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BREEDING AND SELECTING CLONES OF RHODODENDRONS INCLUDING AZALEAS

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(See-Proc. Inter. Plant Prop. Soc. 29:431-436 1979).

Thursday Afternoon, December 11, 1980

The Thursday afternoon session convened at 2:15 p.m. with James Sabo serving as moderator.

MYCORRHIZAE AND THEIR USES IN THE NURSERY

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Symbiotic associations between certain soil fungi and plant roots constitute relationships termed "mycorrhizae". Mycorrhizal roots are observed in nearly all native stands of plants, in all parts of the world (4, 14). In climates ranging from tropical to arctic, woody and herbaceous plants are normally involved in this form of symbiosis. Fungal symbionts in mycorrhizal associations include members of the *Endogone, Ascomycetes and Basidiomycetes* (4, 13).

There are two major types of mycorrhizae, distinguished by the way in which the fungus attaches itself to the root (4, 6, 10). The first classification is the ectomycorrhizal group, and the second is the endomycorrhizal group. In ectomycorrhizal associations, a fungal sheath forms around the exterior of the root and is a distinctive visible feature (10). The fungal sheath consists of divided fungal hyphae, but appears superficially as though it were made of plant cells (6). From this outer sheath, hyphae extend outward into the soil, and also inward around the outer cortical cells of the root. The inward extension is termed a *Hartig*

Net and provides intimate contact for exchange between the two symbionts (6, 10,13).

The second type of mycorrhizae, the endomycorrhizal group, does not have any outwardly visible distinctions, as there is no fungal sheath (4, 6). In this case, however, the hyphae present inside the mycorrhizal root do penetrate the epidermal and cortical cells. The hyphae do not invade the endodermis, stele, or root meristem. Upon infection, the fungi produce structures called arbuscles, which are clusters of fine hyphae, within the cortical cells of the root. As the arbuscles form, starch disappears and the nuclei enlarge within the cell. At some point after this, these formations are digested and the contents absorbed by the plant cell. The nuclei return to the normal size. The formation and dissolution of these structures may serve as the prime mode of exchange between the two symbionts. Endomycorrhizae also contain structures called vesicles, which are ovate to spherical formations containing oil droplets (4). These may remain thin-walled and serve as storage, or become thick-walled and serve as resting spores.

An additional type of mycorrhizae is the ectendo-type, which is an intermediate classification for associations showing characteristics of both ectomycorrhizae and endomycorrhizae (5). Each type of mycorrhizal fungi colonizes the root prior to secondary growth. Upon colonization, the rate of root growth is reduced, and root longevity is extended (4). The results of these interactions are visible and significant increases in overall plant growth.

For woody ornamental species, the endomycorrhizal group has been most studied and is perhaps the most important classification. Although research with endomycorrhizal associations of *woody ornamentals will require expanded effort to thoroughly* explore the subject, increases in height, weight, and survival percentage have been observed for several woody crops (1,7,8,9,11).

Promotions of plant growth by mycorrhizal associations is a well established concept (2,4). These promotions of plant vigor may be mediated by three activites of the fungal symbiont. First, the fungi contribute to plant health through competitive exclusion by lowering root susceptibility to pathogen invasion (2). Colonization by pathogenic fungi, bacteria and nematodes may be reduced by a competition for plant metabolites and exudates, and by way of physical competition by the mycorrhizal hyphae

Secondly, uptake of water may be enhanced in mycorrhizal plants (4). Due to the absorptive surface added by the fungal hyphae and perhaps to more efficient absorption, mycorrhizal plants are observed to have an advantage under moisture stress conditions.

Finally, in a manner similar to moisture uptake, nutrient uptake may also be enhanced (2,4). Increases in absorption of nitrogen, phosphorus, and potassium have been found. The increases in dry weight of mycorrhizal over non-mycorrhizal plants correspond to increases in uptake of N, P, and K. These increases in growth are maximum when comparisons of growth are under conditions of low soil phosphorus (3,4). Thus, it is likely that this component may be more important than other nutrients when considering mycorrhizal interactions. Again, it is believed to be due to the added surface area and perhaps to more efficient uptake by the fungi.

Fungal development and health is also promoted by the mycorrhizal association. The benefits to the fungi begin with the plant exudates from the root (4). The exudates of metabolites, including carbohydrates, are important in mycelial growth and may also function in the initial attraction between the plant root and fungi. The main benefit for the fungi is the use of the root as a carbohydrate source (4).

Research is currently underway at Purdue University, examining the effect of mycorrhizal inoculation on selected woody plant species used in commercial nursery production. The following studies were initiated to investigate some parameters such as spore viability, soil fertility, and temperature which may be useful in evaluating the potential role of mycorrhizal inoculation in the soil management techniques of woody ornamental production.

MATERIALS AND METHODS

The objective of the first experiment was to determine the effects of pasteurized inoculum, containing mostly non-viable spores, on growth of tulip tree, *Liriodendron tulipifera L.* Seedlings were transplanted into 0.98 liter pots (one quart trade designation) containing steam pasteurized medium (2 perlite: 2 peat: 1 soil), fertilized with Osmocote 19-6-12 at a rate of 2 g N/liter. Plants were inoculated with 44,400 spores of *Glomus fasciculatus* (Thaxter) Gerdemann & Trappe per square meter of surface area of the container. In addition to fungal spores and hyphae, the inoculum also contained plant roots and soil. Plants were grown in the greenhouse for 12 weeks and then harvested. Analyses included measurement of height increase, dry weight of shoots and roots, and observation of mycorrhizal infection. Root staining to determine mycorrhizal infection was accomplished by the techniques of Phillips and Hayman (12).

The second experiment examined the effect of inoculation with *G. fasciculatus*, at a rate of 44,400 spores per square meter of container surface area, on tulip trees grown under 3 nutrient

regimes (0, 2, and 4 g N/liter). The experimental plants were grown from seed and transplanted into 3.28 liter pots (one gallon trade designation) containing steam pasteurized medium (2 perlite: 2 peat: 1 soil). Both inoculated and non-inoculated plants were grown under 3 fertility levels supplied by Osmocote 19-6-12 slow release fertilizer, and grown under greenhouse conditions. Analysis of variance was conducted for height increases, dry weight of shoots, and dry weight of roots. Mycorrhizal infection was observed through the use of root stains (12).

The third experiment evaluated the effect of two temperature regimes (40°C day/35°C night and 30°C day/25°C night) on growth of perennial ryegrass, *Lolium perenne* L. Treatments included inoculation with *G. fasciculatus* at rates of 0 or 44,400 spores per square meter of container surface area. Plants were seeded in 0.98 liter pots containing steam-pasteurized medium (2 perlite: 2 peat: 1 soil), and fertilized with Osmocote 19-6-12 slow release fertilizer at a rate of 2 grams N/liter. Plants were grown in a growth chamber at the appropriate temperatures. Analyses included dry weights of shoots, and root staining for observation of mycorrhizal infection (12).

Finally, the effects of incorporation of spores of *G mosseae* into a rooting medium were examined. Cuttings of Regel privet [*Ligustrum obtusifolium var. regelianum* (Koehne) Rehd.] were 16.3 cm long with 8 leaves. The cuttings were stuck either in vermiculite and perlite (1:1) or in the same medium amended with inoculum (3 media:1 inoculum, with 40,800 spores added to a flat 35 × 42.5 × 12.5 cm) or inoculum from the same source but steam pasteurized. The experiment was initiated on September 21, 1980 with cuttings under intermittent mist in a greenhouse with approximately 25% shade. Analyses included fresh weights of roots and enumeration of root initials visibly penetrating the stems of the cuttings. Root staining was conducted for observation of mycorrhizal development (12). Analyses took place on the third and sixth weeks after sticking of cuttings.

RESULTS

Tulip trees, inoculated with steam-pasteurized *G. fasciculatus*, showed minimal mycorrhizal development (less than 2% of cortical cells affected). Upon analysis, the plants inoculated with steam-pasteurized *G. fasciculatus* were not significantly taller and showed no significant increase in mean dry weight of roots or shoots over control plants receiving no treatment (Table 1).

In the fertility study, all non-inoculated plants showed minimal mycorrhizal development, as did inoculated plants grown under unfertilized conditions (0 g N/l). Roots of inoculated plants growing under medium and high fertility levels (2 and 4g N/l)

Table 1. Effect of pasteurized *G fasciculatus* inoculum on height increase, dry weight of shoots, and dry weight of roots of tulip trees

Measurement	Pasteurized inoculum	Control
Height increase (cm)	1 730	1.590
Shoot dry weight (g)	0.708	0.834
Root dry weight (g)	0 750	0.968

were highly mycorrhizal (greater than 50% of cortical cells affected).

At the lowest fertility level (0 g N/l) there was no significant difference in the increase in height of inoculated and non-inoculated plants. At the middle rate (2 g N/l) and high rate (4 g N/l), inoculated plants had significantly greater increases in height than non-inoculated plants. However, there was no real difference between plants receiving the same inoculation treatment in the middle fertility rate (2 g N/l) and the high rate 4 g N/l) (Table 2).

Table 2. Effect of inoculation with *G fasciculatus* on increase in height (cm) of tulip trees grown under three nutrient regimes (0 g N/l, 2 g N/l, and 4 g N/l of Osmocote 19-6-12)

Fertility (g N/l)	Inoculated	Non-inoculated
0	0 84	0.79
2	36.85	13.39
4	40 66	16 42

For dry weight of shoots, there was no difference between inoculated and non-inoculated plants at the 0 g N/l fertility rate (Table 3). At the 2 g N/l rate, shoots of inoculated plants had significantly greater dry weight than those of non-inoculated plants. Dry weights of shoots of inoculated plants grown in soil fertilized at the 4 g N/l rate again were significantly greater than the shoot dry weights of non-inoculated plants. The shoot dry weights of inoculated plants at the 4 g N/l rate were significantly greater than those of inoculated plants at the 2 g N/l rate. However, there was no similar difference between non-inoculated plants at those fertility levels.

Table 3. Effect of inoculation with *G fasciculatus* on shoot dry weights (g) of tulip trees grown under three nutrient regimes (0 g N/l, 2 g N/l, and 4 g N/l of Osmocote 19-6-12)

Fertility (g N/l)	Inoculated	Non-inoculated
0	0 178	0.147
2	7 067	0.671
4	9 406	1 797

For dry weight of roots of plants grown at the 0 g N/l fertility level, there was no real difference between inoculated plants and non-inoculated plants (Table 4). At the 2 and 4 g N/l levels of fertilization, the root dry weights of inoculated plants were significantly greater than those of non-inoculated plants. However, no distinction can be made between the results for the two fertility levels (2 and 4 g N/l).

Table 4. Effect of inoculation with *G fasciculatus* on root dry weights (g) of tulip trees grown under three nutrient regimes (0 g N/l, 2 g N/l, and 4 g N/l of Osmocote 19-6-12).

Fertility (g N/l)	Inoculated	Non-inoculated
0	0.163	0.207
2	2 481	0 481
4	3 100	0 813

The root stains of the temperature project show that there was a small amount (less than 10% cortical cells affected) of mycorrhizal development on inoculated perennial ryegrass plants at the lower temperature studied (30°C day/25°C night). Inoculated plants grown at the higher temperature regime (40°C day/25°C night) exhibited a very small degree of mycorrhizal development, which was less than that developed at the lower temperature regime No mycorrhizal development was noted on the non-inoculated plants.

For the 30°C/25°C treatment, no real difference in dry weights of shoots was detected (Table 5). However, at the higher temperature, the shoot dry weights of the inoculated plants were significantly less than those of the non-inoculated plants.

Table 5. Effect of inoculation with *G mosseae* on dry weight of shoots (g) of perennial ryegrass, grown under two temperature regimes (30°C day/25°C night and 40°C day/35°C night)

Temperature	Inoculated	Non-inoculated
30°C/25°C	2.150	2 210
40°C/35°C	0.120	0 230

From the experiment on the effect of mycorrhizal inoculum on the rooting of Regel privet cuttings, at week 3 after initial propagation, no difference was found among any of the 3 treatments (inoculum amendment, pasteurized inoculum amendment, and control) for number of roots or fresh weight of roots (Table 6 and Figure 1). By the 6th week after initial propagation, the cuttings rooted in media amended with inoculum were highly mycorrhizal, those in media with pasteurized inoculum showed a smaller amount of mycorrhizal development, and those in media with no amendment exhibited virtually no mycorrhizal development.

Table 6. Effect of incorporation of viable *Glomus mosseae* spores and pasteurized spores, into a rooting media, on number and fresh weight of roots of Regel's privet cuttings, as measured on week three after sticking of cuttings

Treatment	Number of roots	Fresh weight of roots (g)
Control	3 00	0 01
Inoculum	0 05	0 00
Pasteurized inoculum	0 00	0 00

On the 6th week, there was no real difference in number of roots found, but there was a separation based on the fresh weights of roots from the 3 treatments (Table 7). The fresh weights of roots from cuttings rooted in media amended with inoculum were significantly greater than those from cuttings rooted in the media with no amendment. The fresh weights of roots from cuttings rooted in the media amended with pasteurized inoculum were not significantly different from either of the other two treatments (Figure 2).

Table 7. Effect of incorporation of viable *G mosseae* spores and pasteurized *G mosseae* spores, into a rooting medium, on number and fresh weight of roots of Regel privet cuttings as measured on week six after sticking of cuttings

Treatment	Number of roots	Fresh weight of roots (g)
Control	4 75	0 21
Inoculum	8 75	0 49
Pasteurized inoculum	6 63	0 34

CONCLUSIONS

From these studies, conclusions relating to the production of woody ornamentals can be drawn. As demonstrated in the experiment on the effect of pasteurized inoculum on growth of tulip tree seedlings, spores and hyphae in the inoculum must not only be present, but also viable if growth increases are to be obtained. Also, inoculation results in important increases in plant growth both at medium (2 g N/l) and high (4 g N/l) fertility levels as shown in the tulip tree study. The shoot: root ratio differs for the 2 treatments, as indicated by the significant increase in dry weights of shoots of inoculated plants at the 4 g N/l level over the 2 g N/l level but lack of a similar increase for the dry weights of roots. More research is needed to determine which fertility level would be superior for establishment and subsequent growth of the mycorrhizal plants.

Not all plants are highly compatible with all species of mycorrhizal fungi. While perennial ryegrass plants grown under

a 30°C/25°C temperature regime did exhibit limited mycorrhizal development, inoculation did not contribute to growth of the plants. In fact, at the higher temperatures (40°C/35°C) growth was actually inhibited, perhaps due to the pathogens which are also introduced with the inoculum. It is important to have a strong plant-fungi compatibility prior to commercial inoculation in a production system.

From this research, no evidence was found to link inoculation with mycorrhizal fungi to promotion of root initiation. Inoculation was shown to result in early development of mycorrhizal roots and increased fresh weight of roots on cuttings of Regel privet. Root fresh weights of cuttings growing in media amended with pasteurized inoculum were intermediate between those growing in media amended with inoculum and those growing in the control medium because of 2 factors. First, the physical role of the amendment (addition of soil, peat, and perlite) may result in an improved rooting media. Secondly, some mycorrhizal development was noted on roots from cuttings rooted in the media amended with pasteurized inoculum, indicating that some spores did survive the steam heat treatment and may have increased root development upon infection. Inoculation in the propagation stage may prove to be most efficient, since it promotes the earliest development of mycorrhizal roots and the subsequent promotions of growth.

Although many woody plants still have not been studied, the concept of growth increases due to the formation of mycorrhizal fungi is well established Evaluation of mycorrhizal inoculation must be considered on a practical and economical basis before the controlled use of mycorrhizal fungi can become a reality for próduction of woody ornamentals. Natural mycorrhizal development is observed under normal production situations, and this factor must be considered when investigating the economical importance of an inoculation program If plants growing in the various media currently in use become inoculated by natural contamination (from the medium, air, and water) in the early stages of plant production, then it may not be necessary or perhaps economically feasible to provide additional inoculation.

While there is little information available on the economical feasibility of inoculation with mycorrhizal fungi in the production of woody ornamentals, certain situations seem to be most suited for inoculation. Such situations may include container production where no soil is included in the growing media; cases where the soil is thoroughly sterilized, as with methyl bromide; and in cases where plants are destined for very harsh growing environments, such as strip mine sites or new highway construction sites.

Recent trends in the production of woody ornamentals have been toward more complete control of the plant environment,

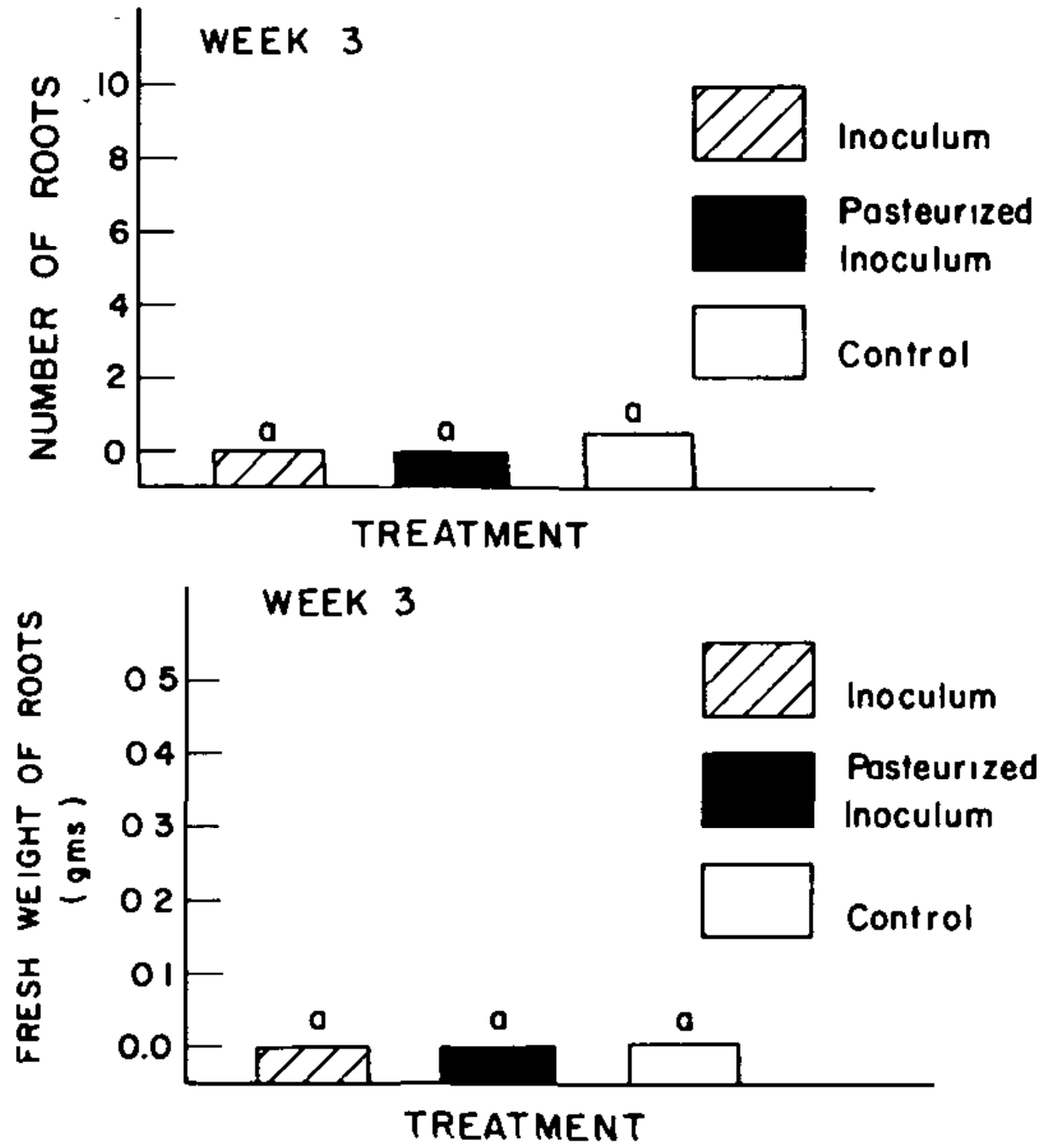

Figure 1. Effect of incorporation of viable *G mosseae* spores and pasteurized *G mosseae* spores, into a rooting medium, on number and fresh weight (g) of Regel privet cuttings as measured on week 3 after sticking of cuttings

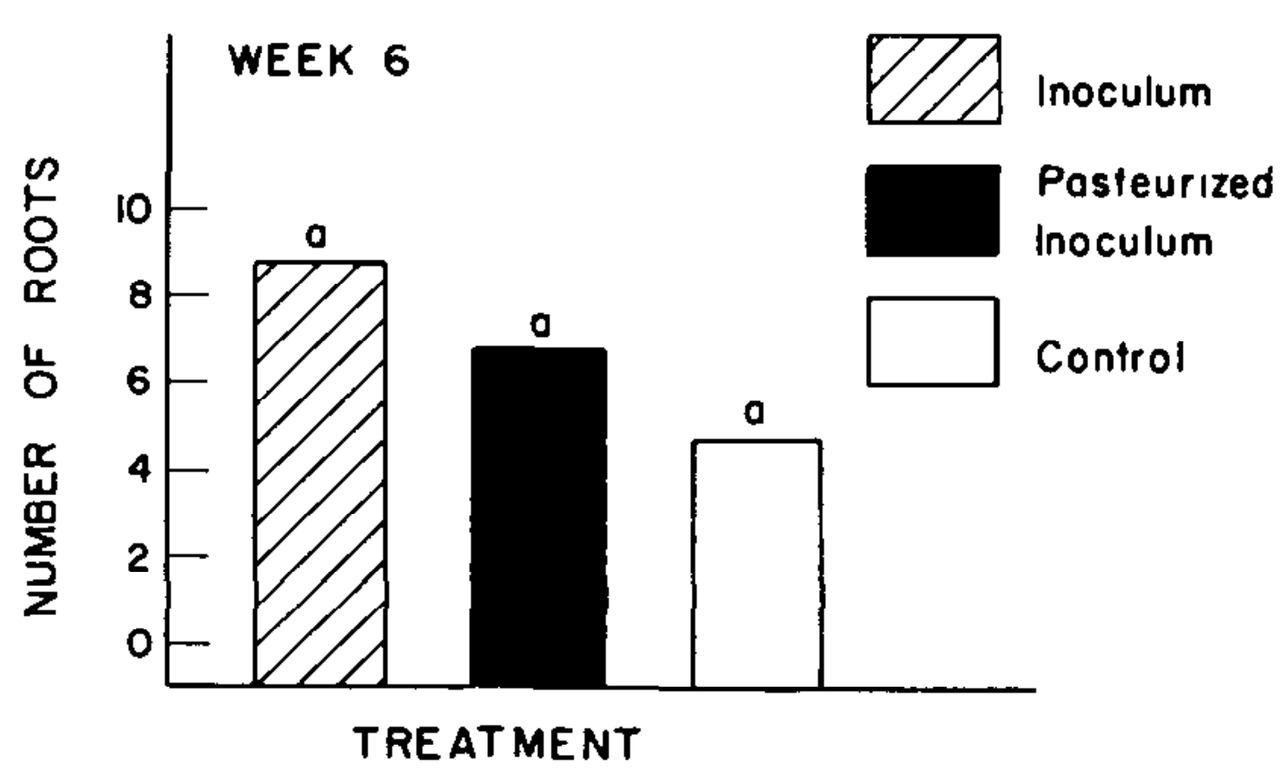

Figure 2. Effect of incorporation of viable *G mosseae* spores and pasteurized *G mosseae* spores, into a rooting medium, on number and fresh weight (g) of Regel privet cuttings as measured on week 6 after sticking of cuttings.

including fertility, temperature, and moisture relations. Plant scientists are now examining the microbiology of the root environment with the same enthusiasm. Nurserymen should be aware of the developments in mycorrhizal research in the near future, as we learn more about the mycorrhizal relations of the commercially important woody plants.

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PETER VERMEULEN: Did you say methyl bromide will destroy mycorrhizae?

STEPHEN VERKADE: Methyl bromide at the normal rate is likely to destroy mycorrhizae.

DALE MARONEK: You find that some spores are resistant to fumigation. Also when you fumigate you fumigate to only a certain level. The roots have the capacity to go below that level and actually pull the mycorrhizae back up into the bed.

INDOOR AND OUTDOOR PROPAGATION OF JUNIPER AND ARBORVITAE

PLATT W. HILL AND BRIAN THOMAS

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In the last 10 years, D. Hill Nursery has moved its growing operation which has blessed us with the opportunity to install new propagating facilites. In 1979, we constructed new greenhouses and growing frames with the idea in mind that we wanted to shift emphasis on production of conifers from inside energy intensive processes to outdoor less energy intensive techniques.

THE INDOOR FACILITIES

Our greenhouses are of the double poly type with fiberglass sidewalls. We root our cuttings in beds 6.5 × 90′ long by 7.5″ tall which are constructed as follows:

1. We lay 1″ of styrofoam at the base of the bed for insulation.
2. The loops of ½″ PVC Pipe for heating are then laid on top of the styrofoam. The PVC pipe is on 6.5″ centers.
3. The sides of the bed are 2 × 8″ boards (Wolmanized), held in place by lengths of ½″ black pipe driven in the ground. The pipes are spaced on 6′ centers and clamped to the 2 × 8″ boards.
4. The heat pipe and styrofoam are then covered with 4″ of pea stone which provides drainage and aids in heat distribution.
5. A layer of woven polypropylene fabric covers the pea gravel. This prevents roots from going into the pea gravel and also prevents the rooting media from infiltrating the pea gravel.
6. We use sand as a rooting medium.
7. We hand mist all of our indoor cuttings.
8. Depending on outside temperatures, we circulate water under the beds at between 105 and 125°F.