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Dear TPN Reader

This is my 15th issue as your editor. I sincerely enjoy working with the authors, learning about the great work so many of you are doing, and seeing each issue evolve from a collection of draft manuscripts to a published masterpiece. It's a lot or work but so rewarding. Every now and then, I receive comments from readers, such as the letter below, that makes it all worthwhile.

> *"I just got the latest issue of TPN, and I figured it was time that I wrote a note of appreciation.*
>
> *This is a marvelous publication. …an exceedingly well designed, written, edited, and illustrated publication.*
>
> *It is exemplary of the kind of professionalism that built the Forest Service, and a reminder that this type of quality is still prized… it is refreshing to read verbs and nouns that denote real things, not just dreams or expectations.*
>
> *Congratulations, and thank you for your excellent work."*

The current issue contains another six articles of practical use for nursery, reforestation, conservation, and restoration activities. Dumroese and Haase (page 4) give an overview of water management guidelines to help prevent and control a variety of pests in greenhouses; Stone and colleagues (page 12) describe results from a restoration study to evaluate development of four species planted on harsh sites; Pike and colleagues (page 17) discuss hardwood seedling production trends in the Northeast United States. with a focus on four fine hardwood species and the implications of declining tree seedling sales at State nurseries; Cram and colleagues (page 25) examined the incidence of internal and external canker development on sweetgum seedlings in response to irrigation and fertilization treatments; Ouallal and colleagues (page 25) studied inoculation with native mycorrhiza on argan seedling quality in Morocco; and South (page 45) describes a stunting phenomenon found in bareroot pine nursery seedbeds following the use of totally impermeable film with spring fumigation.

Best wishes for a pleasant spring and summer planting season,

Diane

Diane L. Haase

Tree Planters' Notes (TPN) is dedicated to technology transfer and publication of information relating to nursery production and outplanting of trees and shrubs for reforestation, restoration, and conservation.

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Editor: Diane L. Haase

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Plant a Tree

by Lucy Larcom

(1824–1893)

He who plants a tree
Plants a hope.
Rootlets up through fibres blindly grope;
Leaves unfold into horizons free.
So man's life must climb
From the clods of time
Unto heavens sublime.
Canst thou prophesy, thou little tree,
What the glory of thy boughs shall be?

He who plants a tree
Plants a joy;
Plants a comfort that will never cloy;
Every day a fresh reality,
Beautiful and strong,
To whose shelter throng
Creatures blithe with song.
If thou couldst but know, thou happy tree,
Of the bliss that shall inhabit thee!

He who plants a tree,—
He plants peace.
Under its green curtains jargons cease.
Leaf and zephyr murmur soothingly;
Shadows soft with sleep
Down tired eyelids creep,
Balm of slumber deep.
Never hast thou dreamed, thou blessed tree,
Of the benediction thou shalt be.

He who plants a tree,—
He plants youth;
Vigor won for centuries in sooth;
Life of time, that hints eternity!
Boughs their strength uprear;
New shoots, every year,
On old growths appear;
Thou shalt teach the ages, sturdy tree,
Youth of soul is immortality.

He who plants a tree,—
He plants love,
Tents of coolness spreading out above
Wayfarers he may not live to see.
Gifts that grow are best;
Hands that bless are blest;
Plant! Life does the rest!
Heaven and Earth help him who plants a tree,
And his work its own reward shall be.

Contents

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Water Management in Container Nurseries To Minimize Pests

R. Kasten Dumroese and Diane L. Haase

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Abstract

Water is the most important and most common chemical used in plant nurseries. It is also the most dangerous chemical used. Insufficient water, excessive water, and poorly timed irrigation can all lead to poor-quality crops and unacceptable mortality. Anticipated future declines of water availability, higher costs to use it, and continuing concerns about irrigation runoff harming the environment also spur interest in making more efficient use of water in nurseries. This article discusses water management in container nurseries and its influence on weeds, insects, and diseases. It also provides guidelines for minimizing pest issues with proper irrigation application and monitoring. This paper was presented at the joint annual meeting of the Western Forest and Conservation Nursery Association and the Intermountain Container Seedling Growers' Association (Troutdale, OR, September 14–15, 2016).

Introduction

Water is essential to a successful nursery, but not just any water will do. Nursery water must be of high quality, readily and consistently available at favorable cost, and well managed (Landis et al. 1990). Unfortunately, costs of obtaining and using water are expected to increase as water becomes less available, especially as restrictions on groundwater withdrawal grow and climatic variablity threatens surface water sources. Moreover, concerns continue about how nursery runoff may harm ground and surface sources of water (Beeson et al. 2004, Fulcher et al. 2016).

Dependable, high-quality water is necessary because it is the chemical applied in the largest quantity to nursery crops. Water is the fundamental component of plants, comprising 80 to 90 percent of the fresh weight and affecting every morphological and physiological attribute, including photosynthesis, nutrient transport, transpiration, growth, and gene expression. Water has a similar essential role for insects and diseases, many of which thrive in high-moisture, nutrient-rich environments. The quantity, quality, and frequency of water applied during each growth stage in the nursery directly affect seedling quality and incidence of pests and disease.

Thus, proper water management is a critical component to meet current nursery objectives of producing high-quality seedlings and limiting the incidence of pests and diseases, while positioning nurseries to meet future challenges of reduced water availability.

Not Enough Water

In typical reforestation and conservation container nurseries, insufficient water can occur two ways: chronically and acutely. Chronic, or long-term, exposure to insufficient amounts of water will reduce overall growth. Acute, or rapid, exposure to insufficient water, such as missing an irrigation event during an extremely hot and dry period, can cause dieback or even mortality. Chronic underwatering, if not satisfactorily diagnosed and remedied, will lead to failure to meet seedling target specifications and thus a decreased number of shippable stock. Chronic underwatering is a much less common problem than chronic overwatering.

Too Much Water

Excessive irrigation is much more common, in our experience, than insufficient irrigation in reforestation and conservation container nurseries and can lead to several chronic problems. Too much water causes excessive growth and succulent tissues that are then difficult to harden off. Excessive watering and subsequent nutrient runoff are expensive, wasting water, fertilizer, and the energy and labor required to apply the water. Nutrient runoff can also pollute groundwater and surface water (Dumroese et al. 1992, Juntunen et al. 2002, Wilson et al. 2010). Furthermore, a wet nursery environment results in favorable conditions for numerous cryptogams, insects, and diseases, as described in the following sections.

Cryptogams

Cryptogams (plants that reproduce from spores instead of seeds, such as algae, moss, and liverworts) flourish in environments with high levels of moisture and nutrients (Khadduri 2011). These pests are resilient because they can also tolerate long, dry periods. Once established in a container, cryptogams impede water infiltration into the growing medium and compete with crop seedlings for water and nutrients (figure 1). Mosses and liverworts can also overtop and smother small seedlings. Excess water in the nursery environment, such as standing water on the greenhouse floor, also promotes the spread of spores. Persistent water on floors can allow algae to develop and make surfaces slippery, which can be a safety hazard. Once established, cryptogams can be difficult to control, especially in smaller volume containers.

Figure 1. Liverworts thrive in the high-moisture, nutrient-rich environment associated with growing seedlings in container nurseries. Once established, they can be difficult to control. Liverworts compete with seedlings for nutrients and can often overtop slower growing crops, reducing seedling quality or even causing mortality. (Photo by R. Kasten Dumroese, 2011)

Insects

Many insects thrive in moist conditions. Adult fungus gnats (*Bradysia* spp. [Diptera: Sciaridae]) can be particularly noticeable, fluttering across the tops of containers in a greenhouse where the growing medium is irrigated excessively. At low population levels, fungus gnats are more of an unflattering nuisance than a problem to plant quality, with the larvae generally feeding on the organic matter found in the substrate. When population densities are high, however, the larvae will consume seedling roots and girdle stems (Wilkinson and Daugherty 1970). Adult fungus gnats are also potential vectors for disease (James et al. 1995). Unfortunately, new insect pests, some of which are resistant to insecticides, are appearing and may present future problems in container nurseries (Rosetta 2017).

Diseases

Warm, wet conditions in forest and conservation nurseries are conducive to the buildup and spread of pathogens (Dumroese and James 2005). Damage from foliar diseases (e.g., rusts and *Botrytis*) and root diseases (such as those in the genera *Fusarium*, *Cylindrocarpon*, and *Phytophthora*) can increase dramatically when too much water is applied (figure 2). In addition to mortality, seedlings can have significant chlorosis and poor root development. Thus, the number of seedlings meeting shipping criteria can be reduced because of failure to meet quality standards, particularly root plug integrity and seedling color. Moreover, infected seedlings may also wilt at the onset of symptoms, causing growers to erroneously conclude that the crop requires even more irrigation.

Sanitation and Water Management

Pest prevention through sanitation as part of an integrated pest management (IPM) program is the prudent first step in pest management (Dumroese 2012). Incorporating vigorous sanitation into the annual production cycle can further enhance the benefits of proper water management to suppress pests. Between crops is the best time to kill spores, seeds, insects, and pathogens. Precrop sanitation practices to disinfest the facility, containers, benches, floors, and equipment are recommended. Container sterilization can be done with steam or hot water. Dumroese et al. (2002) found that containers used for three crop cycles

Figure 2. Excessive irrigation and warm temperatures foster development of root diseases such as *Fusarium*, which can either ruin seedling quality or cause mortality. (Photo by Thomas D. Landis, 1987)

but cleaned annually with hot water yielded 13 percent more Douglas-fir (*Pseudotsuga menziesii* [Mirb.] Franco) seedlings than nontreated containers. Moreover, seedlings from treated containers had 20 percent more biomass and were 10 percent taller than those from noncleaned containers. Disinfesting seeds prior to stratification can reduce pathogen levels before sowing and can be accomplished by imbibing seeds in running water rather than stillwater soaks (James 1987) or by treating seeds with disinfectants such as bleach (Wenny and Dumroese 1987) or hydrogen peroxide (Barnett 1976). Using seeds with high vigor and controlling moisture during stratification will increase germination uniformity and minimize damping off (Karrfalt 2017).

Because water is the most critical environmental factor affecting the incidence, severity, and spread of cryptogams, insects, and diseases, proper management of irrigation frequency, timing, and quantity is paramount to minimize their effect and will also reduce the need for pesticides (Dumroese et al. 1990). Careful attention to the nursery's irrigation regime also has a direct effect on each growth stage of the crop (Mexal and Khadduri 2011). Following are some guidelines for each phase of crop production.

During Germination

Selection criteria for the types of growing media used in the nursery should include consideration of water holding capacity and drainage (Landis et al. 1990). Each growing medium should hold enough water to meet the crop's needs and to optimize water use efficiency but not so much that roots are waterlogged.

Misting for the first week or two after sowing is a typical practice to promote rapid and uniform germination. Frequent misting, however, can result in high-moisture conditions favorable to pests and diseases. In a study to compare the effects of misting frequency on lodgepole pine (*Pinus contorta* Douglas ex Loudon var. *latifolia* Engelm. ex S. Watson) seed germination, no significant differences in germination capacity and speed (table 1) were found among low, medium, or high frequency treatments, although seed zone temperature tended to be lowest with the highest frequency (figure 3), which can reduce germination speed (Pinto et al. 2009). We recommend experimenting with misting frequency to find the minimal amount required to achieve germination objectives. The frequency will depend

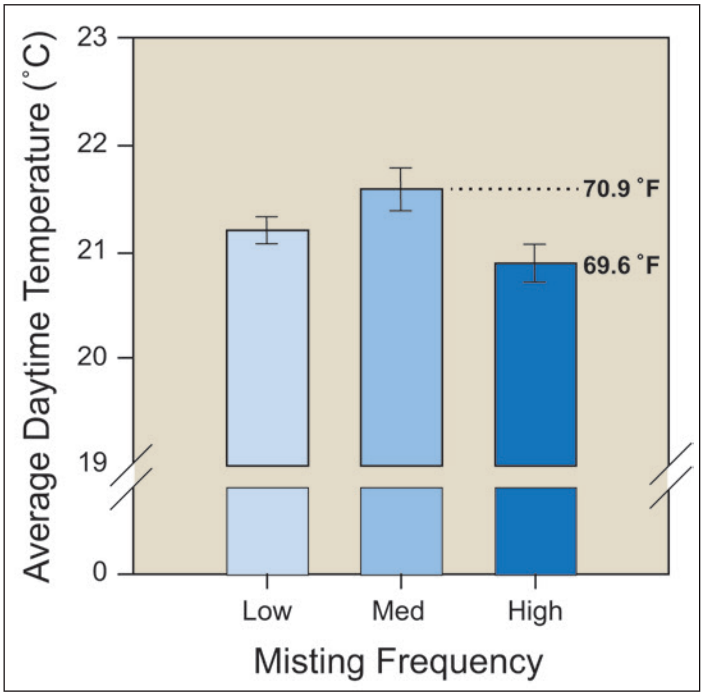

Figure 3. Misting frequency during the germination phase had a significant effect on the temperature at the seed-medium interface, with the most frequent misting reducing the average temperature. (Adapted from Pinto et al. 2009)

Table 1. Average amount of water applied per container cavity and means (± standard errors) for lodgepole pine seed germination parameters among three misting frequencies. Germination rates did not differ significantly among treatments (from Pinto et al. 2009).

Misting treatment	Amount of water per application	Number of applications every 4 days	Total water applied every 4 days	Germination after 21 days (%)	Germination rate (days to 50% germination)
Low	6.7	1	6.7	82.0 (1.0)	9.6 (0.2)
Medium	4.2	2	8.4	84.0 (1.4)	9.4 (0.2)
High	2.1	12	25.4	84.0 (1.4)	9.7 0.2)

on environmental conditions, seed characteristics, and growing medium and may vary from year to year depending on weather conditions. Daily or multi-daily examination can determine the optimum misting frequency to keep the seed-medium interface moist but not wet. Regular observation and experience is part of the art of being a nursery manager.

In addition to misting frequency, other practices—such as acidifying irrigation water, lowering fertilizer rates, using seed coverings, and rogueing infected germinants—will help minimize the incidence of cryptogams, fungus gnats, and diseases (table 2).

During Rapid Growth and Hardening

Irrigation based on a routine schedule is convenient for the nursery staff but can lead to issues with growth, quality, and pests. Efficient irrigation scheduling is based on the plants' requirements, not on a regular schedule (Regan 1994, figure 4). Plant needs can change daily and are determined by species, provenance, plant size, container type and size, growing medium, fertilizer concentration, temperature, sun intensity, ventilation, relative humidity, and other influencing factors. Irrigating early in the day to container capacity results in maximum water availability during warmer, daylight hours and promotes rapid drying to minimize the duration of water on foliage and other surfaces. Good ventilation in the growing area will also keep humidity down and help discourage buildup of pests.

In the next section, Monitoring Water, the use of container weights to determine irrigation frequency is described. Adherence to this technique can provide

Table 2. General guidelines for reducing cryptogams, fungus gnats, and diseases during growth phases at the nursery.

Nursery practice to minimize the indicated pests	Cryptogams	Fungus gnats	Disease
Germination and establishment			
Acidify water	-	-	X
Allow medium surface to dry	X	X	X
Use low fertilizer rates during early development	X	-	X
Add a biological control	-	Parasitic nematodes	*Trichoderma*
Use seed coverings (e.g., grit)	X	-	-
Sanitize (rogue affected plants)	X	X	X
Rapid growth and hardening			
Acidify water	-	-	X
Allow medium surface to dry	X	X	X
Irrigate early in the day	-	-	X
Add a surfactant	-	-	X
Sanitize	X	X	X

Figure 4. Proper irrigation scheduling is based on the needs of the seedlings, not necessarily the convenience to the nursery workers. (Photo by R. Kasten Dumroese, 1995)

opportunities to reduce irrigation frequency without adversely affecting seedling quality. Dumroese et al. (2011) found that the target for irrigating ponderosa pine (*Pinus ponderosa* Lawson & C. Lawson) seedlings could be reduced 15 percentage points during the entire growing season without significantly changing seedling morphology and water-use efficiency. Moreover, that reduction increased the average interval between irrigations from 2 days to nearly 5 days.

Sanitation and other IPM measures (table 2) should continue throughout the growing cycle. The crop should be inspected daily for any problem areas, and all dead and dying seedlings should be removed to minimize infection of adjacent plants and discourage development of *Botrytis* (Haase and Taylor 2012).

Monitoring Water

Daily observation of the growing area for standing water, undesirable growth patterns in the crop caused by variable water availability, and development of weeds, moss, or algae will provide useful feedback for making necessary adjustments or repairs to the irrigation system. In addition, daily monitoring of growing-medium water content or plant water status will guide decisionmaking for irrigation frequency and quantity. Many growers use a visual-tactile method to make a determination of growing-medium moisture based on experience. This technique can provide some information but can have limited usefulness. For example, if the growing medium in the lower portion of the container is quite dry or quite wet, the grower may not make the best irrigation determination or, if the grower is on vacation, the employees may not have the same level of experience to determine proper irrigation frequency. Quantitative methods, such as measuring container weights or xylem water potential, offer repeatable and reliable assessments of current water conditions in the crop.

Container Weights

Monitoring container weights is the most common quantitative approach used for determining irrigation scheduling (Mexal and Khadduri 2011). The standard approach is to irrigate the medium in the container until it reaches container capacity (water begins dripping from the drainage holes), allow the water to drain for several minutes, and then weigh the container to determine its container capacity weight (figure 5). The container is periodically weighed as it dries down to a specified target (e.g., 70 percent of container capacity weight). Target container weights vary by the type of growing medium, container size and type, plant species, and growth stage. When the container weight reaches the target level, it is irrigated again (Dumroese et al. 2015). The target level is chosen based on plant species and growth stage; container weight targets are lower (i.e., drier) during hardening than during active growth. Depending on crop size and greenhouse variability, two or more representative containers are designated for repeated container weight measurements. Weights can be measured by placing the container on a scale (on the bench or a traveling cart) or suspending the container with a handheld scale (figure 5). Note that this approach does not account for the dry weight of the container, medium, or grit and thus does not measure the true water content as would a scientific approach typically used in research (Dumroese et al. 2015). Nonetheless, this monitoring tool is very effective and efficient.

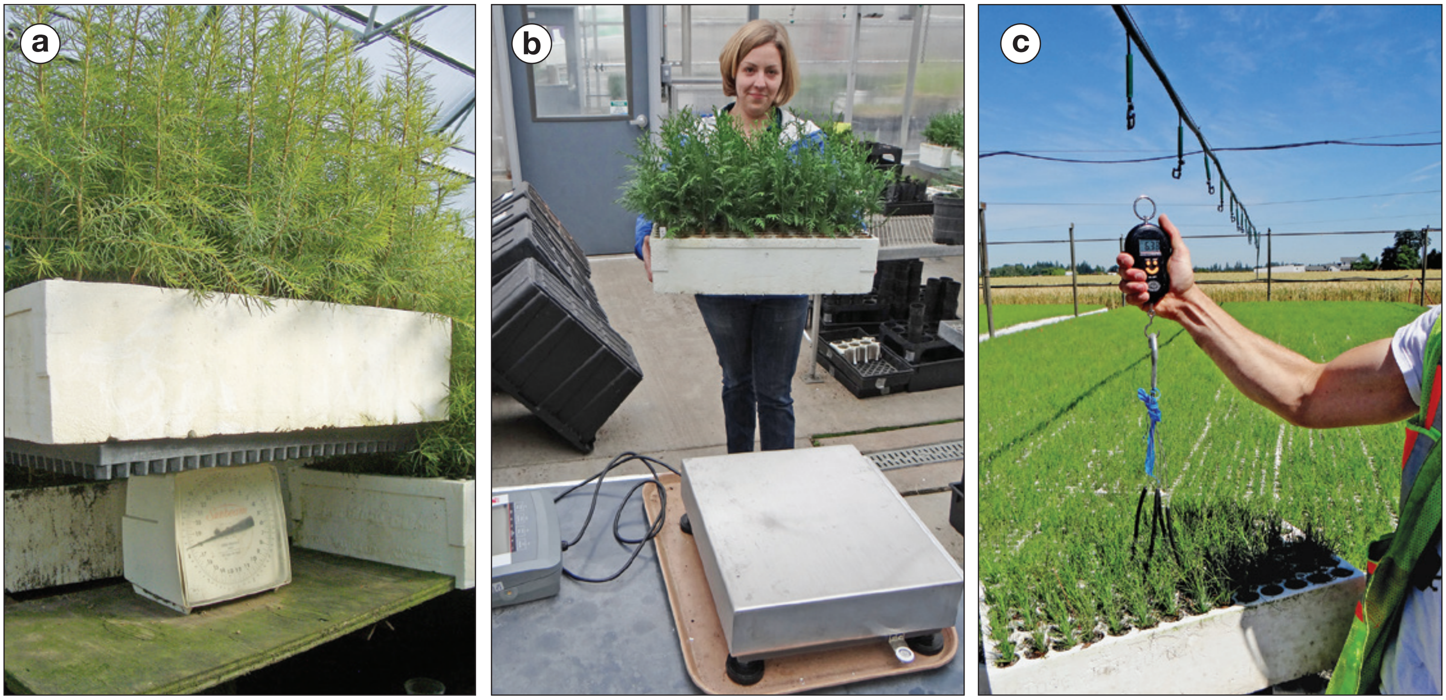

Figure 5. Weighing containers, a common method of monitoring irrigation needs in container nurseries, can be accomplished with (a) dedicated balances in the crop, (b) carrying or carting a balance to different locations in the nursery, or (c) using a hand-held balance. (Photos by R. Kasten Dumroese, 2006, 2015, 2014; from Dumroese et al. 2015)

Xylem Water Potential

Another monitoring method to assist with water management is assessment of xylem water potential (ψ_{xylem}), a direct measure of the plants' water status (Haase 2008). This measurement reflects interactions among water supply, water demand, and plant regulation. In this method, representative plants are assessed in a pressure chamber to determine ψ_{xylem}. Irrigation is then applied when a target ψ_{xylem} threshold is reached.

Other Considerations

Many aspects of the nursery's facility and cultural practice can influence water management requirements to prevent and control cryptogams, insects, and diseases.

Irrigation System

In addition to irrigation timing and amount, the irrigation delivery system influences the effect that water has on pest populations in the nursery. For example, subirrigation is an efficient water delivery system that reduces the amount of water and fertilizer needed to produce plants while also reducing wastewater, nutrient leaching, and the incidence of cryptogams as compared with overhead irrigation (Dumroese et al. 2006, Pinto et al. 2008, Schmal et al. 2011).

Fertigation

In most container nurseries, fertilizer is mixed with the irrigation water to deliver nutrients at a set concentration to the plant. When adjusting watering frequency, one must also adjust the amount of nutrients. Increases or decreases in the stock solution concentration can be made to compensate for watering frequency.

Pesticide Applications

Applying pesticides to the container crop should be aligned with the irrigation schedule. For example, a grower typically applies fertigation followed by a clear water rinse. The rinse could include a foliar fungicide to simultaneously remove the fertilizer salts and maximize the amount of time the pesticide is on the foliage.

Cooling

Irrigation is a common tool for cooling plants under high temperatures. The cooling occurs when the water evaporates (i.e., evaporative cooling). Thus, it is important to be sure that water applied for cooling actually evaporates, so there is no time to create a favorable pest environment. Good ventilation to keep humidity down is critical.

Every Nursery Is Unique

Growing seedlings in a nursery is an art and a science. Although the concepts (science) of water management may be specific, how they are applied (art) may vary because of differences in the facilities, species provenances, tools and supplies, customer expectations, water quality, and environmental patterns of individual nurseries. The philosophy of the managers can further lead to a preference for being either a "wet grower" or a "dry grower." Thus, determining a water management plan that works equally well for all nurseries is not possible (Dumroese and Wenny 1997). Nevertheless, it is essential for nursery managers to annually reevaluate irrigation scheduling, sanitation practices, pest and disease occurrences and their causes, and crop performance. These annual evaluations can be used to improve techniques and yields for future crops.

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Height Growth of Planted Shrubs and Trees in a Semiarid Rangeland in Western Montana

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Abstract

We evaluated height development of restoration species in two studies in the semiarid foothills of the Sapphire Range in western Montana. In one study, ponderosa pine (*Pinus ponderosa* Lawson & C. Lawson) and Rocky Mountain juniper (*Juniperus scopulorum* Sarg.) that were planted on a partially degraded site were given supplemental irrigation for 2 years, after which irrigation was discontinued for a subset of plants. Subsequent height growth did not differ, indicating that irrigation after plants have established and reached a certain level of maturity may not provide an advantage. In the other study, Rocky Mountain juniper, antelope bitterbrush (*Purshia tridentata* [Pursh] DC.), and mountain mahogany (*Cercocarpus ledifolius* Nutt.) were planted on dry, south-facing slopes and measured for annual growth. Plants grew 5 to 30 percent during the first two seasons and 49 to 73 percent in the third season. These results help provide realistic expectations for restoration plantings in semiarid sites.

Introduction

Native plant communities in rangelands across the Western United States have been altered by overgrazing (Fleischer 1994), nonspecific herbicide use (Crone et al. 2009), and conversion to agriculture (Wright and Wimberly 2013). These disturbances can cause soil compaction (Hamza and Anderson 2005), erosion (Montgomery 2007), and invasion by exotic plants (Hobbs and Huenneke 1992). The resulting habitat often supports lower biodiversity and less ecological value (Fleischer 1994) than undisturbed habitats, thereby necessitating revegetation to restore the natural ecology of the landscape (Brennan and Kuvlesky 2005).

One component of restoring rangelands can be increasing shrub and tree cover (Brennan and Kuvlesky 2005). In many areas, topographical features, such as the north sides of hills and draws, create unique microclimates that can favor certain plant species and other biota (Bennie et al. 2008) that may otherwise not be present in a rangeland (Suggitt et al. 2011). Some wildlife species, including birds that rely on these habitats, are in decline (Brennan and Kuvlesky 2005). Increasing shrub and tree cover may create habitat while also establishing wildlife corridors that connect habitat patches and facilitate wildlife travel (Beier and Noss 1998).

Many rangelands in the Intermountain West have arid or semiarid climates that make restoring shrubs and trees difficult. Lack of precipitation often precludes natural establishment in these areas, so introduced plants must often be irrigated during the initial establishment (Bainbridge 2002). Watering plants by hand or installing drip irrigation in areas that might not be easily accessed requires additional labor and cost (Bainbridge 2002). Planted shrubs and trees can also be browsed or damaged by ungulates, requiring the installation of fences, exclosures, or other deterrents (Johnson and Okula 2006, Kimball et al. 2005), further increasing labor and cost. Considering these challenges, it is critical to have realistic estimates about plant growth and survival so that the magnitude of success can be predicted.

The objective of our study was to determine height growth of shrub and tree species on rangeland sites in western Montana. Land managers and restoration practitioners can use these data to estimate growth rates for species planted at similar sites.

Methods

Study Area

We conducted two studies at MPG Ranch (Florence, MT) in the foothills of the Sapphire Range in western Montana (46° 40’ 48” N, 114° 1’ 40” W, 1000 m [~3,300 ft]; mpgranch.com). The area received an average of 20 cm (8 in) of precipitation annually from 2010 to 2015. The topography consists of rangelands and draws. Based on oral history records, much of the area was sprayed with broadleaf herbicides and grazed from 1972 to 2007. As a result, the land suffers from erosion, soil compaction, a high cover of exotic plants, and a low cover of native shrubs and trees. We established two study sites in the area based on habitat type: partially degraded and south-facing slopes.

Partially Degraded Site

The partially degraded site was along a roadside in an area (about 300 m [~1,000 ft] long and 30 m [~100 ft] wide) that had a history of heavy cattle grazing and human disturbance. The site had compacted soils and was invaded by weeds, including tumble mustards (*Sisymbrium* spp.), kochia (*Bassia scoparia* [L.] A.J. Scott), leafy spurge (*Euphorbia esula* L.), and cheatgrass (*Bromus tectorum* L.). Between mid-April and mid-May in 2010 and 2011, we planted Rocky Mountain juniper (*Juniperus scopulorum* Sarg.; n = 265) and ponderosa pine (*Pinus ponderosa* Lawson & C. Lawson; n = 78) seedlings on the site (figure 1).

The Franklin H. Pitkin Forest Nursery at the University of Idaho (Moscow, ID) grew the seedlings as containerized stock (328 cm^3 [20 in^{3-}], with the exception of approximately 10 percent of the ponderosa pine seedlings grown in larger containers) for approximately 15 months. Seeds for ponderosa pine were sourced from the University of Idaho Experimental Forest (Moscow, ID) at an elevation of 945 m (3,100 ft). Rocky Mountain juniper seeds either were sourced from a location in Utah or were Bridger Select from Montana (Scianna et al. 2000). Watershed Consulting (Missoula, MT) planted all the seedlings in microsites that appeared suitable for growth and were spaced a minimum of 1 m (~3 ft) apart. We placed wood chips around the base of each plant and installed plastic exclosures (48 by 117 cm [19 by 46 in]) supported with wooden stakes around each plant to exclude browse by ungulates (figure 1). We placed identification tags on every exclosure.

Figure 1. Rocky Mountain juniper and ponderosa pine were planted near the edge of a road on the partially degraded site. (Photo by Michael McTee, 2015)

During 2010 and 2011, all plants were hand watered approximately every 2 weeks with 3.8 to 11.4 L (1 to 3 gal) of water. In 2012, we installed drip irrigation to all plants, which delivered a rate of 3.8 L (1 gal) of water per hour for 4 to 8 hours every 2 weeks. In 2013, drip irrigation was removed from a randomly selected subset of 7 to 10 plants of each species (table 1) to test whether irrigation influenced height growth after the plants were established. Drip irrigation continued on all other plants through 2015. Plant heights were measured annually at the end of the growing season, in September, from 2012 to 2015 by removing woodchips at the base of the plant and measuring the distance from the soil to the tallest growth leader.

For each species, we tested for differences between the heights of those plants that did or did not receive irrigation. We used a Welches t-test (α = 0.05), because it accommodates for unequal sample sizes and unequal variances among samples (table 1). Statistics were calculated in R (R Core Team 2013). We were unable to calculate survival rates, because the identification tags for many plants were damaged or removed

Table 1. Statistics and sample sizes to compare height development of ponderosa pine and juniper seedlings with and without drip irrigation.

Year	Ponderosa pine			Rocky Mountain juniper		
	*n**	*t*	*p*	*n**	*t*	*p*
2013	59 (8)	-0.52	0.613	139 (10)	-0.91	0.384
2014	51 (7)	-0.65	0.532	122 (10)	0.41	0.689
2015	47 (7)	-0.40	0.701	111 (10)	0.37	0.717

* Sample sizes for number of plants that received drip irrigation after 2013 are followed by the sample sizes for number of plants that did not receive drip irrigation (in parentheses).

by ungulates such that we could not distinguish between study plants and those that were planted solely for restoration. Statistics were calculated for the population of plants that retained tags throughout the study.

South-Facing Slopes

Sites on south-facing slopes consisted of three draws with severe erosion located within 0.6 km (0.4 mi) of each other. The draws received a significant amount of solar radiation and were mostly denuded of vegetation. Between mid-April and mid-May 2013, we planted the slopes with Rocky Mountain juniper (n = 135), antelope bitterbrush (*Purshia tridentata* [Pursh] DC.; n = 88), and mountain mahogany (*Cercocarpus ledifolius* Nutt.; n = 287).

Rocky Mountain juniper seeds were sourced as described previously. Great Bear Nursery (Hamilton, MT) grew the bitterbrush and mountain mahogany seedlings. Bitterbrush seeds were collected from northeastern Washington at approximately 1,800 m (6,000 ft) and were grown for 3 months. Mountain mahogany seeds were collected from the Rocky Mountains and were grown for approximately 1 year. Plant species were randomized across the site, woodchips were placed at their base, and exclosures and identification tags were

Figure 2. South-facing slopes included in the study were steep and dry. (Photo by Michael McTee, 2015)

installed at each plant following the same protocol used in the partially degraded site study (figures 2 and 3). Each plant was placed upslope of logs that were partially buried in the soil to create terraces for slowing soil erosion. Each log was 10 to 20 cm (4 to 8 in) in diameter and 100 to 200 cm (40 to 80 in) in length.

All plants were watered at the time of planting. Thereafter, plants were drip irrigated every 2 weeks at a rate of 3.8 L (1 gal) of water per hour for 4 to 8 hours.

Plant heights were measured at the time of planting and again each fall at the end of three growing seasons, following the same procedure used in the partially degraded site study. We pooled data from the three south-facing slopes for each species for statistical analysis. We compared differences in height development among species with a one-way Analysis of Variance, or ANOVA, with a Tukey's post hoc test (table 2). Statistics were calculated in R (R Core Team 2013).

Results

Partially Degraded Site

Junipers that were drip irrigated increased in height more during the first 2 years (49 and 52 percent, respectively) than during the following 2 years (11 and 14 percent, respectively; figure 4). Ungulate browsing on the leaders of some junipers likely influenced growth in the third and fourth seasons despite plants being protected by exclosures. Ponderosa pine seedlings grew less during the first season (13 percent) than in subsequent growing seasons, in which they increased in height at least 30 percent from the previous year. After the assumed establishment period (2 to 3 years), drip irrigation did not significantly influence height growth of either species (figure 4, table 1).

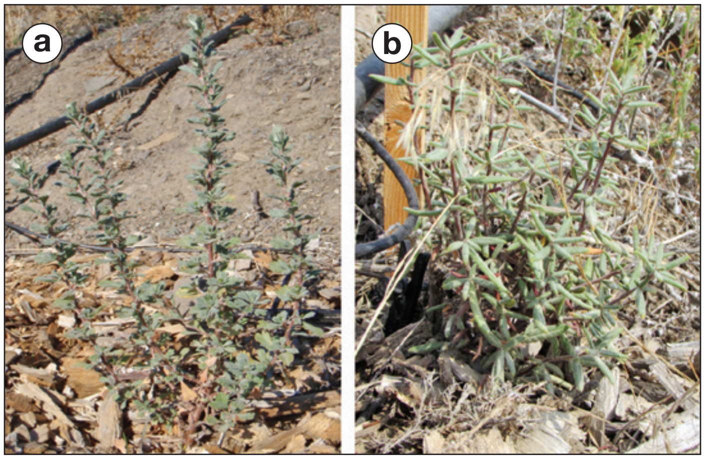

Figure 3. (a) Antelope bitterbrush, (b) mountain mahogany, and (not pictured) Rocky Mountain juniper were planted on south-facing slopes to assess height development. Wood chips were placed around each plant, followed by drip irrigation and an ungulate exclosure (removed for the photos). (Photos by Michael McTee, 2015)

South-Facing Slopes

All three species planted on south-facing slopes roughly doubled in height after three growing seasons (figure 5). Heights increased by 14 to 30 percent

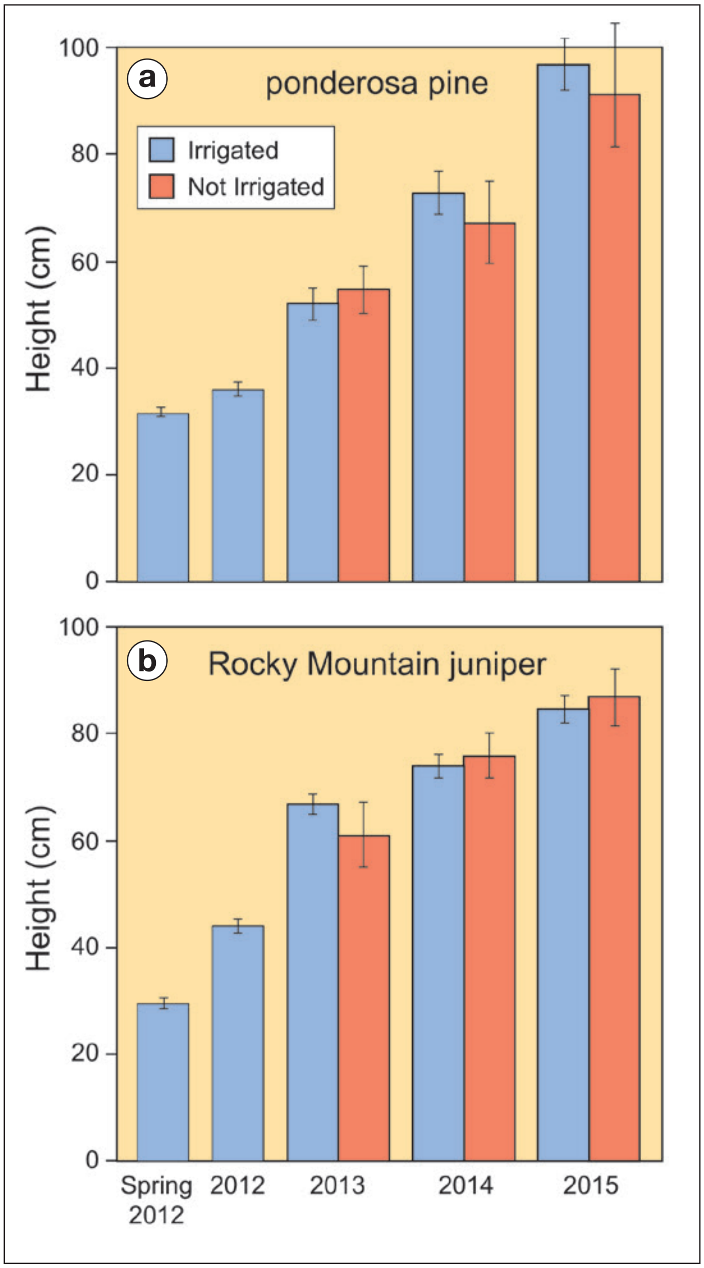

Figure 4. Mean heights of (a) ponderosa pine and (b) Rocky Mountain juniper during four growing seasons. All plants were drip irrigated in 2012. In 2013, we removed drip irrigation from a randomly selected subset of plants and found no irrigation effect thereafter. Error bars represent standard errors.

Table 2. Statistics and sample sizes to compare height development of bitterbrush, juniper, and mountain mahogany during four growing seasons.

Year	Sample sizes			F	P
	Bitterbrush	Rocky Mountain juniper	Mountain Mahogany		
Spring 2013	88	135	287	20.32	<0.001
2013	39	104	171	33.25	<0.001
2014	24	108	136	23.83	<0.001
2015	13	94	113	14.65	<0.001

during the first season, 5 to 14 percent during the second season, and 49 to 73 percent during the third season. Rocky Mountain juniper had an initial height greater than that of the other two species and retained its height advantage throughout the study, although the difference was no longer significant for bitterbrush in the third growing season (table 2). Mountain mahogany were taller than bitterbrush initially but not for the remainder of the study.

Discussion

At the partially degraded site, ponderosa pine had less relative height growth during the first season compared with height growth in subsequent growing seasons. Ponderosa pine grows long taproots that help young trees tolerate drought; therefore, resources during establishment were likely allocated more to the taproot than the aboveground tissues (Wier 2015). Drip irrigation did not influence the height growth of ponderosa pine or Rocky Mountain juniper, which suggests that the added effort and cost of irrigating these species may not be required once plants are established on the site and reach a certain level of maturity. Height measurements, however, do not reflect total biomass, which may have been influenced by irrigation and can affect long-term vigor and growth. The moderate compaction of the site may have inhibited plants from growing as vigorously as if they had been planted in less degraded soils (Ashby 1997).

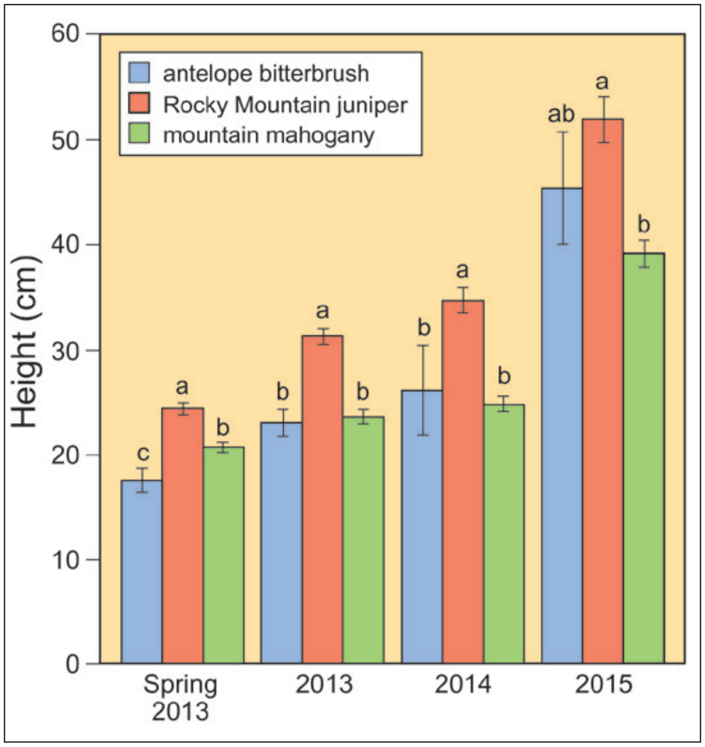

Figure 5. Mean annual heights of antelope bitterbrush, Rocky Mountain juniper, and mountain mahogany during three growing seasons. Error bars represent standard errors. Different letters above bars indicate statistical differences ($p \leq 0.05$).

South-facing slopes in the northern hemisphere receive more solar radiation than slopes of other aspects, resulting in warmer and drier conditions (Desta et al. 2004, Warren 2008). These conditions are unfavorable to plant colonization and establishment in semiarid climates, mainly due to water limitation (Bochet et al. 2009), so we irrigated all shrubs to aid in establishment (Bainbridge 2002). Surviving shrubs will eventually create conditions more conducive for the natural regeneration of other plants due to shading, soil stabilization, and erosion control (Gyssels et al. 2005). Larger plants can also serve as “shrub islands” that can be used for cover by wildlife and young plants (Padilla and Pugnaire 2006, With and Webb 1993). These isolated shrubs may also be a source of seeds in areas where seeds are scarce and natural colonization is rare. Bochet et al. (2009) found that plants cease to colonize south-facing slopes at slope angles greater than 40 degrees in a semiarid ecosystem in eastern Spain. At our south-facing study sites, the slope angles ranged from 5 to 35 degrees, suggesting that some sites may be near the threshold for natural establishment.

Overall, these studies give restoration practitioners some practical data regarding performance of common restoration species in semiarid rangelands of the Intermountain West. Degraded sites within these landscapes present additional obstacles that may be overcome by increasing management efforts, such as by installing drip irrigation, ungulate exclosures, or structures that control erosion. Ultimately, plant success will depend on many factors that may be unique to the site and the timing and patterns of weather during plant establishment.

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Trends in Production of Hardwood Tree Seedlings Across the Northeast United States From 2008 to 2016

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Abstract

Bareroot hardwood seedlings are grown at both State and private nurseries across the 20-State U.S. Department of Agriculture (USDA), Forest Service, Northeastern Area. When propagated as bareroot seedlings, hardwood species such as oak, walnut, and black cherry are better suited for large-scale plantings due to size and cost factors. Here, we report on trends in the production of hardwoods and conifers at State and private nurseries in 2016 and on trends for four fine hardwood species from 2008 through 2016 at State nurseries: red oak (*Quercus rubra* L.), white oak (*Quercus alba* L.), black walnut (*Juglans nigra* L.), and black cherry (*Prunus serotina* Ehrh.). Black walnut exhibited the steepest drop in production relative to the other three hardwood species in spite of having the highest stumpage values of the fine hardwoods. State nurseries are increasingly dependent on private landowners for their market share but may be imperiled by budgetary shortfalls in the future. A decline in seedling demand may be a function of several factors, including declines in Conservation Reserve Program funds or downsizing of markets for timber. We discuss the implications of declining tree seedling sales on State nursery operations and the consumers who depend on them.

Introduction

Forests in the 20 States served by the Northeastern Area State and Private Forestry program of the USDA Forest Service (figure 1), hereafter referred to as the Northeast, provide many services including watershed protection, wildlife habitat production, mineland restoration (Ashby 1996), timber production, and recreational use. Even though the percentage of forest cover in the Northeast has increased since the early 20th century, forests are increasingly in close proximity to, and encroached on by, urban development (Oswalt and Smith 2014). Forests are also increasingly parceled as land ownership patterns change (Butler and Ma 2011). Northeastern forests are also threatened by invasive pests such as chestnut blight (*Cryphonectria parasitica*), Dutch elm disease (*Ophiostoma ulmi*), and emerald ash borer (*Agrilus planipennis*). Invasive plants such as bush honeysuckle (*Lonicera japonica* Thunb.), autumn olive (*Elaegnus umbellata* Thunb.), multiflora rose (*Rosa multiflora* Thunb.), and raspberry (*Rubus* spp.), along with aggressive native pioneers such as tulip tree (*Liriodendron tulipifera* L.) and sugar maple (*Acer saccharum* Marshall), can choke out both natural and artificial tree regeneration (Morrissey et al. 2010). This effect may be further compounded by herbivory from high populations of white-tailed deer (*Odocoileus virginianus*; Kern et al. 2012). The combination of invasive pests and herbivory has created unprecedented gaps in urban and rural woodlands across the Northeast, requiring an increase in management of tree species whose regeneration would otherwise be suppressed.

Disturbance regimes in northeast forests have changed dramatically during the past century, which affects regeneration of plants and trees. Wildfires occasionally still occur in remote forests of the Appalachian Mountains and in the northern Great Lakes regions but not at the high frequency that historical records indicate (Heinselman 1973). Instead, fires are infrequent and may be large in scale, such as the Pagami Creek (2011) and Ham Lake (2007) Fires in Minnesota or the Gatlinburg (2016) Fire in Tennessee. The Northeast is largely devoid of large-scale clearcuts from logging operations, which provide sunlight for regeneration of shade-intolerant species such as oaks *(Quercus* spp.).

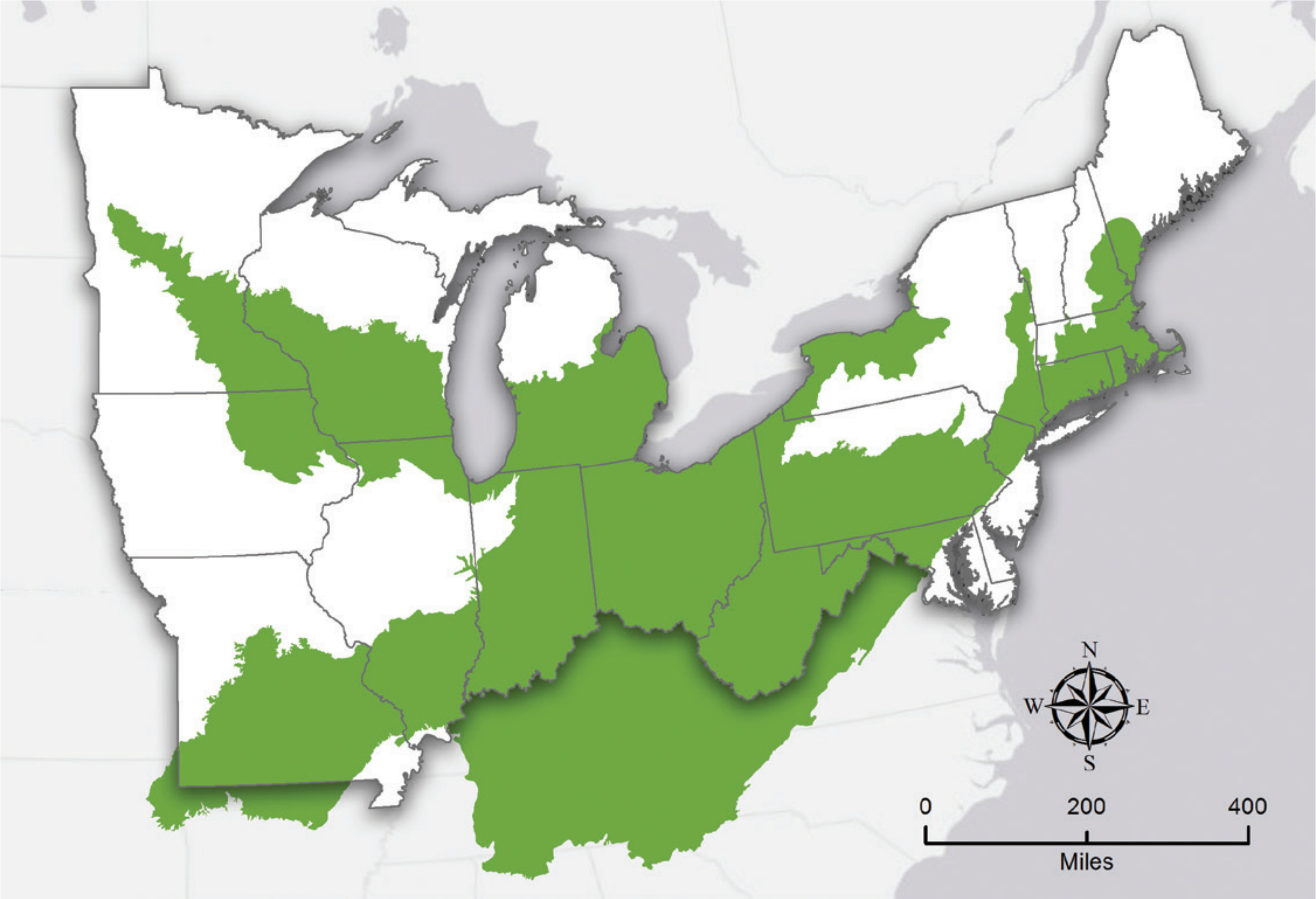

Figure 1. Map of the Northeastern Area of the United States, with the range of central hardwood forest species shown in green.

When naturally occurring seed sources are not available, or regeneration of a particular species is desired, tree planting is necessary to maintain or restore forest cover, especially for oaks, walnuts (*Juglans* spp.), and to a lesser extent black cherry (*Prunus serotina* Ehrh.). The central hardwood region does not have distinct boundaries (Fralish 2003; figure 1), and species associated with these forests span a large portion of the Eastern United States. We focus on hardwood species that are relatively common in this region, have intrinsic wood values, have developed forest products markets, and face a myriad of challenges from invasive plants, insects, and pathogens.

Northern red oak (*Quercus rubra* L.; Sander 1990) and white oak (*Q. alba* L.) are commonly found in forests across the Northeast (figure 1) and are highly prized for their wood quality for furniture and other products. White oak is uniquely suited for barrels used in the crafting of bourbon, an important local industry in Kentucky and, to a lesser degree, Tennessee (Thornberry 2014), as well as for wine barrels. The dense, dark heartwood of black walnut (*Juglans nigra* L.) is used as a high-valued veneer in furniture markets and in the production of other specialty products such as gun stocks. Black cherry is commercially valuable in New York, Pennsylvania, and West Virginia for use in cabinetry and furniture (Burns and Honkala 1990). All four of these species are commonly used for reforestation or afforestation in the central hardwood region (Fralish 2003) and are thus classified as fine-hardwood species based on their timber quality. They are also favored for restoring wildlife habitat, because they represent both soft (black cherry) and hard (oaks, walnut) mast sources for a variety of birds and other wildlife.

The American forest nursery industry began, and rapidly evolved, during the 20th century (Haase 2010). In the early 20th century, State and Federal nurseries were constructed to reforest large swaths of land denuded by timber barons. These nurseries were funded, in large measure, from Federal job programs initiated during the depression in the 1930s (Dumroese et al. 2005). The first State nurseries, located in New York and Pennsylvania, were established in 1902 (Alban and Dix 2013, Verschoor 2007). Subsequently, every State in the Northeast established a nursery, and in most cases multiple nurseries, to meet their reforestation needs. As land was reforested and timber harvesting shifted westward, demand for seedlings declined, resulting in the closure of many States' nurseries. Currently, 13 State and 44 private nurseries in the Northeast produce approximately 95 million seedlings annually (Hernández et al. 2016). These seedlings, sometimes called "conservation grade," are generally small statured, low cost, and lightweight for carrying in a planter's side bag. Conservation-grade seedlings are commonly planted into sites that are commercial or landscape scale for restoration or reforestation.

The private forest nursery industry in the Northeast has steadily gained market share during the past 50 years, as new technologies and efficiencies have streamlined production, so that fewer laborers are needed to run large operations. Advancements in the container nursery sector have also contributed toward increased efficiency of tree planting operations. For example, stout seedlings that contain a soil plug are nimble for tree planters and can be stored for longer periods before planting compared with their bareroot counterparts. Prices between small containerized and bareroot seedlings are often comparable for the same size trees. Hardwood seedlings, however, are usually much larger than conifer seedlings of the same age, as they tend to allocate a larger proportion of resources to root growth. The large, fibrous root systems of hardwoods are critical for the tree's survival after planting. As such, conservation-grade bareroot hardwood seedlings are generally more successful than container stock types (Zaczek et al. 1995), because the container sizes required to contain the immense hardwood roots are necessarily large and bulky. Nonetheless, fewer private nurseries supply bareroot seedlings, as opposed to container seedlings, largely because of the low profitability of selling small, conservation-grade seedlings.

Tree planting has waxed and waned with the U.S. economy and with incentives that drive land conversions to or from agriculture. Conservation Reserve Programs (CRP), offered to private landowners through the USDA Farm Service Agency, are critical resources to supplement costs of tree planting on private, agricultural (i.e., nonforested) land. Tree planting has historically increased sharply with Federal or State incentives such as the Boundary Waters Canoe Area wilderness designation in Minnesota (Reed 1997), the 1929 State Reforestation Act in New York State (Verschoor and Van Dyune 2012), and the Civilian Conservation Corps in the 1930s. Many factors, such as the global economy and changes in the forest products industry that affect stumpage values for harvested trees, influence declines in tree planting. For nonindustrial private landowners, changes in cost-share programs are often a primary determinant of tree planting and management (Hoss 2012). Large-scale shifts in demand for tree seedlings affect public and private nurseries alike.

During lean years, when demand for seedlings is low, private and State nurseries find creative solutions to maintain operations. Larger private nurseries that have a wider market share and an agile operation may focus on new markets that allow them to survive. Production at State nurseries, in contrast, is usually legislatively limited to certain species or stock types for specific uses (i.e., conservation, restoration, or reforestation). Their consumer base is also limited, often to citizens of their State, which limits their ability to expand market share. State nurseries survive because they serve functions other than growing trees for reforestation. For example, they also grow shrubs for restoration and provide free tree seedlings for students and other outreach programs. State nursery facilities are often shared with other State offices, so costs can be shared among other government functions. In addition, some State nurseries host training centers or educational facilities for students and the community on the premises. Other State nurseries have become centers for seed processing and storage for native plants and trees. Despite their intrinsic value to citizens, State nurseries across the country suffered steep fallout from the most recent recession (2008 to 2010); six State nurseries closed permanently in the past decade citing budgetary woes (California, Louisiana, Ohio, Oregon, Texas, and Utah). In the Northeast, State nursery closures have historically been permanent, and land is usually repurposed for other uses. In some States, declining seedling demands have led to consolidation of nurseries. Minnesota consolidated two nurseries into one in 2009, and Wisconsin consolidated three nurseries into one in 2016. Small, private nurseries enter and exit the marketplace with fluctuations in supply and demand and are deeply affected by downturns.

This article reports on trends in tree seedling production from 2008 to 2016 at State nurseries based on annual surveys and discusses the future supply of hardwood tree seedlings across the Northeast based on these trends.

Description of the Annual Survey

The production of tree seedlings at State nurseries across the United States has been reported annually since 2008 (private nurseries were included from 2013 onward) by the Reforestation, Nurseries, and Genetic Resources team, a program of the USDA Forest Service, State and Private Forestry. A survey, conducted by a third party, requests the number of tree seedlings shipped from all forest and conservation nurseries in the country and is collected separately for three main regions: the Northeastern Area, Southern Region, and Western Region. These data are reported annually in *Tree Planters' Notes* (TPN); details regarding the methodology and assumptions used are reported in Harper et al. (2014). For this report, we summarized data from the past 9 years (2008 to 2016) at State nurseries across the Northeast to evaluate temporal trends in seedling production. We also summarized 2016 data for both State and private nurseries. We focused on seedling production at State nurseries because, unlike private nurseries, State nurseries in the Northeast are asked to provide information on species produced in addition to the information provided for the annual report for TPN. Furthermore, we have incomplete datasets for the private sector prior to 2013. Northeastern States that lack a public nursery (Connecticut, Maine, Massachusetts, Rhode Island, and Vermont) are excluded from this summary. The Ohio State nursery closed in 2009 (Zippay 2008), but production was reported for 2008.

Main Findings: Hardwoods Versus Conifers

In general, conifers are more widely grown and planted relative to hardwood seedlings across the Northeastern nursery sector. In 2016, the number of conifer seedlings reportedly shipped by State and private nurseries in the Northeast was almost three times that of hardwoods (figure 2). Hardwood tree seedlings accounted for 22 percent of total production for private nurseries in 2016 and 31 percent of total production for State nurseries for the same year. At State nurseries, hardwood trees are primarily sold as bareroot stock, as opposed to containerized stock of any size (table 1). In contrast, roughly one-third of all hardwood seedlings at private nurseries were sold in some type of container, but the container sizes are not reported.

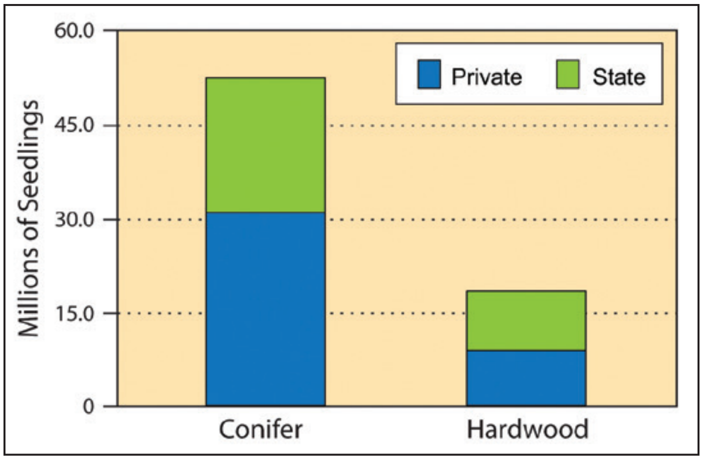

Figure 2. The total number of hardwood and conifer seedlings (bareroot + container) produced by private and State nurseries (Hernández et al. 2016).

Hardwood production also varies within the Northeast which we loosely define as three areas: Mid-Atlantic (Maryland, New Hampshire, New Jersey, New York, and Pennsylvania), Midwest (Illinois, Indiana, Iowa, Missouri, and West Virginia), and Great Lakes (Michigan, Minnesota, Ohio, and Wisconsin) Six other States in the Northeast Area are not included because they do not have State nurseries. The highest production from 2008 to 2016 occurred in the Midwest, followed by the Great Lakes and the Mid-Atlantic (figure 3). Indiana produced the most hardwood seedlings among all States (25 million), followed by Missouri (16 million) and Wisconsin (15 million; figures 3 and 4). Seedlings produced at all State nurseries, except Missouri and West Virginia, are sold only to residents of the State as mandated by State statute. From 2008 to 2016, production of hardwoods and conifers at State nurseries dropped 61 and 47 percent, respectively (figure 5).

Table 1. Survey results for hardwood tree species (bareroot and containerized seedlings) sold at private and public nurseries in the Northeastern Area during fiscal year 2016.

Nursery	Bareroot	Container
Private	6,774,026	2,109,700
Public	9,530,108	1,610
Total	16,304,134	2,111,3310

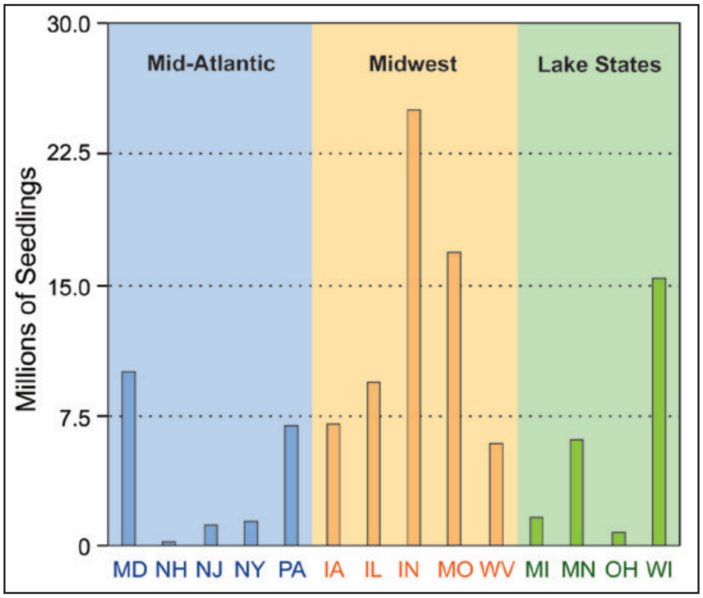

Figure 3. Total production of hardwood seedlings at State nurseries in the Northeast from 2008 to 2016.

Figure 4. Red oaks growing in a bareroot nursery bed in Wisconsin. (Photo by C. Pike, 2016)

Main Findings: Fine Hardwoods

Northern red oak had the highest total production among hardwood species during the 9-year period reported here (figures 4 and 6). The number of red oak seedlings grown at State nurseries dropped 57 percent from a peak of 3.5 million in 2008 to 1.6 million in 2016. White oak seedling production ranked second to red oak in recent years and declined 48 percent during the period. Black walnut production experienced the steepest drop (65 percent) among these four species for this period. Black cherry production is relatively small compared to oak and walnut but also dropped 46 percent, from a peak of 776,000 in 2008 to 418,000 seedlings in 2016 (figure 6).

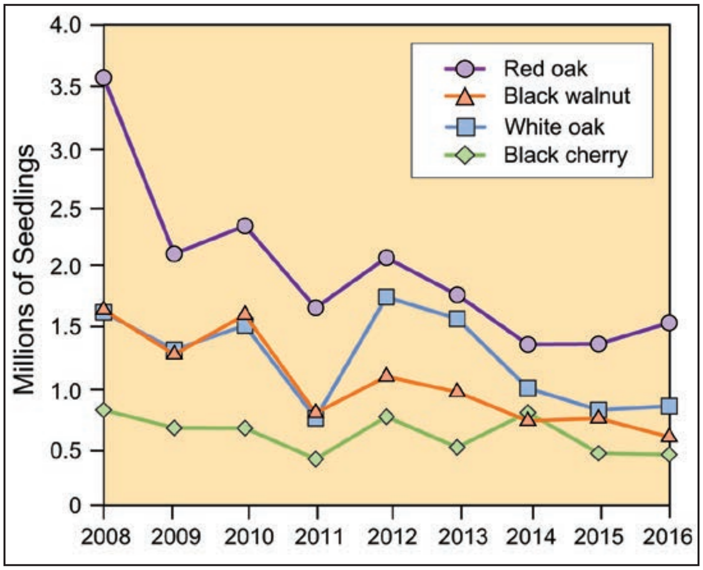

Figure 6. Annual production of four major hardwood species at State nurseries in the Northeast from 2008 to 2016.

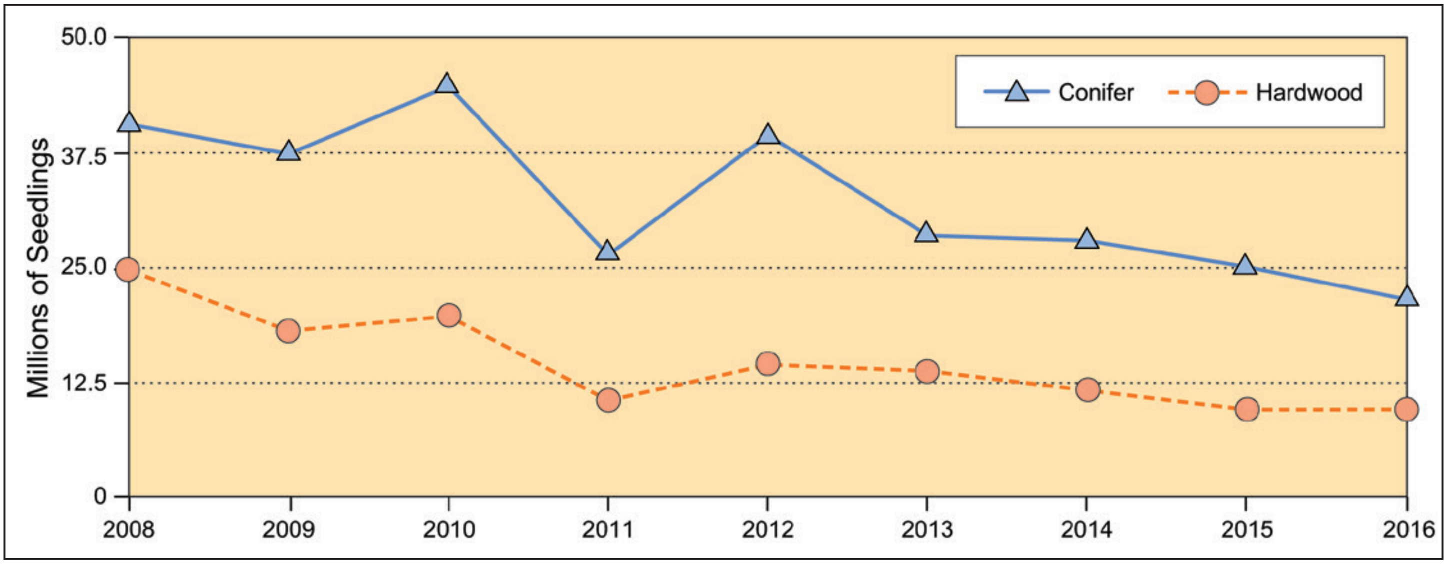

Figure 5. Total number of conifer versus hardwood seedlings shipped annually from 2008 to 2016 at State nurseries in the Northeast.

Discussion

Based on survey results, the number of seedlings produced at public nurseries in the Northeast declined during the 2008-to-2016 period, mirroring the global recession in the United States during that time. The CRP, which reimburses private, nonindustrial landowners for tree planting and management, experienced steep declines in the years prior to 2008 (McDonald 2013), which likely contributed to the substantial downfall in seedling demand in the period following the 2008 market crash.

State and private bareroot nurseries are important suppliers of hardwood seedlings for reforestation and restoration in the Northeast. State nurseries supplied more than one-half (58 percent) of all bareroot hardwood seedlings in 2016, while private nurseries produced 42 percent. Prices per bareroot hardwood seedling are relatively low, ranging from $0.30 per seedling to more than $3, whether purchased from State nurseries or from wholesale private nurseries. Bareroot hardwoods are usually sold as 1-year-old seedlings (1-0) and occasionally as 2-0, because they are comparatively large in stature relative to conifers of the same age. Containerized operations, most of which are run by private nurseries, offer a greater variety of stock ages but at a cost. As the container size increases, the price for consumers increases exponentially. The price increase is high enough that large containerized seedlings, whether hardwoods or conifers, are generally more profitable for private growers than small bareroot seedlings, but they serve different markets. Large, containerized trees (often balled and burlapped) are suitable for small-scale plantings in urban parks and residential areas but are too large (and expensive) for landscape-level reforestation or restoration projects. Both stock types, container and bareroot, are needed to supply markets in the Northeast that benefit future timber markets, create habitat for wildlife, protect waterways, and benefit urban communities.

Several nurseries in the Northeast continue to struggle to find markets for tree seedlings, suggesting that the downturn in tree planting has yet to rebound. Declines in the forest products industry have reduced seedling sales (Oswalt and Smith 2014), but past Federal cost-sharing funds (e.g., CRP) are widely cited for boosting tree planting (Alban and Dix 2013, Auer 2011, Hoss 2012). With decreasing sales (and revenue), budgets for many State nurseries were reduced to critically low thresholds, the effects of which can exacerbate a downturn over a number of years. Collectively, some State nurseries have managed to diversify their output during budgetary lean years. For example, bundles of trees and shrubs are designed for specific purposes such as wildlife habitat, fruit production, nut production, quail habitat (Hoss 2012), or riparian buffers (Alban and Dix 2013). Until demand for tree seedlings rebounds, budgets of many State nurseries will remain at critically low levels.

Nonindustrial private landowners, or family woodland owners, are the largest consumer of tree seedlings grown at State nurseries, according to a 2016 survey conducted by the National Association of State Foresters (NASF 2016). Michigan is the only State nursery in the Northeast where legislative mandates prohibit sales to private landowners. Outside of Michigan, in States with State-run nurseries, landowners are accustomed to purchasing speculatively from available inventory instead of ordering in advance. Landowners are also accustomed to placing small orders (i.e., bundles of 10, 25, 100, or as many as 500 conservation-grade seedlings). This option is available in spite of the added administrative costs of selling small quantities of trees; such small-scale orders are often too expensive to administer for private nurseries. State Soil and Water Conservation District programs do purchase large quantities of seedlings from State or private nurseries for resale to family woodland owners, providing additional outlets for tree seedlings in some States. In general, if a State nursery closes, family woodland owners may have difficulty finding private nurseries that are willing to sell small quantities of speculatively grown, conservation-grade seedlings that are local enough to be reasonably well adapted to their climate.

The fine hardwood species on which we report in this article all experienced a reduction in seedling demand from 2008 to 2016, but the decline in black walnut is particularly noteworthy. Walnut is typically planted for timber because its stumpage value can be twice as high as that of white oak or red oak (Settle et al. 2015), and to a lesser degree, for its edible nuts. It is less desirable for urban plantings because the nuts are messy, and landowners may be concerned about the effects of Juglone, an allelopathic chemical it emits that can inhibit growth of nearby plants (Jose and Gillespie 1998). Black walnut, however, is notoriously site specific and requires high inputs to produce valuable timber. Landowners who aim to improve habitat for wildlife species may favor oak and cherry, because their mast are consumed by a multitude of wildlife and require fewer inputs than walnut. As such, oak and cherry are commonly used for restoration projects with a range of management objectives. In spite of having the highest stumpage value of the fine hardwoods, the extensive inputs for management after planting, and other factors that make it less desirable for woodlands, may have disproportionately affected demand for black walnut seedlings.

Future Direction

Tree planting remains a critical strategy to enhance forest regeneration across rural and urban forests. State and private nurseries alike are key suppliers of tree seedlings to public land managers and family farms but have experienced revenue losses due to declining seedling demand. Consumers' access to conservation-grade seedlings may be compromised if nursery industries fail or State nurseries close. Wildfires, invasive species, and climate change all contribute to shifts in forest age classes across rural and urban environments and should eventually increase demand for tree seedlings. The future for seedling demand for reforestation and restoration projects on private land will also depend largely on the extent that landowner assistance programs, such as the CRP and the Environmental Quality Incentive Program, are authorized in the upcoming Farm Bill.

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Fertilization and Irrigation Effect on Botryosphaeriaceae Canker Development in Intensively Managed Sweetgum (*Liquidambar styraciflua*)

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Abstract

The influence of irrigation and fertilization on the response of sweetgum (*Liquidambar styraciflua* L.) to branch inoculation by species in the Botryosphaeriaceae family, *Botryosphaeria dothidea* (Moug.:Fr.) Ces. & De Not and *Lasiodiplodia theobromae* (Pat.) Griffon & Maubl., was tested over summer and winter seasons for 2 years. Sweetgum planted in South Carolina received irrigation, fertilization, fertilization + irrigation, or no amendments April to September each year. Both Botryosphaeriaceae inoculants caused larger cankers compared with the water agar control with the exception of one winter season, in which *L. theobromae* canker development was insignificant. The irrigation and fertilization amendments, on an individual level, did not directly affect canker development of these Botryosphaeriaceae pathogens on sweetgum; however, an interaction did occur between the amendments and season. By the summer of 2004, plots with the amendments had larger trees than plots with no amendments, leading to more crown closure. The resulting shading of inoculated branches in these plots likely contributed to larger cankers.

Introduction

Sweetgum (*Liquidambar styraciflua* L.) is commercially planted in the midcoastal plains of the Southeastern United States, from Virginia to north Florida and east Texas (Kline and Coleman 2010). A common bottomland hardwood, sweetgum grows well on a variety of sites and has relatively few pest problems (Kormanik 1990). Because of these factors and its excellent hardwood quality, the forest industry considers sweetgum to be an excellent candidate for commercial and bioenergy production (Kline and Coleman 2010).

Although sweetgum is a relatively vigorous tree with few pathogen problems, widespread damage to seedlings from stem cankers and dieback associated with species in the Botryosphaeriaceae family have occurred in nurseries and outplantings in the Southeastern United States (Carey et al. 2004, Cashion 1981, Filer and Randall 1978, Garren 1956, Neely 1968). Unfortunately, considerable past confusion regarding species taxonomy in the Botryosphaeriaceae family means the species identification from past studies must be interpreted with caution (Slippers and Wingfield 2007). Fungi isolated from cankers on sweetgum were previously identified as *Diplodia theobromae* (Pat.) Now. (Garren 1956), *Botryosphaeria ribis* Grossenbacher & Dugar, and/or *B. dothidea* (Moug.:Fr.) Ces. & De Not (Carey et al. 2004, Cashion 1981, Filer and Randall 1978, Neely 1968). *D. theobromae*, identified in 1956 by Garren as associated with sweetgum dieback, would likely be identified today as *Lasiodiplodia theobromae* (Pat.) Griffon & Maubl. (Alves et al. 2008, Goos et al. 1961). The more recent use of DNA-based molecular techniques has proven that *B. ribis* and *B. dothidea* are separate species (Smith and Stanosz 2001). The identification of *B. ribis* as the cause of cankers on sweetgum in older studies is likely accurate given that the anamorph of *B. ribis* is morphologically different than *B. dothidea*, and the authors clearly did not accept earlier attempts to synonymize these species (Slippers et al. 2004, Smith and Stanosz 2001).

In the late 1990s, Botryosphaeria canker occurred on sweetgum seedlings with extensive dieback in nurseries and outplantings near Summerville, SC (Carey et al. 2004). Isolates of *B. dothidea* from sweetgum seedlings in a Summerville, SC nursery (Collector: K. Britton) were included in the molecular and morphological differentiation of *B. dothidea* (Smith and Stanosz 2001). In 2002, predominately *B. dothidea*, and occasionally *Lasiodiplodia theobromae* (Pat.) Griffon & Maubl., were isolated from cankers on dying sweetgum seedlings at an outplanting in Olar, SC (Britton 2002). Cankers and dieback caused by *B. dothidea* and *L. theobromae* are some of the most commonly reported disease problems of woody plant species worldwide (Jurc et al. 2006, Sinclair and Lyon 2005, Slippers et al. 2004, Slippers and Wingfield 2007, Smith et al. 1996, Taylor et al. 2005). *B. dothidea* and L. theobromae are ubiquitous fungi and typically operate as endophytes and opportunistic pathogens of weakened trees (Sinclair and Lyon 2005, Slippers and Wingfield 2007). Stress factors that increase susceptibility of tree hosts to these, and other species in Botryosphaeriaceae, include other pathogens (Maloney et al. 2004), drought, freezes, defoliation (Crist and Schoeneweiss 1975, Wene and Schoeneweiss 1980), competition, and physical damage (Slippers and Wingfield 2007). Of these stress factors, drought is the most cited factor associated with Botryosphaeriaceae disease expression (Desprez-Loustau et al. 2006, Slippers and Wingfield 2007). Many studies have shown drought stress increases host susceptibility of various tree species to both *B. dothidea* (Crist and Schoeneweiss 1975, Ma et al. 2001, McPartland and Schoeneweiss 1984, Pusey 1989) and *L. theobromae* (Lewis and Van Arsdel 1978, Mullen et al. 1991).

Studies on the effects of nutrients on disease development by species in the Botryosphaeriaceae family are limited. One study utilizing young sweetgum, by Garren (1956), found a low-nutrient environment predisposed seedlings to leader dieback after inoculation with *L. theobromae* (=*D. theobromae*). In another study, a boron deficit was related to an increase in *B. ribes* in eucalyptus (*Eucalyptus citriodora* Hook.; Silveira et al. 1998). Increasing tree vigor by reducing nutrient and drought deficits may reduce the effects of Botryosphaeriaceae-related diseases (Brown and Britton 1986). In contrast, high levels of fertilizer and foliar nitrogen have been associated with increased disease development by *Diplodia pinea* (Desm.) Kickx. (Blodgett et al. 2005, Stanosz et al. 2004) and *B. dothidea* (Wilber and Williamson 2008). No studies have reported on the impact of standard fertilization on canker development from Botryosphaeriaceae fungi in sweetgum plantations.

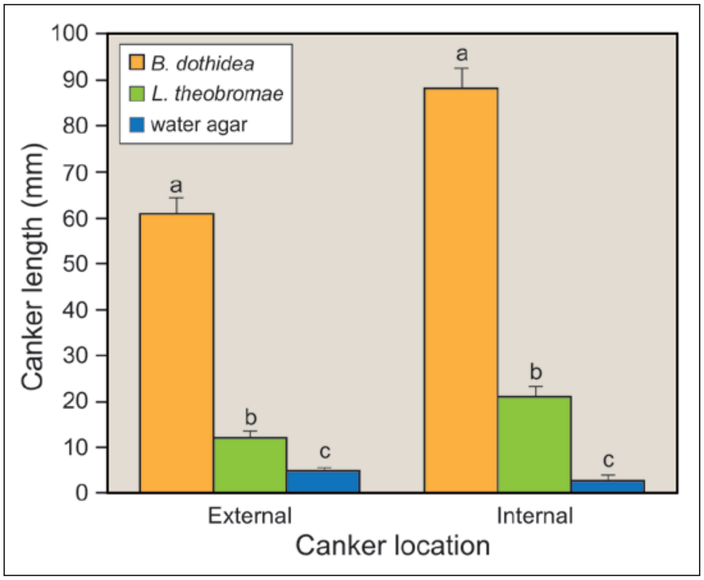

Figure 1. Mean branch canker lengths from *Botryosphaeria dothidea*, *Lasiodiplodia theobromae*, or water agar control inoculations on intensively managed sweetgum (*Liquidambar styraciflua* L.) from November 2002 to 2004. Means of canker length (exterior or interior) sharing a letter are not significantly different at a = 0.05 (Tukey-Kramer test).

Figure 2. Example of cankers on sweetgum (*Liquidambar styraciflua* L.) 6 months after inoculation with (a) *Botryosphaeria dothidea* and (b) *Lasiodiplodia theobromae*. (Photo by Michelle Cram, April 2003).

The primary objective of this study was to measure the response of young sweetgum saplings to inoculation by Botryosphaeriaceae species, *B. dothidea* and *L. theobromae*, under different irrigation and fertilizer amendments used in intensively managed forest plantations. The study also compared winter and summer inoculation to test for potential seasonal effects.

Materials and Methods

Study Site

The study was conducted at the U.S. Department of Energy's Savannah River Site, a National Environmental Research Park near Aiken, SC (33° 23' N, 81° 40' W). A detailed description of the plant material, silvicultural treatments, and experimental design can be found in Coleman et al. (2004) and Coyle et al. (2008). Prior to logging in 1999, vegetation included 14-year-old, pulp-quality loblolly pine (*Pinus taeda* L.) and 38-year-old pole-timber quality longleaf pine (*Pinus palustris* Miller), with an oak (*Quercus* sp.) understory. Following logging, all soil and debris were homogenized to a depth of 30 cm (11.8 in). Temperature and rainfall were recorded continuously during the study period by a weather station (Campbell Scientific, Inc., Logan, UT) installed in a cleared area 50 m (164 ft) west of the site. Total rainfall was 467, 829, 231, and 715 mm (18.4, 32.6, 9.1, and 28.2 in), respectively, for the winter 2002, summer 2003, winter 2003, and summer 2004 seasons. Average air temperatures for the corresponding time periods were 8.7, 21.1, 9.3, and 22.1 °C (47.7, 70.0, 48.7, and 71.8 °F).

Silvicultural Treatments

In spring 2000, open pollinated 1-0 sweetgum seedlings (Westvaco Inc., Summerville, SC) were planted in a randomized complete block design on 0.22-ha (0.54-ac) plots containing 14 rows of 21 trees (294 trees total) planted at 2.5 by 3.0 m (8.2 by 9.8 ft) spacing. Silvicultural amendment treatments were replicated in three blocks and consisted of control, fertilization, irrigation, and fertilization + irrigation. Irrigated treatments received 5.0 mm (0.2 in) water d^{-1} via an automated drip irrigation system. Liquid fertilizer was applied in 26 weekly applications, from the first of April through the end of September each year, via the drip irrigation system to fertilizer and fertilizer + irrigation treatments at 40, 40, 80, 80, and 120 kg N ha^{-1} yr^{-1} (36, 36, 71, 71, and 107 lb N ac^{-1} yr^{-1}) in 2000, 2001, 2002, 2003, and 2004, respectively. The increase of fertilizer corresponded with the increasing nutritional demand of growing trees. All plots except controls received 5.0 mm (0.2 in) water wk^{-1}, which was required to flush the drip irrigation lines. Weed control was applied equally to all plots and included an oxyflourfen (Goal® 2XL, Rohm and Haas Co., Philadelphia, PA) application prior to bud break, and glyphosate (Roundup® Pro, Monsanto Corp., St. Louis, MO) applications as needed throughout the growing season. All pesticides were applied according to label directions.

Inoculation Treatments

Botryosphaeriaceae cultures were obtained from infected sweetgum on a plantation in Olar, SC (33° 17' N, 81° 15' W) in 2002 and stored on silica gel at 20 °C (68 °F). *B. dothidea* was identified by comparing the isolate with type culture *B. dothidea* 97-23 (Smith and Stanosz 2001). The *L. theobromae* isolate, originally identified as its telemorph *Botryosphaeria rhodina* (Cooke) Arx, was verified by internal transcribed spacer sequence with the assistance of Thomas Harrington (Iowa State University, Ames, IA).

The study was conducted over four seasons: the winters of 2002 and 2003 (November to April) and the summers of 2003 and 2004 (April to November). Different subplots of trees within treatment plots were used each season. Inoculum was prepared in the laboratory by placing *B. dothidea* and *L. theobromae* isolates on fresh potato broth dextrose agar with 1 percent lactic acid. For each season, 10 sweetgum trees per silviculture treatment (4) and block (3) were systematically selected for inoculation. Each tree received all three inoculum treatments: *B. dothidea*, *L. theobromae*, and water agar control. Inoculum treatments were randomly applied on the right, center, or left branches of the south side of each tree within 1.0 to 2.0 m (3.3 to 6.6 ft) of the ground. Branches were inoculated by removing a small (3.0 to 5.0 mm [0.1 to 0.2 in] diameter) short side shoot with a sterile razor and placing a 5.0 mm (0.2 in) plug of inoculum or water agar flush on the cut branch surface. The wound was then wrapped with Parafilm®. During the study, 480 sweetgum trees were inoculated.

Measurements

For each season, branches were clipped 26 weeks after inoculation from the inoculation point to approximately 20 to 30 cm (8 to 12 in) above the treatment area, placed in plastic bags at 4.0 °C (39.2 °F), and transported to the U.S. Department of Agriculture (USDA), Forest Service Laboratory in Athens, GA. External and internal canker length was measured on all branch samples within 48 hours. Necrotic or dead tissue that developed from the inoculation was measured along the length of the branch for the external canker. The branches were then cut in half lengthwise with a flame-sterilized knife, and any internal necrotic tissue that developed from the inoculation was measured along the length of the branch for the internal canker.

Data Analysis

Canker length data were log(x + 1) transformed prior to analysis to improve normality and homogeneity of variance. Transformed data were analyzed using PROC MIXED on a split-split plot design of three inoculations by four silvicultural treatments by four seasons with replication by block (3) and estimation based on the residual (restricted) maximum likelihood method (SAS Version 8.01, SAS Institute, Inc., Cary, NC). We examined the main effects of fertilization, irrigation, season, and inoculum, and their interactions using the adjusted Tukey-Kramer test for multiple comparisons in which the experiment-wise error rate was 0.05.

Results

Season and Botryosphaeriaceae inoculum treatments interacted significantly to affect canker length (table 1). Overall, the *B. dothidea* isolate produced greater external and internal canker length than the *L. theobromae* isolate. Both species produced significantly longer cankers (figures 1 and 2) than the water agar controls with the exception of winter 2003, when the *L. theobromae* canker lengths did not differ from the water agar control (table 2). Canker lengths were greater in summer 2004 than all other seasons, but canker lengths in both winters were roughly equivalent (table 2).

Fertilization, irrigation, and the fertilization + irrigation treatments individually did not affect external and internal canker development, nor was there any interaction between inoculum and the amendments (table 1 and figure 3). Similarly, we found no fertilization by season interactions on canker lengths. An interaction between irrigation and season for external canker formation occurred (table 1). In summer 2004, the mean external cankers were larger (P = 0.0457) on irrigated trees (50.6 mm [2.0 in]) compared with those that were not irrigated (27.7 mm [1.1 in]). The internal canker length was affected by an interaction

Table 1. Effects of silvicultural amendment, season, Botryosphaeriaceae inoculum, and their interactions on external and internal canker lengths in intensively managed sweetgum. Significant statistical effects are in bold type.

Canker type	Effect[1]	df[2]	F	*P*-value
External	F	1, 6	0.00	0.9708
	I	1, 6	1.39	0.2826
	FI	1, 6	0.03	0.8698
	S	3, 24	23.44	**<0.0001**
	F × S	3, 24	2.17	0.1180
	I × S	3, 24	4.44	**0.0128**
	FI × S	3, 24	2.32	0.1003
	IN	2, 64	347.40	**<0.0001**
	F × IN	2, 64	0.66	0.5216
	I × IN	2, 64	0.38	0.6851
	FI × IN	2, 64	1.60	0.2104
	S × IN	6, 64	7.32	**<0.0001**
	F × S × IN	6, 64	1.67	0.1437
	I × S × IN	6, 64	0.39	0.8807
	FI × S × IN	6, 64	0.42	0.8603
Internal	F	1, 8	0.39	0.5478
	I	1, 8	0.53	0.4868
	FI	1, 8	0.09	0.7665
	S	3, 88	27.72	**<0.0001**
	F × S	3, 88	0.16	0.9249
	I × S	3, 88	0.99	0.4016
	FI × S	3, 88	3.09	**0.0311**
	IN	2, 88	347.48	**<0.0001**
	F × IN	2, 88	1.05	0.3528
	I × IN	2, 88	0.29	0.7464
	FI × IN	2, 88	0.20	0.8210
	S × IN	6, 88	20.23	**<0.0001**
	F × S × IN	6, 88	1.22	0.3048
	I × S × IN	6, 88	1.44	0.2070
	FI × S × IN	6, 88	0.73	0.6301

[1] F=fertilization; I=irrigation; FI=fertilization + irrigation; S=season; IN=inoculum.
[2] df= degrees of freedom

Table 2. Mean (±SE) external and internal canker length on intensively managed sweetgum over four seasons in South Carolina.*

Season**	Treatment	External stem canker length (mm) (±SE)	Internal stem canker length (mm) (±SE)
Winter 2002	*B. dothidea*	70.6 (± 6.4)a	65.3 (± 7.0)a
	L. theobromae	12.3 (± 0.8)b	19.6 (± 2.3)b
	Water agar control	3.9 (± 0.5)c	0.5 (± 0.2)c
Summer 2003	*B. dothidea*	29.8 (± 3.2)a	72.4 (± 7.5)a
	L. theobromae	5.3 (± 0.3)b	5.0 (± 1.3)b
	Water agar control	2.8 (± 0.2)c	0.9 (± 0.1)c
Winter 2003	*B. dothidea*	54.7 (± 7.9)a	62.8 (± 8.8)a
	L. theobromae	7.7 (± 0.3)b	3.6 (± 0.5)b
	Water agar control	7.6 (± 1.7)b	4.7 (± 1.7)b
Summer 2004	*B. dothidea*	88.6 (± 8.4)a	152.0 (± 9.2)a
	L. theobromae	23.4 (± 4.8)b	57.0 (± 6.7)b
	Water agar control	5.5 (± 0.5)c	6.1 (± 3.3)c

SE = standard error.

* Means by season that share a letter are not significantly different at $\alpha = 0.05$ (Turkey-Kramer test).

** Winter timeframes were from November to April; summer timeframes were from April to November.

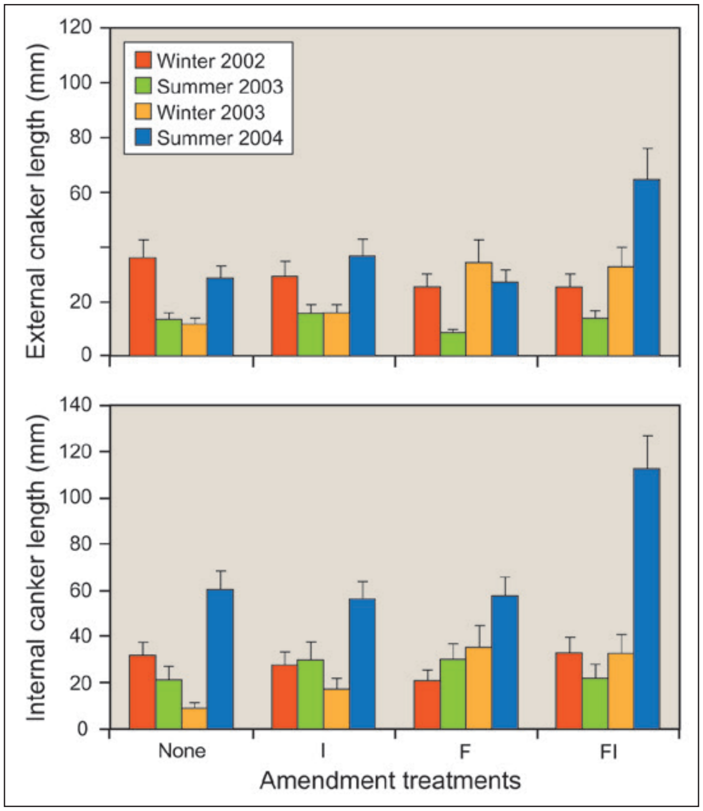

Figure 3. Mean external and internal canker lengths on sweetgum *(Liquidambar styraciflua* L.) by silvicultural amendment (I = irrigation, F = fertilization) and season of inoculation.

between fertilization, irrigation, and season (table 1), but the Tukey-Kramer test for multiple comparisons was unable to separate out individual significant interactions. Internal canker length was similar among treatments within a season, with the exception of the 2004 summer season when lengths were significantly longer, especially for those in the fertilization + irrigation silvicultural treatment (figure 3).

Discussion

The Botryosphaeriaceae isolates used in this study were found to be pathogenic on sweetgum and able to produce cankers regardless of silvicultural amendments or season of inoculation. Previous Botryosphaeriaceae inoculation tests on sweetgum seedlings and saplings have predominately used *B. ribis* Grossenb. & Duggar (Filer and Randall 1978, Neely 1968, Toole 1963). A coppice test using sweetgum seedlings infected with *B. dothidea* led to natural infection of subsequent coppice sprouts 6 months later but no canker symptoms (Cashion 1981). It is likely that *L. theobromae* was the species utilized by Garren (1956) that was found to cause dieback under certain conditions, including when grown in a low-mineral medium. The only other test of *Botryosphaeria* on sweetgum was an unidentified isolate that had no effect on seedlings (Carey et al. 2004). Our inoculations of sweetgum indicate that *B. dothidea* and *L. theobromae* are capable of causing areas of necrotic tissue in sweetgum within 6 months of inoculation. This result is not surprising given that inoculation studies with these Botryosphaeriaceae species also cause cankers on many other tree species (Brown and Hendrix 1981, Chen et al. 2014, Lewis and Van Arsdel 1978, Michailides 1991, Mullen et al. 1991, Peterson 1976, Pusey et al. 1986, Úrbez-Torres et al. 2013). The dieback of sweetgum seedlings in the Olar, SC plantation, where the *B. dothidea* and *L. theobromae* isolates were originally obtained, was likely affected by the presence of these Botryosphaeriaceae species. The report that most of the cankers on dying seedlings at the Olar plantation were associated with *B. dothidea* is likely due to the greater pathogenicity of *B. dothidea* compared with *L. theobromae*, as indicated by our inoculation trials.

Both *B. dothidea* and *L. theobromae* are able to operate as endophytes and latent pathogens affecting a broad range of hosts and geographic areas (Marsberg et al. 2017, Mohali et al. 2005). Although many inoculation studies with these species show that cankers can be induced without the host under stress, the size and number of Botryosphaeriaceae cankers is known to increase with stress conditions (Crist and Schoeneweiss 1975, Lewis and Van Arsdel 1978, Mullen et al. 1991, Pusey 1989). The irrigation and fertilizer applied during this study was designed to meet tree nutritional and moisture needs, with a goal of maximizing tree growth each year (Coleman et al. 2004). Rainfall records at the study site from 2002 to 2004 indicated that both summer and winter seasons received approximately the same amount of rainfall and were not classified as drought conditions according to the Palmer drought index (Palmer 1965). Overall, the addition of irrigation under nondrought conditions either had no effect, or a minimal effect, on sweetgum response to inoculation by Botryosphaeriaceae. Also, no stress factor was related to a nutrition deficit or excessive nitrogen that would lead to increased cankers. The optimization of nutrient and moisture had no direct positive or negative affect on canker size; however, some amendment-by-season interactions led to an increased canker length.

Interactions between amendment and season were associated with increased canker lengths that developed in the summer of 2004. During the final inoculum test period, the sweetgum trees were approaching 4 years in age and ranged from 5.2 to 6.7 m (17.0 to 22.0 ft) in height (Coyle et al. 2008). Young sweetgum trees have a conical growth shape and lower branches on the trees were beginning to touch adjacent trees in the planting. This additional shading may be the cause of the increase in canker lengths found during the summer 2004 season, especially in the internal cankers, compared with the previous seasons (figure 3). The fertilization + irrigation treatment had visibly more crown closure (figure 4), with the greater diameter, height, and aboveground biomass after the 2003 growth season (Coyle et al. 2008). This result, coupled with no clear differences in canker development during 2002 to 2003 in the fertilization + irrigation treatment, indicates that

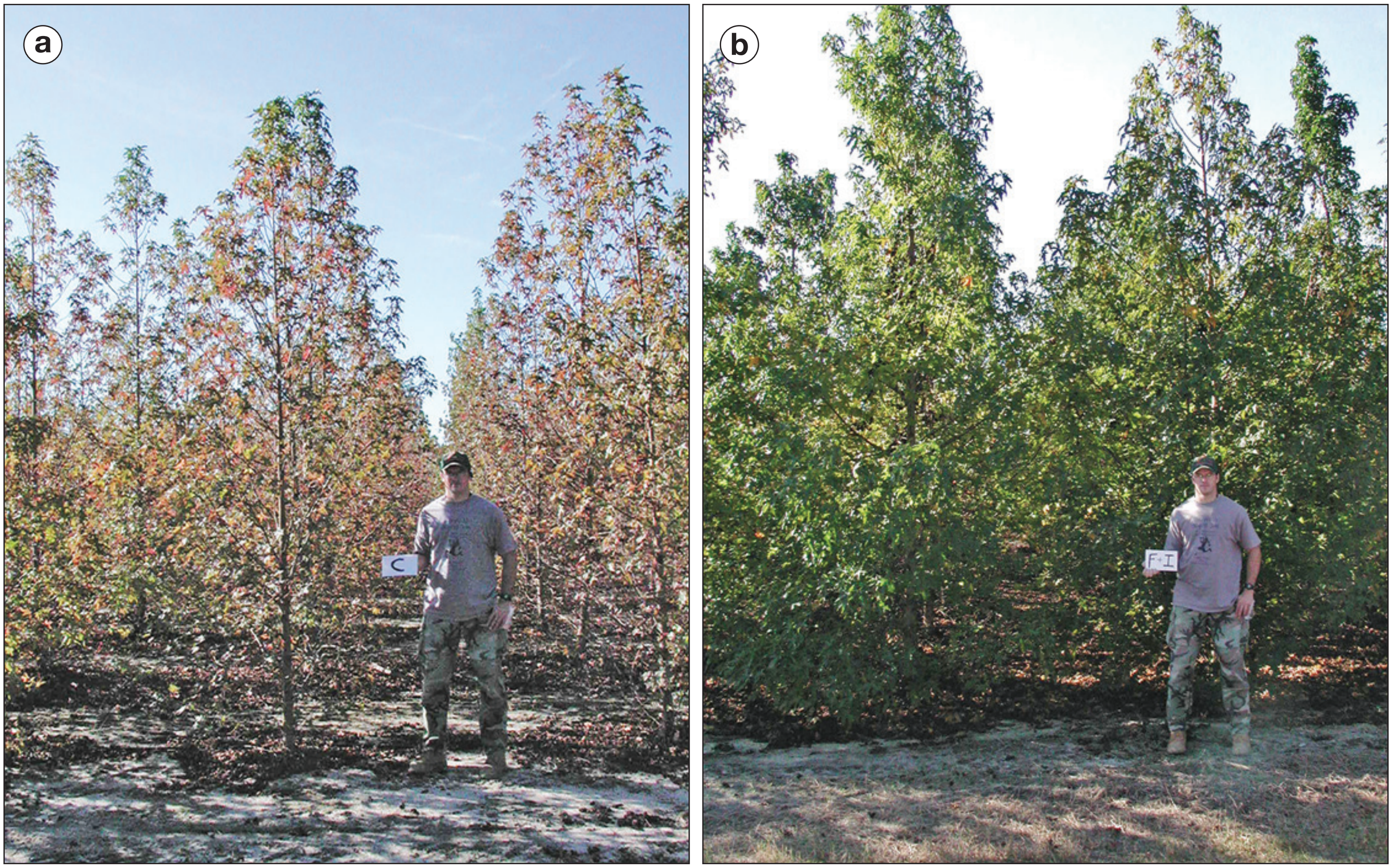

Figure 4. Photos of sweetgum (*Liquidambar styraciflua* L.) plots (a) without amendments and (b) with fertilization + irrigation amendments. (Photos by Michelle Cram, November 2003).

crown closure and shading of inoculated branches in 2004 may have affected canker development. The competition for light of the inoculated branches in the lower canopy is a stress factor that would allow for these pathogens to expand faster (Slippers and Wingfield 2007).

Internal canker length from the *B. dothidea* inoculation tended to exceed external canker length. These results are similar to Brown and Hendrix (1981), in which *B. dothidea* growth was rapid once established in the xylem vessels of apple stems. Brown and Hendrix (1981) also found no differences in *B. dothidea* growth between 15 and 35 °C. This ability of *B. dothidea* to grow under this wide range of temperatures would allow for internal canker growth to continue throughout the winter in the Southern United States. The slowed growth of the exterior canker may be in part due to proliferation of parenchyma cells, which can separate the healthy tissue from the infected tissue (Brown and Hendrix 1981).

Our study indicates that Botryosphaeriaceae species *B. dothidea* and *L. theobromae* are pathogenic on sweetgum and could contribute to dieback of sweetgum seedlings. Results also suggest that these pathogens could accelerate self-pruning of lower canopy branches. The lack of a direct effect by irrigation or fertilization treatments on the canker development is likely due to the lack of a stress-inducing drought period or a nutrient deficiency on our study site. Under normal growing conditions, the application of silvicultural amendments to optimize tree growth does not appear to directly influence Botryosphaeriaceae canker development. To determine if drought or a nutrient deficiency would have an effect on canker development in sweetgum with these Botryosphaeriaceae species would require controlled tests with these stress factors.

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Effects of Native Arbuscular Mycorrhizae Inoculation on the Growth of *Argania spinosa* L. Seedlings

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Abstract

The objective of this study was to assess the effects of native arbuscular mycorrhizal fungi (AMF) harvested from two argan *(Argania spinosa* [L.] Skeels) stands in southwest Morocco (Bouyzakarne and Argana) on the growth of argan seedlings under nursery conditions. Results confirm the strong dependence of argan tree on native AMF for the improvement of seedling quality in nurseries. Native AMF from the two different argan stands differed in their efficiency; inoculum from Bouyzakarne was more efficient than that from Argana and could therefore be used in inoculation programs for the production of argan seedlings in forest nurseries.

Introduction

Argania spinosa (L.) Skeels is an endemic species of Morocco and is part of the United Nations Educational, Scientific and Cultural Organization, or UNESCO, World Heritage since 1998 (Tazi et al. 2003). Argan stands represent an exceptional natural environment and a unique agricultural land. The tree has been widely used in scientific research, as a source of food, and in the development of pharmaceuticals and cosmetic products.

In Morocco, argan trees cover an estimated area of 828,000 ha (M'Hirit et al. 1998). Argan ecosystems are severely threatened by climate change (long periods of drought) and by increasing demographic pressures (collection of fruits, tillage, development of irrigated agriculture, removal of woody materials, browsing, and overgrazing). These pressures are causing a decline of the argan ecosystem (Nouaim et al. 1991). During the last century, about 50 percent of the argan area in Morocco was lost (600 ha/year), and average density decreased from 100 to 30 trees/ha (Abourouh 2007).

Biodiversity and the relationships among organisms are the basis of ecosystem stability, productivity, and resiliency. Morocco has been engaged, as a part of the Moroccan Green Plan, to undertake important steps toward sustainable management of its natural resources. Artificial forest regeneration success in Morocco, however, is currently below 20 percent (M'Hirit and Benchekroun 2006). Conditions of the natural environment are constantly changing and require adjustments in nursery cultural practices to address the shortcomings of outplanted seedlings observed in the field and to improve reforestation success.

In Moroccan argan stands, soils are mostly disturbed and degraded, which can lead to fragmentation of the ecological niche of argan trees and a low mycorrhizal inoculum potential of soils. Mycorrhizal symbiosis is known for improving growth and water nutrition in a range of plant species (Abbas 2014, El Mrabet et al. 2014, Ouahmane et al. 2007a, Smith and Read 2008), and the use of mycorrhizal inoculation in dry areas has been recommended for the development of sustainable farming practices (Duponnois et al. 2011). In the Souss-Massa region (southwest Morocco), argan trees form endomycorrhizal associations mainly with *Glomus* spp. due to its high sporulation rate and strong adaptation to the region's soil and climate (Achouri 1989). In fact, arbuscular mycorrhizal fungi (AMF) belonging to the phylum Glomeromycota are the most widespread group of symbiotic fungi, with 80 percent of land plants forming AMF symbiosis (Smith and Read 2008).

In forest nurseries, argan plants tend to have poorly developed root systems and are, therefore, unable to tolerate drought conditions in the field after planting (Bousselmame et al. 2002). Inoculation of argan seedlings with native mycorrhizae is a potential strategy to improve argan plant quality for reforestation programs in arid and semi-arid areas (Duponnois et al. 2011). The fungal symbiosis improves water and nutrient uptake of argan species and contributes to improved field establishment, particularly in the first months after planting (Echairi et al. 2008; El Mrabet et al. 2014; Nouaim and Chaussod 1994, 1997). These benefits of mycorrhizal root systems are primarily due to the extension of the absorptive surface and the volume of soil explored by fungal hyphae. In a recent study, inoculation with native mycorrhizae positively affected height, basal diameter, biomass, nitrogen (N), and phosphorus (P) concentrations of nursery-grown argan seedlings (El Mrabet et al. 2014). Similar results have been found for other forest species (Boutekrabt et al. 1990, Ouahmane et al. 2007b, Requena et al. 2001).

The mycorrhizal effect on reforestation success is highly dependent on soil and climate origin of the fungal inoculants selected (Abbas 2014). It is, therefore, crucial to examine if endemic sources of symbiotic microorganisms from different argan stands can support and improve the regeneration of argan trees. If they do, then this strategy will not only benefit forest managers but will also support the ambitious program launched by the National Agency for Development of Oasis Areas (ANDZOA) in Morocco to increase argan tree establishment to 5,000 ha by 2020. The objective of this study was to assess the effects of mycorrhizae inocula harvested from two native argan soils in southwest Morocco on the growth of argan trees under nursery conditions.

Material and Methods

Sampling Sites

Two argan forest stands in southwest Morocco (Bouyzakarne and Argana in Guelmim province and Taroudant prefecture, respectively) were selected for this study. These forest stands represent degraded (Bouyzakarne) and nondegraded (Argana) sites (figure 1). The climate of these regions is Mediterranean arid. Table 1 shows some ecological characteristics of these sites.

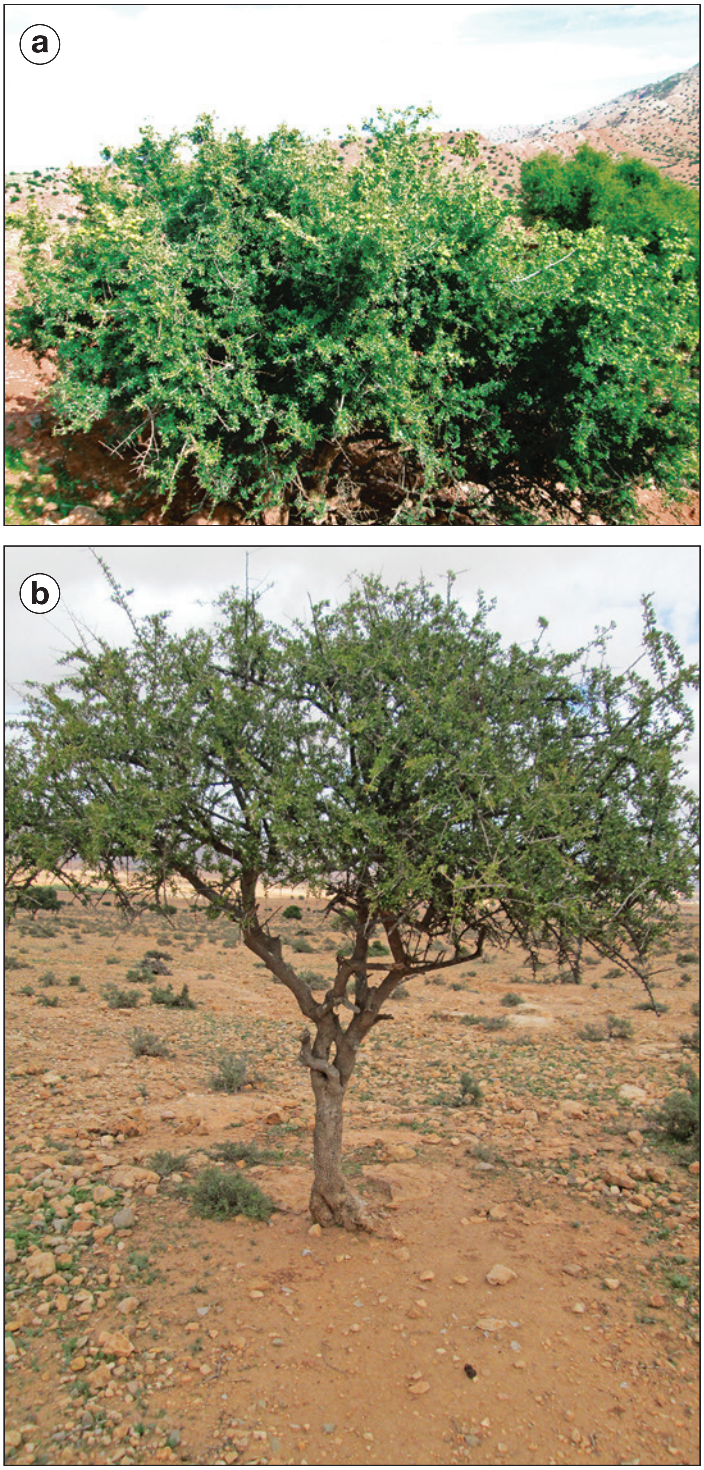

Figure1. (a) Argana and (b) Bouyzakarne stands from which mycorrhizal inoculant was collected for production of seedlings in this study. (Photos by Imane Ouallal, 2013)

Table 1. Ecological characteristics of the two argan sites from which native mycorrhizae were sampled.

Site	Coordinates	Province	Altitude (m)	Tree density /ha	Use	Average annual rainfall (mm)
Bouyzakarne	N 29.1879 W 009.7421	Guelmim-Es Semara	690	5	Pasture	120
Argana	N 30.7544 W 009.1545	Taroudant	735	30	Pasture	226

Soil and Root Sampling

From each forest stand, soil was sampled from the rhizosphere of five mature argan trees representative of the stand. Each sample (1 to 2 kg/tree) was a mixture of six subsamples taken from around each tree at a depth of 20 to 40 cm. Very fine roots, more likely to be colonized and easy to observe under the microscope, were collected at the same time as the soil. The samples were taken in early February 2013 (before the dry season) when the highest microbial activity is expected. The soils were placed in plastic bags and roots were placed in a 1:1:1 glycerol, distilled water, and ethanol solution and stored for 0 to 3 months at 4 °C.

Physical and Chemical Soil Analyses

Physicochemical characteristics of the soil collected at each forest stand were assessed in the soil analysis laboratory at the Forestry Research Centre of Rabat (Morocco) using conventional methods (pH: electrometric method; texture: decantation method; available phosphorous: Olson et al. [1954] method; and organic carbon: Anne [1945] method). Table 2 presents the results of the soil analyses. The soils were sandy and neutral to slightly alkaline at both sites. The Argana soils had good organic matter content (7.88 percent) compared with low organic matter (1.86 percent) found in the Bouyzakarne soil. Similarly, available P was moderate (50 ppm) in the Argana soil and low (27 ppm) in the Bouyzakarne soil.

Mycorrhizae Spore Extraction and Identification

Mycorrhizae spores were extracted by wet sieving as described by Gerdemann and Nicolson (1963). In a 1 L beaker, 100 g of soil was mixed with 0.5 L of tap water and stirred for 1 minute. After 30 seconds of settling, the supernatant (liquid portion above the sediment) was passed through four superimposed sieves with decreasing mesh sizes (500, 200, 80, and 50 µm). This operation was repeated two times. The subsamples from the 200, 80, and 50 µm screens were divided into two tubes and centrifuged for 4 minutes at 5,000 rpm. The supernatant was then discarded, and a viscosity gradient was created by adding 20 ml of 60 percent sucrose solution to each centrifuge tube (Walker et al. 1982). The mixture was quickly stirred, and the tubes were placed into a centrifuge for 10 minutes at 1,000 rpm. Unlike the first centrifugation process, in this step, the supernatant was poured into the sieve mesh of 50 µm, and the substrate was rinsed with distilled water to remove the sucrose. The mycorrhizae spores were then recovered with 5 ml distilled water in an Erlenmeyer flask. Spore identification was based on morphological features: color, size, cell wall structure, and hyphal attachment (INVAM 1997, Morton and Benny 1990). Morphotypes were classified to the genus level and, when possible, to the species level. Endomycorrhizal spores were quantified to estimate the appearance frequency of each species (AFS) and genus (AFG) in 100 g of soil.

Table 2. Soil physical and chemical properties from each argan site (n=5).

Site	Clay (%)	Fine silt (%)	Course silt (%)	Fine sand (%)	Course sand (%)
Bouyzakarne	13.6±0.41	21.7±0.16	10.5±0.78	20.5±1.11	33.8±2.63
Argana	9.6±0.63	21.7±1.02	6.01±0.65	19.75±0.43	40.0±1.15
	pH_{water}	pH_{KCl}	C (%)	Mo (%)	Assimilable Phosphous (ppm)
Bouyzakarne	7.43±0.45	6.0±0.25	1.085±0.96	1.867±0.96	27.0±3.51
Argana	7.75±0.68	4.0±0.13	4.583±1.16	7.883±1.16	50.36±4.09

AFS indicates the percentage of a species relative to other species: AFS = ns/nT × 100.

Where—

ns = number of isolated spores of species X.

nT = total number of spores.

AFG indicates the percentage of one genus relative to other genera: AFG = nG/nT × 100.

Where—

nG = number of spores of the genus X.

nT = total number of spores.

Endomycorrhizal Inoculum Production

Barley (*Hordeum vulgare* L.) was used as an endophytic plant for the production of endomycorrhizal inoculum. Barley seeds were disinfected in a 30 percent H_2O_2 solution for 30 minutes and rinsed several times with sterile distilled water and then planted for 3 months in plastic pots containing the soil collected from degraded (Bouyzakarne) and nondegraded (Argana) argan forests. The barley roots were then harvested to use as inoculum material for production of argan seedlings.

Plant Material and Inoculation

The argan seeds were cleaned and surface disinfected in a 5-percent bleach solution, then rinsed and soaked in warm water for 48 hours. Seeds were then transferred into plastic bags filled with black peat and germinated in the dark at 25 °C for 6 days. The bags were sprayed with water every 2 days. Germinants were transplanted to containers when rootlets were 1- to 2-cm long.

Seedlings were individually transplanted to "WM" shaped containers (3,000 cm^3 volume, 25 cm height, and 12 cm length by 10 cm width) containing a mixture of black peat and sterile sandy soil (1:1 by volume). Fifty seedlings were used as a noninoculated control, 50 seedlings were inoculated with inoculum from the Argana site, and the remaining 50 seedlings were inoculated with inoculum from the Bouyzakarne site. In pots designated for inoculation, a thin layer of mycorrhizal barley roots (about 10 g) was placed just under the radical of the transplanted argan seedlings before transplanting. All plants were kept in a greenhouse and watered every 2 days. The pots were labeled and randomly arranged (figure 2).

Figure 2. Argan seedlings were potted with inocula from two native argan stands or were noninoculated. (Photo by Imane Ouallal, 2014)

Plant Growth and Colonization

Seedling height and stem diameter were measured on all plants 3, 6, and 10 months after inoculation. In addition, shoot and root fresh and dry biomasses were measured on five plants chosen randomly for each inoculant treatment (control, Argana, and Bouyzakarne) on the above dates. The relative mycorrhizal dependency index (RMDI) estimates the need for a plant to be mycorrhizal to achieve maximum growth in a given situation. RMDI was calculated for each of the mycorrhizae treatments from the average dry weight values of mycorrhizal (DWM) plants and nonmycorrhizal (DWNM) control plants as described by Plenchette et al. (1983).

$$RMDI = [(DWM - DWNM)/DWM] * 100.$$

A sample of root fragments from 30 plants of each treatment were rinsed with tap water, clarified, and stained according to the method of Phillips and Hayman (1970). This method involves cutting roots into 1- to 2-cm long segments, submerging them in a solution of 10 percent potassium hydroxide for 45 minutes at 90 ºC, then rinsing them again in cold tap water. Root samples with excess pigment were submerged in 10 percent H_2O_2 to remove the tannin, then rinsed with water again. Root segments were then placed in test tubes containing 100 ml of distilled water and 0.05 g of Trypan blue and incubated at 90 °C for 15 minutes.

The arbuscule (A) content and vesicle (V) content were measured by assigning an index of mycorrhization from 0 to 5 (Derkowska et al. 2008). The method allows for determination of mycorrhizal frequency (MF) and mycorrhizal intensity (MI). Thirty stained root fragments per root sample from each treatment were mounted on an optical microscope slide and assessed under 40X magnification. The mycorrhizal parameters were calculated using Mycocalc software (http://www2. dijon.inra.fr/mychintec/Mycocalc-prg/MYCOCALC.EXE).

MF reflects the colonization percentage of the root system: $MF = 100 \times (N - n0)/N$.

Where—

N = total number of root fragments.

$n0$ = number of nonmycorrhizal root fragments.

MI estimates the proportion of colonized cortex in the root system:

$MI = (95n5 + 70n4 + 30n3 + 5n2 + n1)/N$.

Where—

n = number of fragments with the index 0, 1 2, 3, 4, or 5 of colonization

(according to the scale developed by Derkowska et al. [2008] as follows: n1 = trace; n2 = less than 10 percent; n3 = 11 to 50 percent; n4 = 51 to 90 percent; and n5 = more than 90 percent).

N = total number of root fragments.

A estimates the proportion of the root cortex containing arbuscules:

$A = (100\ mA_3 + 50\ mA_2 + 10\ mA_1)/100$.

Where (using the n and N numbers determined above for MI)—

$mA = (95\ n5A + 70\ n4A + 30\ n3A + 5\ n2A + n1A)/N$.

A = abundance of arbuscules (A_3: 51 to 100 percent; A_2: 11 to 50 percent; A_1: 1 to 10 percent).

nA denotes the number of root fragments for a given n and A (e. g., $n4A_3$ is the number of fragments denoted 4 with A_3).

V estimates the proportion of the root cortex containing vesicles:

$V = (100\ mV_3 + 50\ mV_2 + 10\ mV_1)/100$.

Where (using the n and N numbers determined above for MI)—

$mV = (95\ n5V + 70\ n4V + 30\ n3V + 5\ n2V + n1V)/N$.

V = abundance of vesicles (V_3: 51 to 100 percent; V_2: 11 to 50 percent; V_1: 1 to 10 percent).

nV denotes the number of root fragments for a given n and V (e.g., $n4V_3$ is the number of fragments denoted 4 with V_3).

Statistical Analyses

Normality and homogeneity of variance for all variables were checked. Variables that did not conform to the requirements for parametric tests were log or square-root transformed prior to all analyses (Quinn and Keough 2002, Underwood 1996, Zar 1984). All data were analyzed with SAS software using the Analysis of Variance, or ANOVA, technique, and mean comparisons among treatments were determined using Fisher's least significant difference at the 0.5 level.

Results

Spore Density and Identity

The number of spores per 100 g of dry soil was 57 percent higher on samples from the Bouyzakarne site compared with those from the Argana site (table

Table 3. Spore density and mycorrhizae morphotypes found in soil samples from the two argan sites.

Sites	Number of spores/100 g of dry soil	Morphotypes
Bouyzakarne	88	– *Rhizophagus aggregatus* – *Septoglomus constrictum* – *Glomus* sp. 1 – *Glomus* sp. 2 – *Scutellospora* sp
Argana	56	– *Rhizophagus aggregatus* – *Septoglomus constrictum* – *Glomus* sp. 1 – *Glomus* sp. 2

3). Spore isolation revealed the presence of at least five morphotypes in Bouyzakarne, belonging to the *Glomus* and *Scutellospra* genus and four morphotypes in Argana, belonging only to the *Glomus* genus (table 3). AFS was highest for *Rhizophagus aggregatus* and *Septoglomus constrictum*, and AFG was highest for *Glomus* (table 4).

Plant Growth

Inoculation with native AMF significantly enhanced growth of argan seedlings compared with noninoculated seedlings on every sample date ($p<0.0001$; figures 3, 4, and 5). Additionally, the root-to-shoot ratio for inoculated seedlings was greater for inoculated seedlings compared with control seedlings (figure 5). Seedlings inoculated with mycorrhizae from the Bouyzakarne site tended to be larger compared with those inoculated with mycorrhizae from the Argana site (figures 4 and 5). RMDI was 52.2 and 49.3 percent, respectively, for seedlings inoculated with inoculum from the Bouyzakarne and Argana sites.

Native Mycorrhizal Colonization

Microscopic examination of argan root fragments showed that all samples were densely colonized by AMF with an MF of 100 percent (table 5). MI, V, and A were all significantly higher in roots colonized with inoculum from the Bouyzakarne site compared with those colonized with inoculum from the Argana site (table 5).

Discussion

The success of reforestation and afforestation strategies is strongly dependent on seedling quality. The ecological protocol developed for the current study was based on the formulation of a mycorrhizal inoculum generated from native AMF fungi by colonizing mature argan tree roots. Studies showed that inoculation with selected strains of AMF stimulated the growth of argan

Figure 3. Seedlings inoculated with native mycorrhizae from the (left) Argana or (middle) Bouyzakarne stands grew more than (right) those that were not inoculated. (Photo by Imane Ouallal, 2015)

Table 4. Appearance frequency of mycorrhiza species and genera found in soil samples collected from the Argana and Bouyzakarne sites. Note: *Rhizophagus* and *Septoglomus* have new nomenclature and were formally considered *Glomus* species.

Appearance frequency (%)	*Rhizophagus aggregatus*	*Septoglomus constrictum*	*Glomus* sp. 1	*Glomus* sp.2	*Scutellospora* sp.
Species	32.12	30.55	19	10.03	8.3
Genus	91.7				8.3

plants and improved their nutrient status in nursery conditions (Bousslmame et al. 2002). The use of native fungi is highly recommended as an effective strategy for efficient mycorrhizal inoculation in natural ecosystems (Caravaca et al. 2003a, Duponnois et al. 2011, Johnson et al. 2010, Manaut et al. 2015, Ouahmane et al. 2007a, Requena et al. 2001). Caravaca et al. (2003b) noted that indigenous AMF may be a preferential inoculation strategy compared with commercial inocula to guarantee establishment success of native shrub species in a semi-arid degraded soil.

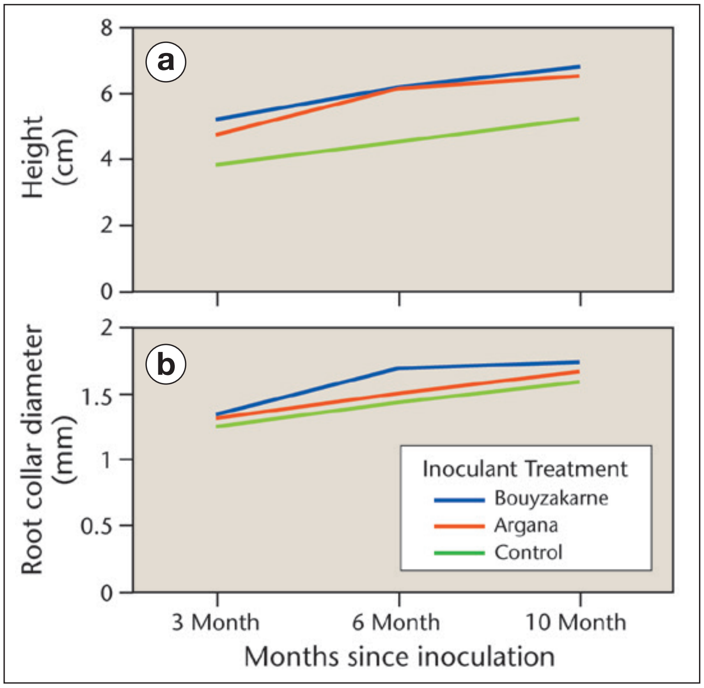

Figure 4. Effect of two native mycorrhizal inoculant treatments on (a) height and (b) stem diameter development of argan seedlings compared with noninoculated control seedlings.

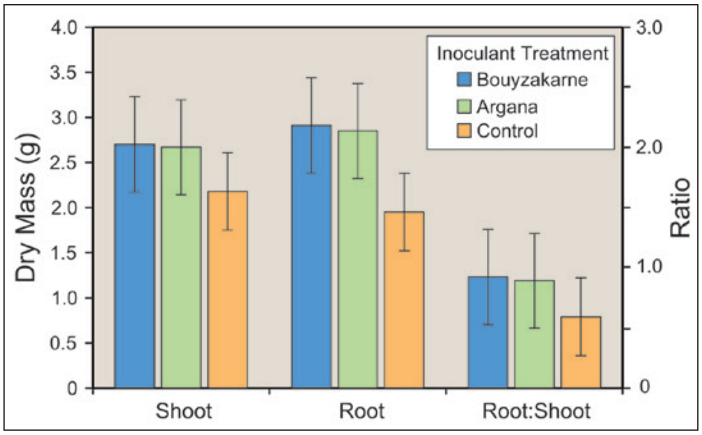

Figure 5. Effect of two native mycorrhizal inoculant treatments on argan seedling biomass development compared with noninoculated control seedlings after 10 months.

All root samples of *Argania spinosa* in this study had endomycorrhizal structures present (vesicles, arbuscules, hyphae), demonstrating the argan species' receptivity to colonization and its dependence on AMF. The presence of these endomycorrhizal structures suggests that argan should be classified as a mycotrophic species (Bousslmame et al. 2002). Nouaim and Chaussod (1994) found that the argan tree is very dependent on mycorrhizal symbiosis with an RMDI of 80 percent, the highest value known for a tree. In our study, RMDI was 52.2 and 49.3 percent, respectively, in response to inocula from the Bouyzakarne and Argana sites.

Both mycorrhizal complexes stimulated argan seedling growth compared with noninoculated control seedlings. El Mrabet et al. (2014) also found that inoculation of argan plants with a native endomycorrhizal inoculum (from a preserved argan forest in the Mesguina mountain at Agadir in southwestern Morocco) resulted in better development of the inoculated plants compared with the controls. This is likely attributable to improved mineral nutrition of AMF-inoculated plants compared with noncolonized plants (Oihabi and Meddich 1996, Ouahmane et al. 2012, Plenchette and Strullu 1996). Inoculation of argan plants by AMF stimulates absorption of macronutrients, especially phosphorus, potassium, and calcium, as well as micronutrients, in particular manganese and copper. Smith and Gianinazzi-Pearson (1988) showed that endomycorrhizal symbiosis favors phosphate uptake. Improved mineral nutrition results in increased biomass production (Bousselmame et al. 2002). Strullu (1991) attributed this effect to the exploration of the fungus' hyphae to a large volume of the substrate, effectively increasing the surface area for exchange and assimilation of minerals in favor of the host. Overall, AMF colonization has a positive influence on growth parameters of many plant species (Dag et al. 2009, Pasqualini et al. 2007, Shokri and Maadi 2009).

Table 5. Mycorrhizal colonization for each of the native inocula treatments (n=30).

Treatment	Mycorrhizal frequency (MF; %)	Mycorrhizal intensity (MI; %)	Vesicle content (V; %)	Arbuscular content (A; %)
Bouyzakarne	100±0	90. 11±2. 87	51. 12±0. 98	32. 14±1. 49
Argana	100±0	76, 14±3. 06	39. 93±2. 65	15. 21±0. 19

The root-to-shoot ratio was higher in inoculated argan seedlings compared with control seedlings. The higher ratio gives plants a better ability to access and uptake water and nutrients, thereby increasing their capacity to withstand abiotic stress, including stresses associated with transplanting (Caravaca et al. 2003a) and salinity (Rinaldelli and Mancuso 1996). Tobar et al. (1994) demonstrated that root system development reflects the degree of AMF efficiency. AMF colonization of subterranean clover (*Trifolium subterraneum* L.) increased the absorptive surface of the root system (root-hair density) and the soil volume that could be explored by the root system, as well as increased P uptake (Hill et al. 2010). Smith and Read (1997) suggest that mycorrhizal symbiosis can improve the quality of the plant root system and, in turn, increase plant survival in the field. Guissou et al. (1998) found that mycorrhizal colonization not only improved stress tolerance in fruit trees but stimulated their growth and mineral nutrition as well.

We found that the inoculation effect of the two native mycorrhizae treatments on biomass, height, and stem diameter was positively correlated to vesicle and arbuscular content, primary points of nutrient exchange between the two symbiotic partners. The arbuscular content of plants inoculated with fungal isolate from the Bouyzakarne site was higher than that of plants inoculated with fungal isolate from the Argana site, which may explain the seedling development differences found between the two isolates. Despite the fact that the Argana forest is better preserved, the isolates from the degraded Bouyzakarne forest resulted in better seedling performance under favorable culturing conditions.

This current study confirms the strong dependence of argan seedlings to native AMF for optimal morphological and physiological development. The use of native AMF can be vital for replanting argan seedlings in its natural environment characterized by very low-water and -nutrient conditions. This must be taken into account for nursery production of argan trees before transplanting to the field for reforestation or silviculture purposes. As demonstrated in this study, however, mycorrhizae colonization of argan plants may vary in efficiency depending on the source.

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Spring Fumigation Using Totally Impermeable Film May Cause Ectomycorrhizal Deficiencies at Sandy Loblolly Pine Nurseries

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Abstract

Stunted 1-0 loblolly pine (*Pinus taeda* L.) seedlings occurred at two bareroot nurseries in 2017. At both nurseries, soil was spring fumigated and covered with a totally impermeable film (TIF). During the summer, the stunted seedlings contained less than 0.11 percent foliar phosphorus, while normal seedlings had more than 0.12 percent foliar phosphorus. The mosaic pattern of stunting was identical to new-ground syndrome, which occurs from an ectomycorrhizal deficiency on newly established pine seedbeds (i.e., on nursery areas not previously in seedling production). Although new-ground syndrome has occurred at several nurseries in the past, 2017 may be the first time spring-fumigation syndrome has occurred at established loblolly pine nurseries. This phenomenon is due to insufficient airborne spores after fumigation and a lack of soil inoculum due, in part, to a deeper fumigation zone resulting from longer periods of exposure to fumigants under TIF. Suggestions for future research directions are provided.

Introduction

Sowing loblolly pine (*Pinus taeda* L.) seed after soil fumigation with methyl bromide (< 500 kg/ha) typically does not result in stunted seedlings at time of lifting (Cram et al. 2007, Davey 1990, Enebak et al. 2013a, Marx et al. 1984). A mycorrhizal deficiency will sometimes occur, however, when pine seed are sown at a new nursery (or new field) for the first time and the fumigated soil is not subsequently recolonized with airborne spores (Hatch 1936, Kessell 1927, Marx et al. 1978, McComb 1938, McComb and Griffith 1946, Molina and Trappe 1984). When this type of ectomycorrhizal deficiency occurs, it may be referred to as "new-ground syndrome" (South et al. 1988). New-ground syndrome has been observed at loblolly pine nurseries in Alabama, Florida, Georgia, Mississippi, North Carolina, Oklahoma, South Carolina, and Virginia.

Growing agricultural crops before sowing conifers can reduce the formation of ectomycorrhiza (Sinclair 1974). At one established loblolly pine nursery in South Carolina, seedbeds were taken out of production and kept in cover crops for 5 years. Five years was apparently enough time to deplete ectomycorrhizal soil inocula; after pines were sown on the area, the new-ground syndrome appeared (figure 1).

Figure 1. Stunted pine seedlings growing in soil where pine seedlings had not been grown for the past 5 years (Cantrell 2017). (Photo by David South, 2008)

The term "old ground" is used for seedbeds formed with soil that has produced an ectomycorrhizal seedling crop within the last 5 years (South et al. 1988). When an ectomycorrhizal deficiency occurs on spring-fumigated soil that produced pines during at least 1 of the previous 5 years, it is called "spring-fumigation syndrome." Except for the occasional occurrence of "new-ground syndrome," ectomycorrhizal deficiencies in spring-fumigated loblolly pine seedbeds are rare (Davey 1990, Marx et al. 1984, South et al. 2016). About one-third of southern pine nurseries currently fumigate soil in the spring (South et al. 2016), and the pine seedlings typically have mycorrhiza colonization by midseason. For example, in 1978, loblolly pine seedlings growing in March-fumigated seedbeds (old ground) at the New Kent Nursery in Virginia had mycorrhiza on 20 to 40 percent of the short roots by July (Marx et al. 1984).

The percentage of short roots that are mycorrhizal by August is typically greater than 5 percent (figure 2). A few ectomycorrhizal deficiencies in loblolly pine seedbeds have been attributed to extended periods of saturated soil and a lack of soil oxygen. But in 2017, we were surprised when spring-fumigation syndrome occurred on "old ground" at two nurseries. These deficiencies occurred on sandy soils that were in production for more than three decades. The objective of this article is to document two cases of spring-fumigation syndrome and to discuss probable causes for these recent cases and potential practices that may avoid similar events in the future.

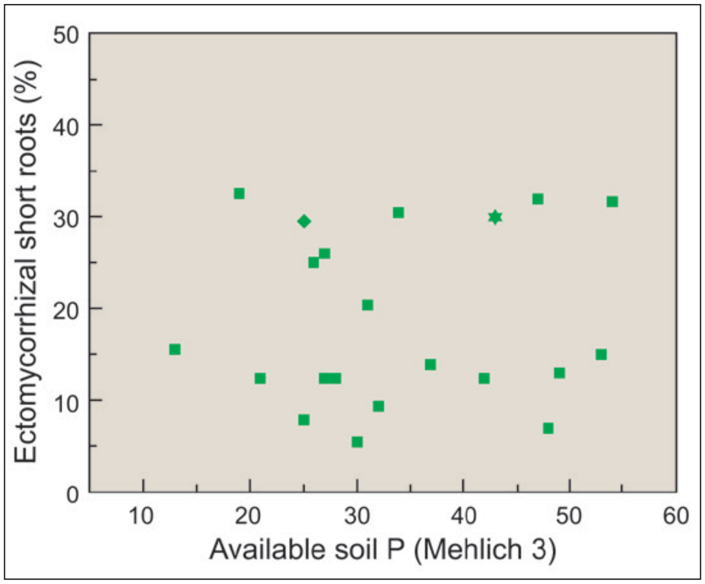

Figure 2. A survey of 21 nurseries (1977 to 1980) determined that 10 to 30 percent of loblolly pine short roots are typically mycorrhizal in the summer (Marx et al. 1984). Seedbeds at one nursery (star) were established on new ground, and seedbeds at another nursery (diamond) were fumigated with 67:33 methyl bromide:chloropicrin. All others were established on soil fumigated with 98:2 methyl bromide:chloropicrin at rates varying from 356 to 672 kg/ha (average 437 kg/ha).

Garland Gray Nursery

When the Garland Gray Nursery (Virginia Department of Forestry, Courtland, VA) was established in 1984, some areas in nonfumigated fields exhibited ectomycorrhizal deficiencies (South et al. 1988). Since then, fields have been fumigated many times without further incidence of stunted pine seedlings until 2017. The nursery's Gray field produced a cover crop in 2014 and produced three crops of 1-0 loblolly pines seedlings since. The Spain and Hughes fields have produced loblolly pine seedlings (using continuous cropping with spring fumigation every 2 years) for the past two decades. Nursery staff knows of no year when fumigation caused stunted pine seedlings in operational seedbeds.

Soil samples were collected from three fields (Hughes, Gray, and Spain) in December 2016 and analyzed for nutrients using the Mehlich 3 extraction method (Waypoint Analytical, Richmond, VA). Because soil phosphorus (P) levels (table 1) were greater than 25 ppm (South and Davey 1983), phosphorus was not applied before sowing. Fields were fumigated on April 12 with methyl bromide

Table 1. Soil characteristics from samples (n = 34) taken December 2016 from three bareroot fields at the Garland Gray Nursery in Courtland, VA.

Field	pH	OM	CEC	P	K	Ca	Mg	S	B	Cu	Fe	Mn	Zn	Na
-	-	%	Meq/100g	ppm	ppm	ppm	ppm	ppm	ppm	ppm	ppm	ppm	ppm	ppm
Hughes	5.0	1.3	1.5	68	20	127	15	11	0.1	0.8	130	6	2.1	13
Gray	5.0	1.4	1.7	78	24	182	21	17	0.1	0.7	117	8.9	2.2	14
Spain	5.3	1.2	1.7	60	22	164	25	7	0.1	0.8	146	4.8	2.9	16

B = boron. Ca = calcium. CEC = cation exchange capacity. Cu = copper. Fe = iron. K = potassium. Mg = magnesium. Mn = manganese. Na = sodium. OM = organic matter. P = phosphorus. pH = potential of hydrogen. S = sulfur. Zn = zinc. ppm = parts per million

(263 kg/ha) and chloropicrin (131 kg/ha) using swept-back shanks at a 25 to 30 cm depth. The soil was covered immediately with a totally impermeable film (TIF) that was later removed on April 19. The average maximum air temperature from April 12 to April 19, 2017, was 25.8 °C, which is 4.6 °C higher than the historical average for April. Loblolly pine seeds were sown on April 27. The fungicide prothioconazole (0.175 kg/ha) was applied on May 17, May 30, and June 12.

Stunted seedlings (figure 3) were observed in all three fields during the third week of July 2017. Foliage samples were collected from each field in July from stunted seedlings and from adjacent normal seedlings. Waypoint Analytical (Richmond, VA) analyzed all six foliage samples, and the results were analyzed using the PROC CORR procedure of statistical analysis software (edition 9, SAS Institute, Cary, NC). A one-sided T-test ($\alpha = 0.05$) was used to test the hypothesis that foliar nutrients were lower in stunted seedlings. It was determined that P was the only element that was significantly lower in stunted seedlings (table 2). This event is the first documented case of spring-fumigation syndrome on pines in Virginia.

To stimulate growth of stunted seedlings, an application of liquid fertilizer was applied on July 20. The application contained 28 kg/ha of P (as phosphoric acid) and 28 kg/ha of nitrogen (urea + ammonium nitrate). An increase in height and a color change were noticed by the end of July, and therefore, a second application (same rate) was made on August 3. The taller seedlings were top pruned August 1–9 (18 cm), August 14–23 (20 cm), September 1–14 (22 cm), and September 18–29 (25 cm). In December, the stunted seedlings were 18 to 25 cm tall, and normal seedlings were 25 to 30 cm tall (figure 4).

Claridge Nursery

Soon after the Claridge Nursery (North Carolina Forest Service, Goldsboro, NC) was established in 1954,

Figure 3. At the Garland Gray Nursery, soil phosphorus (P) in the Spain field ranged from 51 to 77 ppm in December 2016. The field was fumigated in April 2017. In July 2017, stunted seedlings had 0.05 percent foliar P, suggesting delayed mycorrhizal development, while normal seedlings had 0.14 percent foliar P, suggesting typical mycorrhizal development. The seedbeds in this photo have been producing seedlings (without stunting) since 1986. (Photo by Justin Funk, 2017)

Figure 4. Although taller seedlings at the Garland Gray Nursery were top pruned four times, variability in seedling height due to stunting in some seedbeds was still evident in December 2017. (Photo by Justin Funk, 2017)

Table 2. Foliage samples (n = 6) taken July 18, 2017, from stunted and normal seedlings at the Garland Gray Nursery in Courtland, VA. A one-sided T-test indicates phosphorus (P) levels were lower in stunted seedlings than in normal seedlings.

Stock	N	S	P	K	Mg	Ca	Na	B	Zn	Mn	Fe	Cu	Al
-	%	%	%	%	%	%	%	ppm	ppm	ppm	ppm	ppm	ppm
Stunted	1.82	0.097	0.057	0.91	0.100	0.31	0.037	37	52	329	1864	35	1210
Normal	1.89	0.103	0.127	1.06	0.107	0.34	0.030	26	74	319	1247	27	875
LSD	1.5	0.079	0.058	0.607	0.80	0.19	0.009	17.7	39.3	340	2736	13.6	1299

Al = aluminum. B = boron. Ca = calcium. Cu = copper. Fe = iron. K = potassium. LSD = least significant difference (a = 0.05). Mg = magnesium. Mn = manganese. N = nitrogen. Na = sodium. S = sulfur. Zn = zinc. ppm = parts per million

Figure 5. In October 2016, many seedbeds at the Claridge Nursery were inundated by floodwaters from Hurricane Matthew. Rainfall on October 8 exceeded 378 mm, and the nearby Little River crested on October 12. On April 30, 2017, the river crested again, and the nursery was flooded for a second time. During both flooding events, the problem area in Field R was not inundated. (Photo by Drew Hennant, 2016

fumigation trials demonstrated significant gains in seedling production. The Claridge Nursery staff cannot remember any cases of fumigation causing stunted pine seedlings in operational seedbeds before 2017. Although spring fumigation can delay the formation of ectomycorrhiza by a few weeks (Danielson and Davey 1969), the 2017 event is the first documented case of spring-fumigation syndrome on pines in North Carolina.

During the summer of 2016, Claridge Nursery's Field R contained forage radish (*Raphanus sativus* L. var. *niger* J. Kern.). Most of the nursery's 2016 loblolly pine seedling crop was destroyed in October due to flooding from Hurricane Matthew. At that time, Field R was fallow, and most of it remained above the flood (figure 5). Typically, Field R is fumigated in the fall, but the soil remained saturated due to above average rainfall (table 3). As a result, soil fumigation was delayed until early spring. On March 20, 2017, several fields were injected with methyl bromide (358 kg/ha) and chloropicrin (90 kg/ha) using swept-back shanks at a 25 to 30 cm depth. The soil was covered with TIF at the time of fumigation that was later removed on April 3. The average maximum air temperature from March 20 to April 11, 2017, was 22.4 °C, which is 2.5 °C higher than normal. Seeds were sown into Field R on April 11, and the soil was treated with the herbicide pendimethalin (2.1 kg/ha). The fungicide tridimefon (0.42 kg/ha) was applied on May 25 and July 13, and the fungicide prothioconazole (0.156 kg/ha) was applied on May 3, June 14, and June 28.

Stunted seedlings were observed in a section of Field R in July, and photos were taken in August (figure 6). Some of the primary needles had a purple color (Hobbs 1944, Lyle 1969, South et al. 1988). Soil samples (table 4) and foliar samples (table 5) were collected from an area of stunted seedlings and from an adjacent area of normal seedlings. Waypoint Analytical (Wilson, NC) analyzed the samples, and soil nutrients were extracted using the Mehlich 3 procedure.

Table 3. Monthly precipitation totals for locations near the Claridge Nursery (Goldsboro, NC) and Garland Gray Nursery (Wakefield, VA). Values in parentheses are the deviation from historical averages.

Year	Month	Goldsboro, NC	Wakefield, VA
-	-	mm	mm
2016	Aug	65.5 (– 83.5)	18.3 (– 111.7)
2016	Sept	330.5 (+ 178.5)	191.3 (+ 60.3)
2016	Oct	397.2 (+ 321.2)	98.5 (+ 17.5)
2016	Nov	17.8 (– 60.2)	25.1 (– 71.9)
2016	Dec	76.4 (– 5.6)	51.1 (– 36.9)
2017	Jan	70.3 (– 22.7)	104.4 (+10.4)
2017	Feb	27.7 (– 59.3)	17.3 (– 56.7)
2017	March	85.8 (– 16.2)	130.0 (+ 24.0)
2017	April	115.1 (+ 30.1)	84.3 (– 9.7)
2017	May	143.8 (+ 47.8)	127.0 (+ 23.0)
2017	June	102.1 (+ 4.1)	100.8 (– 0.2)
2017	July	39.9 (– 101.1)	106.7 (– 15.3)

Table 4. Soil samples (n = 2) taken near stunted or normal seedlings growing in Field R in August 2017 at the Claridge Nursery in Goldsboro, NC.

Stock	pH	OM	CEC	P	K	Ca	Mg	S	B	Cu	Fe	Mn	Zn	Na
-	-	%	Meq/100g	ppm	ppm	ppm	ppm	ppm	ppm	ppm	ppm	ppm	ppm	ppm
Stunted	5.4	0.4	3.7	203	41	298	41	8	0.3	1.2	199	27	3.5	18
Normal	5.4	0.4	5.7	155	27	220	36	8	0.2	0.7	148	14	1.2	19

B = boron. CEC = cation exchange capacity. Cu = copper. Fe = iron. K = potassium. Mg = magnesium. Mn = manganese. Na = sodium. OM = organic matter. P = phosphorus. pH = potential of hydrogen. S = sulfur. Zn = zinc. ppm = parts per million

Table 5. Foliage samples taken from stunted or normal seedlings in August 10, 2017 at the Claridge Nursery in Goldsboro, NC.

Stock	N	S	P	K	Mg	Ca	Na	B	Zn	Mn	Fe	Cu	Al
-	%	%	%	%	%	%	%	ppm	ppm	ppm	ppm	ppm	ppm
Stunted	3.04	0.17	0.09	1.45	0.11	0.3	0.04	128	73	827	539	10	520
Normal	1.91	0.16	0.16	1.35	0.14	0.41	0.03	112	56	475	474	10	331

Al = aluminum. B = boron. Ca = calcium. Cu = copper. Fe = iron. K = potassium. Mg = magnesium. Mn = manganese. N = nitrogen. Na = sodium. P = phosphorus. S = sulfur. Zn = zinc. ppm = parts per million

Figure 6. The soil at the Claridge Nursery had more than 150 ppm phosphorus (P) in August 2017. Stunted seedlings had 0.09 percent foliar P, suggesting insufficient mycorrhizae. The normal seedlings had 0.16 percent foliar P, suggesting sufficient mycorrhizal development. (Photo by McClain Davis, 2017)

To stimulate growth of stunted seedlings, a solution of ammonium polyphosphate was applied to all seedlings on August 28 (21.5 kg P/ha and 14.8 kg N/ha). A second treatment (same rate) was applied on September 4. A substantial change in the size and color of stunted seedlings was noticed by the middle of September. The taller seedlings were top pruned on August 28 (20 cm), September 15 (25 cm), September 25 (30 cm), and October 10 (35 cm). Stunted seedlings were typically shorter than the top-pruning height, and most never achieved the same height. In December, normal seedlings were 35 cm tall, while stunted seedlings were 20 to 30 cm tall (figure 7).

Discussion

Spore and Mycelia Inoculum in Pine Seedbeds

Ectomycorrhizal deficiencies in loblolly pine seedbeds are due to a lack of airborne spores and insufficient soilborne inoculum. Even on new ground, airborne inoculum may be sufficient for loblolly pine to have mycorrhiza in midseason (Marx et al. 1984). Sufficient airborne inoculum (figure 8) is why most new southern pine nurseries (established after 1978) produced a successful first crop of mycorrhizal pine seedlings. Spring fumigation 3 weeks before sowing in April, however, may not allow enough time for airborne spores to uniformly cover treated soil (South et al. 1988).

When bareroot 1-0 pine seedlings are harvested, roots and mycorrhiza will be stripped during the lifting process, providing the source of soilborne inoculum for succeeding crops (Henderson and Stone 1970). Spring fumigation in subsequent years may eliminate inoculum in the topsoil (0 to 25 cm), but inocula typically remain viable in soil below the fumigation zone. Therefore, once the taproot reaches viable inoculum, the roots become infected with ectomycorrhiza (even when airborne spores are lacking). Because roots can reach the inoculation zone, ectomycorrhiza can usually be detected on roots 6 to 10 weeks after seedling

Figure 7. The taller seedlings at the Claridge Nursery were top pruned four times; stunted seedlings were generally shorter than the pruning height. Variability in seedling size was still evident in December 2017. (Photo by McClain Davis, 2017)

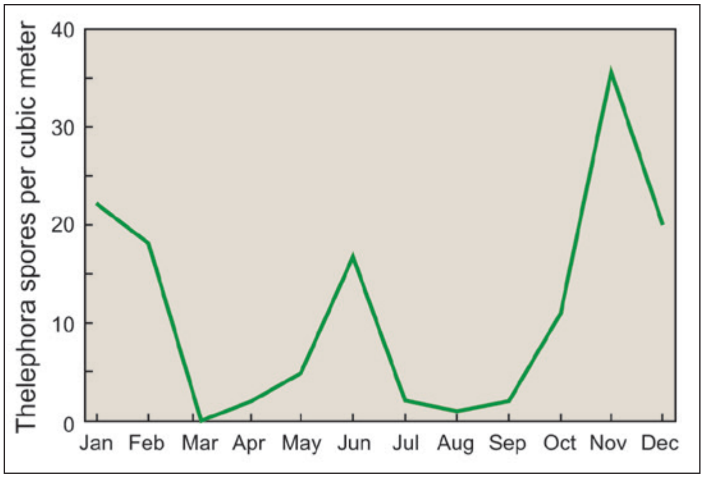

Figure 8. The average number of airborne spores (Thelephora spp.) per cubic meter of air sampled at Mérida, Spain (adapted from Trejo et al. 2013).

emergence (Marx et al. 1976, Molina and Trappe 1984). The expectation that sufficient inoculum will persist after spring fumigation is so high that most managers of loblolly pine nurseries see no need to use either vegetative inoculum (Marx et al. 1978, Mitchell and South 1992), basidiospores (Marx et al.1979), or extra P fertilizer on spring-fumigated soil (South et al. 1988).

Effect of Tarp Permeability on Soil Mycorrhiza

In the past, soil fumigation included the use of low-density polyethylene plastic tarps, and the occurrence of stunted, purple loblolly pine seedlings was rare. Fumigation in the spring was common (Boyer and South 1984), and the rate of fumigant sometimes exceeded 500 kg/ha (Marx et al. 1984). For example, no stunting occurred even when a nursery (where soils were 94 percent sand) was spring fumigated (March 1978; average maximum air temperature 23 °C) with 549 kg/ha of a 67:33 mixture of methyl bromide and chloropicrin (Marx et al. 1984). Methyl bromide and chloropicrin typically would escape through the polyethylene plastic (Qin et al. 2011, Wang et al. 2005), but the use of TIF, a relatively new technological innovation (Enebak 2013, Enebak et al. 2013b), effectively increases the fumigation dosage (i.e., concentration over time), because it retains the fumigant in the soil for longer periods of time (Weiland et al. 2013). Longer retention times plus warmer temperatures (figure 9) likely contributed to killing mycelia in deeper depths of sandy soils and caused the stunting at the Garland Gray Nursery and Claridge Nursery in 2017.

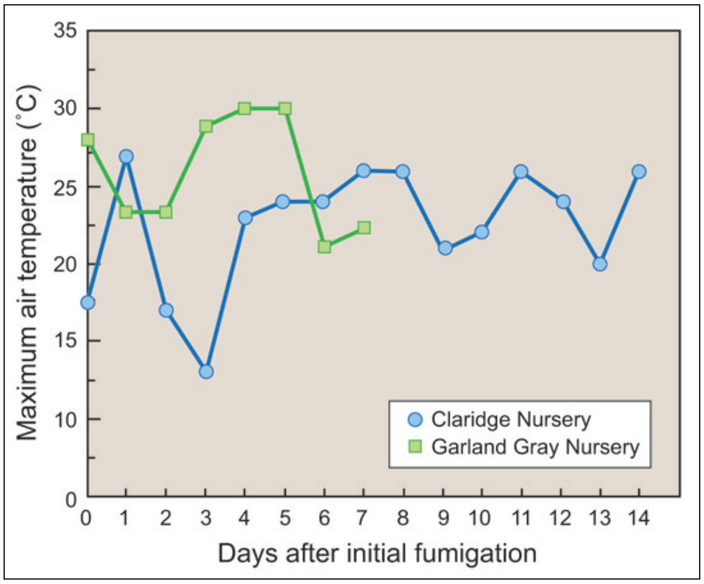

Figure 9. Maximum air temperatures during the time the totally impermeable film tarp was in place at the Claridge Nursery (dots) and the Garland Gray Nursery (squares). The 30-year average maximum temperature for April is 23.0 °C at Goldsboro, NC and 21.2 °C at Wakefield, VA.

For half a century, it was believed that ectomycorrhizal deficiency was more likely to occur when fumigating sandy soils (Stone et al. 1966). Indeed, fumigation is more effective on sandy soils (Collins et al. 2006, Lembright 1990). For example, no stunting occurred when TIF was used to fumigate silt loam seedbeds in Alabama (Enebak et al. 2013a) or when less sandy fields at the Claridge Nursery were fumigated in the spring of 2017.

How To Avoid Spring-Fumigation Syndrome

Fumigating in the fall is the simplest way to lower the risk of producing nonmycorrhizal seedlings in July. Even if deep fumigation eliminates the soil inoculum in lower root zones, airborne spores would inoculate the soil after the TIF was removed. Fall fumigation also allows nursery managers and contractors more flexibility to decide when to fumigate. Fall fumigation cannot be used, however, when pine seedlings are still in the fields.

When spring fumigation is required, managers may need to consider lowering the fumigant rate when the soil contains more than 84 percent sand. Effective pathogen control has been achieved using lower rates under TIF (Enebak et al. 2013b). A less expensive, high-density polyethylene (HDPE) film could be used at rural locations. For example, in Georgia, a loamy sand was fumigated with 263 kg/ha of methyl bromide plus 130 kg/ha of chloropicrin (the maximum air temperature in early April was 31 °C), and the soil was covered with HDPE (Enebak et al. 2011). This treatment did not produce stunted seedlings with purple primary needles.

Research Needs

Detection

Nursery managers who choose to fumigate in the spring need a quick, nondestructive way to detect nonmycorrhizal seedlings during the first week of July. One simple method is to monitor seedlings for reddish-purple color on tips of older primary needles (Lyle 1969). For some genotypes, however, this might be more difficult than in the past. Research at the Auburn University Southern Forest Nursery Management Cooperative determined that certain pesticides will make pine seedlings greener. For example, the herbicide pendimethalin (South and Hill 2009) and the fungicide prothioconazole (Starkey and Enebak 2011, Starkey et al. 2013) will cause loblolly pine seedlings to be greener than normal. Both chemicals were applied to seedbeds at the Claridge Nursery, and stunted seedlings still exhibited some purple primary needles.

Phosphorus Monitoring

Foliar analyses can be used to evaluate the level of P in young seedlings (Potvin et al. 2014, Rousseau and Reid 1990). When needles of young, stunted pine seedlings contain less than 0.11 percent P, then the seedlings are likely nonmycorrhizal. When pine seedlings are stunted for other reasons, the needles of mycorrhizal seedlings will have more than 0.11 percent P (table 6).

Seedlings that are 14 weeks old can have 0.07 percent foliar P, and 2 weeks later, they can have 0.17 percent foliar P (Rousseau and Reid 1991). For this reason, it is important to sample seedlings as soon as stunting or purple needles are detected. Sampling after stunted seedlings have formed mycorrhiza may be too late to detect a difference in foliar P.

Table 6. Published examples of foliar phosphorus (P) concentrations in stunted conifer seedlings from various nurseries and growth chamber studies. A lack of ectomycorrhiza is indicated when foliar P values are less than 0.11 percent.

Species	Nursery Location	P (%)		Reference
		Stunted	Normal	
Pinus resinosa Ait.	Growth chamber	0.04	0.13	Campagna and White (1973)
Pinus taeda L.	Growth chamber	0.05	0.33	Rousseau and Reid (1990)
Picea glauca (Moench) Voss	Quebec	0.05	0.16	Campagna and White (1973)
Pseudotsuga menziesii (Mirb.) Franco	Oregon	0.05	0.17	Trappe and Strand (1969)
Pinus taeda L.	Greenhouse	0.07	0.11	Ford et al. (1985)
Pinus thunbergii Parlat.	Greenhouse	0.07	0.12	Shi et al. (2017)
Pinus taeda L.	Alabama	0.07	0.15	South et al. (1988)
Pinus strobus L.	Iowa	0.07	0.16	McComb and Griffith (1946)
Pinus strobus L.	Greenhouse	0.07	0.19	Hatch (1936)
Pinus strobus L.	New York	0.08	0.13	Mitchell et al. (1937)
Pinus elliottii Engelm.	Greenhouse	0.08	0.14	Lamb and Richards (1971)
Pinus virginiana Mill.	Iowa	0.10	0.18	McComb (1938)
Pinus banksiana Lamb.	Michigan	0.12*	0.28	Potvin et al. (2014)
Pinus ponderosa Dougl. Ex Laws.	Idaho	0.15*	0.18	Morby et al. (1978)

* Chlorotic and or stunted 1-0 seedlings might be due to too much water (Idaho) or too much organic matter (Michigan).

Phosphorus Fertilizers

To reduce the risk of stunted seedlings due to endomycorrhzal deficiencies (Cram and Fraedrich 2015, South et al. 1980), some managers apply P fertilizers to hardwood seedlings soon after germination is complete in the spring (South et al. 2016). Since P deficiency is rare in pine seedbeds, routine fertilization with P is not practiced. Once an ectomycorrhizal deficiency is detected, however, several types of P fertilizers may be used to stimulate the growth of stunted seedlings. These fertilizers include phosphoric acid (South et al. 1988), superphosphate (Stone et al. 1966), triple super phosphate (Henderson and Stone 1970), ammonium phosphate, diammonium phosphate, potassium phosphite, and ammonium polyphosphate (Claridge Nursery). Research could determine which fertilizer would cause stunted seedlings to grow as rapidly as those treated with phosphoric acid.

Fungicide Effects

The fungicide triadimefon will affect the formation of ectomycorrhiza (Kelley 1982, Marx et al. 1986, South and Kelley 1982) but not enough to stunt seedlings in the nursery or to affect growth after outplanting (Rowan and Kelley 1986). As a result, triadimefon is used on loblolly pine seedlings to reduce the need to cull diseased seedlings. Researchers demonstrated that the fungicide prothioconazole can increase the number of root tips and may increase seedling growth (Starkey and Enebak 2011). Although the direct effects on ectomycorrhizal roots are not known, eight applications of prothioconazole did not stunt loblolly pine seedlings in Mississippi (Starkey et al. 2013). Multiple applications of prothioconazole on fumigated soil at the Claridge Nursery did not cause stunted seedlings on other spring-fumigated fields. Future research could determine if prothioconazole has any effect on the formation of ectomycorrhiza.

Influence of Flooding

Flooding can reduce the level of viable endomycorrhiza inoculum and, therefore, cause a P deficiency in the following year (Ellis 1998). Although several spring-fumigated fields at the Claridge Nursery flooded after fumigation in April, surviving seedlings in these fields were not stunted. Ten weeks of flooding will reduce ectomycorrhiza (Stenström 1991), but little is known about the effects of flooding on the viability of soil inoculum. Research might determine if a “postflood syndrome” also exists for ectomycorrhizal fungi.

Conclusions

For more than five decades, spring fumigation with methyl bromide and chloropicrin did not cause stunting on “old ground” until the 2017 incidences described here. Using TIF with spring fumigation (390 to 448 kg/ha) on sandy and loamy sand soils may result in purple, stunted seedlings. Where feasible, nursery managers should consider fumigating in the fall (Enebak et al. 1990, Hansen et al. 1990, Molina and Trappe 1984) to allow enough time for ectomycorrhizal spores to cover treated soil. When seedbeds are fumigated in the spring using TIF, managers may need to check for signs of purple primary needles in early July and to test foliage for P deficiencies.

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