Vegetative Propagation of American Elm (*Ulmus Americana*) Varieties from Softwood Cuttings[1]

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Abstract

Softwood cuttings of American elm varieties 'Jefferson', 'New Harmony', 'Princeton', 'R18-2', 'Valley Forge', and a tissue-cultured non-transformed control clone (BP-NT) were rooted using three different treatments to determine which method would be most suitable for small-scale propagation. The treatments included aeroponic chambers, an intermittent-mist bench in a greenhouse, and Grodan rootplugs soaked in a nutrient solution. The rootplug treatment had the highest percentage of rooted shoots (44%) followed by the intermittent-mist bench treatment (20%) and lastly by the aeroponics chambers (10%). The rooted cuttings from the rootplug treatment also looked substantially healthier and had more fresh growth four weeks after potting than the other two treatments. The Grodan rootplug treatment is recommended, but additional testing can be useful to improve the overall rooting percentage.

Index words: American elm, Dutch elm disease, *Ophiostoma ulmi*, rooting, softwood cutting, *Ulmus Americana*.

Significance to the Nursery Industry

This study observed the effects of various rooting treatments on the propagation of American elm. These treatments may be used as a model for commercial nursery growers interested in propagating similar varieties or new ones they produce through breeding or genetic engineering. Transgenic American elms that are engineered to carry anti-fungal genes would allow for true American elms to express resistance to Dutch elm disease.

Introduction

The American elm once graced urban areas throughout the eastern United States with its unique vase-like shape, turning city streets into green cathedrals of shade. These trees were not only preferred for their looks, but also their tolerance of compact, wet or poorly oxygenated soils and de-icing salts, making them an ideal urban species (3). The introduction of the pathogens responsible for Dutch elm disease (DED) almost wiped out the population of native American elm in two waves, the first in the 1920s by *Ophiostoma ulmi* and the second in the 1970s by the more virulent *Ophiostoma novo-ulmi*. More than 77 million trees were estimated to have been killed (5).

Dutch elm disease is a vascular wilt disease induced by the fungi *O. ulmi* and *O. novo-ulmi* attacking the tree's vascular tissues. Elm bark beetles transmit the fungus. When the beetles feed on the branches of trees, spores are introduced into the plant's vascular tissue. The fungal hyphae proliferate in the xylem, inducing tylosees formation and blocking water flow upwards thereby inducing the characteristic wilting symptoms (2). The fungus can also be transferred through the elm's natural root grafts into neighboring trees.

Restoring the American elm to its former range and glory has been a shared goal of many research groups over the past decades. Breeding programs have sought to develop elms that would display resistance to the fungal pathogens while still retaining the desired traits of the elms' specific growth habit and tree form. Early efforts in breeding were unsuccessful since all native North American elm species are susceptible to Dutch elm disease. Later programs began to examine crossings between North American species with European and Asian species (9, 10, 13).

Different varieties of interbred elm species have shown variable resistance to both Dutch elm disease and elm yellows, another important elm pathogen. R18-2 was developed at Cornell University and exhibited a good vase-like growth habit, and although it was moderately resistant to DED, it was found to be susceptible to elm yellows (11). 'Princeton', 'New Harmony' and 'Valley Forge' were developed by the USDA Forest Service and showed higher levels of resistance through a reduction of foliar dieback when inoculated with the pathogen (12). The 'Jefferson' variety is a full American elm, tetraploid, and exhibits high resistance to DED (7). BP-001 NT is a wild-type American elm clone from a commercial seedlot which was put into tissue culture to be used for genetic engineering research.

Once a potentially resistant clone has been developed, either through breeding or genetic transformation, increasing the number of ramets as quickly as possible will be critical for inoculation studies. If the clone demonstrates strong resistance, rapid multiplication will speed up its release as a new variety. Multiplication through tissue culture has the potential to make unlimited numbers of ramets, but is labor intensive and expensive compared with rooted cuttings. Very few methods of elm propagation have been reported. Aeillo and Graves (1) had success with rooting Pioneer elms by subirrigating through a perlite substrate without adding mist. Townsend and Douglass (12) used 'rooted stem cuttings' but no mention is made as to how they were grown. In this paper we examined three methods of softwood cutting propagation for the American elm.

Materials and Methods

Cuttings. Two identical trials were performed; each used three replicates of five cuttings per rooting treatment. In 2010, bud-break occurred several weeks earlier than usual, so greenwood cuttings approximately 6 cm long were taken first in late April and then in early May. The varieties 'Jefferson', 'New Harmony', 'Valley Forge', 'Princeton', 'R18-

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2', and a wild type control clone, BP-001 NT, were used. BP-001 NT was put into aseptic tissue culture, and brought back out through rooting without being transformed. Donor trees, approximately four years old, were grown on plots at the SUNY- ESF Lafayette Road Experiment Station, Syracuse, NY. The cuttings were wrapped in wet paper towels, placed in plastic bags, and brought back to the SUNY-ESF greenhouse. The cuttings were gently washed under running tap water and re-cut with a razorblade at a 45-degree angle immediately before the hormone dip. The basal end of each cutting was dipped to a depth of 2 cm (0.75 in) in a 14 mM IBA Clonex® Rooting Compound (Hydrodynamics, Lansing, MI) before being inserted into the treatment media. In addition to IBA, Clonex® Rooting Compound contains a gelling agent that makes the solution quite sticky, allowing it to adhere readily to the stem.

Aeroponic treatment. For the first rooting treatment, three replicates of five softwood cuttings of each clone were placed in three Power Cloner 45® aeroponic chambers (American Agritech, Tempe, AZ). All but the top 2–3 leaves were removed from each shoot, and the neoprene collars were placed halfway up the shoot. The reservoir was filled with 15 liters of nutrient solution containing 1% Clonex® Clone Solution (Hydrodynamics, Lansing, MI) and 1% Hydroguard (Botanicare, Tempe AZ). The solution's pH was adjusted to 5.5 using 1.0 N KOH; the solution was made fresh and replaced weekly. The humidity dome was sprayed with distilled water to increase humidity and then placed over the shoots. The chambers were placed on a light bench where they received approximately 40 µmol of illumination from Vita-Lite 5500K 40 Watt fluorescent bulbs (Duro-Test Lighting Inc. Philadelphia, PA). After three weeks, the number of rooted plantlets was recorded. The plantlets were then potted (see below) and moved to the greenhouse.

Rootplug treatment. Standard commercial planting trays (10 ½" × 20 ⅞") were prepared by soaking them in a 10% bleach solution for 15 minutes followed by three 5 minute rinses with distilled water. This helped remove possible algae and fungi from the trays. 90 Grodan 'A-OK' 1.5 in Starter Rootplugs (Rockwool International A/S, Hedehusene, Denmark) were placed in the standard size planting trays and thoroughly soaked in 1% Clonex® Rooting Solution (Hydro-Dynamics International, Lansing, MI) and 1% Aquashield (Botanicare, Chandler, AZ). Additional Clonex® Rooting Solution was added to the trays to a depth of 1 cm. Three replicates of five pre-dipped shoots of each clone were immediately inserted into the moistened Grodan rootplugs and covered with a tight-fitting clear plastic tray cover that was lightly misted with distilled water. The trays were placed on a light bench as described above for three weeks, at which time the number of rooted plantlets was recorded and the plantlets were potted (see below) and moved to the greenhouse.

Mist bench treatment. Ninety 7.5 cm (3 in) square pots were filled with moistened Perlite and placed under an intermittent-misting system in the greenhouse. Three replicates of five pre-dipped shoots of each line were inserted into the Perlite and 15 seconds of mist was applied every two minutes twenty-four hours a day. The shoots received only natural light, and were undisturbed for three weeks then checked for rooting. Rooted plantlets were then potted (see below).

Potting. All healthy rooted plantlets were potted in a mixture of peat:perlite:vermiculite (2:1:1 by vol) and placed under the mist bed for three days to improve acclimatization. They were then transferred to a greenhouse bench and watered as needed. Survivors at the end of the summer were planted at the SUNY-ESF Lafayette Road Experiment Station.

Results and Discussion

The only treatment to induce rooting in more than half the cuttings in a clone line was the Grodan rootplug treatment; 'Jefferson', 'New Harmony', and 'Princeton' all rooted more than 50% of the time (Fig. 1). The overall rooting percentage of the rootplug treatment was 44%. There was a large amount of variation between clones as well. The rootplug treatment, which worked well for 'Princeton', performed poorly with 'R18-2'. The mist bed, however, was significantly better for 'R18-2' than 'Princeton'. The mist bed treatment was more consistent than the other two treatments, but had a low overall rooting percentage of 20%. The aeroponic treatment worked for BP-NT, 'Jefferson' and 'New Harmony' but not at all for 'Princeton', 'R18-2' and 'Valley Forge'. Averaged over all the clones, the aeroponics treatment achieved 10% rooting percentage.

Univariate analysis of variance (ANOVA) showed that there was a significant difference in the number of plants rooted per rooting treatment, $F = 13.10$, p-value < 0.001. The differences between clone line, $F = 2.4$, p-value $= 0.043$ and the interaction between rooting treatment and clone line, $F = 2.4$, p-value $= 0.014$ were both significant at $\alpha = 0.05$.

A month after potting, the plantlets were grouped by treatment and photographed. Fig. 2 shows the dramatic differences between the cuttings four weeks post-potting. The mist bed cuttings lacked fresh new growth, as did the more spindly aeroponics cuttings. The rootplug cuttings, by contrast, were lush and vigorously putting out new growth.

The best rooting method overall for elm softwood cuttings was the Grodan rootplug treatment. The other two methods did not fail completely; however, they would need to be refined further in order to be considered reasonable treatments. While the aeroponic system had been used previously with great success, in this experiment it was very prone to algal and fungal overgrowth, even when using the Hydroguard (Botanicare, Tempe, AZ), a 'beneficial bacteria'

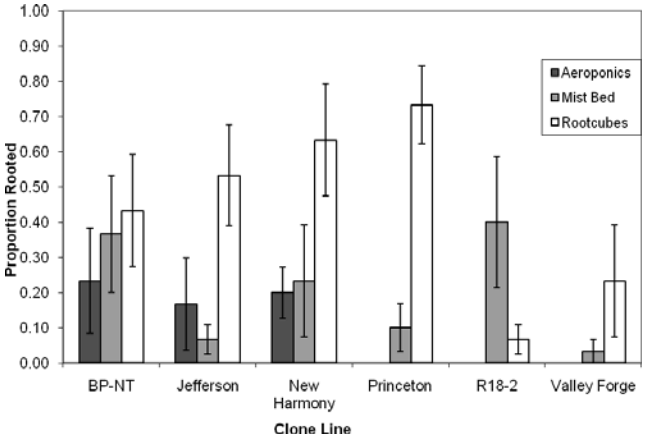

Fig. 1. Propagation of cuttings rooted by rooting treatment and clone line. Error bars are +/– one standard error of the mean.

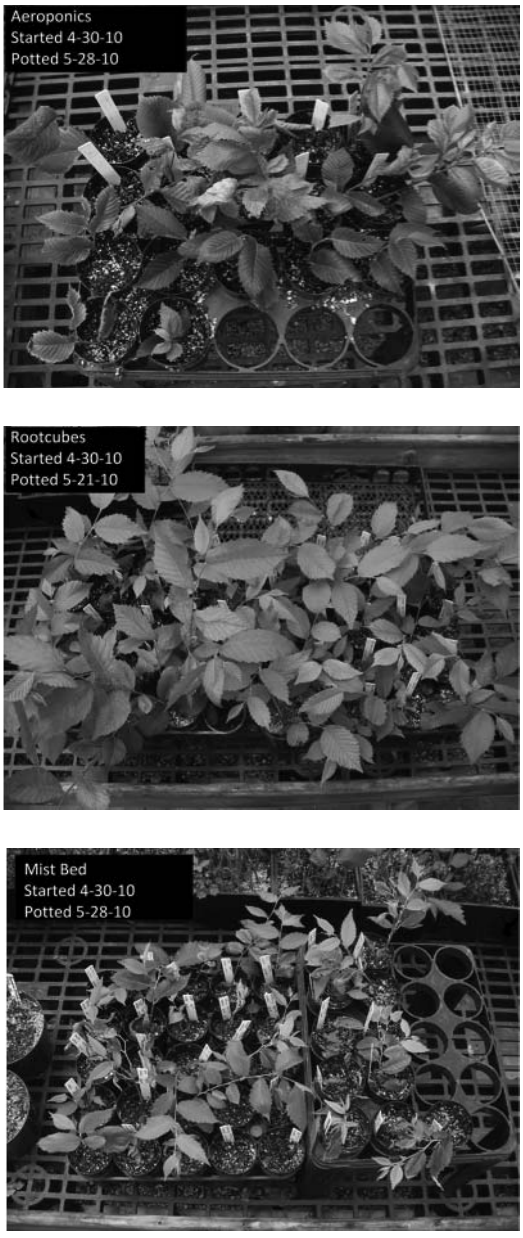

Fig. 2. Rooting treatments four weeks post-potting in aeroponics (a), rootcubes (b), and the mist bed (c).

mix. The tank solution became cloudy and green with algae very quickly and a weekly solution change may not have been often enough. Also, dipping the cuttings in the rooting compound most likely had little to no effect, as it would have been quickly washed off by the rather forceful and continuous spray hitting the roots. Including a rooting hormone directly in the tank solution would have been a better choice.

While the two trials were performed only three weeks apart, the physiology of the cuttings differed substantially. At the first cutting date, new growth ranged from 4 to 6 cm (1.5 to 2.5 in) in length and leaves were still less than one-half of full size. By the second cutting date, most of the shoots were partially lignified and at least ¾ of the leaves on the shoots had reached full size. Because there were substantial differences in flushing date among the clones, there were also differences in rooting rates for each clone between the two dates. 2010 had quite an early, warm spring, so the leaves flushed out at least three to four weeks earlier than expected. For example, New Harmony did not root at all in the first aeroponics trial when its new growth was very small compared to the other varieties, but three weeks later the cuttings were much larger. It was the only variety to root in the second trial aeroponic treatment; the other varieties had become too large and had already begun to harden off. This discrepancy between rooting success and the time at which cuttings were taken has been noted previously when two sequential trials of rooting *Ulmus* 'Pioneer' cuttings on a mist bed gave rooting percentages of 25% from April 17th cuttings and 79% from June 21st cuttings (1). However, in their second trial it took eight weeks for 55% of the 'Pioneer' elms to root; our study only ran for three weeks. A longer rooting time may have increased the low rooting percentage of the mist bed cuttings.

Another factor that led to the recommendation of the rootplug method was the vigor of the cuttings post-potting. The mist bed and aeroponic cuttings were removed from their substrates and potted essentially bare-root. In contrast, the roots of the rootplug cuttings were protected by the rootplug itself, which was directly planted into the potting mix. This protective effect has been seen in other studies using rockwool rootplugs (6, 8).

Of the three treatments studied, the Grodan rootplugs are recommended as the method to be used. Other small scale rooting systems should be tested as well, since 44% rooting is hardly sufficient for a species as purportedly easy-to-root as elm. Direct sticking methods are another option, where cuttings are dipped in a rooting hormone then inserted into potting mix. Other 'cells' of substrates, such as peat or coir plugs, could be tested against the rockwool plugs. The aeroponic system was a success in early trials, so if contamination of the tank solution can be reduced, it could be a good choice as well.

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