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SEEDLING GROWTH AND MYCORRHIZAL FORMATION IN CLEARCUT AND ADJACENT, UNDISTURBED SOILS IN MONTANA: A GREENHOUSE BIOASSAY*

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ABSTRACT

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In a series of greenhouse bioassays, tree seedling growth and root-tip development were compared among soils from two areas that had been clearcut and site prepared 16 years earlier and adjacent, undisturbed forest. Seedlings grown in soil from logged areas were shorter and had fewer root tips than those grown in undisturbed forest soil; however, effects on seedling weights were not consistent among species or experimental series. Fertilizing with nitrogen eliminated size differences due to soil source. In Douglas-fir [*Pseudotsuga menziesii* (Mirb.) Franco], the only species tested in sterilized soils, sterilization of logged soils resulted in increased seedling size and root-tip formation, whereas sterilization of unlogged soils had either no or a negative effect.

INTRODUCTION

Removing logging residues through broadcast burning or root raking is common practice in many areas to prepare sites for reforestation and reduce fire hazard. Perry and Rose (1982) reviewed effects of these practices on site productivity. Root raking concentrates nutrients on a small proportion of the site and has been shown to result in decreased productivity (Ballard, 1978; Glass, 1976). Broadcast burning may volatilize from 30—90% of the nitrogen (N) in litter and upper soil layers, depending on the heat of the burn, and produce long-term changes in soil biological properties (Bissett and Parkinson, 1980). Little work has been done on the effects of broadcast burning on site productivity.

At least two problems are inherent in studies relating site perturbation to future productivity (see the discussion by Stone, 1979). First, observations of growth patterns on logged and unlogged areas are necessarily confounded because their environments are so different. Comparing soil nutrients

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of disturbed and undisturbed areas can be useful (for example, White, 1974); however, plant nutrition depends on factors other than only the simple presence of nutrients. Mycorrhizal fungi form an important link in the nutrient chain not only by physically extending the plant root but also by solubilizing mineral elements (Bowen, 1973; Graustein et al., 1977). Recent research has shown that other rhizosphere microorganisms may also play a role (Kloepper et al., 1980; Wilde et al., 1980; Powell et al., 1980). Impacts of site preparation on these organisms may well influence site productivity (see the review by Harvey et al., 1976) but may not be detectable by standard nutrient analyses.

Second, our understanding of recovery processes is poor. How fast, if at all, will nutrient losses be recouped through natural inputs? How quickly do populations of soil organisms return to normal? Predicting N recovery is particularly difficult because we know little about the magnitude of N fluxes (inputs and losses) through paths such as symbiotic and free-living N fixation, sorption on soil particles (Malo and Purvis, 1964), denitrification, and leaching (Vitousek and Melillo, 1979).

The research reported here consisted of three separate experiments. All involved bioassays (pot tests) of soils disturbed to various levels 13—16 years earlier. Bioassays may be subject to artifacts and do not necessarily extrapolate to field conditions (Peterson, 1971; Mead and Pritchett, 1971). But they are a more realistic assessment of nutrients available to plants than conventional chemical analyses (Stone, 1979), allow detection of effects not related to simple presence of nutrient elements (e.g., alteration of soil biology), and permit comparison of soils from disturbed and undisturbed areas to be made under controlled conditions. We compared

(1) growth of three tree species and domestic wheat in N-fertilized and unfertilized replications of the various soil-disturbance types;

(2) root-tip formation on the three tree species grown in unfertilized replications of the soil-disturbance types; and

(3) growth and root-tip formation of one of the tree species in sterile and nonsterile soils.

Our objectives were to determine

(1) if seedling growth differed in disturbed and undisturbed soils, and if this difference was associated with N availability;

(2) if root-tip formation, and, therefore, a seedling's ability to take up nutrients, differed in disturbed and undisturbed soils; and

(3) if elimination of soil biological activity through sterilization affected seedling growth and root-tip formation.

METHODS

Study sites

Study sites were in southwest Montana, on a high (2200 m), level plain adjacent to the west boundary of Yellowstone National Park. Soils are loamy sands formed from alluvial deposits of obsidian and rhyolite (Stermitz et al., 1974). Forests are pure, uneven-aged lodgepole pine (*Pinus contorta* var. *latifolia* Engelm.), with site index (height in meters at 100 years of age) of 12. Climate is continental mountain, with cold winters and a short growing season. Precipitation for June through August averaged 147 mm from 1962 through 1975 (U.S. Weather Bureau, 1962—1975).

Two 80- by 500-m strips, approximately 3 km apart, were clearcut in May 1963. On roughly 75% of each strip, logging slash was piled in windrows by bulldozers (W). Soil present in the windrows suggests that at least some topsoil was scraped away during piling. W areas are sparsely covered with various grasses and forbs, scattered bitter brush (*Purshia tridentata*), an N-fixing shrub, and about 1300 young (1—13 years old) lodgepole pine per ha. On the remaining area, tree limbs and foliage were piled by hand into numerous, small windrows and burned, creating approximately 2-m-wide strips covered with ash, charcoal, and charred branches and stems (BB), as well as intervening unburned strips from which tree crowns had been removed by hand (NBB). In contrast to W and BB areas, duff and litter layers of the NBB strips were essentially undisturbed. BB areas had no tree stocking and NBB areas, about 650 young lodgepole pine per ha.

Experiment 1

In September 1976, we collected soil (not including surface litter) to a depth of 15 cm from three randomly selected points in BB and W areas of each clearcut strip as well as from adjacent, undisturbed forest (UF). Soils from each treatment were composited and bioassays conducted with seedlings of lodgepole pine, Douglas-fir [*Pseudotsuga menziesii* (Mirb.) Franco], Engelmann spruce (*Picea engelmannii* Parry ex Engelm.), and domestic wheat (*Triticum* sp.). Seed from a single species was sown in 8-inch pots containing UF, BB, or W soil. Half these pots were fertilized once every 2 weeks with 50 ppm NH_4NO_3 dissolved in irrigation water (distilled); the other half served as controls. Each experimental unit (species × soil source × N amendment) was replicated five times. Pots were placed within a single greenhouse bench in a completely random design and plants grown under natural daylengths extended to 20 h with six gro-lux lamps. Wheat was harvested 1 month after seed germinated; trees (averaging nine per pot in UF and BB soils, eight per pot in W) were harvested 6 months after seed germinated. The height of each tree seedling was recorded and ovendry weight determined for tops and roots in each pot for both wheat and trees.

Experiment 2

In September 1978, soil samples were collected from ten randomly chosen points in each of the three treatment areas of strip A and its adjacent, undisturbed forest. All samples were bioassayed for mycorrhizae and analyzed for total N, water-soluble NO_3, total KCl-extractable NH_4 (Black, 1965), mineralizable N (Keeney and Bremner, 1966), and total carbon (by Leco W-12 carbon analyzer). For mycorrhizal bioassays, we mixed two parts sieved soil with one part each steam-sterilized peat and vermiculite. Individual seedlings of the three previously used tree species were grown for 4 months (Engelmann spruce for 6 months) in pine-cell-size Ray-Leach tubes filled with this soil mixture. Each soil-collection point was potentially represented by five randomly placed Ray-Leach tubes, each with one seedling. Because of mortality, however, particularly among Engelmann spruce, the number of seedlings per soil-collection point varied. In some cases, all five seedlings died; thus, the number of replications (collection points) per cell (soil-disturbance type × species) varied from 5—10. Seedlings were grown in a greenhouse with natural daylengths extended to 16 h with sodium vapor lights. We used a dissecting microscope to count mycorrhizae and squash mounts and thin sections when necessary to verify presence of the Hartig net.

Experiment 3

In August 1980, a final soil collection was made from five randomly chosen points in the BB and W areas of strip B and its adjacent, undisturbed forest. Soil from each treatment was composited as in experiment 1, and half was steam sterilized. Five Douglas-fir seedlings were grown in sterile and five in nonsterile soil from each disturbance type. Growing conditions were as in experiment 2. Seedlings were lifted 4 months from planting; mycorrhizal and total root tips were counted as in experiment 2; and top and root weights were determined separately after drying for 48 h at 30°C.

Analyses

For experiment 1, average seedling height and weight were calculated per pot, and this value was used as a replicate. (Degrees of freedom for seedling weights were less than those for heights because of loss of some samples.) Analysis of covariance was run for height, top weight, and root weight, using number of seedlings per pot as a covariate. Analysis of variance was run for the other two experiments, using soil-collection points (experiment 2) and individual seedlings (experiment 3) as replicates. For experiments 1 and 3, in which heights and root weights were distributed lognormally, analysis was performed on log-transformed values. In all other cases (top weights, root tips, heights in experiment 2), data were distributed normally and therefore not transformed for analysis.

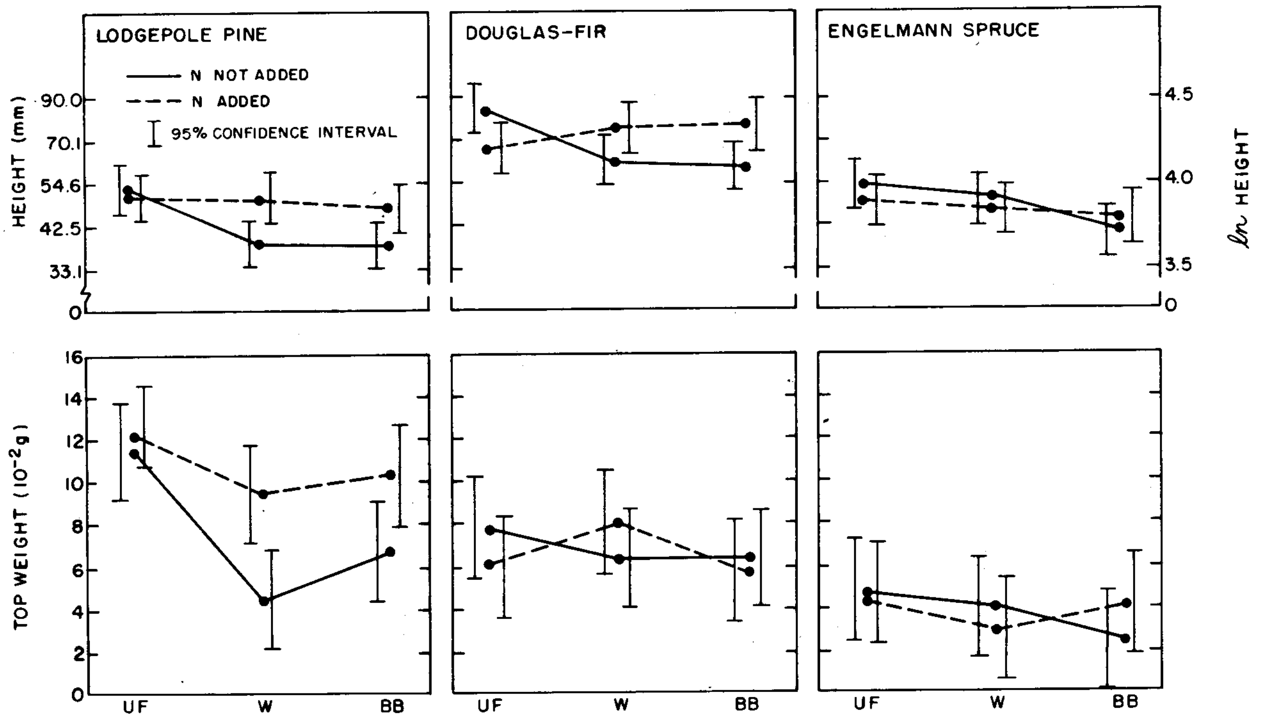

Fig. 1. Seedling height and top weight in N-fertilized and unfertilized soils collected from logged and windrowed (W) and logged and broadcast-burned (BB) areas and adjacent, undisturbed forest (UF) (experiment 1).

RESULTS

Experiment 1

Without fertilization, seedling heights were lower in disturbed than undisturbed soils; however, when N was added, there was no difference (Fig. 1). Analysis of covariance (Table I) shows no significant interaction between species and soil, although Engelmann spruce heights were little affected relative to the other tree species and were not increased by the added N.

TABLE I

Analysis of covariance for experiment 1[a]

	Height		Top weight		Root weight	
Source	d.f.	MS	d.f.	MS	d.f.	MS
Soil (*D*)	2	0.22**	2	25.80*	2	0.10
Species (*S*)	2	1.64**	2	169.04**	2	13.85**
Nitrogen level (*N*)	1	0.06	1	16.40	1	0.01
$D \times S$	4	0.06	4	16.00*	4	0.29
$D \times N$	2	0.22**	2	9.29	2	0.15
$S \times N$	2	0.06	2	21.96**	2	0.23
$D \times S \times N$	4	0.03	4	5.90	4	0.08
Error	71	0.03	61	6.20	61	0.14

[a] Height and root weight were log transformed for analysis.
*, **Significant at 5% and 1% level, respectively.

Top weights were significantly different among soils (Table I), but this was largely due to the strong negative response of lodgepole pine seedlings to disturbed soils; in the other tree species, little or no top weight reduction occurred in disturbed soils. Root weights were unaffected in all tree species.

Biomass of wheat was higher in W and BB than UF soils and was positively correlated ($r^2 = 0.99$) with soil pH.

Experiment 2

For all tree species, height and number of total and mycorrhizal root tips per seedling were lower in soils from clearcut strip A than in those from adjacent, undisturbed forest (Table II, Fig. 2). Differences in the percentage of mycorrhizal tips, however, were small or nonexistent.

Experiment 3

Seedlings grown in nonsterile W and BB soils were generally smaller and

TABLE II

Analysis of variance for experiment 2

Source	d.f.	MS Height	 Total root tips	 Mycorrhizal root tips
Soils (*D*)	3	143.98**	30460**	23640**
Species (*S*)	2	3189.50**	14973**	22213**
D × *S*	6	35.81	1576	1221
Error	71	20.57	1644	1190

**Significant at 1% level.

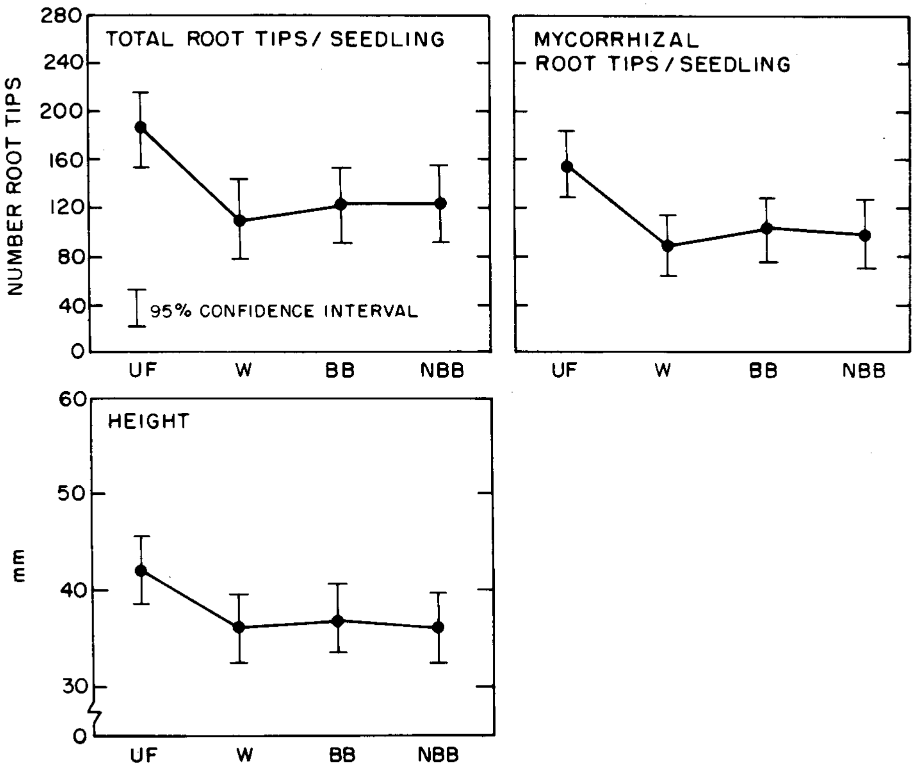

Fig. 2. Number of root tips and height of seedlings grown in soils collected from strip A and adjacent, undisturbed forest (experiment 2). Values are means of the three tree species. (UF, W, and BB as in Fig. 1; NBB = logged and neither windrowed nor broadcast burned.)

TABLE III

Analysis of variance for experiment 3[a]

Source	d.f.	MS Height	MS Stem weight	MS Root weight	MS Total root tips
Sterility (*S*)	1	0.26**	445.82**	1.42*	19.50
Soil (*D*)	2	0.01	46.61	0.13	72.59*
S x *D*	2	0.17**	607.05**	2.17**	86.10*
Error	22	0.03	18.94	0.24	19.11

[a] Height and root weight were log transformed for analysis; root-tip numbers were square-root transformed.

*, **Significant at 5 % and 1% level, respectively.

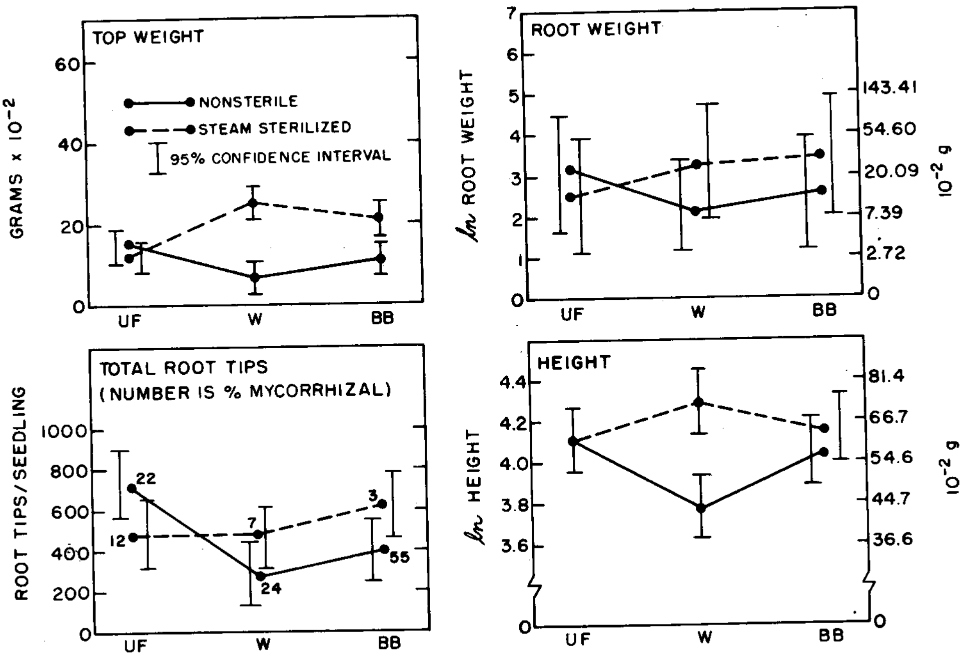

Fig. 3. Weight, height, and number of root tips of Douglas-fir seedlings grown in sterile and nonsterile soils collected from strip B and adjacent, undisturbed forest (experiment 3). (UF, W, and BB as in Fig. 1).

formed fewer root tips than those grown in nonsterile UF soil (Table III, Fig. 3). However, sterilization reversed this relationship, generally increasing seedling growth and root-tip formation in soils from logged areas but having either no or a negative effect on seedling performance in undisturbed soil.

TABLE IV

Chemical analyses of soils from disturbed and undisturbed sites[a]

	Total N (%)		Mineral-izable N (ppm)		NH_4 (ppm)		NO_3 (ppm)		Carbon (%)		pH	
Treatment	$\overline{X}$	SE	$\overline{X}$	SE	$\overline{X}$	SE	$\overline{X}$	SE	$\overline{X}$	SE	$\overline{X}$	SE
UF	0.146a	0.011	14a	1.2	11a	1.1	0.33a	0.10	2.97a	0.50	5.03a	0.23
W	0.102b	0.011	5b	1.0	7bc	0.8	0.23a	0.07	3.22a	0.52	5.29a	0.19
BB	0.145a	0.011	9c	1.6	9ab	0.8	2.19b	0.69	3.27a	0.57	5.34a	0.23
NBB	0.180a	0.030	9bc	1.7	6c	0.9	0.66a	0.18	—	—	5.18a	0.11

[a] Means followed by the same letter are not significantly different at the 5% level.

Soil chemical analyses

Chemical analyses tended to support indications of N deficiency in disturbed soils. Mineralizable N was lowest in W, highest in UF, and intermediate in BB and NBB soils (Table IV). Total soil carbon (C) was not different among UF, W, and BB soils (NBB carbon was not tested); however, W areas were lower in total N. Thus, the C/N ratio of W soils was higher than that of UF and BB soils. Nitrate levels were quite high in BB soils.

DISCUSSION

Accelerated nutrient loss and short-term reduction of nutrient capital after severe scarification or whole-tree logging are well documented. Work by Glass (1976) and Ballard (1978) indicate that, at least in some cases, these effects may persist long enough to influence wood yields.

Our data show the following:

(1) Sixteen years after logging, seedling heights were lower in soils collected from logged strips than in those from adjacent, unlogged areas. However, differences in seedling weights between logged and unlogged soils varied among species and experiments. The stronger effect on Douglas-fir weight seen in experiment 3 as compared to experiment 1 may relate to different experimental parameters; in experiment 1, seedlings were grown several to a pot but in experiment 3 they were grown singly, eliminating seedling competition.

(2) Seedling root-tip production was lower in soils from the two logged strips than in those from adjacent, undisturbed forest. We hypothesized that differences in root-tip formation among soils were due to differences in presence of mycorrhizal inocula. If so, root-tip formation in soils from logged and unlogged areas should not differ when the soils are sterilized (experiment 3). Although this was the case, the nature of the response was not as we would have predicted if availability of mycorrhizal inocula were the primary causal factor in root-tip formation.

Other work suggests that soil organic material may play a role in mycorrhizal root-tip formation. Meyer (1980) saw reductions in number of mycorrhizal root tips on burned sites in the Oregon Cascade Mountains but not on logged sites where woody slash had not been burned, although this effect again varied with tree species. Harvey et al. (1979) found that most mycorrhizae were associated with humus and decayed wood in Montana soils. In contrast, Alvarez et al. (1979) found that growth and development of mycorrhizae in some California soils were favored by the absence of organic material. However, in our study, the quantity of soil organic material was not correlated with root-tip formation, nor was the C/N ratio. Thus, the reason for reduction in root-tip formation in disturbed soils remains unclear. Because tips are the primary absorbing root surfaces, reduced seedling height in W and BB soils may have been due to the decreased ability of roots to absorb nutrients as well as reduced nutrient availability.

(3) Lower mineralizable N in disturbed soils, coupled with positive seedling response to added N, suggests that effects on growth were N related. Seedling response to soil sterilization, however, complicates this interpretation. Soil disturbance has been clearly shown to impact microbial populations (Widden and Parkinson, 1975; Bissett and Parkinson, 1980; Perry and Rose, 1982); in some cases, this may affect subsequent tree growth (Klock and Benson, 1975; Jalaluddin 1968, as cited in Ahlgren, 1974). In one Oregon clearcut, Perry and Rose (1982) found results similar to those presented here: seedling root-tip production increased when broadcast-burned soils were sterilized but decreased when undisturbed soils were sterilized. The burned area of that Oregon site revealed no bacterial production of siderophores, proteins that play an important role in both plant nutrition and protection (S.L. Rose and D.A. Perry, unpublished data, 1981). The results of experiment 3 do not necessarily indicate that soil biological factors influenced seedling response. Although we steam sterilized, which disrupts soil physical structure less than autoclaving, mechanical effects may well have influenced our results.

These experiments should be interpreted cautiously. Where soils from the various areas were pooled before bioassay (experiments 1 and 3), results apply only to the tested soils. Also, no single experiment was designed such that effects of generalized logging and slash disposal treatments were tested. Our data refer to certain areas on the ground and reflect effects due to both treatment (logging and slash disposal) and inherent differences in location. However, the area within which soils were collected is a glacial outwash plain with no obvious environmental discontinuities, and it seems probable that the results of our assays accurately reflect differences between logged and unlogged portions of these two sites.

These data add to the growing body of knowledge which suggests that windrowing and hot broadcast burns should be avoided where possible. We cannot say for certain that similar effects will be manifested in field-grown seedlings, as tree growth under natural conditions depends on many factors that are not reflected in a greenhouse bioassay. The strong, negative response of lodgepole pine height and weight in the bioassay of disturbed soils suggests that yields of this species may, in fact, be affected. Reduction in root-tip formation, if it occurs in the field, may influence a seedling's ability to compete for nutrients, its tolerance to drought (Duddridge et al., 1980), and its resistance to root pathogens (Marx, 1969).

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