Hormone. Woods liquid rooting hormone with active ingredients of indole-3-butyric acid (1.03%) and naphthalene acetic acid (0.66%) is used at 1 part hormone to 20 parts water for both hardwood and softwood. The cuttings are dipped for 3 to 5 sec.

Availability of Cuttings. Cuttings are taken from dedicated stock and from production plants in the nursery.

Method of Propagation.

Softwood Cuttings. These are taken from current seasons growth, 4 to 6 inches long with three nodes. Cuttings are taken, made, and bundled into groups of 25 in the field. Bundles are placed into blueberry lugs, when lugs are full they are transferred in a refrigerated truck. Several times per day, cuttings are transported to the greenhouse and stored in a cooler. Cuttings are misted and cooler temperature is maintained between 36 and 38°F. At sticking time, cuttings are removed from the cooler and a fresh cut is made. Cuttings are dipped into hormone and placed back into a blueberry lug. The cuttings are brought to a sticking crew and are stuck one per cell.

Hardwood Cuttings. Hardwood cuttings are taken in much the same manner. Cuttings 6 to 8 inch with 3 to 4 nodes are cut, made, and bundled into groups of 25. Cuttings are taken to a cooler in blueberry lugs and stored at 36 to 38°F. Cuttings are removed from the cooler, a fresh cut is made, and then they are dipped in rooting hormone. We do stick multiple cuttings per cell—two per 21-cell and three per 18-cell tray.

It is very important to use protective fungicides to minimize disease in the wet, humid conditions under mist. We have found that applications of Terrachlor-75 W.P. within 2 days after sticking reduces disease problems. Other fungicides are used on a 21-day schedule. They include a rotation of Cleary 3336, BannerMax, Terrazole, and Chipco.

RESULTS AND DISCUSSION

Our sales forecast drives our propagation schedule with demands at many different time frames. To accomplish those demands we have stretched our softwood production from April through September, and our hardwood production from November through March. This has allowed us to provide finished cell liners for our facilities in Michigan, North Carolina, Tennessee, and Louisiana. This program has allowed us maximum use of our facility and fulfills the demands of our sales needs.

Forcing Epicormic Sprouts on Branch Segments of Adult Hardwoods for Softwood Cuttings©

J. W. Van Sambeek
North Central Research Station, USDA Forest Service, 202 Natural Resource Building, University of Missouri, Columbia, Missouri 65211-7260 U.S.A.

John E. Preece
Department of Plant, Soil, and General Agriculture, Southern Illinois University, Carbondale, Illinois 62901-4415 U.S.A.

Mark V. Coggeshall
Center for Agroforestry, 203 Natural Resource Building, University of Missouri, Columbia, Missouri 65211-7270 U.S.A.

Branch segments cut from basal limbs of transitional or adult hardwood trees were forced in the greenhouse to initiate shoot growth from latent buds for the production of softwood cuttings. Forcing in February, March, and April produced 10 to 15 visible buds or elongating shoots per meter of branch wood, which was more than twice the number during any other month. On average from January through August, two to four shoots per meter of branch wood exceeded 4 cm in length and could be harvested as softwood or greenwood cuttings. During this period, the red and white oaks, white ash, and honeylocust yielded more sprouts than did black walnut, several walnut hybrids, hickory, pecan, and chestnut. Sugar maple was the least productive of the twelve hardwood species evaluated. Number of sprouts declined with increasing age of the trees. Manually watering trays daily or maintaining trays under intermittent mist throughout the day yielded more sprouts than continuous mist for 30 min each day or use of humidity domes. All four moisture regimes resulted in the production of more shoots than treatments with continuous bottom flooding.

INTRODUCTION

Vegetative propagation of hardwoods using cuttings taken from adult (flowering) trees generally yields few if any rooted cuttings unless stock plants are severely cut back to force epicormic or stump sprouts from latent or dormant buds. The meristem of most epicormic buds develops in the axils of the leaves during shoot and branch elongation and remain dormant within the bark (Kramer and Kozlowski 1979). Fontaine et al. (1999) indicated that the vascular cambium produces the new cells making up the vascular trace that subtends each bud allowing the dormant meristem to retain its juvenile traits. Dormant buds on basal branches cut from phenotypically superior trees or new cultivars as part of normal tree maintenance could be an alternative source of shoots for explants for in vitro culture or softwood cuttings for rooting if the buds could be forced to elongate into epicormic sprouts. In this study, we quantify epicormic sprout production on branch wood using several greenhouse-forcing environments (moisture regime and light level) to determine response related to the time when branch wood was cut, tree species, and tree age.

MATERIALS AND METHODS

Plant Materials. From Sept. 1994 to April 2001, one or more basal branches or the smaller of competing forks were intermittently harvested from over 160 5- to 40-year-old trees of 12 hardwood species. Trees were mostly open-grown trees growing in research arboreta, yards, or plantations. Height of basal branch cut and estimated tree age were recorded. Branch wood ranging from 2 to 10 cm in diameter was divided into 24-cm-long segments. Freshly cut branch wood from each tree was divided equally among each forcing environment being tested that year. Three to six segments were placed horizontally approximately 5 cm apart across plastic 1020 perforated trays filled with enough horticultural-grade coarse perlite to cover the lower half of each segment.

Forcing Environments. For most experiments, light in the greenhouse consisted of ambient light (approximately one-half of full sunlight) from the fall to the spring supplemented with low intensity sodium vapor lamps to maintain a 16-h photoperiod. During the summer, woven black polyethylene shade fabric was suspended over the benches reducing light to 30% of full sunlight. To test for etiolating effects, 80% shade fabric was suspended over selected benches reducing light to 10% of full sunlight. Perlite within the trays was kept moist by either (1) daily hand watering between the branch segments with the equivalent of 5 cm of precipitation across the tray, (2) covering trays with plastic humidity domes and hand watering between segments two or three times a week with 5 cm of water, (3) continuous misting for 30 min each morning yielding 5 to 10 cm of precipitation, (4) intermittent mist throughout the daylight hours using the same schedule as used to root cuttings (6 sec of mist every 6 or 10 min), or (5) continuously flooding the bottom 1 cm of nonperforated trays in addition to treatment 1 or 3 above.

Measurements and Analysis. Length of each live bud (< 0.6 cm long), sprout (> 0.5 cm long), and harvestable shoot (> 4 cm long) on each branch segment was recorded bi-weekly as well as length of dead or harvested sprouts used in the rooting trials. Information for each tray was summarized by source tree and forcing environment to determine percentage of branch segments producing buds or sprouts; number of days to first visible bud; number of buds, sprouts, and harvestable sprouts; length of the longest sprout or shoot; and combined length of branch segments. Data from more than 400 trays (experimental units) was subjected to analysis of variance using the General Linear Model (SAS Institute, Cary, North Carolina) for a completely random experimental design with unequal replication. The entire data set was used for time of year analysis. A condensed data set was used for all other analyses that excluded branch wood cut from September through December when epicormic sprouting is poor. The 5% t-test values were calculated as the $p = 0.05$ t-value for the error degrees of freedom multiplied by the square root of the expression two times the error mean square divided by the mean number of replications per treatment.

RESULTS AND DISCUSSION

When averaged across 10 species, different tree ages, and four forcing environments, two to four harvestable shoots (> 4 cm long) were forced per meter of branch wood from January through August (Fig. 1). There is a slight depression in sprouts per meter of branch wood if cut immediately after trees have flushed. The few harvestable sprouts produced from September through December occurred primarily on branch wood cut from two species, i.e., honeylocust (*Gleditsia*) and pecan (*Carya illinoinensis*). Unlike the number of harvestable shoots per meter of branch wood, the total number of visible buds and elongating sprouts varied considerably from January through August. Branches cut in February, March, and April had more than double the number of visible buds and sprouts than branch wood cut in the other 5 months. The oaks (*Quercus* sp.), white ash (*Fraxinus americana*), and honeylocust had the highest percentage of segments producing sprouts yielding between 14 to 17 sprouts per meter of branch wood when cut between January and August (Table 1). Branch wood from black walnut (*Juglans nigra*), several walnut hybrids, chestnut (*Castanea*), pecan, and other hickories (*Carya*) averaged between 6 and 10 sprouts per meter of branch wood. Sugar maple (*Acer saccharum*) had the highest percentage of segments failing to produce any sprouts and produced fewer sprouts per meter of branch wood than any of the other tested species. The high number of sprouts on oak branch wood was expected because most oaks naturally produce epicormic sprouts when trees are suddenly exposed to light following thinning (Schlesinger, 1986). The high number of sprouts on white ash was unexpected because this species does not produce many epicormic sprouts when dense forest stands are heavily thinned. The sugar maple results are somewhat surprising because sugar maple in dense stands shows an intermediate tendency to produce epicormic sprouts with heavy thinning. Henry and Preece (1997a, 1997b) also found sugar maple to be relatively unresponsive to greenhouse forcing of epicormic sprouts.

The number of days under forcing conditions before the first latent buds started to enlarge spanned from 2 weeks for honeylocust to more than 6 weeks for some of the hickories (Table 1). Most species required between 3 and 4 weeks. There is a trend for the number of days to first visible bud to decrease from 4 weeks in February to 2 weeks in April and May and then to gradually increase again to 4 weeks in September. Although the oaks and honeylocust produced the longest sprouts, each

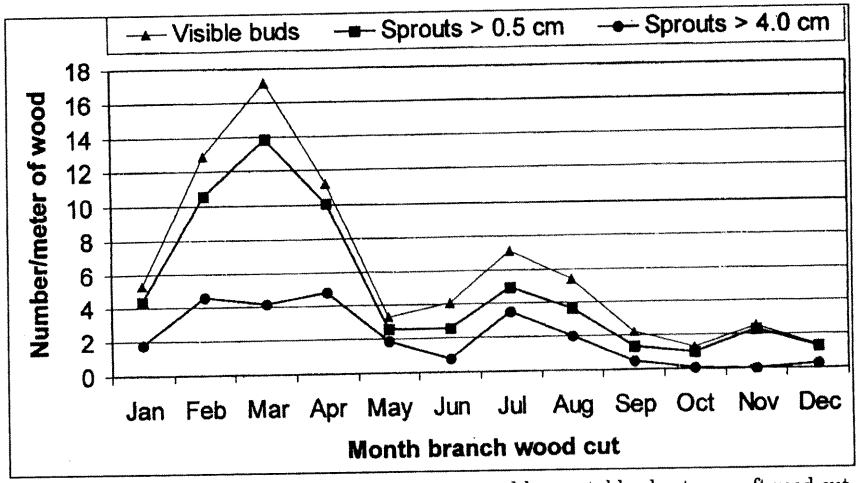

Figure 1. Mean number of visible buds, sprouts, and harvestable shoots as softwood cuttings per meter of branch wood for 12 hardwood tree species by the month the wood was cut from 5- to 40-year-old trees and placed under greenhouse forcing environments. The 5% t-test values for each line are 6.2 buds, 5.6 sprouts, and 2.8 shoots.

Table 1. Epicormic sprout productivity under greenhouse forcing on branch segments for twelve hardwood species cut from adult trees from January through August.

Variables evaluated	Trays[1] (no.)	Segments with sprouts (%)	Days to visible buds (days)	Sprouts per meter		Length longest sprout (cm)
				> 0.5 cm (no.)	>4.0 cm (no.)	
TREE SPECIES[2]:						
White oak species	30	84	21	15.8	9.1	14.2
Red oak species	22	84	21	17.0	11.9	17.0
Black walnut	131	66	27	9.6	2.4	5.0
Walnut hybrids	43	67	22	6.7	2.9	8.7
Chestnut	26	62	29	7.1	3.0	8.2
Pecan	24	62	26	7.8	0.8	3.5
Hickory	14	49	45	10.3	4.5	9.0
Sugar maple	10	31	34	1.7	6.5	5.1
White ash	53	74	24	14.0	4.0	7.2
Honeylocust	22	93	12	15.3	7.5	21.6
Significance[3]:	----	**	**	**	**	**
5% t-test values:	----	15	6	4.7	2.1	2.7
TREE AGE:						
5 to 10 years	135	79	25	14.7	4.7	9.2
10 to 20 years	55	82	27	11.1	5.3	9.1
20 to 30 years	52	51	29	6.6	2.7	6.7
30 to 40 years	108	54	25	6.7	1.8	5.6
Significance:	----	**	ns	**	**	ns
5% t-test values:	----	13	5	4.1	2.2	2.9

[1] A tray consisted of 3 to 6 pieces of branch wood (24 cm long) from one tree placed under one of several forcing environments. Tray responses were used in the ANOVA and separation of means procedures.

[2] Except for honeylocust, species are listed as to potential to naturally produce epicormic sprouts when trees in dense stands are suddenly exposed to light following thinning (Schlesinger 1986).

[3] ns, *, and ** used to designate nonsignificant or significant at the P <0.05 or 0.01, respectively.

showed a different growth pattern. Sprouts of the red and white oak species showed episodic growth with rapid stem elongation followed by leaf expansion. In contrast, sprouts of honeylocust showed indeterminate growth with continuous elongation of stems and leaves until either the tips desiccated, developed black tip necrosis, or shoots on more apical branch wood produced flowers. Most of the other species showed determinate growth in elongating stems and leaves until setting a terminal bud. Only pecan failed to produce harvestable shoots that could easily be used as softwood or greenwood cuttings.

Both the percentage of branch segments producing buds and the number of elongating sprouts decreased with increasing tree age (Table 1). In contrast, the position along a branch or the main bole from which the branch wood was cut showed no consistent pattern for either the number of segments producing sprouts or the number of sprouts per meter of branch wood. Preliminary studies using stem segments of 10- to 15-year-old white oak and black walnut yielded differences between species and trees within species, but not for height within each tree for number of sprouts per meter of stem wood. Likewise, the diameter of the branch wood showed no consistent pattern for either the number of segments producing sprouts or the number of sprouts per meter of branch wood when analyzed across the ten species. Henry and Preece (1997b) reported a species dependent response among maple species on effects of branch wood diameter on epicormic sprouting that might explain the inconsistent response across the ten hardwoods in this study.

The traditional method of manually watering daily between the branch segments generally resulted in the most sprouts and the longest sprouts being produced (Table 2). Using intermittent mist throughout the daylight hours produced similar results. There was a trend for branch wood from upland species to produce more sprouts by manual watering and bottomland species to produce more sprouts under intermittent mist. Choice of species may explain why Preece et al. (2002) reported that intermittent mist was a more effective forcing environment than manually watering daily. Forcing with 30 min of continuous mist each day resulted in the fewest sprouts per branch segment being produced, especially harvestable sprouts, suggesting this technique was the least effective at removing toxic exudates from the perlite medium. Using humidity domes to cover the trays to reduce both water use and labor requirements was slightly more effective than daily misting. Forcing environments that retained approximately 1 cm of standing water in the bottom of the trays favored high microbial populations and few sprouts (Van Sambeek and Preece, 1999; Preece et al., 2002).

Under light between 10% and 30% of full sunlight, no differences were found in number of branch wood segments producing sprouts or number of sprouts initiated (Table 2). Bassuk et al. (1986) reported that 90% shade was adequate to grow stock plants to produce etiolated softwood cuttings with enhanced rooting. In our study, 90% shade achieved with a 50% reduction in light level through the greenhouse roof and 80% shade fabric resulted in minimal elongation of internodes or leaves on harvestable shoots.

Many of the longer shoots (>10 cm) were harvested as softwood or greenwood cuttings, treated with various auxin formulations, and inserted in several different medium before placing under intermittent mist or within a subirrigation system (Van Sambeek et al., 1998b; Coggeshall and Van Sambeek, 2001). Without the use of fungicides, a high percentage of the softwood cuttings were quickly infected with stem-rot-causing fungi or black tip necrosis (Coggeshall and Van Sambeek

Table 2. Effect of moisture regime and light level on forcing epicormic sprouting of branch wood from January through August.

Treatments	Trays (no.)	Segments with sprouts (%)	Visible buds per meter (no.)	Sprouts > 0.5 cm per meter (no.)	Sprouts >4.0 cm per meter (no.)	Longest sprout (cm)
MOISTURE REGIME:						
Water daily	26	88	18.4	15.9	10.1	11.6
Intermittent mist	34	79	17.2	14.9	7.5	11.0
Daily 30 min mist	76	68	13.4	10.8	3.4	7.1
Humidity domes	74	69	12.4	11.1	4.2	9.2
Significance[1]:	---	ns	*	ns	**	*
5% t-test values:	---	13	4.6	4.2	2.3	3.0
LIGHT LEVEL:						
10 % full sunlight	40	65	9.2	8.3	2.8	7.0
30 % full sunlight	37	67	6.8	6.2	2.3	7.3
Significance:	---	ns	ns	ns	ns	ns
5% t-test values:	---	16	3.0	2.8	1.3	2.0

[1]ns, *, and ** used to designate nonsignificant or significant at the P <0.05 or 0.01, respectively.

2001; Preece et al., 2002). Although our rooting results have been discouraging, successive rooting of cuttings from greenhouse-forced epicormic sprouts have been reported for several species of maples, white ash, northern red oak, and European birch (Cameron and Sani 1994; Harner 1989; Henry and Preece 1997a; Van Sambeek et al., 1998b).

SUMMARY AND CONCLUSIONS

Although epicormic buds on temperate hardwoods are largely dormant meristematic tissues, these buds do respond to environmental signals and annually enter a state of dormancy in the fall when meristems cannot be forced to initiate new growth. The decreasing number of days required to produce visible buds when forcing during late winter suggests cold temperatures can gradually break this type of dormancy. The required cold period is species dependent and can be as short as a couple months, i.e. honeylocust and pecan, to 5 months for some black walnut cultivars.

With the exception of white ash and sugar maple, the number of sprouts that could be forced on branch wood follows the same trend as the number of epicormic sprouts produced on stems when trees in dense stands are heavily thinned and suddenly exposed to sunlight (Schlesinger, 1986). The number of harvestable sprouts was relatively constant from January through August; however, there were a high percentage of visible buds that failed to elongate suggesting there is a potential for many more harvestable shoots. Henry and Preece (1997b) demonstrated that increasing the length of the branch wood segments to 40 or 45 cm for maples would increase the number of harvestable cuttings. Although many of visible buds did not elongate sufficiently to make harvestable shoots, many buds resulted in short sprouts that could be utilized as explants for in vitro culture when forced under conditions with minimal direct contact with water (Van Sambeek et al., 1998a). Because epicormic sprouts arise from inactive meristems produced during the juvenile phase, we expect these explants will establish more easily in vitro, cuttings will root more easily, and cloned plants will show fewer somaclonal changes than shoots from more adult meristems.

Our results indicate greenhouse-forcing of epicormic sprouts on branch wood has several distinct advantages over more traditional approaches for obtaining softwood cuttings. Forcing sprouts on branches removed during regular pruning yields a higher value-added product from prunings than traditional methods of disposal as mulch. Trees and shrubs are not deformed by being radically pruned or hedged to produce softwood cuttings. For species that require actively growing cuttings, there is an 8-month window in which to produce softwood cuttings compared to a few months on field-grown stock plants. This should give the greenhouse manager greater flexibility in scheduling staff and use of facilities. The ability to produce softwood cutting in late winter while field-grown material is still dormant means more growing days on rooted cuttings before onset of fall dormancy. This may be especially important for species such as the oaks, maples, and walnuts where cuttings must produce a growth flush following rooting to overwinter successively and break bud vigorously the following spring (Shreve 1974; Hartmann et al., 1997).

Acknowledgments. The authors express our appreciation for staff assistance and use of facilities at the Tree Improvement Center, Carbondale, Illinois and the Horticulture and Agroforestry Research Center, New Franklin, Missouri. Special thanks go to Nadia Navarrete-Tindall, a postdoctoral cooperator, for completing data collections when authors

were unavailable. The authors wish to thank Bill Reid for providing the pecan branches and Sandra Anagnostakis and Ken Hunt for providing the chestnut branches. This work was partially funded by the USDA Forest Service and USDA Agricultural Research Service, Dale Bumpers Small Farms Research Center, Booneville, Arkansas. The results presented are the sole responsibility of the principal investigators and may not represent the policies or positions of the funding agencies.

LITERATURE CITED

Bassuk, N, B. Maynard, and **J. Creedon.** 1986. Stock plant etiolation and banding for softwood cutting propagation: Working towards commercial application. Comb. Proc. Intl Plant Prop. Soc. 36:599-604.

Cameron, A. D. and **H. Sani.** 1994. Growth and branching habit of rooted cuttings collected from epicormic shoots of *Betula pendula* Roth. Tree Physiol. 14:427-436.

Chen, P. Y. S., R. E. Bodkin, and **J. W. Van Sambeek.** 1995. Variation in pin knot frequency in black walnut lumber cut from a small provenance/progeny test,. pp. 488. In: Gottschalk, K. W. and S. L. C. Fosbroke (eds.), 10th Central Hardwood Forest Conf., U.S.D.A. Forest Service General Tech. Report NE-197, Radnor, Pennsylvania.

Coggeshall, M. V. and **J. W. Van Sambeek.** 2001. Development of a subirrigation system with potential for hardwood tree propagation. Comb. Proc. Intl. Plant Prop. Soc. 51:443-448.

Fontaine, F., E. Kiefer, C. Clement, M. Burrus, and **J. L. Druelle.** 1999. Ontogeny of proventitious epicormic buds in *Quercus petrea.* II. From 6 to 40 years of the tree's life. Trees 14:83-90.

Hackett, W. P. 1985. Juvenility, maturation, and rejuvenation in woody plants. Hort. Rev. 7:109-155.

Harner, R. 1989. Production and use of epicormic shoots for vegetative propagation of mature oak. Forestry 61:305-316.

Hartmann, H. T., D.E. Kester, F. T. Davies, Jr., and **R. L. Geneve.** 1997. Plant propagation Principles and practices. 6th Ed. Prentice Hall, Upper Saddle River, New Jersey.

Henry, P. H. and **J. E. Preece.** 1997a. Production and rooting of shoots generated from dormant stem sections of maple species. HortScience 32:1274-1275.

Henry, P. H. and **J. E. Preece.** 1997b. Production of shoots from dormant species of *Acer* as influenced by length and caliper of stem sections. J. Environ. Hort. 15:153-156.

Kramer, P. J. and **T. T. Kozlowski.** 1979. Physiology of woody plants. Academic Press, New York, New York.

Preece, J. E., J. W. Van Sambeek, P. H. Henry, and **J. Zaczek.** 2002. Forcing the tissue. Amer. Nurseryman 196(7):26-30, 32, 34.

Schlesinger, R.C. 1986. Pruning Appalachian hardwoods. pp. 221-227. In: Smith, H. C. and M. C. Eye (eds.). Guidelines for managing immature Appalachian hardwood stands. Soc. Amer. Foresters Publ. 86-02, Morgantown, West Virginia.

Shreve, L.W. 1974. Propagation of walnut, chestnut, and pecan by rooted cuttings. Pp. 20-23. In: Polk, R.B., ed., Proceedings, 8th Central hardwood forest tree improvement conference. University of Missouri, Columbia, Missouri.

Van Sambeek, J. W., L. J. Lambus, and **J. E. Preece.** 1998a. Production of epicormic sprouts on branch segments of adult black walnut for in vitro culture. Ann. Rpt. Northern Nut Growers 88:93-104.

Van Sambeek, J. W., N. E. Navarrete, and **J. E. Preece.** 1998b. Rooting softwood cuttings from forced stem segments of adult white ash. HortScience 33:503.

Van Sambeek, J.W. and **J. E. Preece.** 1999. Forcing environment affects epicormic sprout production from branch segments for vegetative propagation of adult hardwoods. Comb. Proc. Intl. Plant Prop. Soc. 49:399-401.

Micropropagation of Trillium Species©

Sherry Kitto
Department of Plant and Soil Sciences, College of Agriculture and Natural Resources, University of Delaware, Newark, Delaware 19717-1303 U.S.A.

INTRODUCTION

I work on native herbaceous perennials that I believe are garden worthy. Garden worthy, for me, describes a plant that is easy to establish and maintain in a garden. I think trilliums are garden worthy. Based on personal experience with trilliums in my own garden, I think of trilliums as being very forgiving garden plants as they are easy to establish, easy to maintain, and are tolerant of neglect. Most of the trilliums that are presently on the market do not begin to tap the available diversity in foliage variation, flower color, flower form, or plant form found in the genus or the individual species. There is an abundance of variation present in *Trillium discolor* leaf variegation (Fig. 1) and in *T. grandiflorum* flower form (Fig. 2) and color (Fig. 3). This is one reason why I decided to work with trilliums a few years ago. I am interested in being able to develop micropropagation protocols so that superior trilliums can be made generally available. Tissue culture of trilliums allows for the generation of large amounts of clonal material and the subsequent development of reliable, repeatable protocols.

A second reason for me to work with trilliums is related to the University of Delaware being located close to Mt. Cuba Center where a good friend and colleague, Jeanne Frett, is employed. I am fortunate that Mt. Cuba Center is a ready source of trillium plant material, which is essential to my tissue culture research program.

MATERIALS AND METHODS

For the micropropagation experiments, the plants used include one seedling clone of *T. discolor* and two seedling clones of *T. grandiflorum*. Mother cultures were maintained on Murashige-Skoog (1962)-based media supplemented with sucrose, glycine, and the growth regulators, BA and 2,4-D for rhizome proliferation, and IBA for root generation experiments. Media were liquid or gelled with Phytagar.

RESULTS

Some species are easier to clean up and establish in sterile culture. We have not been able to establish *T. grandiflorum* 'Quick Silver' after 4 years compared to *T. maculatum, T. rugelii,* and *T. decumbens* that were established on the first try. Leaf, ovary, and stem tissue explants from early spring growth have been the most reliably responsive in vitro.

A new protocol for surface disinfesting plant material has improved the ease with which we have been able to establish trilliums in vitro. Dr. Alice Waegel, a microbiologist at Neumann College, Aston, Pennsylvania, who worked in my laboratory Fall 2001, developed this protocol. This protocol involves placing the plant material in a medium that will encourage the germination and growth of the microbial contaminants during a 12 to 24-h period followed by a bleach treatment. This cycle of microbial growth followed by a bleaching death can be repeated any number of times and has greatly facilitated my work with trilliums. We now know that we