Influence of Water-soluble Plant Diffusates on Root Initiation and Growth in *Chionanthus virginicus* and *Vigna radiata* Stem Cuttings

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INTRODUCTION

White fringetree (*Chionanthus virginicus*) is an important and valuable plant for the nursery and landscape industries. Many consider white fringetree a premier specimen plant due to its bold foliage, refinement, dignity, and bloom characteristics (Dirr, 1990). White fringetree is extremely difficult to propagate by stem cuttings and is more commonly propagated by seed, which normally requires up to two years for germination. To date, there are no scientific reports of successful propagation with stem cuttings. Surveys in 1988 and 1995 ranked white fringetree as one of the most difficult plants to propagate (Gamstetter and Gulick, 1996). The surveys showed that although the tree was considered "unavailable," it was one of the top plants in market demand. Propagation difficulties make it an expensive plant in the trade and reduce its usage. By increasing the production of white fringetree, we could lower the cost and satisfy market demands.

Treatments of aqueous diffusates from terminal stems of easy-to-root plants have been known to influence rooting of stem cuttings. Previous research indicated the presence of at least four root-promoting substances, termed "cofactors," in diffusates from easy-to-root softwood cuttings (Girouard and Hess, 1964; Kawase, 1971). In difficult-to-root plants, these cofactors are postulated to be either missing or limited. Exogenous application of these missing cofactors to a difficult-to-root cutting may increase rooting success. The limited amount of research in this area supports this hypothesis. Further results have shown that cofactors have a strong synergistic effect with auxins on root formation of cuttings (Girouard and Hess, 1964; Kawase, 1970, 1972; LeClerc and Chong, 1983). Three experiments were conducted using either black locust (*Robinia pseudoacacia*) or contorted willow (*Salix* 'Erythroflexuosa') water diffusates as a pretreatment on stem cuttings of white fringetree.

PROPAGATION OF WHITE FRINGETREE USING PLANT DIFFUSATES

Experiment 1. The experiment was initiated on 19 Feb. 1996. Fifteen uniform, dormant 3-year-old seedlings were selected and brought into the greenhouse. Plants were then transplanted into 2-gal containers using amended pine bark as a medium. Each plant was fertilized with 20 g of Osmocote (14N-14P-14K). In addition, plants were weekly fertilized with 200 ppm Peters 20N-20P-20K. Ambient lighting was supplemented with 150-W incandescent lamps to provide a 16-h photoperiod. Air temperature controls were set at 28C (82F) days and 17C (62F) nights. Cuttings were harvested 12 weeks later on 10 May 1996. Cuttings were stripped to the four

upper leaves and double wounded. Ten cuttings were placed in a willow diffusate (10 g willow diffusate per 100 ml H_2O) for a 24-h soak. Five other cuttings were treated with 3.0% IBA in talc and inserted in the medium. On the following day, the 10 cuttings that soaked in the willow diffusate were divided into two groups. Five of these cuttings were inserted into the medium and the other five were treated with 3.0% IBA in talc and inserted in the medium. The cuttings were arranged in a completely randomized design with five replications.

All cuttings were inserted in Dyna-flats (10 cm × 35 cm × 50 cm [4 inches × 10 inches × 20 inches]) with holes. Flats were filled with a mixture of moistened shredded peat and sand (3 : 1, v/v) to a depth of approximately 7 cm (3 inches), and placed in a propagation greenhouse. This greenhouse was covered with off-white corrugated fiberglass. The cuttings received ambient light and no heat. Cuttings received 15 sec of mist every 12 min from 6:30 AM to 7:30 PM. White fringetree cuttings were harvested after 70 days on 19 July 1996, and evaluated for rooting. Four out of five cuttings propagated using the willow diffusate followed by IBA rooted. Three of the four cuttings rooted heavily, with over 35 roots and an average root length of 8.3 cm (3.3 inches). The fourth cutting rooted moderately well, with 12 roots and an average root length of 8.3 cm (3.3 inches). The only other treatment that induced rooting was the 3.0% IBA with 20% rooting; rooting was poor, with only five roots and an average root length of 6.0 cm (2.5 inches).

Experiment 2. The experiment was conducted on 9 July 1996. This experiment was designed to test an additional easy-to-root plant diffusate, black locust. This experiment used conditions similar to the previous experiment except that the diffusate concentration was higher (680 g per 3.8 liters H_2O [24 oz gal^{-1} H_2O] per 180 cuttings). This experiment was arranged as a completely random design using a 3 × 3 factorial arrangement of treatments with IBA talc (0%, 0.8%, 3.0%) and diffusates (a tap water control, locust and willow diffusates). There were 20 replications per treatment. Cuttings were maintained under a 3.0-mm (0.125 inches) polyethylene tent and provided with intermittent mist. After 76 days on 25 Sept. 1996, white fringetree cuttings were harvested and evaluated for rooting.

Out of 180 cuttings, only one produced roots. This cutting, which had been treated with black locust diffusate followed by 3.0% IBA, produced only four roots. However, over 60% (12 out of 20) of the cuttings treated with either diffusate and followed by IBA developed callus on the wounded area. Cuttings treated with IBA alone developed callus at a rate of 5% (1 out of 20). In some species, callus formation may be a precursor to adventitious root formation (Hartmann et al., 1997). These results suggest that the diffusates may be beneficial in promoting roots of white fringetree, even though these cuttings failed to root. A contributing factor may have been the age and health of the stock plant. The plant was in its mature phase and showing signs of decay and deterioration. Generally, the more juvenile the plant the higher the success rate of rooting. Also, in general, the healthier the plant the better the chances for rooting.

Experiment 3. This experiment was conducted on 9 May 1997. Water diffusates were made using black locust and contorted willow, as described in Experiment 1, at two strengths: 1× (454 g per 3.8 $liters^{-1}$ H_2O [16 oz gal^{-1} H_2O]) and 2× (908 g 3.8 $liters^{-1}$ H_2O [32 oz gal^{-1} H_2O]) per 80 cuttings. Cuttings came from four different sources: (A) 4-year-old juvenile plants in 2-gal containers forced in a greenhouse on

17 Feb. 1997, similar to plants in Experiment 1; (B) juvenile containerized plants forced 4 weeks later than Source A; (C) juvenile plants grown in containers under outdoor nursery conditions; and (D) 5-year-old field-grown plants. In addition to 3.0% IBA in talc, full-strength Dip'N Grow (1.0% IBA and 0.5% NAA) was tested.

Cuttings were collected on 9 May 1997, and placed in diffusates for a 24-h steeping. After steeping, cuttings were treated with either 3.0% IBA in talc or a 5-sec quick-dip in Dip'N Grow. Cuttings were then randomly inserted into the same medium as Exp. 1. The experimental design was a completely random design using a $2 \times 2 \times 2 \times 4$ factorial with ten replications. Cuttings were maintained under similar conditions as in Exp. 2 except a polyethylene wall was placed across the propagation bench to deflect the air flow created by the exhaust fans. This was done to prevent the cuttings from drying out. After 73 days on 21 July 1997, white fringetree cuttings were harvested and evaluated for rooting.

A numerical rating scale of five classes was set up for the experiment, where 1 = no roots, 2 = poorly rooted, 3 = average number of roots, 4 = above-average number of roots, and 5 = heavily rooted. Cuttings evaluated at 3, 4, and 5 would be commercially usable. Two independent judges subjectively evaluated the cuttings for rooting by making the closest match to the representative cutting for each class. Data were subjected to analysis of variance and means separated using Duncan's multiple range test at the 5% level.

The three best rooting treatments came from cuttings taken from source-A (4-year-old juveniles forced early). When treated with willow diffusate (2× strength) and Dip'N Grow source-A cuttings had 90% rooting and a rooting scale of 3.7±1.3. Cuttings from source-A treated with willow diffusate (1× strength) and Dip'N Grow had 80% rooting and a rooting scale of 3.5±1.6, while cuttings treated with locust diffusate (1× strength) and Dip'N Grow had 70% rooting and a rooting scale of 2.9±1.5. Of the remaining 29 treatments, 15 treatments produced insufficient roots and the other 14 treatments failed to root.

The two most important rooting factors in Expt. 3 were cutting source and auxin source. Full-strength Dip'N Grow clearly gave better results than 3.0% IBA in talc. Although the first three cutting sources were from the same lot of containerized seedlings, their handling affected propagation results. The lot that was first brought into the greenhouse (17 Feb. 1997) and forced into growth rooted best. We attribute this to the physiological age and maturation of the cuttings. There may be a narrow physiological window where rooting is possible in these still somewhat juvenile fringetrees.

MUNG BEAN ROOTING BIOASSAYS

Four standard mung bean (*Vigna radiata*) bioassays were used to partially characterize and verify the effects of the diffusates. Diffusates were made from chopped, frozen locust or willow terminal stems placed in deionized (DI) water (10 g 300 ml^{-1} H_2O) and stirred for 24 h. This was used to test the effects on rooting of mung bean cuttings of either locust diffusate (LD) or willow diffusate (WD) (5 ml of diffusate with 10 ml DI water containing 8 ppm IBA). A second test used ethyl acetate extracts of each diffusate at pH 3.0 and 7.0 to determine the polar nature of the diffusates. The third tested decreasing serial dilutions of both diffusates. Diffusates were prepared by adding the following amounts of WD or LD to the respective test tubes and bringing the volume up to 15 ml using DI water: 33.3% (5.0 ml), 25.0% (3.75 ml),

16.7% (2.5 ml), 3.3% (0.5 ml), and 0% (15.0 ml H_2O). The fourth test used a silica gel thin-layer chromatography of LD and WD and their extracts at pH 3.0 to characterize indole compounds.

Mung bean seeds were grown in flats containing moistened Pro Mix BX in a growth chamber at 28C (82F) with an 18-h photoperiod for 7 days. On Day 7, cuttings were harvested by cutting off the seedlings' root systems 4.0 cm (1.5 inch) below the cotyledon node. The mung bean bioassay consisted of one mung bean stem cutting per test tube with solution. There was 15 ml of solution per test tube, which was monitored daily and replenished with DI water as needed. After the fourth day, all solutions were discarded and replaced with only fresh DI water. The number of roots were counted and recorded on the Day 10. All mung bean bioassays were replicated three times and their averages reported.

RESULTS OF MUNG BEAN ROOTING BIOASSAYS

Mung bean cuttings treated with locust or willow diffusate in combination with IBA, stimulated the production of roots more than IBA or either diffusate alone. These results suggest that LD and WD have an additive effect with IBA on rooting of mung bean cuttings. These results are similar to Hess's (1964) and Kawase's (1970, 1971) work in which diffusates with indol-3yl-acetic acid (IAA) showed an increased number of roots on mung bean cuttings. This additive effect of the diffusates could be caused by their ability to stimulate rooting and/or enhance the activity of IBA (Hess, 1964; Kawase, 1970, 1971; LeClerc and Chong, 1983).

Ethyl acetate extracts of each diffusate at pH 3.0 produced more roots than extracts at pH 7.0. The pH 3.0 ethyl acetate extracts of both locust and willow diffusates produced a rooting response in the mung bean bioassay equal to or greater than the straight diffusate. At pH 3.0, essentially all acidic components in the diffusate were protonated, making them considerably less polar and thus extractable by the ethyl acetate phase. Under conditions of neutrality (pH 7.0), the majority of ionizable compounds containing acidic groups were negatively charged and therefore not soluble in ethyl acetate. Thus, the compounds remained in the aqueous phase, perhaps accounting for the lower root-stimulating ability of the pH 7.0 extracts. Therefore, we deduce the root-stimulating compounds in the diffusates were ionizable compounds containing acidic groups.

Both diffusates at 33.3% concentration produced the highest mean number of roots. Diffusates at 25.0% concentration were second in root production, followed by 16.7% and 3.3% concentrations, and each was significantly different. Results of these experiments show that both diffusates induced rooting in mung bean cuttings in a linear fashion as concentration increased. Since the root stimulating effect with increasing diffusate concentration was linear and did not plateau, the concentrations required to elicit maximum root production were not established. None of the concentrations had an effect on root length, as all roots were similar (1.4 cm).

Silica gel thin-layer chromatography of LD and locust extract at pH 3.0 showed no detectable color bands when tested for indoles. WD showed five detectable color bands, which were pink and rose in character at Rf 0.05, 0.25, 0.35, 0.68, and 0.93. Willow extract at pH 3.0 showed four similarly colored bands at Rf 0.24, 0.38, 0.54, and 0.73. These colors may indicate the presence of indoles in the WD and willow extract at pH 3.0.

CONCLUSION

Results of this research support the use of easy-to-root plant diffusates followed by auxins to increase rooting of very difficult-to-root plants such as white fringetree. The mung bean bioassay demonstrated that root-promoting substances are in locust and willow diffusate and their pH 3.0 ethyl acetate extracts. Both willow diffusate and willow extract at pH 3.0 tested positive for indoles, but these were not identified. Easy-to-root plant diffusates as postulated by Hess (1959) and Kawase (1970, 1971, 1972) may be the missing ingredients needed to help overcome rooting failure in difficult-to-root plants.

This work is the first to show that locust and willow diffusates followed by auxins can influence rooting of white fringetree. Further work is currently under way using various types of locust and willow diffusate followed by conventional auxin treatments on white fringetree and other woody plant species. As of 25 Aug. 1997, all the rooted white fringetree cuttings are in healthy condition and are producing a flush of new growth.

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