

Distribution and metabolism of ^{14}C imidacloprid in *Fraxinus* spp.

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Trunk or soil injection of systemic insecticides is often a preferred method for controlling insect pests in landscapes because it minimizes potential spray drift, applicator exposure and impacts on non-target organisms. Recent field trials and anecdotal evidence indicate that imidacloprid, applied as either a soil drench or trunk injection, can significantly reduce emerald ash borer (EAB), *Agrilus planipennis* Fairmaire (Coleoptera: Buprestidae) populations in ash trees and canopy dieback associated with EAB. The persistence and translocation of imidacloprid in ash trees, however, is not well understood. In this study we used radiolabeled ^{14}C imidacloprid to assess the distribution, persistence and movement of imidacloprid in green ash and white ash trees following trunk injection. We also determined EAB mortality in bioassays of adults beetles fed leaves from trunk-injected trees.

The specific objectives of the study were to: 1) Determine the translocation and persistence of ^{14}C imidacloprid over time in trunk-injected white and green ash trees; 2) Determine the effects of water availability on imidacloprid translocation and distribution; 3) Determine the mortality and knockdown of EAB adults fed leaves from injected trees and; 4) Identify the major metabolites of imidacloprid in ash trees.

On June 14, 2004, we injected 20 container-grown green ash (*Fraxinus pennsylvanica*) trees (7.6 cm dbh) and 20 white ash (*F. americana*) trees (10.1 cm dbh) with 6 ml of imidacloprid (10% a.i.). The trunks of the trees were injected at 15 cm above ground level via two injection ports on opposite sides of the tree (Figures 1 & 2). Each tree received 25 μCi of ^{14}C -imidacloprid in a ratio of 1:2400 (labeled:non-labeled imidacloprid). After injection, half of the trees were kept well watered (3.8 cm irrigation per week) and half were subjected to water stress (1 cm irrigation per week). Imposition of water stress was verified with periodic measurements of soil water content and leaf gas exchange (Figure 3). We collected leaf, twig, trunk and root samples 0, 2, 7, 21, 60, 105 and 150 days after treatments (DAT). After 105 DAT five trees from each species were covered with netting to collect litterfall samples (Figure 4). All samples were brought to the lab; oven dried, ground, weighed and oxidized in biological tissue oxidizer. The resultant $^{14}\text{CO}_2$ was trapped in scintillation cocktail and radioactivity was determined by scintillation counting. A subsample of fresh leaves was collected 21 and 60 DAT for bioassays on adult EAB.

A subsample of fresh leaves was collected 21 and 45 DAT for bioassays on adult EAB. Single leaves were put in a vial with distilled water, which was placed in a cup. Four beetles (10 days old) were introduced in the cups. The beetles were kept at 28°C, 50% relative humidity, photoperiod of 16:8 (L:D) while inside the cups. Mortality and "knock down" were assessed at 24, 48 and 72 hours. Beetles unable to stand on their legs and walk a distance equal to their own body length were counted as knocked down.

Topical bioassays. Technical grade insecticide was diluted with acetone. Five doses that resulted in more than 0 percent and less than 100 percent mortality based in preliminary assays were used. Four beetles (about 10 days old) were treated with 1 μl of solution on the ventral area of the abdomen with a 50 μl microsyringe connected to a microapplicator. The control beetles were treated with 1 μl of acetone only. Three to five replications per concentration were performed. After treatment, beetles were placed in cups and fed ash leaves and kept at 28°C, 50 percent relative humidity, photoperiod of 16:8 (L:D). Mortality and "knock down" were assessed at 24, 48 and 72 hours after treatment.

Radioactivity in leaves increased steadily from 2 DAT to DAT 60 (Figure 5). Through 21 DAT radioactivity in twigs and roots was not significantly different from zero. Radioactivity in leaves collected after leaf-fall was as high or higher than samples collected at 60DAT, indicating little re-translocation of

imidacloprid or imidacloprid metabolites from leaves before leaf-fall. Specific activity was somewhat lower in white ash than green ash reflecting a dilution in the larger trees. Initial results did not indicate a significant effect of water stress on ¹⁴C imidacloprid movement. We are continuing processing and analysis of later sample dates and trunk tissues.

A high percentage of knock down in EAB adults was observed 24 hours after treatment (40% at 20 d and 36% at 45 days). Beetle mortality was less than knock down (11% at 20 days, and 17% at 45 d). However, three days after exposing the adults to the foliage the mortality increased. Translocation of labeled and unlabeled imidacloprid was effective to control adults of EAB. The percent of knock down plus dead beetles was 71 percent 20 d after treatment and 77 percent at 45 d after treatment. Other sub lethal effects, including reduced feeding and slowed movement, were also observed. These effects may severely affect the fitness of surviving beetles. The LD50 for the adults of EAB was 7.1 ng/beetle, which confirms that EAB is very susceptible to imidacloprid as compared to other insect species.



Figure 1. David Mota-Sanchez injects ¹⁴C labeled imidacloprid into the trunks of ash trees at the MSU Horticulture Teaching and Research Center.



Figure 2. Ash trees taking up ¹⁴C imidacloprid via trunk injection.

Gas exchange of green and white ash under varying soil moisture

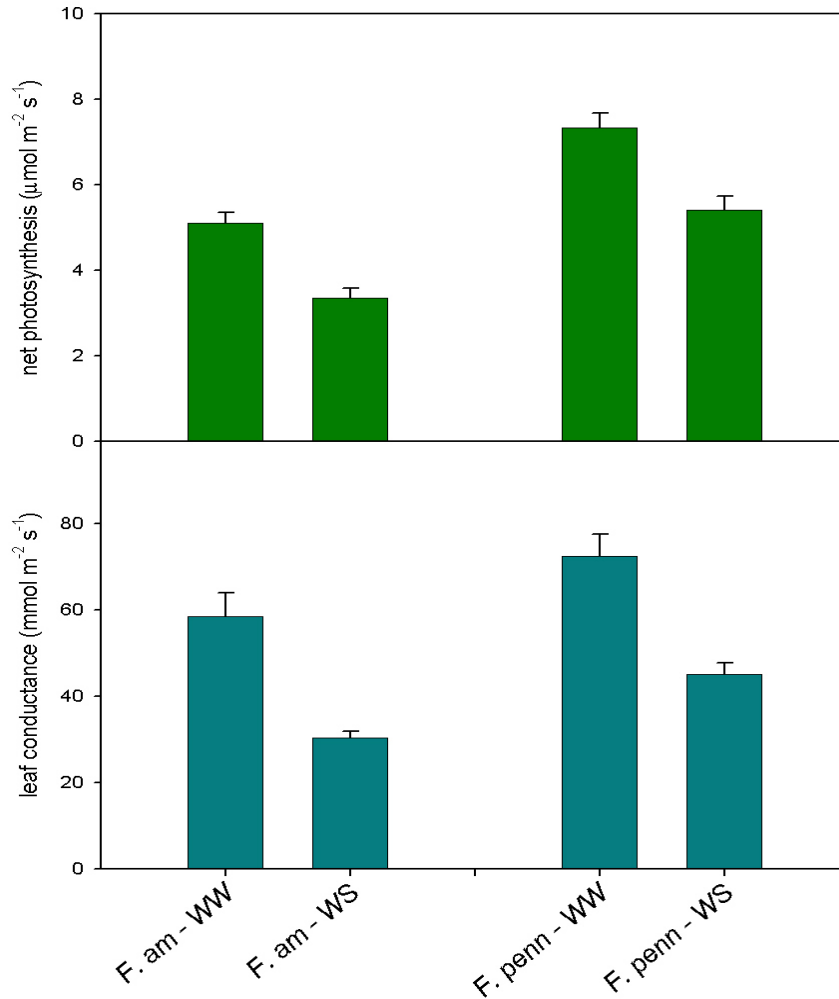


Figure 3. Leaf gas exchange of white ash and green ash trees under two levels of water stress. F. am = Fraxinus Americana, F. penn = Fraxinus pennsylvanica, WW = Well watered (1" of irrigation per week), WS = Water stress (1/4" irrigation per week)



Figure 4. Ash trees netted for litterfall collection.

Distribution of ^{14}C Imidacloprid in *Fraxinus* spp.
60 days after trunk injection & litterfall

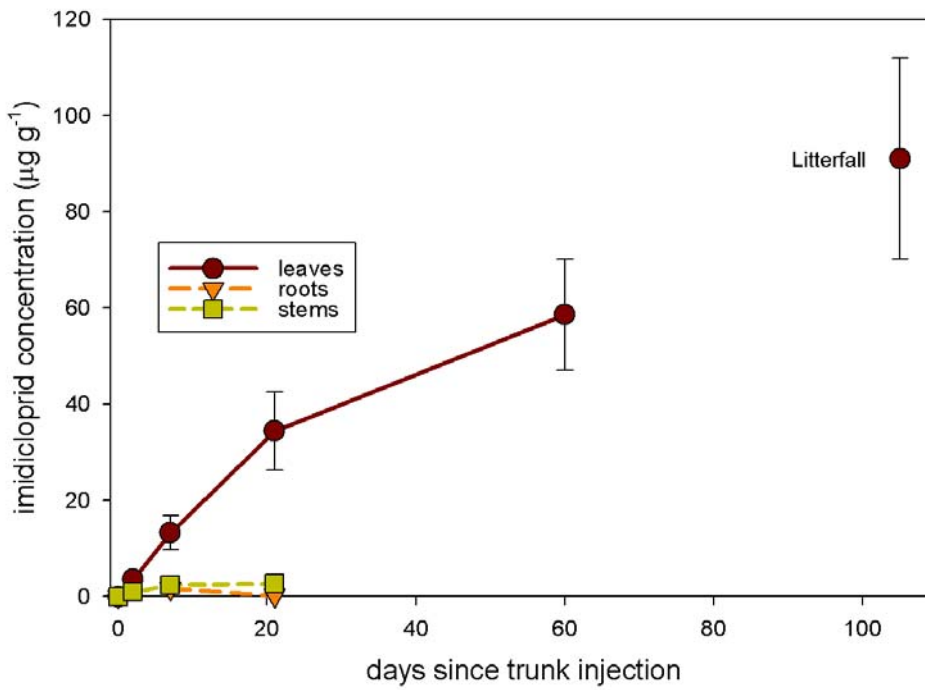


Figure 5. Distribution of imidacloprid after trunk injection by plant part. Last sampling point indicates concentration in leaf litterfall collected from netting trees.