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Root Deterioration of Black Walnut Seedlings During Overwinter Storage in Wisconsin

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Black walnut seedlings suffered root damage resulting from subfreezing soil temperatures during overwinter storage in nursery- and heeling-in beds. This problem can be avoided either by mulching beds in the fall or by winter storage of bareroot seedlings in shipping bags at 3° C.

In recent years, the demand for quality black walnut (*Juglans nigra* L.) has resulted in an increase in walnut plantings throughout the Midwestern United States. To ensure proper density and uniformity, new plantations are established with 1-year-old (1-0) nursery transplants, rather than by direct seeding. In Wisconsin alone, 150,000 to 300,000 black walnut seedlings are distributed annually.

One of the limiting factors in the production of 1-0 walnut seedlings is losses from root rot. Root rots may be a problem both in the seedbed during the growing season and in overwinter storage of the trees. Seedling root rot caused *by Cylindrocladium* spp. is a serious problem in nurseries in the Southeastern United States *(1, 6)*, while *Phytophthora citricola* Sawada is considered to be the major root pathogen of walnut seedlings in Indiana *(3, 5)*. So far, in Wisconsin, seedling root rots caused by these fungi, though present, have not produced extensive damage *(7)*.

In contrast, root deterioration of black walnut seedlings during overwinter storage has resulted in heavy mortality in Wisconsin in some years. Previous studies in other States indicate that both environmental *(8)* and biotic agents *(3, 4)* may be involved in storage losses. The purpose of this study was to evaluate several methods of overwinter storage and to identify factors contributing to seedling root deterioration during storage.

Materials and Methods

One-year-old black walnut seedlings, raised at the Wilson State Forest Nursery, Boscobel, Wis., were used in all studies. Seedlings were grown from open-pollinated nuts collected in southern Wisconsin and northern Iowa. Fall-lifted seedlings were undercut approximately 20 centimeters below the soil surface the first week in November of each year. Undercut seedlings were mechanically lifted, hand-graded, and bundled into groups of 20 or 25 trees. Those trees that had a taproot of less than 20 centimeters in length or a root system showing discoloration were culled before bundling. Spring-lifted seedlings were undercut and lifted the first or second week in April.

During the winter of 1980-81, fall-lifted trees were stored either in heeling-in beds or in heavy paper shipping bags. The heeling-in method consisted of placing the bundled, graded seedlings in trenches, 30 to 45 centimeters deep, dug in the nurserybed. The roots were covered with soil and the seedlings stored in this manner until they were lifted in the spring. Five bundles of 20 seedlings each were placed in a heeling-in bed and then mulched to a depth of 40 to 60 centimeters with marsh hay. Five other bundles were placed in a heeling-in bed that was not mulched. Other fall-lifted bundled trees were placed in shipping bags and stored overwinter in a large walk-in cooler at 3° C. The relative humidity inside the bags remained above 90 percent throughout the storage period.

Several root treatments were applied to bundled, bareroot seedlings before bagging. Bundled seedlings were root-dipped for 1 minute in a fungicide suspension of benomyl (Benlate 50% WP at 6 g active ingredient/l) or captafol (Difolatan 4F at 5 g active ingredient/l). Another set of untreated bundles was placed into bags containing wetted marsh hay. Bundled seedlings not treated or root-dipped in water only served as controls. Each of the five treatments was replicated in five bags, with 20 seedlings per bag.

In 1981-82, the same treatments were used except that each treatment was replicated four times, with four bundles of 25 seedlings in each bag. Also, another treatment was added. The roots of four bundles were packed with wetted marsh hay, then covered with corrugated paper. The seedling tops remained exposed during storage in the cooler.

When the bags were opened in the spring, each bag was rated on a 1-to-3 scale for the amount of root and top mold present. Those bags having little or no superficial mold buildup on the bundled seedlings were graded as 1.0; those having a moderate amount of molding were graded as 2.0; and those having heavy mold on both roots and tops were graded as 3.0.

Further studies were conducted in 1981-82 to determine the effect of mulching and storage method on the incidence of root rot. Three adjacent plots (13 m^2) were established in a portion of the walnut nurserybed in late October. Trenches were dug between the rows of seedlings, and 16 bundles (25 trees/bundle) of fall-lifted seedlings were heeled-in per plot. One plot was then mulched to a depth of 40 to 60 centimeters with marsh hay. The second plot received no mulch. A sturdy polyethylene tent, supported by a wooden framework, was built over the third plot. The sides of the tent were open: that there was no impairment of air movement through the plot. The purpose of the tent was to prevent snow deposits, thereby simulating conditions in the bed during winters without snow cover.

Soil temperatures in each plot were monitored by thermistors buried 13 centimeters beneath the soil surface. Minimum and maximum temperatures for 5-day intervals from November 28, 1981, through April 3, 1982, were recorded with a CR 21 data logger (Campbell Scientific, Logan, Utah).

Fall-lifted, bundled seedlings were also stored in bags at 3° C or at —2° C. In the case of frozen storage, bags were held 2 months at 3°C before placement at —2° C. Four bags, each containing four bundles of 25 trees, were placed at each storage temperature.

All trees were examined the first week in April. The incidence of root rot and overall appearance of the trees after storage were recorded. For some treatments, seedlings were outplanted in the nursery. Seedling survival was recorded in August 1982.

Results

During the winter of 1980-81, there was no accumulation of snow on the nurserybeds. Seedlings that were left over winter in the beds and then lifted in April had extensive root rot. Surveys of the seedbed showed that 60 percent of the seedlings had complete taproot mortality, and many of the remaining trees had varying degrees of root deterioration. When pulled from the ground, injured roots were rubbery and discolored. The cortex of rotted roots was water-soaked and light brown to black. The discoloration never extended beyond the root collar region. This was in sharp contrast to the pearl-white cortex of healthy roots. Isolations were made from the rotted tissue, but no fungi or bacteria were isolated consistently. Because seedling mortality in the nursery was so high and the identification of healthy trees was so uncertain, the entire stock of over 150,000 black walnut seedlings was condemned.

Fall-lifted seedlings placed in heeling-in beds without mulch also had a high percentage (75 percent) of root rot and a low survival rate (14 percent) after outplanting (table 1). Mulching of the heeling-in beds reduced root rot incidence to 7 percent. Seedlings that were bagged and stored at 3° C had almost no root rot. Root treatments before bagging had no influence on the incidence of root rot. However, one problem associated with bag storage was the epiphytic colonization of roots and stems by fungi during the winter. When the bags were opened in the spring, we noted a sparse, unsightly covering of mycelium on the roots, but roots were still sound and the superficial mycelium did not affect seedling survival after outplanting. Both the benomyl and captafol root-dip treatments prevented molding on the roots and reduced molding of the stems.

Table 1—*Effect of storage method and root treatments on root rot incidence, superficial molding, and survival of black walnut seedlings after outplanting*

Storage method[1]	Treatment	1980-81 Root rot[2]	1980-81 Survival[3]	1981-82 Root rot	1981-82 Mold index[4]
		%	%	%	
Bagged, 3° C	No-dip	2	98	0	2.5
Bagged, 3° C	Water dip	0	96	1	2.5
Bagged, 3° C	Benomyl dip	0	100	0	1.0
Bagged, 3° C	Captafol dip	1	97	0	1.0
Bagged, 3° C	Root bundle	—[5]	—	0	1.5
Heeling-in	Straw mulch	7	87	—	—
Heeling-in	No mulch	75	14	—	—

[1]Seedlings were fall-lifted, bundled, and stored either in paper shipping bags in coolers at 3° C or placed in heeling-in beds in the nursery.
[2]Mean percentage of root rot for five replicates (20 seedlings/replicate) in 1980-81 and four replicates (100 seedlings/replicate) in 1981-82.
[3]Mean percentage of survival of seedlings after outplanting for five replicates.
[4]Superficial molding of roots and stems rated on 1-3 scale, where 1.0 = slight or no molding of the roots in each bag, 2.0 = approximately 50 percent of the seedlings showing some mold, and 3.0 = heavy molding of all seedlings.
[5]— = not available.

Root treatments of bagged seedlings in 1981-82 gave results similar to those in 1980-81 (table 1). Almost no root rot was found in any treatment; however, the molding of bagged seedlings was heavy in the no-dip and water-dip treatments. Molding was prevented by root-dip treatments with benomyl or captafol.

In the winter of 1981-82, the entire nursery was covered with snow from the first week in January to the second week in March. Snow depths ranged from 22 to 38 centimeters. Spring-lifted seedlings that had been mulched with marsh hay or had snow cover survived the winter in excellent condition (table 2). Samples taken throughout the seedbed showed less than 1 percent of the seedlings had discolored roots. These appeared to be carry-over infections of *P. citricola* during the growing season. Root rot was not found in those heeling-in plots that had been covered by snow or hay and snow.

Soil temperatures in both the mulched and nonmulched plot with snow cover were similar (fig. 1). Soil temperatures dropped to the freezing point in early December. In the nonmulched plot, minimum temperatures dropped to —4.5°C in mid-December, but then increased as snow cover accumulated on the plot. Soil temperatures remained constant and near 0° C in both the mulched and nonmulched plot throughout much of the winter.

In contrast, soil temperatures in the bare-ground plot had great temperature fluctuations throughout the winter (fig. 1). Soil temperatures dropped to —14°C in Feb-

Table 2—*Effect of storage method on the incidence of root rot during overwinter storage in 1981–82*

Storage method	Treatment	Root rot	Survival	Stem necrosis
		%	%	%
Bagged	3° C	0[1]	—[2]	5.6
Bagged	−2° C	0	98.0	1.6
Heeling-in	Mulch + snow	0	—	5.0
Heeling-in	Snow only	0	98.0	1.4
Heeling-in	Bare ground	74.4	31.2	4.2
Spring-lift	Mulch + snow	0	—	5.3
Spring-lift	Snow only	0	—	4.6
Spring-lift	Bare ground	59.6	—	1.2

[1]Percentages are averages from four replicates containing 100 seedlings per replicate.
[2]— = not available.

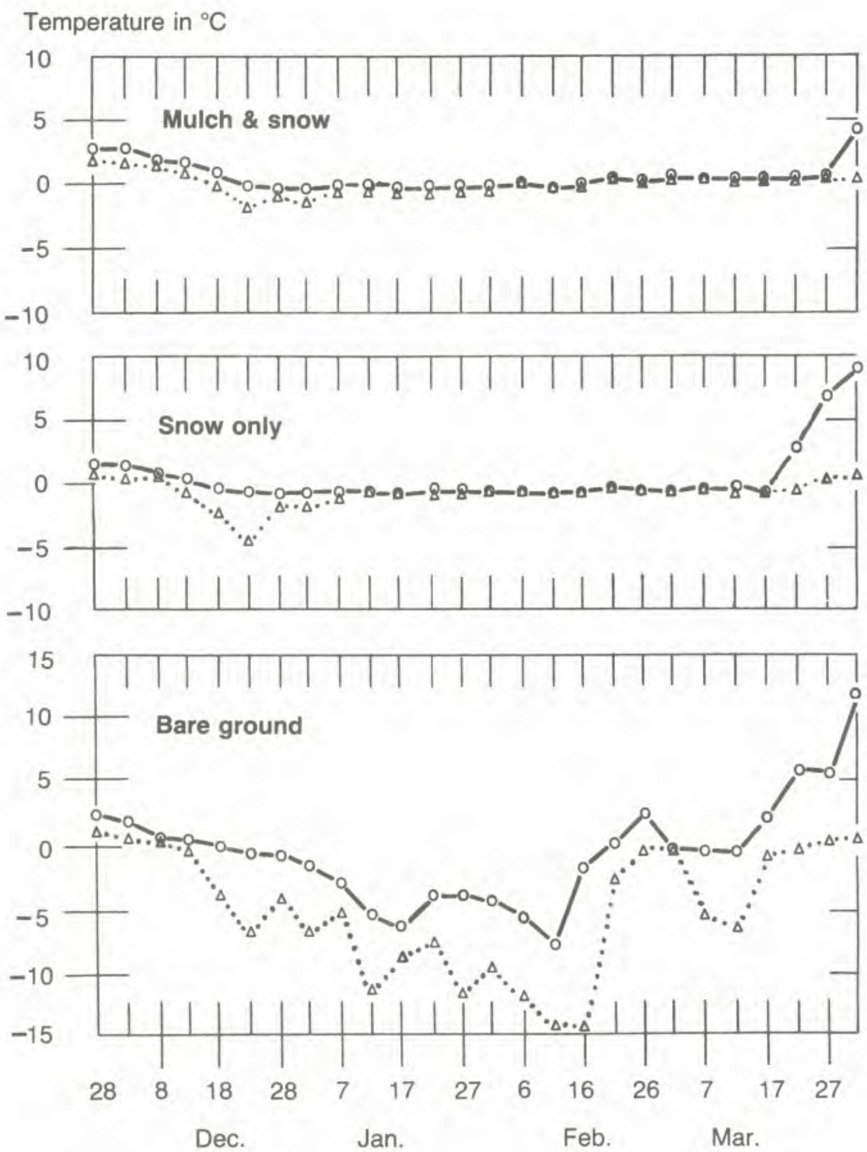

Figure 1—*Five-day minimum and maximum soil temperatures for nurserybed plots having mulch plus snow cover, snow cover only, or bare ground during the winter of 1981–82. Soil temperatures were taken at a depth of 13 centimeters.*

ruary. Trees overwintered in this plot had a high incidence of root rot (table 2). An average of 74 percent of the fall-lifted seedlings in the bare-ground heeling-in bed suffered root mortality.

Seedlings that were bagged and stored at 3°C or —2°C had no root rot (table 2). It was noted that, in all treatments, some seedlings had discoloration of the taproot where it had been cut upon lifting. This discoloration did not extend above 1 or 2 centimeters from the base of the root.

In the 1981-82 experiment, 3.5 percent of all seedlings had discoloration of the stem. These seedlings were found in all storage treatments, indicating that the damage occurred before fall lifting. No fungi or bacteria were isolated consistently from the discolored areas.

Discussion

In past years, overwinter storage of black walnut seedlings in nurserybeds or heeling-in beds resulted in extensive root rot. Our results indicate that root deterioration during overwinter storage in Wisconsin is caused principally by cold temperature damage to the roots. In both years, root damage was confined to those seedlings stored outdoors in nonmulched plots. Soil temperatures at a depth of 13 centimeters in the non-mulched areas often dropped below —10°C during January and February.

Young *(8)* found that bareroot walnut seedlings, exposed to —12°C for 30 minutes, developed root rot during overwinter storage. He also found a high incidence of root rot in both mulched and nonmulched heeling-in beds in sandy soil in an Iowa nursery. This is similar to the results we obtained in Wisconsin. It is still not known whether the roots are injured by low temperatures or by fluctuations in temperatures in the nurserybed, which may result in freezing and thawing of root tissue during storage.

Our results suggest alternative methods for storage of black walnut seedlings during the winter months. Root rot can be avoided by mulching the beds in the fall to a depth of 20 centimeters with marsh hay. Other mulches also would be suitable. Spring-lifting of mulched beds and immediate distribution of seedlings are recommended where cold storage facilities are not available. Because Wisconsin is located along the northern limits of the geographic range of blackwalnut, some loss in nursery stock should be expected. Trees that are susceptible to early frost or are not winter-hardy will be killed. We believe that the stem discoloration found in 1981-82 probably was caused by an early frost in the fall, since a biotic agent was not isolated and the damage was found in all treatments. However, Green *(2)* has reported a discoloration of walnut stems caused by *Phomopsis elaeagni* and has suggested that this pathogen maybe responsible for top damage previously ascribed to frost injury. Further studies and isolations should be made to confirm the cause of tip dieback in Wisconsin.

We believe that the best method of storage in Wisconsin is fall-lifting and storage of seedlings indoors at a constant temperature. Moreover, fall-lifting and cold storage may be more convenient for nursery managers, enabling them to avoid the spring rush in lifting other tree species. Bareroot seedlings can be stored in sealed shipping bags at 3°C throughout the winter with little or no root rot. Seedlings dc; develop epiphytic molding during storage in bags, but it does not appear to affect survival. Both the benomyl and captafol dips prevented molding of the roots. Green and Plourde *(4)* previously reported reduction in molding by the use of captafol. While molding did not affect survival, it was unsightly and could reduce the marketability of bagged seedlings. Top molding could also be prevented by wrapping the roots in wetted straw and corrugated paper, but leaving the tops exposed.

Results from frozen storage experiments at —2° C are promising. Seedlings stored below freezing had no root rot or superficial mold. Survival after outplanting was also very high. Frozen storage might eliminate the need for fungicide dips to prevent molding. Care should be taken to avoid fluctuating temperatures. Previous experiments *(7)* have indicated that cold storage at —5°C results in a decrease in survival over seedlings stored at 3°C.

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