SURVIVAL AND GROWTH OF DEEP-PLANTED, IN-LEAF GRAFTS IN A GERMPLASM REPOSITORY OF CANKER-RESISTANT BUTTERNUT

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Butternut (*Juglans cinerea* L.), highly valued for its timber and nuts, occurs as widely scattered trees or isolated stands throughout the Central Hardwood region (Rink 1990). The introduced fungus *Sirococcus clavigignenti-juglandacearum* has rapidly cankered, girdled, and killed most of the butternut trees; however, a few trees that may be resistant to the fungus have been found (Furnier and others 1999). A program to graft scionwood from putatively canker-resistant trees and establish germ-plasm repositories within the natural range of butternut has been initiated as one of several conservation measures (Ostry and others 1994, 2002). Although butternut easily grafts to seedling rootstocks of black walnut (*J. nigra* L.) and heartnut (*J. ailanthifolia* Carr.), the overwintering of containerized grafts has been problematic.

The objectives of our study were
1) to evaluate the feasibility of mid-summer field-planting of in-leaf grafts,
2) to establish a germplasm repository in the southern part of the natural range, and
3) to test for differences in virulence of the pathogen as found in the Central Hardwood region.

Black walnut seedlings to be used as rootstocks were obtained from the Minnesota state tree nursery and Forrest Keeling Nursery in Elsberry, MO, and planted into 10-cm-square x 30-cm-tall plastic pots. In March 1994, scionwood from 31 putatively canker-resistant butternuts from Minnesota, Wisconsin, New York, and Connecticut were side grafted on the basal stem of the rootstocks (table 1). In mid-June, successful grafts were moved to open shadehouses. In mid-July 1994, in-leaf grafts were transported from Minnesota to the Tree Improvement Center near Carbondale, IL, which is located 10 km west of the Southern Illinois University-Carbondale campus.

Approximately, 2 and 6 weeks before establishing the planting, the area was broadcast sprayed with glyphosate to deaden the tall fescue sod. Planting holes, 60- to 75-cm deep on a 2.5- x 2.5-m spacing, were prepared with a tractor-mounted 20-cm-wide posthole digger in relatively moist Hosmer silt loam soil with a 45- to 60-cm-deep fragipan. To minimize any "clay pot" effects, the packed sidewalls of planting holes were cut with a tile spade and dropped into the bottom of each hole. Grafts were planted using soil from the borings with the rootstock root collar near the soil surface or deeper. Two to five grafts of each clone were randomly assigned to each block of eight rows of 13 trees.

Landscape fabric, 60-cm-square, was placed around each graft and covered with a 10-cm-deep layer of pine/hardwood mulch for weed control. Weed control in 1995 consisted of cultivation between the rows and spot application of glyphosate and simazine in 1996 and 1997. To protect grafts from rabbit and deer damage, a 20-cm-diameter x 75-cm-tall cylinder of 1.2-cm-square hardware cloth was placed over each graft and anchored with a bamboo pole. A wire stake with a stamped aluminum tag identified each graft as to clone number.

Overhead irrigation was provided for 24 to 48 hours after planting and weekly thereafter so that the precipitation averaged 2.5 cm per week. Many grafts settled deeply into the planting holes with many having graft unions below the soil line. In June and July 1995, basal sprouts on rootstocks were removed, but were allowed to grow thereafter if the scion had died. Survival of rootstock and scion, presence of cankers,

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Table 1.—*Clone number; ortet location by state and county, diameter at breast height (cm), appearance of cankers on putatively resistant trees; number of grafts planted; second, fourth, and seventh year survival of grafts on live rootstocks; and seventh year height, diameter at ankle height, and stems on butternut grafts in the southern Illinois germplasm repository*

Clone	Ortet location		Ortet	Cankers	Total	Surviving grafts on live rootstocks			Seventh Year[a]		
no.	State	County	DBH	present	Grafts	Yr 2	Yr 4	Yr 7	Ht.	DAH	Stems
			-cm-		-no-	-%-	-%-	-%-	-m -	-cm-	-#-
1	WI	Dunn	41.7	Maybe	16	50	14	14	2.6	5.0	1.0
5	MN	Rice	36.8	No	8	43	29	29	1.5	2.0	2.0
6	MN	Houston	44.2	No	16	21	11	0	xxx	xxx	xxx
20	WI	Rock	33.5	No	20	67	33	36	2.6	5.0	1.5
21	WI	Rock	35.1	No	12	27	17	17	1.9	4.3	4.0
22	WI	Rock	34.5	No	8	43	43	43	3.0	6.4	1.5
23	WI	Rock	32.3	No	8	50	50	60	1.6	2.2	1.6
29	WI	Pepin	41.9	One	16	33	22	12	2.3	4.4	1.0
32	WI	Marquette	30.5	No	12	45	0	0	xxx	xxx	xxx
34	WI	Pepin	46.0	Maybe	16	44	11	11	1.8	2.8	2.0
35	WI	Pepin	18.0	Small	16	54	38	38	1.4	3.4	2.0
36	WI	Burnett	31.0	Few	20	18	10	11	1.4	2.0	1.0
37	WI	Burnett	33.0	Branch	20	56	27	27	1.6	2.9	2.0
38	WI	Burnett	18.8	No	12	17	13	14	1.0	1.4	3.0
39	WI	Burnett	17.5	No	12	36	13	13	1.4	4.8	2.0
50	WI	Price	42.4	No	20	59	42	33	1.7	3.2	1.9
51	WI	Forest	46.7	No	20	50	30	20	1.0	2.1	1.2
52	WI	Forest	63.5	No	12	67	56	38	1.6	3.7	2.0
55	WI	Forest	46.1	No	12	50	0	0	xxx	xxx	xxx
56	WI	Langlade	36.8	No	16	38	13	14	1.2	3.3	2.0
57	WI	Langlade	36.3	Yes	8	17	0	0	xxx	xxx	xxx
61	WI	Rock	28.4	No	20	47	40	40	2.2	4.8	2.1
63	WI	Rock	29.2	No	8	29	14	0	xxx	xxx	xx
64	MN	Wabasha	44.5	One	8	57	25	33	2.2	6.3	1.5
69	MN	Hennepin	35.1	No	16	63	53	40	1.8	3.0	1.4
70	WI	Burnett	38.6	No	12	33	14	14	2.0	4.8	2.5
71	WI	Burnett	38.9	No	8	43	20	20	3.3	7.8	1.5
95	NY	Jefferson	30.2	No	8	50	50	50	xxx	xxx	xxx
98	NY	Jefferson	41.9	No	12	73	22	11	1.7	3.0	1.5
101	CT	Hartford	>30	No	16	64	60	60	3.3	6.8	1.6
104	CT	Hartford	>30	No	8	83	80	80	2.8	7.8	2.7

[a]Height, diameter, and number of stems does not include grafts repeatedly mowed off along the north border of the planting.

height, and diameter at ankle height were determined in fall of 1995, 1996, 1997, and 2001. In 1995 and 1997, we determined the origin of live stems relative to the soil surface. The morphology of dormant terminal buds was used to determine species in 1997 and 2001.

Survival after the second growing season of deep planted walnut rootstocks (graft union more than 10 cm deep) with and without live butternut scions was less than survival of walnut rootstocks with graft unions less than 10 cm deep or above the soil surface (table 2).

Survival differences of walnut at different planting depths were no longer significant after the fourth growing season. Williams (1974) previously reported that deep planting of black walnut had no effect of seedling survival.

After two growing season, survival of the black walnut rootstocks ranged from 50 to 100 percent among the 31 clones. Walnut rootstock survival decreased slightly through the seventh growing season when it varied from 42 to 94 percent among the clones. Half of the repository was located in a draw that frequently had standing

water in the spring or after irrigation. Survival of walnut rootstocks in the draw averaged 43 percent compared to 95 percent for rootstocks on a south-facing slope and ridge in the other half of the repository. Presumably, the deep planting holes cut through the fragipan produced unacceptable growing conditions for the black walnut rootstocks considered intolerant of poorly drained soils.

Butternut scion mortality after two growing seasons was higher on surviving deep-planted walnut rootstock than on rootstocks with graft union slightly buried or still above the soil line (table 2). Scions continued to be lost through the fourth growing season; however, differences in mortality no longer existed for planting depth of the walnut rootstock, which can partially be explained by too few surviving butternut grafts.

After two growing seasons, butternut scion mortality on the live walnut rootstocks ranged from 17 to 83 percent among the 31 clones (table 1). After four growing seasons, scion survival among clones ranged from 0 to 80 percent. Only 8 of 27 clones showed additional mortality through the seventh growing season.

Although source trees for some clones had cankers, no cankers have been observed on the butternut saplings in the repository. A few grafts show repeated annual shoot die-back and sprouting from unknown causes. Five grafts exhibit visual symptoms of witch's broom, which is severely affecting some butternut hybrids in an adjacent planting. In one of the four blocks, the ambrosia beetle (*Xyloborus germanus*) has caused dieback of several butternut stems and resprouting of black walnut rootstock; however most attacks have been on black walnut saplings that were allowed to develop after the scion died.

After seven growing seasons, deep planted walnut rootstock on which the scion had died had more rapid basal sprout growth than sprout growth on walnut rootstocks where the grafting scar was above the soil line or slightly buried (table 3). Likewise, butternut scions on deep planted walnut rootstocks had greater shoot growth than successful butternut grafts where the graft union was buried less than 10 cm deep or above the soil line. Overall, height growth of butternut scions was more rapid than walnut sprout growth on failed grafts. This is consistent with previous reports that butternut has more rapid stem growth than black walnut (Schroeder 1972). Many of the butternut grafts had multiple stems in contrast to the sprouted black walnut rootstock that had self-thinned in most cases to a single stem.

After seven growing seasons, height varied greatly among the 27 surviving clones ranging from 1.0 to more than 3.3 m (table 1). Likewise, diameter growth also varied greatly among the 27 clones ranging from 2.0 to 7.8 cm in diameter-at-ankle height. Too few grafts survived for most clones for any statistical comparisons among clones. Scionwood from the more vigorous clones in the Illinois germplasm repository was recently harvested for inclusion in a new repository being established by the Hardwood Tree Improvement and Regeneration Center in northern Indiana.

In summary, we did establish a germplasm repository of putatively canker-resistant butternut clones. The high mortality of the walnut rootstocks and of butternut scions on surviving

Table 2.—*Survival of black walnut rootstocks and butternut scions on live walnut rootstocks*

Species	Scion location[1]	Survival[2] Yr 2	Yr 4	Yr 7
		%		
Walnut	Above	88 a	71 a	67 a
	0 to 10	88 a	74 a	71 a
	>10 cm	81 b	71 a	70 a
Butternut	Above	57 a	36 a	35 a
	0 to 10	48 ab	34 a	29 a
	> 10 cm	39 b	21 a	21 a

[1]Location of graft union or scar above soil line, 0 to 10 cm deep, or deeper than 10 cm.
[2]Means with column and species followed by different letters are different at p = 0.05 percent.

Table 3.—*Stem height for black walnut rootstocks without live scions and for butternut scions grafted on live walnut rootstocks*

Species	Scion location[1]	Height[1] Yr 2	Yr 4	Yr 7
		m		
Walnut	Above	0.4 a	0.7 a	1.4 b
	0 to 10	0.4 a	0.7 a	1.3 b
	>10 cm	0.5 a	0.8 a	1.5 a
Butternut	Above	0.4 a	0.7 a	1.6 c
	0 to 10	0.4 a	0.8 a	2.0 b
	> 10 cm	0.5 a	0.9 a	2.3 a

[1] Means with column and species followed by different letters are different at p = 0.05 percent.

walnut rootstocks suggests mid-summer field-planting of in-leaf grafts with irrigation is not a good alternative to the traditional overwintering of grafts in coolers and subsequent spring planting. Moist soil conditions of the poorly drained soils in this respository, however, can explain the high mortality of the walnut rootstocks in the years following planting. High failure rates for butternut grafts on walnut rootstocks has also been observed in repositories in Vermont and Wisconsin that were established with spring planting of dormant stock (Ostry and Moore 2001). Because no genetic variation has been found within fungal isolates from cankered butternut (Furnier and others 1999), we should not expect to see changes in virulence to the pathogen among the putatively resistant clones used in this repository.

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