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Early Season Softwood Cuttings Effective for Vegetative Propagation of *Juglans cinerea*

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***Abstract. Juglans cinerea* L. (butternut) is a hardwood species valued for its wood and edible nuts. Information on the vegetative propagation of this species is currently unavailable. Our objective was to determine the conditions necessary for successful stem-cutting propagation of butternut. In 1999 and 2000, 10 trees (each year) were randomly selected from a 5- and 6-year-old butternut plantation located in Rosemount, Minn. Hardwood stem cuttings were collected in March, April, and May. Softwood cuttings were collected in June and July. K-IBA at 0, 29, or 62 mM in water and IBA at 0, 34, or 74 mM in 70% ethanol were tested for root induction on cuttings. The basal end of cuttings were dipped in a treatment solution for 10 to 15 seconds, potted in a peat : perlite mixture, and placed in a mist bed for 5 to 8 weeks. Rooted cuttings were gradually hardened off from the mist bed, allowed to initiate new growth, overwintered in a controlled cold-storage environment, and then outplanted to the field. For hardwood cuttings, rooting was greatest for those taken in mid-May (branches flushed out), 22% with 62 mM K-IBA and 28% with 74 mM IBA. Softwood cuttings rooted best when taken in June (current season's first flush of new growth or softwood growth 40 cm or greater) and treated with 62 mM K-IBA (77%) or 74 mM IBA (88%). For 1999, 31 out of 51 rooted softwood cuttings (60.8%) survived overwintering in cold storage and acclimatization to the field. For 2000, 173 out of 186 rooted softwood cuttings (93%) survived overwintering and acclimatization to the field. Chemical names used: indole-3-butyric acid-potassium salt (K-IBA); indole-3-butyric acid (IBA).**

Juglans cinerea L. (butternut) is a hardwood species native to the northeastern United States and adjacent Canada, ranging from New Brunswick to Georgia and west to Minnesota and Arkansas (Rink, 1990). It is valued for its wood and edible nuts (Ostry and Pijut, 2000). Quality butternut wood commands a high market price for many uses, including furniture, cabinets, veneer, paneling, specialty products, and carving. In addition, the wood can be stained to resemble black walnut. The edible nuts are oily and sweet, consumed by humans, and are an important food source for wildlife. *Juglans cinerea* is considered to be one of the most winter hardy (USDA Hardiness Zone range of 3 to 7) of the *Juglans* species (Dirr, 1990). Butternut is not a common species, and is seldom found in pure stands (Rink, 1990). Butternut canker disease, caused by the fungus *Sirococcus clavigignenti-juglandacearum* N.B. Nair, Kostichka and Kuntz (Nair et al., 1979), was first observed in 1967 and is causing widespread mortality, and is threatening native butternut tree survival (Cummings Carlson, 1993; Nair, 1999; Orchard et al., 1982; Ostry, 1997, 1998; Tisserat and Kuntz, 1984). The fungus, believed to be introduced (Furnier et al., 1999), causes perennial cankers on all aboveground parts of the tree. All age and size classes of trees are susceptible. The cankers cause the wood to turn dark brown to black in an elliptical pattern, which reduces the quality of the wood and renders many stands unmarketable. Eventually, the girdling effect of multiple coalescing cankers kills the tree.

Butternut is propagated easily from seed (Brinkman, 1974), but the canker fungus is also seed-borne (Orchard, 1984; Orchard et al., 1981). Vegetative propagation will be required to produce clones of genotypes selected for resistance to butternut canker disease. Grafting to black walnut rootstock (Ostry and Pijut, 2000) and in vitro culture (Pijut, 1997) of butternut are successful, but both are time-consuming processes with variable success. There are few reports of vegetative propagation of butternut through cuttings (Pijut and Barker, 1999). Rooting is reported for other *Juglans* species: *J. hindsii* (Lee et al., 1977), *J. microcarpa* (Shreve, 1990), *J. nigra* (Carpenter, 1975; Farmer, 1971; Farmer and Hall, 1973; Shreve, 1972; Shreve and Miles, 1972), *J. regia* (Gautam and Chauhan, 1990), *J. sinensis* (Kwon et al., 1990), and hybrids (Reil et al., 1998; Serr, 1964).

The objective of this study was to determine the conditions necessary for successful hardwood or softwood cutting propagation of butternut. Preliminary studies in 1998 with hardwood cuttings collected in May resulted in 12.5% rooting when cuttings were treated with 29 mM K-IBA, but only three out of six plants survived acclimatization to the field (Pijut and Barker, 1999). Softwood cuttings collected in June and July resulted in 63% to 70% rooting when cuttings were treated with 62 mM K-IBA or 74 mM IBA, but again only three out of 68 plants survived overwintering and acclimatization to the field. Based on these observations, for the years 1999 and 2000, we examined the effects of propagation time, type of cutting (hardwood vs. softwood), and K-IBA or IBA treatment on rooting percentage, number and length of roots regenerated, and overwintering survival. Success in propagating unselected, but canker-free, trees could be applied to propagating older, elite, disease-resistant clones, especially as selected grafted trees become established in plantations.

Materials and Methods

Hardwood cuttings. In 1999 and 2000, 10 trees (10 different trees each year) were selected from a 5- and 6-year-old butternut field plot located in Rosemount, Minn. Hardwood cuttings were collected at specific growth stages: dormant (30 Mar. 1999 and 29 Mar. 2000); budbreak (27 Apr. 1999 and 2 May 2000); and branches flushed out (13 May 1999 and 19 May 2000). Six to 12 cuttings (25 cm in length) were taken from each tree at each collection date. Two years of growth were pruned from the remaining branches (after last hardwood collection date) to encourage sprouting. Cuttings were placed in polyethylene bags, held on ice, and transported to the St. Paul greenhouse, where the cuttings were processed the same day. Stems were recut to between 20 and 23 cm in length. The basal 3 cm of cuttings were treated by dipping for 10 to 15 s in either 0, 29, or 62 mM K-IBA dissolved in deionized water or 0, 34, or 74 mM IBA dissolved in 70% ethanol. Cuttings were inserted vertically to a depth of 5 to 7 cm in Deepots™ (D40) (Stuewe and Sons, Corvallis, Ore.) containing a moist medium of 1 perlite : 1 peat (v/v). Cuttings in Deepots™ were placed under intermittent mist (15 s every 18 min) on a greenhouse bench with bottom heat maintained at 27 °C. Twelve hours of supplementary irradiance (from 0600 to 1800 HR) were provided by high-pressure sodium lamps (60 $\mu mol \cdot m^{-2} \cdot s^{-1}$ at bench top), and greenhouse temperature was maintained at 22 ± 2 °C. After 6 to 8 weeks, cuttings were harvested and number of cuttings rooted, number of roots per cutting, and individual root lengths were recorded. Data were analyzed using statistical programs for categorical data (Rugg, unpublished), log linear modeling (SPSS, 1998), and logistic regression (Mehta

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and Patel, 1996). The number of roots per cutting were square root transformed and the data analyzed using linear regression. The data for root lengths were log transformed and the data analyzed using linear regression.

Rooted cuttings were transplanted in Treepots™ (Tall One) (Stuewe and Sons) containing a moist medium of Sunshine SB-40 bark mix (Sun Gro Horticulture, Bellevue, Wash.), with 14N–14P–14K Nutricote® timed-release fertilizer, and returned to intermittent mist for 1 week. Rooted cuttings were then acclimatized to greenhouse benches and allowed to initiate new shoot growth. In late October or early November, rooted cuttings were placed in a cooler environment (15 °C or less) and lower light to induce dormancy. After a month, containerized cuttings were placed in polyethylene bags and moved to a controlled cold-storage environment (3 to 4 °C in darkness) for 4 to 5 months. After overwintering, the pots were returned to the greenhouse (March/April, the following year), and allowed to acclimatize to this environment. Pots were hand-watered as needed until budbreak, after which the pots were placed on an automatic drip irrigation system until they were field-planted in June.

Softwood cuttings. In 1999 and 2000, the same 10 trees (each year) as used for hardwood cuttings were used for softwood cuttings. Softwood cuttings were collected at specific growth stages: current season's first flush of new growth (8 June 1999 and 12 June 2000); softwood growth 40 cm or greater (15 June 1999 and 23 June 2000); shoots beginning to become lignified (1 July 1999 and 7 July 2000); and shoots starting to set bud (22 July 1999 and 19 July 2000). Six to 12 cuttings (40 to 45 cm in length) were taken from each tree at each collection date. All but two leaves were removed. Softwood cuttings were handled and processed as described for hardwood cuttings, except no bottom heat was used in the mist bed. After 5 to 6 weeks, cuttings were harvested and rooting data recorded. Data collection and statistical analyses were the same as described for hardwood cuttings. Rooted cuttings were maintained, allowed to initiate new growth, overwintered, and planted in the field as described for hardwood cuttings.

Results

Hardwood cuttings. There was no yearly difference ($P > 0.85$) in percent rooting success of hardwood cuttings. Rooting success did not vary by growth stage (collection date) in 1999 ($P > 0.45$) or 2000 ($P > 0.85$). Analysis of the data pooled across years found no differences by date of collection ($P > 0.40$). K-IBA or IBA were equally effective in promoting rooting ($P = 0.38$). There was a significant effect of the auxin concentration ($P = 0.007$). The controls (deionized water or 70% ethanol) were not different ($P = 0.11$) from the low concentration (29 mM K-IBA or 34 mM IBA), but did significantly differ ($P = 0.005$) from the high concentration (62 mM K-IBA or 74 mM IBA) (Table 1). The low and high concentrations did not differ ($P = 0.24$) from each other

Table 1. Effects of time of collection and rooting treatment concentration on rooting percentage, root count, and root length of *Juglans cinerea* hardwood cuttings[z].

Rooting treatment	Date of collection[y]					
	1999			2000		
(mM)	30 Mar.	27 Apr.	13 May	29 Mar.	2 May	19 May
			Rooting (%)			
0 K-IBA	0 a[x]	0 a	0 a	0 a	0 a	11.1 a
29 K-IBA	0 a	7.7 ab	10.5 ab	9.1 a	6.3 ab	16.7 ab
62 K-IBA	0 a	0 b	10.5 b	0 a	12.5 b	22.2 b
0 IBA	0 a	0 a	0 a	0 a	0 a	11.1 a
34 IBA	0 a	0 ab	0 ab	0 a	6.3 ab	11.1 ab
74 IBA	0 a	0 a	18.8 b	0 a	6.3 b	27.8 b
			Mean number of roots[w]			
0 K-IBA	---	---	---	---	---	1.0 a
29 K-IBA	---	1.0	1.0 a	1.0	4.0 a	7.0 a
62 K-IBA	---	---	6.5 a	---	1.5 a	4.8 a
0 IBA	---	---	---	---	---	2.0 a
34 IBA	---	---	---	---	7.0 a	1.0 a
74 IBA	---	---	5.0 a	---	2.0 a	3.1 a
			Mean root length (mm)[w]			
0 K-IBA	---	---	---	---	---	8.0 a
29 K-IBA	---	20	30.5 a	22	21.5 a	23.4 a
62 K-IBA	---	---	15.2 a	---	7.3 a	9.4 a
0 IBA	---	---	---	---	---	13.7 a
34 IBA	---	---	---	---	17.9 a	8.0 a
74 IBA	---	---	21.1 a	---	5.0 a	11.2 a

[z]Average sample size for each collection date and rooting treatment concentration, n = 14.
[y]Hardwood cutting growth stages: dormant (30 Mar. 1999 and 29 Mar. 2000); budbreak (27 Apr. 1999 and 2 May 2000); and branches flushed out (13 May 1999 and 19 May 2000).
[x]Mean separation within a column by LSD, $P \leq 0.05$. Letters indicate significant differences among means within a column for a given variable. "---" indicates that no mean was calculated because no observations were available.
[w]Means are per rooted cutting.

Table 2. Effects of time of collection and rooting treatment concentration on rooting percentage, root count, and root length of *Juglans cinerea* softwood cuttings.[z]

Rooting treatment	Date of collection[y]							
	1999				2000			
(mM)	8 June	15 June	1 July	22 July	12 June	23 June	7 July	19 July
				Rooting (%)				
0 K-IBA	0 a[x]	5.6 a	0 a	0 a	37.5 a	17.9 a	10.7 a	23.1 a
29 K-IBA	12.5 b	15.8 b	25.0 a	3.6 a	37.5 b	73.1 b	42.9 b	50.0 b
62 K-IBA	37.5 b	20.0 b	20.8 a	0 a	44.4 b	76.9 b	42.9 b	57.7 b
0 IBA	12.5 a	5.3 a	0 a	0 a	37.5 a	25.0 a	10.7 a	19.2 a
34 IBA	12.5 b	26.3 b	25.0 b	0 a	37.5 b	67.9 b	39.3 b	50.0 b
74 IBA	25.0 b	15.8 b	25.0 b	0 a	87.5 b	75.0 b	46.4 b	46.2 b
				Mean number of roots[w]				
0 K-IBA	--- a	14.0 a	--- a	---	3.0 a	3.5 a	1.7 a	3.8 a
29 K-IBA	1.0 b	6.3 b	2.7 b	3.0	7.0 b	9.7 b	3.9 b	8.0 b
62 K-IBA	3.3 c	6.6 c	10.1 c	---	13.8 c	7.3 c	13.3 c	11.2 c
0 IBA	1.0 a	3.0 a	--- a	---	3.0 a	3.1 a	1.7 a	3.0 a
34 IBA	27.0 c	16.0 c	14.4 c	---	9.7 c	20.6 c	10.7 c	12.2 c
74 IBA	14.0 c	16.3 c	20.6 c	---	18.7 c	21.0 c	7.4 c	6.0 c
				Mean root length (mm)[w]				
0 K-IBA	--- a	23.2 a	--- a	---	29.0 a	42.0 a	35.3 a	24.2 a
29 K-IBA	5.0 a	28.4 a	24.4 a	11.7	13.8 a	40.2 a	22.2 a	38.7 a
62 K-IBA	23.0 a	14.7 a	14.3 a	---	26.5 a	32.5 a	24.2 a	30.9 a
0 IBA	17.0 a	17.7 a	--- a	---	12.7 a	17.5 a	18.0 a	58.7 a
34 IBA	29.6 a	27.6 a	16.1 a	---	26.2 a	41.0 a	24.1 a	22.1 a
74 IBA	19.9 a	22.6 a	27.8 a	---	16.7 a	36.1 a	24.5 a	20.9 a

[z]Average sample size for each collection date and rooting treatment concentration, n = 21.
[y]Softwood cutting growth stages: current season's first flush of new growth (8 June 1999 and 12 June 2000); softwood growth 40 cm or greater (15 June 1999 and 23 June 2000); shoots beginning to become lignified (1 July 1999 and 7 July 2000); shoots starting to set bud (22 July 1999 and 19 July 2000).
[x]Mean separation within a column by LSD, $P \leq 0.05$. Letters indicate significant differences among means within a column for a given variable. "---" indicates that no mean was calculated because no observations were available.
[w]Means are per rooted cutting.

in rooting success, but a logistic regression on these data showed that a linear response to concentration accounts for nearly all of the variability in the data. The greatest rooting success was with 62 mM K-IBA (22.2%) and 74 mM IBA (27.8%) when hardwood cuttings were collected when the branches had flushed out (mid-May). There was no difference in the number of roots regenerated per hardwood cutting ($P > 0.1$). No differences ($P = 0.02$) were observed for root lengths except for year. For 1999, six out of eight rooted cuttings (75%) survived overwintering in cold storage and acclimatization to the field. For 2000, six out of six rooted cuttings (100%) survived overwintering in cold storage and acclimatization to the field.

Softwood cuttings. In 1999, the first three sample growth stages (collection dates) did not differ ($P > 0.95$), but the fourth sample growth stage was significantly lower ($P < 0.003$) from the others in rooting success (Table 2). In 2000, sample development stages showed no change in rooting success ($P > 0.40$). Auxin type did not affect rooting success ($P = 0.79$). However, there was a significant additive effect on rooting success of the year softwood cuttings were collected ($P < 0.0001$), as well as the auxin concentration of rooting treatment used ($P < 0.0001$). Cuttings propagated in 2000 had greater rooting success than those in 1999 (Table 2). Using K-IBA (29 or 62 mM) or IBA (34 or 74 mM) improved rooting success over the control, but there was no difference ($P = 0.39$) between the treatment levels. The greatest rooting success of 76.9% (62 mM K-IBA) and 87.5% (74 mM IBA) was achieved when softwood cuttings were taken in June (Fig. 1A). Using K-IBA or IBA produced more roots than controls ($P < 0.0001$), but the auxin concentrations did not differ in number of roots produced ($P = 0.43$). There were no differences between the controls (water vs. 70% ethanol) in number of roots produced ($P = 0.69$). In addition, the high concentrations of both K-IBA and IBA produced a similar number of roots ($P = 0.11$). However, the 34 mM IBA treatment resulted in significantly greater root numbers ($P = 0.0001$) than in the similar concentration of K-IBA. For K-IBA, the low concentration (29 mM) was not statistically different from either the control ($P = 0.14$) or the high concentration ($P = 0.08$). However, the high concentration produced more roots than the control ($P = 0.006$). Using K-IBA produced a linear dose-response relationship for number of roots produced. Softwood cuttings have a uniformly higher number of roots produced than do hardwood cuttings. Roots produced in 2000 were significantly ($P = 0.008$) longer (30 cm) than in 1999 (21 cm). Cuttings, from the current season's first flush of new growth (20.7 cm) and shoots beginning to become lignified (23.2 cm) development stages, had shorter roots than the softwood growth 40 cm or greater development stage (33.5 cm). There was no difference in root length from cuttings from the first, third, and fourth sample development stages. Mean root length was similar for all treatments and controls. For 1999, 31 out of 51 rooted softwood cuttings (60.8%) survived overwintering in cold storage (Fig. 1B) and acclimatization to the field. For 2000, 173 out of 186 rooted softwood cuttings (93%) survived overwintering and acclimatization to the field.

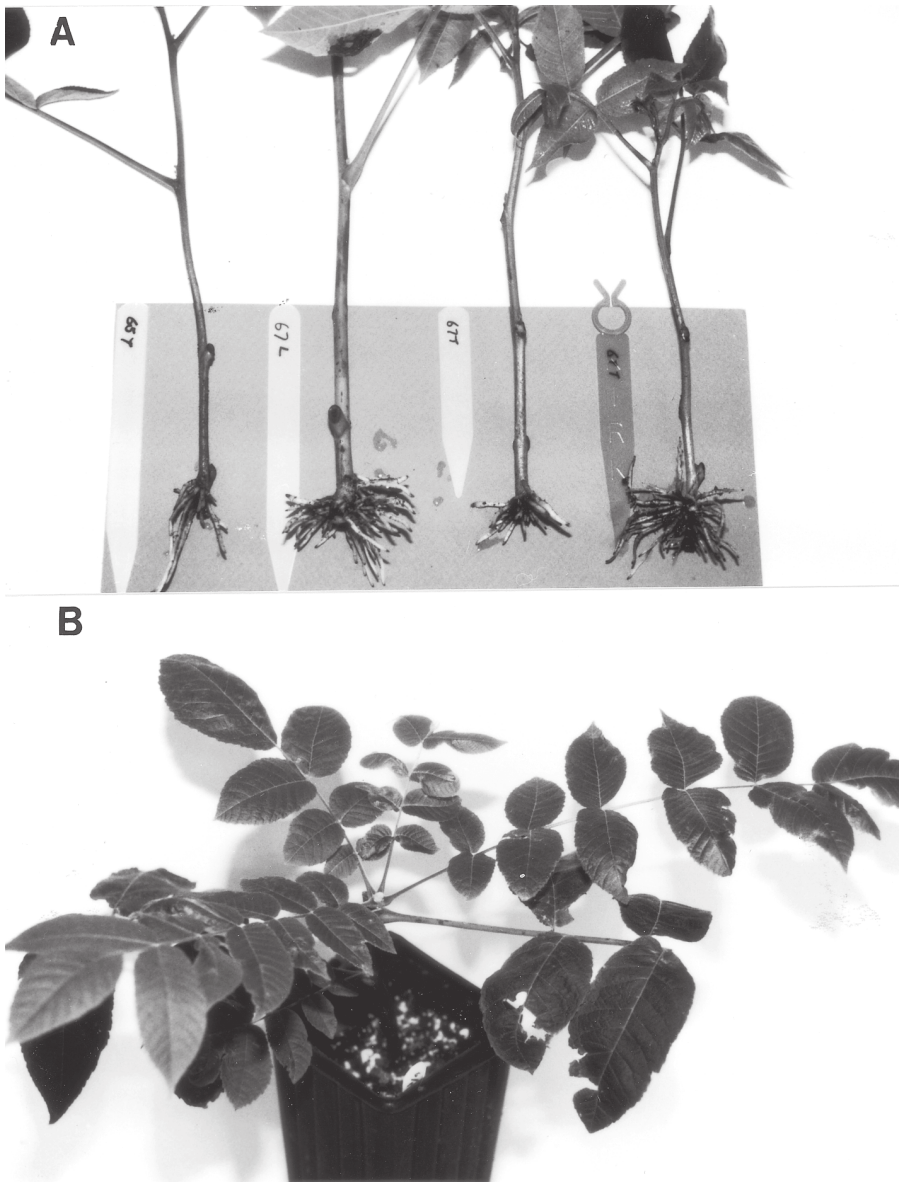

Fig. 1. (**A**) Rooted softwood cuttings of *Juglans cinerea* after 4 weeks under mist (from left to right: 34, 74 mM IBA, and 29, 62 mM K-IBA). (**B**) Butternut plant (softwood cutting, June collection, 62 mM K-IBA) ready for transplanting to the field.

Discussion

Propagation of 5- and 6-year-old *J. cinerea* from hardwood cuttings collected in mid-May (branches flushed out in the spring) was successful (10.5% to 27.8%), although at a low percentage. The greatest rooting success of 22.2% and 27.8% was achieved when hardwood cuttings were treated with 62 mM K-IBA or 74 mM IBA. Rooted hardwood cuttings were successfully overwintered in cold storage and planted to the field the following year. Propagation of other *Juglans* species by hardwood cuttings has also been investigated. Hardwood cuttings of 'Paradox' walnut (*J. hindsii* x *J. regia*) root with variable success (30% to 80%) after a quick dip in 20 to 39 mM IBA or a 24-h soak in 1 to 1.5 mM IBA (Reil et al., 1998; Serr, 1964). Dormant cuttings taken from 4- to 5-year-old hedges of *J. regia* rooted (14.5%) when treated with 74 mM IBA (Gautam and Chauhan, 1990). Carpenter (1975) reported 60% to 70% rooting of hardwood cuttings taken from mature black walnut trees (*J. nigra*), when soaked in ethephon for 6 h, but shoots did not elongate following this treatment and no survival was reported.

Softwood cuttings of butternut rooted better than hardwood cuttings. Softwood cuttings rooted (3.6% to 87.5%) at all collection dates and rooting treatment concentrations tested. Rooting success ranged from 15.8% to 87.5% when June cuttings (current season's first flush of new growth or softwood growth 40 cm or greater) were treated with 62 mM K-IBA or 74 mM IBA. The greatest rooting success (76.9% and 87.5%) was achieved when softwood cuttings collected in June 2000 were treated with

62 mM K-IBA or 74 mM IBA. Rooted softwood cuttings were successfully overwintered and transplanted to the field. Propagation of other *Juglans* species by softwood cuttings is also successful. Cuttings taken from adventitious shoots of eastern black walnut, *J. nigra* (from 1- to 135-year-old material), rooted 80% to 100% when treated with 25 to 49 mM IBA (Shreve, 1972; Shreve and Miles, 1972). Rooting (0 to 100%) of juvenile black walnut (2-year-old seedlings) occurs after extensive etiolation and auxin treatments (Farmer, 1971; Farmer and Hall, 1973). Lee et al. (1977) reported that cuttings taken from adventitious shoots of a 5-year-old *J. hindsii* tree had a significantly greater number of roots produced as a result of pretreatment with 2 N sulfuric acid prior to a 10-s dip in 15 mM IBA. Shreve (1990) reported rooting (percentage not stated) *J. microcarpa* (river walnut) softwood cuttings by treatment with 34 mM IBA and without the use of a mist bed.

Juglans species are, for all practical purposes, recalcitrant to routine, commercial-scale, vegetative propagation. However, successful propagation of *J. cinerea* on a commercial scale can be achieved if the type of cutting (softwood), date of collection (early season), auxin concentration (62 mM K-IBA or 74 mM IBA), and greenhouse parameters (mist bed, supplemental lighting, etc.) are carefully considered. To our knowledge, this is the first report on the successful vegetative propagation and transplantation of butternut to the field. Plantings in the field will be monitored for growth and survival over the next few years.

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