# A New Strategy to Control Hemlock Woolly Adelgid (*Adelges tsugae* Annand) using Imidacloprid and an Arboricultural Method to Gauge Hemlock Health

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## Abstract

Imidacloprid, a systemic insecticide is labeled for use against Hemlock Woolly Adelgid (HWA) and armored scales. In this study we applied imidacloprid by soil and/or tree injection, neither of which is, in of itself unique. What is new is combining both as a strategy for insect management and tree protection. Designed as a two year study, applications to trees were made in 2007. Evaluations include HWA and Elongate Hemlock Scale (EHS) densities, tree response and imidacloprid residues. An outcome is a method for the forest manager to gauge hemlock health. In 2007 results, 94.2% of hemlocks were categorized as being in poor to very poor health (little or no growth or dieback); 52.2% of hemlocks had symptoms of dieback. Low HWA densities (mean of 0.41 HWA/cm of twig) and poor tree condition categorize very late stage infestation. HWA survival is most closely associated with new growth (17.5% of trees); the potential of re-emergence therefore exists. EHS was present in the study trees as well (mean of 1.15 EHS/needle) with highest numbers observed in three-year old needles. Protecting the extant canopy is critical to tree recovery; therefore EHS is a management priority. Successful treatment methods are those that aid hemlock recovery and provide protection over time. Combining tree and soil injection is a new strategy that may provide the forestry manager with key advantages, such as quick and simple applications for near and long term tree protection.

## Keywords

Imidacloprid, Hemlock Woolly Adelgid, Tree Microinjection, Soil Injection, Hemlock Health

## Introduction

Imidacloprid (1-[(6-chloro-3-pyridinyl)methyl]-N-nitro-2-imidazolidinimine), a neo-nicotinoid insecticide is labeled for use in Hemlock Woolly Adelgid (*Adelges tsugae*) (Homoptera: Adelgidae) management and suppression of armored scales. It may be applied as a canopy spray, to the soil or injected directly into the tree. For forest and woodland applications, the latter two methods offer the most versatility. For example, the Kioritz soil injector, and the Arborjet QUIK-jet tree injection equipment are light weight, easy to carry, use low volumes of concentrate and are simple to use. Typically, the forest manager will choose one application method, but there may be significant advantages to combining soil and tree injection as a strategy for near term therapeutic and longer term tree protection.

As part of this study, we developed a simple method to help quantify hemlock (*Tsuga canadensis* (L) Carriere) response that may be used by the forestry manager. This method is based on the impact HWA has on twig growth. McClure suggested an inverse relationship between HWA pressures and hemlock growth (1991). This growth loss occurs with increasing HWA pressure and may take 3-4 or more, years. Ward (1991) suggested an HWA threshold of 25-30 HWA/100 needles as negatively impacting twig growth. We use 2.0 HWA/cm twig growth (Doccola et al 2007). In hemlock, we counted needles per cm and calculated a mean of 7.2; or 2.8 HWA/10 needles, which is comparable to HWA densities reported by Ward. HWA feed in the xylem ray parenchyma cells (McClure, 1987) on twigs at the needle base, symplastic tissue rich in carbohydrates and other essential nutrients. As carbohydrates are siphoned off by insect feeding, less is available to the tree for basic life functions (i.e., growth, metabolism, reproduction, storage and defense) (Shigo, 1989). As infestation progresses, twig lengths decrease, followed by loss of new growth altogether. Photosynthetic capacity, and therefore carbohydrate manufacture is reduced. The capacity of the tree to meet existing and new mass requirements becomes limited. Unchecked, HWA can compromise photosynthetic capacity further; below a critical level, twigs dieback and shed needles. As the resource becomes depleted, the HWA numbers also drops. These hemlock responses to HWA infestation may be used by the forestry manager as indication of treatment efficacy and hemlock health.

Elongate Hemlock Scale (*Fiorinia externa*) (Hemiptera: Diaspididae) infestation exasperates hemlock decline by increasing needle shedding (2). Alleviating pest pressures by therapeutic treatments makes tree recovery possible. Of additional concern in woodlands are inconsistent patterns or low levels of precipitation. New shoot growth and root uptake of soil applied imidacloprid are dependent upon adequate soil moisture.

This study compares three treatment methods and three formulations for tree protection to answer the questions, A. Does method of application and formulation of imidacloprid make a difference to outcome? B. Can methods and formulations be combined to advantage? C. Is there sufficient activity and protection provided in hemlock?

**Methods**

Sixty four hemlocks were treated with the insecticide formulations containing the active ingredient, imidacloprid for HWA and EHS infestations. Eight treatments were assigned in a complete randomized block design. Study trees ranged from 10.6 to 34.7" DBH (26.5 to 86.8 cm) with a mean DBH of 19.6" (49 cm). The eight treatments were a. controls, b. IMA-jet microinjection (0.15 g A.I./DBH") (0.06 g A.I./cm DBH), c. IMA-jet Micro-Infusion™ (0.3 g A.I./DBH") (0.12 g A.I./cm DBH), d. MERIT Tree Injection, low rate (0.15 g A.I./DBH") (0.06 g A.I./cm DBH), e. MERIT Tree Injection, high rate (0.3 g A.I./DBH") (0.12 g A.I./cm DBH), f. IMA-jet microinjection + MERIT soil injection (1.45 g A.I./DBH") (0.6 g A.I./cm DBH), g. IMA-jet Micro-Infusion™ + MERIT soil injection, and h. MERIT soil injection. Treatments were applied on August 29-30, 2007 at the Biltmore Estate, Asheville, N.C., U.S.A. The Tree

I.V. and the QUIK-jet injector are methods of Micro-Infusion™ and tree microinjection, respectively. The QUIK-jet injector delivers a fixed, measured dose per injection site. The number of application sites into the sapwood varied depending upon the method used. The configuration of the injection site was standardized using a 0.375" (9 mm) Brad point bit. We drilled 1.5" (3.75 cm) into the sapwood to create a 2.5 $cm^3$ capacity site. The Tree I.V. is equipped with 4 injector tips, and sites were located every 8" of stem circumference. The Tree I.V. delivered the higher volume dosages by trunk injection. 16 trees were treated using this method. The QUIK-jet injector applied the lower volume dosages. Sites were located every 6" of stem circumference, using the QUIK-jet injector. 32 trees were treated using this method. Tree injection formulations of imidacloprid were applied in concentrate as formulated Only IMA-jet was applied by Tree I.V. at the 6 mL/DBH"(2.4 mL/cm DBH) rate. IMA-jet at the 3 mL/DBH" (1.2 mL/cm DBH), MERIT Tree Injection at 0.75 or 1.5 mL/DBH" (0.3 – 0.6 mL/cm DBH) were applied by QUIK-jet injector. Soil applications were applied using the Kioritz soil injector. Soil applications were made 12-36" (30-90 cm) of the tree bole. Each MERIT 75 WSP 1.6 oz (45.4 g) packet was mixed in 32 oz (960 mL) water. Thus prepared, the mixture supplies 1.2 oz of A.I. (34.1 g), sufficient to treat a 23.5" DBH (58.8 cm) tree at the 1.45 g A.I./DBH" (0.58 g A.I./cm DBH) rate. 24 trees were treated by soil injection.

In November, 2007 (70 DAT) twig samples were taken from the study trees. Four branches were cut from the mid to upper tree canopy by aerial lift truck, each between 40 to 45 cm in length, and shipped to Arborjet, Inc, Woburn, MA, U.S.A. for evaluation. Thirty-two (32) additional branch samples from 8 non-infested, healthy hemlocks were cut for comparison. Samples were held at 40°F (4.5°C) for the extent of the bioassay assessments. Branches were cut to 30 cm length. Each sample was scanned using a Canon Color Image Scanner (CanoScan 8800F) and identified with the tree ID number and year. The digital images were inserted in a Micro-Soft PowerPoint file and tip growth was determined on a percentage basis, adapting the Webb et al method (2003). In infested twig samples, dieback, no growth, or new growth may be observed. Here dieback is defined as the loss of current year twig growth and needle drop in one-year old or older twigs. We quantified tree response by assigning a numeric value, such as -1, 0, or +1, respectively. The positive numeric values are further assigned a value that corresponds to percent tip growth observed. Percent was determined by number of tips with growth divided by the total counted x 100. We assigned a -1 value to samples with dieback, 0 value to samples with no growth and 1 to samples with <10% tip growth, 2 to 10-25% , 3 to 26-50%, 4 to 51-75%, and 5 to >75% tip growth. The mean of the 4 samples generated a tree rating. The number of trees with each rating was tallied and percent trees in each health category, calculated. Scans will be made of twigs sampled annually for three years to record tree response. Of 4 branches taken, one was selected at random for bioassay assessments; three were forwarded to the USDA-FS/Villa Nova for imidacloprid residue analyses. One terminal and two laterals (3) x 3 years of growth x 64 treatments generated 192 analyses. Annual twig growth (ATG) was measured in cm and recorded for the most current three increments (e.g.., 2007, 2006, 2005 growth). ATG is the internodal length measured from terminal bud or bud scar to bud scar. Current season twigs are light orange-brown, whereas three year old twigs are dark brown or gray (1). In non-infested hemlock, we cut to three bud scars for our sample. However, in infested twigs with no growth or dieback, we count back from the terminal bud, two bud scars. Dendrochronological checks were made to assist in dating infested twigs. A transverse section of the xylem was made by scalpel and the number of growth rings counted. In healthy samples, one year old xylem was formed in the current year (e.g., 2007), whereas one year old xylem in twigs with no growth was formed the year prior (e.g., 2006). In samples with dieback (needle loss), we assumed, a., terminal growth was formed the year prior, and b., dated that wood to the previous year. In this case, twig samples to two-year old wood (i.e., formed in 2005 or earlier) were evaluated. Dendrochronological checks were cross referenced to twig color. Therefore, light orange-brown twigs with one-year growth ring were formed in the current year; darker brown twigs with one-year growth ring were formed the year prior or those with 2 year growth rings were formed three years prior.

HWA and EHS scale infestations were assessed by microscopic examination. Each branch generated 5 branchlets, each of which were assessed from the most current twig. Three growth increments were assessed when current growth was present. When samples had no current year growth, the last two growth increments were assessed. For HWA, the number of live sistens was counted. Dead HWA on twigs were ignored. The number of HWA/cm was calculated. All EHS life stages present on needles were counted. The number of EHS was counted on 1 cm of needles of twig. The needles per cm were also tallied. The number of EHS was multiplied by the increment length with intact needles. EHS per year was determined by multiplying EHS by length of growth increment. EHS divided by number of needles was also calculated.

## Results

### Tree Treatments and Application Efficiency

Two applicators completed the (56 treated and 8 controls) treatments in two days. Application time varied with the method used. In terms of speed of application, the QUIK-jet injector was fastest, the Kioritz injector was intermediate and the Tree I.V., the slowest. Mean application times for the methods were 2, 5 and 10 minutes, respectively. The difference in time is a function of volume and tree (high) or soil (low) resistance to formulation absorption. For example, the Tree I.V. delivered 18 mLs/ injection site or 7.2x the volumetric capacity of the injection site. The uptake time is dependent upon tree transpiration and absorption into restrictive sapwood tracheids. The dosages applied by QUIK-jet injector, on the other hand were 0.6, 1.2 or 2.4x that of the volumetric capacity of the injection site. Therefore, QUIK-jet applications may be expected to be 3-12xs faster than Tree I.V. Soil injections met with little or no resistance to application. The Kioritz injector however is limited to 5 mL per stroke, and therefore requires numerous pump strokes to complete an injection, for example to deliver 960 mLs requires 192 pump strokes, which accounts for the intermediate time of application.

**HWA Densities and Annual Twig Growth**

Because of the high percent of dieback we observed in hemlock branches, only a sub-sample of 7 controls and 6 randomized treatments, were assessed for HWA and ATG. Eight (8) non-infested trees are included for comparison of annual twig growth. This data is presented in the Table 1.

Table 1

| | **1 year twigs** | | | **2 year twigs** | | | **3 year twigs** | | |
|---|---|---|---|---|---|---|---|---|---|
| | Twig length (cm) | HWA (n) | HWA/cm | Twig length (cm) | HWA (n) | HWA/cm | Twig length (cm) | HWA (n) | HWA/cm |
| Means UTC (n=35) | 0.92 | 0.29 | 0.32 | 3.74 | 1.17 | 0.31 | 4.73 | 0.67 | 0.14 |
| Means Treatments (n=30) | 1.88 | 2.57 | 1.37 | 4.51 | 0.73 | 0.16 | 4.53 | 0.57 | 0.13 |
| Means Non-infested (n=31) | 8.84 | --- | --- | 12.10 | --- | --- | 11.58 | --- | --- |

Statistical analyses were conducted in Minitab version 15. Statistical significance was observed for the number of HWA/linear cm for 2007 at 95% CI (p=0.034), but no significance was found in 2006 or 2005 twigs between treatments and controls. The number of HWA/linear cm ranged from a low of 0.13 to 1.37. Interestingly, the HWA in treatment trees were higher than in the controls, probably due to limited sample size. The highest numbers of HWA/cm were in current year twigs with the greatest growth. Low densities were observed on older twigs of either treated trees or controls. Twig measurements were made at the end of the growing season, treatments were in late August, and therefore this observation of increased growth is not a treatment effect. One-way ANOVA of 2007 twig length compared treatments, controls, and non-infested (healthy) hemlock indicate strong significance (p=0.000 at a 95% CI) between healthy and infested mean twig growths. Mean HWA/100 needles was calculated at 4.05, well below the threshold as described by Ward. The low HWA numbers and reduced twig growth are indicative of a remnant HWA population and late infestation. HWA infestation is likely older than the 3 years of growth analyzed. Subsequent twig checks to 5 year-old twigs, suggest that the trees first were infested in 2003.

**EHS Scale on Hemlock needles**

Mean numbers of EHS on untreated controls increase with twig age (p=0.002) at 95% CI. There were no significant differences in treatments (n=168) or control trees (n=24) suggesting that EHS pressures are the same across all treatments. Dieback from scale in hemlock has been reported when density reaches 10 EHS/needle (2). Mean scale density was low (0.09/needle) in the 2007 sample but growth was absent in 57.97% of the samples evaluated due to HWA infestation. Mean EHS densities were higher in 2006 and 2005 needles, with 0.93 and 2.43/needle, respectively. Second year (2005) needles represent the highest percentage of intact biomass; protecting extant needles is therefore critical to tree survival.

**Needle Density**

The number of needles per cm was also determined. Infested trees had mean needle density of 2.16, 7.37, 7.86/linear cm for 2007, 2006 and 2005 twig increments, respectively (n=69). Needle density was significantly lower in 2007 compared to the two years previous growth (p=0.000) due to HWA limiting growth. Non-infested trees had needle densities per year of 9.97, 6.74, and 4.87/linear cm, respectively (n=31). Mean 3-year needle density for non-infested trees was 7.19/linear cm.

**Precipitation in Asheville, N.C.**

Hemlock growth is sensitive to available moisture. Onken (1994) found that there was a direct relationship between the amount of rainfall that trees receive in a given year and the amount of new growth in the next year. The chart below summarizes monthly rainfall data in 2006 for Asheville, NC (3). The mean annual precipitation was 48.29", 103% of normal. The fall months in particular, were above normal. 2007 growth in non-infested trees was 8.8 cm compared to 1.4 cm in HWA infested trees. Based on this data, the trees received adequate moisture for growth in 2007. Infestation rather than moisture limited twig growth in the study trees. Monthly totals were also tallied for 2007; the mean annual precipitation was 34.39", 74.06% of normal. In non-infested hemlock, 2007 twig growth was less (though not statistically significant) than the previous two years (means of 8.8, 12.1, 11.6 cm, resp.). These drier conditions are not encouraging for hemlock recovery in 2008.

Table 2. 2006 Monthly Precipitation Data for Asheville, N.C.

| Rainfall month | rainfall (inches) | normal (inches) | departure from normal | % of normal |
|---|---|---|---|---|
| January | 3.58 | 4.06 | -0.48 | 88% |
| February | 2.55 | 3.83 | -1.28 | 67% |
| March | 0.91 | 4.59 | -3.68 | 20% |
| April | 4.58 | 3.50 | 1.08 | 131% |
| May | 1.69 | 4.42 | -2.73 | 38% |
| June | 5.16 | 4.38 | 0.78 | 118% |
| July | 2.81 | 3.87 | -1.06 | 73% |
| August | 7.12 | 4.30 | 2.82 | 166% |
| September | 7.80 | 3.72 | 4.68 | 210% |
| October | 2.93 | 3.18 | -0.25 | 92% |
| November | 4.52 | 3.82 | 0.70 | 118% |
| December | 4.64 | 3.40 | 1.24 | 136% |
| Yearly totals | 48.29 | 47.07 | 1.22 | 103% |

**Hemlock Health Scale**

Three basic conditions were observed in infested trees and compared to non-infested trees; these were dieback, no growth, or growth. Hemlock growth varies with tree age, environmental conditions and pest pressures. For this reason we grouped tree response into broad, descriptive categories, see Table 3.

Table 3 Hemlock Health Scale

| Numeric Scale | Observation of terminal (% annual twig) growth | Tree Health Descriptive |
|---|---|---|
| -1 | no growth and needle loss | very poor, dieback |
| 0 | no growth | very poor, no growth |
| 1 | <10% tip growth | poor |
| 2 | 10-25% | poor to below normal growth |
| 3 | 26-50% | below normal to normal growth |
| 4 | 51-75% | normal to healthy growth |
| 5 | >75% | healthy, normal growth |

The 2007 results appear in Table 4. 94.2% of the trees are in poor to very poor condition: 52.2% of infested hemlock rated very poor, with dieback, 30.4% rated very poor, with no growth and 11.6% rated poor. Healthy, normal growth was observed in non-infested trees, only.

Table 4.Hemlock Condition in 2007

| Tree Rating | Dieback | No Growth | Tip Growth <10% | 10-25% | 26-50% | 51-75% | >75% |
|---|---|---|---|---|---|---|---|
| | -1 | 0 | 1 | 2 | 3 | 4 | 5 |
| Totals (n) | 36 | 21 | 8 | 3 | 1 | 0 | 0 |
| % | 52.2% | 30.4% | 11.6% | 4.4% | 1.5% | 0.00% | 0.00% |

**Discussion**

This is the first of three evaluations. This paper includes preliminary evaluation of method, tree condition and a hemlock health scale that may be used by the forestry manager to assess tree condition. Three application methods and three formulations were used either alone or in combination for therapeutic and protective activity in hemlock. The QUIK-jet microinjections were simple and fast (2 minutes/tree), but designed for low volume applications. This is an efficient method because the injection site is configured to accept the low volume applied and therefore independent of tree uptake (i.e., transpiration) rate. The dosages applied are designed for insecticidal but not necessarily for long term activity. The Tree I.V. applications took more time (10 minutes/tree) to apply because uptake of large volumes depends on tree transpiration rate. Larger dosages were designed to deliver a higher concentration of active ingredient for increased insecticidal activity, especially in larger diameter (>19" DBH) (>47.5 cm DBH) trees. The larger dosage exceeds the volumetric capacity of the injection site; therefore uptake rate is dependent upon absorption into the axial tracheary elements. However, uptake of large volumes in restrictive tracheid trees can be slow (Cruziat et al., 2002). The advantage of applying a systemic insecticide directly into the sapwood is that activity against the pest is at least in theory, quicker than when soil applied. The Kioritz soil injector applied MERIT 75WSP simply and relatively quickly (5 minutes/tree). Soil application of imidacloprid has been demonstrated to show insecticidal activity over time (McClure, 1987), even years (Cowles et al, 2006). However, time to activity is generally slow. Imidacloprid moves slowly in soils because of its high octanol-water partition coefficient (0.57) and relatively low water solubility (0.5g/L) (4). Though less likely to leach in soils; it may be only slowly taken up into trees. Combining tree and soil injection may provide the forestry manager with advantages, such as quick and simple applications for immediate and long term titration of imidacloprid into the sapwood tissues. At the time of writing, no imidacloprid residue data was available for the samples taken at 70 DAT.

The low HWA numbers and greatly reduced twig growth are indicative of a remnant HWA population and late infestation. HWA infestation is likely older than the 3 years of growth analyzed. Although a low (17.5%) percentage of trees had growth, this HWA reservoir has the potential to re-infest trees. 87.5% of the trees evaluated were treated with imidacloprid, of these, 47.9% of hemlocks had little or no growth; while 52.1% had dieback symptoms. Webb et al reported that trees with little growth, but no dieback recovered quickly producing dense foliage following imidacloprid treatment compared to trees with dieback, which recovered, but at a slower rate.

Of concern is EHS in the compromised hemlock. Although the numbers were less than 10 EHS/needle, EHS pressures were evident across treatments. The highest EHS pressures were in the 3-year old needles (mean of 2.43 EHS/needle), the lowest in current year needles (0.09 EHS/needle). The low EHS density in current growth is a function of tip dieback. EHS in the 2-year old growth was 0.93/needle. Second year (2005) needles represent the highest percentage of intact biomass; protecting extant needles is therefore critical to tree survival. Also of concern is soil moisture. Although 2006 precipitation was 103% of normal, 2007 was drier (74.06% of normal). In non-infested hemlock, 2007 twig growth was less (though not statistically significant) than the previous two years (means of 8.8, 12.1, 11.6 cm, resp.). Hemlock recovery is at least in part dependent on adequate soil moisture, especially in treatments of soil injected imidacloprid.

A possible source of error may be in the determination of the age of wood evaluated in samples with dieback. Although we counted growth rings (dendrochronology), cross referenced twig color and presence or absence of axillary buds (i.e., origin of current year growth), dieback may have progressed more slowly than assumed in the analysis. Although we standardized counting back two bud scars in these samples, they may have been older. Incidental checks of HWA pressure on samples date to 5 years, therefore it is possible that the twigs cross checked to two growth rings may have dated back to four, rather than to three, years.

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