Production and Evaluation of Eastern Hemlocks (*Tsuga canadensis*) Potentially Resistant to the Hemlock Woolly Adelgid (*Adelges tsugae*)

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Abstract. As the hemlock woolly adelgid (HWA) has spread throughout the forests of the northeast, it has killed countless eastern hemlocks while possibly sparing a small minority of trees with some degree of innate resistance. There are, as yet, no published records of HWA resistance in T. canadensis, but on rare occasions, a relatively healthy tree (referred to as 'putatively resistant') is found amidst a devastated stand. As HWA susceptibility is influenced by many factors including plant nutritional status and prior attack by HWA and other insects, we chose to vegetatively propagate cuttings from putatively resistant forest trees in order to grow and evaluate these plants for HWA resistance under standardized greenhouse conditions. We found that a combination of IBA and NAA rooting hormones gave the best rooting results of cuttings taken in mid winter. When 6-month old rooted plants were inoculated with adelgids, there was much lower settlement on putatively resistant plants than on control plants (collected from T. canadensis growing in northern MA).

Keywords. Hemlock woolly adelgid, Adelges tsugae, Tsuga canadensis, vegetative propagation, pest resistance.

Introduction. The hemlock woolly adelgid (HWA), Adelges tsugae Annand, has become a serious pest of the native North American eastern hemlock species Tsuga canadensis (L.) Carriere and T. caroliniana Engelm.. Several Tsuga species resist HWA including western hemlock, T. heterophylla (Raf.) Sarg.; mountain hemlock, T. mertensia (Bong.); Chinese hemlock T. chinensis (Franch.) E. Pritz.; and Japanese hemlocks (T. diversifolia (Maxim.) Mast., and T. sieboldii Carriere) (McClure 1992, Pontius et al. 2002) Although these resistant species can be quite useful in managed landscapes, they are not suitable for replacing eastern hemlocks in forested settings. If HWA-resistant eastern hemlocks could be identified and propagated, they would be useful for both managed landscapes and stand-level reforestation of HWA-devastated areas. To date no one has demonstrated HWA resistance in eastern hemlocks. Although a few relatively healthy trees can be found in otherwise devastated areas, it is unclear if their health is linked to innate resistance or growing conditions (McClure and Chea 1992, Pontius et al. 2006, Preisser et al. 2008). HWA resistance should ideally be evaluated under standardized conditions in order to isolate the effect of innate plant resistance. Although it is possible to collect and grow seed from putatively resistant plants, this approach is problematic since the resulting seedlings may have at least one susceptible parent. For documenting resistance levels among selected clones, vegetative propagation quickly yields a population of progeny that are genetically identical to parent plants. Del Tridici (1985) and Jetton et al. (2005) successfully propagated hemlocks from branch cuttings and Butin et al. (2007) experimentally inoculated hemlocks with adelgids – two techniques that are central to our research. In this paper we discuss the selection, propagation, maintenance, and evaluation of hemlocks that may be resistant to the HWA. Materials and Methods Rooting Trial One. As part of a larger research effort (Preisser et al. 2008), we surveyed 142 hemlock stands in CT and MA in 2005 for potentially HWA-resistant eastern hemlock trees. In forest stands where the mortality rate of mature hemlocks exceeded 95%, we sought mature (>10 m) trees that appeared healthy and largely free of HWA and the elongate hemlock scale Fiorinia externa (Marlatt). We report data on six putatively resistant hemlocks identified in our 2005 survey – two trees at each of three sites near the towns of East Haddam, Madison, and Old Lyme in CT. We also sampled a tree from the Arnold Arboretum (Jamaica Plain, MA) that was identified as potentially resistant by Peter Del Tredici.

Table 1. Trees from which cuttings were taken in 2005 and 2006.

Location	Site Code	Coordinates
Jamaica Plain, MA	AA	42.29° N 71.12° W
East Haddam, CT	BB 1	41.46° N 72.33° W
East Haddam, CT	BB 2	41.46° N 72.33° W
Madison, CT	M 1	41.38° N 72.63° W
Madison, CT	M 2	41.38° N 72.63° W
Old Lyme, CT	OL 1	41.37° N 72.37° W
Old Lyme, CT	OL 2	41.37° N 72.37° W

Cuttings were taken in late July 2005 and in late January 2006 using a 4m pole pruner to clip healthy terminal growth from branches at varying heights. Cuttings were placed in plastic bags with wet paper towels and kept refrigerated at 4.4°C for a few days until they could be treated. Cuttings were trimmed to a uniform length of new growth: ~8 cm. for July cuttings and ~25 cm for January cuttings. The bottom 1/3 of each cutting was stripped of foliage, wounded on one side and the end cut at a 45°
angle with a grafting knife. Each cutting was then dipped in one of five rooting hormone treatments for five seconds. The rooting treatments consisted of the following combinations of IBA (indole-3-butyric acid) and NAA (1-napthaleneacetic acid): (1) Hormex© 45 (4.5% IBA) powder; (2) 1:1 aqueous dilution of Dip 'N Grow® (1% IBA) and 0.5% NAA) with water; (3) 1% aqueous solution of KIBA (potassium salt of IBA); (4) Hormodin® #3 (0.3% IBA) powder; and (5) no hormones (control).
The number of cuttings receiving each treatment varied according to the amount of usable plant material collected from each tree (Table 2).

Table 2. Cuttings taken in 2005 and 2006

	Summer cuttings			Winter cuttings		
Site Code	Taken	Treated	# of cuttings	Taken	Treated	# of cuttings
AA	NA	NA	NA	2/7/2006	2/8/2006	82
BB 1	8/5/2005	8/6/2005	22	1/28/2006	1/30/2006	85
BB 2	8/5/2005	8/6/2005	20	1/28/2006	1/30/2006	73
M 1	7/25/2005	7/26/2005	106	1/17/2006	1/20/2006	59
M 2	7/25/2005	7/26/2005	73	1/17/2006	1/20/2006	28
OL 1	7/25/2005	7/26/2005	67	1/17/2006	1/20/2006	25
OL 2	7/25/2005	7/26/2005	58	1/17/2006	1/20/2006	67

These cuttings were then stuck in 12cm-deep square flats (36cm by 36cm) filled with rooting media (2:1 horticultural perlite:milled peat moss by volume) and placed in a propagation bed in a greenhouse at the University of Rhode Island's East Farm (Kingston RI). The propagation bed measured 1.5 m wide by 30 m long, and had bottom heat and plastic-sheeting sides. There was also a misting system controlled by a leaf wetness gauge that averaged ~10 seconds of mist every 10 minutes. The greenhouse maintained a minimum temperature of 12°C while the cuttings received a constant bottom heat of 21°C, After 3 months we began weekly fertilization with 200ppm of 20-20-20 Peter's soluble fertilizer. The cuttings were kept under mist for another 2 months before potting.

Five months after taking cuttings, the plants were removed individually from the rooting media and the number of roots counted. Rooted plants were transplanted into 1.95l pots (15.2cm tall 13.3cm square), filled with Sun GroMetro Mix® 510 growing media and fertilized with a low level of Osmocote® fertilizer (1.0mg/m2 of standard release 19-6-12) in preparation for HWA resistance trials (described below).

Rooting Trial Two. Based upon the results of trial one, we repeated the rooting experiment using branches from the same CT trees as above. We also used samples of new plant material provided by cooperators in PA, and NJ (Table 3).

Table 3. Trees from which cuttings were taken in 2007.

LOCATION	SITE CODE	COORDINATES	TAKEN	TREATED
East Haddam, CT	BB 1	41.46°N 72.33°W	1/30/2007	2/1/2007
East Haddam, CT	BB 2	41.46°N 72.33°W	1/30/2007	2/1/2007
Pelham, MA	C 1	42.36°N 72.43°W	2/3/2007	2/5/2007
Pelham, MA	C 2	42.36°N 72.43°W	2/3/2007	2/5/2007
Madison, CT	M 1	41.38°N 72.63°W	2/1/2007	2/1/2007
Madison, CT	M 2	41.38°N 72.63°W	2/1/2007	2/1/2007
Walpack, NJ	NJ 1	41.13°N 74.91°W	1/26/2007	1/29/2007
Walpack, NJ	NJ 2	41.13°N 74.91°W	1/26/2007	1/29/2007
Walpack, NJ	NJ 3	41.13°N 74.91°W	1/26/2007	1/29/2007
Walpack, NJ	NJ 4	41.13°N 74.91°W	1/26/2007	1/29/2007
Walpack, NJ	NJ 5	41.13°N 74.90°W	1/26/2007	1/29/2007
Old Lyme, CT	OL 1	41.37°N 72.37°W	1/30/2007	2/1/2007
Old Lyme, CT	OL 2	41.37°N 72.37°W	1/30/2007	2/1/2007
Drums, PA	PA 1	41.07°N 75.92°W	2/12/2007	2/19/2007

One hundred cuttings per tree were treated with Dip 'N Grow® the best rooting treatment identified in the previous trail. The plants used in this trial were propagated in the same greenhouse and maintained as in trial one. Cuttings were trimmed to 25cm and treated as in the previous season with one exception:

because some of the cuttings were infested with elongate hemlock scale, all cuttings were dipped into a 1% mixture of horticultural oil insecticide (Sunspray™ Ultra-fine) and allowed to dry before rooting treatment.

Rooting treatments for trial two included: (1) a 1:1 ratio of Dip 'N Grow® and water; (2) a 1:2 ratio of Dip 'N Grow® and water (3) and no-hormone tap water control. The 100 cuttings per tree were divided into eight five-cutting groups for each of the two concentrations of hormone (=80 total cuttings), and four five-cutting groups for the control (=20 total cuttings). Each group was placed randomly within a flat. All of the cuttings were inserted into identical flats of the same rooting media and maintained in the same location, under the same conditions of the first trial. Six replicates were used in each flat to maintain equal spacing within the flat.

The cuttings were allowed 5 months to develop roots before being removed from the media for examination and transplantation in late June, 2007. Prior to being transplanted into individual pots, each surviving cutting was rinsed and given a rating of 0-3 as a combined measure of root number, root length, and overall size of the root system. A 0 rating indicates that the cutting was still alive at the end of five months but did not produce any roots. A 1 rating of 1 indicates the cutting developed few short roots in fair condition. A 2 rating indicates a denser root system of 5-10 main roots with many root hairs. Finally, a 3 rating indicates a dense root system of greater that 10 highly branched main roots densely covered with root hairs (Fig. 1).

Figure 1. Root ratings 0 (left) through 3 (right). HWA exposure: In May 2006 the successfully-rooted cuttings from rooting trial one were individually potted in 15.3 cm tall x 14 cm square pots with Sun Gro® Metro Mix 510 growing media. Plants were maintained in the greenhouse under ambient light and temperature and fertilized weekly through a proportioner set to deliver 200 ppm of nitrogen using a liquid 20-20-20 fertilizer. At the same time, we also potted HWA-resistant western hemlocks and HWA-susceptible T. canadensis. The western hemlocks used were two year old bare rooted seedlings shipped from Western Maine Nurseries in Fryeburg, ME. The control eastern hemlocks used were randomlyselected seedlings collected from the Cadwell Memorial Forest (Pelham, MA).

In April 2006 HWA-infested branches were cut from eastern hemlock trees on the URI Kingston campus, and from Saint Patrick Cemetery in Fall River, MA. The branches were placed in 20L buckets of water to keep the cut ends submerged and held at 4.4°C to delay crawler emergence. In early June these infested branches were used to inoculate five plants from each plant source (only two western hemlocks) using the protocol described in Butin et al. (2007). Inoculated plants included the six putatively-resistant CT trees, one tree from the Arnold Arboretum, and the western hemlock and eastern hemlock controls. In these inoculations an 8cm twig of heavily HWA-infested hemlock foliage was inserted into a florist's water pic and twist-tied to the stem of each potted hemlock. HWA eggs hatched within a few days and all plants were initially exposed to immense numbers of crawlers. Inoculated plants were held in the greenhouse until mid-August 2006 when they were examined with a hand lens and settled adelgids were counted.

Results

Trial 1. By mid-May 2006 the successfully-rooted cuttings had developed a root mass adequate for transfer into individual pots. January cuttings were more successful than the smaller July cuttings. Most cuttings from the July collection failed to develop roots even after eight months in the mist bed, and the few that did root died several weeks after being potted. The best rooting results were obtained from January cuttings with 1:1 solution of Dip 'N Grow® and Hormex© 45 powder treatments, both of which gave over 60% rooting success for some trees. However, the Dip 'N Grow® treatment yielded the best rooting overall (Table 4).

Table 4. Results of 2006 winter cuttings (number rooted/number treated).

SITE CODE	HORMEX© 45	1:1 DIP 'N GROW®	1% KIBA	HORMODIN® 3	CONTROL
AA	0/18	12/18	2/18	0/10	0/18
BB 1	11/17	14/17	6/17	8/17	1/17
BB 2	9/15	8/15	5/15	10/16	2/12
M 1	2/14	6/15	0/15	0/15	NA
M 2	5/7	4/7	5/7	0/7	NA
OL 1	3/7	1/6	2/6	0/6	NA
OL 2	0/17	3/17	0/17	0/16	NA
Totals	30/95	48/95	20/95	18/87	3/47
% Rooted	31.58%	50.53%	21.05%	20.69%	6.38%

Trial 2. The second trial had a slightly lower overall rooting success rate than trial one. The 1:1 concentration of Dip 'N Grow® in 2007 resulted in 35.9% (Table 5) vs. 50.5% in the previous year. Only one of six CT trees (M2) had rooting success exceeding the 2006 average. Although the difference between the two hormone concentrations was negligible, both hormone treatments significantly increased percent rooting compared to the control group (Fig. 2). The same relationship among treatments can be seen in root ratings (Fig. 3.)

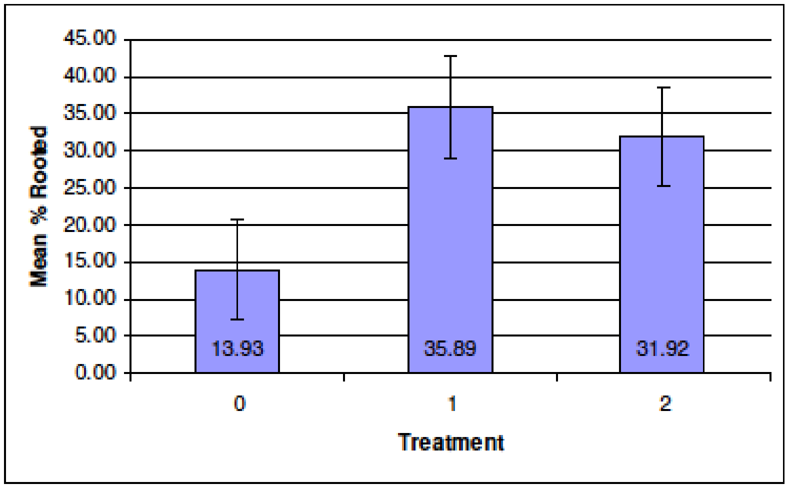
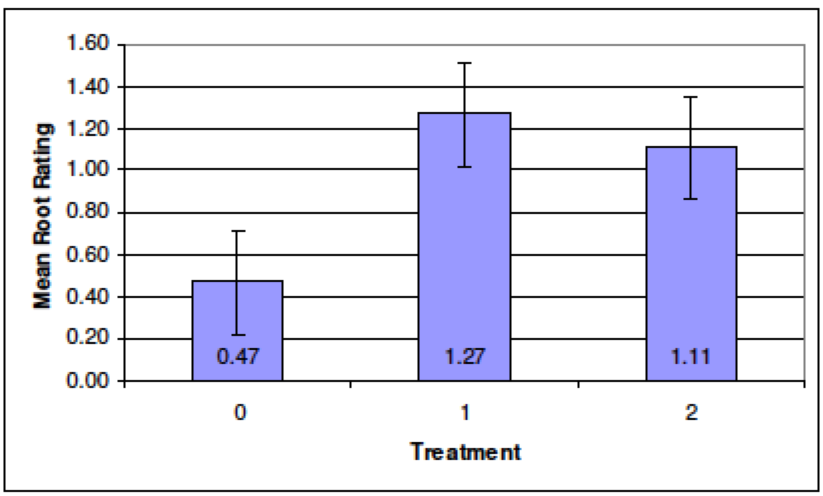


Fig. 2. Mean number of cuttings rooted. Treatment 0 = control, Treatment 1 = 1:1 ratio of Dip 'N Grow® to water, Treatment 2 = 1:2 ratio of Dip 'N Grow® to water.



Hemlock Adelgid Exposures. By August 2006 there were substantial differences in numbers of settled adelgids among the plant groups (Fig. 2). There were far fewer adelgids on the putatively resistant eastern hemlocks from CT than on the fieldcollected controls. However, the plant from Arnold Arboretum appeared quite susceptible to HWA. The western hemlock had the fewest settled adelgids – slightly lower, on average, than the CT plants (Fig. 4). Due to mortality of some of the plants between the time of inoculation and examination, numbers of replicates among treatments are not equal and one of the trees could not be evaluated.

Discussion

Rooting. Our research shows that winter cuttings are an efficient way to propagate T. canadensis, with average rooting success ranging from about 30% to 50% when using the most effective rooting hormone. All hormone treatments increased rooting success relative to untreated controls, with Dip ‘N Grow® (IBA + NAA) being the most effective. It remained equally effective when diluted 1:1 and 1:2 with water. Del Tredici (1985) also had good success using IBA for rooting T. canadensis. Jetton et al. (2005) found no benefit from using NAA alone as a rooting treatment. Thus we are confident that IBA is important for rooting T. canadensis and it appears to be more effective when combined with NAA as in the commercial Dip ‘N Grow® product.

Table 5. Results of 2007 winter cuttings. Treatment 0 = control, Treatment 1 = 1:1 ratio of Dip ‘N Grow® to water, Treatment 2 = 1:2 ratio of Dip ‘N Grow® to water. Root ratings (described in text) range from 0 – no roots through 3 – extensive roots.

	Plant Source														
Percent Rooting															
Treatment	BB1	BB2	C1	C2	M1	M2	NJ1	NJ2	NJ3	NJ4	NJ5	OL1	OL2	PA1	TRT. MEAN
0	0.00	25.00	15.00	10.00	5.00	10.00	0.00	10.00	30.00	20.00	10.00	25.00	20.00	15.00	13.93
1	20.00	40.00	7.50	12.50	47.50	55.00	30.00	37.50	40.00	35.00	40.00	42.50	27.50	67.50	35.89
2	35.00	57.50	25.00	10.00	46.67	45.00	17.14	15.00	20.00	20.00	27.50	57.50	35.56	35.00	31.92
Source Mean	18.33	40.83	15.83	10.83	33.06	36.67	15.71	20.83	30.00	25.00	25.83	41.67	27.69	39.17	27.25
Root Rating															
Treatment	BB1	BB2	C1	C2	M1	M2	NJ1	NJ2	NJ3	NJ4	NJ5	OL1	OL2	PA1	TRT. MEAN
0	0.00	0.83	0.63	0.50	0.25	0.38	0.00	0.25	0.60	1.00	0.38	0.75	0.31	0.63	0.47
1	0.67	1.15	0.50	1.00	1.20	1.57	1.06	1.13	1.93	1.31	1.96	1.30	1.28	1.67	1.27
2	0.88	1.25	0.91	0.56	1.42	1.07	1.14	0.96	1.33	1.13	1.29	1.38	0.97	1.22	1.11
Source Mean	0.52	1.08	0.68	0.69	0.96	1.01	0.73	0.78	1.29	1.15	1.21	1.14	0.85	1.17	0.95

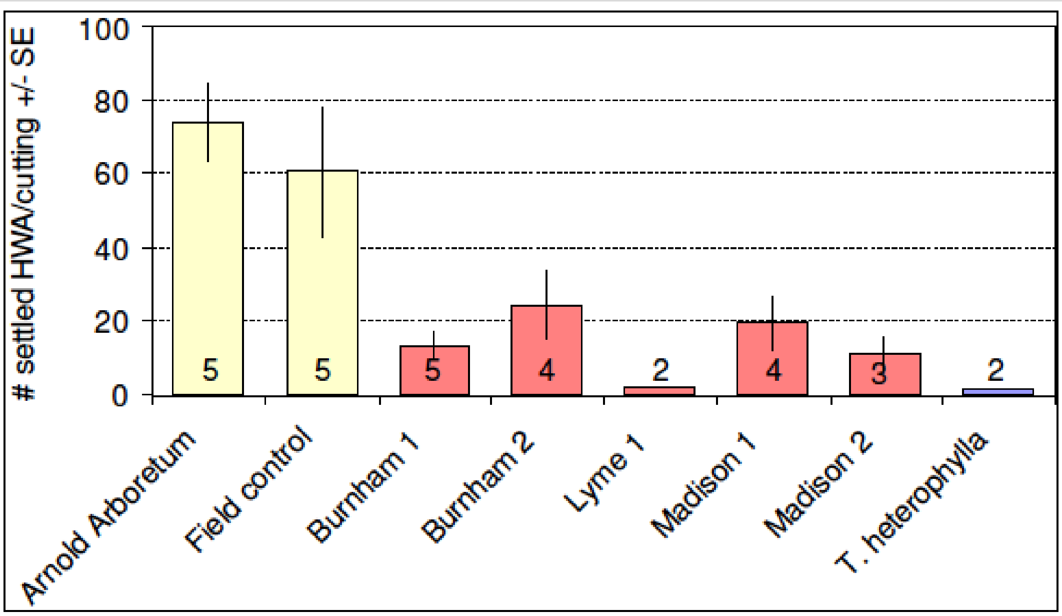


Fig. 4. Average number of settled adelgids per plant. Numbers in the columns refer to the number of rooted cuttings used in the evaluation of each tree.

Error bars represent standard errors. We had no success with summer cuttings – the few that rooted quickly died. However, Jetton et al (2005) had relatively good success (41% rooting of T. canadensis) with relatively small (3-6 cm) June cuttings. Although Del Tredici (1985) obtained better rooting success with January cuttings than with those taken in June, he pointed out in the same paper that summer cuttings of T. canadensis “might well prove to be the most economical way to produce healthy plants.” Summer cuttings develop a good flush of growth in the spring following an overwintering chill period. Winter cuttings generally don’t develop a good growth flush for about 18 months. Summer cuttings also do not require the use of bottom heat. Thus summer cuttings may warrant additional investigation – perhaps with more shade and better protection from summer heat than we provided in 2005.

Several factors other than timing and hormone treatment also contribute to the success of rooting hemlock cuttings. Larger cuttings did considerably better than the small summer cuttings, likely because of a larger reserve of carbohydrates, greater surface area for root development, and more leaf surface area for photosynthesis (Hoad and Leakey 1996). The presence of elongate scale on some of the experimental trees at the time of collection may have also played a role in reducing energy reserves to produce roots. Recent summer droughts in CT may have also affected rooting success (Hartmann et al. 2002). Furthermore, the trees used in this experiment were chosen for their potential resistance to HWA rather than their overall vigor, a factor which could have reduced rooting success.

Fertility management is crucial for plants used in adelgid experiments. Although a minimal amount of fertilizer is needed to maintain plant growth and vigor in pots of soilless media, overfertilizing promotes HWA attack (McClure 1991) and can mask innate plant resistance. Pontius et al. (2006) hypothesize that high N and P favor HWA attack while C and K inhibit these insects. Thus, we have opted for a moderate rate of fertilizer. After three months in the mist bed, cuttings are fertilized weekly with 200ppm of 20-20- 20 soluble fertilizer until potting. Subsequently, each plant receives 1.5 teaspoons of Osmocote® Plus 15-9-12 controlled release fertilizer. This dose provides 5-6 months of feeding at the lowest dosage listed on the label.

Adelgid Exposures. The adelgid transfer method of Butin et al 2007 was quite effective in establishing HWA on our test plants and all plants were quickly exposed to large numbers of crawlers. By the time we counted these adelgids six weeks after inoculation, there were substantial differences in adelgid populations among the various trees. Our results suggest that the putatively resistant trees from CT may be HWA-resistant and that these resistance levels may approach those of western hemlocks. This interpretation is supported by the fact that rooted T. canadensis cuttings from the Arnold Arboretum tree received identical treatment but became heavily infested. While exciting, these are only preliminary results from trials that were intended more to develop the process than to evaluate the trees. We started with only five plants from each clone and there was plant mortality during the trial. The relatively high mortality in CT plants (7/25) compared to the field controls and Arnold Arboretum plants (0/10) may reflect a greater stress on these plants which may have reduced adelgid survival. Other factors such as past infestation history on those sites may have also played a role in adelgid settlement in this trial. The real significance of this work is that we have a process to clonally propagate trees and evaluate them for HWA resistance.

In continuing tests we are maintaining rooted plants for an additional year before testing them for adelgid resistance. Following rooting, these plants are potted and maintained outdoors under partial shade and provided a moderate level of fertilizer. They are overwintered in an unheated greenhouse under a protective blanket in preparation for adelgid inoculation in the following spring. In addition to propagating putatively resistant trees, we are similarly propagating and maintaining several T.

canadensis clones started from plants on the northern edge of the current HWA distribution. These plants, which have never been exposed to HWA, will serve as controls in future tests. This additional time should allow plants to recover from the trauma of rooting and transplanting and minimize any carryover effects from growing conditions prior to our taking cuttings. Our evaluation of clonally-produced plants maintained under identical conditions eliminates the environmentally-induced variability in HWA/host plant interactions, allowing a very rapid and accurate assessment of resistance. If useful levels of resistance are found, we may refer back to our repository of potted clonal material for further experimentation and distribution.

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This entry was posted on Friday, June 25th, 2010 at 2:40 pm and is filed under Hemlock Woolly Adelgid.