MEASUREMENT OF IMIDACLOPRID IN XYLEM FLUID FROM EASTERN HEMLOCK *(TSUGA CANADENSIS)* BY DERIVITIZATION/GC/MS AND ELISA

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ABSTRACT

Imidacloprid is a nonvolatile insecticide and its direct quantification is not possible by gas chromatography. In order to ascertain imidacloprid levels in soil and trunk injection treated trees, a sensitive and selective method has been developed using GC/MS to measure the imidacloprid levels in xylem fluid exudates. In May 2005, a stand of hemlock trees in West Virginia were treated by soil injection and trunk injection, while certain trees were selected as untreated controls. Terminal branches from four cardinal points within the crown, mid-crown, and base of the tree were collected and placed in a stainless-steel pressurized cylinder to exude the xylem fluid from the branch cutting. An internal standard, $C^{13}D_3$-imidacloprid, was added to the xylem fluid. Following C_{18}-SPE cleanup of the xylem fluid, imidacloprid and the internal standard are derivatized at the imidazole nitrogen to form the semi-volatile, heptafluorobutyryl derivatives. Derivatized imidacloprid was separated using a splitless injection on a Varian Factor 4 VF-5ms column (30 m × 0.25 mm i.d × 0.25 µm film). The derivatized imidacloprid and internal standard are quantified by mass spectrometry using *m/z* 407 and *m/z* 411, respectively.

A commercially available competitive enzyme-linked immunosorbent assay (ELISA) for imidacloprid provides an attractive alternative to analyzing large numbers of of xylem fluid samples as in the current study. Advantages of the ELISA include its sensitivity (working range of 0.2-6 ppb), simplicity, ease of use, and inexpensive cost; however, disadvantages can arise when structurally simlar, imidacloprid metabolites are detected as false-positives by ELISA. We observed and quantified the cross-reactivities of nine known imidacloprid metabolites. Furthermore, a 1:20 dilution was necessary to eliminate cross-reactivity from the xylem fluid matrix raising the ELISA limit of detection to 4 ppb. Over the course of the next several years, imidacloprid in approximately 4000 xylem fluid samples will be quantified by ELISA with 10 percent of the samples confirmed by GC/MS analysis. Concurrent with the insecticide analysis, population levels of adelgids are being studied with the intent of determining imidacloprid action levels and an insecticide reapplication time period. Results will be presented that address the discrepancy in imidacloprid levels due to the cross-reactivity in ELISA, a comparison of the imidacloprid treatment methods used and the persistence of imidacloprid in treated trees.