analysis. Taps were inserted at least six inches below or 12 inches above trunk injection sites for the trunk injection treatment groups, and about 3 feet above the ground for the soil application treatment group. No special consideration was given to place the tap a specified lateral distance from holes left from trunk injection. However, as injection sites were no more than ~6 inches apart, around the circumference of the trunk, taps could be no more than 3 inches lateral distance from injection sites.

The concentrations of imidacloprid and its important insecticidal metabolites were performed at Villanova University. Imidacloprid and two of its insecticidal metabolites were quantified, because their combined concentrations are treated as "imidacloprid" by regulators (U.S. National Archives and Records Administration 2010). The quantification method used high performance liquid chromatography coupled with tandem mass spectroscopy (HPLC/MS/MS) (Fig. 1). This method first separates chemicals carried in liquid solution by their affinity for a stationary adsorbent coating particles packed into a column. Compounds are identified first by their retention time in this packed column. As each compound leaves the column, it is charged with a high voltage from a spray tip. Each compound has a mass-to-charge ratio that is dependent on the molecular weight of the compound. Next, this ionized compound is fragmented and these fragments have unique mass-to-charge ratios due to the chemical structure of the original compound. Thus, the combination of retention time, and parent and fragment mass-to-charge values allow for unambiguous identification of target molecules. There were a total of 160 samples analyzed for 3 compounds, for

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a total of 480 chemical determinations from this experiment. An additional 19 trees had been measured in preparation for this experiment. These were tapped along with the experimental trees and the sap samples analyzed for imidacloprid and imidacloprid metabolite residues, for an additional 76 samples and 228 determinations.

From these laboratory analyses, it is clear that soil application and trunk injection are equivalent with respect to introducing imidacloprid into maples. In each treatment group, there were one or two trees (9 – 18% of each group) for which there were no detections of imidacloprid over the course of the sap harvest season; there were no differences among treatments with respect to the proportion of non-detectably contaminated trees. There were no detections of imidacloprid from the trees designated as untreated controls, making this group significantly different from the other three groups (Fisher's Exact test on numbers of trees with and without detections, P = 2.2 × 10-6) (Microsoft Research 2014). The concentrations of imidacloprid and metabolites detected among the three treatment groups varied widely and required logarithmic transformation prior to statistical analysis; the concentrations of imidacloprid and its metabolites were equivalent among these groups (Table 1). Maximum detection of imidacloprid in the trunk injection treatments were 2,580 ppb when the tap was placed above the injection site and 982 ppb when the tap was placed below the injection site. The maximum detection for imidacloprid for the soil treatment was 246 ppb in sap.

Finding statistically equivalent concentrations for imidacloprid contamination when the taps were placed above and below trunk injections sites was unexpected. We planned this comparison because imidacloprid (and other neonicotinoid insecticides) are stated to be acropetalar, that is, they are xylem mobile and move upwards and outwards in plants (Sur and Stork 2003). If they exclusively moved upwards, then sap drawn from taps placed in maple trees below trunk injections sites for insecticide treatments should never experience contamination with the insecticide. Our experiment clearly disproves exclusive upward movement, and the placement of taps in any trees treated with imidacloprid, either through soil or trunk injection methods, clearly can be expected to yield contaminated sap. Unfortunately, as other research has demonstrated, xylem conducts materials in trees both upwards and downwards (Tattar and Tattar 1999), and even limited downward movement can cause contamination of sap in trunk-injected trees when taps are placed below injection sites.

Downward movement of imidacloprid following trunk injection may be more dramatic, however. An example of possible extreme downward translocation and transport through a root graft was detected in Tree #5, an untreated tree (but not chosen to be used among the 12 replicates of the main experiment), which was 50 feet away from Tree #3, which was trunk injected. Tree #5 had imidacloprid present at 0.441, 2.62, 76, and 42.6 ppb in the four sap sampling dates, and olefin at

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1.4 ppb detected on April 11. This was a unique instance of imidacloprid and metabolite detection in an untreated tree, but the degree of contamination is remarkable, if indeed it resulted from downward translocation and movement across a root graft.

Laboratory experiment

We wished to compare the concentrations of imidacloprid and its metabolites in fresh sap, versus sap that was boiled to produce syrup. This is an important question, because if cooking sap to make syrup destroyed the residues, this would mitigate imidacloprid contamination. To study the effects of sap processing on these chemicals, a commercial, uncontaminated syrup (Trader Joe's, Monrovia, CA) was diluted 50-fold to simulate sap. Analytical standards of imidacloprid, imidacloprid olefin, dihydroxy imidacloprid and 5-hydroxy imidacloprid were added to triplicate samples of 50 ml each of diluted syrup to yield 150 ppb concentrations. A small quantity was analyzed by HPLC/MS/MS, and then the samples were heated until evaporated to the consistency of syrup. The cooked samples were re-diluted to the starting volume, and the residues re-tested. The final concentrations were compared with the initial concentrations to calculate changes resulting from cooking.

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From this experiment, we found that imidacloprid decreased in concentration by 3.6%, which was within the margin of measurement error. The dihydroxy imidacloprid was not recovered, indicating that it was completely converted; the imidacloprid olefin residues increased in these samples, demonstrating that the dihydroxy metabolite is converted to the olefin compound. The 5-hydroxy imidacloprid concentrations decreased by about 35%, and for these samples the olefin metabolite concentration also increased. Therefore, since imidacloprid and its olefin metabolite are stable during cooking to produce maple syrup from sap, and the less stable metabolites are converted in part or totally to the olefin metabolite, we can expect that processing sap to make syrup will only serve to concentrate imidacloprid and its metabolites to approximately the same degree as the reduction in liquid volume.

Conclusions

Imidacloprid and its metabolites, which are grouped together and are considered to be equivalent for regulatory purposes, are readily detected in maple sap from trees treated either through soil application or from trunk injection. Small differences that we measured related to the time of sampling, over the four week sap collection

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Table 1. Contamination (parts per billion [ppb], mean ± se) of maple sap in 2014 with imidacloprid and its chief insecticidal metabolites following soil or trunk injections in 2013 of imidacloprid, following USDA Asian longhorned beetle treatment protocols.

				Concentrations found in sap*		
Treatment	tap placement	n	dbh (in.)	imidacloprid	5-hyroxy	olefin
Untreated	-	16	14.1 ± 0.8	0 ± 0	0 ± 0	0 ± 0
Soil drench	-	15	14.5 ± 0.3	18 ± 5	2.0 ± 0.4	2.7 ± 0.7
Trunk inject	above injection	13	14.7 ± 0.6	102 ± 51	1.9 ± 0.9	2.2 ± 1.2
Trunk inject	below injection	12	14.8 ± 0.5	56 ± 24	1.1 ± 0.4	1.1 ± 0.4

*The only significant differences among treatment groups were between the untreated control and the remaining three treatment groups. Repeated measures analysis of variance performed on the treated groups, on log-transformed concentrations ($F \leq 1.5$; $df = 2, 22$; $P > 0.2$).

period, and related to differences in tree diameter, have no practical significance to maple syrup producers, because no detection of imidacloprid can be tolerated in maple products. Furthermore, cooking sap to produce syrup or candy will concentrate illegal residues of imidacloprid and its metabolites. Because imidacloprid can persist for multiple years in tree tissues (Cowles et al. 2006), the only practical option for maple syrup producers will be to permanently exclude trees from harvesting sap, if they ever are treated with imidacloprid.

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