BLACK WALNUT MYCORRHIZAE NOT SUPPRESSED BY HIGH SOIL FERTILITY

by
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Abstract - High soil fertility did not prevent mycorrhizae infection of black walnut seedlings but inoculation was required after seedbeds were fumigated.

Additional keywords: MC2, Juglans nigra, endomycorrhizae, nursery seedbed, fumigation.

Black walnut (Juglans nigra L.) seedlings grown at a southern Indiana nursery were examined annually from 1975 to 1978, and were found devoid of vesicular-arbuscular mycorrhizae (VAM). [2]/ Although mycorrhizae were not detected, the 1-0 black walnut seedlings grown at the nursery were larger than those grown in most, if not all, other nurseries. When transplanted, however, few of the seedlings grew the first year. Lack of mycorrhizal infection, due to seedbed fumigation and high seedbed fertility (Kormanik et al. 1977), was suspected to be the cause. This paper reports the results of two greenhouse studies and a seedbed pot study that were conducted to determine if the lack of mycorrhizae was caused by high seedbed fertility.

GREENHOUSE STUDY A

Methods

Eight combinations of soil from the upper 6 inches of a nursery seedbed fumigated with 450 pounds of bromomethane (MC2) per acre, white silica sand from a northern Illinois source, and fertilizers were combined to formulate the potting media to grow black walnut seedlings. The addition of mycorrhizal inoculum to these created the 16 treatments listed below:

1. Silica sand.
2. Silica sand + 1 NPK.
3. Silica sand + 2 NPK.
4. 3/4 silica sand + 1/4 nursery soil.
5. 1/2 silica sand + 1/2 nursery soil.
6. 1/4 silica sand + 3/4 nursery soil.
7. Nursery soil.
8. Nursery soil + 1 NPK.

9-16. Similar to 1 through 8 with mycorrhizal inoculum added.

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[2] Personal correspondence from E. Hacskaylo, Beltsville, MD, and W.C. Bryan, Athen, GA, concerning their examinations of seedlings for mycorrhizal infection, are on file in Bedford, IN.

The nursery soil - Princeton fine sandy loam - requires constant attention to maintain minimal levels of organic matter and favourable fertility. When tree seedlings are harvested in the spring, the bare ground is fertilized with 600 pounds per acre of a 1:1 mixture of ammonium nitrate and 12-12-12 before a manure crop of sordon (a hybrid of sorghum and sudan grass) is sown. The following spring, just prior to plowdown, 500 pounds of 12-12-12 are applied to hasten decomposition prior to fumigation. Top dressing is seldom applied to the walnut seedbeds.

The 16 treatments were replicated three times in a randomized complete block design. The seedlings were grown in 5-gallon plastic pots that were filled with about 48 pounds of the soil formulation. Before treatment, the silica sand contained 25 to 50 pounds of P205 per acre and no potassium or calcium, while the nursery soil contained 296 pounds of P205, 172 pounds of K_20, and 500 pounds of calcium per acre. Pots were fertilized immediately after the seed was sown with ammonium nitrate, calcium phosphate, and potassium chloride in a 1-3-5 ratio - that is, 1 pound of N, 3 pounds of P205, and 5 pounds of K20. On a per-acre basis, the 1 NPK treatment was 40 pounds of N, 120 pounds of P_20_5, and 200 pounds of K_20. The 2 NPK treatment was 80, 240, and 400 pounds of N, P205, and K20, respectively.

On January 6, three germinated black walnut seeds were sown in each pot, and additional germinated seeds were sown 2 weeks later in pots that contained fewer than three seedlings. To provide 16-hour days, daylight was supplemented by fluorescent and incandescent lights. Thermostats in the greenhouse were set to maintain a 20^0C nighttime temperature; temperature was allowed to fluctuate upward during the day.

The pots were thinned March 6 to leave the two tallest seedlings in each pot. The experiment was terminated and the seedlings recovered from the pots about 3 months after the first seed was sown. The seedlings were measured, and the roots were examined for mycorrhizal infection using a modified version of the clearing and staining technique of Phillips and Hayman (1970).

Results

Two-thirds of the seedlings grown in inoculated pots were mycorrhizal, while none of those grown in non-inoculated pots were infected (table 1). All seedlings in pots containing inoculated, pure nursery soil (treatments 15 and 16) were mycorrhizal. It was especially meaningful, since we were interested in the effects of fertility on mycorrhizal infection, that formation of mycorrhizae was not hindered by the addition of the NPK in treatment 16. And the non-inoculated counterparts, treatments 7 and 8, were not infected. Soil type had little effect on seedling infection, so soil fertility did not inhibit infection. Seedlings were not infected, though, unless inoculated, which indicated that the fumigation had eradicated inoculum in the seedbed.

Seedling height was related to soil type rather than mycorrhizal infection. The tallest seedlings were grown in the two unfertilized nursery soils (treatments 7 and 15), one inoculated, the other not.

Table 1. Mycorrhizal infection and seedling size by soil formulation and mycorrhizal treatment.

Treatment	Infection[1/] Pot 1	Pot 2	Pot 3	Seedling size Diameter (mm)	Height (cm)	Weight Root (gm)	Stem (gm)
				Not Inoculated			
1. Silica	N	N	N	5.3	19.5	35.4	15.5
2. Silica + 1 NPK	N	N	N	5.3	16.5	39.8	16.2
3. Silica + 2 NPK	N	N	N	6.0	18.5	30.3	15.9
4. 3/4 Silica + 1/4 Nursery	N	N	N	6.0	23.7	43.8	23.8
5. 1/2 Silica + 1/2 Nursery	N	N	N	5.3	22.8	32.0	19.2
6. 1/4 Silica + 3/4 Nursery	N	N	N	6.3	24.0	42.2	31.8
7. Nursery	N	N	N	6.0	30.8	33.9	27.2
8. Nursery + 1 NPK	N	N	N	5.7	21.8	45.1	35.3
Mean	0 of 24			5.7	22.2	37.8	23.1
				Inoculated			
9. Silica	Y	N	N	4.7	20.7	28.0	16.2
10. Silica + 1 NPK	Y	Y	Y	5.7	25.0	57.3	18.6
11. Silica + 2 NPK	N	Y	Y	6.7	23.7	65.4	33.1
12. 3/4 Silica + 1/4 Nursery	N	Y	N	4.7	20.7	26.7	19.3
13. 1/2 Silica + 1/2 Nursery	N	Y	N	5.3	26.7	27.2	24.2
14. 1/4 Silica + 3/4 Nursery	N	Y	Y	5.3	20.8	24.7	20.2
15. Nursery	Y	Y	Y	6.3	31.5	34.0	36.8
16. Nursery + 1 NPK	Y	Y	Y	6.0	26.0	46.6	34.4
Mean	16 of 24			5.6	24.3	38.7	25.4

[1] Y = Mycorrhizae found; N = no mycorrhizae found.

Differences in seedling height, attributable to soil type, were highly significant statistically, but mycorrhizal inoculation had no significant effect on seedling height. Root weight was greatest when pure silica sand was fertilized and inoculated (treatments 10 and 11). Neither soil nor inoculation differences were significant but the interaction between the two was significant at the 5-percent level. Stem weight differences among soil types were significant but inoculation had no significant effect on stem weight.

GREENHOUSE STUDY B

Methods

Because black walnut seedlings seemed difficult to inoculate in previous attempts, a second greenhouse experiment, using about 6 pounds of soil in 2-quart plastic pots, was conducted concurrently with Study A to compare relative infectivity of sordan and walnut. The same sources were used for the silica sand and the nursery soil as in Study A. Objectives of the study were to: (1) determine if the endomycorrhizal spores found in the seedbed after fumigation were viable, (2) determine if autoclaving is a more effective eradicant and more conducive to reestablishment of mycorrhizae than fumigating with MC2, and (3) find out if walnut seedlings are more difficult to infect than sordan.

Study B included the 16 treatments listed below:

	Black walnut		Sordan	
Fumigated nursery soil.	1	9	2	10
Fumigated nursery soil + 1 NPK.	3	11	4	12
Autoclaved nursery soil.	5	13	6	14
1/2 Fumigated + 1/2 silica sand.	7	15	8	16

Treatments 1 through 8 were inoculated, while treatments 9 through 16 were similar but not inoculated.

Each treatment was replicated three times in a randomized complete block design. Fertilizer sources and the 1-3-5 ratio, the MC2 rate, and the rates of inoculum used **in** Study A were used for Study B, also. Soil was autoclaved 1/2 hour at 121°C and 15 pounds of pressure. On January 6, either three germinated walnut seeds or about 25 sordan seeds were sown in each pot. More germinated seeds were sown 2 weeks later if fewer than 2 walnut seedlings had developed in a pot. And because mice ate some of the sordan seed and seedlings, more sordan seed was sown February 1.

About 90 days after the initial seeding, the walnut and sordan seedlings were recovered from the pots, measured, and the roots cleared and stained for examination for mycorrhizal infection.

Results

Only 25 percent of the inoculated pots produced infected walnut seedlings (Table 2). None of the walnut seedlings grown in non-inoculated pots were mycorrhizal.

Inoculation of the sordan seedlings was more effective. Sordan seedlings in 11 of the 12 inoculated pots were mycorrhizal. Seedlings in three of the 12 non-inoculated pots were mycorrhizal.

Both fumigation with MC2 and autoclaving were effective eradicants of any mycorrhizal inoculum that might have been present.

Table 2. Mycorrhizal infection for black walnut and sordan by soil and mycorrhizal treatments.

Treatment	Black walnut: Treatment number	Black walnut: Infection[1] Pot 1	Pot 2	Pot 3	Black walnut: Diam. (mm)	Black walnut: Height (cm)	Sordan: Treatment number	Sordan: Infection[1] Pot 1	Pot 2	Pot 3
					Inoculated					
Fumigated	1	0	L	0	3.8	21.7	2	M	L	L
Fumigated + 1 NPK	3	0	0	L	4.2	28.7	4	M	0	L
Autoclaved	5	0	0	0	4.0	24.2	6	L	M	L
1/2 Fumigated + 1/2 silica sand	7	0	L	0	3.5	24.3	8	L	M	M
Treatment summary		3 of 12			3.9	24.7		11 of 12		
					Not Inoculated					
Fumigated	9	0	0	0	4.0	23.0	10	0	0	0
Fumigated + 1 NPK	11	0	0	0	3.8	21.7	12	L	0	0
Autoclaved	13	0	0	0	3.7	20.2	14	0	L	0
1/2 Fumigated + 1/2 silica sand	15	0	0	0	3.7	20.8	16	0	L	0
Treatment summary		None of 12			3.8	21.4		3 of 12		

[1] Infection: 0 = none, L = light, and M = medium

Samples from the silica sand and nursery soil, taken after the fumigation and autoclave treatments, were examined for spores. There were no spores in the silica sand and no difference was detected in the appearance of the dead spores resulting from the two methods of soil sterilization. There was no difference in number of infected seedlings nor degree of infection of seedlings grown in fumiaged and autoclaved soils.

These results demonstrate that sterilized soil must be inoculated for mycorrhizal infection and that it is more difficult to inoculate walnut than sordan. Although seedlings grown in inoculated pots were slightly taller than those grown in non-inoculated containers, the differences were not statistically significant.

SEEDBED POT STUDY

Methods

Because growth differences in the greenhouse studies were small, a pot study was conducted in a nursery seedbed in the spring of 1979. Fumigated nursery soil and silica sand from the same sources used in Studies A and B were used to fill forty 5-gallon pots to produce the treatments listed below:

1. Not inoculated.
2. Silica sand.
3. Silica + 1 NPK.
4. Nursery soil.
5. Nursery soil + 1 NPK.

Treatments 5 through 8 were similar to 1-4 but inoculum was added.

Pots were fertilized using the same 1-3-5 ratio used in Studies A and B. Inoculation was done April 17 with about 2 ounces per pot of the soil and sordan root source used for Studies A and B. Also, about 16 ounces per pot of heavily mycorrhizal chopped walnut, yellow-poplar, and white ash roots, and soil from their rhizospheres, were added to the top 4 inches in each pot. Each treatment was replicated five times in a randomized complete block design.

Ten germinated walnut seeds were sown in each pot April 30. One week later three additional germinated seeds were sown in each pot and all pots were mulches with sphagnum moss to help conserve moisture. The sphagnum was not fumigated and probably became a source of mycorrhizal contamination for the non-inoculated pots. Also, to help conserve moisture, shade screens were erected.

On June 5, pots that contained nursery soil were thinned to three seedlings per pot. Seedlings growing in silica weren't developing as well as those in the nursery soil, so four seedlings were left in each pot. The experiment was terminated after about 60 growing days. The seedlings were recovered from the pots, measured, and examined for mycorrhizal infection.

Results

Ninety-eight percent of the walnut seedlings in the inoculated pots were mycorrhizal (Table 3). However, 39 percent of the seedlings in the non-inoculated pots were infected. Since contamination was not a problem in Studies A and B, where sphagnum wasn't used, it is probable that the sphagnum contaminated the non-inoculated pots.

Although seedlings in some non-inoculated pots were infected, there was a big difference in degree of infection among treatments. By assigning values of 1 for no infection through 6 for very heavy mycorrhizal infection, it can be seen that inoculation greatly increased the degree of infection. Mean index values were 1.9 and 4.7 for the

Table 3. Mean height and diameter of walnut seedlings by soil and mycorrhizal treatments.

Treatment	Degree of infection[1/]	Diameter (mm)	Height (cm)
	Not Inoculated		
1. Silica	2.5	4.6	22.7
2. Silica + 1 NPK	1.4	4.8	22.9
3. Nursery	2.6	6.2	33.6
4. Nursery + 1 NPK	1.0	6.1	31.4
$\bar{X}$	1.9	5.4	27.6
	Inoculated		
5. Silica	4.5	4.7	23.1
6. Silica + 1 NPK	3.9	4.8	22.6
7. Nursery	5.3	6.1	28.7
8. Nursery + 1 NPK	5.1	6.3	33.8
$\bar{X}$	4.7	5.5	27.1
Grand mean	3.3	5.4	27.4

[1] Degree of infection was determined by assigning numerical values as follows: None = 1; very light = 2; light = 3; moderate = 4; heavy = 5; and very heavy = 6. Each treatment value index is the mean for 15 seedlings.

non-inoculated and inoculated seedlings, respectively. Inoculation of seedlings grown in the nursery soil, treatments 7 and 8, was 100 percent effective and there was a high degree of infection.

Seedlings growing in nursery soils, regardless of mycorrhizal treatment, were tallest and largest in diameter, but inoculation did not affect seedling size.

CONCLUSIONS

The primary objective of the studies was to find out if high fertility levels in nursery seedbeds were responsible for the absence of mycorrhizae in the walnut seedlings. The results show that fumigation with MC_2, rather than high fertility, is the probable cause. Walnut and sordan seedlings grown in fumigated soil inoculated with mycorrhizal soil and roots developed mycorrhizae, regardless of fertility levels tested.

Inoculation success was about equal in soil sterilized by fumigation and autoclaving, so either method should be satisfactory for normal greenhouse and research work. Plant material had a great effect on the success of inoculation. A higher degree of infection was achieved with sordan than with black walnut.

Growth results from these studies support the findings of Ponder (1981); that is, there were no significant height differences between mycorrhizal and non-mycorrhizal walnut seedlings. However, it is possible that our seedlings were not inoculated with the most compatible, most beneficial mycorrhizal species, or that size differences would emerge over a longer time period.

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