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Protocol Note

Soil type affects *Pinus ponderosa* var. *scopulorum* (Pinaceae) seedling growth in simulated drought experiments[1]

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- *Premise of the study:* Effects of drought stress and media type interactions on growth of *Pinus ponderosa* var. *scopulorum* germinants were investigated.
- *Methods and Results:* Soil properties and growth responses under drought were compared across four growth media types: two native soils (dolomitic limestone and granite), a soil-less industry standard conifer medium, and a custom-mixed conifer medium. After 35 d of growth, the seedlings under drought stress (reduced watering) produced less shoot and root biomass than watered control seedlings. Organic media led to decreased root biomass, but increased root length and shoot biomass relative to the mineral soils.
- *Conclusions:* Media type affected root-to-shoot biomass partitioning of *P. ponderosa* var. *scopulorum*, which may influence net photosynthetic rates, growth, and long-term seedling survival. Further work should examine how specific soil properties like bulk density and organic matter influence biomass allocation in greenhouse studies.

Key words: greenhouse; growth media; *Pinus ponderosa* var. *scopulorum*; ponderosa pine; seedling growth; soil.

Plant-available water within any soil or growth medium is affected by multiple factors including bulk density, porosity, texture, and volume of soil accessible to the root system. In mineral soils, the degree of weathering of parent material can affect a soil's bulk density and porosity by altering soil particle size and texture (percent sand, silt, and clay) (Foth, 1990). Soils or growth media with a high percentage of organic matter (greater than 12%) typically have lower bulk density and higher soil porosity, water-holding capacity, and soil aggregation than mineral soils with less than 12% organic matter (Scott, 2000), but aggregation may be minimal in native conifer soils with sandy texture (Azuaje et al., 2012). Soil properties such as soil texture, organic matter, and pH can also influence nutrient availability (Havlin et al., 2005). Growth media composed largely of organic material like peat ("organic media," such as Pro-Mix) differ from mineral soils because their characteristics are influenced by the large component of organic matter, rather than by the soil texture (Foth, 1990). Media high in organic matter may have high cation-exchange capacities, but may not be able to retain potassium and other cations due to a low percentage of silt and sand particles. Consequently, the type of growth medium used in research may influence plant growth and response to experimental treatments.

Little literature exists on the effects of using organic media as compared to use of mineral soils similar to those of the native environments on seedling growth, and thus use of nonnative media may not lead to accurate predictions of plant response to stress in a natural environment. Many studies on drought tolerance of conifers occur in greenhouses and use organic media (Aranda et al., 2010) or a mineral soil–organic media mixture (Ladjal et al., 2007), while other studies (McMillin and Wagner, 1995) do not indicate the type of media used in the experiment. Other studies that have included native soil parameters have not examined soil depths that commonly occur in native environments (Torreano and Morris, 1998). Shallow soils may contain less water, resulting in reduced heat diffusion and greater soil temperatures (Scott, 2000) that can affect root development and architecture and, ultimately, seedling survival (Del Campo et al., 2007a). Soil qualities like texture and depth will affect water infiltration, retention, and availability, as well as nutrient supply and availability (Scott, 2000), and may affect seedling survival and root development.

Most mountain conifers from western North America do not natively grow in soils with organic matter greater than 12% (Scholes and Nowicki, 1998) but are considered generally tolerant of drought stress (Richardson and Rundel, 1998). However, water stress and soil type may interact to influence root growth (Howard, 2003). For example, ponderosa pine (*Pinus ponderosa* P. Lawson & C. Lawson) seedling growth and root nitrogen levels are reduced under dry conditions (Dumroese et al., 2011). Young ponderosa pine (*Pinus ponderosa* var. *scopulorum* Engelm.) survival in dry environments can be affected by seedling root length and planting depth (Pinto et al., 2011). Consequently, root growth potential can be a good predictor of seedling survival, at least in another western

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conifer such as Aleppo pine (*Pinus halepensis* Mill.) (Del Campo et al., 2007b). This experiment examined seedlings of ponderosa pine, a widespread and economically valuable conifer, found throughout submontane forests in western North America (Richardson and Rundel, 1998; Howard, 2003). Given the potential differences in pine seedling growth in traditionally used organic media in the greenhouse vs. native mineral soils under drought conditions, we investigated the influences of growth medium and drought on soil surface temperature, root architecture, and biomass accumulation in ponderosa pine seedlings.

METHODS AND RESULTS

Four specific growth media were compared in this experiment: two organic media and two mineral soils. The two organic media included a standard conifer mix (Fafard 52 Mix; Conrad Fafard Inc., Agawam, Massachusetts, USA) consisting of Canadian sphagnum peat moss (30%), processed pine bark (55%), and a mix (15%) of vermiculite, perlite, starter nutrients, wetting agent, and dolomitic limestone, and a custom mix of 1 : 1 : 3 (v/v) perlite, steam-sterilized loamy sand, and Fafard 52 (Kilgore, 2007). Native mineral soil material was cleared of undecomposed leaf litter and collected to 15 cm depth from dolomitic and granitic parent materials within the current elevational range of ponderosa pine in the Santa Catalina Mountains of southern Arizona. Dolomitic soil was collected within 5 m of 32°26.877′N, 110°46.013′W, 2446 m elevation, and granitic soil was collected within 5 m of 32°23.489′N, 110°41.662′W, 2167 m elevation. All specific media were analyzed by the Soil Testing Laboratory at Michigan State University (MSU) for pH, phosphorus, potassium, and calcium content (Table 1). Organic matter and cation-exchange capacity were measured for the two mineral soils. The organic matter for the standard and custom mix is based on product supplied analysis. Eighty rhizotrons measuring 20 × 20 × 1.5 cm (James et al., 1985) were constructed from clear acrylic plexiglass and tubing, with four adjacent rhizotrons within one structure. Rhizotrons were sterilized in a 10% bleach solution, rinsed, and air-dried. The unsterilized growth media were randomly assigned to each of the four rhizotrons within a structure and added to 19 cm depth. The media were not sterilized to prevent alterations in nutrient availability that can result from sterilization (Ferriss, 1984; Heinrich and Patrick, 1986). Due to insufficient quantity of the native mineral soils for full replication, 20 rhizotrons each contained the standard or the custom mix, 13 rhizotrons contained dolomitic soil, and 15 rhizotrons contained granitic soil. To minimize the influence of the unfilled rhizotron cells, medium-grit crushed granite was used to fill the remaining 12 rhizotrons but was not included in the final analysis. Half-sibling seeds (n = 100) from a single ponderosa pine tree ("MTL 011," 2752 m elevation) in the Santa Catalina Mountains were surface-sterilized in a 10% bleach solution for 5 min, rinsed, and imbibed in deionized water for 24 h. One randomly selected pregerminated seed (moist, dark conditions for 3 d) was placed on the soil surface of each rhizotron, with the radical entering the soil. Paperboard was placed between the rhizotrons to minimize the effect of light on the roots, and the rhizotrons were set at an 11° angle from vertical to encourage root growth along the translucent wall. To minimize spatial temperature variation, rhizotrons were contained in an open-topped box constructed of 2.5-cm-thick polystyrene insulation.

Plants were grown under natural light from February to April 2008 in a greenhouse at East Lansing, Michigan, USA (42.72°N, 84.47°E, 260 m elevation) at a day/night temperature of 27/24°C. Each rhizotron received 150 mL of tap water at planting and 50 mL water every 3 d until initiation of the drought treatment 15 d after planting. Rhizotrons receiving the drought vs. control watering treatment were randomly assigned within each media treatment. Control seedlings were watered every 4 d with 50 mL water, while drought-stressed seedlings received 5 mL water 10 d after drought initiation (25 d after planting). Soil surface temperatures (Model 42529 Wide Range IR Thermometer; Extech Instruments Corporation, Nashua, New Hampshire, USA) were measured for all rhizotrons on four consecutive days (31 Mar–3 Apr) two weeks into the drought period. The experiment was terminated 20 d after drought initiation (day 35 of the experiment) to prevent root binding in the well-watered rhizotrons because of rapid taproot development, and because of severe drought stress in the droughted seedlings. Seedlings were removed from the disassembled rhizotrons, roots were rinsed in tap water, and the roots were scanned at 1600 × 3200 dpi (Perfection 1640SU Scanner; Epson Corporation, Long Beach, California, USA). Images were digitally cropped and similarly enhanced for clarity (Photoshop CS2; Adobe Systems Incorporated, San Jose, California, USA). Root length and surface area were calculated by Root Image Analyzer at the Root Image Processing Laboratory at MSU. After root scanning, each seedling was separated into root and shoot portions, dried for 24 h at 70°C, and weighed. Data by media type were tested for evidence of nonnormality ($P < 0.05$) using the Shapiro–Wilk test and transformed only if such evidence decreased for all media types for a given variable. Root length and root surface area were log transformed before ANOVA, while root length and root : shoot biomass ratio were square-root transformed for analysis by soil type (organic vs. mineral). Differences in soil temperature, root length, root surface area, root biomass, shoot biomass, and root : shoot biomass ratio were determined by two-way ANOVA with media and watering treatment as fixed factors and with $P < 0.05$ indicating significance. Data were analyzed examining each specific medium separately and also by comparing the two organic media to the two mineral soils. Interactions were not significant ($P > 0.1$) and thus excluded from the results. All statistical analyses were conducted in IBM SPSS Statistics Version 19.0.0 (IBM Corporation, Armonk, New York, USA).

The physical and chemical properties of the growth media markedly varied (Table 1). The soil acidity was similar in the standard (pH 6.9) and custom (pH 6.2) organic media mixes and native dolomitic soil (pH 6.4) but was much lower (pH 5.5) in the native granite soil. Exchangeable potassium and calcium content followed a similar pattern to pH and were most available in the organic media (standard and custom mix). Potassium and calcium are essential plant nutrients involved in many cell processes such as stomatal control of transpiration, cell membrane, and cell wall stability (Matoh and Kobayashi, 1998; Epstein and Bloom, 2005). Organic matter content was higher in the standard and custom media mixes than the native granitic (1.5%) and dolomitic (5.2%) mineral soils. The standard medium contained approximately 85% organic matter, and the custom mix was approximately 50% organic matter because 60% of its volume was the Fafard 52 with the remaining volume of perlite and steam-sterilized loamy sand (Kilgore, 2007). There was no evidence of mycorrhizal colonization observed on the seedling roots after harvest despite the medium and soil not being sterilized. Soil surface temperature was not influenced by media type ($F < 0.606$, $df = 3$, $P > 0.6$) even though organic matter content was greater in the standard and custom mix media (varied by 0.2 to 0.4°C between media). However, surface temperature increased by 2.4°C under drought treatments for all media when compared to the control ($F > 7.037$, $df = 1$, $P < 0.02$).

Table 1. Properties of organic media and mineral soil used in the greenhouse drought experiment.

Specific media	Media source	Soil type and texture[a]	pH	CEC (cmol$^+$/kg)	P (mg/kg)	K (mg/kg)	Ca (mg/kg)	OM (%)[b]
Standard media	Conrad Fafard Inc., Agawam, Massachusetts, USA	organic	6.9	—	65	340	2083	85
Custom mix	East Lansing, Michigan, USA	organic	6.2	—	103	493	1207	51
Native dolomite	32°26.877′N, 110°46.013′W, 2446 m elev.	mineral, sandy loam (67.6% sand, 28.2% silt, 4.2% clay)	6.4	15.0	60	167	1629	5.2
Native granite	32°23.489′N, 110°41.662′W, 2167 m elev.	mineral, loamy sand (80.0% sand, 17.1% silt, 2.9% clay)	5.5	3.5	11	61	330	1.5

Note: Ca = calcium; CEC = cation exchange capacity; K = potassium; OM = organic matter; P = phosphorus.

[a] No texture and cation-exchange capacity are listed for the organic media because they are not mineral soils.

[b] Organic matter percentages from the standard and custom mix are reported based on manufacturer's product analysis.

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Average surface temperature across media for the control was 28.7°C, whereas the temperature of drought treatments across media was 31.1°C.

Root length was not affected by specific media ($F = 2.466$, $df = 3$, $P = 0.074$), but the roots from seedlings grown in organic media (230.3 mm, one standard error of the mean [SEM] = 14.3 mm) were longer ($F = 5.051$, $df = 1$, $P = 0.029$) than those grown in mineral soils (185.0 mm, SEM = 19.3 mm). Root surface area was not affected by specific media ($F = 1.371$, $df = 3$, $P = 0.263$) or media type ($F = 2.786$, $df = 1$, $P = 0.101$). Drought did not reduce root length ($F = 2.634$, $df = 1$, $P = 0.111$) or root surface area ($F = 1.151$, $df = 1$, $P = 0.289$). Because the majority of the roots in this study were classified by the processor as the smallest category of root size, fine differences in radial root growth may not have been detected. In larger rhizotrons, Torreano and Morris (1998) observed no difference in final root distribution by visual examination and soil cores in the drought-stressed and control loblolly pine seedlings. However, Ludovici and Morris (1996) did observe reduced pine root density (25%) via soil cores and surface area (28%) via leaf area meter in drought-stressed loblolly pine seedlings over two growing seasons. A combination of longer time and larger soil volume in our experiment may have led to detectable differences in root length and surface area in drought-stressed ponderosa pine seedlings.

Seedlings allocated less biomass to shoots and more to roots in the mineral soils as compared to the organic media (Fig. 1). In mineral soils relative to organic media, total seedling biomass was 14% less ($F = 4.987$, $df = 1$, $P = 0.03$),

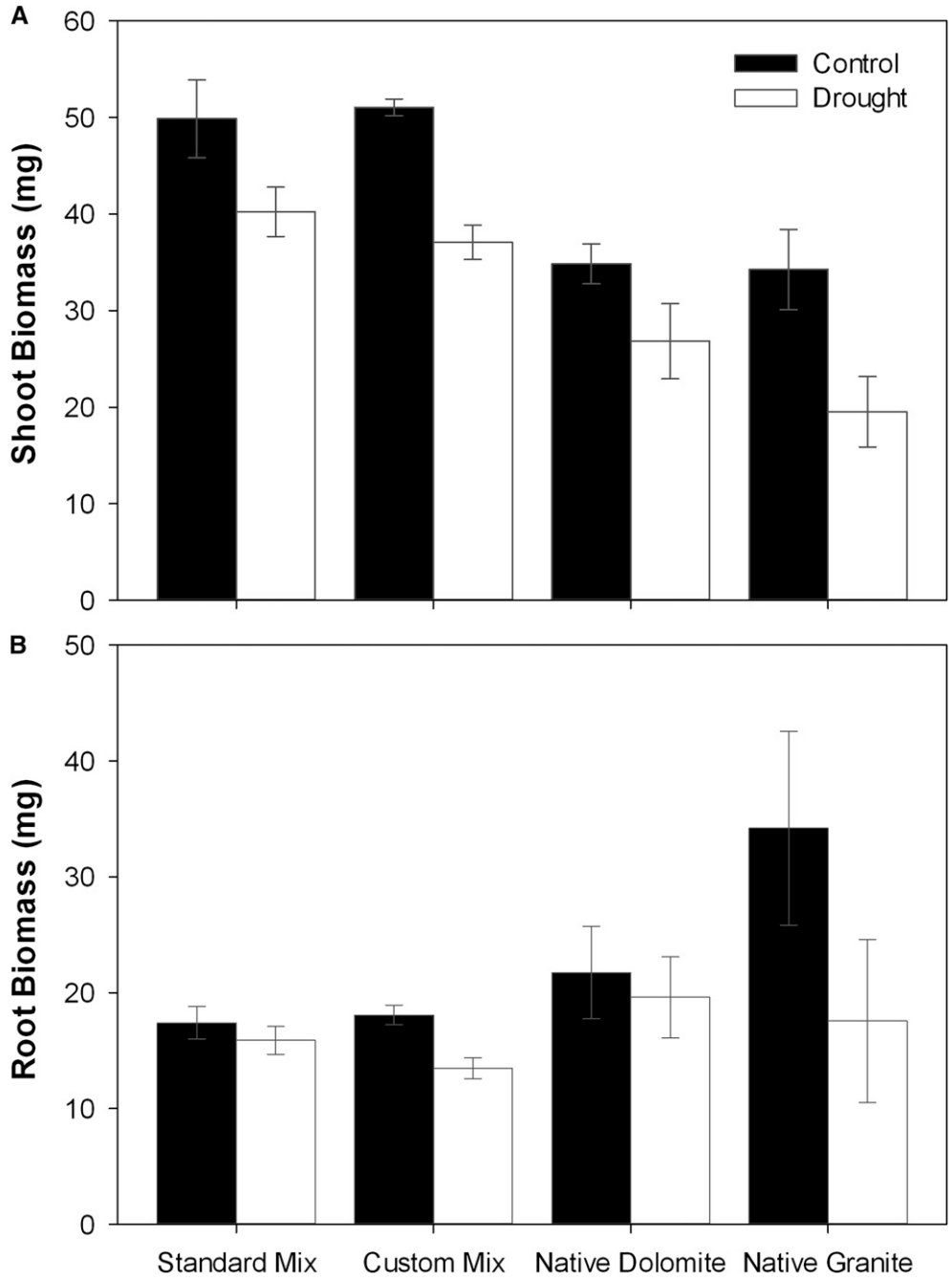


Fig. 1. Shoot (A) and root (B) biomass of 35-d-old, greenhouse-grown ponderosa pine seedlings grown in organic media (standard mix and custom mix) and mineral soil (native dolomite and native granite) under differing hydrologic regimes. Seedlings grown in organic media had higher shoot biomass ($P < 0.001$), while those grown in native granitic soil had higher root biomass ($P < 0.02$). The drought treatment resulted in lower shoot ($P < 0.001$) and root ($P = 0.008$) biomass regardless of growing medium. Error bars represent one standard error of the mean (SEM).

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shoot biomass was 35% less ($F = 55.498$, $df = 1$, $P < 0.001$), and root biomass was 42% more ($F = 8.881$, $df = 1$, $P = 0.004$), but the control seedlings in the granitic soil were mainly responsible for this difference. Shoot biomass was greater in organic vs. mineral media, but root biomass was similar among the seedlings grown in organic and dolomitic media with only the seedlings grown in granitic soil having higher biomass. The resulting root : shoot biomass ratio was 2.1 times greater in mineral than organic soils ($F = 74.6$, $df = 1$, $P < 0.001$). Seedlings in the mineral soils were expected to have higher root : shoot biomass ratios, but shorter root length and no detected differences in surface area suggests increased radial growth occurred that was not detected.

Drought-stressed seedlings in organic media produced as much shoot biomass as the non-drought-stressed seedlings in the mineral media. Drought treatment resulted in 27% less total seedling biomass ($F = 20.417$, $df = 1$, $P < 0.001$), with equal reductions in shoot and root biomass relative to the control treatment (Fig. 1). However, a greater yet not significant (media type by drought treatment interaction, $F = 1.459$, $df = 1$, $P = 0.233$) reduction in root biomass occurred in the mineral soils. The root : shoot biomass ratio was unaffected by drought ($F = 0.144$, $df = 1$, $P = 0.706$) because of equal reductions in root and shoot biomass, but mineral soils still exhibited a root : shoot biomass ratio 2.1 times greater than that in organic soils ($F = 74.6$, $df = 1$, $P < 0.001$).

CONCLUSIONS

Researchers considering greenhouse studies of seedling growth should consider the effects of media type when conducting drought tolerance research in conifer seedlings. The use of an organic medium resulted in differential shoot and root biomass as well as root : shoot biomass ratio when compared to mineral soils. While a similar response to drought was observed in this study regardless of media type, future studies should examine the long-term effects of using native soils rather than organic media on seedling growth and survival under drought conditions. Differences in seedling growth due to media type resulted in significant changes in biomass allocation and root length in this experiment. In future studies, researchers should consider using native soils in greenhouse experiments examining drought response of conifer seedlings due to their varying effects on seedling growth.

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