Control of a Molding-Root Rot Complex of Black Walnut Seedlings in Storage

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ABSTRACT

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Losses as high as 30% of black walnut seedlings in winter storage in Indiana nurseries are caused by a molding-root rot complex. Several fungi are involved in the molding phase. Species of *Fusarium* and *Pythium* are associated with the root rot, which is distinct from that caused in the seedbed by *Phytophthora citricola*. Losses in cold storage were significantly reduced by packing seedlings in plastic-lined bags rather than by moist packing. A fungicide (captafol) root dip before storage reduced losses further. Seedlings outplanted after these treatments displayed 98–100% survival and generally better growth than controls. Seedlings that had molded or sustained decay of as much as 25% of the taproot survived at rates of 70–88%.

Black walnut (*Juglans nigra* L.) is a highly valued species of the American deciduous forest for both lumber and veneer. It is in high demand in the United States and abroad because of its rich wood color, its durability, and the ease with which it is worked. Nearly one-third of the veneer-quality walnut logs harvested annually in the United States come from Indiana (2). Because of the high demand and declining supplies of marketable black walnut trees, nursery production of seedlings for replanting has increased dramatically in state-owned and private tree nurseries in Indiana and elsewhere in the eastern United States (7).

In Indiana, walnut seedling production is often curtailed by losses in the seedbed and in storage and transit. Seedbed losses are due mainly to the root rot caused by *Phytophthora citricola* Sawada (6,8). Further losses as high as 30% caused by a molding-root rot complex occur during winter storage and in transit. Symptoms include a dense, superficial molding of the fleshy primary root of the seedlings, and in some cases, soft, watery necrosis and decay of root tissues (5). This reduces storage inventory and often necessitates costly regrading before shipment. The molding-root rot complex may also develop in transit, especially if shipment is delayed, leading to rejection of the shipment at its destination.

Green and Plourde (5) demonstrated the association of a number of fungi with this disease complex. Species of *Fusarium, Trichoderma, Gliocladiopsis, Penicillium, Zygorhynchus,* and others were involved, but their frequency and pathogenicity were unknown. On the basis of symptoms, the root rot phase of the disease complex was distinct from the root rot caused by *P. citricola* in the seedbed. Losses can be reduced both in cold storage and the heeling-in yard by a fungicide root dip before winter storage, but little is known of conditions that favor disease development (5).

The objectives of this study were to determine the roles of the various fungi in the disease complex, the effect of packing methods used for cold storage of seedlings on disease incidence, and the survival of walnut seedlings with the root molding phase of this disease complex after outplanting. A preliminary report has been published (4).

Nursery practices. Black walnut seedlings are lifted in autumn and stored over the winter in cold storage (1–5 C) or in heeling-in yards. Before storage, the seedlings are graded and tied 25 per bundle. For cold storage, 10 bundles are either packed in moist sphagnum moss or wrapped in a moist, nonwoven fabric (CONWEB, Conweb Corp., St. Paul, MN) and then in polyethylene-coated, laminated paper (FLXOL, H. P. Smith Co., Chicago, IL). Bundles stored in heeling-in yards are placed upright in trenches, covered with soil to a depth slightly above the root collar, and left undisturbed until spring (March), when they are lifted (as needed) for shipment.

The molding-root rot complex develops most rapidly in late winter or early spring in the heeling-in yards as soil temperatures increase to 4–8 C or higher. In cold storage, disease increases after the coolers are opened in spring and there are fluctuations in temperature and humidity. If regrading is necessary after winter storage, the nurseryman culls all seedlings with root rot or superficial molding of roots.

MATERIALS AND METHODS

Fungi were isolated from the surfaces of infected roots and from the margins of root lesions in the internal tissues of 50 seedlings. For surface isolation, roots were washed and rinsed several times with sterile, deionized water. Mycelium and tissue samples were taken directly from the root surface. To sample internal tissues, roots were washed, blotted dry, dipped in 70% ethyl alcohol, and surface-sterilized with 1% NaOCl for 5 min. The surface tissue was removed with a sterile blade and tissue samples were taken from the margins of necrotic lesions.

Tissue samples were incubated on the following media: 2% water agar; potato-dextrose agar (PDA) containing 30 μg of aureomycin sulfate and 200 μg of Tergitol NPX per liter (10); PDA with 50 μg of neomycin, 35 μg of penicillin G, and 100 μg of pimaracin per liter (3); and V-8 juice nutrient agar with 100 μg of neomycin, 10 μg of chloromycetin, and 10 μg of endomycin per liter (9). The latter two media are for isolation of *Phytophthora* spp. and pythiaceous fungi, respectively.

Fungi isolated from both surface and internal root tissues were inoculated singly into the roots of dormant, 1-yr-old walnut seedlings as follows: Roots were washed, blotted dry, and inoculated by placing a block of agar ≅0.5 cm^2) with mycelium of the test fungus in a wound cut at an angle into the root cortex. After inoculation, the wound was sealed with petroleum jelly to reduce desiccation. Inoculated seedlings (10 per isolate) were wrapped in moist CONWEB fabric and plastic-coated, laminated paper as described earlier and incubated in a controlled-climate room (7.5 C) for 10 wk. After storage, inoculated seedlings were examined for growth of the test organism on the roots and for root symptoms as follows: The inoculation was rated positive for surface molding if the test organism had grown on the root surface beyond the wound area where the inoculation was made. The roots were then cut lengthwise and the internal root tissue examined for development of the soft, watery decay associated with the root rot phase of this disease complex.

Incidence of the molding-root rot complex in cold storage was evaluated in relation to two methods of packing seedlings for winter storage with and without a fungicide root dip. Bundles of graded walnut seedlings were packed in either large, laminated paper bags with a plastic liner (Bemis Co., Des Plaines, IL)

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and closed by stitching or in moist CONWEB fabric and wrapped in plastic-coated, laminated paper as described. Before packing, 50 bundles in each treatment were root-dipped in captafol (Difolatan 4F, 1 L/100 L of water) and 50 bundles were packed without the root dip. The treatments, each consisting of five packages (10 bundles per package), were placed in cold storage at a nursery in November 1981 and rated for disease incidence in March 1982. The experiment was repeated in 1982–1983.

In March 1982, after the various storage treatments were rated for incidence of the molding-root rot complex, outplantings were made to evaluate the survival and growth of seedlings. The planting consisted of graded, apparently healthy seedlings from the four storage treatments and diseased seedlings with superficial root molding or with molding-root rot involving no more than 25% of the lower root.

The seedlings (100 per treatment) were planted in a nursery in March 1982 and maintained weedfree by hand cultivation. In September 1983, these plantings were rated for seedling survival and growth. Seedlings with root surface molding only and molding-root rot were also lifted and their root systems compared.

RESULTS AND DISCUSSION

The fungi isolated from surface and internal root tissues of walnut seedlings showing symptoms of the molding-root rot complex and the frequency of isolation are shown in Table 1. Only isolates of *Fusarium* and *Pythium* were routinely obtained from the internal root tissues. All others were limited primarily to the superficial molding phase of the disease complex. Isolates of *Phytophthora* were obtained only twice from the necrotic root tissues.

When roots of dormant black walnut seedlings were wound-inoculated with isolates of these fungi, including the unknowns, the symptoms produced were primarily the same as those on infected seedlings from storage. That is, only isolates of *Fusarium, Pythium,* and *Phytophthora* induced root necrosis and decay, whereas all other isolates were limited to superficial growth in the wound area on the seedling roots. The isolates of *Phytophthora*, identified as *P. citricola*, produced symptoms similar to the root rot that occurs in seedbeds.

The isolates of *Fusarium* identified were *F. roseum* Link and *F. solani* (Mart.) Sacc. emend. Snyd. & Hans.; the isolates of *Pythium* identified included *P. irregulare* Buism., *P. ultimum* Trow, and *P. vexans* de Bary.

When the nursery cold storage facilities were opened in early March 1982 and in March 1983, significant suppression of the incidence of both the surface molding and the molding-root rot phases of this disease complex was evident in treatments in which seedlings were either bagged for storage, root-dipped in the fungicide captafol, or both compared with the standard storage method (Table 2).

The method of packing seedlings for cold storage was important in the incidence of this disease complex, especially the molding phase. The moist, nonwoven fabric wrap used commercially apparently maintains high humidity and moisture that favor disease development. Roots of seedlings packed in the plastic-lined bags appeared drier, but there was no evidence of desiccation of even the fibrous roots.

The fungicide dip treatment suppressed or completely prevented both the molding and the root rot phases of this disease, regardless of the packing method used for storage.

Apparently healthy walnut seedlings and seedlings with root molding only or the molding-root rot complex were compared for survival and growth after two growing seasons in the field (Table 3). The seedlings (100/treatment) were first rated for percent survival and then for height growth, based on a scale of 1–4, where 4 = greatest growth, 3 = 90%, 2 = 75%, and 1 = 50% or less of greatest growth.

There were no significant differences in survival or growth of the apparently healthy seedlings from the two packing methods, with or without the fungicide root dip. This suggests that even though the seedling roots in the plastic-lined bags were somewhat drier than those from the moist CONWEB packing, there were no adverse effects on field survival.

There was a significant reduction in survival of seedlings that showed root molding at the time of transplanting and a further reduction in survival of seedlings with as much as 25% of the primary root necrotic (root rot) at the time of transplanting. The surviving seedlings in both of these treatments also showed a significant suppression of growth. When these surviving seedlings were lifted and the roots examined, most had produced callus that delimited the necrotic area. In many cases, adventitious roots were produced from the callus.

These results confirm the findings of Green and Plourde (5) that this storage disease complex is caused by a number of fungi and that the root rot phase is distinct from that in the seedbed caused by *Phytophthora citricola*. Two species of *Fusarium, F. roseum* and *F. solani*, and three species of *Pythium, P. irregulare, P. ultimum,* and *P. vexans*, were associated with the root rot phase of this disease complex. Berry (1) previously reported the isolation of *F. episphaeria* from diseased walnut seedlings from the seedbed, but *Pythium* spp. have not previously been associated with root rot of black walnut seedlings either in the seedbed or in storage. In cold storage,

Table 1. Frequency of isolation of fungi associated with the molding-root rot complex of black walnut seedlings in winter storage

Genera of fungi	Root tissue	
	Surface	Internal
Fusarium	3[a]	27[a]
Pythium	0	19
Phytophthora	0	2
Trichoderma	13	2
Zygorhynchus	16	0
Gliocladiopsis	7	0
Penicillium	21	0
Rhizopus	8	2
Others	9	1

[a]Isolations per 50 diseased seedlings.

Table 2. The effect of packing method of black walnut seedlings for winter cold storage on the incidence of a molding-root rot disease complex

Treatment	Root symptoms[w]	
	Molding (%)	Molding-root rot (%)
CONWEB + FLXOL		
wrap[x]	21.3 a[y]	3.1 a
+ captafol root dip[z]	4.8 b	0.8 b
Plastic lined bags	3.0 bc	0.9 b
+ captafol root dip	0.0 c	0.0 b

[w]Data are an average of the percentage of seedlings showing either the molding phase or the molding-root rot complex in the 2 yr of this study.
[x]Moist, nonwoven fabric + plastic-coated, laminated paper wrap.
[y]Captafol 4F (1 L/100 L of water) root dip.
[z]Within columns, means followed by different letters are significantly different ($P = 0.05$) according to Duncan's multiple range test.

Table 3. Survival and growth of outplanted healthy and diseased black walnut seedlings after winter storage using different packing methods and fungicide root dip

Storage treatment	Survival[u] (%)	Growth class[v]
Apparently healthy seedlings		
CONWEB + FLXOL		
wrap[w]	98 a[x]	3.7 a
+ captafol root dip[y]	100 a	3.8 a
Bagged[z]	100 a	4.0 a
+ captafol root dip	100 a	3.8 a
Diseased seedlings		
Surface mold only	88 b	3.4 b
Molding-root rot complex	70 b	3.2 b

[u]Survival after two growing seasons.
[v]Growth class: 4 = greatest height growth, 3 = 90%, 2 = 75%, and 1 = 40% or less of greatest growth.
[w]Moist nonwoven fabric and plastic-coated, laminated paper wrap.
[x]Within columns, averages with no letter in common are significantly different ($P<0.05$) using pairwise treatment comparison on chi-square.
[y]Captafol 4F (1 L/100 L of water) root dip before storage.
[z]Plastic lined, laminated paper bag with closure.

losses from this molding-root rot complex can be effectively reduced by modifying the packing method, and it may not be necessary to use the fungicide root dip. Also, the use of the bagging method for cold storage is less costly in both materials and labor than other methods used.

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