



# TreeAzin, A Systemic Bioinsecticide containing Azadirachtin for the Control of an Invasive Woodboring Beetle, the Emerald Ash Borer.

Blair Helson, Dean Thompson, Gard Otis, Nicole McKenzie, Joe Meating

## Introduction

Extracts containing the safe, natural product, azadirachtin, from the neem tree (*Azadirachta indica*) are known to possess systemic activity on several defoliating and leafmining insects when injected into trees. A new liquid formulation named TreeAzin, containing 5% azadirachtin has been developed specifically for trunk injections. Low volumes of TreeAzin can be injected quickly and completely into trees with a new, high output, commercial tree delivery method (Picture 1).

Picture 1. TreeAzin Injection of an Ash Tree with the EcoJect System



In 2002, the emerald ash borer (*Agrilus planipennis*) (EAB) (Picture 2), an invasive woodborer from China, was discovered in Windsor, Ontario, Canada. This serious pest has killed millions of native ash (*Fraxinus spp*) trees in both Canada and the USA.

Picture 2. Emerald Ash Borer Larva in Gallery and Adult



In an effort to find a solution to protect high value ash trees from damage by EAB, the potential of TreeAzin was assessed by determination of:

- uptake and dissipation of azadirachtin as it relates to levels and duration of residues in foliage.
- efficacy on larvae in small and large trees.
- effects on adult mortality, female fecundity and fertility following ingestion of ash leaves containing azadirachtin.

## Methods and Results

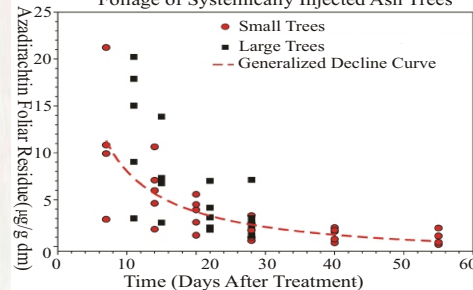
### Azadirachtin Foliar Residues

Five small, 2.2-cm dia. potted green ash trees and 5 large, 22-cm dia. ornamental trees were injected with 55 mg and with 250 mg azadirachtin per cm dia. respectively in June. Leaves were collected and analysed for azadirachtin residues with a liquid chromatograph mass spectrometer.

- Azadirachtin was rapidly taken up and translocated in both small and large trees (Figure 1).
- The highest concentrations (mean = 11 µg/g and 13 µg/g, small and large trees respectively) were observed within 7 and 11 days.

- Concentrations dissipated in a slow curvilinear pattern. Patterns in small and large trees were similar. Estimated DT 50 values were 21 days and 22 days for small and large trees.

Figure 1. Dissipation of Azadirachtin Residues in Foliage of Systemically Injected Ash Trees



## Efficacy

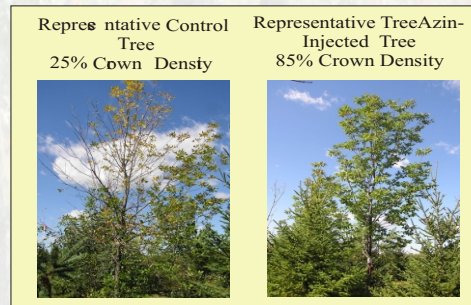
Small, 8-cm dia. green ash trees (n=10) and large, 37-cm dia. trees (n=7) in areas known to be infested with EAB were injected with 100 mg (low rate) or 250 mg (high rate) azadirachtin per cm dia. Efficacy was assessed by comparing adult emergence holes and foliar crown densities on treated and control trees one year after treatment.

- For small trees, significantly fewer (100%) new exit holes were found on treated trees at both rates than on control trees (Table 1).
- The mean crown density of trees injected with TreeAzin was significantly higher than the untreated trees (Picture 3).

Table 1. Exit Holes and Crown Densities of Small Ash Trees Injected with TreeAzin 28, 30 June

| Rate mg. aza /cm di | New Exit Hole Mean # ± SD | Crown Density Mean % ± SD |
|---------------------|---------------------------|---------------------------|
| 0                   | 12.7 ± 15.5a              | 52.5 ± 40.2x              |
| 100                 | 0 ± 0b                    | 94.0 ± 4.6 y              |
| 250                 | 0 ± 0 b                   | 88.5 ± 22.7y              |

Picture 3. Crown Densities of Untreated and Injected Small Green Ash Trees



- For large trees, exit holes in branches from treated trees were significantly reduced 87% at the high rate and 82% at the low rate compared to control trees (Table 2).
- New exit holes on trunks were significantly reduced at the high rate (96%) but not at the low rate, largely due to one tree with 130 exit holes.

- Crown densities of treated large trees were higher than controls but differences were not significant, probably due to advanced infestation in these trees.

Table 2. Exit Holes and Crown Densities of Large Ash Trees Injected with TreeAzin 12 July 2005

| Rate mg aza. /cm dia. | Branches Exit Holes Mean ± SD | Trunk Exit Holes Mean ± SD | Crown Density Mean % ± SD |
|-----------------------|-------------------------------|----------------------------|---------------------------|
| 0                     | 11.1 ± 16.5 e                 | 38.6 ± 32.7 s              | 23.6 ± 25.4 x             |
| 100                   | 2.0 ± 4.1 f                   | 24.3 ± 47.2 s              | 37.9 ± 32.1 x             |
| 250                   | 1.4 ± 2.9 f                   | 1.7 ± 4.1 t                | 49.2 ± 25.0 x             |

## Adult Mortality, Fecundity, Fertility

Mating pairs of male and female EAB were fed for 3 days on ash leaves treated systemically with a 5 ppm solution of azadirachtin (n=72) or distilled water only (n=71). Each pair was observed regularly for mortality, egg production and egg hatch.

- Azadirachtin did not significantly reduce survival of either males or females.
- Azadirachtin did not reduce the numbers of females laying eggs (Table 3).
- Azadirachtin significantly reduced the fecundity of females by 66% and the fertility of females by 98%.

Table 3. Oviposition, Fecundity and Fertility of EAB Females fed on Leaves Treated Systemically with Azadirachtin

| Treatment | Egg Laying Females % | Eggs Laid/Female Mean # ± SD | Hatched Eggs/Female Mean # ± SD |
|-----------|----------------------|------------------------------|---------------------------------|
| Control   | 32.4 ns              | 36.5 ± 39.9 a                | 5.4 ± 12.8 x                    |
| Aza       | 32.4 ns              | 12.3 ± 17.7 b                | 0.09 ± 0.4 y                    |

## Conclusions

- Azadirachtin is rapidly taken up and effectively translocated in green ash trees following trunk injections of TreeAzin.
- Toxicologically significant concentrations are likely to occur in injected trees throughout the larval and adult feeding period.
- Early summer trunk injections with TreeAzin at 250 mg azadirachtin/cm dia. at early stages of infestation should provide very effective control of EAB larvae and tree protection for one season.
- Injections with 100 mg azadirachtin /cm dia. should provide acceptable control of EAB larvae and tree protection as prophylactic treatments.
- Trunk injections with azadirachtin may result in reductions in fertility and fecundity of adults feeding on leaves of injected trees.

**Trunk injections of TreeAzin is an effective, highly targeted new tool for the management of Emerald Ash Borer and has great potential for other wood boring beetles.**

### Acknowledgements

We thank John McFarlane, Teresa Buscarini, Lyndsey Desjardins and Rob Krause for their technical assistance. Financial support - the Canadian Food Inspection Agency, Ontario Ministry of Natural Resources and Natural Resources Canada