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Establishment of Coastal Douglas-fir Orchards

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Abstract

Successful and cost-effective establishment of coastal Douglas-fir orchards is crucial for delivering genetic gain from tree improvement programs to the forest growers of western Oregon and Washington. Aspects of site selection, site preparation, grafting, planting, and early aftercare are described.

Introduction

Coastal Douglas-fir is the most important timber species in western Oregon and Washington. The total reforestation need for this species for these two States currently exceeds 60 million seedlings per year. About 50 yr from the establishment of the very first seed orchards (Wheat 1969), and long after many of the grafted clonal orchards were established in the 1980s and 1990s, this demand is met almost entirely from seed orchard seed, which is now the industry standard.

There are several reasons to compile information now on establishing coastal Douglas-fir orchards in a usable and accessible format:

- Each year, older first-generation Douglas-fir seed orchards are being phased out, and new 1.5-generation or second-cycle production orchards and third-cycle breeding orchards are being established rapidly. For example, a survey of orchardists indicated that about 25,000 grafts were made in winter 2008–2009 in Oregon and Washington; at planting densities of 100 to 400 grafted trees per acre, that would translate to 62 to 250 ac of orchards (Jayawickrama, unpublished data).
- The ability of genetic improvement programs to deliver substantial gains has been established through realized genetic gain trials (e.g., St. Clair and others 2004) and comparison of full-sib crosses with woods-run lots in advanced-generation trials.
- In a competitive world wood market, forest growers need to keep costs under control. Seed costs being a component of reforestation expenses, information that helps reduce or manage seed production costs will benefit forest growers in the Pacific Northwest.
- The economic viability of a seed orchard depends on seed production. Effective and efficient management that shortens the time interval to the first seed crop, increases seed production per acre, or both will increase the profitability of the orchard.
- As better genetic material becomes available from breeding and testing programs, the time value of money even at moderate discount rates creates a strong incentive to replace production from older, lower gain orchards with seed from new, higher gain orchards as soon as reasonably possible.
- After a boom in the 1970s to 1980s, investment in seed orchard research has greatly diminished; at the same time, many experienced orchardists from that era either have retired or will soon retire. Yet very little has been published on the establishment of coastal Douglas-fir orchards, especially recently. For example, the Industrial Forestry Association (IFA) Tree Improvement Newsletter, a serial that documented advances in cooperative tree improvement, was discontinued 24 yr ago.

It is therefore desirable to compile the research and operational knowledge available at this time of transition to aid a new generation of seed orchardists, who will undoubtedly operate with smaller budgets and higher expectations than their predecessors. This article documents the establishment of an orchard up to the beginning of stimulation and seed production.

Expected Seed Production per Acre and Sizing New Orchards

Just as expected demand and production capacity are determined before an industrial plant is designed, it is necessary to project future seed needs and seed production before establishing an orchard. The first of these is far from trivial, with harvest rates and locations fluctuating in ways hard to predict due to variation in log prices, leading to increased harvests as log prices increase and the opposite as prices decrease; land acquisition and sales; and catastrophic events, such as summer fires and winter storm blowdown. Seed needs from a given orchard can also depend on availability of alternative appropriate sources of seed. The second is equally challenging because precise predictions of seed production (i.e., a seed production model) have not been published. Overestimating future seed production can result in shortages of high-gain seed for many years, with a resulting high opportunity cost; at the other extreme, underestimating production can result in building excess orchard capacity and incurring unnecessary costs.

With an initial planting density of 200 ramets per acre (480 ramets ha^{-1}), we can expect seed production on a productive site to begin around 5 yr^{-1} from grafting and to increase to about 25 lb $ac^{-1}yr^{-1}$ or 27 kg $ha^{-1}yr^{-1}$(50 lb ac^{-1} or 54 kg ha^{-1} per harvest, assuming stimulation of the orchard every 2 yr) between age 10 and age 15 from grafting. (A ramet is a grafted tree, obtained by grafting a branch or *scion* of a desirable tree onto the base of a seedling called a *rootstock.*) Planting at this spacing will result in the orchard needing to be rogued at around age 10. The maximum harvests reported from mature orchards (20 yr or older) are about 80 lb ac^{-1} (87 kg ha^{-1}) per harvest. This kind of production is not guaranteed and will not occur every year or on every site. In rare years such as 2005, seed production can drop to almost zero on a regional scale (typically due to weather conditions that hamper development or pollination of cones).

Planning the Orchard Life-Cycle

As is the case for any major business investment, it is advisable to calculate the expected useful/productive life of the orchard at the design stage. The cumulative total cost of seed production (total costs incurred/total seed production) decreases dramatically from the first harvest to the large crops seen by age 15 or so. This trend would encourage organizations to keep orchards going for 20 yr or more; some orchards have, in fact, stayed in production for 40 yr. Staying with a mature orchard also postpones the high costs of replacing an orchard (felling, site preparation, grafting, etc.). The opposing consideration is that newer, higher gain genetic material will come available, at least in areas served by active breeding programs, motivating us to phase out older orchards.

These two factors, essentially cost versus gain, need to be balanced. Each organization building a new seed orchard, either singly or in a cooperative, operates under its own specific business model (discount rate, willingness to take risk, etc.). Decisions on how large the orchard should be and how long it is run will vary by organization. In some cases, strategies that result in larger short-term costs could reduce the overall cost per pound of high-gain seed.

One sensible compromise is to establish new orchards adjacent to existing mature orchards of the same or roughly equivalent breeding zone, gradually eliminating most of the older orchard after about 15 yr, but roguing and leaving enough of it to provide pollen for the young orchard. This does not rule out the possibility of short-lived orchards, though they will be the exception rather than the norm; it would seem logical to keep establishment costs as low as possible in such orchards if large, late harvests are not obtained.

Site Location of New Orchards

Characteristics of successful sites. Orchards have been established on a wide variety of sites, with Douglas-fir seed produced successfully on most of them. Problems have mainly been on (1) sites very close to the ocean, where frequent fog and heavy rain appear to interfere with pollination, leading to disappointing seed production; (2) frost-prone sites, where early establishment has been poor and subsequent seed production is affected by damage to flowers; (3) remote high-elevation sites, where access can be very difficult due to snow; (4) very droughty sites with no irrigation; and (5) areas completely out of the normal range of Douglas-fir. Early efforts to locate orchards outside the Douglas-fir zone (e.g., southern California) have been abandoned. In contrast to these problematic sites, notable successes have been seen in mild agricultural sites, such as the Willamette Valley, and in rain-shadow areas on the eastern Olympic peninsula.

Soils are an important factor in siting an orchard. For example, a well-drained soil with moderate fertility would be far superior to a poorly drained soil retaining lots of water throughout the fall and winter. Little can be done to modify soil conditions at reasonable costs. The only moderately cost-effective treatments are ameliorating compaction by subsoiling, improving drainage by tiling, and incorporating organic matter in the planting spots. Even these could cost thousands of dollars for an orchard.

Because Douglas-fir pollen is ubiquitous, pollen contamination affects all orchards west of the Cascade Mountains to a lesser or greater degree and needs to be addressed in orchard management.

Options in siting new orchards. Currently, one important option for industrial forest growers is locating suitable acreage of mature timber within their ownership, preferably with relatively flat terrain and within easy access to an office belonging to the organization. The main advantages of such an arrangement are (1) no upfront land purchase costs; (2) little or no interference by residential neighbors on operations such as spraying for cone and seed insects; (3) relative ease of access; and (4) ability to control the management of the surrounding timber lands, such as harvesting standing mature timber and replacing with young plantations (preferably with a different species to create a pollen buffer, or with high-gain orchard seedlings). The main potential disadvantages include (1) increased risk of damage from wildfire; (2) high cost of land clearing and site preparation; (3) some degree of pollen contamination; (4) lack of viable irrigation options; (5) terrain too rough for tractor-based activities, such as mowing or spraying; and (6) damage from deer, elk, mountain beaver, and rabbits, unless expensive fencing, control measures, or both are used.

Another option is purchase of agricultural land in a designated farming area with no residential neighbors and tight zoning restrictions that prevent urbanization. Such a site can have several advantages.

- Irrigation may be possible if there is adequate ground water and water rights.
- A pool of labor and contractors accustomed to agricultural work may be available.
- The ground is typically flat to gently undulating.
- High fertility can lead to rapid early growth.
- Likelihood of damage from wildfire is very low.

Disadvantages can include the following.

- There may be high purchase costs of such land and ongoing expenses, such as property taxes.
- Low-lying areas may flood.
- Heavy clay soils can be simultaneously waterlogged in winter (requiring drain tiling) and droughty in summer.
- Droughty soils can cause establishment problems for 1-yr-old rootstock seedlings (these problems can be overcome with larger seedlings and/or irrigation).
- There can be problems from gophers or periodic epidemics of voles on such sites, causing mortality to the seedlings and requiring intensive control.
- The pressure to sell such orchards for development can become very high, especially if the zoning changes, parts of the owning company are sold, or both.

Regardless of which option is preferred, some precautionary measures are mandatory. It is important to examine the soils and topography for water drainage issues, as well as incidence of root rot or other diseases in ex-forest sites. It is advisable to dig soil pits and conduct soil tests to determine clay content and fertility. Propensity for ponding of cold air, which can result in frequent damaging frosts, must also be checked.

A third option is buying an existing Douglas-fir orchard, roguing and modifying it as needed. This will often be expensive but can expedite arriving at the desired production of high-gain seed.

Site Preparation and Infrastructure

If the cleared timber land option described above is chosen, the land will need to be cleared following timber harvest (figures 1, 2). Removing stumps can sometimes be expensive (over \$1,000 ac^{-1} on occasion), but avoids the operational and safety issues of trying to drive equipment (tractors, lifts) over tree stumps and uneven ground. Cutting off the stump close to the ground is sometimes suggested as a cost-saving measure, but this is unlikely to be enough; the ground will still be uneven, and holes in decaying stumps can be a hazard. One option is to push the trees over with heavy equipment such as a loader (rather than severing them), as the root wad usually comes out of the ground quite easily. The trees can then be moved to a landing and bucked to appropriate length, or the material can be chipped and hauled from the site.

Low-budget operations where no equipment will be driven on the orchard have managed to proceed without removing the stumps. In the case of former agricultural land, subsoiling to break up hardpans from years of farming or grazing livestock will usually be beneficial (figure 3). If stumps are removed, the site will need to be leveled to fill in the resulting holes and the whole surface will need to be smoothed and tilled.

Intensive operations, especially large ones, will usually add some or all of the following infrastructure: (1) perimeter fences; (2) a well and pump house; (3) irrigation lines to the individual ramets; (4) all-weather gravel roads to key locations; (5) a cone-drying shed; (6) an equipment shed; (7) an office; (8) windbreaks for coastal areas, such as Whidbey Island, with consistent and strong winds; and (9) some measure of security (gate alarms, video surveillance cameras, on-site hosts, etc.). In contrast, small low-budget orchards have been established with very few of these amenities; in such instances, orchard equipment has to be hauled in each time work is planned.

Figure 1. Clearing an area previously under trees.

Figure 2. Area cleared for a new orchard.

Figure 3. Using a winged subsoiler to break a hardpan in a previously cultivated property.

Wire perimeter fences to about 8 ft (2.4 m) in height, costing up to $4 per ft, are mandatory to control deer and elk damage for forest-established orchards (figure 4); however, some orchards developed in agricultural areas have managed successfully without them. Animal protection tubing can provide some protection, but will be less effective than a big-game perimeter fence.

Irrigation is worth considering in some detail (figures 5 and 6). Many orchards have been established and managed successfully without irrigation, especially on more mesic sites with deep, well-drained soil. There will obviously be a cost saving as a result. In addition to installation costs, there is ongoing maintenance, addressing things such as clogged nozzles and driplines chewed through by coyotes. However, irrigation can (1) improve survival and establishment in very dry areas or on droughty or shallow soils; (2) help grow the foliage and crown needed to start producing large numbers of cones, thereby reducing the delay to onset of seed production; and (3) be used to delay bloom in the orchards (through overhead emitters)—

Figure 4. Big-game fence built around orchard in forest.

Figure 5. Rolling out dripline.

Figure 6. Details on a dripline near a ramet.

though in practice this has rarely been used in Oregon or Washington. The irrigation system must be able to deliver several gallons per ramet, usually in one session, at least once a week. A properly designed irrigation system (clean well water, proper chlorination and filtration system) will have very little problem with clogged emitters. Backflow prevention is required to prevent fertilizer or chemicals being pulled back into the water supply.

Genetic Quality

Genetic material adapted to the area of reforestation and with the highest genetic quality available to the forest grower naturally should be used. Most of the timber land area in western Oregon and Washington is included in first-generation and second-cycle cooperative tree improvement programs from which members can obtain genetic material (Jayawickrama 2005).

Use of Graft-Compatible Rootstock

The use of graft-compatible rootstock is now standard for coastal Douglas-fir. In Oregon and Washington, these are derived from Don Copes' breeding program based at the Forest Service Pacific Northwest Research Station in Corvallis (Copes 1999). The compatible parents have also been grafted at several other orchards, and there are vigorous efforts to produce adequate supplies of compatible rootstock seed. Incompatibility has been greatly reduced; it has not gone away, however, and a low level of mortality (1 or 2 percent per year) should be expected. Graft maintenance, especially in the first few years after grafting, is prudent for orchard success. In order to obtain good results, high-quality seedlings (good caliper, vigorous lateral branches), trained grafters, and good maintenance are needed.

Grafting Methods

Douglas-fir orchards are established using two rootstock growing approaches: pot-grafting and field grafting. Both methods utilize cleft grafting techniques and cost about $3–5/graft for the actual grafting. When properly executed, both produce high-quality grafted stock.

Pot-grafting. Many organizations use pot-grafting (figure 7) almost exclusively to establish new orchards. In this method, containerized rootstock seedlings are grafted and outplanted after various lengths of time in greenhouses, shadehouses, or even cold storage. Advantages include the following.

- Grafting can be done indoors in sheltered conditions and at a convenient working height, a situation usually preferred by grafters.
- The planning horizon to obtain rootstock of graftable size is shorter.
- Graft unions can heal in controlled, mild conditions.
- There is a long grafting window, starting in December and continuing to as late as early April, if the rootstocks are kept from flushing.
- Orchard ground is not tied up while rootstock is growing to graftable size.
- Because the orchard can be started with a healthy graft at each planting spot, there are fewer problems with first-year mortality leaving vacant spaces in the orchard.

Figure 7. Successful potgrafts.

- Survival of large potted grafts is higher than that of smaller rootstock seedlings in some situations.
- All the ramets of a single clone can be grafted and labeled as one batch, reducing chances for error.
- Graft maintenance in the field is usually minor.

The key difference, compared to field-grafting, is that pot-grafting gives a higher probability of fully establishing an orchard on a known schedule. Greenhouse grafting can go from graft-compatible seed to grafted seed orchard in the ground in 20 mo to 2 yr. Field grafting cannot provide such a short or reliable time-frame. Depending on the situation, disadvantages can include (1) high repotting costs and growing charges for greenhouse use, if done at contract prices (as high as $10 per graft); (2) lack of interest by greenhouse operations in taking on the extra effort and responsibility of growing large stock, providing facilities for grafting, and maintaining grafts; (3) plagiotropism (although one organization reports no issues with plagiotropism when using pot-grafts); and (4) larger logistical effort to transport and plant potted grafts.

There are several variations on the pot-grafting theme.

1. One-year-old container seedlings (515A, 615A, or similar) can be potted into a larger container in fall; they can also be lifted in winter and kept in cold storage until 2 wk before grafting, then taken out, potted and grafted.
2. The grafted ramets can be moved outdoors in the spring after the risk of frost is over; this reduces cost and prevents the trees from growing too tall in the first year. Conversely, they can be grown in the greenhouse that season, if it is possible to prune and stake them.
3. One organization is testing grafting on 1-yr-old container seedlings (e.g., 515A, 615A), keeping them in cold storage, and outplanting them the same winter; this avoids the expense of potting the grafts and growing them in a greenhouse or nursery but involves accepting lower survival and first-year growth.
4. Another organization uses 1015A container rootstock, grafted after 1 yr and outplanted in fall of the first growing season after grafting; the organization's experience is that Douglas-fir doesn't like to be in a container very long.

Staking and top-pruning (pruning of scion with excessive growth) may be needed if growth rates are not closely monitored in the greenhouse. If the grafts are sufficiently vertical and firm, staking can be avoided (this also improves safety, since stakes can injure workers' eyes). With good timing, we would aim to top-prune the graft just once.

Field-grafting. A few organizations prefer field-grafting. Advantages include (1) rapid and healthy scion growth resulting from grafting on large, vigorous rootstock; (2) avoiding the high cost of growing potted grafts in greenhouses; (3) few issues with plagiotropism or large spindly grafts; (4) avoiding post-grafting transplanting shock, and (5) easy graft maintenance, because graft unions are relatively high above the ground (though, if the ramets are managed as short-hedged trees, a substantial proportion of the ramet would not be usable for seed production).

Disadvantages include (1) a long planning horizon to obtain rootstock of graftable size; (2) poor survival of planted rootstock due to drought or herbicide damage; (3) tying up valuable orchard ground while rootstock is growing to graftable size; (4) cold wet conditions and an inconvenient height when grafting outdoors, resulting in slower production and higher costs; (5) the need for grafters to carry scions from many clones through the orchard and ensure the right scion is grafted on a given location; (6) reduced grafting success if severe weather, such as very cold temperatures or desiccating winds, follows grafting; (7) a shorter grafting window, from February to April; and (8) potential issues withvacant spaces in the orchard resulting from first-year mortality.

For field grafting, 1-yr-old container seedlings (515A, 615A or similar) can be outplanted in place and grown under a suitable cultural regime. Such seedlings will be ready for grafting after 2 yr in the field. It may be possible to reduce this period to 1 yr by using very large container stock (815A, 1015A), but there is less experience grafting on such seedlings in Oregon and Washington. Grafting too early in the winter can lead to poor survival, as the scion cannot obtain nutrients and water from the rootstock until the graft union heals, and such healing does not take place until spring.

Spacing

A variety of spacing regimes are reasonable, depending upon such considerations as the following:

- Acreage available: if more land is available, wider spacing is more feasible.

- Fixed cost per acre per year of orchard maintenance and ownership: the higher the cost, the greater the incentive to reduce spacing and maximize production per acre.
- Spacing between existing irrigation lines, if any.
- Equipment to be used (pickups, trailers, tractors and sprayers, translators, lifts): the larger the equipment, the wider the spacing needed.
- Expected life of orchard: the longer we expect to need it, the wider the spacing needed.
- Urgency to obtain seed: the greater the urgency, the greater the reason to pack in more ramets per acre until production per ramet reaches an acceptable level.
- Expected future management: development of large tree-form ramets [the default approach, with top heights from 30 to 50 ft (9.1 to 15.2 m)] requires wider spacing than does management of hedged orchards kept as low as 9 ft (2.7 m) (less frequent, but still a viable alternate).

There is no single "right" orchard spacing. Initial densities have ranged from 50 to 500 ramets ac^{-1} (0.4 ha^{-1}) and a great variety of spacings have been used [36 ft × 24 ft (10.9 m × 7.3 m), 18 ft × 12 ft (5.5 m × 3.6 m), 9 ft × 12 ft (2.7 m × 3.6 m), etc.]. Even more complex spacings have been used [e.g., three rows 8 ft (2.4 m) apart separated by about 15 ft (4.5 m) for large equipment access]. If herbicides are not available to control grass and weeds within rows, the orchard may need to be mowed in two directions, requiring standardized spacing [12 ft × 12 ft (3.6 m × 3.6 m), 15 ft ×15 (4.5 m × 4.5 m), etc.] to make equipment operation easier. A north-south orientation of the wider alley is generally preferred to allow better sunlight penetration.

Development of holding orchards (temporary establishment of ramets in one location with the intent to move them later to a permanent location) can reduce costs when annual per-acre orchard maintenance and ownership costs are high. For example, a holding orchard can be as tight as 6 ft (1.8m) within and between rows [1,200 ramets ac^{-1} (500 ramets ha^{-1})] for a few years. If the costs of moving ramets (figure 8) from the holding orchard to the final location are high, however—very much the case if ramet movement is contracted out and if the ramets are large—these cost savings will be reduced. For example, moving 500 ramets at $50 per ramet will cost $25,000. It is advisable to develop holding orchards as close to the location of the permanent orchard as possible to reduce tree-moving costs. Moving the ramets may shock the tree into flowering; this might be exploited by proper scheduling.

Orchard Design

Related trees will tend to have similar phenology, and crosses between relatives will tend to have reasonable seed set; these two factors will work to produce some amount of inbred seed unless steps are taken to reduce it. It is therefore desirable that ramets of related clones (sibs, parent-offspring) are physically distant from each other to reduce related matings. In contrast, separating ramets of the same clone will be a lower priority because selfs are much less likely to produce viable seed. Several software programs are available to optimize separation of related clones.

Planting and Early Aftercare

Whether established as pot-grafts or field-grafts, orchard plantings have many similarities to planting of progeny tests. These are expensive, high-value seedlings, so planting quality is very important to obtain a successful orchard (figure 9). Excellent record-keeping of where each ramet is planted is imperative because loss of identity or misidentification of ramets is very detrimental. Each ramet should be tagged with parent identification and Row/Column address. This information has to be mapped and entered into an orchard database that must be updated continually as changes occur from mortality, regrafting, etc. If a permanent irrigation system is not installed, temporary irrigation using water tanks may improve survival and early establishment on hot, dry sites.

Figure 8. Moving a ramet from a holding orchard to permanent location with a treespade.

Grass cover, such as perennial ryegrass, is typically established to reduce erosion and allow for easier year-round vehicular and equipment access. The use of slow-growing varieties will reduce the need for future mowing, although the grass has to be aggressive enough to compete with weeds and moss. There may be instances, however, where grass cover is not required (e.g., no vehicles will be driven over the orchard), or is even undesirable (e.g., areas with very low rainfall or other droughty sites with no irrigation, where grass cover will use all available moisture). One approach would be to develop sod only after the ramets are well established and have built up a strong root system. As mentioned before, it is customary to spray herbicide in the rows while trees are dormant; this reduces some of the competition for water from grass or other ground vegetation in the establishment phase. Where herbicides cannot be used, mulch matting [3 ft × 3 ft (0.91 m × 0.91 m) or 4 ft × 4 ft (1.2 m × 1.2 m)] can provide a suitable alternative.

Orchard mowing is commonly necessary to permit access, provide a measure of fire prevention, and help control noxious weeds. Although the idea of grazing livestock inside seed orchards, thereby taking advantage of lush grass and reducing the need to mow, can seem very attractive, livestock will destroy young grafts. Sheep, cattle, and horse grazing has been permitted in older established orchards, but requires very good cooperation with the permittee to ensure that grazing is closely controlled to limit damage. Livestock can cause problems even for mature orchards with large ramets by compacting the soil and damaging the roots, disturbing tree tags, and spreading noxious weeds.

Ramets require periodic maintenance during the first and second season after grafting, such as trimming the “ears” (any loose rootstock stem separating above the graft union), removing the first branch whorl above the graft union, and scoring bark to reduce and delay the incidence of incompatibility. Rootstock foliage is removed progressively as the scion foliage grows and becomes capable of supporting the entire graft. It is obviously important that all rootstock foliage is eliminated before seed production begins. Early rootstock pruning also extends the safe herbicide spraying period, as the chemical no longer contacts tree foliage. Painting the graft union and lower stem with light-colored latex paint can reduce damage due to sunscald.

Figure 9. A recently established orchard.

Agricultural areas such as the Willamette Valley can experience difficulties due to periodic population explosions of voles; the voles often use the container media of the potgrafts and vegetation control matting as habitat, damaging the grafts. Tilling the entire new orchard block (except for the area immediately surrounding the grafts) is one way to control or eliminate voles. At some point tilling would have to cease, as it would damage the root systems of the ramets and prevent establishment of sod. Trapping has also been used, but results have been mixed; this technique appears to be best suited for control of small populations. Several chemicals are registered for control of voles. Zinc phosphide, chlorophacinone, and diphacinone can be used in Washington and Oregon. (More details are available on the Washington State University Extension Web site (http://gardening.wsu.edu/library/tree012/tree012.htm) and the Oregon Department of Agriculture Web site (http://www.oregon.gov/ODA/PEST/docs/pdf/meadowmouse.pdf).

Orchards should be monitored periodically through soil and foliar testing to ensure nutrient health, and site-specific fertilizer mixes should be applied to ensure vigor and growth. Nutrition requirements can be met by either broadcasting granular fertilizers or injecting soluble nutrients into irrigation water.

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REFERENCES

Copes, D.L. 1999. Breeding graft-compatible Douglas-fir rootstocks (*Pseudotsuga menziesii* (Mirb.) Franco). Silvae Genetica. 48: 188–191.

Jayawickrama, K.J.S. 2005. Genetic improvement: boosting plantation productivity in the Pacific Northwest. Western Forester. May/June: 10–11.

St. Clair, J.B.; Mandel, N.L.; and Jayawickrama, K.J.S. 2004. Early realized genetic gains for coastal Douglas-fir in the Northern Oregon Cascades. Western Journal of Applied Forestry. 19: 195–201.

Wheat, J.W. 1969. Forest tree improvement work advances in Douglas fir region. Forest Industries. 96(5): 38–40.

Nursery Quality and First-Year Response of American Chestnut *(Castanea dentata)* Seedlings Planted in the Southeastern United States

REFEREED PAPER

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Abstract

We examined nursery seedling quality and 1-yr field performance of American chestnut [*Castanea dentata* (Marshall) Borkh.] seedlings planted in Alabama (AL study) and Tennessee (TN study). Root-collar diameter (RCD) had the highest correlations to nursery seedling quality and first-year field performance for both studies. Survival was low in the Alabama study (18 percent) due to ink disease, caused by *Phytophthora cinnamomi*, and rabbit damage. Seedling growth was not affected by genetic family in either study, and seedling size class and silvicultural treatment did not affect growth at the AL site. On the TN site, survival was 86 percent; relative height and diameter growth were negatively related to nursery height, indicating planting shock was a factor. Restoration of blight-resistant American chestnut will depend on early establishment success, which can be affected by factors not easily controlled.

Introduction

The American chestnut [*Castanea dentata* (Marshall) Borkh.] was an important component of the eastern hardwood forest for thousands of years until a blight-causing fungus, *Cryphonectria parasitica* (Murrill) Barr, was introduced in the early 1900s (Delcourt and others 1998, Anagnostakis 2001). Loss of the American chestnut has resulted in large-scale shifts in species composition in forests of the Southeastern United States, particularly in well-drained upland stands where the species was most competitive (Braun 1950, McCormick and Platt 1980, Stephenson and others 1991). Remnant chestnut trees manage to stay alive through stump and root sprouting and occasionally live long enough to flower and bear fruit, providing genetic material for reintroduction efforts. Blight-resistant trees are produced through backcross breeding with the resistant Asian chestnut (*Castanea mollissima* Blume or *C. crenata* Siebold & Zucc.), in hopes of breeding for the Asian resistance while maintaining the desired form and other silvical characteristics of the American chestnut (Hebard 2001).

The American Chestnut Foundation (ACF) will have limited numbers of resistant hybrid chestnut trees available in 2009 for research testing (Hebard 2001), but little information is available on how seedlings will respond in "real world" forest conditions. The first years of hardwood plantation establishment are the most crucial, because this is when mortality is generally highest and seedlings are most vulnerable to biotic and abiotic factors. Successful establishment of American chestnut will require information on effects of nursery seedling quality, genetics, and silvicultural requirements of the species, yet no or very limited testing of these factors has been conducted. Chestnut may have silvicultural requirements similar to oak (*Quercus* spp.) with the ability to persist in the absence of disturbance, but stimulated growth and photosynthesis with increased light (McNab and others 2003, McCament and McCarthy 2005, Wang and others 2006, Joesting and others 2007). To our knowledge, no study has specifically examined how nursery seedling quality, together with early genetic and silvicultural treatments, affects growth and survival of American chestnut.

Our objectives were to determine:

- The best indicator of nursery seedling quality for nursery managers.
- The effects of nursery seedling quality and genetic family on first-year growth and survival in the field.

- First-year growth and survival differences in seedlings planted into contrasting light environments created through silvicultural prescriptions.

Methods

Our research was conducted at two study areas: Jackson County, Alabama (AL study), and the Cherokee National Forest in Polk County, Tennessee (TN study).

Site-specific descriptions, seedling production, and study design. *Alabama study*. The AL study is located on properties owned by Stevenson Land Company and by the Alabama Department of Conservation and Natural Resources, State Lands Division, on the escarpment of the Mid-Cumberland Plateau (Smalley 1982). The site is characterized by generally short, moderately steep side slopes, with elevations averaging 1,600 ft (488 m). Soils were field tested by the Natural Resources Conservation Service, U.S. Department of Agriculture (USDA), and identified as loamy, derived from sandstone with a small component of residuum from limestone. Dominant tree species before silvicultural manipulations consisted of oaks, sugar maple (*Acer saccharum* Marsh.), and hickories (*Carya* spp.); remnant American chestnut sprouts were present in the understory.

Five silvicultural treatments were implemented in the fall of 2001 (Schweitzer 2004). For this study, we examined the oak shelterwood and the clearcut treatments. The oak shelterwood treatment consisted of an herbicide injection (imazapyr) designed to remove 25 percent of the total basal area by treating trees >1 in (2.5 cm) in diameter at breast height (dbh) and in a suppressed or intermediate social position. The oak shelterwood method creates transient conditions (typically lasting a few years) where light-seeded species [e.g., yellow poplar (*Liriodendron tulipifera* L.)] are restricted in their ability to establish and grow, but where enough light penetrates the forest floor to stimulate growth of desirable regeneration, e.g., oak (Loftis 1990). The clearcut treatment, used to regenerate shade-intolerant species, involved removing all trees >4 in (10.2 cm) dbh by chainsaw felling and grapple skidding along predesignated trails.

Photosynthetically active radiation (PAR) was measured in each treatment unit three times during the 2006 growing season with an AccuPar Linear Par ceptometer, model PAR-80 (Decagon Devices, Inc., Pullman, WA). The PAR measurements taken inside the treatment units were compared to PAR measurements taken at the same time in adjacent areas receiving full sun.

Seedlings for planting were grown from nuts gathered from seven open-pollinated, pure American chestnut families. Seedlings were grown 1 yr in nursery beds managed by the Alabama Chapter of TACF in Muscle Shoals, AL. Seedlings were watered as needed throughout the growing season and were fertilized once with 13-13-13 fertilizer at approximately 25 lb ac^{-1} (28 kg ha^{-1}).

Nursery managers sometimes use visual grading of hardwood seedlings by quick assessments of overall seedling size to improve seedling quality (Oswalt and others 2006, Tennessee Department of Agriculture 2006). To mimic these methods, we visually graded seedlings into “Large” and “Small” size classes by assessing their overall size (Clark and others 2000).

A total of 288 seedlings was planted in February 2006 on a 5 × 5 foot (1.5 × 1.5 m) spacing into the oak shelterwood and clearcut treatments (three replications of each) in a randomized-block, split-plot design. Silvicultural treatment was the whole plot factor; a 7 × 2 factorial (family × seedling grade) was the subplot factor.

Planting spots in the clearcut units were cleared with machetes before planting, and herbicide (glyphosate) was applied in April 2006 to sprouting vegetation within a 3-ft (0.9 m) radius around each planted seedling. Care was taken to protect planted seedlings from herbicide drift. Deer Away® animal repellent was applied twice during the growing season to a rope fence surrounding the plantings.

Tennessee study. The TN study area is located on the Ocoee Ranger District of the Cherokee National Forest, within the Blue Ridge Mountains Section of the Central Appalachian Broadleaf Forest Province (Bailey 1995). The planting site was 1,240 ft (372 m) in elevation on a site previously dominated by planted Virginia pine (*Pinus virginiana* Mill.) and shortleaf pine (*Pinus echinata* Mill.) decimated by the southern pine beetle (*Dendroctonus frontalis*) in summer 2005. All dead trees were harvested or downed by chainsaw felling, and the site was prescribed-burned in November 2005.

Seedlings were obtained from nuts grown from seven open-pollinated pure American families located at the American Chestnut Foundation Meadowview Seed Orchard in Meadowview, VA. Seedlings were grown for 1 yr and received regular fertilization and irrigation throughout the growing season according to protocols developed by Kormanik and others (1994).

We planted 167 seedlings on an 8 × 8 ft (2.4 × 2.4 m) spacing in February 2006, using an incomplete block design. Three to four trees were planted within each incomplete block, with a single tree plot for each family. Single tree plots were used to reduce variation in family performance between incomplete blocks, as compared with using multiple tree plots. We used an incomplete block design because the space required for a complete block composed of all seven families would have been too large to assume constant variability within a block. Deer repellent was not used in the TN study.

Seedling measurements and damage assessment. Seedlings were lifted in February for the AL study and in January 2006 for the TN study. After lifting, we measured seedling root-collar diameter (RCD) to the nearest 0.001 in (0.1 mm) approximately 1 in (2.5 cm) above the root collar, using a digital caliper. We measured total height from the root collar to the top of the terminal bud to the nearest 0.4 in (1 cm), using a standard height pole. We counted the number of first-order lateral roots (FOLR), defined as a root connected to the main tap root and at least 0.04 in (1 mm) diameter at the proximal end. Because FOLR count can be subjective, the same person counted roots on seedlings for each study separately.

Because planting height differs from nursery height due to differences in planting depth of each tree, total height and ground-line diameter (GLD) were measured immediately after planting. Height and GLD were measured again when trees were dormant in October 2006. Relative height and GLD growth were computed as the 1-yr growth increment divided by initial planting height or GLD, respectively.

We recorded damage from deer (*Odocoileus virginianus*), rabbit (*Sylvilagus floridanus*), chestnut blight, and ink disease, caused by *Phytophthora cinnamomi*. Deer browse was identified as a jagged cut to the main stem; rabbit damage was identified as a clean-cut, angled clipping of the main stem or as gnawing on the outer cambium layer (University of Nebraska Cooperative Extension 1994). Chestnut blight was identified as a sunken and dried spot on the stem, sometimes accompanied by orange fruiting bodies. Ink disease was identified by wilted leaves followed by tree death and accompanied by an inky blue exudate on the roots (Crandall and others 1945). Ink disease was confirmed through testing by the Connecticut Agriculture Experiment Station in New Haven.

Data analysis. All data were analyzed in SAS version 9.1 (SAS 2004). An error level of 5 percent was used to indicate significance in all tests. For the AL study, *t*-tests were conducted to determine if large and small seedlings differed significantly in nursery seedling characteristics. We excluded seedlings damaged by deer browse, rabbit browse, and ink disease from statistical analysis involving first-year field measurements, excluding computations of raw means, because these factors influence seedling growth responses to independent variables being tested.

Pearson correlation coefficients were used to test the linear relationships among nursery seedling characteristics and between these characteristics and first-year height and GLD. We were interested in determining if initial nursery seedling characteristics could predict total first-year absolute height and GLD, which, along with survival, are the primary criteria for successful seedling establishment (Ward and others 2000). Growth may be a better measure than absolute size in determining seedling response to biotic and abiotic factors during early plantation establishment, however, because absolute size largely depends on initial seedling size in the early years (Dey and Parker 1997). Therefore, linear regression analysis (PROC REG) was used to determine the usefulness of initial seedling characteristics in predicting first-year absolute height, relative height growth, absolute GLD, and relative GLD growth. The best regression models were identified by stepwise selection procedures. Graphical analysis of residuals conducted to assess normality and homogeneity of variance assumptions found no problems with these assumptions; therefore, variables were not transformed.

We conducted logistic regression (PROC LOGISTIC) to determine if initial nursery seedling characteristics (both studies) and seedling size class (AL study only) could be used to predict the probability of survival. We used analysis of variance (ANOVA; PROC MIXED) to test for fixed effects of silvicultural treatment (AL study only), family (both studies), and size class (AL study only) and their interactions on relative height, relative GLD growth, and survival. Relative, rather than absolute, height and GLD growth were used in the ANOVAs in order to understand how independent variables affect growth of seedlings while controlling for effects of initial seedling size. For both studies, replication (AL study) and incomplete block (TN study) were random. If fixed effects were significant, least-square means were computed and mean separations performed with the PDIFF option.

Results and Discussion

Seedling quality and growth. Nursery seedling quality depended on study site. Seedlings used in the AL study were approximately 22 in (57 cm) shorter and 0.2 in (4.6 mm) smaller in RCD than seedlings in the TN study (table 1). RCD was a good indicator of nursery seedling quality and was highly correlated with both height (R=0.76, AL; R=0.75, TN) and number of FOLR (R=0.69, AL; R=0.62, TN). These results are similar to the findings of Ruehle and Kormanik (1986) and Clark and others (2000), who found high correlations between RCD and other nursery characteristics for northern red oak (*Quercus rubra* L.). Number of FOLR had the lowest correlations with both height (R=0.56, AL; R=0.47, TN) and RCD. The similarity in correlation coefficients among studies suggests that RCD may be the best indicator of nursery seedling quality, regardless of nursery production methods. This finding is important to nursery and forest managers because RCD has been shown to be a good predictor of growth 2 yr after planting for other hardwood species (Dey and Parker 1997, Jacobs and others 2005).

Seedlings planted at the AL site, regardless of size class, were below minimum standards for other species of hardwoods (Johnson 1981). Nursery height, RCD, and number of FOLR were all significantly larger in the Large size class than in the Small in the AL study (table 1). Despite their relatively small size, variation in quality among nursery seedlings in the AL study was large enough to allow us to visually distinguish two grades of seedlings (Clark and others 2000) that may perform differently after several years in the field (Kormanik and others 2002, Jacobs and others 2005, Oswalt and others 2006).

Height and RCD measurements taken in the nursery were not significantly different from those taken at planting, but individual measurements could be as much as 9 in (23 cm) different in height and 0.2 in (5.1 mm) different in diameter because of differences in planting depth and occasional stem damage during transportation to field. Researchers should not use nursery measurements as a surrogate for measurements taken at planting because use of the former may erroneously reduce correlations to subsequent field growth. Additionally, distinguishing between nursery and planting measurements was important for computing accurate measurements of first-year growth.

For all seedlings in the AL study, relative height growth was 32 percent and relative GLD growth was 19 percent

Table 1. Nursery seedling characteristics, planting measurements, and first-year measurements and survival, expressed as arithmetic means (standard error), for American chestnut seedlings planted in Jackson County, Alabama (AL), and the Cherokee National Forest, Tennessee (TN). Results for the AL study are for two seedling size classes and for all seedlings combined. Nursery measurement means for the AL Large and Small seedlings followed by the same letter do not differ significantly (t-test, p<0.05).

	AL			TN
	Large	Small	All	
Nursery measurements				
Height (in)	19.5 (0.4)a	12.5 (0.3)b	16.0 (0.3)	38.0 (0.9)
(cm)	50 (1)	32 (1)	41 (1)	98 (2)
Root-collar diameter (in)	0.35 (0.01)a	0.25 (0.01)b	0.30 (0.01)	0.48 (0.01)
(mm)	8.9 (0.2)	6.3 (0.1)	7.6 (0.1)	12.2 (0.3)
Number of first-order lateral roots	9 (<1)a	6 (<1)b	8 (<1)	7 (<1)
Measurements at planting				
Height (in)	19.0 (0.4)	12.5 (0.3)	16.0 (0.3)	38.0 (0.9)
(cm)	48 (1)	32 (1)	40 (1)	98 (2)
Ground-line diameter (in)	0.34 (0.01)	0.25 (0.01)	0.30 (0.01)	0.44 (0.01)
(mm)	8.8 (0.2)	6.4 (0.2)	7.6 (0.1)	11.3 (0.2)
First-year field measurements				
Height (in)	22.0 (1.2)	14.0 (0.8)	17.5 (0.8)	44.0 (0.9)
(cm)	56 (3)	37 (2)	45 (2)	113 (2)
Ground-line diameter (in)	0.34 (0.01)	0.29 (0.01)	0.31 (0.01)	0.57 (0.01)
(mm)	8.8 (0.3)	7.3 (0.4)	7.9 (0.3)	14.7 (0.3)
Relative height growth (percent)	36 (5)	30 (5)	32 (4)	21 (2)
Relative ground-line diameter growth (percent)	14 (5)	23 (5)	19 (4)	33 (3)
Survival (percent)	15 (3)	21 (3)	18 (2)	86 (3)

(table 1). Seedlings in both size classes grew more in height than they did below ground, indicating that the smaller nursery stock from AL may have grown stems at the expense of the root system. For the larger seedlings in the TN study, in contrast, seedlings grew more in relative GLD than in relative height. Growth of seedlings at the TN site was similar to that reported by Struve and Joly (1992), who found that hardwoods devote growth to the root system just after the first flush.

Percent full sunlight in the clearcut and the oak shelterwood was 82 and 11 percent, respectively, corresponding to 504 and 57 μmol m^{-2} s^{-1} PAR, respectively. Despite the large differences in amount of available sunlight between silvicultural treatments, we detected no differences in relative growth among silvicultural treatments, families, and seedling size class in the AL study or among families in the TN study. American chestnut seedlings have exhibited increased growth in higher light areas than in lower light (McCament and McCarthy 2005, Wang and others 2006), but this difference was not significant in the AL study, likely due to the low numbers of surviving seedlings. We conducted a *post-hoc* ANOVA and removed silvicultural treatment from the model. In these analyses, neither family nor seedling size class affected relative height and GLD growth.

Survival, disease, and animal damage. Survival in the AL study was low (table 1); mortality was primarily related to ink disease, documented on 51 percent of trees (table 2). Chestnut blight and ink disease were more common in the oak shelterwoods than in the clearcuts, which contradicts a previous study that found chestnuts were more susceptible to chestnut blight in clearcuts than in undisturbed areas (Griffin and others 1991). American chestnuts show little to no resistance to ink disease, which, unlike chestnut blight, destroys the root system and kills the entire tree (Anagnostakis 2001, Rhoades and others 2003). The disease is not easily treated in the field, so planting chestnuts for reforestation should be avoided where the pathogen exists until resistant seedlings can be produced; however, TACF is only in the early stages of breeding for ink disease resistance.

Animal damage was documented on 34 percent of trees in the AL study and was an additional factor controlling survival, particularly in clearcut units (table 2). Rabbit browse was greater in clearcuts than in oak shelterwoods, while deer browse occurrence was similar for both treatments. Our results indicate that open canopy areas may leave seedlings more vulnerable to predators, especially small mammals. Deer repellent as applied in the AL study was not effective; however, the repellent was not applied directly to the stem because it damages newly developing leaves in oak (Scott Schlarbaum, unpublished data).

First-year survival was higher in the TN study than in the AL study (table 1), likely due to the lack of animal damage and disease. No ink disease was documented at this site, and chestnut blight and deer browse were documented on only 9 percent and 3 percent of trees, respectively (table 2).

Survival probability could not be explained by seedling size class or initial nursery seedling measurements by logistic regression (χ^2=1.8, p=0.77, AL; χ^2=5.5, p=0.14, TN), contradicting studies that have found seedling size to be positively related to survival (Zaczek and others 1996, Kormanik and others 2002, Spetich and others 2002).

Relationships of nursery characteristics to first-year growth. Nursery seedling height, RCD, and FOLR were all positively correlated to first-year absolute height and GLD (table 3). For the AL study, only RCD was a significant predictor of relative height growth, but the overall model was not significant (P>0.05); relative RCD growth could not be explained by the variation in nursery seedling size. For the TN study, initial nursery seedling characteristics were significant, but weak predictors of relative height or GLD growth (R^2<0.27; table 4). This result was similar to the work of Jacobs and others (2005), who found low predictive power of initial seedling characteristics to growth increment.

Table 2. Occurrence of animal browse and disease of American chestnut seedlings planted at two field studies in Jackson County, Alabama (AL), and at the Cherokee National Forest, Tennessee (TN). The AL results are shown for seedlings planted in clearcut and oak shelterwood silvicultural treatments and for all seedlings combined.

	AL			TN
	Clearcut	Shelterwood	All	
	Percent			
Rabbit browse	55	1	28	0
Deer browse	6	5	6	3
Ink disease	45	56	51	0
Chestnut blight disease	9	20	15	9

Variation in absolute height was best explained by nursery height for the AL study (R^2=0.74) and by both nursery height and RCD for the TN study (R^2=0.89). Variation in absolute GLD was best explained by nursery RCD (R^2=0.40, AL; R^2=0.41, TN) (table 4). A higher number of FOLR has been shown to improve field performance after outplanting (Schultz and Thompson 1990, Ward and others 2000, Kormanik and others 2002), but in this study FOLR was the variable least correlated with first-year height and GLD and could not significantly explain variation associated with absolute size, relative growth, or survival in either study. Our results agree with others who have found that early seedling size after planting is strongly related to nursery seedling height or diameter (Dey and Parker 1997) and that number of FOLR may not correlate well with field performance until after a few years of establishment (Ward and others 2000).

The negative relationship between relative height growth and GLD to nursery seedling height in the TN study (table 4) indicates that taller nursery seedlings may be more likely to experience planting shock. Johnson and others (1984) found that shoot dieback was more noticeable in large 1-0 oak seedlings than in smaller seedlings, which they attributed to shoot dehydration during cold storage. Although we tried to minimize stress from shoot dehydration by using a root wetting agent after lifting, the seedlings for the TN site were in cold storage for a month before planting, increasing the opportunity for shoot dehydration. Bareroot hardwood seedlings typically grow slowly the first 1–2 yr after planting while they overcome planting shock and roots become established (Struve 1990), and we expect seedlings to increase growth rate in subsequent years. Future measurements and analysis will help us determine if the benefit of growing and planting larger seedlings will outweigh the increased stress of planting shock in larger seedlings, as it does in oak species (Johnson 1981).

Conclusions

Preliminary results from these two studies indicate that factors not specifically tested *a priori*, such as animal damage, disease, and planting shock, can control early establishment success of artificially regenerated American chestnut seedlings. Results on the response of American chestnut to genetics, seedling size class, and silvicultural treatment were not clear due to low survival and plant-

Table 3. Pearson correlation coefficients and associated p-values for the linear relationship between nursery seedling characteristics and first-year height and ground-line diameter for American chestnut seedlings planted in Jackson County, Alabama (AL), and at the Cherokee National Forest, Tennessee (TN).

First-year measurements	Nursery seedling characteristic					
	Height		Root-collar diameter (RCD)		Number of first-order lateral roots (FOLR)	
	AL	TN	AL	TN	AL	TN
Total height	0.86 (<0.001)	0.96 (<0.001)	0.71 (<0.001)	0.75 (<0.001)	0.37 (0.026)	0.46 (<0.001)
Ground-line diameter (GLD)	0.42 (0.009)	0.49 (<0.001)	0.63 (<0.001)	0.64 (<0.001)	0.33 (0.048)	0.45 (<0.001)

Table 4. Regression models used to explain variation in first-year seedling performance of American chestnut using initial nursery characteristics for two field studies in Jackson County, Alabama (AL), and the Cherokee National Forest, Tennessee (TN).

Linear regression model	R^2	F	P
AL study			
Height (in)=1.59 + 1.13(HtNurs)	0.74	100.74	<0.0001
Height (cm)=4.06 + 1.13(HtNurs)			
GLD (in)=0.11 + 0.68(RCDNurs)	0.40	23.62	<0.0001
GLD (cm)=2.92 + 0.68(RCDNurs)			
TN study			
Height (in)=11.25 + 0.76(HtNurs) + 8.29(RCDNurs)	0.89	558.08	<0.0001
Height (cm)=28.81 + 0.76(HtNurs) + 0.83(RCDNurs)			
GLD (in)=0.21 + 0.77(RCDNurs)	0.41	96.04	<0.0001
GLD (mm)=5.42 + 0.779(RCDNurs)			
RHtG=30.34 – 0.26(HtNurs) + 0.59(RCDNurs)	0.26	23.54	<0.0001
RGLDG=65.52 – 0.31(HtNurs)	0.08	12.04	0.0007

Height=absolute first-year height; GLD=absolute first-year ground-line diameter growth; HtNurs=nursery seedling height; RCDNurs=nursery seedling root-collar diameter; RHtG=relative first-year height growth; RGLDG=relative first-year ground-line diameter growth.

ing shock. The results from this study, not surprisingly, indicate that first-year seedling size is largely a function of nursery seedling size, and that first-year height growth will be minimal (21–32 percent). Managers may need to provide provisional protection to seedlings from animals, disease, and competing vegetation until seedlings can overcome planting shock. Once established, American chestnuts are expected to have higher growth rates than other hardwoods (Ashe 1911, Jacobs and Severeid 2004, McEwan and others 2006), so additional efforts to protect seedlings may only be required for the first couple of years.

Higher-quality nursery seedlings, like those used in the TN study, may initiate root growth more rapidly than lower quality seedlings, like those used in the AL study. Our results indicate that RCD may be the best criterion for selecting high-quality seedlings; however, planting shock may be related to larger size at planting, resulting in decreased stem growth. American chestnut seedlings should be planted immediately after lifting from the nursery to minimize shoot dehydration and planting shock stress.

Although family was not a significant effect, we speculate that family differences in growth and survival will become important after seedlings establish a strong root system (Zobel and Talbert 1984). Because resistance to chestnut blight can be inherited, tracking family identification in chestnut restoration efforts will provide the ability to link field performance to resistance. Testing early field performance of pure American chestnut will provide researchers and managers with important information to improve success of blight-resistant field plantings that will be established in 2009.

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REFERENCES

Anagnostakis, S.L. 2001. The effect of multiple importations of pests and pathogens on a native tree. Biological Invasions. 3: 245–254.

Ashe, W.W. 1911. Tennessee State Geological Survey Bulletin. O-B. Nashville, TN: Baird-Ward Printing Company. 35 p.

Bailey, R.G. 1995. Description of the Ecoregions of the United States. 2nd ed., rev. Misc. Pub. 1391. Washington, DC: U.S. Department of Agriculture (USDA). 108 p. + map.

Braun, E.L. 1950. Deciduous Forests of Eastern North America. Philadelphia, PA: The Blakiston Co. 596 p.

Clark, S.L.; Schlarbaum, S.E.; and Kormanik, P.P. 2000. Visual grading and quality of 1-0 northern red oak seedlings. Southern Journal of Applied Forestry. 24: 93–97.

Crandall, R.S.; Gravatt, G.F.; and Ryan, M.M. 1945. Root disease of *Castanea* species and some coniferous and broadleaf nursery stocks, caused by *Phytophthora cinnamomi*. Phytopathology. 35: 162–180.

Delcourt, P.A.; Delcourt, H.R. 1997. The influence of prehistoric human-set fires on oak-chestnut forests in the Southern Appalachians. Castanea. 63: 337–345.

Delcourt, P.A.; Delcourt, H.R.; Ison, C.R.; Sharp, W.E.; and Gremillion, K.J. 1998. Prehistoric human use of fire, the eastern agricultural complex and Appalachian oak-chestnut forests: Paleoecology of Cliff Palace Pond, Kentucky. American Antiquity. 63: 263–278.

Dey, D.C.; Parker, W.C. 1997. Morphological indicators of stock quality and field performance of red oak (*Quercus rubra* L.) seedlings underplanted in a central Ontario shelterwood. New Forests. 14:145–156.

Griffin, G.J.; Smith, H.C.; Dietz, A.; and Elkins, J.R. 1991. Importance of hardwood competition to American chestnut survival, growth and blight development in forest clearcuts. Canadian Journal of Botany. 69: 1804–1809.

Hebard, F.V. 2001. Backcross breeding program produces blight-resistant American chestnuts (Virginia). Ecological Restoration. 4: 252–254.

Jacobs, D.F.; Severeid, L.R. 2004. Dominance of interplanted American chestnut (*Castanea dentata*) in southwestern Wisconsin, USA. Forest Ecology and Management. 191: 111–120.

Jacobs, D.F.; Salifu, K.F.; Seifert, J.R. 2005. Relative contribution of initial root and shoot morphology in predicting field performance of hardwood seedlings. New Forests. 30: 235–251.

Joesting, H.M.; McCarthy, B.C.; and Brown, K.J. 2007. The photosynthetic response of American chestnut seedlings to differing light conditions. Canadian Journal of Forest Research. 37: 1714–1722.

Johnson, P.S. 1981. Nursery stock requirements for oak planting in upland forests. In: Northeastern Area Nurserymen's Conference. Broomall, PA: USDA Forest Service, Northeastern Area State and Private Forestry: 1–19.

Johnson, P.S.; Novinger, S.L.; and Mares, W.G. 1984. Root, shoot, and leaf area growth potentials of northern red oak planting stock. Forest Science. 30: 1017–1026.

Kormanik, P.P.; Sung, S.S.; and Kormanik, T.L. 1994. Irrigating and fertilizing to grow better nursery seedlings. In: Northeastern and Intermountain Forest and Conservation Nursery Associations; 1993 August 2–5; St. Louis, MO. General Technical Report RM-243. Fort Collins, CO: USDA Forest Service, Rocky Mountain Research Station: 115–121.

Kormanik, P.P.; Sung, S.S.; Kass, D.; and Zarnock, S.J. 2002. Effects of seedling size and first-order lateral roots on early development of northern red oak on a mesic site: eleventh-year results. In: Outcalt, K.W., editor. 11th Biennial Southern Silviculture Research Conference; 2001 March 20–22; Knoxville, TN. General Technical Report SRS-48. Asheville, NC: USDA Forest Service, Southern Research Station: 332–337.

Loftis, D.L. 1990. A shelterwood method for regenerating red oak in the southern Appalachians. Forest Science. 36: 917–929.

McCament, C.L.; McCarthy, B.C. 2005. Two-year response of American chestnut (*Castanea dentata*) seedlings to shelterwood harvesting and fire in a mixed-oak forest ecosystem. Canadian Journal of Forest Research. 35: 740–749.

McCormick, J.F.; Platt, R.B. 1980. Recovery of an Appalachian forest following the chestnut blight or Catherine Keever, You were right! American Midland Naturalist. 104: 264–273.

McEwan, R.W.; Keiffer, C.H.; and McCarthy, B.C. 2006. Dendroecology of American chestnut in a disjunct stand of oak-chestnut forest. Canadian Journal of Forest Research. 36: 1–11.

McNab, W.H.; Patch S.; and Nutter, A.A. 2003. Early results from a pilot test of planting small American chestnut seedlings under a forest canopy. Journal of the American Chestnut Foundation. 16: 32–41.

Oswalt, C.M.; Clatterbuck, W.K.; and Houston, A.E. 2006. Impacts of deer herbivory and visual grading on the early performance of high-quality oak planting stock in Tennessee, USA. Forest Ecology and Management. 22: 128–135.

Rhoades, C.C.; Brosi, S.L.; Dattilo, A.J.; and Vincelli, P. 2003. Effect of soil compaction and moisture on incidence of phytophthora root rot on American chestnut (*Castanea dentata*) seedlings. Forest Ecology and Management. 184: 47–54.

Ruehle, J.L.; Kormanik, P.P. 1986. Lateral root morphology: a potential indicator of nursery seedling quality. Research Note SE-344. Asheville, NC: USDA Forest Service, Southeastern Forest Experiment Station. 6 p.

SAS Institute Inc. 2004. SAS/STAT User's Guide, Version 9. 2nd ed., rev. Cary, NC: SAS Institute Inc.

Schultz, R.C.; Thompson, J.R. 1990. Nursery practices that improve hardwood seedling root morphology. Tree Planters' Notes. 3: 21–32.

Schweitzer, C.J. 2004. First-year response of an upland hardwood forest to five levels of overstory tree retention. In: Conner, K., editor. 12th Biennial Southern Silvicultural Research Conference. 2003 February 24–28; Biloxi, MI. General Technical Report SRS-71. Asheville, NC: USDA Forest Service, Southern Research Station: 287–291.

Smalley, G.W. 1982. Classification and evaluation of forest sites on the mid-Cumberland Plateau. General Technical Report SO-38. New Orleans, LA: USDA Forest Service, Southern Forest Experiment Station. 58 p.

Spetich, M.A.; Dey, D.C.; Johnson, P.S.; and Graney, D.L. 2002. Competitive capacity of *Quercus rubra* L. planted in Arkansas Boston Mountains. Forest Science. 48: 504–517.

Stephenson, S.L.; Adams, H.S.; and Lipford, M.L. 1991. The present distribution of American chestnut in the upland forest communities of Virginia. Bulletin of the Torrey Botanical Club. 118: 24–32.

Struve, D.K. 1990. Root regeneration in transplanted deciduous nursery stock. HortScience. 25: 266–270.

Struve, D.K.; Joly, R.J. 1992. Transplanted red oak seedlings mediate transplant shock by reducing leaf surface area and altering carbon allocation. Canadian Journal of Forest Research. 22: 1441–1448.

Tennessee Department of Agriculture, Division of Forestry. 2006. Seedling Catalog [Brochure]. pp. n.a.

University of Nebraska Cooperative Extension. 1994. Prevention and Control of Wildlife Damage. Hygnstrom, S.E.; Timm, R.M.; and Larson, G.E., eds. Lincoln, NE. pp. n.a.

Wang, G.G.; Bauerle, W.L.; and Mudder, B.T. 2006. Effects of light acclimation on the photosynthesis, growth, and biomass allocation in American chestnut (*Castanea dentata*) seedlings. Forest Ecology and Management. 226: 173–180.

Ward, J.S.; Gent, M.P.; and Stephens, G.R. 2000. Effects of planting stock quality and browse protection-type on height growth of northern red oak and eastern white pine. Forest Ecology and Management. 127: 205–216.

Zaczek, J.J.; Steiner, K.C.; and Bowersox, T.W. 1996. Northern red oak planting stock: 6-year results. New Forests. 13: 175–189.

Zobel, B.; Talbert, J. 1984. Applied Forest Tree Improvement. Prospect Heights, IL: Waveland Press, Inc. 505 p.

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Root Morphology and Growth of Bare-Root Seedlings of Oregon White Oak

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Abstract

Root morphology and stem size were evaluated as predictors of height and basal-area growth (measured at groundline) of 1-1 Oregon white oak (*Quercus garryana* Dougl. ex Hook.) seedlings planted in raised beds with or without an additional irrigation treatment. Seedlings were classified into three root classes based on a visual assessment of the fibrosity of their root systems. Early-season and late-season height and basal-area growth differed significantly among root classes after accounting for initial seedling size. Irrigation significantly increased late-season height growth, but not the other growth increments. Recommendations are made for grading seedlings to increase the growth of those that are outplanted.

Introduction

Interest has been increasing in recent years in restoring or recreating Oregon white oak (*Quercus garryana* Dougl. ex Hook.) savannas and woodlands in the Pacific Northwest. Oak savannas and woodlands were an important part of the historical landscape, especially in the Willamette Valley of Oregon (Sprague and Hansen 1946, Habeck 1962, Thilenius 1968), the Puget Lowlands of Washington (Crawford and Hall 1997, Thysell and Carey 2001, Foster and Shaff 2003), and eastern Vancouver Island, British Columbia (Gedalof and others 2006). Much of the area that was historically occupied by oak has been altered by agricultural and urban development and by succession to dense, conifer-dominated forests.

In some cases, land managers are planting oak to replace trees that have been lost or to create new stands in areas that were not historically occupied by oak. Planting often requires a significant investment in labor, browse protection, and control of competing vegetation (Devine and others 2007). High-quality seedlings are needed to ensure a high rate of survival and acceptable growth. Oak species in general are known for their high degree of variation in morphology and growth rates (Long and Jones 1996, Dey and Parker 1997). Grading seedlings so that only those with a high growth potential are planted can improve the success of restoration projects.

Although little information is currently available on the production of Oregon white oak seedlings, studies of other species have shown that root morphology strongly affects growth and survival after outplanting. Seedlings with fibrous root systems generally have the best growth and survival (Davis and Jacobs 2005). The number of first-order lateral roots (FOLR) >1 mm (0.04 in) diameter at the junction with the taproot has proven a useful quantitative measurement for predicting growth in many studies (Ruehle and Kormanik 1986, Thompson and Schultz 1995, Dey and Parker 1997, Schultz and Thompson 1997). Qualitative visual assessments of root morphology have also proven effective for identifying seedlings with high growth potential. An advantage of this approach is that seedlings can also be graded quickly on the basis of root morphology, sometimes in combination with measurements of stem height and diameter (Clark and others 2000, Oswalt and others 2006).

Nursery practices such as undercutting and root-wrenching encourage lateral root development and discourage the development of deep roots that are lost when the seedlings are lifted (Schultz and Thompson 1997, Andersen 2004). These nursery practices are important to oaks and other species that otherwise have a strong tendency to form a deep taproot.

The purpose of this study was to evaluate how root morphology, seedling size, and the growing environment affect the growth of bare-root Oregon white oak seedlings. Root morphology was qualitatively assessed on 2-yr-old seedlings that were then outplanted into raised beds. Growth was measured over one growing season; subsets of seedlings were destructively sampled for biomass measurements and determination of the initial timing of root growth. Because the ability of roots to adequately move water and nutrients from the soil into the stem is a primary factor affecting the success of recently planted seedlings (Grossnickle 2005, Jacobs and others 2005), an irrigation treatment was imposed to create conditions of high and low soil moisture. Seedling growth in the nursery during the growing season before outplanting and growth after outplanting were also summarized in order to evaluate strategies for culling seedlings with low growth potential.

Methods

Planting regime and seedling measurements. Seedlings were grown from acorns collected from two trees in Thurston and Mason Counties, Washington. Small and unsound acorns were discarded. The large and sound acorns were sown by hand in October 2004 at the Washington Department of Natural Resources Webster Nursery (lat 46.95° N, long 122.96° W) near Olympia, WA. Initial sowing densities ranged between 29 and 86 seedlings/m^2 (between 3 and 8 seedlings/ft^2); sowing density did not affect seedling size at the end of the first growing season (data not shown). Seedlings were undercut June 2, 2005, lifted in January 2006, and held in cold storage until they were transplanted into different nursery beds at a density of 29 seedlings/m^2 (3 seedlings/ft^2) in April 2006. A sample of seedlings (n=70) was selected for destructive sampling for biomass measurements at the end of the 2005 growing season. Seedlings were irrigated and fertilized while in the nursery beds during the 2005 and 2006 growing seasons.

Lifting and transplanting were not planned cultural treatments; rather, they were done because the original nursery beds were not going to be irrigated in 2006. Transplanting did provide an opportunity to impose an additional root treatment with the intention of increasing the range of root morphologies. Tap roots were pruned to a length of 15 cm (6 in) on approximately one-half of the seedlings right before they were transplanted in April 2006. About one-half of the pruned seedlings and all unpruned seedlings were root-wrenched in August 2006 to encourage the development of fibrous root systems. Seedling height and basal diameter were measured once monthly during the 2006 growing season. Seedlings were lifted in January 2007 and placed in cold storage.

Between March 14 and 16, 2007, seedlings (n=273) were removed from storage, gently rinsed with water, tagged, photographed, and planted. Seedlings were assigned to one of three classes based on their root morphology (figure 1); those with the most fibrous root systems were assigned to root class 1, and those with the least fibrous systems, to root class 3. Six of the 273 seedlings died during the growing season: 5 in root class 3 and 1 in root class 2. All mortality occurred during the first half of the growing season.

Seedlings were planted at a density of 11 seedlings/m^2 (1 seedling/ft^2) in eight raised beds [4.9 m (16 ft) long × 1.2 m (4 ft) wide × 0.6 m (2 ft) deep] located adjacent to the Webster Nursery at the Olympia Forestry Sciences Laboratory, Forest Service. The raised beds were used so that roots could easily be extracted for future sampling. The raised beds were constructed in early 2007 and filled with a well-drained sandy-loam soil. Four of the eight raised beds were assigned to the irrigation treatment; a drip irrigation system maintained high soil moisture throughout the growing season.

Another 29 seedlings were planted in large pots filled with the same soil and buried in two adjacent raised beds. These seedlings were excavated between April 30 and May 25 (i.e., 6–10 weeks after planting) to quantify new root growth at the beginning of the growing season. After

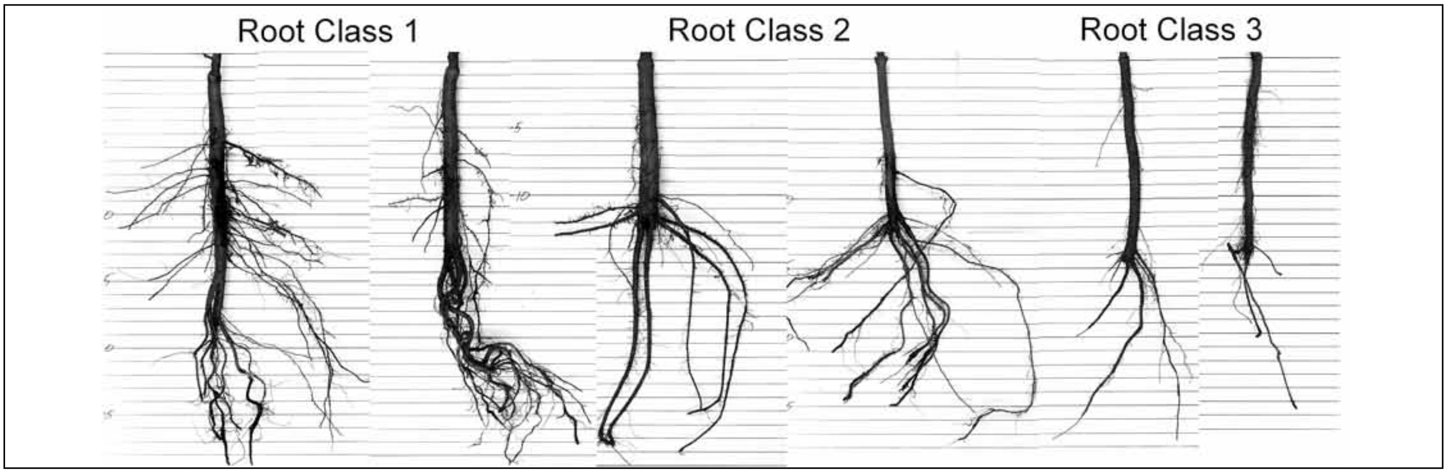

Figure 1. Examples of Oregon white oak seedlings that were classified into root class 1 (high root fibrosity; left two seedlings), root class 2 (moderate root fibrosity; middle two seedlings), and root class 3 (low root fibrosity; right two seedlings). The image is a composite of photographs of individual seedlings. Horizontal lines show 1 cm (0.4 in) gradations.

excavation and washing, the light-colored, succulent new roots were clipped from the seedlings, the remaining, older roots were divided into the small and large root categories, and all samples were oven-dried and weighed.

Seedling heights and diameter at groundline were measured shortly after planting, in the middle of the growing season, and at the end of the growing season. Basal area was calculated from diameter [basal area = $\pi (d/2)^2$] for growth comparisons. Early-season (May 1–July 31) and late-season (August 1–October 31) growth increments were calculated.

In order to quantify differences in seedling morphology among root classes, a set of seedlings was randomly selected before planting (n=56) and at the end of the growing season (n=33) for additional measurements. The number of FOLR [≥1mm (0.04 in) diameter] was counted and each seedling was partitioned into the shoot, small roots [≤5 mm (0.2 in) diameter], and large roots, and then oven dried and weighed. The small and large root categories generally divided the root systems into lateral roots and the taproot, but they also provided a criterion for classifying woody roots without a simple taproot.

Data analysis. The effects of root class and irrigation on seedling height and basal-area growth were tested using the general linear model with initial height or basal area as a covariate (Neter and others 1996). Statistically significant differences for all tests are reported at α=0.05. The Bonferroni joint-estimation procedure (Neter and others 1996) was used for multiple comparisons (e.g., among root classes) to maintain the type 1 error rate. Interactions between root class and irrigation were also tested, but these terms were not statistically significant and were dropped from the final models.

In order to examine strategies for culling seedlings with low growth potential, size-class transition rates were calculated for the 2006 growing season (when the seedlings were in nursery beds) and the 2007 growing season. Within each growing season, seedlings were classified into an initial size class (based first on height and second on diameter at the beginning of the growing season) and a final height class (based on measurements at the end of the growing season). Transition rates among size classes were calculated as the percentage of seedlings in each initial size class that was assigned to each of the final size classes. Size classes were designated so that one-third of the seedlings fell into each class based on height and basal diameter. For example, the small size class included the shortest one-third of seedlings with ties broken first by basal diameter and then at random if basal diameters were also equal.

Results

About one-half of seedlings were assigned to root class 1, indicating a high degree of root fibrosity (table 1). The remaining seedlings were about equally divided between root classes 2 and 3. The root class assignments were not significantly associated with nursery treatments (χ^2=6.0, df=4, p=0.20); however, the two wrenched treatments had greater percentages of seedlings assigned to class 1 (46 and 56 percent) than the unwrenched treatment (40 percent).

Seedlings assigned to the three root classes differed in several morphological traits (table 2). At the time of planting, seedlings in root class 1 were significantly taller than those in the other root classes and had a greater mean diameter than those in root class 3. Seedlings in root class 1 had significantly greater large root mass, small root mass, and number of FOLRs than those in the other two root classes. Differences between root classes 2 and 3 were not statistically significant for these measurements, but seedlings in root class 2 were larger in height and diameter than those in root class 3. Differences between root classes generally increased during the growing season. For example, the mean height difference between seedlings in root classes 1 and 3 was about 9 cm (3.5 in) at the beginning of the growing season and 24 cm (9.4 in) at the end.

All three root classes had approximately equal root-shoot ratios (range, 3.4–3.8) at the beginning of the growing season. In comparison, the mean root-shoot ratio of seed-

Table 1. Percentages of Oregon white oak seedlings assigned to each root morphology class by nursery treatment.

Treatment	Root Class		
	1	2	3
Pruned–unwrenched	40	31	29
Pruned–wrenched	46	29	25
Unpruned–wrenched	56	20	24
All seedlings	48	26	26

Table 2. Summary of morphological traits by root morphology class at the beginning and end of the 2007 growing season. Means followed by different letters indicate significant differences (α = 0.05) among root classes at the beginning or end of the growing season.

Measurement[1]	Beginning of season			End of season		
	Root class			Root class		
	1	2	3	1	2	3
Height (cm)	23.6a	19.1b	14.8c	53.0a	36.7b	28.8c
Diameter (mm)	7.3a	6.7a	5.8b	12.4a	11.2b	9.2c
Large-root mass (g)	6.2a	3.6b	2.9b	29.8a	13.3b	8.9b
Small-root mass (g)	3.5a	1.9b	1.4b	8.6a	5.4ab	2.9b
Stem mass (g)	3.3a	1.8ab	1.4b	27.5a	10.1b	4.7b
Root-shoot ratio (g/g)	3.5a	3.4a	3.8a	1.6b	2.6ab	3.4a
FOLR (n)	8.6a	5.0b	4.4b	18.4a	11.0b	7.7b

[1] Height and diameter were measured on all seedlings (n=273, beginning of season; n=267, end of season). Large root mass [>5 mm (0.2 in) diameter], small-root mass, stem mass, root-shoot ratio, and number of first-order lateral roots (FOLR) were measured on two sets of seedlings that were destructively sampled at the beginning (n=56) or the end (n=33) of the growing season.

lings from the same crop that were sampled after their first growing season was 4.1 (n=70 seedlings sampled in 2005). Root-shoot ratios were lower in all three root classes at the end of the growing season than at the beginning. The decline in root-shoot ratio was much greater in root class 1 than in the other two root classes, and root-shoot ratio was significantly lower at the end of the growing season in root class 1 than in root class 3.

Early root growth was significantly correlated with the mass of older small roots [≤5 mm (0.2 in) diameter] in seedlings that were excavated in late April and May (figure 2). Seedlings in root class 1 had significantly more new root mass than those in root class 3. The mass of new roots in root class 2 was intermediate between root classes 1 and 3. New root mass was not significantly correlated with sampling date. Early root growth was mostly due to the elongation of existing first- and higher-order roots, rather than the emergence of new lateral roots. Root elongation was already underway, but most seedlings had not yet broken bud, when seedlings were first sampled on April 30.

Root classes differed significantly in height and basal area growth (figure 3), but late-season height growth was the

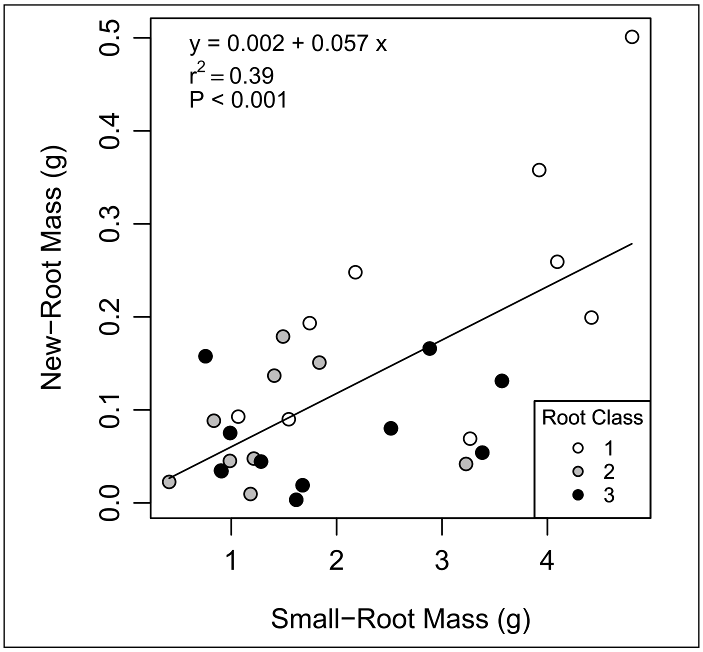

Figure 2. The growth of new roots sampled between April 30 and May 25. New-root mass (y-axis) was significantly correlated with the mass of older small roots (x-axis), but it was not correlated with sampling date. The mass of new roots differed significantly between the three root morphology classes, with the greatest mass in root class 1.

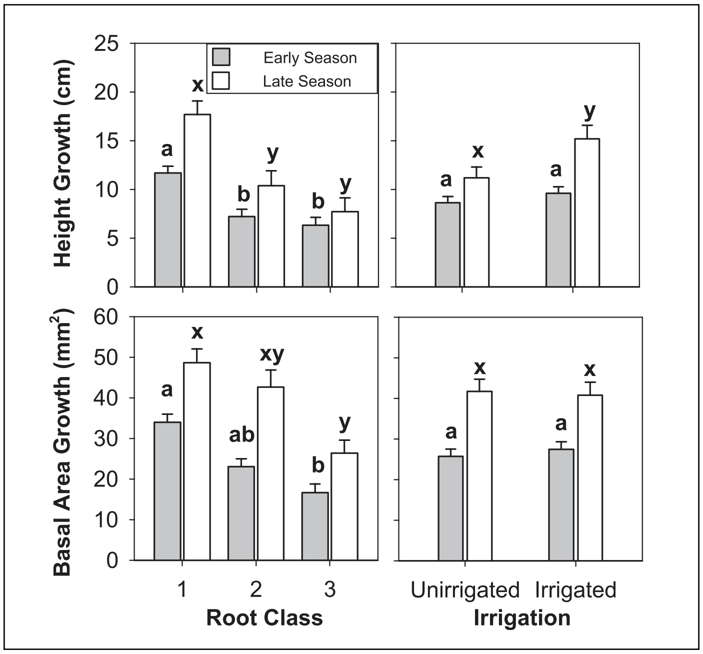

Figure 3. Mean height and basal-area growth for the three root morphology classes and the irrigation treatments. Early-season growth (grey bars) includes growth through July 31; late-season growth (white bars) includes growth from August 1 to October 31. Different letters over the bars indicate significant differences (α=0.05) among treatments (a and b for early-season growth; x and y for late-season growth).

only growth increment that was increased by irrigation. The difference in late-season height growth between irrigation treatments [4 cm (1.6 in)] was small relative to the difference between root classes 1 and 3 [10 cm (3.9 in)]. Both height and basal area growth were greater in the second half of the growing season than in the first. Total height growth of seedlings in root classes 1, 2, and 3 averaged 29, 18, and 14 cm, respectively. Total basal area growth of seedlings in the three respective classes was 83, 66, and 43 mm^2 (0.12, 0.10, and 0.07 in^2).

In addition to root class, initial height and basal area were statistically significant predictors of height and basal-area growth, respectively. Both predictors were positively correlated with growth. The percentage of observed variation in growth that was explained by the statistical models was low in all cases, with r^2 between 0.11–0.20.

The size-class transition rates calculated for the 2006 and 2007 growing seasons indicated that slow-growing seedlings may be identified for culling after the first growing season (figure 4). During 2006 (the second growing season in the nursery), only 10 percent of seedlings that were initially in the small size class [≤5 cm (2 in) tall] grew into the large size class [≥18 cm (7 in) tall]; thus, culling these seedlings would have had little effect on the number of large seedlings at the end of the growing season. The likelihood of seedlings transitioning between the small and large size classes diminished after outplanting. Only 3 percent of seedlings in the small size class transitioned into the large size class, while 73 percent remained in the small size class. Size-class transition rates could not be directly calculated between growing seasons because seedlings were not tagged before lifting; however, based on the transition rates for the individual years, only 19 percent of seedlings that were in the small size class after the first growing season would be expected to reach the large size class 1 yr after outplanting. About 54 percent of seedlings in the large size class at the end of the first year in the nursery would be expected to remain in the large size class 1 yr after outplanting.

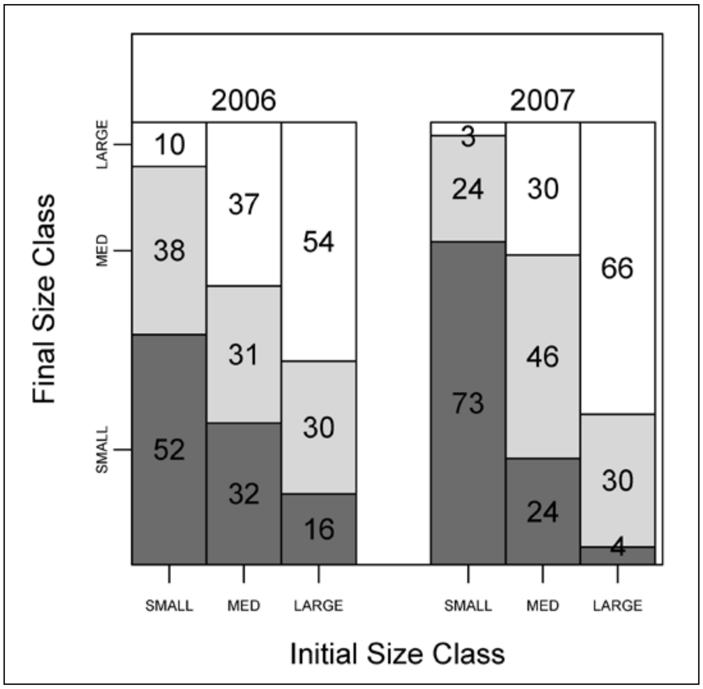

Figure 4. Size-class transition rates of Oregon white oak seedlings during the 2006 growing season (in nursery beds) and 2007 growing season (outplanted in raised beds). Size classes were designated based on seedling height and basal diameter so that each initial and final size class contained one-third of the seedlings measured during each growing season.

Discussion

The growth of Oregon white oak seedlings is highly variable, but it is affected by initial seedling size and root morphology. Seedling size (height or basal area) and root class were significant predictors of growth in linear models that included both variables, indicating that both size and root class are separate indicators of seedling vigor. The visual assessment of root morphology was valuable for identifying seedlings that had relatively high root mass and a high number of FOLR. Seedlings in root class 1 also had relatively high new root growth in the early growing season, which is essential for successful seedling establishment (Grossnickle 2005).

Root class was not associated with nursery treatments that were imposed at the time of transplanting or late in the second growth season; however, these treatments might have been more effective if all seedlings had not already received some root management in the first season (undercutting in June) and again when they were lifted in January. Although it was not statistically significant, there was some evidence that root pruning reduced the percentage of seedlings in root class 1, possibly due to the removal of some FOLR (Kaczmarek and Pope 1993). Root pruning may not offer additional benefits in nursery regimes that already include undercutting to reduce the length of the taproot and root wrenching to increase root fibrosity.

Irrigation increased late-season height growth but did not significantly affect the other growth increments. Oregon white oak has a determinant growth habit, and early-season growth may have been more strongly affected by the number of leaf primordia in the bud at the beginning of the growing season than by soil moisture. Late-season height growth presumably reflected additional flushes from terminal buds formed during the growing season. Nevertheless, the magnitude of the irrigation effect was small compared to growth differences among root classes. Possibly the effect of irrigation would have been greater if there had been less summer rainfall. Precipitation in May through September 2007 was 185 mm (7.3 in), which is slightly less than the long-term average [203 mm (8.0 in)]. However, several rain events during the growing season helped to maintain soil moisture. The total precipitation in July was more than twice the average due to one large rain and several smaller ones.

Although the pattern of decreasing soil moisture during the growing season that is typical of the Pacific Northwest did not occur over the course of this study, Oregon white oak are often planted in dry and nutrient-poor soils and typically must compete with other vegetation for soil moisture and growing space (Devine and Harrington 2007; Devine and others 2007). The survival and early growth of seedlings depend on their ability to quickly recover from the stress caused by lifting, storage, and planting and their ability to develop a root system that can adequately supply the seedling with water and nutrients (Rietveld 1989; Grossnickle 2005). Seedlings in root class 1 likely benefited from their high initial root area and the high root growth potential that was observed among those seedlings that were excavated at the beginning of the growing season.

Seedlings in root class 1 had a lower root-shoot ratio at the end of the growing season than those in root classes 2 and 3; however, root-shoot ratio should not necessarily be viewed in this case in terms of a trade-off between root and shoot growth. Seedlings in root class 1 had considerably greater above- and below-ground growth after outplanting than those in the other root classes. These seedling were clearly vigorous, and the decrease in root-shoot ratio was the result of the normal changes in morphology (e.g., greater above-ground biomass) that occurs as trees grow larger (Monk 1966).

Culling may be an important tool for improving the vigor of Oregon white oak seedlings at the time of outplanting. Oregon white oak is a slow-growing species in general (Hibbs and Yoder 1993), and some of the seedlings used in this study grew particularly slowly. Growing conditions over the life of the seedling were generally very favorable; however, at the end of this study approximately 25 percent of the 3-yr-old seedlings were still ≤24 cm (9.4 in) tall. In this study and related nursery trials, we have observed that the growth of some seedlings appears to stagnate early and the seedlings remain small over multiple growing seasons. Identifying and removing slow-growing seedlings at an early age may help to reduce losses later.

Conclusions

As is the case with many other tree species, root morphology is an important factor in determining the quality of Oregon white oak seedlings. Although growth was not tested across the same range of growing conditions that seedlings encounter in operational outplantings, seedlings with fibrous root systems should have the best growth and survival under most growing conditions. Nursery managers should consider applying root-management treatments to encourage development of fibrous root systems. When practical, Oregon white oak seedlings should be graded based on a combination of height, diameter, and root morphology to increase the percentage of outplanted planted seedlings that have a high growth potential. It may also be effective to sow acorns at very high densities [e.g., >100 acorns/m^2 (9 acorn/ft^2)] and then heavily cull seedlings during or after the first growing season. Land managers should likewise select large seedlings with fibrous root systems, when available, for outplanting.

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REFERENCES

Andersen, L. 2004. Field performance of *Quercus petraea* seedlings grown under competitive conditions: influence of prior undercutting in the seedbed. New Forests. 28: 37–47.

Clark, S.L.; Schlarbaum, S.E.; and Kormanik, P.P. 2000. Visual grading and quality of 1-0 northern red oak seedlings. Southern Journal of Applied Forestry. 24: 93–97.

Crawford, R.C.; H. Hall. 1997. Changes in the South Puget Sound landscape. In: Dunn, P.; Ewing, K., eds. Ecology and Conservation of the South Puget Sound Prairie Landscape. Seattle, WA: Nature Conservancy: 11–15.

Davis, A.S.; Jacobs, D.F. 2005. Quantifying root system quality of nursery seedlings and relationship to outplanting performance. New Forests. 30: 295–311.

Devine, W.D.; Harrington, C.A. 2007. Release of Oregon white oak from overtopping Douglas-fir: effects on soil water and microclimate. Northwest Science. 81: 112–124.

Devine, W.D.; Harrington, C.A.; and Leonard, L.P. 2007. Post-planting treatments increase growth of Oregon white oak (*Quercus garryana* Dougl. ex Hook.) seedlings. Restoration Ecology. 15: 212–222.

Dey, D.C.; Parker, W.C. 1997. Morphological indicators of stock quality and field performance of red oak (*Quercus rubra* L.) seedlings underplanted in a central Ontario shelterwood. New Forests. 14: 145–156.

Foster, J.R.; Shaff, S.E. 2003. Forest colonization of Puget Lowland grasslands at Fort Lewis, Washington. Northwest Science. 77: 283–296.

Gedalof, Z.; Pellatt, M.; and Smith, D.J. 2006. From prairie to forest: three centuries of environmental change at Rocky Point, Vancouver Island, British Columbia. Northwest Science. 80: 34–46.

Grossnickle, S. 2005. Importance of root growth in overcoming planting stress. New Forests. 30: 273–294.

Habeck, J.R. 1962. Forest succession in Monmouth Township, Polk County, Oregon since 1850. Proceedings of the Montana Academy of Sciences. 21: 7–17.

Hibbs, D.E.; Yoder, B.J. 1993. Development of Oregon white oak seedlings. Northwest Science. 67: 30–36.

Jacobs, D.F.; Salifu, K.F.; and Seifert, J.R. 2005. Growth and nutritional response of hardwood seedlings to controlled-release fertilization at outplanting. Forest Ecology and Management 214: 28–39.

Kaczmarek, D.J.; Pope, P.E. 1993. The effects of pruning treatments and initial seedling morphology on northern red oak seedling growth. In: Proceedings, 9th Central Hardwood Forest Conference; 1993 March 8-10; West Lafayette, IN. General Technical Report NC-161. St. Paul, MN: U.S. Department of Agriculture (USDA), Forest Service, North Central Forest Experiment Station: 436–446.

Long, T.J.; Jones, R.H. 1996. Seedling growth strategies and seed size effects in fourteen oak species native to different soil moisture habitats. Trees–Structure and Function. 11: 1–8.

Monk, C.D. 1966. Root-shoot dry weights in loblolly pine. Botanical Gazette. 127: 246–248.

Neter, J.; Kutner, M.H.; Nachsheim, C.J.; and Wasserman, W. 1996. Applied Linear Statistical Models. 4th ed. Boston: WCB McGraw Hill.

Oswalt, C.M.; Clatterbuck, W.K.; and Houston, A.E. 2006. Impacts of deer herbivory and visual grading on the early performance of high-quality oak planting stock in Tennessee, USA. Forest Ecology and Management. 229: 128–135.

Rietveld, W.J. 1989. Transplant stress in bareroot conifer seedlings: its development and progression to establishment. Northern Journal of Applied Forestry. 6: 99–107.

Ruehle, J.L.; Kormanik, P.P. 1986. Lateral root morphology: a potential indicator of seedling quality in northern red oak. RN-SE-344. USDA Forest Service, Southeastern Forest Experiment Station, Asheville, NC. 6 p.

Schultz, R.C.; Thompson, J.R. 1997. Effect of density control and undercutting on root morphology of 1+0 bareroot hardwood seedlings: five-year field performance of root-graded stock in the central USA. New Forests. 13: 301–13,314.

Sprague, F.L.; Hansen, H.P. 1946. Forest succession in the McDonald Forest, Willamette Valley, Oregon. Northwest Science. 20: 89–98.

Thilenius, J.F. 1968. The *Quercus garryana* forests of the Willamette Valley, Oregon. Ecology. 49: 1124–1133.

Thompson, J.R.; Schultz, R.C. 1995. Root system morphology of *Quercus rubra* L. planting stock and 3-year field performance in Iowa. New Forests. 9: 225–236.

Thysell, D.R.; A.B. Carey. 2001. *Quercus garryana* communities in the Puget Trough, Washington. Northwest Science. 75: 219–235.

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Improving Root Growth and Morphology of Containerized Oregon White Oak Seedlings

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Abstract

We conducted four trials to determine if we could alter root morphology of containerized Oregon white oak seedlings in order to potentially improve their performance in restoration plantings. Early pruning of the radicle produced a branched taproot, though with fewer branches than reported for other oak species. Pruning the taproot at 15 cm (6 in) promoted greater taproot branching than radicle pruning but did not increase formation of lateral roots. Nontransplanted seedlings grown in tall containers (2.83 L; 0.75 gal) responded to air-pruning with increased lateral root growth and minimal circling of roots. Inoculation with soil containing ectomycorrhizal fungi substantially improved shoot- and root-growth response to fertilization.

Introduction

The range of Oregon white oak (*Quercus garryana* Dougl. ex Hook.) extends from southern California to southern British Columbia. It is the only species of *Quercus* native to northern Oregon, Washington, and British Columbia. Oregon white oak occupies sites ranging from rock outcrops to riparian zones, although its extent has been reduced significantly during the past 150 yr by agriculture, urbanization, and lack of regular fire. In recent years, interest in restoring oak-associated plant communities and in landscaping with native species has resulted in the planting of Oregon white oak seedlings on a variety of private and public lands. Bareroot and container-grown Oregon white oak seedlings are produced by numerous commercial nurseries in California, at least 30 in Oregon, and several in Washington and British Columbia.

Many native Oregon white oak sites are relatively harsh, with shallow or coarse-textured soils, dense vegetative competition, and limited soil water availability during the dry summers characteristic of its range. Access to soil water on these sites is related to early growth of planted seedlings (Devine and others 2007); thus, development and morphology of seedling roots will likely influence performance. The extra costs associated with containerized seedlings (compared to bareroot seedlings) might be justified on these dry sites, as survival rates may be higher and the window of time for planting may be greater than for bareroot seedlings (Wilson and others 2007). Additionally, restorationists or landowners planting relatively few oak seedlings or planting at low density may be willing to accept the higher per-seedling cost for the benefits of container stock.

Container-grown Oregon white oak seedlings ideally have root systems with many fibrous lateral roots and a straight taproot that ends at the base of the container. The lateral roots increase the total root surface area, which, in turn, is related to greater potential for water and nutrient uptake, reduced planting stress, and improved post-planting survival and growth (Schultz and Thompson 1996, Grossnickle 2005). The taproot grows rapidly following germination in the fall. Given the droughty growing-season soil conditions in much of the species' range, this taproot may be an evolved strategy for acquiring water from deeper soils with relatively more available moisture in summer. In a standard pot, this taproot grows straight to the bottom and circles repeatedly, creating a 'pot-bound' seedling.

Oak seedlings planted with this root morphology perform poorly (McCreary 1996) and may never fully recover. To prevent this and other root deformities, techniques such as air-pruning or chemical pruning often are used for tree seedlings grown in containers. In several oak species, pruning the newly emerged radicle promotes branching of the taproot (Barden and Bowersox 1989, McCreary 1996, Tilki and Alptekin 2006). These branches grow down, with the appearance of multiple taproots of similar size and morphology. We refer to these branches as multiple taproots in this report. If we assume that lateral roots originate from each taproot at a similar frequency regardless of the number of taproots, a seedling with multiple taproots would have a greater root surface area than a seedling with a single taproot.

A wide variety of container types also has been developed to improve seedling root morphology (Owen and Stoven 2008). Various container designs and root treatments have been tested on other western oak species in California (McCreary 2001) and on oaks in eastern North America (Landis 1990). Inoculating container-grown seedlings with mycorrhizal fungi also may significantly increase growth and nutrient uptake (Dixon and others 1984, Mitchell and others 1984). The effects of root manipulation treatments on containerized Oregon white oak, however, have not been documented. In this study, we examined the effects of radicle and root pruning, air pruning, and mycorrhizal inoculation on development of root systems of containerized Oregon white oak seedlings.

General Methods

Four trials (2003–2006) examined the root- and shoot-growth responses of Oregon white oak seedlings to various treatments. This section describes the general practices used; further details appear in the pertinent sections. Trials were conducted in a lath-walled building under a corrugated, translucent white roof in Olympia, WA. Sunlight was unobstructed except in early morning and late afternoon, when trees blocked direct sunlight. January and July temperatures in Olympia average 3 °C and 17 °C (38 °F and 63 °F), respectively.

Sowing practices. Acorns were collected from regional sources and refrigerated until sowing in October or November. Before sowing, acorns were placed in water for 24 h; those that floated were discarded. Sound acorns were sown horizontally at a 1–2 cm (0.4–0.8 in) depth in Tall One 2.83-L (0.75-gal) Treepots^{TM}, 10 cm (4.0 in) square at the top and 36 cm (14.2 in) deep (Stuewe & Sons, Inc., Corvallis, OR). Potting medium was a 2:1:1 volumetric ratio of mixed peat moss: coarse perlite: vermiculite. Seedlings were irrigated as needed. Water-soluble fertilizer (20 N-20 P_2O_5-20 K_2O) with micronutrients (The Scotts Company, Marysville, OH) was added at the manufacturer's recommended rate during the growing season.

Statistical treatment. Sample size ranged from approximately 30 to 100 seedlings per treatment, depending on the number of treatments. Randomized block designs were used; blocks were designated based on bench and distance from the lath wall. All data were analyzed with the Mixed Procedure in SAS (SAS Institute 2005). Results are reported with a minimum confidence level of 95 percent.

Radicle Pruning

We tested the effect of radicle pruning on Oregon white oak acorns that we germinated in November 2003 in a tray of moist vermiculite at 20 °C (68 °F). After the radicle had emerged from each acorn and reached a length of at least 2.5 cm (1 in), the acorn was randomly assigned to the pruned or the unpruned treatment. In the pruned treatment, the radicle was clipped 1 cm (0.4 in) from the acorn; the unpruned treatment was an undisturbed control. Both treatments were then sown in pots and grown until November 2004.

After 1 yr, 47 percent of the seedlings in the pruned treatment had multiple taproots, compared to 3 percent of the seedlings in the unpruned treatment. The number of taproots averaged 1.8 ± 1.0 (standard error) in the pruned treatment and 1.1 ± 0.5 in the unpruned treatment. The multiple taproots originated where the radicle had been clipped. The average combined length of all taproots per seedling was significantly greater in the pruned treatment (92 cm; 36 in) than in the unpruned (63 cm; 25 in).

Seedlings in the pruned treatment formed somewhat fewer taproots than other oak species, which averaged 3 to 4 taproots in response to similar radicle-pruning treatments (Barden and Bowersox 1989, McCreary 1996). Pruning the radicle had no significant effect on seedling shoot weight, root weight, stem diameter, or stem height after 1 yr. Similarly, shoot growth of other oak species has shown little response to radicle pruning (Bonner 1982, Barden and Bowersox 1989, McCreary 1996). On harsh sites where early root-soil contact influences survival, however, the potentially greater root surface area resulting from multiple taproots may increase establishment success.

Root Pruning

Undercutting oak seedlings in the nursery bed at a 15- to 20-cm (6- to 8-in) soil depth increases frequency of lateral roots, which is positively related to post-planting survival and growth (Schultz and Thompson 1996). We examined the effects of severing the taproot of containerized Oregon white oak seedlings to determine how root morphology was affected. We tested three treatments on 4-mo-old oak seedlings in March 2003: (1) seedling removed from potting medium, taproot severed at a 15-cm (5.9-in) depth, and seedling repotted, (2) seedling removed from potting medium and repotted, and (3) seedling undisturbed. Comparison of treatments 1 and 3 indicated the effect of taproot pruning and repotting, while comparison of treatments 2 and 3 isolated effects due to repotting alone.

Pruning taproots of 4-mo-old seedlings resulted in an average of 2.8 ± 1.2 taproots at a depth of 30 cm (12 in), compared with 1.1 ± 0.3 taproots in the undisturbed treatment, and 1.0 ± 0.3 taproots in the repotted treatment. In all pruned seedlings, the multiple taproots originated from the point at which the original taproot was cut. Average diameter of taproots at a 30-cm depth (pot bottom) varied with the number of taproots (figure 1), indicating that individual taproots were smaller as the number of taproots increased. Pruning the taproot had no effect on seedling total dry weight, shoot dry weight, shoot: root ratio, stem diameter, or stem height after 1 year. Pruning the taproot also did not affect the dry weight of lateral roots above or below the 15-cm depth. While the method of root pruning used in this trial would not be practical on a large scale, our results suggest that changes in morphology, but not dry weight, of Oregon white oak roots may result from treatments that sever the taproot, such as undercutting or pruning at the time of transplanting.

Air-Pruning

When seedling taproots are pot-bound, the pot-bound portion is typically pruned at planting. This results in the loss of both a substantial portion of taproot biomass and associated starch reserves and the lateral roots that originate from the pruned section. To prevent this taproot deformity, we began using air-pruning (Landis 1990) on our Oregon white oak seedlings and initiated a trial to document its effects on the species. We compared two container types: a Tall One Treepot™ and a Tall One Treepot™ with the base removed. In the air-pruned treatment (i.e., the containers without bases), containers were placed on galvanized hardware cloth [0.25-in (0.6-cm openings] with a layer of newspaper between the pot and the hardware cloth to retain the medium (McCreary 2001). The hardware cloth was suspended on a wood frame 2.5 cm (1.0 in) above the surface of the bench to create an air gap.

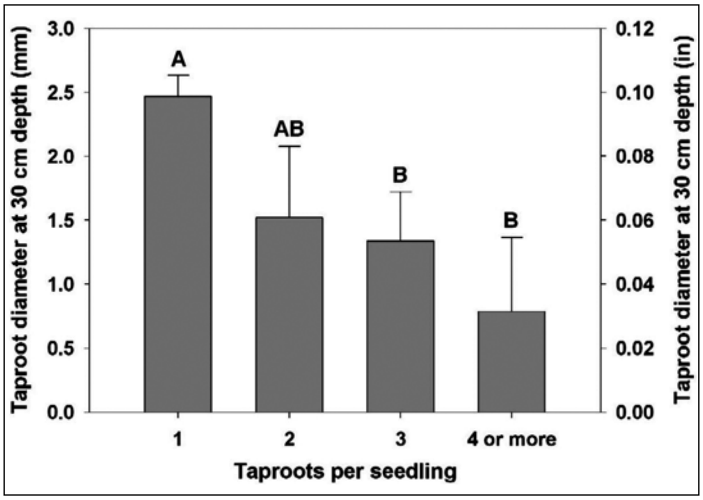

Figure 1. Average taproot diameter at a 30-cm (12-in) soil depth for Oregon white oak seedlings with various numbers of taproots. Bars with the same letter do not differ at the 95-percent confidence level.

After the first and second years of growth, seedling height and stem diameter were similar for seedlings in the intact pot and the air-pruned treatment. After the first year, total seedling dry weight and taproot weight did not differ significantly between treatments. Dry weight of lateral roots was 62 percent greater for air-pruned seedlings, however, indicating that air-pruning increased growth of these roots (figure 2). This lateral root growth did not come at the expense of taproot growth, as taproot weight did not differ between treatments. The average total weight of roots circling the pot base was greater in the intact pot (0.29 g; 0.0102 oz) than in the air-pruned treatment (0.01 g; 0.0003 oz) after 1 yr. After 2 yr, there was a visible concentration of lateral roots in the lower portion of the containers in both treatments (figure 3); however, these roots typically circled the pot base in the intact-pot treatment but were more likely to either terminate or grow horizontally for only a short distance in the air-pruned treatment.

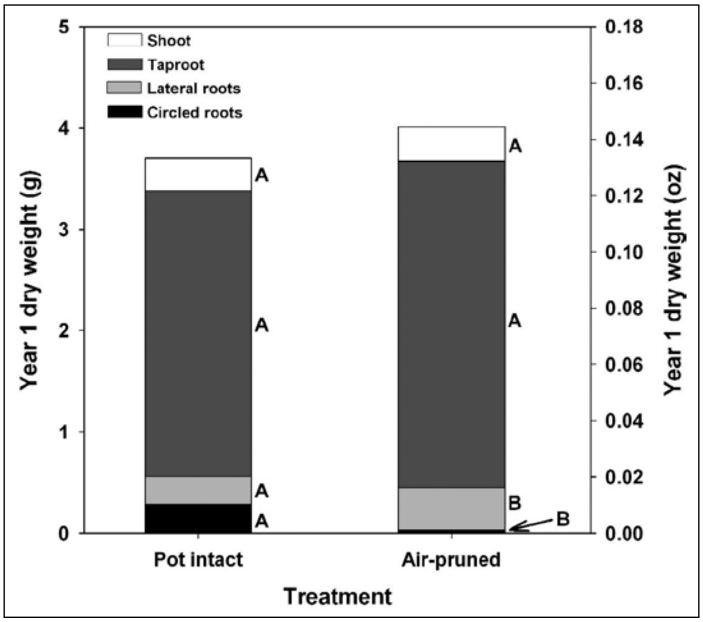

Figure 2. Dry weight after 1 yr, by component, of Oregon white oak seedlings grown in pots with the base intact or in pots with the base removed to promote air-pruning. Bars with the same letter and representing the same component do not differ at the 95-percent confidence level.

Figure 3. Two-yr-old Oregon white oak seedlings grown in intact pots (two seedlings on left) and in pots with the base removed to promote air-pruning (two seedlings on right). Note the difference in lateral root development. Photos by Diana Livada.

In an ongoing study in which Oregon white oak seedlings were transplanted from air-pruning D16 Deepot™ cells (Stuewe & Sons, Inc.) to Tall One Treepots™, 1-yr-old air-pruned transplants produced greater aboveground biomass growth and a more fibrous root system below the point of air-pruning (personal communication, Timothy Harrington, Forest Service, Olympia, WA). Similarly, in nursery stock of blue oak (*Quercus douglasii* Hook & Arn.) in California, seedlings started as an air-pruned plug had greater shoot height, root weight, and root system fibrosity at the time of planting than did 1+0 container seedlings or 1+0 bareroot stock (McCreary and Lippitt 2000).

Inoculation With Mycorrhizal Fungi

In December 2004, we started a 2-yr trial to determine whether growth of containerized Oregon white oak seedlings in fertilized potting medium was limited by a lack of mycorrhizal fungi. We used a factorial combination of inoculation (yes/no; abbreviated +IN/-IN) and fertilization (yes/no; abbreviated +FER/-FER).

The inoculum consisted of mineral soil taken from a local stand of Oregon white oak trees. The largest trees in the stand were approximately 80 yr old, and the stand had likely been prairie or savanna before their establishment. The soil was a gravelly sandy loam of the Spanaway series (Soil Survey Staff 2008). We used soil from a 5- to 15-cm (2- to 6-in) depth because density of fine oak roots was high in this interval. Gravel was screened out, and soil (including fine root fragments) was homogenized before inoculation. For the +IN treatment, we removed potting medium to a depth of approximately 6 cm (2 in) from the center of the filled pot and added 120 cm^3 (7.3 in^3) of the inoculum soil. We then added a 2-cm (0.8 in) layer of potting medium above the inoculum soil and planted the acorn in this medium, at a depth of 1 cm (0.4 in). Acorns in the -IN treatment were planted similarly but with no inoculum soil. This inoculation method was based on the assumption that seedlings would begin to form lateral roots (i.e., roots that are potentially mycorrhizal) while the inoculum was still viable; an alternative approach is to inoculate after lateral roots are present.

The +FER treatment received our standard NPK fertilizer; the -FER treatment received none. All treatments were irrigated equally. After 1 yr of growth, 50 percent of the seedlings in each treatment combination were randomly selected for destructive sampling.

After the first and second years of growth, we compared the -IN-FER and the +IN-FER treatments to determine whether the inoculum affected seedling growth in the absence of fertilization, possibly due to the nutrient content or water-holding capacity of the inoculum soil. There were no significant effects on seedling height, stem diameter, or first-year dry weight between the treatments (figure 4); thus, there was no evidence that the physical or chemical properties of the inoculum soil influenced seedling growth. In all analyses of seedling height and diameter (yr 1 and 2), seedling shoot and root components, and total dry weight, the interaction term for the inoculation and fertilization treatments was significant ($P<0.05$). This interaction can be summarized by three trends: (1) inoculation alone did not produce a growth response; (2) fertilization alone produced a negligible to intermediate growth response; (3) inoculation plus fertilization produced a growth response significantly greater than the other treatments. These growth results suggest that the soil inoculum facilitated the fertilization response. We infer that this was due to an absence of mycorrhizal fungi among seedlings that were grown in potting medium without inoculum soil. Conversely, we would expect a reduced or absent inoculation effect on growth of containerized seedlings planted in medium that had already been “contaminated” by native soil.

Following the second growing season, we identified the mycorrhizas present on the roots of four seedlings from each treatment combination, using a microscope and DNA analysis (see Frank and others 2006). On all of the seedlings examined in the +IN treatment group, we identified the presence of ectomycorrhizas associated with *Cenococcum geophilum*, a common and widespread

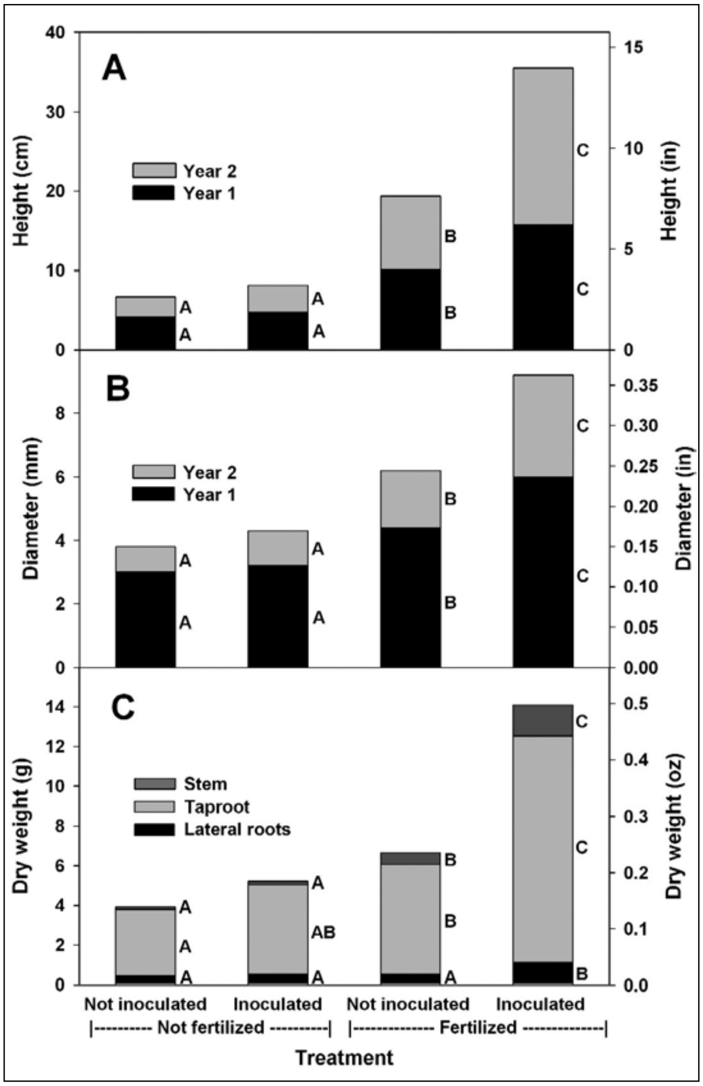

Figure 4. Two-year height (A) and stem diameter (B) responses and 1-yr dry weight growth response (C) for Oregon white oak seedlings treated or not treated with fertilization, mycorrhizal inoculation, or both. Bars with the same letter and representing the same year or seedling component do not differ at the 95-percent confidence level.

fungal species previously reported on Oregon white oak in southern Oregon (Valentine and others 2002, 2004). On all of the -FER seedlings, but not on the +FER seedlings, we observed the ectomycorrhizal *Hebeloma* sp. that has been reported previously in association with *Quercus* (Cairney and Chambers 1999). Only one of the four seedlings in the -IN+FER treatment was infected (ectomycorrhizal *Tomentella* sp.).

Mycorrhizal inoculation has been used in production of containerized seedlings for decades, but the mycorrhizal communities associated with Oregon white oak have only been studied in recent years (Valentine and others 2004, Moser and others 2005, Southworth and others 2009). Although research on inoculation with individual species of mycorrhizal fungi would be necessary to better understand their potential influences on Oregon white oak seedlings, we can report that *Cenococcum geophilum* was associated with seedlings that had a significantly improved response to fertilization, compared with seedlings that were not infected with this species. Although soil was used as inoculum in this trial, once the species of mycorrhizal fungi that are most beneficial for containerized Oregon white oak are identified, seedling inoculation might be achieved more efficiently using commercially available isolates, collected spores, or fungal cultures (Castellano and Molina 1989).

Implications

On the basis of information from other oak species, we assumed that the optimal root morphology for Oregon white oak seedlings is a combination of fibrous lateral roots and one or more straight taproots. Our trials suggest that air-pruning and ectomycorrhizal inoculation (if seedlings are grown in fertilized potting medium) are the most promising methods of improving growth and root morphology of containerized Oregon white oak seedlings. Our nontransplanted seedlings grown in tall (36 cm; 14 in) containers responded to air-pruning with increased lateral root growth and minimal circling of roots. Pruning new radicles or the taproot increased taproot branching but did not increase growth of lateral roots.

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REFERENCES

Barden, C.J.; Bowersox, T.W. 1989. The effect of root pruning treatments on red oak seedling root growth capacity. In: Rink, G.; Budelsky, C.A., tech coords. 7th Central Hardwood Forest Conference; 1989 March 5–8, Carbondale, IL. General Technical Report NC-132. St. Paul, MN: U.S. Department of Agriculture (USDA), Forest Service, North Central Forest Experiment Station: 115–119.

Bonner, F.T. 1982. The effect of damaged radicles of pre-sprouted red oak acorns on seedling production. Tree Planters' Notes. 33: 13–15.

Cairney, J.W.G.; Chambers, S.M., eds. 1999. Ectomycorrhizal fungi: key genera in profile. New York: Springer-Verlag. 370 p.

Castellano, M.A.; Molina, R. 1989. Mycorrhizae. In: Landis, T.D.; Tinus, R.W.; McDonald, S.E.; and Barnett, J.P., eds. The Container Tree Nursery Manual, Volume 5. Agricultural Handbook 674. Washington, DC: USDA Forest Service: 101–167.

Devine, W.D.; Harrington, C.A.; and Peter, D.H. 2007. Oak woodland restoration: understory response to removal of encroaching conifers. Ecological Restoration. 25: 247–255.

Dixon, R.K.; Garrett, H.E.; Cox, G.S.; Marx, D.H.; and Sander, I.L. 1984. Inoculation of three *Quercus* species with eleven isolates of ectomycorrhizal fungi. I. Inoculation success and seedling growth relationships. Forest Science. 30: 364–372.

Frank, J.L.; Barry, S.; and Southworth, D. 2006 Mammal mycophagy and dispersal of mycorrhizal inoculum in Oregon white oak woodlands. Northwest Science. 80: 264–273.

Grossnickle, S.C. 2005. Importance of root growth in overcoming planting stress. New Forests. 30: 273–294.

Landis, T.D. 1990. Containers: types and functions. In: Landis, T.D.; Tinus, R.W.; McDonald, S.E.; and Barnett, J.P., eds. The Container Tree Nursery Manual, Volume 2. Agriculture Handbook 674. Washington, DC: USDA Forest Service: 1–39.

McCreary, D.D. 1996. The effects of stock type and radicle pruning on blue oak morphology and field performance. Annals of Forest Science. 53: 641–648.

McCreary, D.D. 2001. Regenerating rangeland oaks in California. Oakland, CA: University of California Sierra Foothill Research and Extension Center. 62 p.

McCreary, D.D.; Lippitt, L. 2000. Blue oak mini-plug transplants: how they compare to standard bareroot and container stock. Native Plants Journal. 1: 84–89.

Mitchell, R.J.; Cox, G.S.; Dixon, R.K.; Garrett, H.E.; and Sander, I.L. 1984. Inoculation of three *Quercus* species with eleven isolates of ectomycorrhizal fungi. II. Foliar nutrient content and isolate effectiveness. Forest Science. 30: 563–572.

Moser, A.M.; Petersen, C.A.; D'Allura, J.A.; and Southworth, D. 2005. Comparison of ectomycorrhizas of *Quercus garryana* (Fagaceae) on serpentine and nonserpentine soils in southwestern Oregon. American Journal of Botany. 92: 224–230.

Owen, J.; Stoven, H. 2008. Searching for the perfect pot. Digger Magazine. March 2008: 40–45, 57.

SAS Institute. 2005. The SAS System: SAS OnlineDoc®, Version 9, HTML format [CD-ROM]. Cary, NC.

Schultz, R.C.; Thompson, J.R. 1996. Effect of density control and undercutting on root morphology of 1+0 bareroot hardwood seedlings: five-year field performance of root-graded stock in the central USA. New Forests. 13: 297–310.

Soil Survey Staff. 2006. Official Soil Series Descriptions [online]. Available from http://soils.usda.gov/technical/classification/osd/index.html [accessed 14 February 2008]. USDA-Natural Resources Conservation Service.

Southworth, D.; Carrington, E.M.; Frank, J.L.; Gould, P.J.; Harrington, C.A.; and Devine, W.D. 2009. Mycorrhizas on nursery and field seedlings of *Quercus garryana*. Mycorrhiza. 19: 149–158.

Tilki, F.; Alptekin, C.U. 2006. Germination and seedling growth of *Quercus vulcanica*: effects of stratification, desiccation, radicle pruning, and season of sowing. New Forests. 32: 243–251.

Valentine, L.L.; Fiedler, T.L.; Haney, S.R.; Berninghausen, H.K.; and Southworth, D. 2002. Biodiversity of mycorrhizas on Garry oak (*Quercus garryana*) in a southern Oregon savanna. In: Standiford, R.B.; McCreary, D.; and Purcell, K.L.; tech. coords. 5th Symposium on Oak Woodlands: Oaks in California's Changing Landscape; 2001 October 22–25; San Diego, CA. General Technical Report PSW-184. Albany, CA: USDA Forest Service, Pacific Southwest Research Station: 151–157.

Valentine, L.L.; Fiedler, T.L., Hart, A.N.; Petersen, C.A.; Berninghausen, H.K.; and Southworth, D. 2004. Diversity of ectomycorrhizas associated with *Quercus garryana* in southern Oregon. Canadian Journal of Botany. 82: 123–135.

Wilson, E.R.; Vitols, K.C.; and Park, A. 2007. Root characteristics and growth potential of container and bare-root seedlings of red oak (*Quercus rubra* L.) in Ontario, Canada. New Forests. 34: 163–176.

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Seed Diseases and Seedborne Pathogens of North America

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Abstract

Seedborne pathogenic fungi can greatly affect seed quality and cause diseases that impact seedling production in nurseries. Management strategies for the control of various seedborne diseases are based on the epidemiology of the diseases and the biology of the host and pathogen. This paper provides a brief review of seedborne fungal problems that affect conifer seeds and discusses established and potential control practices.

Introduction

Forest-tree seed diseases and diseases related to seedborne pathogens are primarily caused by fungi. Numerous species of fungi are associated with forest tree seeds (Anderson 1986a, Mittal and others 1990), but many are saprophytes that do not adversely affect the performance of seeds sown in nurseries (Mittal and Wang 1987). Losses to seedborne pathogens include reduced seed germination, increased damping-off, and mortality of older seedlings in nursery beds. The effect of seedborne pathogens on seed and seedling production can go unnoticed until extreme germination failures have occurred in seedbeds (Fisher 1941, Epners 1964) or losses have occurred in containers (Campbell and Landis 1990). The extent to which seedborne pathogens cause losses in nursery beds is often difficult to separate from other causes of poor germination, such as damping-off by soilborne fungi. Knowledge of the biology of seedborne pathogens and practices for their management and control can help seed orchard and nursery managers reduce seed and seedling losses.

Types of Seedborne Pathogens

Pathogenic fungi can infect seeds internally and destroy the endosperm and the embryo or contaminate the seeds and affect seedling germination and development. In this paper, seedborne pathogens are defined as any infectious agent carried on the seeds, internally or externally, that has the potential to cause disease in either seeds or the developing plants. A current list of seedborne pathogens of relative importance to forest orchards and nurseries in North America is provided in table 1.

Certain seedborne pathogens primarily cause disease of seeds and have minor effects on other developmental stages of trees. Examples include diseases caused by *Lasiodiplodia theobromae* (Fraedrich and others 1994) and *Caloscypha fulgens* (Epners 1964). *Lasiodiplodia theobromae* is responsible for "black seed rot," which causes destruction of slash pine (*Pinus elliottii var. elliottii*) seeds in the Southern United States (Miller and Bramlett 1979). This fungus also causes shoot dieback of slash and loblolly (*P. taeda*) pine seedlings in nurseries in Georgia and Florida (Rowan 1982), but the shoot dieback has limited effects on production, and it is not certain if seedborne inoculum is a major factor in the disease. *Caloscypha fulgens* causes a seed rot in pine, spruce (*Picea* spp.), and fir (*Abies* spp.) seedlots in Canada and the Northern United States (Sutherland and others 1987). This fungus is particularly important because it can spread from diseased to healthy seeds during stratification and after seeds are sown in nursery beds during cool, moist conditions (Salt 1974).

Other seedborne pathogens can also be responsible for diseases that affect other developmental stages of plants, such as damping-off, shoot dieback, and cankers. Included in this category are *Sirococcus conigenus, Diplodia pinea*, and several *Fusarium* spp.

Sirococcus conigenus, found primarily in the northern latitudes of North America, causes a shoot blight that affects numerous conifer species, including pines, firs, spruces, and hemlock (*Tsuga* spp.) (Sutherland and others 1987). In container nurseries, this pathogen causes diseases of spruce seedlings, with seedborne inoculum thought to be the primary source of infection (Sutherland and others 1981).

Diplodia pinea causes shoot blight and cankers that are devastating to many pines (Sinclair and Lyon 2005). This

Table 1. Seedborne pathogens of North American forest tree species and references.

Pathogen	Host(s)	Disease	Reference
Caloscypha fulgens[1] (Pers.) Boudier	*Abies grandis* [(Dougl.) Lindl.], *Pseudotsuga menziesii* [(Mirb.) Franco], *Picea glauca* [(Moench) Voss], *Picea engelmannii* (Parry), *Picea stichensis* [(Bong.) Carr.], *Pinus contorta* (Dougl.), *Pinus resinosa* (Ait.), *Pinus. sylvestris* (L.), *Pinus strobes* (L.), *Tsuga heterophylla* [(Raf.) Sarg.]	Seed disease	Epners 1964; Salt 1970, 1974; Sutherland 1979
Fusarium spp.	Conifers	Seed disease, cotyledon blight, damping-off	Fisher 1941, Pawuk 1978, James and others 1989, Axelrood and others 1995
Fusarium circinatum Nirenberg and O'Donell (syn. *F. subglutinans* f.sp. *pini)*	*Pinus elliottii* (Engelm.) *var. elliottii, Pinus taeda* (L.), *Pinus palustris* (Mill.), *Pinus radiata* (D. Don)	Seed disease, damping-off, shoot dieback, cankers	Miller and Bramlett 1979, Barrows-Broaddus and Dwinell 1985, Runion and Bruck 1988, Storer and others 1998
Fusarium oxysporum (Schlecht.)	*Pseudotsuga menziesii, Pinus palustris*	Root rot, seed disease, damping-off	Pawuk 1978, Graham and Linderman 1983, Axelrood and others 1995
Fusarium moniliforme var. *moniliforme* (Sheld.)	*Pinus elliottii* var. *elliottii, Pinus taeda, Pseudotsuga menziesii*	Seed disease and damping-off	Mason and Van Arsdel 1978, Huang and Kuhlman 1990, Axelrood and others 1995
Fusarium proliferatum (Matsushima) Nirenberg	*Pinus elliottii* var. *elliottii*	Damping-off	Huang and Kuhlman 1990
Lasiodiplodia theobromae (Pat.) Griff. & Maubl. (syn. *Diplodia gossypina)*	*Pinus elliottii* var. *elliottii*	Seed disease	Miller and Bramlett 1979
Sirococcus conigenus (DC.) P. Cannon & Minter, (syn. *S. strobilinus)*	*Picea sitchensis, P. glauca, P. engelmannii*	Seed disease and top dieback	Sutherland and others 1981
Diplodia pinea (Desmax.) J. Kickx fil., (syn. *Sphaeropsis sapinea)*	*Pinus elliottii* var. *elliottii*	Associated with seed damage	Fraedrich and others 1994
Trichothecium roseum [Link]	*Picea glauca*	Damping-off	Mittal and Wang 1993

[1] *Caloscypha fulgens* is the perfect state of *Geniculodendron pyriforme*.

pathogen is associated with diseased seeds of slash pine (Fraedrich and Miller 1995) and loblolly pine (Fraedrich, unpublished data) and is also a seed disease of some Central American pine species (Rees and Webber 1988). In addition, *D. pinea* has been reported to infect seeds of *P. rigida* Mill. and *P. albicaulis* Engelm. at the Montreal Botanical Gardens, a location outside the natural range of both pine species (Vujanovic and others 2000). *Diplodia pinea* is a periodic problem in some northern nurseries, but inoculum from sources other than seeds is considered more important (Palmer and others 1988). Nonetheless, the association of *D. pinea* with seeds provides a means by which this pathogen may become established in new locations.

Fusarium spp. are widespread in their distribution, and many are associated with seeds of conifer species (Anderson 1986a, Mittal and others 1990). *Fusarium circinatum* (syn. *F. subglutinans* f. sp. *pini*), the pitch canker fungus, is a highly virulent pathogen that can infect reproductive and vegetative stages of many pine species (Dwinell and others 1985). The pitch canker fungus has long been known to be a seedborne pathogen in the Southern United States and, since the late 1980s, as a seedborne contaminant of Monterey pine (*P. radiata*) in California (Dwinell and Fraedrich 2000). The potential transport of this pathogen via infested seedlots is a serious concern nationally and internationally.

Other species of *Fusarium* that can cause seedborne diseases include *F. oxysporum*, *F. moniliforme*, and *F. proliferatum*. The pathogenicity of isolates within these species ranges from highly virulent to nonpathogenic; therefore, the level of contamination by a *Fusarium* sp. does not always correspond to development of seedborne diseases (Pawuk 1978, Graham and Linderman 1983, Axelrood and others 1995). In several studies, damping-off caused by pathogenic isolates of *F. oxysporum*, *F. moniliforme,* and *F. proliferatum* has been shown to increase greatly following heat stress (Huang and Kuhlman 1990, Axelrood and others 1995).

The presence of certain fungi on seeds is often significant because it may indicate problems with the quality of the seedlot due to improper handling and storage of both cones and seeds. Seedborne fungi such as *Aspergillus* spp., *Mucor* spp., *Penicillium* spp., *Rhizopus* spp., and *Trichoderma* spp. have reduced germination of conifers in some laboratory tests (Fisher 1941, Gibson 1957, Mittal and Wang 1993). However, these fungi were associated with seeds that were damaged (Gibson 1957), of low vigor (Mittal and Wang 1993), or grown in an environment that favors fungal over seedling growth (Fisher 1941). Agarwal and Sinclair (1997) regard many of these fungi as "storage fungi" that may be involved in deterioration of seeds during storage.

Detection of Fungus-Damaged Seeds and Determination of Pathogens Associated With Seeds

The detection of seedborne pathogenic fungi and seed diseases is an important aspect of disease management. Determining the presence of seedborne pathogens allows managers to apply the appropriate controls or modify management practices to avoid the problem in the future.

The presence of diseased seeds in seedlots cannot be reliably detected by visual examination. Radiographic assays of seeds (figure 1) provide an efficient, nondestructive method to determine internal seed damage (Karrfalt 1983). Internal seed contents can be examined by cutting the seed open (figure 2) and looking for mycelium or symptoms of disease (Sutherland and others 1987).

Seedborne pathogens can also be present on seeds without obvious disease symptoms or signs. The presence of pathogenic fungi on seeds is most often determined through laboratory culture and identification. Samples of seeds are placed on various media and the fungi that grow from the seeds are evaluated (Anderson 1986b). Although this technique is widely employed, it is time consuming and may not detect pathogens at low levels. Competitive saprophytic fungi on seeds are an additional problem because they can obscure the presence of a pathogen. For some fungal species, such as *F. oxysporum*, evaluation of isolates from seeds on living seedlings is necessary to determine pathogenicity (Littke 1997).

In recent years, research has been developing more sensitive and less time-consuming techniques to detect pathogens in seedlots. An immunological assay (ELISA test) has been developed to detect *S. conigenus* in spruce seedlots (Mitchell and Sutherland 1986). This type of test is highly specific, less time consuming, and affords greater accuracy because sample size can be greatly increased (Sutherland and others 1987). The development of assays to detect other seedborne pathogens such as *F. circinatum* and *D. pinea* could prove very beneficial. These techniques could be especially useful in seed certification programs where seeds are to be shipped internationally or used in areas outside the known range of specific pathogens.

Pathogen Establishment and Disease Development

Many factors are related to the establishment of pathogens on and inside seeds and the development of seed diseases. Contamination and infection of seeds by pathogens can occur in all phases of seed production. For many diseases,

Figure 1. Radiographs of seeds can be a useful tool for the detection of internal seed problems, including infection by pathogenic fungi (Photo courtesy of Thomas Miller).

Figure 2. The mycelial growth of seedborne pathogens is often readily detected in seeds with internal infections (photo by Stephen Fraedrich).

however, the relationships between pathogens and seed production are not well understood.

Cone collection practices influence seedborne diseases caused by *L. theobromae* on slash pine (Fraedrich and others 1994) and *C. fulgens* on spruce seeds (Sutherland 1979). These fungi become established on cones when they have been in contact with the ground for extended periods. *Caloscypha fulgens* spreads among seeds in storage or in the field under cold wet conditions (Sutherland and others 1987). Disease development caused by *L. theobromae* is also strongly affected by the degree of cone maturation when cones are shaken from trees. Cones that are collected prematurely (specific gravity >0.89) have a higher incidence of disease than more mature cones with lower specific gravities (Fraedrich and others 1994).

Sirococcus conigenus becomes established in seedlots when older cones are inadvertently included in the cone harvest (Sutherland and others 1981). This seedborne pathogen spreads to seeds before germination and can result in post-germination infection. The conditions that favor this pathogen include high humidity, low light, and cool temperatures ranging from 10 to 20 °C (Sutherland and others 1987).

Several factors have been linked to the development of *Fusarium*-related diseases of seeds and seedlings of conifers; however, our understanding of the epidemiology of these diseases is limited (Kuhlman and others 1982). The method by which *Fusarium* spp. becomes established internally in seeds is still uncertain. Inoculations of strobili with *F. circinatum* during pollination failed to demonstrate that seeds become infected at the time of pollen receptivity (Miller and others 1987). Assessments of seedlots for *F. circinatum* suggest that this pathogen is more likely to be associated with seeds of longleaf pine (*P. palustris*) produced in intensively managed seed orchards than in unmanaged seed production areas (Fraedrich, unpublished data). The use of fertilizers has been suggested as a factor involved in the greater occurrence of *F. circinatum* in orchard seeds. Fertilization has been linked with an increase in pitch canker on slash, loblolly, and Virginia (*P. virginiana* Mill.) pines (Wilkinson and others 1977, Fraedrich and Witcher 1982); however, a direct link between fertilization, *F. circinatum* infection, and seed contamination has not been reported.

Fresh wounds provide infection courts for *F. circinatum* (Miller and Bramlett 1979, Barrows-Broaddus 1990). Various agents can wound reproductive structures, including insects, storm damage, and cone handling (Dwinell and others 1985). Insects can also vector *F. circinatum* (Hoover and others 1996) and have been associated with seed deterioration (Bramlett and others 1977). The levels of seed contamination by *Fusarium* spp. varies by collection date and by orchard (Fraedrich and Miller 1995, Littke 1997). Contamination can also vary by tree clone or family (Kelley and Williams 1982, Rockwood and others 1988, Carey and others 2005), but information is lacking on the regulation of susceptibility to *Fusarium*-related seed and seedling diseases through genetic improvements.

Management and Control of Diseases Caused by Seed and Seedborne Diseases

Strategies for management of seed disease and seedborne pathogens focus on prevention of disease and contamination or on remedial procedures to reduce contamination. The type of problem and the causal agent determine the applicability of various pest management approaches. Generally, no single method will provide complete control of any specific seedborne disease; control is best achieved through an integrated pest management approach (Agarwal and Sinclair 1997). For some seedborne problems, little information is available on the biology of the pathogen and epidemiology of the diseases that they cause. Thus, recommendations for disease prevention and control may not always be readily available.

Cone Collection and Management. Some seed and seedborne diseases are linked to cone collection practices, and modification of practices can help to prevent disease losses. *Sirococcus conigenus* is a problem on spruce seeds only when old cones are included in fresh cone collections (Sutherland and others 1981). The simplest method of controlling this pathogen is to avoid collecting old cones. Yearly collection of spruce cones has probably helped keep seedlots free of *S. conigenus* in Canada (Sutherland and others 1987).

In western North America, *C. fulgens* has been found commonly in seeds from cones collected from the ground and from squirrel caches (Sutherland 1979). In the Southern United States, infection of slash pine seeds by *L. theobromae* is also most prevalent in cones that are collected from the ground (Fraedrich and others 1994). Collecting cones directly from trees eliminates *C. fulgens* from seedlots (Sutherland and Woods 1978) and significantly reduces *L. theobromae* from slash pine seeds (Fraedrich and others

1994). Since slash pine seeds are usually collected by shaking trees and collecting the cones from the ground, managers can reduce *L. theobromae* in the seeds by collecting cones with a specific gravity of less than 0.89. Variation in cone maturation among individual pine clones or families is an important consideration when establishing the appropriate times to collect cones (Zoerb 1969, Fraedrich and others 1994). Seed viability can also decrease when cones are collected in advance of cone maturity, or when cones are stored at incorrect temperatures or seed moisture contents (Barnett 1997). Collection times can be extended for a few weeks for some species, such as loblolly pine. However, seed viability in species such as longleaf pine decreases during cone storage when cones are collected at a specific gravity <0.80; therefore, cones should be collected only when mature and stored for no more than 4 to 5 wk (Barnett 1997).

The effect of cone collection and management practices on the establishment of *Fusarium* spp. with conifer seeds is somewhat less clear than with other fungi such as *C. fulgens* and *L. theobromae*. The association of *F. circinatum* with seeds and cones does vary somewhat among collection times and clones within an orchard (Kuhlman and others 1982, Dwinell and Fraedrich 1997, Carey and others 2005), but the expression of the disease is associated with wounding and has been linked to high levels of fertilization. Therefore, disease incidence is not necessarily correlated with resistance.

Many managers protect pine reproductive structures in orchards from insects with regularly scheduled insecticide sprays, thus minimizing wounding and the presence of possible vectors of *F. circinatum*. Managers can also try to limit wounding due to mechanical damage. When an outbreak of pitch canker does occur in an orchard, managers may be able to reduce seed infestation and loss by avoiding heavily infested trees during cone collection. Carey and others (2005) found a correlation among pitch canker ratings of longleaf pine clones, the percentage of seed infested, and seedling mortality. They concluded that clones exhibiting a high incidence of pitch canker should be removed from seed collections.

There is some evidence that *Fusarium* levels on seeds increase with cone storage (Fraedrich and Miller 1995) and that *Fusarium* spp. infest seeds mostly after seed processing (Littke 1997). Littke (1997) reported that faster cone-drying schedules reduced *Fusarium* levels on seeds of Douglas-fir [*Pseudotsuga menziesii* (Mirb.) Franco] following extraction. Faster cone drying may be a benefit when storage is not needed for seeds to mature. Fortunately, when *Fusarium* contaminates seed, more than 90 percent is on the surface and can be controlled by seed treatments (James 1986, Dwinell and Fraedrich 1997, Littke 1997).

Seed Treatments. Various types of seed treatments can control seedborne pathogens. These treatments may include chemical, physical, and mechanical control. Seed treatments have the potential to damage seeds; therefore, seed treatments should be used only when the gain in germination and seedling survival is greater than the potential loss. Chemical treatments, in particular, can be toxic to seeds and should be used with caution (Vaartaja 1964, Runion and others 1991).

Chemical Seed Treatments. Seed treatments currently used in the United States for control of seedborne pathogens are thiram and seed disinfectants. Thiram (tetramethylthiuram disulfide) is commonly used in nurseries as a bird and animal repellent, as well as a fungicide. In particular, thiram has been effective against *F. oxysporum* on ponderosa pine and Douglas-fir seeds (Littke 1997), and against *Fusarium* spp. on longleaf pine seeds (Barnett and Varela 2003). Thiram can have a toxic effect on seeds of many conifer species (Belcher and Carlson 1968, Dobbs 1971, Runion and others 1991), especially at high dosages (Bloomberg and Trelawny 1970). Its benefit as a bird/animal repellent and fungicide, however, often outweighs the relatively small toxic effect on germination under operational conditions (Abbott 1958, Nolte and Barnett 2000). Other fungicides not specifically labeled for use on tree seeds have been tested with some positive results, but so far have not provided significantly better control of fungal infestation or germination than thiram or the disinfectants (Barnett and others 1999, Barnett and McGilvray 2002, Barnett and Varela 2003, Allen and others 2004).

Disinfectants such as sodium hypochlorite (i.e., the active ingredient in bleach), hydrogen peroxide, and hydrogen dioxide (Zero Tol®) can be used to reduce fungal contamination and improve seed germination. Hydrogen peroxide has long been known to eliminate seedborne mycoflora and to stimulate seed germination (Trappe 1961). A 30-percent concentration of hydrogen peroxide can be effective for increasing germination of conifer seeds (Riffle and Springfield 1968, Barnett 1976, Barnett and McGilvray 2002) and can virtually eliminate seedcoat contamination of longleaf pine seeds by *F. circinatum* and other *Fusarium* spp. (Fraedrich 1997). Concentrations of 3-percent hydrogen peroxide have also been effective for reducing seedborne contamination of conifers by various *Fusarium* spp. (Dumroese and others 1988, Ocamb 1995, Littke 1997, Hoefnagels and Linderman 1999). Poststratification treatments with 3-percent hydrogen peroxide are particularly effective for eliminating seedborne inoculum and maintaining high germination in Douglas-fir seedlots (Dumroese and others 1988).

Prestratification seed treatment with 2-percent sodium hypochlorite also reduces *Fusarium* contamination of Douglas-fir seeds (Dumroese and others 1988) and can increase germination in several Western conifers (Wenny and Dumroese 1987). Pretreatment of seeds briefly with ethyl alcohol can increase the efficacy of sodium hypochlorite in some agricultural crops (Sauer and Burroughs 1986), and this practice may have applications for seeds of some conifers. Hydrogen dioxide (Zero Tol®) is a surface sterilant that is registered as a fungicide for tree seeds. Application of hydrogen dioxide on longleaf pine seed has been found to reduce *Fusarium* contamination without inhibiting germination (Allen and others 2004).

Physical Seed Treatments. Water rinses over 24 to 48 h can be used to reduce seed pathogens and improve germination (Riffle and Springfield 1968, Wenny and Dumroese 1987, Littke 1997). Stratification can increase the presence of fungi in some seedlots and a running water (2 h) imbibition treatment can decrease surface contamination (Axelrood and others 1995). However, water rinses will not eliminate contaminating fungi from seeds (Riffle and Springfield 1968, Littke 1997). Seedlots suspected of being contaminated with a seedborne pathogen should also be treated with a disinfectant or fungicide (Campbell and Landis 1990).

Heat treatments have been used to control certain seedborne pathogens without affecting seed viability. Heat treatments include hot water, aerated steam, and microwave radiation. Heat treatments have been used on seeds of agricultural crops in numerous studies (Agarwal and Sinclair 1997). Microwave hot-water treatments have controlled *Fusarium* spp. on Douglas-fir seeds without significantly affecting germination (James and others 1988). Heat treatments may have potential use as a seed treatment for other conifer seeds but will require additional research.

Mechanical Seed Treatments. Various mechanical methods have been used to remove dead and fungus-damaged seeds from healthy, viable seeds in order to increase germination of seedlots and reduce inoculum of seedborne pathogens. Specific gravity tables can be used to separate fungus-damaged seeds from seedlots (Karrfalt 1983). This system has been used by several organizations with good results. The proper calibration of the specific gravity table for individual seedlots is important to minimize the loss of good seeds while rejecting fungus-damaged seeds. The IDS (Incubation, Drying, Separation) system is another procedure for separating viable from filled-dead seeds (Simak 1984, Karrfalt 1997). The procedure is based on the differential drying of viable and filled dead seeds, and the resulting separation of these seeds according to their differences in weight and density. The IDS system has been used successfully to remove damaged seed from seedlots of various conifers (Donald 1985, Downie and Wang 1992, McRae and others 1994) and *Plantanus* x *acerifolia* (Ait.) Willd. (Falleri and Pacella 1997).

Summary

Compared to seedborne disease problems of agricultural crops, research on seedborne pathogens that affect production of forest-tree species has been very limited in North America. One possible reason is that diseases from seedborne pathogens often go undiagnosed under operational conditions. There are many causes of poor germination, and determining if a seedborne pathogen is a factor can be difficult and time consuming. Testing seed germination is often a first step in determining if a seedborne pathogen is a problem. Confirming the presence of a pathogen usually requires the services of a pathologist.

Some seedborne pathogens and diseases can be avoided by modifications in cone collection practices. Treating seeds with disinfectants and thiram can reduce seedcoat contamination by pathogens and increase germination. Mechanical separation techniques can remove diseased seeds and improve seedlot quality. In some cases, diseases caused by seedborne pathogens are a constant problem, and seed treatments are routinely used by managers. Seed efficiency could be increased through further research on avoiding pathogen establishment and preventing disease development, as well as developing more effective seed treatments.

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REFERENCES

Abbott, H.G. 1958. Application of avian repellents to eastern white pine seed. Journal of Wildlife Management. 22: 304–306.

Agarwal, V.K.; Sinclair, J.B. 1997. Principles of Seed Pathology. 2nd ed. Boca Raton, FL: CRC Press, Inc. 539 p.

Allen, T.W.; Enebak, S.A.; and Carey, W.A. 2004. Evaluation of fungicides for control of species of *Fusarium* on longleaf pine seed. Crop Protection. 23: 979–982.

Anderson, R.L. 1986a. Check list of microorganisms associated with tree seeds in the world, 1985. General Technical Report SE-39. Asheville, NC: U.S. Department of Agriculture (USDA), Forest Service, Southeastern Forest Experiment Station.

Anderson, R.L. 1986b. New method for assessing contamination of slash and loblolly pine seeds by *Fusarium moniliforme* var. *subglutinans*. Plant Disease. 70: 452–453.

Axelrood, P.E.; Neumann, M.; Trotter, D.; Radley, R.; Shrimpton, G.; and Dennis, J. 1995. Seedborne *Fusarium* on Douglas-fir: Pathogenicity and seed stratification method to decrease *Fusarium* contamination. New Forests. 9: 35–51.

Barnett, J.P. 1976. Sterilizing southern pine seeds with hydrogen peroxide. Tree Planters' Notes. 27: 17-24.

Barnett, J.P. 1997. Longleaf pine seed quality: can it be improved? In: Landis, T.D.; South, D.B., eds. National Forest and Conservation Nursery Associations; 1996 June 25–27; Gatlinburg, TN. General Technical Report PNW-389. Portland, OR: USDA Forest Service, Pacific Northwest Research Station: 183–186.

Barnett, J.P.; McGilvray, J.M. 2002. Improving longleaf pine seedling production by controlling seed and seedling pathogens. In: Outcalt, K.W., editor. 11th Biennial Southern Silvicultural Research Conference; 2002 March 20–22; Knoxville, TN. General Technical Report SRS-48. Asheville, NC: USDA Forest Service, Southern Research Station: 45–46.

Barnett, J.P.; Pickens, B.; and Karrfalt, R. 1999. Longleaf pine seed presowing treatments: effects on germination and nursery establishment. In: Landis, T.D.; Barnett, J.P., eds. National Forest and Conservation Nursery Associations; 1998 July 13–17; Lafayette, LA. General Technical Report SRS-25. Asheville, NC: USDA Forest Service, Southern Research Station: 43–46.

Barnett, J.P.; Varela, S. 2003. Producing high-quality slash pine seeds. In: Riley, L.E.; Dumroese, R.K.; and Landis, T.D., eds. National Forest and Conservation Nursery Associations; 2002 July 15–18; Gainesville, FL. General Technical Report RMRS-28. Ogden, UT: USDA Forest Service, Pacific Northwest Research Station: 52–56.

Barrows-Broaddus, J. 1990. Colonization of cones and seed of loblolly pine following inoculation with *Fusarium subglutinans*. Plant Disease. 74: 1002–1005.

Barrows-Broaddus, J.; Dwinell, L.D. 1985. Branch dieback and cone and seed infection caused by *Fusarium moniliforme* var. *subglutinans* in loblolly pine seed orchard in South Carolina. Phytopathology. 75: 1104–1108.

Belcher, J.; Carlson, L.W. 1968. Seed-treatment fungicides for control of conifer damping-off: laboratory and greenhouse tests, 1967. Canadian Plant Disease Survey. 48(2):87–52.

Bloomberg, W.J.; Trelawny, J. 1970. Effect of thiram on germination of Douglas-fir seed. Phytopathology. 60: 1,111–1,116.

Bramlett, D.L.; Belcher, E.W.; Debarr, G.L.; Hertel, G.D.; Karrfalt, R.P.; Lantz, C.W.; Miller, T.; Ware, K.D.; and Yates, H.O. 1977. Cone analysis of southern pines. General Technical Report SE-13. Asheville, NC: USDA Forest Service, Southeast Forest Experiment Station and Southeastern Area State Private Forestry. 32 p.

Carey, W.A.; Oak, S.W.; and Enebak, S.A. 2005. Pitch canker ratings of longleaf pine clones correlate with *Fusarium circinatium* infestation of seeds and seedling mortality in containers. Forest Pathology. 35: 205–212.

Campbell, S.J.; Landis, T.D. 1990. Managing seedborne diseases in western forest nurseries. Tree Planters' Notes. 41(4): 3–7.

Dobbs, R.C. 1971. Effect of thiram-endrin formulations on the germination of jack pine and white spruce seed in the laboratory. Tree Planters' Notes. 22: 16–18.

Donald, D.G.M. 1985. The separation of full dead seed from live seed in *Pinus elliottii*. In: South, D.B., editor. International Symposium on Nursery Management Practices for the Southern Pines; 1985 August 4–9; Montgomery, AL. Auburn University, Department of Research Information: 83–88.

Downie, B.; Wang, B.S.P. 1992. Upgrading germinability and vigor of jack pine, lodgepole pine, and white spruce by the IDS technique. Canadian Journal of Forest Research. 22: 1,124–1,131.

Dumroese, R.K.; James, R.L.; Wenny, D.L.; and Gilligan, C.J. 1988. Douglas-fir seed treatments: Effects on seed germination and seedborne organisms. In: Landis, T.D., editor. Western Forest Nursery Council, Intermountain Nursery Association, and Forest Nursery Association of British Columbia; 1988 August 8–11; Vernon, British Columbia. General Technical Report RM-167. Fort Collins, CO: USDA Forest Service, Rocky Mountain Forest and Range Experiment Station: 155–160.

Dwinell, L.D.; Fraedrich, S.W. 1997. Recovery of *Fusarium subglutinans* f.sp. *pini* from shortleaf pine cones and seeds. In: James, R.L., editor. 3rd meeting of the IUFRO Working Party S7.03-04 (Disease and Insects in Forest Nurseries); 1996 May 19–24; Orlando-Gainesville, FL. Report 97–4. USDA Forest Service, Northern Region, Forest Health Protection. 42–47.

Dwinell, L.D.; Fraedrich, S.W. 2000. Contamination of pine seeds by *Fusarium circinatum.* In: Lilja, A.; Sutherland, J.R., eds. Fourth Meeting of the IUFRO Working Party 7.03.04 (Diseases and Insects in Forest Nurseries); 1999 July 25–28; Helsinki, Finland. Research Paper 781; Vantaa, Finland, Finnish Forestry Research Institute: 75–82.

Dwinell, L.D.; Barrows-Broaddus, J.; and Kuhlman, E.G. 1985. Pitch canker: a disease complex. Plant Disease. 69: 270–276.

Epners, Z. 1964. A new psychrophilic fungus causing germination failure of conifer seeds. Canadian Journal of Botany. 42: 1,589–1,604.

Falleri, E.; Pacella, R. 1997. Applying the IDS method to remove empty seeds in *Platanus* x *acerifolia*. Canadian Journal of Forest Research. 27: 1,311–1,315.

Fisher, P.L. 1941. Germination reduction and radical decay of conifers caused by certain fungi. Journal of Agricultural Research. 62: 87–95.

Fraedrich, B.R.; Witcher, W. 1982. Influence of fertilization on pitch canker development on three southern pine species. Plant Disease. 66: 938–940.

Fraedrich, S.W. 1997. Seedborne diseases of southern pines and developing strategies for their control. In: Landis, TD, South DB, eds. National Forest and Conservation Nursery Associations; 1996 June 25-27; Gatlinburg, TN. General Technical Report PNW-389. Portland, OR: USDA Forest Service, Pacific Northwest Research Station: 75–81.

Fraedrich, S.W.; Miller, T. 1995. Mycoflora associated with slash pine seeds from cones collected at seed orchards and cone processing facilities in the southeastern USA. European Journal of Forest Pathology. 25: 73–82.

Fraedrich, S.W.; Miller, T.; and Zarnoch, S.J. 1994. Factors affecting the incidence of black seed rot in slash pine. Canadian Journal of Forest Research. 24: 1,717–1,725.

Gibson, I.A.S. 1957. Saprophytic fungi as destroyers of germinating pine seeds. East African Agricultural Journal. 22: 203–206.

Graham, J.H.; Linderman, R.G. 1983. Pathogenic seedborne *Fusarium oxysporum* from Douglas fir. Plant Disease. 67: 323–325.

Hoefnagels, M.H.; Linderman, R.G. 1999. Biological suppression of seedborne *Fusarium* spp. during cold stratification of Douglas fir seeds. Plant Disease. 83: 845–852.

Hoover, K.; Wood, D.L.; Storer, A.J.; Fox, J.W.; and Bros, W.E. 1996. Transmission of the pitch canker fungus, *Fusarium subglutinans* f. sp. *pini*, to Monterey pine, *Pinus radiata*, by cone- and twig-infesting beetles. Canadian Entomologist. 128: 981–994.

Huang, J.W.; Kuhlman, E.G. 1990. Fungi associated with damping-off of slash pine seedlings in Georgia. Plant Disease. 74: 27–30.

James, R.L. 1986. Occurrence of *Fusarium* on conifer tree seed from northern rocky mountain nurseries. In: Landis, T.D., ed. Western Forest Nursery Council and Intermountain Nursery Association; 1986 Aug 12–15; Tumwater, WA. General Technical Report RM-137. Fort Collins, CO: USDA Forest Service, Rocky Mountain Forest Experiment Station: 109–114.

James, R.L.; Dumroese, R.K.; Gilligan, C.J.; and Wenny, D.L. 1989. Pathogenicity of *Fusarium* isolates from Douglas-fir seed and container-grown seedlings. University of Idaho, College of Forestry, Wildlife and Range Sciences, Idaho Forest, Wildlife and Range Experiment Station. Station Bulletin 52. 10 p.

James, R.L.; Gilligan, C.J.; Dumroese, R.K.; and Wenny, D.L. 1988. Microwave treatments to eradicate seedborne fungi on Douglas-fir seed. USDA Forest Service, Forest Pest Management, Report 88–7. 8 p.

Karrfalt, R.P. 1983. Fungus-damaged seeds can be removed from slash pine seedlots. Tree Planters' Notes. 34: 38–40.

Karrfalt, R.P. 1997. Upgrading seeds with IDS: a review of successes and failures. In: Landis, T.D.; South, D.B., eds. National Forest and Conservation Nursery Associations; 1996 June 25–27; Gatlinburg, TN. General Technical Report PNW-389. Portland, OR: USDA Forest Service, Pacific Northwest Research Station: 183–186.

Kelley, W.D.; Williams, J.C. 1982. Incidence of pitch canker among clones of loblolly pine in seed orchards. Plant Diseases. 66: 1,171–1,173.

Kuhlman, E.G.; Dianis, S.D.; and Smith, T.K. 1982. Epidemiology of pitch canker disease in a loblolly pine seed orchard in North Carolina. Phytopathology. 72: 1,212–1,216.

Littke, W. 1997. Seed pathogens and seed treatments. In: Landis, T.D.; South, B.D., eds. National Forest and Conservation Nursery Associations; 1996 Aug 20–22; Salem, OR. General Technical Report PNW-389. Portland, OR: USDA Forest Service, Pacific Northwest Research Station: 187–191.

McRae, J.B.; Bergsten, U.; and Lycksell, S. 1994. The use of the IDS-treatment on southern pine seeds and its effect of seed cost and efficiency in the seed. In: Landis, T.D.; Dumroese, R.K., eds. Northeastern /Southern Forest Nurserymen's Conference; 1994 July 11–14; Williamsburg, VA. General Technical Report RM-257. Fort Collins, CO: USDA Forest Service, Rocky Mountain Forest and Range Experiment Station: 73–79.

Mason, G.N.; Van Arsdel, E.P. 1978. Fungi associated with *Pinus taeda* seed development. Plant Disease Reporter. 62: 864–867.

Miller, T.; Blakeslee, G.M.; Bramlett, D.L.; and Matthews, F.R. 1987. The effects of using pollen contaminated with conidia of *Fusarium moniliforme* var. *subglutinans* on control-pollinated strobili of slash pine. In: 19th Southern Forest Tree Improvement Conference; 1987 June 16–18; College Station, TX. Southern Forest Tree Improvement Committee. No. 41. College Station, TX: Texas Agriculture Experiment Station: 232–239.

Miller, T.; Bramlett, D.L. 1979. Damage to reproductive structures of slash pine by two seed-borne pathogens: *Diplodia gossypina* and *Fusarium moniliforme* var. *subglutinans*. In: Proceedings of the Flowering and Seed Development in Trees: A Symposium. 1978 May 15–18; Jackson, MS. Washington, DC: U.S. Government Printing Office: 347–355.

Mitchell, L.A.; Sutherland, J.R. 1986. Detection of seed-borne *Sirococcus strobilinus* with monoclonal antibodies in an enzyme-linked immunosorbent assay. Canadian Journal of Forest Research. 16: 945–948.

Mittal, R.K.; Anderson, R.L.; and Mathur, S.B. 1990. Microorganisms associated with tree seeds: world checklist 1990. Information Report PI-X-96. Chalk River (Ontario) Canada: Forestry Canada, Petawawa National Forestry Institute. 57 p.

Mittal, R.K.; Wang, B.S.P. 1987. Fungi associated with seeds of eastern white pine and white spruce during cone processing and seed extraction. Canadian Journal of Forest Research. 17: 1,026-1,034.

Mittal, R.K.; Wang, B.S.P. 1993. Effects of some seed-borne fungi on *Picea glauca* and *Pinus strobes* seeds. European Journal of Forest Pathology 23: 138–146.

Nolte, D.L.; Barnett, J.P. 2000. A repellent to reduce mouse damage to longleaf pine seed. International Biodeterioration & Biodegradation. 45: 69–174.

Ocamb, C.M. 1995. Chemical treatment of eastern white pine seeds for removal of *Fusarium* propagules. Phytopathology. 85: 1129.

Palmer, M.A.; McRoberts, R.E.; and Nicholls, T.H. 1988. Sources of inoculum of *Sphaeropsis sapinea* in forest tree nurseries. Phytopathology. 78: 831–835.

Pawuk, W.H. 1978. Damping-off of container-grown longleaf pine seedlings by seedborne Fusaria. Plant Disease Reporter. 62: 82–84.

Rees, A.A.; Webber, J.F. 1988. Pathogenicity of *Sphaeropsis sapinea* to seed, seedlings and saplings of some Central American pines. Transactions of the British Mycological Society. 91: 273–277.

Riffle, J.W.; Springfield, H.W. 1968. Hydrogen peroxide increases germination and reduces microflora on seed of several southwestern woody species. Forest Science. 14: 96–101.

Rockwood, D.L.; Blakeslee, G.M.; Lowerts, G.H.; Underhill, E.M.; and Oak, S.W. 1988. Genetic strategies for reducing pitch canker in slash pine. Southern Journal of Applied Forestry. 12: 28–32.

Rowan, S.J. 1982. Tip dieback in southern pine nurseries. Plant Disease. 66: 258–259.

Runion, G.B.; Bruck, R.I. 1988. Effects of thiabendazole-DMSO treatment on longleaf pine seed contaminated with *Fusarium subglutinans* on germination and seedling survival. Plant Disease. 72: 872–874.

Runion, G.B.; Kelley, W.D.; and Land, D.H. 1991. Effects of triadimefon and thiram seed treatments on emergence of southern pines. Seed Science & Technology. 19: 57–66.

Salt, G.A. 1970. Conifer seedling pathology. In: Report on Forest Research for the Year End. London, England: Forestry Commission, Her Majesty's Stationery Office: 174–175.

Salt, G.A. 1974. Etiology and morphology of *Geniculodendron pyriforme* gen. Et. sp. nov., a pathogen of conifer seeds. Transactions of the British Mycological Society. 63: 339–351.

Sauer, D.B.; Burroughs, R. 1986. Disinfection of seed surfaces with sodium hypochlorite. Phytopathology. 76: 745–749.

Simak, M. 1984. A method for removal of filled-dead seeds from a sample of *Pinus contorta*. Seed Science & Technology. 12: 767–775.

Sinclair, W.A.; Lyon, H.H. 2005. Diseases of Trees and Shrubs. 2nd ed. Ithaca, NY: Cornell University Press. 660 p.

Storer, A.J.; Gordon, T.R.; and Clark, S.L. 1998. Association of the pitch canker fungus, *Fusarium subglutinans* f. sp. *pini*, with Monterey pine seeds and seedlings in California. Plant Pathology. 47: 649–656.

Sutherland, J.R. 1979. The pathogenic fungus *Caloscypha fulgens* in stored conifer seeds in British Columbia and relation of its incidence to ground and squirrel-cache collected cones. Canadian Journal of Forest Research. 9: 129–132.

Sutherland, J.R.; Woods, T.A.D. 1978. The fungus *Geniculodendron pyriforme* in stored Sitka spruce seeds: Effects of seed extraction and cone collection methods on disease incidence. Phytopathology. 68: 747–750.

Sutherland, J.R.; Lock, W.; and Farris, S.H. 1981. *Sirococcus* blight: a seed-borne disease of container-grown spruce seedlings in Coastal British Columbia forest nurseries. Canadian Journal of Botany. 59: 559–562.

Sutherland, J.R.; Miller, T.; and Quinard, R.S., eds. 1987. Cone and Seed Diseases of North American Conifers. Publication No. 1. Victoria, British Columbia: North American Forestry Commission.

Trappe, J.M. 1961. Strong hydrogen peroxide for sterilizing coats of tree seed and stimulating germination. Journal of Forestry. 59: 828–829.

Vaartaja, O. 1964. Chemical treatment of seedbeds to control nursery diseases. Botanical Review. 30(1): 1–91.

Vujanovic, V.; St-Arnaud, M.; and Neumann, P.J. 2000. Susceptibility of cones and seeds to fungal infection in a pine (*Pinus* spp.) collection. European Journal of Forest Pathology. 30: 305–320.

Wenny, D.L.; Dumroese, R.K. 1987. Germination of conifer seeds surface-sterilized with bleach. Tree Planters' Notes. 38(3): 18–21.

Wilkinson, R.C.; Underhill, E.M.; McGraw, J.R.; Pritchett, W.L.; and Schmidt, R.A. 1977. Pitch canker incidence and fertilizer insecticide treatment. Progress Report 77-1. Gainesville, FL: University of Florida, Institute of Food and Agricultural Sciences. 4 p.

Zoerb, M.H. Jr. 1969. Clonal variation in time of cone ripening in loblolly pine. Rincon, GA: Woodlands Research Note 22. Woodlands Research Department, Union Camp Corporation. 3 p.