METHOD FOR THE EXTRACTION AND ANALYSIS OF IMIDACLOPRID RESIDUES IN PLANT MATERIAL BY ENZYME-LINKED IMMUNOSORBENT ASSAY (ELISA)

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Abstract

Residues of the pesticide imidacloprid in plant tissue are typically determined by extraction of the sample in an organic solvent followed by analysis of the pesticide residue by either HPLC or GC-MS. These methods, while accurate, are expensive and time consuming to perform and are not convenient for the processing of large numbers of samples. An Enzyme Linked Immunosorbent Assay (ELISA) is commercially available which can rapidly analyze large numbers of samples with great sensitivity and at reduced cost. Presently, this assay is restricted to the determination of imidacloprid residues in aqueous samples. We have successfully utilized this method to measure imidacloprid concentrations in the xylem sap of pesticide treated trees. In order to expand the capabilities of our residue analysis, we set out to develop techniques whereby the ELISA assay could be utilized to quantify residues from the solvent-extracted phloem or leaves of imidacloprid-treated trees. Our objective is to develop a rapid and reliable method for measuring imidacloprid concentrations in specific plant tissues targeted and fed upon by plant pests such as the Asian longhorned beetle (ALB) or the emerald ash borer (EAB).

The percentage recovery of imidacloprid added to ash phloem tissue ranged from 89 to 119% over a range of 48 to1600 ng of added pesticide. All of the pesticide residue recovered was found in the initial methanol extraction and subsequent wash. No detectable imidacloprid was found in the second methanol extraction. The LOQ of imidacloprid from the plugs under the extraction conditions we utilized (4 ml initial methanol extraction and 40 X dilution of the sample with distilled water prior to ELISA analysis) was approximately 48 ng. A dilution of tissue extracts less than 40 X yielded positive matrix effects on the assay, resulting in false positive values. The efficiency of recovery of imidacloprid from methanol extracted Norway maple leaves was excellent (93.5 and 94.5% respectively for the two concentrations of imidacloprid tested). Unlike the phloem extracts, however, only 90 to 92% of the added imidacloprid was recovered in the first extraction and subsequent wash step. The remaining 8 to 10 % of the added imidacloprid was recovered in the second methanol extraction. This suggests that a more strenuous extraction technique may be required to quantitatively recover imidacloprid from leaf tissue in a single extraction procedure.

Our results demonstrate that a simple methanol extraction can be coupled with a commercially available ELISA kit to provide a suitable screening method for the quantitative determination of imidacloprid in the phloem or leaf tissue of ash and maple trees. In addition, as employed, the method does not require any extensive sample clean-up leading to a considerable savings in time and cost.