Tree Planters' Notes

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Spring 2020

Dear TPN Reader

This issue contains six articles on a variety of topics. Donnelly (page 4) adds to TPN's ongoing State-by-State series and gives us an overview of Connecticut's past and present tree planting; Masairi and colleagues (page 19) describe the beneficial effects of inoculating olive trees with mycorrhiza in Morocco; Krishnapillai and colleagues (page 29) describe a process for creating growing media from coconut husks to be used for plant production in tropical environments; Brennan and Jacobs (page 39) share their protocol for seed propagation of butternut; Benomar and colleagues (page 51) examine budset phases among white spruce seed sources; and Dumroese and colleagues (page 61) describe uses for biochar in field and nursery operations.

I hope you find this issue interesting and useful.

Diane L. Haase

Until you dig a hole, you plant a tree, you water it and make it survive, you haven't done a thing. You are just talking.

— Wangari Maathai

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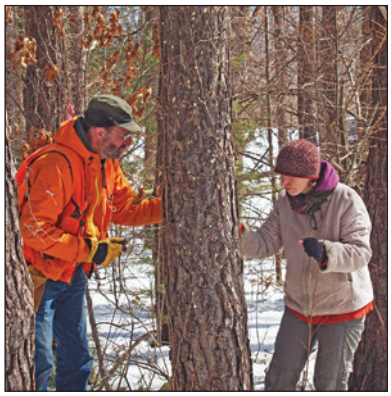

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Connecticut's Forest – A Legacy of Change in Today's Forest

Chris Donnelly

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Abstract

Over the past four centuries, the forest of Connecticut has undergone significant changes. From the early 1600s, when Native American land-use practices included fire and agricultural clearing of the forest, on through the colonial period, and then the years of trade and industrial development, the forest has been heavily shaped by human society. Many of these practices, particularly those throughout the 19th century, were not beneficial to the forest. At the start of the 20th century, the new State forestry program sought to take on the challenges of restoring Connecticut's forests head-on through practices based on scientific management and productive use of the forest. Before it closed in 2005, Connecticut's State Forest Nursery had a main role in the recovery of the forest. While forest land acreage has more than doubled in size from its nadir in the first half of the 19th century, the challenges to forest management in Connecticut remain immense, as Connecticut's foresters rise to meet these challenges.

Introduction

Connecticut is a small State of 3.6 million ac (1.5 million ha), of which about 3.1 million ac (1.3 million ha) is land. Roughly rectangular, the State is 110 mi (177 km) long and 70 mi (112 km) wide, with its southern edge being the shore of Long Island Sound. Based on the 2010 census, Connecticut is the third smallest State, ranks 29th in population, and fourth in population density. The State has three major geologic regions—the eastern and western highlands, each composed of older, metamorphic rock, and a central valley, largely composed of basalt overlain by sandstone. The soils are largely glacially derived. The Connecticut River bisects the State, almost directly through the center.

Climatically, Connecticut has been described as northern continental grading into subtropical, as one travels from the higher elevations in the northern corners toward the shoreline. In Hartford, the average high/low temperatures are 84 ˚F and 63 ˚F (29 ˚C and 17 ˚C) in July and 35 ˚F and 16 ˚F (2 ˚C and -9 ˚C) in January. Average annual precipitation is 46 in (117 cm), distributed evenly throughout the year.

The present forest of Connecticut (figure 1) is described by the U.S. Department of Agriculture, Forest Service as oak-hickory, although it is converting more to a mixed hardwood forest increasingly dominated by maple (*Acer* sp.), beech (*Fagus* sp.), and birch (*Betula* sp.). The land area of the State is currently about 58 percent forested, down from a recent peak of 65 percent in the 1950s (Butler 2017), and 73 percent of the land is under tree canopy, including that of the individual trees in urban areas (Nowak and Greenfield 2012).

This article assumes that the majority of Connecticut's forests were established in or around the first decade of the 20th century. But before we can discuss the 20th century, the following sections give an overview of Connecticut's forests in the centuries before 1900.

The Native American and Colonial Periods

The first steady incursion of Dutch and English immigrants into the land that was to become Connecticut began in the early 17th century. Before that time, these lands were inhabited by several Native American Tribes. It is estimated that these lands were approximately 95 percent forested prior to European immigration. Living in the midst of this forest, the Native Americans were largely migratory and

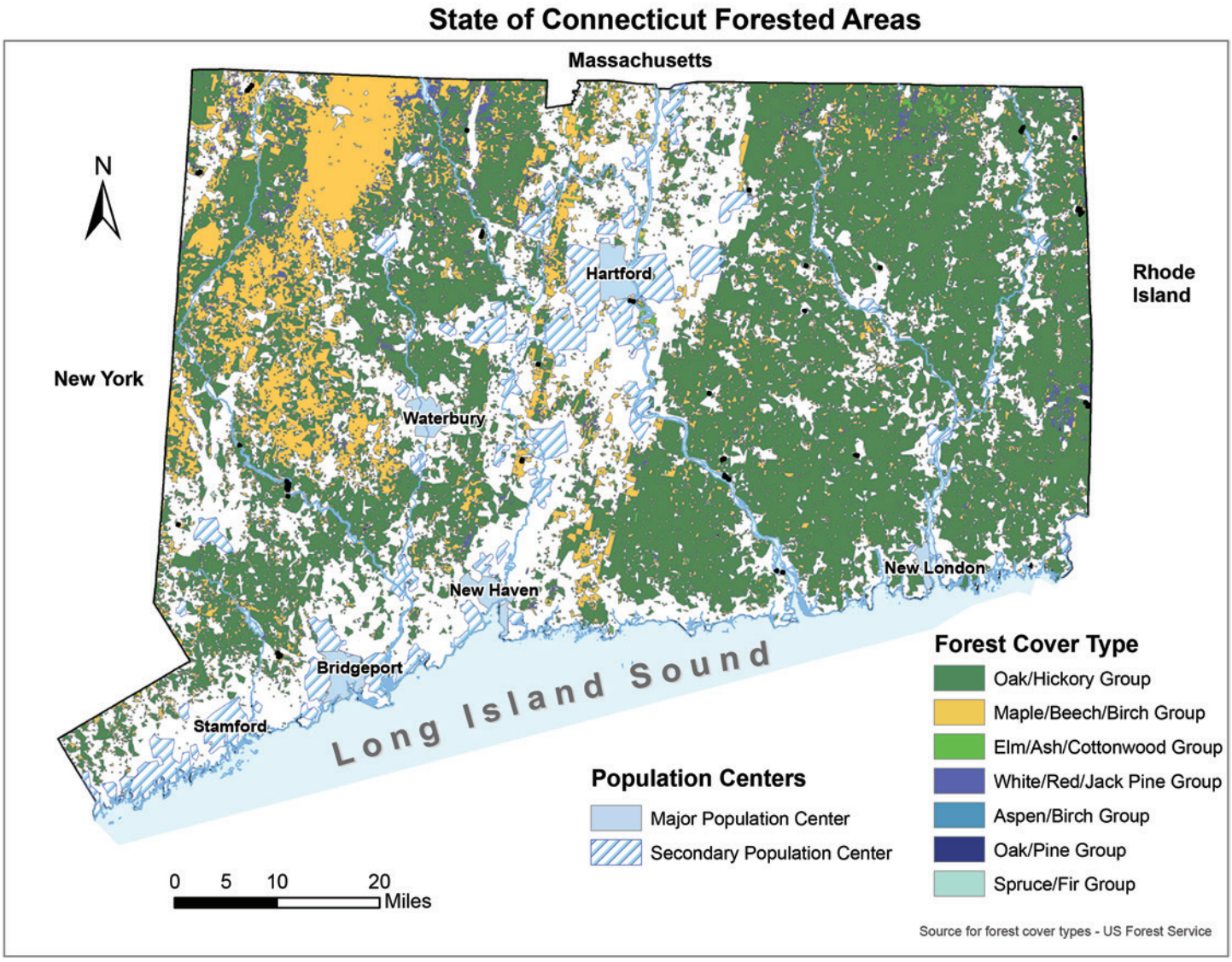

Figure 1. Overview of Connecticut's forests. Map created by Chris Donnelly using USDA Forest Service Forest Inventory and Analysis and Forest Health data for the Northeastern United States, available through Databasin.org.

territorial in their way of life. They practiced rotating agriculture and used fire for land clearing. Fire was also used to clear underbrush for forestscaping, with the planned regrowth fostering an increase in game animals such as turkey and deer. Early Europeans frequently commented on the open, park-like condition of Southern New England forests that resulted from these well-established Native American forest management practices. The Native Americans' seasonal cycle of land use was reflected in the mosaic quality it gave to the natural forested ecosystem.

Once they arrived, the European settlers who came to New England were not migratory. For the most part, they sought to build a way of life centered on individual property ownership, with the maintenance of livestock a key feature. Despite major differences, however, in important ways these early European settlers and the Native Americans were fundamentally similar. The settlers also lived a life highly connected to the land, dependent on the seasons and what the local landscapes had to offer. In New England, the focus of the settlers tended to be less on the individual accumulation of wealth and more on the establishment of a community, one that would carry over across generations. Forests were critical for providing wood for building, fuel, and household items such as bowls, furniture, and farm implements. The forests also provided materials for fences that, perhaps as much as anything, signified the major landscape changes.

These settlers did not clear all forests to get to the soil below. Township records for colonial Southern New England suggest that tilled land for corn, and

Figure 2. Cathedral Pines in Cornwall, CT; one of Connecticut's few remnant old-growth forests. (Photo by Chris Donnelly 2018)

later potatoes, was usually no more than about 10 percent of a typical farm. In addition, there was land for pasturage, meadows for growing hay, orchards, and woodlands. Perhaps 30 to 50 percent of early farms were left in forest to provide for household needs.

While these settlers lived in close association with the land, they were also prepared to make large changes to facilitate their way of life. They were willing to eliminate ecosystem features for which they saw no particular need. Wetlands were regularly cleared and drained and tilled fields were fertilized with manure to improve their fertility. Old-growth forests (figure 2), along with populations of wolves, beavers, and deer, diminished.

Fortunately, records exist that allow a glimpse back into early forests of this region. As New England was settled and property boundaries marked out, witness trees were established to define these boundaries. These early records of landownership survive in extensive numbers, serving as a de facto survey of forest composition at the time when the boundaries of the first colonial properties were set. In a comprehensive review of these witness trees within New England, Cogbill et al. (2002) found that both oaks (*Quercus* sp.) and hickories (*Carya* sp.) were about twice as common as they are today (table 1).

Due to changing land-use practices, shifts in species composition would be expected to occur in the early years after European settlement. For example, the use of European tools such as the axe would have had an influence. Pollen records suggest that oaks declined following European settlement, perhaps due to preferential harvesting, while the amount of chestnut increased, likely benefiting from steady seed production along with its being a prolific stump sprouter (Brugam 1978, Foster 1995).

Trade and Transportation – Connecticut Forests in the Nineteenth Century

After the colonial period, major changes in land use continued. Developments in trade, transportation,

Table 1. Witness tree genera as compared to recent FIA tree populations.

Genus	Genus proportions (%) of colonial witness trees	Current forest composition (%) based on FIA data*
Oak (*Quercus* sp.)	60	28
Hickory (*Carya* sp.)	10	5
Chestnut (*Castanea* sp.)	9	0
Maple (*Acer* sp.)	4	29
Beech (*Fagus* sp.)	3	3
Pine (*Pinus* sp.)	3	5
Ash (*Fraxinus* sp.)	3	3
Hemlock (*Tsuga* sp.)	3	7
Witness trees were typically recorded by current common name and not recorded as to species. For instance, 'oak' would have been recorded and not necessarily 'red oak' or 'white oak'.	From Cogbill et al. (2002). These percentages are based on the combined Connecticut and Rhode Island records.	*Only trees 7" dbh and over are included in this table, based on the assumption that smaller diameter trees would not have been used as witness trees. Data source: Butler et al. 2012 – Table_CT-10

FIA = Forest Inventory and Analysis

industry, and energy all had their influence. It is estimated that the forest cover in Connecticut reached its lowest point sometime between 1825 and 1850, driven partly by the craze in raising Merino sheep, but also due to population increases (Foster 2017, Harper 1918). By 1825, canals and then railroads led to new trading patterns. This opening of the States and territories further west released some of the growing population pressure in rural areas.

It was the industrial revolution, however, that was the main story. By 1850, industry and manufacturing had replaced farming as the economic mainstay in Connecticut, though farms were still necessary to provide such goods as fresh vegetables and milk. The new economic center shifted from the higher-elevation rural settlements to the factory centers along the many fast-flowing rivers and streams as well as the coastal and central cities and towns from which goods were sent and received.

The return of farmland to woodland largely happened on its own as farmers planted fewer crops and gradually abandoned all but the best pastures in favor of imported feed for their livestock (Foster et al. 2008). With the rise of the new industrial centers, these re-growing forests became an important source of fuel for factories. Initially, firewood was used, but it was heavy and costly to transport. Charcoal, produced by burning hardwoods in oxygen-starved conditions, became a prime forest product and a key companion of industrial growth (figure 3). Charcoaling remained a main provider of energy for manufacturing until the early 1900s, by which time charcoal had been largely replaced by coal.

By the end of the 19th century, stone walls, wells, cellar holes, and remnants of charcoal mounds were scattered throughout the fields and woods of Connecticut. Beaver, wolves, and turkey had been eliminated, and black bear and white-tailed deer were nearly gone. The passenger pigeon had become totally extinct. It has been suggested that the loss of this bird had a major impact on the forest. As the massive flocks moved through the forests during the spring, they ate huge quantities of beechnuts, chestnuts, and acorns. Because white oak (*Quercus alba* L.) germinates in the fall, its acorns were not available during these migrations. Without the passenger pigeon, white oak lost this advantage, further affecting forest composition (Faison 2014).

Figure 3. Charcoal production was an important fuel source until the early 1900s. (a) In the early stages of charcoal production, several cords of wood are piled around a central pole. (b) The wood is then covered with dirt to restrict air flow as the wood is slowly burned in oxygen-deprived conditions. (Photo a courtesy of Yale University archives and photo b courtesy of the State of Connecticut Library archives)

The First Half of the Twentieth Century

Connecticut's forests were in poor condition at the start of the 20th century (figure 4). Austin Hawes, Connecticut's third State Forester, described the condition of the State's forested land in those years as follows:

> For a generation the portable sawmills had been eating further and further up the hillsides removing timber which had been inaccessible for the old water powered mills. The demand for railroad ties, poles and posts resulted in practically uniform clear cutting, and the slash from these

Figure 4. A view within Meshomasic State Forest— Connecticut's first State Forest— in 1906 shows the unhealthy condition of forestland at that time. (Photo courtesy of the State of Connecticut Library archives)

> operations made tinder, which resulted in great forest conflagrations. Almost every slope was covered with unsightly scars where gaunt fire-killed trees stood out against the horizon. The evergreen trees, pine and hemlock particularly, had suffered from repeated fires and natural reproduction of these species had been almost eliminated so that the woods were becoming more and more patches of hardwood brush (Hawes 1957, p. 22).

Hawes served as State Forester from 1904 until 1909 and again from 1921 until his retirement in 1944. Early on, Hawes set his sights on two major goals: reestablishing the forests as a healthy and productive use of the land and instilling in the public an appreciation of forestry and forest management as essential to maintaining this productive and useful landscape. For the latter, Hawes and his colleagues needed to appeal to farmers. In 1900, the majority of Connecticut's forestland was owned by farmers. As described by Henry S. Graves, director of the Yale Forest School (as it was known at the time), in a 1907 address to the Connecticut Forestry Association:

> General talk about forestry is not needed so much as information on how to practice it. Farmers and other owners do not want to hear about the protective influence of forests on stream flows, but how to plant trees and how to increase their rate of growth. Experience has shown me in my own work that I can accomplish more with an owner in the educational line by a few hours walk in the woods, than by writing a half dozen books. The Connecticut farmer must be his own forester (Graves 1907, p. 37).

One of Hawes's early research efforts was a tally of existing plantations and how they came into being. Several plantations, primarily for white pine (*Pinus strobus* L.), existed prior to the State's forestry program, most notably the Shaker Plantation, established in Enfield in 1876. In 1905, Hawes oversaw the establishment of the Rainbow Plantation, located very near to where the Bradley International Airport is today. This plantation was used until 1943 for research purposes and as a source of seedlings for private- and State-owned lands. In 1903, Connecticut established its first State Forest in Portland, followed by its second, 2 years later, in Union.

From 1901 to 1921, the Station Forester of the Connecticut Agricultural Experiment Station (CAES) also served as the Connecticut State Forester. Thus, the Rainbow Plantation was a CAES-owned operation. In 1921, the General Assembly of Connecticut voted to establish the State Forester as a separate position, distinct from that of the CAES Station Forester. As a result, the State Forester reported to the State Park and Forest Commission, while the Station Forester continued to provide outreach advice to landowners and distribute seedlings from the Rainbow Plantation. In 1924, the United States Congress passed the Clarke-McNary Act, partly for the purpose of helping States provide assistance to private forest landowners. This Act led to the appointment, in 1926, of Connecticut's first Extension Forester. This position was affiliated with the University of Connecticut, the State's land-grant university. After that, the focus of the Station Forester was more centered on research. The Station, however, continued to provide seedlings to private landowners until its nursery was closed. As for the State Forester, that office continued to have a role in outreach through the previously established service forestry program. The State Forester was also given full responsibility for the growing State Forest system.

Establishment of the Connecticut State Nursery

In 1905, there were just two State Forests, totaling 1,400 ac (565 ha). In 1921, there were five, totaling 4,452 ac (1,800 ha). Then, within a year, the total number of acres had increased to 7,260 ac (2,935 ha), and to 11,500 ac (4,650 ha) by 1925. As a consequence, the General Assembly voted in 1925 for $5,000 to establish a nursery on State lands. In explaining this vote, Hawes wrote, "Besides the desirability of increasing the percentage of softwoods in the state, there was always more interest in planting than in any other aspects of forestry" (Hawes 1957, p. 86). The State Nursery opened in 1928 in People's Forest in Barkhamsted to produce seedlings for planting on State lands (figure 5). It was not until 1945, following CAES's closure of the Rainbow Plantation Nursery, that this State Nursery began to provide seedlings to qualifying private landowners.

In August 1955, the State Nursery at People's State Forest flooded. In December, the legislature voted to

Figure 5. The first Connecticut State Nursery, located in Barkhamsted, within the People's State Forest was established in 1928. (Photo courtesy of the State of Connecticut Library archives circa 1950)

allocate $36,000 to re-establish the nursery in Pachaug State Forest in Voluntown. In approving this funding, the legislature anticipated that the nursery would be able to produce up to 2 million seedlings annually for meeting both public and private forest planting needs throughout the State. The new nursery was also expected to provide seedlings to environmental and conservation organizations, and to towns and schools for Arbor Day events.

Species Selection for Tree Planting in Connecticut

At the Rainbow Plantation, 17 hardwood and 16 conifer species were planted as trials to determine which species grew well in Connecticut and could be recommended for planting. These trials indicated the potential value of certain non-native species such as red pine (*Pinus resinosa* Aiton). From the start, however, the two mainstay species for planting in Connecticut were expected to be eastern white pine and American chestnut (*Castanea dentata* [Marshall] Borkh.). In fact, the first two sites chosen as State Forests, Portland in 1903 and Union in 1905, were selected primarily due to their perceived ability to grow chestnut and white pine, respectively.

Interest in chestnut was particularly high. This species is a fast-growing tree of good form with high decay resistance and strong, versatile wood that can be readily sawn into good-quality lumber. Its nuts are also valuable mast for wildlife. In many parts of its range, chestnut meal was a major component of the local diet for people as well as wildlife. Because it sprouted readily and grew rapidly on cleared sites, 25 to 50 percent of the stems in Connecticut's re-growing forest were reported to be chestnut by the early 1900s (figure 6).

The plans for chestnut as the centerpiece of Connecticut forestry took a huge hit, however, when chestnut blight was discovered in 1905 on the grounds of the Brooklyn Botanical Gardens in New York. In 1907, the blight was found in Connecticut. By 1911, it was clear that the future forests in the State would have to go forward without chestnut as a major component (figure 7).

About that time, things looked almost as bad for eastern white pine. In 1900, an exotic fungus, the white pine blister rust, had been imported from Europe. Fortunately, efforts to control this disease throughout the century proved effective and the species was saved. The main control tool used was the near-eradication in the wild of all gooseberry (*Ribes* sp.), the alternate host to the rust.

Despite the obstacles, seedling production and tree planting contributed significantly to reforestation in Connecticut. Estimated forest cover increased from 38 percent in 1900, to 46 percent in 1920, and to 65 percent by 1952. According to Hawes, "A summary made in 1929 of all forest plantings which had been done in the state over the past approximately twenty years was 16,600 acres, of which 1,690 acres were in state forests and 4,725 acres belonging to Water and Power Companies. The balance was on private

Figure 6. Chestnut, a species that readily sprouts after fire, was an important component of Connecticut forest land in the early 20th century. The initials refer to Walter L. Mulford, the first Connecticut State Forester. (Photo courtesy of the Connecticut Agricultural Experiment Station archives)

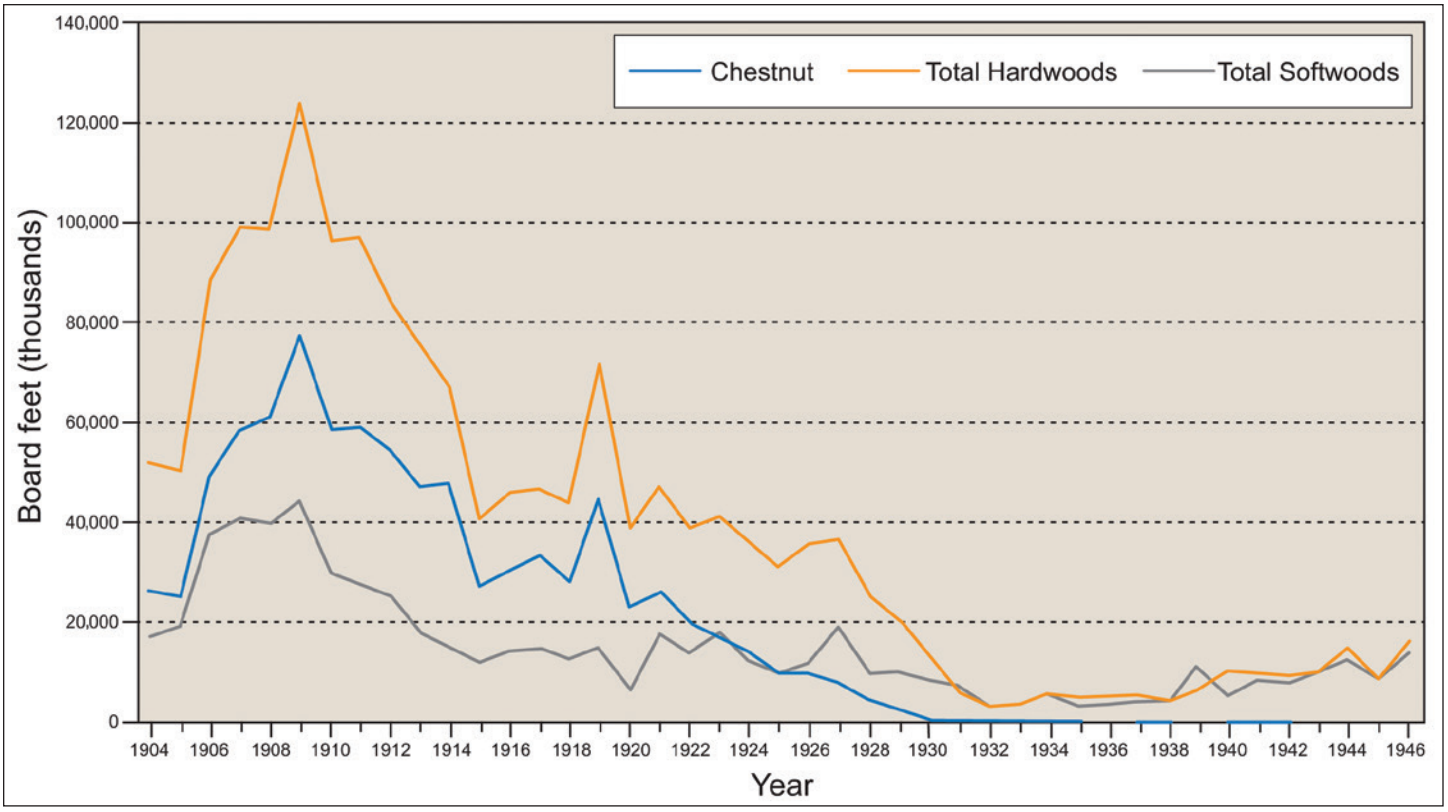

Figure 7. Lumber production in Connecticut, 1904–1940. (Source: Steer 1948)

holdings" (Hawes 1957, p. 86). Using the 1925 State Forest acreage as a guide, these comments by Hawes suggest that 10 to 15 percent of State Forest lands had been hand-planted using nursery stock. Hawes also noted that 1,117,000 seedlings, mostly conifers, had been planted on the State Forests between 1922 and 1928. Reviewing these forest plantings as a whole, he commented, somewhat ruefully, that "some of these areas had been destroyed due to fire or been suppressed by lack of care" (Hawes 1957, p. 86).

Other Challenges

In 1938, a major hurricane caused enormous damage in Connecticut (figure 8). Hawes estimated that 20 percent of the State's timber volume and 100,000 street trees were lost in this storm, a number that easily would have been higher if most of the forest had not been in young stands. Hawes reported, "While the forests of Connecticut have been in a deplorable condition ever since the death of the chestnut in the early years of the present century, the timber loss through the hurricane was undoubtedly less than it would have been except for this fact" (Hawes 1939, p. 16).

Despite the Great Depression, Hawes described the 1930s as the "Golden Age of Forestry in Connecticut" (Hawes 1957) due to the activities of the Civilian Conservation Corps (CCC) (figure 9). Since road building using CCC funding was proscribed by Federal law, the CCC workers established extensive "truck trails." The goal was a mile of 'trail' for each 500 ac (202 ha) of forest. These workers were also active in implementing

Figure 8. The Keney Park in Hartford, CT, designed for public recreation by Frederick Law Olmstead, was one of many heavily damaged by the 1938 hurricane. Log salvage was one approach to removing the downed trees. (Photo courtesy of Keney Park Sustainability Project 1938).

Figure 9. This footbridge was constructed by the Civilian Conservation Corps workers in the American Legion State Forest. (Photo courtesy of the State of Connecticut Library archives circa 1935)

timber stand improvement measures throughout the State forests, including efforts to minimize gypsy moth impacts, another pest problem that had found its way into Connecticut.

The Second Half of the Twentieth Century Through 2020

In the second half of the 20th century, the focus of forestry in Connecticut shifted towards management of hardwood forests and an increased reliance on natural regeneration. For the most part, hardwoods with some conifers intermixed are the native vegetation in Connecticut. Hardwood forests tend to occur whether or not the landowner invests in their establishment. Planting extensive stands of conifers means upfront costs and long-term risk. Red pine, for example, growing south of its natural range, proved susceptible to the red pine scale, virtually eliminating it as a timber crop and taking the investment of many landowners with it. Forestland ownership also changed with farmers owning less and less of the land and new landowners bringing new values. Many of these new landowners did not see the forest as something needing investment until the trees had grown to a certain size.

Forest Management

In a study of Connecticut's forest program, MacDonald (1969) described four phases of forest policy from 1900 to 1968. The early phase was an appeal to farmers, with reforestation and forest plantings as key features. The second phase focused on the establishment of a forest products industry in the State. The third phase moved recreational aspects of forests into the forefront (figure 10), with hunting, fishing, camping, hiking, and management of parks guiding both State forestland acquisition and overall forest policy (see also Chapman 1935). Finally, by the 1960s, the forest gained recognition as an important component of the State's environment (figure 11). This culminated in 1971 when the State forestry program was included within Connecticut's newly established Department of Environmental Protection.

Figure 10. By the 1930s, recreational opportunities became increasingly important factors in State forest policy. Development of the automobile is credited for encouraging more visitors to the forest seeking recreation, a trend that continues to this day. (Photo by Chris Donnelly 2008)

In 1962, Public Act 490 passed the legislature, reducing the tax burden on farmlands and forestlands of at least 25 ac (10 ha) in size. However, the Act did not place any management or harvesting requirements on forestland owners. This continues to present a challenge to foresters throughout the State, as this statute provides no incentive for forest management beyond keeping the land as forest for the tax break. This factor may limit forest landowners from seeking additional advice from forest professionals.

At the same time, there were several factors working in favor of sound forest management. For one, New Haven, CT, happens to be the home of the Yale School of Forestry (renamed, in 1972 the Yale School of Forestry and Environmental Studies). This school produces a regular crop of graduate students, many of whom take advantage of Connecticut's forests to explore basic aspects of hardwood silviculture and stand development (e.g., Oliver 1978). Secondly, Connecticut forests proved capable of producing high-quality timber, especially oak, which continues to attract great interest from Europe and China. Thirdly, many individuals, families, and corporations that own the forests are often highly motivated towards conservation and maintaining the forests as forests, to be intrinsically valued for what they are.

An extensive study of Connecticut's early 21st century woodland owners provides a clear contrast between the prototypical farmer of Hawes's early years and current forest landowners (Tyrell 2015). For example, by the early 2000s, the typical woodland owner has more formal education than the average Connecticut resident. In addition, Connecticut woodland owners show a strong conservation ethic and place a high value on a woodland-owning lifestyle, which means protecting privacy, nature, wildlife habitat, beauty, scenery, and biological diversity. The study also shows, however, that the number of woodland owners who receive management advice from forestry professionals is relatively low.

State Nursery Closure

In the mid-1960s, the State Nursery was still going strong, producing about 1.8 million seedlings in a typical year. About two-thirds of seedlings went to private landowners, one-sixth went to the State of Rhode

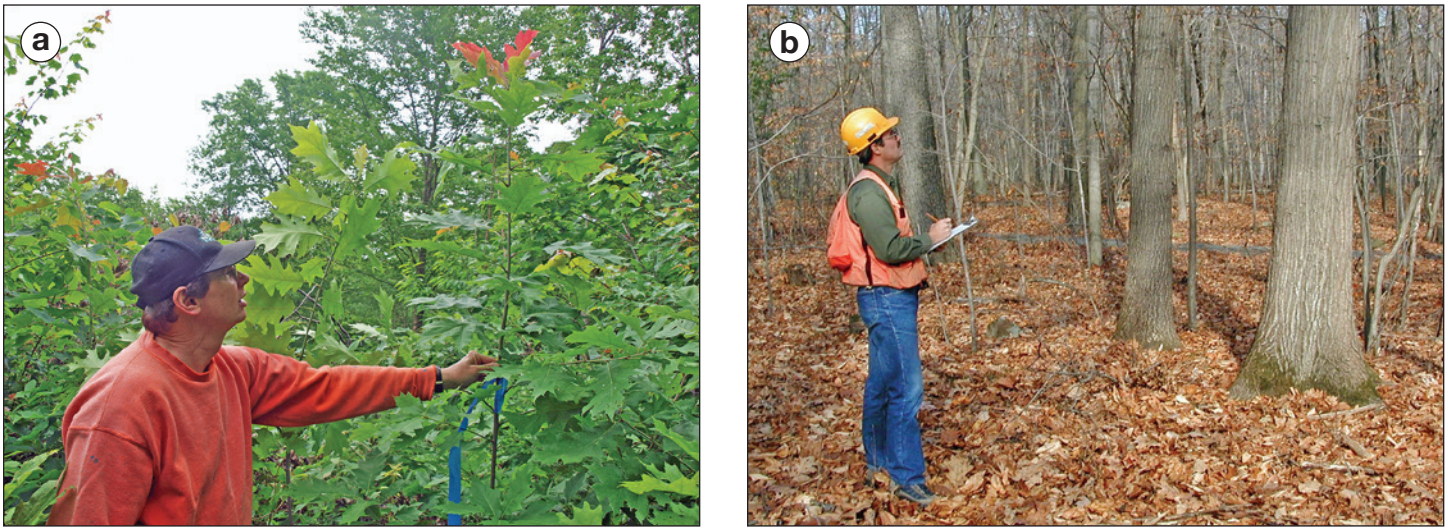

Figure 11. Forests in Connecticut are recognized as environmentally important. (a) State Lands Forester Ed McGuire inspects young red oak growing on State forest property. (b) Service Forester Rob Rocks inspects a thrifty red oak tree growing on private land. (Photos by Chris Donnelly 2012, 2014)

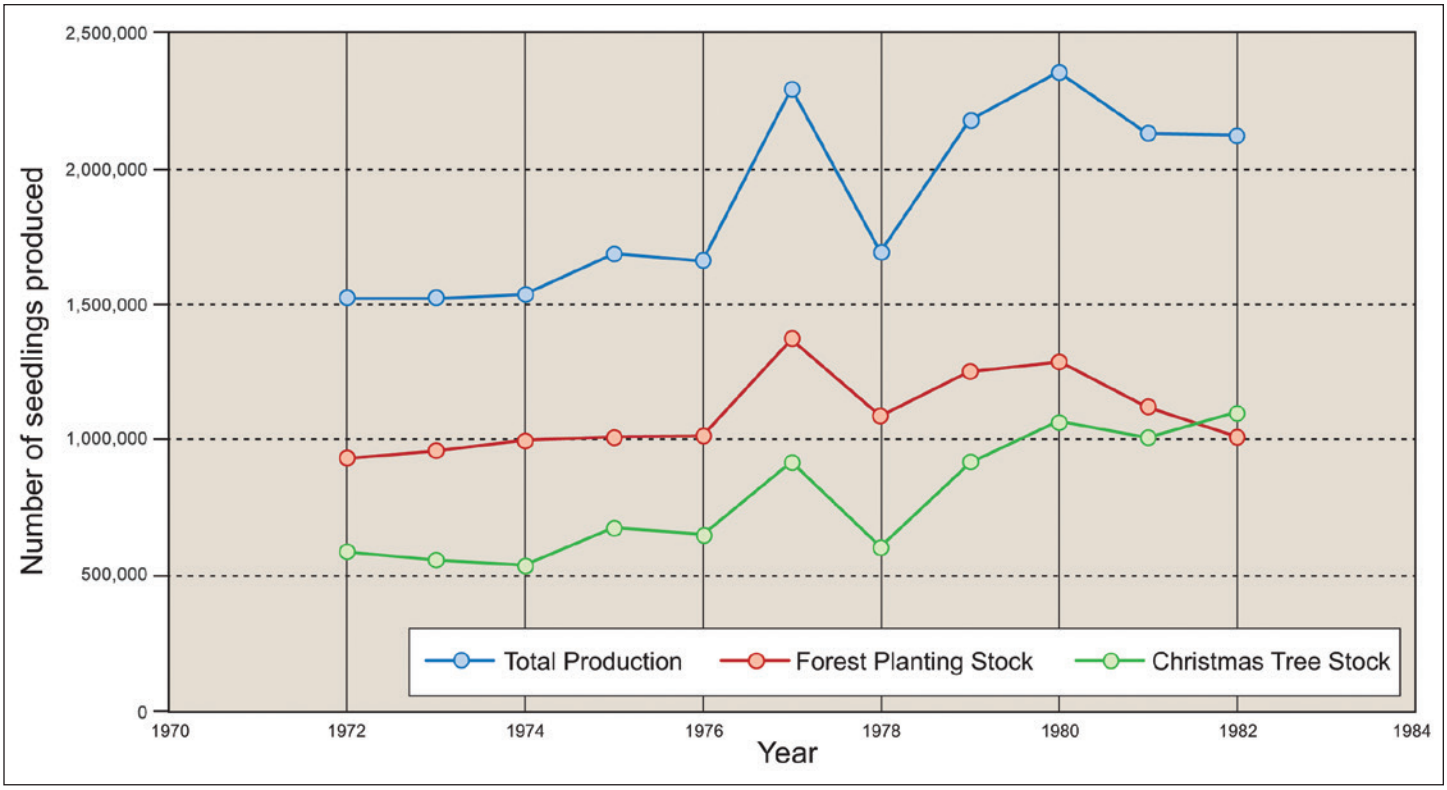

Figure 12. Seedling production at the Connecticut State Nursery changed over time until more than half of production was for Christmas trees. (Source: Cubanski 1988)

Island as the nursery took on a regional role, and one-sixth went to the State forests. By the mid-1980s, demand for seedling stock from the nursery exceeded production. Much of this increase in demand, however, came from Christmas tree growers as demand for forest planting stock was declining (figure 12). In part, this was due to white-tailed deer. Deer thrived in Connecticut's rebounding forests and, by the 1970s, had become a scourge for those who sought to underplant nursery stock. Deer, it appears, preferentially feed on nursery seedlings. The changing demands and other factors made running the State Nursery complicated and, in some ways, controversial. As a result, the State Nursery was closed in 2005. Many foresters view this closure as the loss of an important tool, especially as there are limited replacement sources for seedling stock within the State.

Current Challenges and Strategies for Forest Management in Connecticut

In the last few decades, the risks faced by specialized ecosystems have received greater consideration. One such ecosystem is the pitch pine-scrub oak barrens that occur on dry, sandy soils in association with fire (figure 13). Since these barrens are often considered to be poor for agricultural use but good for development, approximately 95 percent of these barrens within Connecticut have been lost (Gluck 2015). A long history of wildland fire suppression is also a factor. State Land foresters have led efforts to increase the amount of pitch pine (*Pinus rigida* Mill.) in the State's forests, through controlled burns, seedling planting (when they were still available from the State Nursery), and direct sowing of seeds harvested from existing trees.

Figure 13. Pitch pine regeneration in the pitch pine scrub oak barrens within Wharton Brook State Park in Wallingford, CT. These seedlings were first released by an overstory harvest in 2015. A controlled burn planned for that year was cancelled after an outbreak of southern pine beetle in the park, also in 2015. A major windstorm in 2018 further opened the canopy. (Photo by Chris Donnelly 2019)

Another concern is the balance of age classes within the State's forests. Because much of the forest initiated from large-scale, contemporaneous events, such as heavy logging in the early 1900s, the demise of the chestnut, and the 1938 hurricane, the forests are largely even-aged and many stands are of the same age. As stated in Wharton et al. (2004, p. 32):

> In Connecticut forests today, a beneficial mix of stand size classes may not exist. A disproportionate area – 69 percent of the timberland area – is in mature stands. In addition, there is an unusually small amount of regenerating stands, which comprise only 6 percent of timberland. The overall nature of tree growth, a decline in the abandonment of farmland, and reduced timber harvesting activities, have contributed to produce a forest comprised predominantly of mature stands and with a deficit of regenerating stands.

The extent of this problem became apparent when a major gypsy moth outbreak, combined with drought, occurred in eastern Connecticut from 2015 to 2017 (figure 14). The drought interfered with the activation of the maimaiga fungus that normally keeps the gypsy moth in check. In 2017, the combination of extensive repeated defoliation and drought stress led to large-scale tree mortality, especially for oaks in this part of the State (figure 15).

In response to the sudden loss of so much mature forest canopy, Connecticut's State Land foresters are considering four aspects of these oak-dominated stands (Evans 2019):

1. Encouraging and maintaining natural regeneration. Advanced regeneration of a mix of oak seedlings is somewhat hit or miss in these stands. The seedbank, however, is very good, with hick-

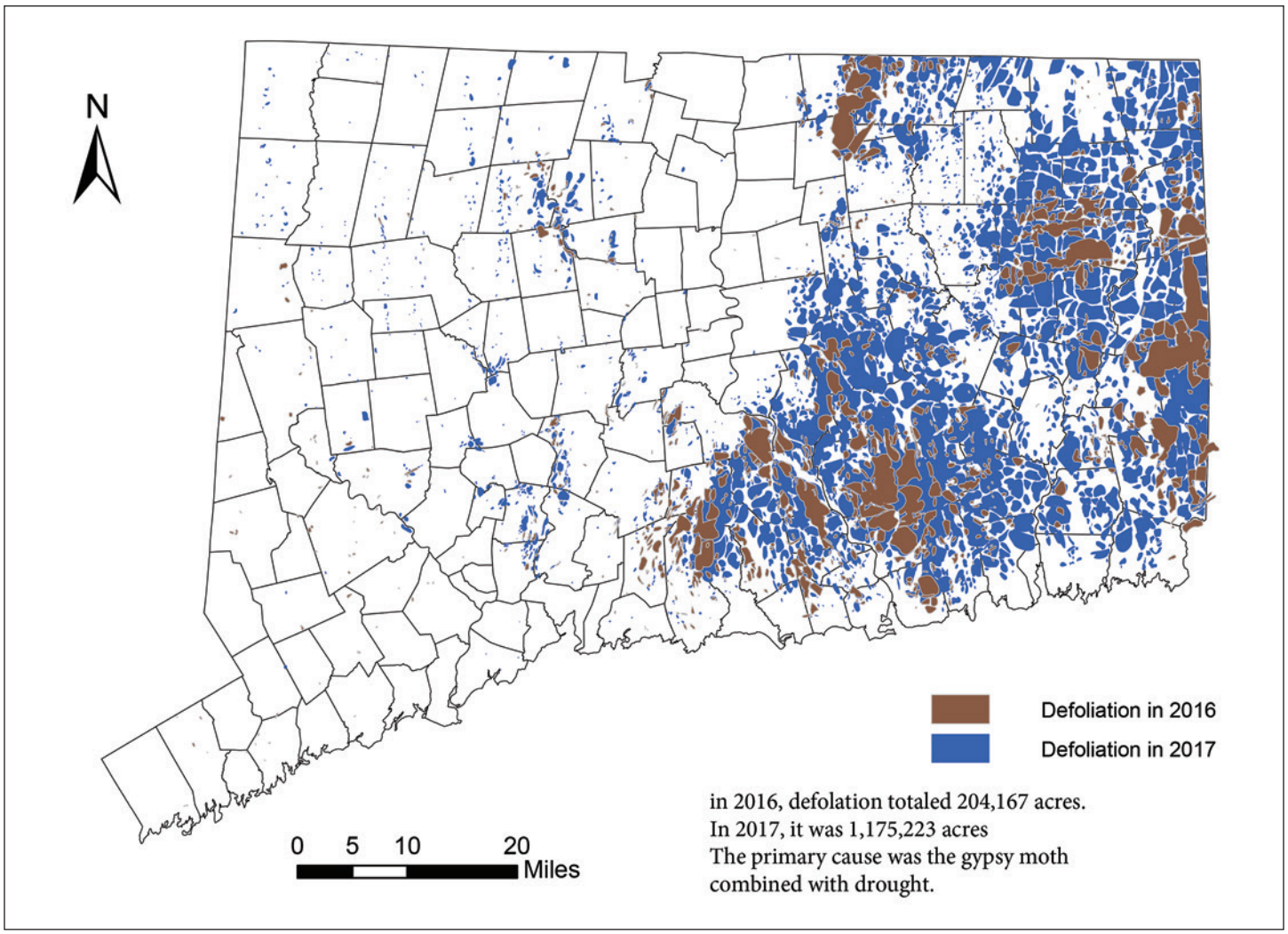

Figure 14. Overview of defoliation in Connecticut in 2016 and 2017. Map created by Chris Donnelly using data provided by the Connecticut Agricultural Experiment Station.

Figure 15. Oak forest defoliation occurred due to the combination of gypsy moth and drought. Photo was taken in August 2017, when re-foliation of oaks should have occurred. (Photo by Chris Donnelly)

ory, tulip poplar (*Liriodendron tulipifera* L.), black cherry (*Prunus serotina* Ehrh.), and other hardwoods all present, even in places where oak regeneration is limited. The fall of 2018 proved to be a good seed-crop year for white pine, adding an additional desirable seed source to the mix (figure 16).

2. Limiting opportunities for invasive plant species. Heavy regrowth is important for minimizing the incursion of invasive plant species. Invasive plants are a serious problem, hindering regeneration and causing additional forest-use problems, such as increased exposure to Lyme disease due to the relationship between Japanese barberry (*Berberis thunbergii* DC.) and the blacklegged deer tick (Williams et al. 2009). Experience indicates that shade from the regrowth can work to restrict or exclude invasive plants. In these stands, the number of invasive plants is relatively low in their interior, likely due to shading. Judicious herbicide treatments of plants such as Japanese stiltgrass (*Microstegium vimineum* [Trin.] A. Camus) also help.

Figure 16. White pine seedlings released through the removal of a low-quality, hardwood overstory. While not from a stand affected by gypsy moth, this is a good example of a "catch" of pine seedlings. (Photo by Chris Donnelly 2007)

3. Increased potential for larger fires. These areas have been subject to significant wildfires in the past, when the forest stands were younger and conditions were similar to what they are now. It will be important to make needed preparations should such fires occur, such as mowing areas of heavy shrub growth along roads to improve access for fire crews.

4. Harvesting in areas where damage is heaviest. In unmanaged areas, mid-story trees in the stratified, even-aged forest are often suppressed American beech (*Fagus grandifolia* Ehrh.), black birch (*Betula lenta* L.), and red maple (*Acer rubrum* L.). Releasing these species can yield a result similar to what happens following a high-grade operation, in which trees of low value and poor form come to dominate the stand. For this reason, even though the moth- and drought-killed oaks are of only modest value, due to their condition, their harvest may be justified by the simultaneous removal of this new, low-value overstory, in order to trigger germination of the diverse seedbank mentioned earlier.

Reflecting Back and Looking Forward

Are the forests of Connecticut better off in the 21st century than they were at the start of the 20th? For many people, this is a glass half full or half empty question. Certainly, there are many facts on the glass half full side. Among these are:

- 58 percent of the State is forested (Butler 2017).
- A profitable lumber industry is established within the State.
- More than 150 foresters and 300 other forestry professionals are currently certified through a rigorous examination process by the State's Department of Energy and Environmental Protection.
- The State has 32 State forests, covering more than 169,000 ac (68,400 ha; about 5.5 percent of the State land area).

With regard to the "glass half empty" outlook, factors include:

- Loss or diminished status of key forest trees such as chestnut and ash
- Extensive forest regeneration problems caused by invasive plants and deer
- Frequent outbreaks of exotic insect and disease problems (figure 17)
- The current unmanaged condition of many public and private forests
- The continued conversion of forestlands to subdivisions.

Indeed, the challenges to management of Connecticut's forests remain immense. Even with these concerns, however, Connecticut's forests are a long way from the "unsightly scars" and "gaunt fire-killed trees" referenced by Austin Hawes. The progress Connecticut has made is testimony to the solid vision and hard work of many people (figure 18), including the early State foresters, the many State Forestry staff over the years who dedicated their careers to bringing back the forests, the forest workers such as those associated with the CCC who helped shape the forest acre by acre, and the forest landowners and public policy makers, who helped to define a structure that has allowed a remarkable ecological turnaround to occur.

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Figure 17. Current challenges to forests in Connecticut include concerns about new insect and disease pests. State Lands Forester Jerry Milne and Connecticut Agricultural Experiment Station Entomologist Claire Rutledge explore an unusual outbreak of southern pine bark beetle in a mixed pine stand in Connecticut. (Photo by Chris Donnelly 2015)

Figure 18. A volunteer helps plant disease-tolerant American elms in the Connecticut River floodplain in an effort to improve the genetic resources of that species. This project is being led by the Nature Conservancy with support from such agencies as Connecticut DEEP and the USDA Forest Service. (Photo by Chris Donnelly 2012)

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The author thanks the Division of Forestry Staff, especially Emery Gluck, Dan Evans, Doug Emmerthal, and Jerry Milne, whose contributions helped shape this narrative, and Jeff Ward of the Connecticut Agricultural Experiment Station and Thomas Worthley of the University of Connecticut, who both have done much to keep awareness of the past present to those who work in Connecticut's forests. The author also recognizes the archives housed at the Yale University Library, the Connecticut State Library, the Connecticut Forest and Park Association, and the Connecticut Agricultural Experiment Station. Thanks also to Marielena Lima, who as a resource assistant at DEEP helped gather much of the material used in the writing of this report.

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Effect of Endomycorrhizal Inoculum on the Growth and Protection of Olive Plants Against *Phytophthora palmivora*

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Abstract

This study demonstrated beneficial effects of the symbiotic relationship between mycorrhizal arbuscular fungi (AM) and olive trees. Young olive trees were treated with a composite mycorrhiza treatment with and without inoculation of the pathogenic *Phytophthora palmivora*. A non-inoculated control was also included. Mycorrhizal plants had greater morphology compared with non-mycorrhizal plants. Of particular interest was the fact that plants inoculated with the pathogen, in the presence of mycorrhizae, had much higher growth compared with those that were inoculated with the pathogen only, indicating disease resistance due to mycorrhizal colonization. A total of 36 mycorrhizal fungal species were isolated from the rhizosphere of mycorrhizal olive plants with a spore count of 121 spores/100 g soil, compared with 27 species and a spore density of 67 spores/100 g in the rhizosphere of plants inoculated with both *Phytophthora palmivora* and mycorrhizae. Species frequency also varied between the two treatments.

Introduction

The olive tree (*Olea europaea* L.) is a characteristic species of the Mediterranean landscape (Dahbia 2009) and plays a very important socio-economic and environmental role in several countries of this region (Abousalim et al. 2005). Olive trees are integral for the maintenance of ecological continuity, the reduction of greenhouse gas production, the fight against erosion, the valorization of agricultural land, and sustainability of tree populations in mountain areas (Angles 2016). In Morocco, the olive production area has increased from 773,000 ha in 2009 to more than 1,000,000 ha in 2016 (Sadiki 2016) with nearly 1,500,000 metric tons of olives generated per year (El Mouhtadi et al. 2014). In addition, olive production actively contributes to the income of 7 million families and the settlement of the rural population by creating more than 11 million working days (MAMVA 1996). Olive cultivation, however, is experiencing several problems such as pests and diseases (Chliyeh et al. 2014a, Zouiten et al. 2001) and environmental stresses under prolonged spring and summer drought (Khabou et al. 2009, Meddad 2010, Semane et al. 2017).

The rhizosphere of olive tree roots forms a large reservoir of biological diversity, including mycorrhizal fungi that establish a symbiotic relationship with olive roots (Kachkouch et al. 2014). These arbuscular mycorrhiza (AM) improve assimilation of mineral nutrients and benefit growth of the host plant in poor soils (Chliyeh et al. 2016, Plenchette 2005). For example, uptake of nutrients with low mobility and low concentration in the soil solution, such as phosphorus, iron, zinc, and copper, can be increased in the presence of symbiotic microflora, especially mycorrhizal fungi (Duponnois et al. 2005, Gianinazzi et al. 1982, Smith and Read 1997). Mycorrhizae also allow plants to better withstand environmental stresses such as salinity, drought, and even some pathogenic soil microorganisms (Caravaca et al. 2003, Dahbia 2009, Meddad 2010, Rosendahl and Rosendahl 1991, Schreiner et al. 1997, Selosse et al. 2004). These telluric pathogens include *Phytophthora palmivora*, a fungal agent responsible for root rot of olive trees, which was recently encountered in different Moroccan olive groves (Chliyeh et al. 2013, 2014b; Msairi et al. 2017).

The objective of our study was to evaluate the effects of a composite endomycorrhizal inoculum (originating from the rhizosphere of olive trees) on the growth of young olive trees and to determine if the inoculum protected against *Phytophthora palmivora*.

Materials and Methods

Mycorrhizal Inoculum

A composite endomycorrhizal inoculum was prepared from the roots of mycorrhizal olive plants. The inoculum contained multiple endomycorrhizal species, 22 of which were identified morphologically. All of the 22 identified species have been isolated previously from the rhizosphere of olive trees in different regions of Morocco (Chliyeh et al. 2016). Barley (*Hordeum vulgare* L.) was used as a host plant to multiply the composite mycorrhizal inoculum. Barley seeds were disinfected with 5 percent sodium hypochlorite for 2 minutes, then germinated in plastic pots filled with a mixture of sterile sand and endomycorrhizal inoculum. After 4 weeks of culture, the barley roots were excised, rinsed 3 times with distilled water, and cut into 1- to 2-mm long fragments. These root fragments were used as the endomycorrhizal inoculum.

Pathogen Inoculum

Fungal pathogen inoculum was produced using an isolate of *Phytophthora palmivora* obtained from the dried twigs of an olive tree growing in Morocco's Sidi Kacem region. The inoculum was cultivated for 14 days on oatmeal agar plates (60 g oatmeal, 12.5 g of agar, and 1 L of distilled water). The mycelium was then transferred to sterile Petri dishes containing 20 ml of sterile distilled water and incubated overnight at 28 °C in the light. Subsequently, the dishes were cooled for 5 min at -20 °C to induce zoospore release. The inoculum concentration was adjusted to 10^6 zoospores/ml.

Inoculation of Young Olive Trees

In May 2015, a total of 24 young olive trees (18-months old), grown at a nursery in the Meknes region, were excavated from their substrate. Roots were rinsed under running water to remove soil, after which trees were divided into groups of six and randomly assigned to four treatments (control, pathogen inoculum, mycorrhizal inoculum, or pathogen+-mycorrhizal inoculums). For the control treatment, seedlings were transplanted to pots filled with disinfected Mamora sand. For the pathogen inoculum treatment, trees were soaked for 6 hours in a spore suspension of *Phytophthora palmivora* (10^6 zoospores/ml) and subsequently planted in pots containing the disinfected Mamora sand. For the mycorrhizal inoculum treatment, trees were transplanted to pots containing the disinfected Mamora sand and 3 g of endomycorrhizal barley root fragments incorporated into the top of the pot. For the pathogen+mycorrhizal inoculum treatment, six plants were soaked for 6 hours in the spore suspension of *P. palmivora*, then transplanted to pots containing the disinfected Mamora sand and fragments of mycorrhizal barley roots. Pots for all treatments were 30 cm in diameter and 40 cm deep with a volume of 27 liters. After transplanting, all pots were transported to the university greenhouse and seedlings were watered regularly with distilled water.

After 6 months of culture, the olive plants from all four treatments were severed at the base of the stem. Roots were rinsed with tap water, then the root and aerial parts were dried on absorbent paper for 6 hours under ambient laboratory conditions. Morphology was assessed on each plant by measuring stem height, root biomass, and number of leaves, twigs, and buds.

Spore Extraction

After 6 months of greenhouse cultivation, olive plants were excavated from the pots and mycorrhizal spores were extracted from the growing medium of the two treatments that included mycorrhizal inoculum according to the wet sieving method described by Gerdemann and Nicolson (1963). A sample was collected from each of the 6 pots of each treatment inoculated with mycorrhizae to determine the mycorrhizal population associated with the olive tree in the presence and absence of the pathogen. In a 1 L beaker, 100 g of each composite soil sample was immersed in 0.5 L of tap water and stirred for one minute with a spatula. After 10 to 30 seconds of decantation, the supernatant was passed through four superposed sieves with a decreasing mesh size (500, 200, 80, and 50 microns). This operation was repeated twice. The contents recovered after passing through the different sieves were divided into two

tubes and centrifuged for 4 min at 9000 rpm. The supernatant was discarded and a viscosity gradient was created by adding 20 ml of a 40-percent sucrose solution to each centrifuge tube (Walker et al. 1982). The mixture was rapidly stirred and the tube was returned to the centrifuge for 1 min at 9000 rpm. In contrast to the first centrifugation step, the supernatant was poured into the sieve with a mesh size of 50 microns. The resulting substrate was rinsed with distilled water to remove sucrose, and then disinfected with an antibiotic solution (streptomycin). The spores were then recovered with a little distilled water in a flask.

Spore identification was performed according to the species descriptions provided by the International Cultural Collection of Mycorrhizal Arbuscular Vesicular Fungi (INVAM 2017) and by following both the classification of Redecker et al. (2013) and the criteria proposed by Schenck and Smith (1982), Schenck and Perez (1987), and Morton and Benny (1990).

Statistical Analyses

Seedling morphology data were analyzed using analysis of variance (ANOVA) for a completely randomized design. Significant differences among the four treatments were determined using the least significant difference test at the 5 percent threshold. Data were analyzed using Statistica software (StatSoft Inc.).

Results

Mycorrhizal inoculation had a positive effect on olive plant shoot morphology after 6 months of greenhouse cultivation (table 1). Plants inoculated with *Phytophthora palmivora* without the mycorrizal inoculum showed disease symptoms of dieback, leaf drop, decay, and root system degradation (figure 1). In contrast, plants inoculated with both *P. palmivora* and mycorrhizal fungi showed no signs of disease and had significantly greater height, number of branches, number of leaves, and root biomass compared with plants inoculated with *P. palmivora* only (table 1). AM fungal species isolated from mycorrhizal soils without the presence of *P. palmivora* showed an association of 36 species with a spore density of 121 spores/100 g of soil (table 2,

Figure 1. The effect of an endomycorrhizal inoculum on root and shoot development of olive plants; I: plant inoculated with *Phytophthora palmivora*, M: mycorrhizal plant, C: control plant. (Photo by S. Msairi 2015)

Table 1. The effect of a composite endomycorrhizal inoculum on the growth parameters of olive plants inoculated with *Phytophthora palmivora*. Within a column, means followed by the same letter are not significantly different at the 0.05 level.

Inoculation treatment	Number of branches	Number of leaves	Number of buds	Root mass (g)	Height (cm)
Phytophthora palmifora	9.0 c	101.0 d	13.3 a	13.1 c	53.0 b
Phytophthora palmifora + mycorrhizae	27.0 a	258.3 c	27.0 a	44.6 b	67.4 a
Mycorrhizae	18.0 b	381.3 a	15.3 a	96.9 a	74.1 a
Control (no inoculation)	29.8 a	338.6 b	11.8 a	75.1 ab	76.5 a

Table 2. The identification of the isolated mycorrhizal fungi from the rhizosphere of mycorrhizal olive trees. Photos of each are shown in figure 2.

Photo number	Name	Number of spores	Shape	Color	Spore surface	Average size (µm)
1	*Acaulospora* sp.	3	globular	clear yellow	smooth	32
2	*Acaulospora* sp.	7	oval	dark yellow/ clear brown	smooth	31
3	*Acaulospora* sp.	2	globular	brown	irregular	28
4	*Acaulospora colossica*	19	oval	clear brown	irregular	23
5	*Acaulospora foveata*	15	globular	dark yellow	smooth	40
6	*Acaulospora gedanensis*	3	oval	dark yellow	irregular	45
7	*Acaulospora mellea*	14	globular	clear yellow	smooth	38
8	*Acaulospora morrowiae*	9	globular	yellow	irregular	25
9	*Acaulospora nicolsonii*	3	globular	yellow	irregular	22
10	*Acaulospora scrobiculata*	41	globular	clear yellow	smooth	38
11	*Claroideoglomus etunicatum*	24	oval	dark brown	granular	30
12	*Gigaspra* sp.	1	oval	dark yellow	smooth	50
13	*Gigaspora* sp.	1	globular	yellow	irregular	31
14	*Gigaspora margarita*	27	globular	dark yellow	granular	35
15	*Glomus ambisporum*	18	oval/ globular	dark brown	irregular	36
16	*Glomus aureum*	9	globular	clear brown	smooth	30
17	*Glomus clarum*	30	globular	dark yellow	granular	37
18	Glomus constrictum	4	globular	dark brown	irregular	25
19	*Glomus deserticola*	30	globular	brown	irregular	50
20	*Glomus glomerulatum*	6	globular	dark yellow	smooth	38
21	*Glomus heterosporum*	30	globular	dark brown	irregular	45
22	Glomus hyderabadensis	6	globular	brown	smooth	42
23	*Glomus intraradices*	39	oval/ globular	brown	irregular	30
24	*Glomus leptotichum*	4	oval	yellow	smooth	45
25	*Glomus macrocarpum*	17	globular	yellow/ brown	irregular	32
26	*Glomus margarita*	3	oval	clear brown	granular	34
27	*Glomus microcarpum*	5	globular	yellow	smooth	36
28	*Glomus mosseae*	18	globular	clear brown	granular	27
29	*Glomus versiforme*	66	globular	clear brown	irregular	55
30	*Glomus walker*	6	globular	clear brown	smooth	32
31	*Pacispora* sp.	3	globular	dark yellow	granular	34
32	*Pacispora* sp.	3	globular	yellow/green	granular	35
33	*Scutellospora* sp.	6	globular	dark brown	granular	28
34	*Scutellospora gilmorei*	6	globular	transparent/ light green	granular	24
35	*Scutellospora heterogamma*	2	oval	brown	granular	63
36	*Scutellospora savannicola*	4	oval	clear brown	irregular	29

figure 2). These species were distinguished on the basis of morphological criteria representing 6 genera with the dominant AM fungal species being *Glomus versiforme, G. intraradices, Acaulospora scrobiculata, G. clarum,* and *G. deserticola*. In contrast, species isolated from mycorrhizal soils in the presence of *P. palmivora* showed an association of 27 species with a spore density of 67 spores/100 g of soil with the dominant AM species being *Acaulospora scrobiculata, A. genensidas, Scutellospora nigra, Glomus radiates,* and *Gigaspra margarita* (table 3, figure 3).

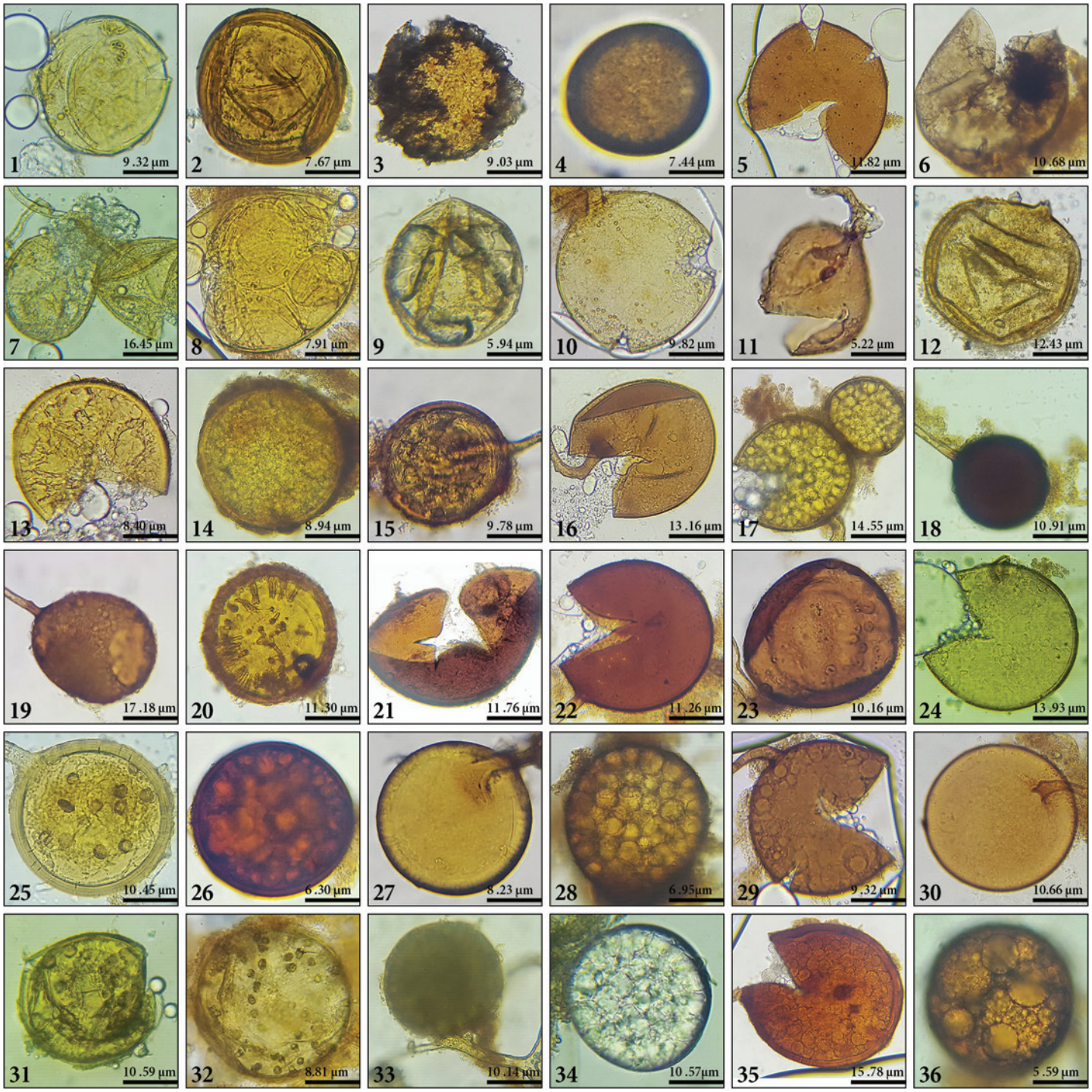

Figure 2. Species of endomycorrhizal fungi that were isolated from the rhizosphere of mycorrhizal olive plants; numbers correspond to table 2. (Photos by S. Msairi 2015)

Table 3. Identification of mycorrhizal fungi isolated from the rhizosphere of mycorrhizal olive plants and inoculated with *Phytophthora palmivora*. Photos of each are shown in figure 3.

Photo number	Name	Number of spores	Shape	Color	Spore surface	Average size (µm)
1	*Acaulospora* sp.	4	globular	dark yellow	irregular	22
2	*Acaulospora adenticulate*	4	globular	yellow	granular	7
3	*Acaulospora colombiana*	2	oval/ undetermined	clear brown	granular	47
4	*Acaulospora foveata*	9	globular	brown	irregular	37
5	*Acaulospora genensidas*	22	globular	yellow	granular	25
6	*Acaulospora lacunose*	14	oval	brown	granular	30
7	*Acaulospora laevis*	6	globular	dark yellow	granular	44
8	*Acaulospora nicolsonii*	9	oval	clear brown	irregular	27
9	*Acaulospora scrobiculata*	40	globular	brown	irregular	29
10	*Ambispora leptoticha*	2	oval	yellow	smooth	30
11	*Gigaspora* sp.	6	globular	green	granular	45
12	*Gigaspora* sp.	3	globular	yellow	smooth	31
13	*Gigaspora* sp.	11	globular	yellow	granular	33
14	*Gigaspora margarita*	17	globular	brown	smooth/ granular	40
15	*Glomus* sp.	5	globular	dark yellow/ clear brown	granular	21
16	*Glomus* sp.	7	globular	brown	smooth	35
17	*Glomus* sp.	3	oval	transparent clear brown	smooth	25
18	*Glomus aureum*	9	globular	yellow	smooth	26
19	*Glomus clarum*	10	globular	brown	granular	31
20	*Glomus fecundisporum*	15	globular	brown	granular	30
21	*Glomus macrocarpum*	10	globular	yellow	smooth	48
22	*Glomus mosseae*	8	oval	brown	granular	35
23	*Glomus radiatus*	19	globular	yellow/ brown	smooth	32
24	*Glomus rubiforme*	4	oval/ undetermined	transparent clear brown	smooth	43
25	*Pacispora scintillans*	3	globular	yellow	smooth	48
26	*Scutellospora* sp.	5	globular	brown	granular	29
27	*Scutellospora nigra*	21	globular	dark brown	smooth	30

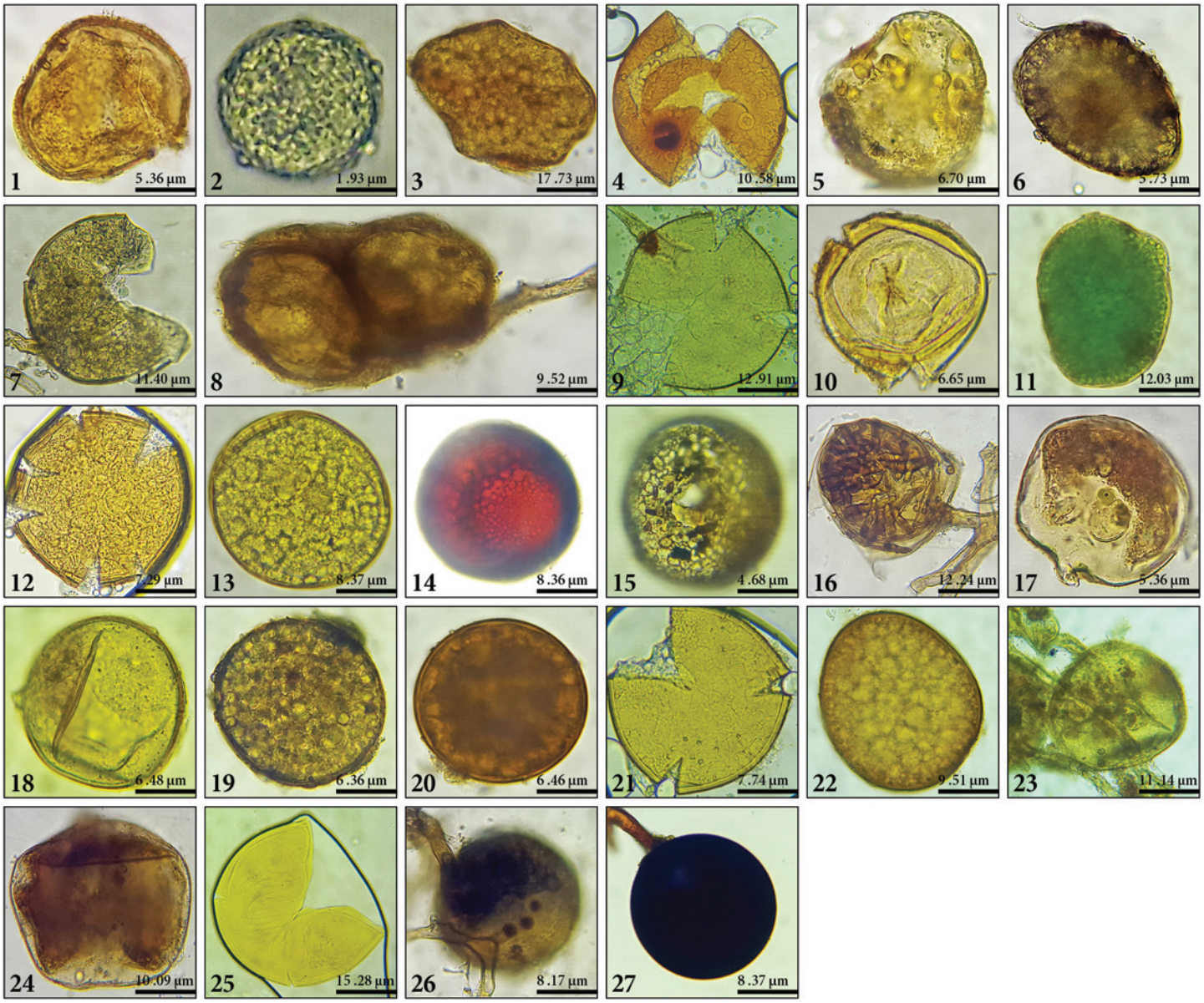

Figure 3. Species of endomycorrhizal fungi were isolated from the rhizosphere of olive plants that were inoculated with *Phytophthora palmivora* in the presence of mycorrhizae; numbers correspond to table 3. (Photos by S. Msairi 2015)

Discussion

Mycorrhizae inoculation resulted in good root colonization and improved shoot and root growth of young olive trees compared with non-mycorrhizal plants (control). These results are similar to research reported by Chliyeh et al. (2014) in which inoculation of olive plants with a composite endomycorrhizal inoculum showed good establishment of mycorrhizal symbiosis and improved growth compared with non-inoculated controls. Other studies have also noted that olive trees growing on a substrate containing endomycorrhizal fungi had improved growth compared with controls (Meddad et al. 2010, Semane et al. 2017). According to these authors, all studied growth parameters of mycorrhizal plants, including number of leaves, number of buds, height, root biomass, and shoot biomass, were higher than those of non-mycorrhizal control plants. Favorable effects of mycorrhizae have also been reported in other plant species such as the argan tree (*Argania spinosa*) (Sellal et al. 2017), rice (*Oryza sativa* L.) (Bernaola and Stout 2019), leek (*Allium porrum* L.) (Hibilik et al. 2018, Tran et al. 2019), sorghum (*Sorghum bicolor* [L.] Moench), carrot (*Daucus carota* L. var. *sativus* Hoffm.) (Kim et al. 2017), and common reed (*Phragmites australis* [Cav.] Trin. ex Steud.) (Liang et al. 2018).

In a study on sweet cherry (*Prunus avium* L.), plants inoculated with *Glomus intraradices* and *G. caledonium* showed a positive effect on foliage formation, dry weight, and stem diameter in mycorrhizal plants compared with control plants (Cordier 1996). The main effect of *G. intraradices* was on plant dry weight, while that of *G. caledonium* was on plant stem diameter. The increased growth of olive trees grown in mycorrhizal soil is likely due to increased access to soil water and nutrients. Mycorrhizal fungi can be considered biofertilizers, bio-regulators, and bio-protectants (Gianinazzi et al. 2010).

AM fungi in our study also showed a positive effect against disease caused by *Phytophthora palmivora*. Other studies have shown a positive effect of mycorrhiza on the growth and protection of plant species against certain root pathogens, including *Phytophthora* (Bärtschi et al.1981, Cordier et al. 1998, Duponnois et al. 1993, Duponnois and Cadet 1994, Guillemin et al. 1994). Similar to our study, the pathogen *P. cinnamomi* did not cause negative effects on fresh biomass development of sweet cherry roots pre-colonized by the AM fungi *Glomus mosseae*, whereas a decrease in root growth was observed in non-mycorrhizal plants (Cordier 1996).

Studies have been conducted to better understand the bioprotection mechanisms of mycorrhizae. In tomato, AM bioprotection against *Phytophthora parasitica* is related to a reduction of pathogen propagation in the mycorrhizal plants' root systems, and to a resistance of cells containing arbuscules (Cordier et al. 1998). This resistance may be related to the activation of defense responses in host tissues (Benhamou et al. 1994, Gianinazzi 1991), or to the expression of certain defense-related genes in the cells containing the arbuscules (Blee and Anderson 1996; Gianinazzi-Pearson et al. 1992, 1996; Harrison and Dixon 1994; Lambais and Mehdy 1995).

Dalpé et al. (2005), identified five interacting mechanisms of mycorrhizae as biocontrol agents. Some mechanisms are directly related to the plant, either through growth stimulation by increasing nutrient supply and better plant health, or morphological transformation at the root level, or by induction or suppression of defense mechanisms, especially those involving multiple enzymes. Other mechanisms act on the parasite through direct competition with mycorrhizal fungi related to the availability of nutrients, sites of infection, and soil structure and quality, through a modification of the microflora and an increase in the rate of the organic matter (content).

Conclusion

The olive tree is a highly mycotrophic species and forms a positive mycorrhizal association with several AM species. These mycorrhizal fungi not only stimulate shoot and root growth but can have a remarkable protective effect against root rot caused by *Phytophthora palmivora*. This protective activity can be exploited to attenuate the progressive extension of *P. palmivora*, which could constitute a real danger for the olive tree and for the crops in the vicinity. The introduction of mycorrhizae as a biological control agent in agricultural practices can contribute to the development of a sustainable agriculture by reducing the application of chemical pesticides.

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Locally Produced Cocopeat Growing Media for Container Plant Production

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Abstract

In our pursuit of finding a local alternative to using topsoil for plant production in containers, we concluded that coconut husk can be easily processed into an ideal growing medium. On Yap, Federated States of Micronesia, coconuts are abundant. The coconut kernel is used for food, fodder, and other purposes, but the spongy pericarp (husk) is a byproduct. Over the years, we standardized a method to make a suitable growing medium (cocopeat). This product is not only useful for growing plants but also can be used for soil remediation and other agricultural purposes. This article describes the process we use to create the medium, along with a description of its properties and uses.

Introduction

For small-scale growers in Yap, field-based plant cultivation for gardening, reforestation, and land restoration has always been a challenge due to the inherent properties of native soils. Depleted nutrients and pH-dependent accumulation of soluble aluminum pose serious challenges for field-based agriculture in degraded volcanic red soils. Two upland soil types are prevalent on Yap Proper: upland soils underlain by volcanic material (oxisols in the U.S. Department of Agriculture [USDA] classification) (figure 1) and upland soils underlain by schist (a metamorphic rock) (alfisols in the USDA classification) (figure 2) (Smith 1983). These soils have unique properties and, therefore, need special management practices if used for plant cultivation. The volcanic, red soils are the most degraded and the least fertile soils in Yap. Nutrient contents and the ability to hold nutrients are very low in these soils due to their low cation exchange capacity. These physical, chemical, and biological limitations of degraded soils create challenges for both natural forest regeneration and plant cultivation (figure 3). For example, schist-derived soils are made up of a particular type of

Figure 1. In Yap, soils such as this Gagil series underlain with volcanic material are highly degraded and low in fertility. (Photo by Murukesan V. Krishnapillai)

Figure 2. Upland soils over schist, such as this in the Weloy series, are prevalent on Yap proper and are challenging for reforestation and plant cultivation. (Photo by Robert Gavenda, retired, USDA Natural Resources Conservation Service)

Figure 3. Degraded soils often result in nutrient-stressed, slow-growing plants as seen on this site with planted *Calophyllum inophyllum* L. (Photo by J.B. Friday)

shrink/swell clay particles and become sticky when wet and hard when dry. When wet, water movement slows in these soils, thereby making it challenging to grow plants. Because of these inherent soil challenges, plant production in containers has been promoted in Yap as a climate change adaptation strategy for displaced atoll communities residing in marginalized environments.

Growing media or substrates for container production are composed of solid materials that can be used individually or in mixtures. Media directly affect root system development and function. A good-quality growing medium provides sufficient anchorage or support to the plant, serves as a reservoir for nutrients and water, allows oxygen diffusion to the roots, and permits gaseous exchange between the roots and the atmosphere (Abad et al. 2002; Argo 1998a, 1998b; Gruda et al. 2013). Topsoil is commonly used as a component of growing media in many tropical nurseries. Using topsoil, however, can be problematic and hinder successful production of seedlings for field establishment. Problems associated with using topsoil in nurseries include shortages and sustainability of quality topsoil, compaction, introduction of weed seeds or pathogens, poor drainage, and insufficient nutrients. In addition, containers filled with soil are heavy and bulky for handling and transportation. Thus, other suitable growing media are needed for plant production.

Cocopeat as a Growing Medium

A wide selection of growing media is available in the market. The choice of which medium to use depends on a grower's financial and technical capabilities (Gruda et al. 2013). In tropical regions, most growers use substrates that are locally available because they are inexpensive and reliable. To cope with soil challenges in Yap and to cultivate plants in containers, cocopeat planting medium has been successfully produced. Coconut coir pith (also known as cocopeat or cocopith) is one of the renewable resources widely available in the tropics. Coconut palms (*Cocos nucifera* L.) are abundant in the Pacific Islands (figure 4), where they are extremely important for daily subsistence and also have significant economic and cultural values. Coconut plays a central role in islanders' diets and is thus vital for food security, health promotion, and sustainable livelihoods. As a versatile, raw material that supports both household and wider societal needs—from housing, to transport, to cultural production—coconut is a valuable resource woven into the very fabric of Pacific society and daily life.

On Yap, the inner kernels of coconuts (endosperm) are largely exploited for pig feed and, to a limited extent, coconut oil. The spongy mesocarp (husk) is left as a byproduct. Coconut husk is made up of natural fibers called coir along with parenchymatous, spongy material called coirpith that binds the fibers in the husk (figure 5). Being made up of sclerified tissue, coconut fiber does not retain much water. In a growing medium, however, fibers create aeration through porosity in the coir and provide structure to prevent compaction. These characteristics are important for a healthy root zone. The coirpith acts like micro sponges where the moisture is stored. The fiber and pith (cocopith) together make a great growing medium with an excellent air-to-water ratio.

Figure 4. Coconut palms are abundant in many tropical islands and provide a good source of food, animal feed, oil, and other products. (Photo by J.B. Friday)

Figure 5. The coconut consists mainly of an inner endosperm and outer mesocarp (husk). (Photo by Murukesan V. Krishnapillai))

Raw cocopith has a high carbon (C) to nitrogen (N) ratio (112:1) and high lignin content and can result in immobilization of plant nutrients. This inhibitory effect can be eliminated, however, by using partially decomposed coir pith. Decomposition of coconut husks reduces the C:N ratio to about 30:1, which is ideal for use as an organic growing substrate. Cocopith has many desirable characteristics (table 1), making it an ideal medium for various horticultural uses. These characteristics include high moisture retention capacity, high potassium content, low bulk density (0.18 g/cm^3) and particle density (0.8 g/cm^3) and high cation exchange capacity enabling it to retain high amounts of exchangeable potassium (K), sodium (Na), calcium (Ca), and magnesium (Mg). These characteristics also make cocopith ideal for use as a mulch and soil amendment, especially for dry and sandy areas with low water retention.

Table 1. Chemical and physical properties of partially decomposed cocopith.

Property	Partially decomposed cocopith
Lignin (%)	28.5
Cellulose (%)	25.8
Organic carbon (%)	29.0
Nitrogen (%)	0.26
Phosphorus (%)	0.01
Potassium (%)	0.76
C:N ratio	30:1
Calcium (%)	0.47
Magnesium (%)	0.41
Copper (ppm)	4.20
Iron (ppm)	0.08
Manganese (ppm)	17.0
Zinc (ppm)	9.8
pH	5.6 – 6.0
EC (millimhos/cm)	0.3 – 0.6
CEC (meq/100 g)	40 - 100

Sources: Alexander and Bragg 2014, Awang et al. 2009, Cahyo et al. 2019, Carlile et al. 2015, Coir Board 2016, Gruda 2019, Holman et al. 2005, Kalaivani and Jawaharlal 2019, Londra et al. 2018, Noguera et al. 2000, Paramanandham et al. 2013, Prasad 1997, Robbins and Evans 2011, Sengupta and Basu 2016.

Cocopith resembles *Sphagnum* peat moss, the most common potting medium used in horticulture, but offers many advantages as a growing medium (table 2). With the demands of commercial horticulture and resulting reduction in *Sphagnum* peat availability due to despoiling of ecologically important peat bog areas, cocopeat has become internationally recognized as an ideal soil amendment and component of soilless container media for the horticultural industry.

While various commercial products are available, the local abundance of coconuts on Yap allows for on-site processing of coconut husks into a suitable growing medium. An average coconut tree produces 150 to 180 coconuts per year, ensuring a continuous supply of husks. One coconut yields approximately 100 g (0.22 lb) of cocopeat, thus making it an affordable and sustainable product.

Cocopith Preparation Method

Both fresh and partly decomposed coconut husks are suitable for preparing quality growing media (figure 6). Coconut husks begin to decompose in about 2 to 3 months under humid, tropical conditions.

Figure 6. Coconut husks will decompose in 2 to 3 months in tropical conditions, making them an ideal source of growing medium for plant production. (Photo by Murukesan V. Krishnapillai)

Table 2. Comparison between cocopith and *Sphagnum* peat.

Characteristics	Cocopith	Sphagnum peat
pH	5.5 – 6.5	3.9 – 4.3
Water holding capacity	6 to 11 times its dry weight; coirpith is composed of spongy parenchyma cells and will hold up to 80 percent water once the excess drains away.	4 to 8 times its dry weight
Rewetting time	Very rapid because cocopith's sponge-like parenchyma structure has the ability to absorb large quantities of water very quickly	Considerably slower than cocopith; becomes hydrophobic once dried.
Longevity	Approximately 3 to 5 years owing to high lignin content which inhibits bacterial and fungal breakdown and thus allows cocopith to decompose much more slowly than traditional peat moss.	6 months to 1 year depending upon the quality of product.
Air-filled porosity	Quality cocopith can retain high (~96 percent) air porosity while also holding large quantities of water without becoming waterlogged.	Sphagnum peat has 71-95 percent air porosity. Over time, however, air porosity can decrease due to breakdown, thereby decreasing oxygen to the roots.
Shrinkage	Due to its high lignin and cellulose structure, cocopith does not shrink	Sphagnum peat can shrink away from sides of the container if allowed to get too dry resulting in water draining down the sides.
Cation Exchange Capacity (CEC)	Coirpith has a high CEC ratio of 40 to 100 meq per 100 g, thus nutrients are not leached away but are held to release to the plant as required.	Sphagnum peat has a CEC of 55 to 200 meq per 100 g
Sustainability	Coconut husks are always available as a waste product wherever coconut palms are present.	Peat bogs take at least 25 years to renew.

Sources: Holman et al. 2005, Londra et al. 2018, Prabhu and Thomas 2002, Rezanezhad et al. 2016, Shanmugasundaram et al. 2014, Wellock et al. 2011, WSU 2018, Xiong et al. 2017.

Though fresh husks result in a long-lasting product, coarse fibers need to be screened out before using. Soft, partially decomposed husks are ideal for shredding into a usable medium (figure 7). A commercially available chipper-shredder of at least 10 horsepower is recommended to shred the coconut husks (figure 8). We use a Troy-Bilt Model CS 4325 Chipper Shredder (Troy-Bilt LLC, Valley City, OH). Before feeding into the chipper-shredder, husks must be chopped into small (1- to 2-in [2- to 5-cm]) pieces

Figure 7. (a) Fresh, (b) partially decomposed, and (c) fully decomposed coconut husks can all be shredded to create a suitable substrate for forest and agriculture plants. (Photos by Murukesan V. Krishnapillai)

(figure 9). By chopping the husks before shredding, the final mix usually consists of 10 to 20 percent short fibers and 80 to 90 percent parenchymatous pith ranging in size from fine dust to granules (up to 5 mm [0.2 in])(figure 10). If using fresh husks, a mesh screen may be used to separate coarse fibers from the shredded mix (figure 11).

After shredding, we thoroughly mix 3 parts shredded cocopith with 1 part commercial composted chicken manure (figure 12). In Yap, this gives an excellent growing medium for both forest seedling production and vegetable production. Unlike commercially available cocopeat bales or briquettes, cocopith extracted from freshly sourced husks does not contain excessive salt levels, does not require rehydration, and contains sufficient coarse fibers to maintain adequate aeration. When using cocopeat made from fresh husks, however, there is a likelihood of nitrogen drawdown. Therefore, adding slow-release or organic fertilizers in addition to the composted chicken manure is advised.

Figure 8. Coconut husks can be shredded using a commercially available chipper-shredder. (Photo by Diane L. Haase)

Uses for Cocopeat

Cocopeat can be mixed with soil and other media components to make suitable mixes for plant propagation. It is widely used in agriculture, horticulture, and restoration for the production of flowers, vegetables, trees, shrubs, and forbs (Alzrog et al. 2013, Ayesha et al. 2011, Bagci et al. 2011, Barrett et al. 2016, Cahyo et al. 2019, Erwan et al. 2013, Gohil et al. 2018, Ilahi and Ahmad 2017, Khan et al. 2019, Kumarasinghe et al. 2015, Rose and Haase 2000, Rubio et al. 2011, Soltani and Naderi 2016, Sutari et al. 2018, Tariq et al. 2012, Udayana et al. 2017, Xiong et al. 2017). Cocopeat media can be used in various container types as well as vertical gardening structures (figure 13).

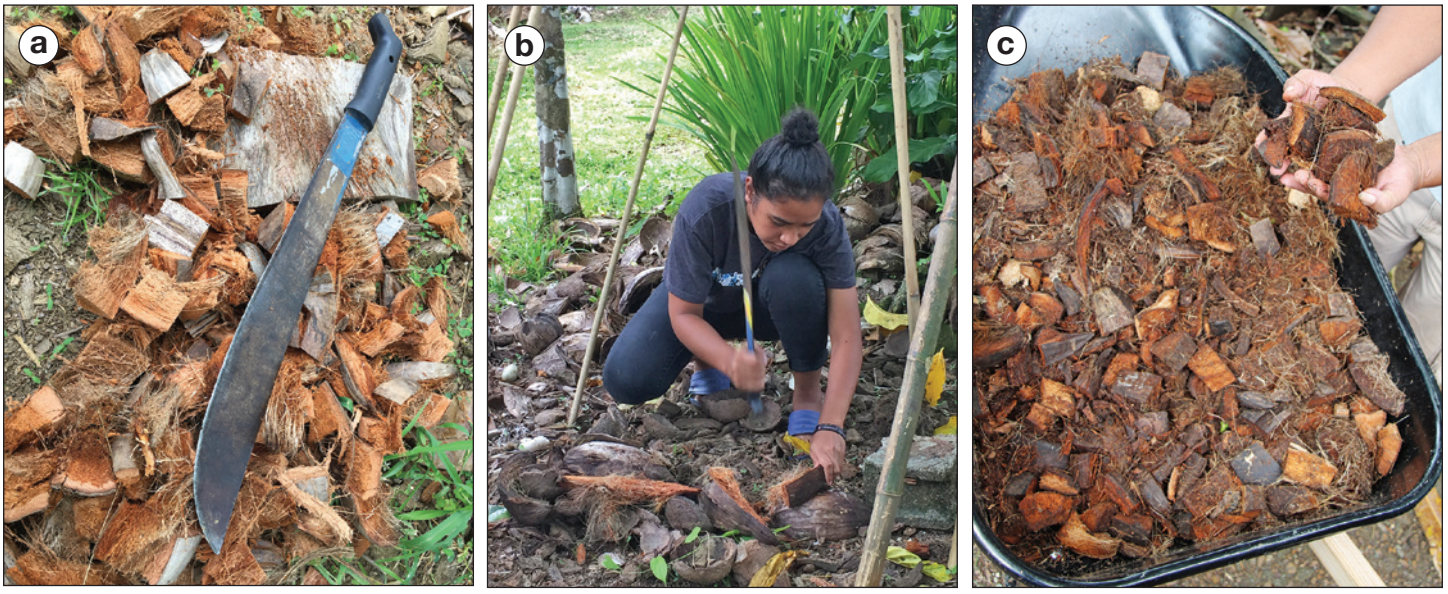

Figure 9. To ensure uniformity and optimum fiber size, composted coconut husks should be (a and b) chopped into (c) small pieces before shredding. (Photos on left and in middle by Diane L. Haase, photo on right by J.B. Friday)

Figure 10. After shredding, both (a) fresh and (b) composted husks can be used in a growing mix for plant production. (Photos by Diane L. Haase)

Figure 11. A mesh screen is often needed to separate coarse fibers from shredded fresh husks (Photo by Diane L. Haase)

Figure 12. Shredded coconut husks can be mixed with chicken manure to create an excellent medium for growing forest seedlings or agricultural plants. (Photo by Diane L. Haase)

Because it is relatively resistant to harmful microbial and fungal growth, it is an ideal medium for germinating seeds (Hyder et al. 2009). Increasingly, cocopeat is used for roof, patio, and kitchen gardening. Cocopeat and coir fibers are also used to make pots for growing plants. These pots can be arranged on screens in vertical gardening and can even be hung on balconies. The versatility and quality of cocopeat supports various community programs on Yap (figure 14).

In addition to growing plants, cocopeat has many other uses. It is an excellent bedding for the growth of earthworms for vermiculture (Patil et al. 2017). Cocopeat is also used as bedding in animal farms, poultry sheds, and pet houses to absorb animal waste. It can be used as an oil absorbent on slippery floors.

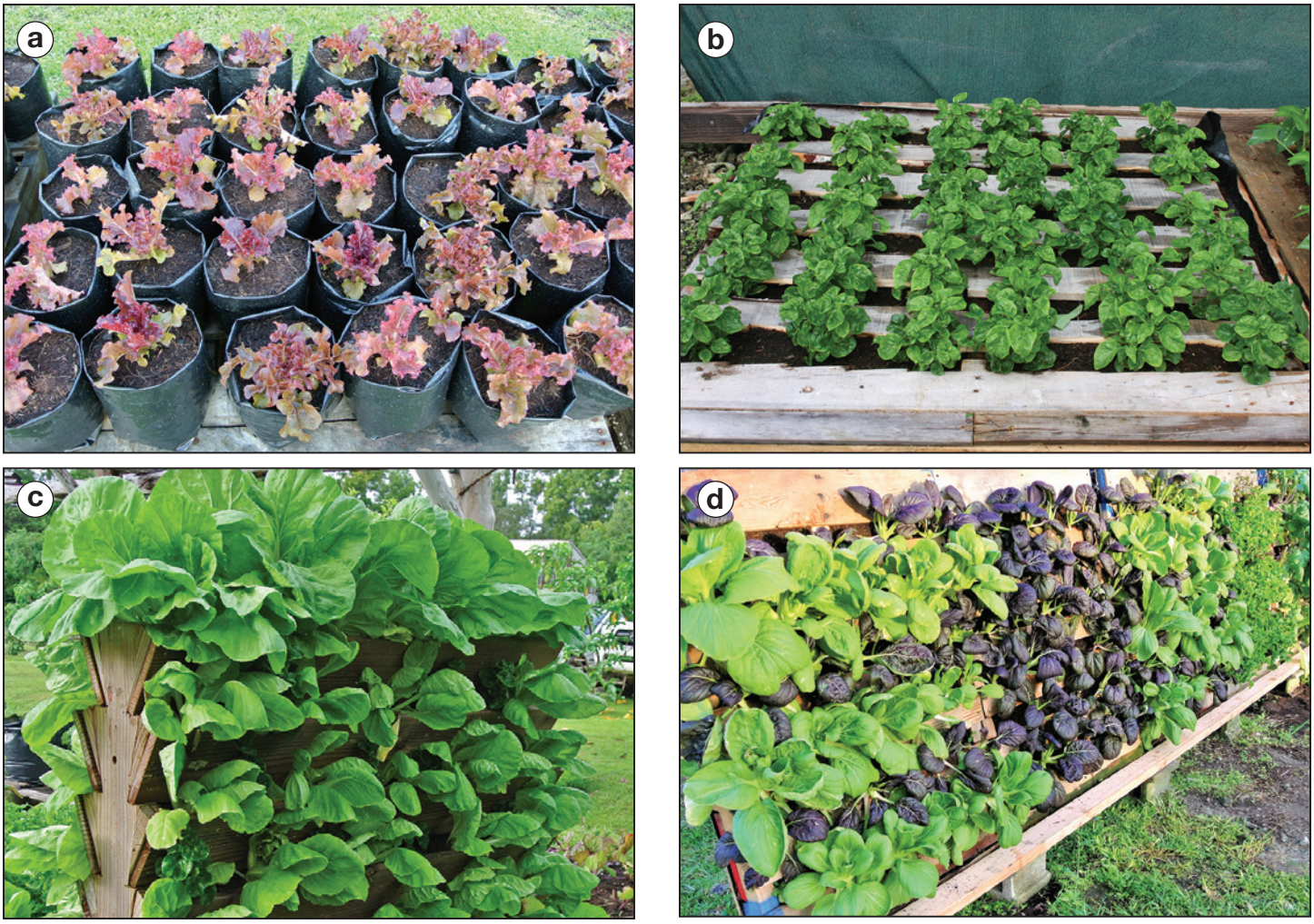

Figure 13. Cocopeat media can be used in a variety of containers including (a) polybags and (b) pallet planters. It can also be used in (c) vertical planters such as a (d) salad wall. (Photos by Murukesan V. Krishnapillai)

Cocopeat can be used as a soil conditioner and is widely used as a mulch for soil remediation (Santiago and Santhamani 2010, Udayana et al. 2017). It helps conserve water, slow evaporation, and reduce nutrient leaching. Bioengineering managers incorporate a mixture of coir and cocopeat into land-stabilization structures used to prevent soil erosion, sediment runoff, and land degradation. The high tensile strength of coir and cocopeat can be used on steep surfaces to inhibit heavy water flow and debris movement.

Conclusions

Growing medium is an important step to successful plant production in containers. In our pursuit of finding a local alternative to soil, we concluded that coconut husk, locally processed into cocopeat, is an ideal medium for growing plants in various container types. The favorable chemical and physical properties of cocopeat are a determining factor in its ability to support quality plant development (Awang et al. 2009, Ilahi and Ahmad 2017, Nazari et al. 2011, Paramanandham et al. 2013, Udayana et al. 2017, Xiong et al. 2017). In addition to reducing exploitation of peatland, there is an increasing emphasis on using alternatives to *Sphagnum* peat-based media for container production. Cocopith is an ideal alternative in many tropical locations, given the abundance of coconut palms and the fact that it is a byproduct that would otherwise be wasted. Use of these organic byproducts for plant production, mulch, soil remediation, disease suppression, and other purposes results in a renewable and environmentally sustainable system. In a world of increasing soil scarcity and climate uncertainty, soilless cultivation has much to contribute towards a truly green industry which minimizes waste while improving productivity and efficiency of plant production.

Figure 14. Production of cocopeat growing medium results in a sustainable resource to support forestry and agriculture projects for Yap's communities. (Photos by Murukesan V. Krishnapillai)

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Seed Propagation Protocol for Pure and Hybrid Butternut (*Juglans cinerea* L.)

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Abstract

Butternut (*Juglans cinerea* L.) is a native, eastern North American hardwood tree with economic and ecological value. It is severely threatened by butternut canker disease, which is rapidly killing the species range-wide. Hybrids of butternut and butternut canker-resistant Japanese walnut (*Juglans ailantifolia* Carr.) have been proposed as an alternative to planting pure butternut. Information on pure and hybrid butternut seed harvest, preparation, stratification, germination, planting, and initial seedling care is lacking. Methods and results are described from a project growing these species at Purdue University, forming a seed propagation protocol for the species. Germination was first observed 14 days after stratification. After 17 days, 64 percent of seeds germinated using the current method. Alternate methods to those used in this project are provided when possible, so growers can tailor protocols at different scales.

Introduction

Butternut (*Juglans cinerea* L.) is a medium-sized, exceptionally cold-hardy (USDA zone 3) hardwood tree native to Eastern North America (Dirr 2009, Rink 1990). The economically valuable wood of this species is easily worked and rot-resistant, making it ideal for furniture, paneling, veneer, and carving (Goodell 1984, Michler et al. 2005, Ostry et al. 1994). Butternut also holds ecological value as a mast species, providing energy-rich food for wildlife (and humans) with its large, oily kernels (Ostry et al. 1994). However, butternut canker disease, caused by the fungus *Ophiognomonia clavigignenti-juglandacearum* ([Nair, Kostichka, & Kuntz] Broders & Boland), has caused rapid declines in butternut populations since its discovery in 1967 (Broders and Boland 2011). The species is now classified as "endangered" by the International Union for Conservation of Nature (Stritch and Barstow 2019) and is listed under Canada's Species At Risk Act (SARA) (Environment Canada 2010). In the United States, butternut has a conservation status of either "critically imperiled," "imperiled," or "vulnerable" in 21 States (NatureServe 2019). While butternut was never as widely produced as the closely related black walnut (*Juglans nigra* L.), the severity and prevalence of butternut canker disease has recently made butternut less viable for nurseries to produce and sell.

Butternut is readily able to hybridize with Japanese walnut (*Juglans ailantifolia* Carr.) and the resulting hybrids have naturalized in some parts of butternut's range (Hoban et al. 2009). Researchers have only recently begun comparing the biology and performance of pure and hybrid butternuts. Crystal and Jacobs (2014) found that the hybrids were intermediate to butternut and Japanese walnut in terms of drought and flood stress tolerance. Morphologically, the hybrids have shown great variability and can hold the phenotypical features of either of the progenitor species (Crystal et al. 2014). The hybrids have also shown initial tolerance to butternut canker disease (Boraks and Broders 2014, Orchard et al. 1982), and are now being proposed by some as a possible alternative for butternut restoration (Boraks and Broders 2014, Michler et al. 2005).

Detailed and illustrated guidelines on the care of pure and hybrid butternut seeds and seedlings would aid in both restoration and research efforts, while also making it easier for growers to propagate and increase butternut in the landscape. This article contains seed-propagation protocols for pure and hybrid butternut, including information on seed harvest, preparation, stratification, germination, planting, and initial seedling care. Pure and hybrid butternut seedlings were recently grown at Purdue University (West Lafayette, IN) for

a project comparing their cold tolerances and phenology. Specific details from the seed propagation portion of the project are recorded here, but alternative methods are also included for use by growers at different scales with varying resources.

Step 1: Seed Harvest, Preparation, and Stratification

Harvesting

Harvest butternut and hybrid butternut fruits after ripening in autumn, preferably before they fall to the ground (Bonner 2008, Woeste et al. 2009, Young and Young 1992). For our project, fruits were harvested from September to October 2017 from the U.S. Department of Agriculture National Germplasm Repository (NCGR) and from six orchards of the Hardwood Tree Improvement and Regeneration Center (HTIRC) at Purdue University (table 1). Fruits were stored in plastic ventilated bags to allow airflow (figure 1).

Fruits can be planted directly into the ground (direct seeding) immediately after harvest or after removal of the green husks. As it requires fewer steps, direct seeding can be more efficient, allowing you to skip stratification and pre-germination, and may be most useful for large-scale plantings. Stratification and pre-germination, however, allow for more control over the entire process, protection from predation, and the ability to screen out nonviable seeds and unhealthy seedlings prior to planting. If direct planting the seeds, make sure the fruits are covered with a 1- to 2-in (2.5- to 5-cm) layer of soil and consider using screens to protect the planted seeds from rodent predation (Bonner 2008). See step 3 in this article for information on site selection and seedling care if direct planting.

Table 1. Butternut and hybrid butternut seed and germination information for Purdue University project. Seeds were harvested in fall 2017 and germinated in spring 2018.

Accession	Name	Orchard	Species	Origin	Quantity	Avg. Wt/nut (g)	No. Germ.	% Germ.
PI 666982	CJUG 1. 002 PL: Ayres	NCGR (Corvallis, OR)	butternut	MI	42	12.5	40	95.2
PI 666983	CJUG 4. 002 Chamberlin	NCGR (Corvallis, OR)	butternut	NY	33	13.0	33	100.0
PI 666987	CJUG 9. 001 PL: Herrick	NCGR (Corvallis, OR)	butternut	IA	29	22.7	24	82.8
PI 666992	CJUG 14. 001 PL: Booth	NCGR (Corvallis, OR)	butternut	NY	53	9.1	44	83.0
# 719	Part: 9906 OS-23 Slocums Woods	HTIRC (Walla Walla, WA)	butternut	WI	39	13.9	37	94.9
# 856	Hadley #1' Dave Hadley	HTIRC (West Lafayette, IN)	butternut	MI	40	12.8	35	87.5
03-713	Prog. OS-14 - #2097	HTIRC (West Lafayette, IN)	butternut	WI	37	11.4	31	83.8
PI 666997	CJUG 42. 001 Collier #2	NCGR (Corvallis, OR)	butternut	WV	34	16.8	32	94.1
# 968	Haberle # 1	HTIRC (West Lafayette, IN)	butternut	KY	40	15.5	36	90.0
# 979	Rickey #2 - Chilicothe	HTIRC (West Lafayette, IN)	butternut	OH	40	14.3	30	75.0
# 1073	Maxwell #5	HTIRC (West Lafayette, IN)	butternut	OH	40	12.3	37	92.5
# 1090	Hoosier #2	HTIRC (Huntingburg, IN)	butternut	IN	40	18.3	39	97.5
# 1083	Part: 9903 Indiana -Hoosier # 3/HNF	HTIRC (Walla Walla, WA)	butternut	IN	40	20.0	40	100.0
# 701	11th Road Hyb. Marshall Co	HTIRC (Plymouth, IN)	hybrid	IN	86	11.4	71	82.6
# 1093	Kellogg Comp. Hyb	HTIRC (West Lafayette, IN)	hybrid	MI	43	13.7	29	67.4
OS-222	'LaCrosse' Hybrid	HTIRC (West Lafayette, IN)	hybrid	WI	40	14.9	29	72.5
HYB 212	'Vrana' Fulton Co.	HTIRC (Plymouth, IN)	hybrid	IN	84	15.1	78	92.9
# 2033	Prog. No. 1-OS-191 / HTI #750	HTIRC (Wanatah, IN)	hybrid	IA	20	16.9	7	35.0
# 1000	Norristown # 2	HTIRC (West Lafayette, IN)	hybrid	IN	42	12.1	31	73.8
# 696	'Bountiful' grafts	HTIRC (Vera, MO)	hybrid	MO	79	12.3	77	97.5
Total					**901**		**780**	**86.6**

Figure 1. Freshly harvested butternut and hybrid butternut seeds placed in ventilated plastic bags prior to preparation for stratification. (Photo by A.N. Brennan 2017)

Husk Removal

While not necessary, removing the husks before stratifying and storing the seeds is helpful for preventing mold growth (Bonner 2008, Woeste et al. 2009). Remove the husks when they are firm, yet slightly soft; after this point, they can become too soft and quite difficult to remove (Bonner 2008, Young and Young 1992). We removed husks in our project within approximately 1 month of harvest.

Remove the major portion of the husk using any form of abrasion that can safely remove the husks without cracking the shell (Hartmann et al. 2002, Woeste et al. 2009). Possible methods conducted on a hard surface (driveway, garage floor, etc.) include: pounding with a metal rake (figure 2a), running over with a light- to mid-weight vehicle (figure 2b), and stomping and twisting while wearing hard-soled shoes (figure 2c).

Figure 2. Husks of butternut and hybrid butternut seeds can be removed by (a) pounding with a metal rake, (b) running over with a light- to mid-weight vehicle, (c) stomping and twisting while wearing hard- soled shoes, and (d) repeated abrasion over a raised metal grill-like structure that also allowed the husks to fall through to the floor. A garden hose was used in a, b, and c to contain the seeds and prevent them from rolling away. (Photos by A.N. Brennan 2017)

Figure 3. Power-washing can be used to remove the final bits of husk from butternut and hybrid butternut seeds. (Photo by A.N. Brennan 2017)

The husk can also be manually peeled off. Another method is to remove by repeated abrasion over a raised, metal grill-like structure that allows the husks to fall through, but the seeds to remain above (figure 2d). Throughout the husking process, a garden hose or similar object can be used to set a perimeter and provide a barrier to prevent seeds from rolling away (figure 2). Be advised that skin and clothes that come in contact with the husk and seed during this process are likely to become stained. Once the majority of the husk is removed, a power-washer or garden hose can be used to remove remaining bits of husk, but is not necessary (Woeste et al. 2009) (figure 3).

Rogueing and Sanitization

Within a few weeks of husk removal, prepare the seeds for stratification. To rogue out nonviable seeds, submerse the seeds in water and discard those that float (Woeste et al. 2009). For our project, we sanitized seeds in November 2017 with a 1:10 bleach:water solution to help prevent fungal and bacterial growth (Fraedrich and Cram 2012, Reil et al. 1998). Dip and swoosh batches of seeds using a large colander in a bucket of the bleach solution for approximately 15 seconds (figure 4a) followed by a 15-second rinse under plain water (figure 4b).

Stratification Preparation and Storage

For our project, we placed cleaned seeds in moist, but not wet, sand (just enough so no water could be

Figure 4. Sanitizing butternut seeds prior to stratification can be accomplished by (a) placing them in a colander and immersing for 15 seconds in a 1:10 bleach:water solution followed by (b) rinsing under plain water. (Photos by A.N. Brennan 2017)

squeezed out by hand from a fistful of sand) (figure 5a and b). Other stratification media, such as peat, sphagnum moss, or vermiculite can also be used (Reil et al. 1998, Woeste et al. 2009). Ensure that each seed is completely surrounded by the medium (figure 5c) and that a small amount of airflow can pass through the container—enough so that the seeds can respire, but not enough to dry out the medium (Woeste et al. 2009). We

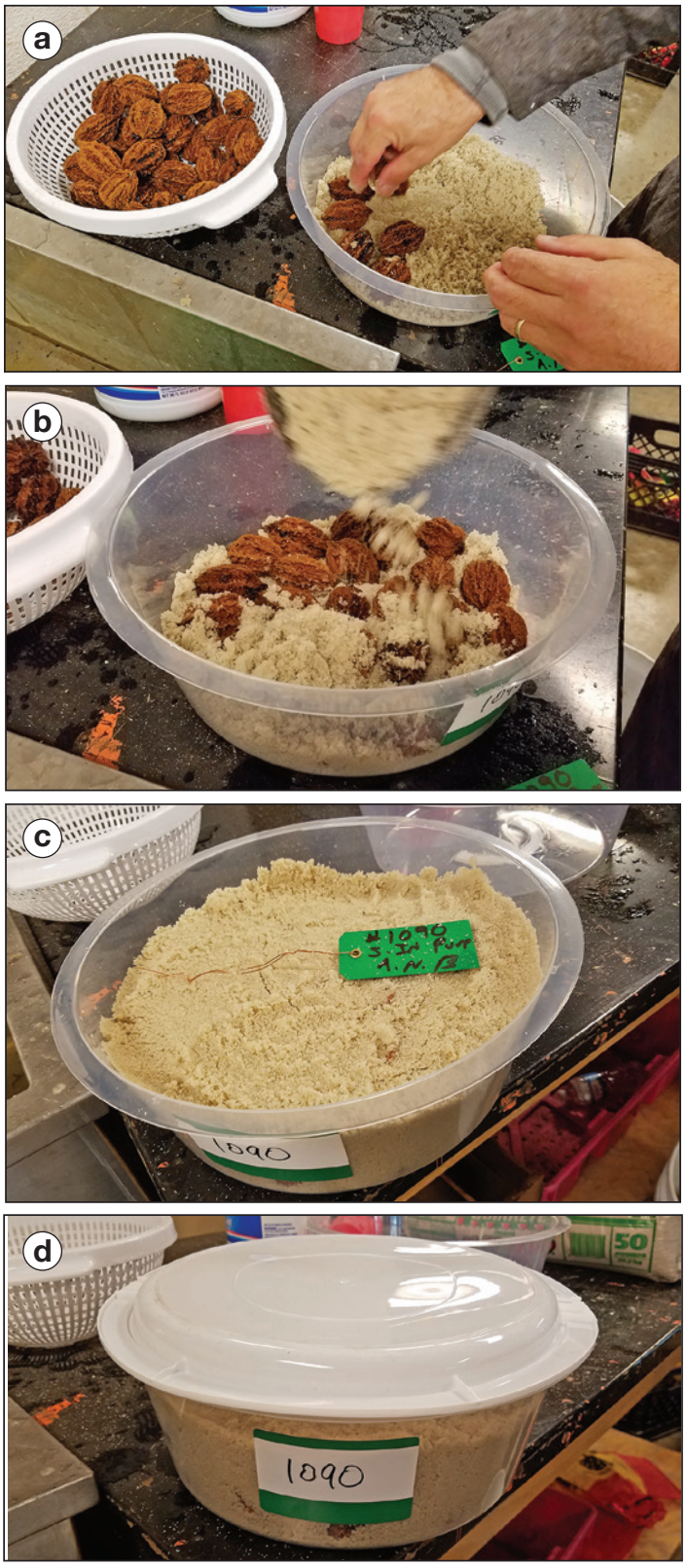

Figure 5. To prepare for stratification, sanitized butternut seeds can be (a) placed in a single layer on a shallow layer of moist sand using inverted cake containers. (b) Seeds should be covered with another shallow layer of sand, ensuring that each seed is surrounded by the moist sand. (c) This process is repeated for three layers of seeds. (d) The finished container should be covered with a loose-fitting lid to allow for a small amount of air circulation. Small holes can also be drilled near the top to further aid in circulation. (Photos by A.N. Brennan 2017)

accomplished this by drilling small holes (3/32-in [2.4-mm] drill bit) into inverted cake-storage containers with loose-fitting lids (figure 5d). If preparing multiple seed batches, make sure to appropriately label containers.

Store the seed containers in a cool area, such as a cooler or well-insulated garage or shed, just above freezing (34 to 41 °F [1 to 5 °C]) for stratification (Bonner 2008, Woeste et al. 2009). Juglans seeds are very attractive to wildlife, so ensure they are stored such that wildlife cannot access them (Bonner 2008, Woeste et al. 2009). For our project, seeds were stored in a walk-in cooler at 37 to 41 °F (2.8 to 5.0 °C) (figure 6).

Stratification Duration and Monitoring

Stratify the seeds for 90 to 120 days (Bonner 2008, Young and Young 1992). We stratified the seeds for our project for 120 days and removed them from cool conditions in mid-March 2018. Check seeds weekly throughout the stratification period for mold growth and to ensure the sand is not drying out. If mold growth does occur, discard the moldy sand, re-sanitize the

Figure 6. Butternut and hybrid butternut seeds packed in moist sand in inverted and non-airtight containers and stored in a walk-in cooler for stratification. (Photo by A.N. Brennan 2017)

Figure 7. Brown staining (circled) in the moist sand surrounding butternut seeds after 45 days in stratification is suspected to be leached tannins from the seed and leftover husk pieces. (Photo by A.N. Brennan 2017)

Figure 8. To ensure seed health in the middle of stratification, a small sample of butternut seeds were cracked open to reveal healthy, cream- to nearly white-colored endosperm. (Photo by A.N. Brennan 2017)

affected seeds as described previously, and replace them in a new batch of moist sand. Other techniques, such as the application of fungicides or hydrogen peroxide can also be used, although fungicides may negatively affect germination and should be used with caution (Cram and Fraedrich 2012). If the sand is too dry, add just enough water to keep the sand moist, but not wet. In our project, we noticed dark-brown staining in the sand surrounding some of the seeds (figure 7). We took a small sample of seeds from different batches, including from those where the surrounding sand was stained, and cracked them open with a hammer to check the endosperm health. All endosperms from the samples looked healthy: bright cream to nearly white and a bit "gummy" (figure 8). Given this, we suspected the brown staining to be leached tannins from the seed itself, particularly from any bits of remaining husk.

Step 2: Seed Germination

Upon completion of the stratification period in early spring, seeds can be planted directly into the ground or moved to warmer conditions for pre-germination before planting. Germinating the seeds in ideal conditions before planting into pots or in the field will encourage more expedient and uniform germination and allow for selection of the most viable and healthy seedlings.

Germination Container and Medium Selection

Use moderately shallow, broad containers or trays, at least 7-in (17.8-cm) deep to ensure adequate depth for fast-growing roots. We used plastic storage containers (16.75-in length by 11.88-in width by 7.00-in height [42.5-cm by 30.2-cm by 17.8-cm]) and drilled nine small holes in the bottom of each container to allow for drainage of excess water (figure 9).

Fill the trays a little more than halfway with moist, but not wet, sand, peat, perlite, vermiculite, or soil,

Figure 9. Plastic storage containers are useful for germinating butternut and hybrid butternut seeds prior to planting. In this example, small holes were drilled in the bottom to allow for drainage. (Photo by A.N. Brennan 2018)

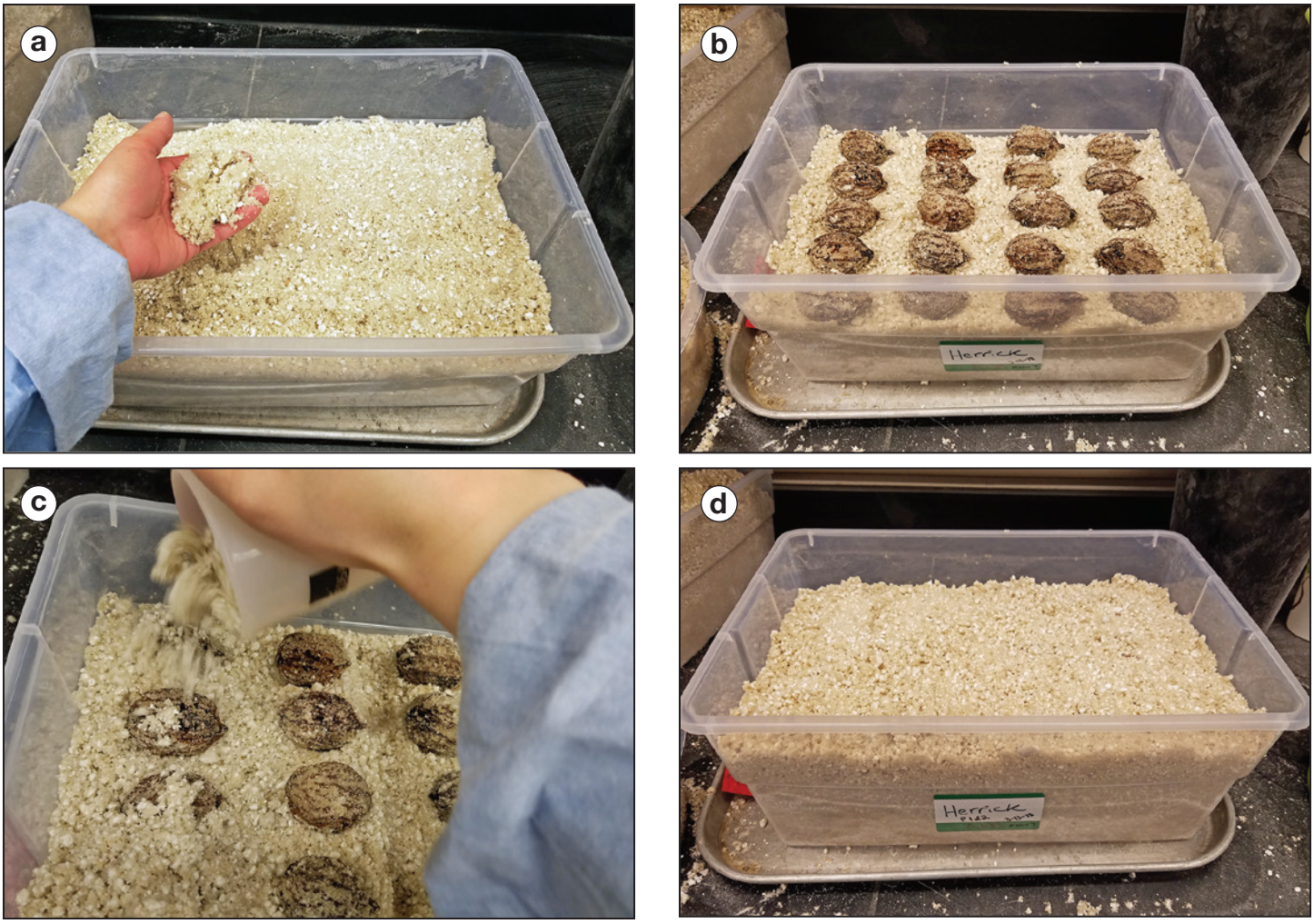

Figure 10. (a) A germination tray prepared for butternut seeds with a moist germination medium of 50:50 sand:perlite. (b) The seeds are placed on top of the medium lengthwise, on their sides. (c and d) Seeds are then covered with a shallow layer of medium. (Photos by A.N. Brennan 2018)

exclusively or in a combination (Bonner 2008). We used a 50:50 sand:perlite mixture (figure 10a).

Preparing Seeds for Germination

Place the seeds in the substrate-filled trays. Lay each seed on its side, lengthwise (figure 10b). Butternuts have hypogeal (underground) germination, so it is important to then cover the seeds with a shallow layer (approximately 1 in [2.5 cm]) of substrate (figures 10c and 10d) (Rink 1990). Make sure there is enough room for the radicle (first seedling root) to emerge and grow downwards until transplanting or outplanting (otherwise, when the radicle reaches the bottom of the container, it will grow horizontally and "tangle" with other roots, making it difficult to extract for planting).

Label the container to identify the seed batch and cover it to help retain moisture but still allow a small amount of airflow. We used the loosely fitting lids that came with the storage containers (figure 11), though other covers, such as loosely applied plastic wrap or tightly fitting lids with small holes drilled into them, could also be used.

Germination Conditions

Place the seed trays into warm conditions (68 °F [20 °C] up to 86 °F [30 °C]) (Bonner 2008, Young and Young 1992). Light is optional for germination of *Juglans* species (Bonner 2008, Young and Young 1992). A greenhouse or growth chamber is ideal for providing warm, consistent temperatures, but if neither of these is available, germination heating mats can be used. These mats take up a small amount of space and are relatively inexpensive and easy to obtain from online vendors. *Juglans* seeds can also be germinated at room temperature, although it

Figure 11. Covered germination trays of butternut seeds in a growth chamber. (Photo by A.N. Brennan 2018)

will take longer and may not be as uniform. In our project, we placed the seed trays into growth chambers (figure 11) with 8 hours of 86 °F (30 °C) day temperature alternated with 16 hours of 68 °F (20 °C) nighttime temperature (Bonner 2008). No light was used.

Check the germination containers every 4 days to ensure a consistently moist, but not wet, medium; add water as needed. At the same time, monitor for germination and fungal growth (the bleach sanitation method described previously will help prevent this). If serious fungal growth occurs, consider discarding the affected seeds or try treating them with a hydrogen peroxide solution (Fraedrich and Cram 2012). A general fungicide is also an option but could negatively impact germination (Fraedrich and Cram 2012).

Germination

Seeds begin to germinate by cracking open at the main seam along the length of the shell. Soon afterwards, the radicle emerges from the crack (figures 12a-c) followed by the hypocotyl hook (curved stem that breaks through the surface of the growing medium) (figure 12d). The hook will straighten so that the epicotyl (terminal shoot) is on top (figure 12e). Seeds from the same family tend to germinate at a similar time, though there can be some variation in developmental speed (figure 12f).

In our project, germination was first observed after 14 days (late March). Generally, 50 to 80 days are

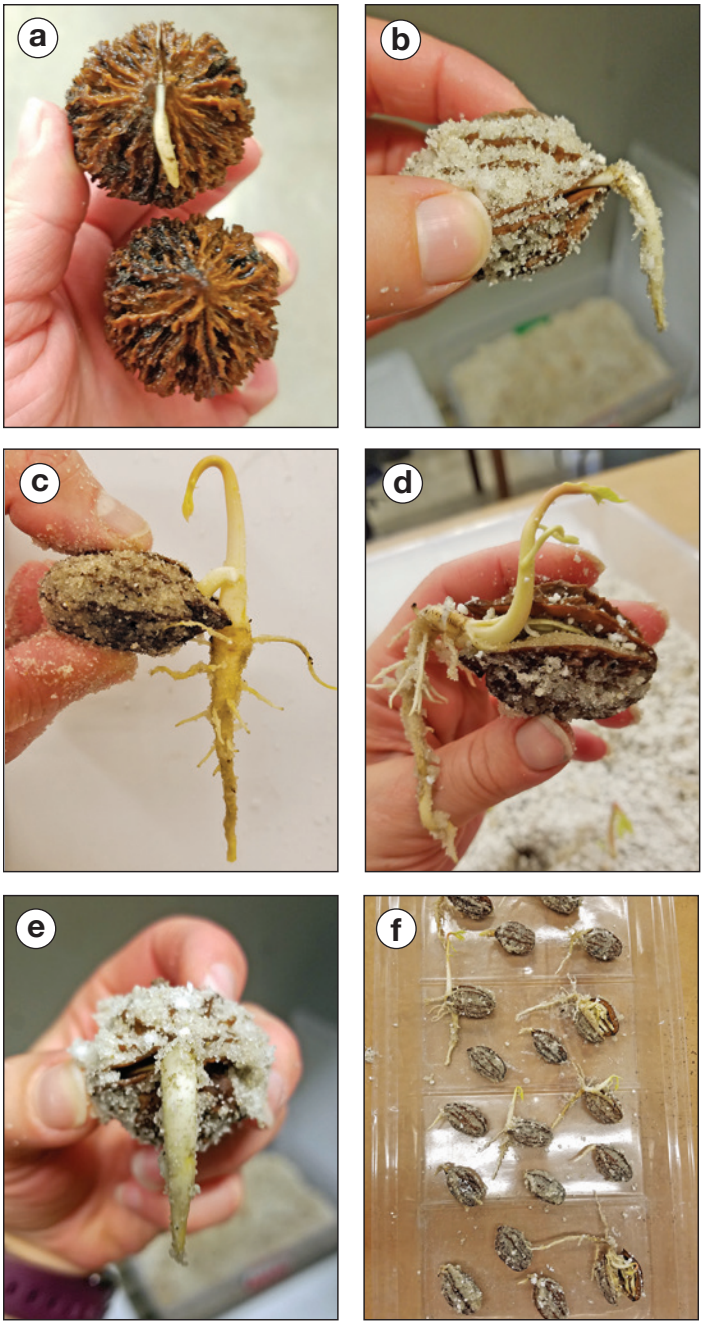

Figure 12. Germination of pure and hybrid butternut seeds begins with (a) a crack along the seam of the shell (b and c) from which the radicle will emerge. After the radicle emerges, (d) the hypocotyl hook will push out of the seed and the growing medium. (e) Eventually, the hypocotyl will straighten so that the epicotyl is pointing upwards. While related seeds will tend to germinate at a similar time, there is still some variation, (f) which can be seen by the different developmental stages of seeds of the same family. (Photos by A.N. Brennan 2018)

required for the majority of seeds to germinate and a germination rate of about 65 percent is expected (Bonner 2008, Young and Young 1992). Our method, however, resulted in a majority of seeds (64 percent) germinating by 17 days and 86.6 percent germinated within 45 days (table 1).

Step 3: Planting the Seedlings

Planting in the Field

Once the radicle is visible, germinated seeds can be carefully removed and planted directly in the ground or into pots. If planting directly in the ground, well-drained, rich loamy soils are ideal for butternut, but the species may also tolerate rocky, dry soils (Cogliastro et al. 1997, Rink 1990). Butternuts are shade-intolerant and must be planted in full sun (Rink 1990). Care must also be taken to protect the young seedlings from herbivore damage (particularly deer) by using fencing or tree shelters (Woeste et al. 2009). Once butternut seedlings are planted in the field, they generally require very little maintenance as long as the previously listed conditions are met. If the seedlings are planted on a particularly dry site or during a dry year, it is advisable to check if additional watering is required every few weeks during the first year of establishment.

Planting in Pots

If planting butternut germinants into pots, start with 1-gal (3.8 L) or larger tree pots. We use TP414 "Tall One" pots (Stuewe & Sons, Inc., Corvallis, OR). Depending on individual growth rates, the seedlings may need to be transplanted into larger pots later in the growing season. Fill the pots with a coarse, well-draining medium that is predominantly bark and/or coir mixed with peat, perlite, and/or vermiculite, and a wetting agent. We used Metro-Mix 560 (Sun Gro Horticulture Distribution, Inc., Agawam, MA) for our project.

Plant the pre-germinated seedlings, radicle pointed down, about 1- to 2-in (2.5- to 5-cm) deep (Bonner 2008), so that the medium just lightly covers the seed shell (figure 13). A layer of vermiculite or perlite can also be added to the top of the pots to help retain moisture and prevent weed growth. Immediately after planting the germinated seeds, water well with unfertilized water (until water drains out the bottom).

Place the pots into a rack or other support structure (such as milk crates or inverted cow panels on supports) that will keep the long, narrow tree pots in an upright position. For our project, pots were placed in a greenhouse on a metal grid supported by a wooden frame and legs (figure 14). Butternut and butternut hybrid seedlings grow very quickly (figure 15), so will need to be spaced apart as they grow to accommodate the vigorous growth.

Figure 13. Germinated butternut seeds, not yet covered, placed in pots (bottom right corner) and seeds that have already been covered with a shallow layer of potting mix (top left corner with red markers). (Photo by A.N. Brennan, 2018)

Step 4: Culturing Seedlings in Pots During the First Growing Season

Irrigation

Allow the medium to dry out somewhat, but not completely, between watering sessions. For our project, plants were watered when the medium turned from dark brown/nearly black (freshly watered) to light brown and felt dry below the top 1 to 2 in (2.5 to 5 cm). Regularly monitor the top few inches of the medium and check the moisture level from the bottom of the pots. Monitoring moisture levels is especially important until a deeper root system develops beyond the first few inches of growing medium. It is also important not to overwater, which can encourage damping-off. This fungal disease, especially prevalent in seedlings, causes the base of the stem to rot and the seedling to collapse (James 2012).

Figure 14. (a) Metal grids supported by a wooden frame and legs were used to support (b) containers of butternut and hybrid butternut seedlings. The seedlings were spaced more widely as they grew. (Photos by A. N. Brennan, 2018)

Figure 15. Butternut seedlings, (a) 1 week post-germination and (b) 6 weeks post-germination. (Photos by A. N. Brennan, 2018)

Fertilization

Once seedlings have grown their first two or three true leaves, begin fertilizing them once a week. For field-grown hardwood seedlings, fertilization can be beneficial, but is not required for survival (Jacobs et al. 2005). If growing the seedlings in pots, however, fertilization is important due to the closed nature of the growth system. Pay special attention to the amount of nitrogen added. At least once monthly, irrigate beyond field capacity with clear water to rinse the substrate, thereby preventing salinization buildup.

Since there is currently no literature describing fertilizer regimes for butternut or hybrid butternut, we used the recommended nitrogen rate (luxury consumption point) for the closely related black walnut (Nicodemus et al. 2008) which is 1,200 mg N/seedling by the end of the growing season. The fertilizer concentration in our greenhouse fertigation water was 150 mg N/L. Thus, to apply 1,200 mg N/seedling by the end of the growing season, we needed to apply a total of 8 L (or 8,000 ml) fertigation per plant. By dividing the total fertigation needed by the 22 weeks in the growing period (May to September), we determined that the application rate should be 365 mL of fertigation water per seedling each week.

Conclusion

Using our seed propagation methods, we found that overall, pure and hybrid seeds were both able to germinate quickly and uniformly. Eighty-six percent of the seeds germinated in 45 days; however, 64 percent had germinated by day 17, illustrating that this method can be used to germinate a majority of the seeds in just over 2 weeks. Our methods were also successful in producing strong, healthy seedlings, with all surviving through their first growing season (the duration of the project). The methods implemented in our project were designed specifically for our own research efforts, but additional methods were also provided to allow the protocol to be versatile for a variety of purposes and scales. This protocol is a valuable tool for butternut land managers and researchers wishing to use genetically diverse, seed-propagated material, while also supporting efforts to evaluate the suitability of hybrid butternuts as an alternative to the pure species.

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Variation in Bud Set Process Among Eight Genetically Improved White Spruce Seed Sources From Eastern Canada

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Abstract

A strong differentiation of bud set among natural populations may lead to limited adaptive capacity of seed sources during assisted population migration. The present study aimed to fill gaps regarding the dynamic nature of bud set and its variation among genetically improved white spruce (*Picea glauca* [Moench] Voss) seed sources used in the reforestation program in Québec, Canada. Bud set phases of seedlings from eight white spruce seed sources were monitored during the first growing season on a test plantation site. Results showed that bud set phases were interdependent but did not vary significantly among seed sources. Bud set timing was unrelated to the latitude or longitude of geographic origin. The lack of significance in bud set timing among tested seed sources may indicate low potential risk associated with the transfer of southern seed sources to the northern locations.

Introduction

Bud set is a complex physiological process representing the transition phase from active growth to dormancy (Cooke et al. 2012). Because bud formation is accompanied by growth cessation, the timing of bud set represents a trade-off between the active growth duration and the cold hardiness acquisition (Aitken et al. 2008, Howe et al. 2003, Savolainen et al. 2007). Natural selection has led to genetic differentiation and clinal variation in phenological traits such as bud set in boreal and temperate tree species. The resulting local adaptation enables populations to synchronize bud set with local climate conditions (Aitken et al. 2008, Beaulieu et al. 2004, Howe et al. 2003, Savolainen et al. 2007). For this reason, one may expect limited adaptation to climate change due to strong genetic control of phenological traits, and that this would be more obvious under restricted phenotypic plasticity and epigenetic memory to bud set expression. Assisted population migration (APM), which aims to move tree populations to sites where the future climate is similar to that of their origin, has been proposed as a forest management practice that can potentially mitigate the adverse effects of climate change on forest plantations (Aitken and Whitlock 2013, Pedlar et al. 2011). This practice is already implemented for some tree species (O'Neill et al. 2008, Pedlar et al. 2012). Therefore, the assessment of bud set process and its variation among genetic populations is of great interest for successful APM.

In indeterminate species, bud set initiation is induced by a critical photoperiod and low temperature (Cooke et al. 2012, Maurya and Bhalerao 2017). In contrast, environmental cues (photoperiod and temperature) are not as essential for the initiation of bud formation for determinate species (Bigras and D'aoust 1992, Cooke et al. 2012). For these species, bud set is initiated once the elaboration of all pre-formed stem units (during the last growing season) is completed (Cooke et al. 2012, El Kayal et al. 2011). However, temperature, photoperiod, and their interactions may nevertheless affect onset and duration of the bud set process (Bigras and D'aoust 1992, El Kayal et al. 2011, Hamilton et al. 2016, Singh et al. 2017). Several studies showed that short-day or blackout treatments resulted in the induction of bud formation, cessation of height growth, and significant increases in carbohydrate content, root

nutrient contents, and root dry mass (Colombo et al. 2001, Lamhamedi et al. 2013).

White spruce (*Picea glauca* [Moench] Voss), a determinate species, is one of the most commercially important tree species in Canadian boreal forests (Beaulieu et al. 2009). Genetic variation in the timing of bud set has been found among natural populations of white spruce in the eastern part of the boreal forest (Li et al. 1993, 1997; Jaramillo-Correa et al. 2001). A similar trend was observed for the sympatric black spruce (*Pica mariana* [Mill.] BSP) (Beaulieu et al. 2004, Perrin et al. 2017) and for interior spruce (*Picea glauca engelmannii* complex) in Western Canada (Liepe et al. 2016).

Reforestation programs in Eastern Canada typically use genetically improved stock (up to 90 percent in the province of Quebec) and until now, no results on the investigation of the genetic variation in the timing of bud set was reported for this type of material under plantation site conditions. Also, bud set has generally been assessed only as a single stage or an index (Bousquet 1984, Jaramillo-Correa et al. 2001, Li et al. 1993) and sometimes inferred from growth cessation. Growth studies, however, have shown that bud set is a complex and dynamic sequence of events (Cooke et al. 2011, El Kayal et al. 2011, Perrin et al. 2017, Rohde et al. 2011). It remains unknown whether these events are highly correlated, and if they involve independent environmental cues and different levels of genetic control (Perrin et al. 2017).

The present study is part of a research project on assisted migration initiated in 2013 by the Quebec Ministry of Forests, Wildlife, and Parks to assess local genetic adaptation and phenotypic plasticity of functional traits of various genetically improved white spruce seed sources. The overall goal is to refine their deployment under climate change (Benomar et al. 2015, 2016, 2018; Otis Prud'homme et al. 2018; Villeneuve et al. 2016). Results reported so far have shown the existence of clinal variation in height growth, which was partially driven by the trade-off between photosynthetic rate and water use efficiency, mediated by genetic variation in stomatal conductance. Furthermore, genetically improved white spruce seed sources in Quebec exhibit a similar level of phenotypic plasticity for several functional traits. In the present study, we focus on bud phenology by (1) assessing the variation in bud set phases among eight genetically improved white spruce seed sources and its association with height growth and geographical origin, and (2) examining the level of interdependency among bud set phases.

Material and Methods

Genetic Material and Experimental Design

The study was carried out using eight white spruce seed sources from six first-generation and two second-generation clonal seed orchards (table 1).

Table 1. Geographic coordinates of the centroid of plus-trees (i.e., the location of the parent trees) that make up the eight white spruce seed sources used in this study.

Seed source	Locality	Latitude (°N)	Longitude (°W)	Target ecological region*
SO1-1	Wendover	46.39	71.94	2b, 2c
SO1-2	Fontbrune	46.43	75.74	1, 2, 3, 4
SO1-3	Baby	47.75	78.47	3aS, 4a, 4b, 4c, 5a, 5b, 5cT
SO1-4	Desroberts	48.76	77.86	5a, 5b, 5cS, 5cT, 6a, 6c, 6e
SO1-5	Robidoux	48.55	65.59	4g, 4h, 5h, 5k
SO1-6	Falardeau	48.54	71.73	4c, 4d, 4e, 5dM, 5dT095, 5dT096, 5dT097, 5dT098, 5dT099, 5cM, 5cT
SO2-1	Berthierville	46.08	73.18	1, 2, 3, 4aT037, 4bM, 4bT039, 4bT041
SO2-2	Sainte-Luce	48.35	68.35	4, 3dS, 3dT, 5cM, 5dM, 5eT, 5h

*Ecological regions are described in Saucier et al. (2009).

Figure 1. Seedlings performance and bud set timing were evaluated at the Watford plantation site (Photo by Mohammed S Lamhamedi, April 2016).

The first-generation seed orchards are the most commonly used for reforestation in Québec, Canada, and were established about 30 years ago using phenotypically plus-trees selected in local natural forests from distinct regions. The second-generation seed orchards were established more recently using grafts of plus-trees selected from the top-performing, open-pollinated families from across Québec and Ontario and assessed in a series of genecological tests (Beaulieu et al. 2009).

Open-pollinated seeds were collected from each seed orchard for 2 consecutive years (2008 and 2009). Seedlings were produced from the mixed seed collections for each seed orchard in the State forest nursery of St-Modeste Québec, Canada (47.50 °N, 69.23 °W) using Québec's standard nursery cultural practices (Lamhamedi et al. 2006, Villeneuve et al. 2016).

A genetic field test was established using a randomized complete block design with four blocks; each block being partitioned into eight plots in which the eight seed sources (SO) were assigned randomly. The size of each plot was about 730 m2 (0.18 ac) and contained 144 trees (12 by 12 rows of trees) (figure 1). The plantation site was located in the Eastern Canadian forest near the locality of Ste-Rose de Watford, Québec, Canada (46.30°N, 70.40°W) in the sugar maple-yellow birch domain on loamy soil. The 2-year-old seedlings were planted at 2.25 m by 2.25 m (7.38 by 7.38 ft) spacing during the last week of May 2013.

Prior to outplanting (in 2012 at the end of the second nursery growing season), characteristics were assessed on a sample of 15 seedlings from each seed orchard. Average values ranged from 33.5 cm (1.1 ft) to 41.9 cm (1.36 ft) for height, 11.9 g (0.41 oz) to 15.5 g (0.55 oz) for total dry mass, and 1.5 to 1.68 percent for shoot nitrogen concentration.

Additional information regarding soil characteristics of the planting site and morphophysiological variables under forest nursery and site conditions can be found in Otis Prud'homme et al. (2018) and Villeneuve et al. (2016). At the end of the first growing season at the planting site, survival averaged 98 percent (Villeneuve et al. 2016).

Bud Set Monitoring

Apical bud set was monitored in summer 2013 from July 7 (DOY 188) to September 11 (DOY 254). Monitoring was done every 2 days except for the last 2

weeks, when data were collected every 3 days. Monitoring observations were made on the 15 central seedlings within each plot for a total of 480 seedlings (15 trees x 4 blocks x 8 seed sources). Five bud set phases were defined according to Dhont et al. (2010): phase 1 (white bud); phase 2 (beige bud); phase 3 (brownish bud); phase 4 (visible brown bud); and phase 5 (opaque brown, clearly visible bud). Bud set phase was assessed using binoculars for the two first phases and by visual inspection for the later phases. Photos showing the different bud phases are described and illustrated by Dhont et al. (2010).

For each seed orchard within each block, the timing of each bud set phase was estimated as the time at which 50 percent of seedlings reached that phase (BS50). The cumulative frequency of bud set was calculated using the following logistic regression curve:

$$Y = \frac{1}{1 + e^{a(X - BS_{50})}} \quad \textbf{1.}$$

where Y is the cumulative frequency of X, X is the timing of seedling bud set within the plot, and a is a parameter representing the rate of the process.

The time at which 25 (BS25) and 75 (BS75) percent of seedlings reached each bud set phase was also estimated as in equation (1).

Climate Data During the Sampling Year

A weather station was installed on the plantation site after planting. The station was supplied with a shielded air temperature and relative humidity sensor (HMP50, Vaisala, Helsinki, Finland), PAR sensors (Li 190 Campbell Scientific, Logan, UT, USA), pluviometer, and soil temperature sensors (figure 2). Data were recorded hourly throughout the growing season using a datalogger (CR10X, Campbell Scientific, Logan, UT, USA).

The data were used to estimate the accumulation of chilling hours (ACHs) according to Lamhamedi et al. (2005) as:

$$ACHs = \sum_{j=182}^{j=300} \cdot \sum_{i=1}^{i=24} (T_r - T_h) \; and \; (T_r - T_h) > 0 \quad \textbf{2.}$$

where Tr, is the reference temperature (=5°C), Th is the hourly temperature, i is the hour of the day,

Figure 2. A permanent meteorological station was installed April 2013 at the Watford plantation site (Photo by Mohammed S. Lamhamedi, July 2013)

and j is the day of the year. The July 1 (DOY=182) was used as the starting day for calculation of ACHs which corresponds to one week before bud set measurements began.

Statistical Analyses

Data were analyzed using the SAS/STAT software version 9.4 (SAS Institute, Cary, NC, USA). Bud set (BS50) was subjected to repeated-measures analysis of variance (Proc Mixed) using the following linear mixed model:

$$Y_{SPB} = \mu + \beta_s + \beta_P + \beta_{SP} + v_B + e \quad \textbf{3.}$$

Where Y_{SPB} is the timing of bud set; μ is the grand mean; β_p is the fixed effect of seed source; β_s is the fixed effect of bud set phase; β_{SP} is the fixed effect of the interaction between seed source and bud set phase; v_B is the random effect of block; and e is the residual error.

Four covariance structures were tested: Heterogeneous Banded Toeplitz (TOEPH), Autoregressive(1) (AR[1]), Compound Symmetry (CS), and Heterogeneous AR(1) (ARH[1]). The latter was chosen because it had the lowest Akaike information criterion (AIC) value. Comparisons of means were performed using the Tukey's range test, and differences were considered significant at $P < 0.05$. The relationships between bud set phases and between the first bud set phase and geographic variables (latitude and longitude) of seed source origins were tested using Proc Corr.

Results

Mean daily temperature from July to mid-September averaged 17.1 ± 3.2 °C (62.8 ± 5.7 °F) on the plantation site with a coefficient of variation of 19 percent (figure 3a). The accumulation of chilling

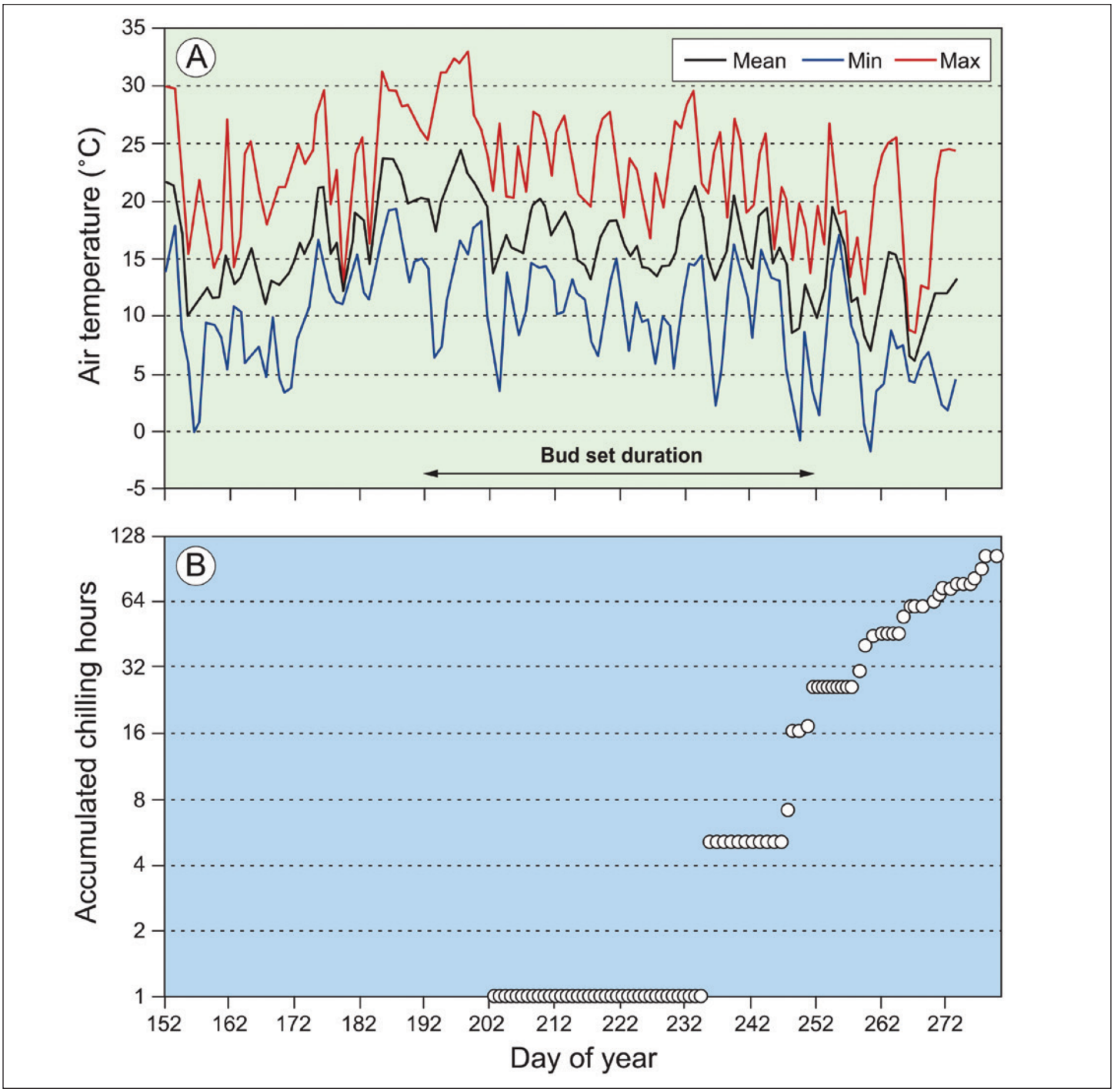

Figure 3. (a) Mean, minimum, and maximum daily air temperature and (b) accumulation chilling of hours during the first growing season at the Watford plantation site. July 1 (DOY=182) was used as the starting day for calculation of chilling hours.

Table 2. The effect of seed source on bud set for white spruce seed sources from the first- and second-generation seed orchards at the end of the first growing season in the Watford forest plantation site.

		BS50		BS25		BS75	
	DF	F value	P value	F value	P value	F value	P value
First-generation seed orchards (n=6)							
Seed source	5	1.70	0.185	1.17	0.360	1.92	0.141
Phase	4	2484.60	<0.001	1725.19	<0.001	2096.55	<0.001
SS*P	20	0.83	0.669	0.79	0.713	1.17	0.305
Second-generation seed orchards (n=2)							
Seed source	1	1.56	0.258	2.10	0.197	0.56	0.483
Phase	4	561.97	<0.001	375.85	<0.001	331.73	<0.001
SS*P	4	0.89	0.487	0.75	0.567	0.86	0.501
Combined first- and second-generation seed orchards							
Seed source	7	1.74	0.124	1.17	0.358	1.52	0.210
Phase	4	3009.53	<0.001	2040.10	<0.001	2190.60	<0.001
SS*P	28	1.07	0.397	0.76	0.791	0.90	0.610

DF = degrees of freedom

hours (ACHs) during the monitoring period reached only 4 hours (figure 3b). The mean July temperature in 2013 was 19.9 °C (67.8 °F), which was 1.5 °C (2.7 °F) above climate normals (1981 to 2010). Also, 2013 was the warmest and wettest year from 2010 to 2017 at the plantation site.

Average bud set initiation (BS50) was DOY 200 ±2 (July 19), and the first phase was completed within 12 days. The average date of the last phase, which corresponds to complete bud development was DOY 240 (August 28). None of the five bud set phases differed by seed source (tables 2 and 3). Similarly, BS25 and BS75 for the five phases were similar among seed sources. Bud set duration averaged 38 days (from the first to the fifth phase) and did not differ among seed sources (P=0.32). Intra-seed source variance for each bud set phase was similar between first- and second-generation seed orchards and averaged (2.3 day). This variance was also unrelated to both latitude and longitude ranges of plus-trees of the six first-generation seed orchards (table 3, figure 4).

The occurrence of each bud set phase was dependant on the occurrence of the previous phase (table 4). The strength of this interdependency was higher for the first

Table 3. Mean day of year (DOY± standard deviation) when 50 percent of seedlings from each of the eight seed sources reached each bud set phase (BS50).

Source	Phase 1	Phase 2	Phase 3	Phase 4	Phase 5
SO1-1	200±2.91	204±2.02	215±2.93	225±2.19	239±2.35
SO1-2	200±1.51	205±0.91	214±1.43	225±0.47	237±1.08
SO1-3	200±1.28	205±1.09	213±1.64	225±0.83	238±1.18
SO1-4	201±1.19	204±1.48	214±2.03	225±0.24	237±0.92
SO1-5	199±2.15	203±1.35	213±3.1	223±2.31	236±3.13
SO1-6	199±0.68	203±1.51	212±1.26	224±1.53	236±2.01
SO2-1	199±1.55	204±2.52	213±2.18	225±0.81	237±2.36
SO2-2	201±1.41	205±1.79	214±2.64	226±1.58	237±4.07

For all phases, means were similar at α=0.05.

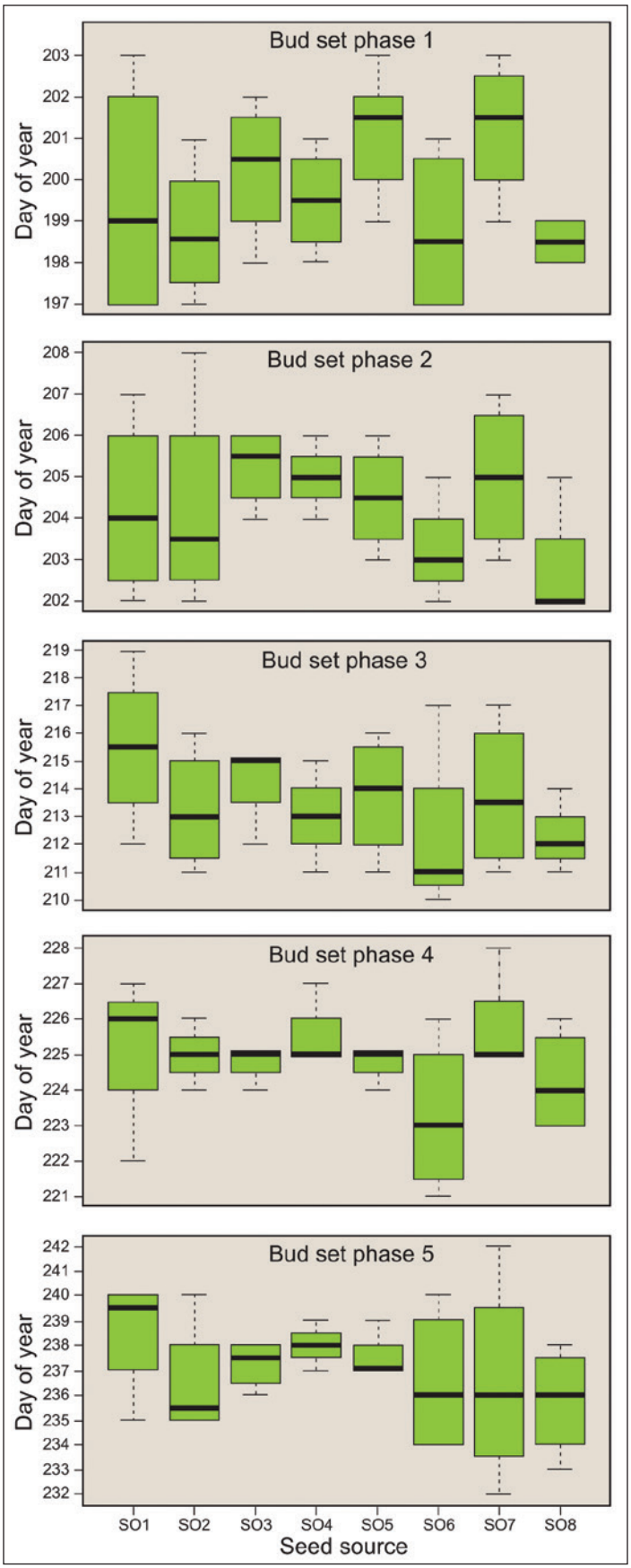

Figure 4. Timing of the five bud set phases of seedling of eight seed sources of white spruce during the first growing season in forest plantation of Watford. The timing of each phase corresponds to the day of the year at which 50 percent of seedlings reached the stage.

three phases and was marginal for the last two phases (table 4). The timing of the first bud set phase was unrelated to the latitude and longitude of seed source origins (figure 5). The relationship between bud set and growth during the first growing season was marginal (P=0.07).

Discussion

The present study was part of a research project motivated by the urgent need to mitigate climate change effects. The project aimed to fill physiological knowledge gaps to design robust climate-based seed transfer systems for commercial forest tree species in Quebec, Canada. Phenological traits are part of functionals traits involved in tree fitness through the synchronization of a tree's growth cycle with its local environment (Cooke et al. 2012, Savolainen et al. 2004). Our results showed a lack of variation in bud set phenophases among the eight white spruce seed sources most used in Quebec for its reforestation program. Unexpectedly, the variation observed in growth traits during the juvenile phase (Benomar et al. 2016, Otis Prud'homme et al. 2018, Villeneuve et al. 2016) was only marginally explained by the bud set timing.

Clinal variation for phenological traits has been found for several boreal tree species including white and black spruces (Beaulieu et al. 2004; Hurme et al. 1997; Li et al. 1993, 1997; Perrin et al. 2017). In contrast, seed sources in the present study were similar in their bud set timing. These contrasting results may be related to: i) the combined effect of large within-population variation for the measured traits and small ecological distance between tested seed sources; ii) the effect of selection pressure; and/or iii) abnormal climatic conditions at the planting site during the

Table 4. Matrix of phenotypic correlation coefficients between the timing of the five bud set phases of the eight white spruce seed sources tested. Significant correlations (P<0.05) are in bold and marginally significant correlations (P<0.1) are underlined.

	Phase 1	Phase 2	Phase 3	Phase 4	Phase 5
Phase 1		**0.70**	0.39	0.42	0.10
Phase 2			**0.72**	0.53	0.44
Phase 3				<u>0.68</u>	**0.82**
Phase 4					<u>0.62</u>
Phase 5					

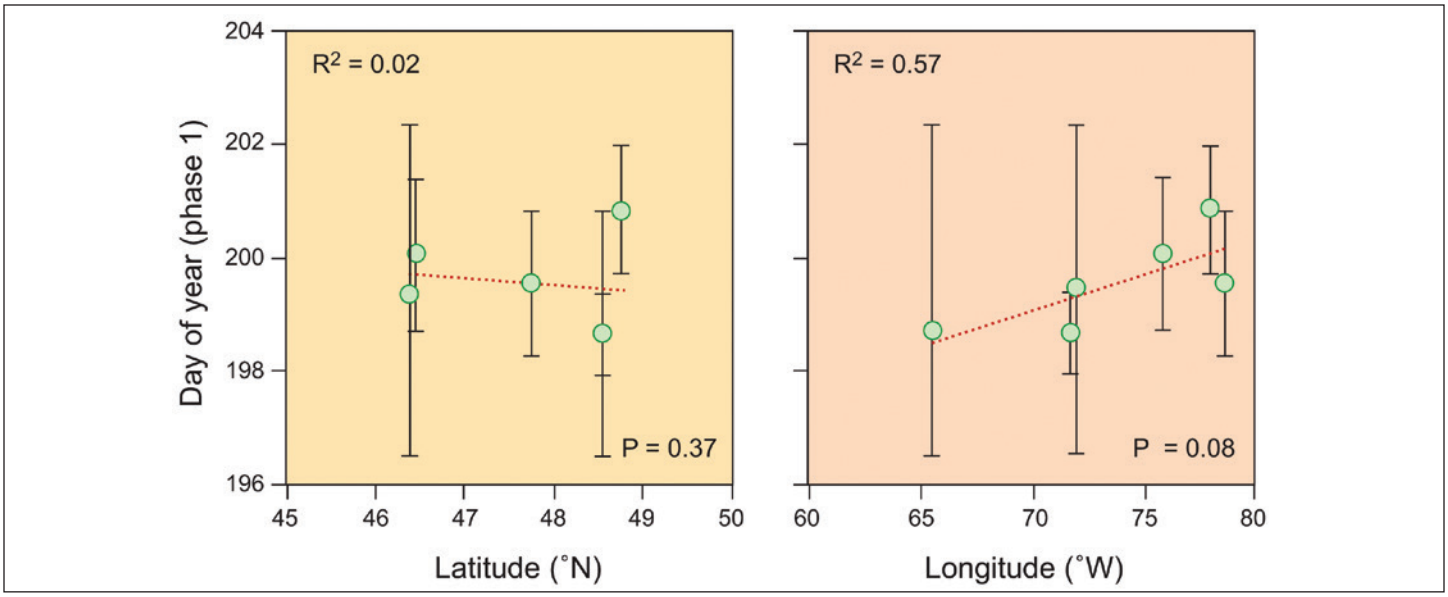

Figure 5. Timing of the first phase of bud set plotted against the latitude and longitude of seed source origin. The correlations were not significant at P<0.05. Second generation seed orchards (SO2-1 and SO2-2) were excluded from the regression analysis because they were composed of trees representing multiple widespread provenances from Québec and Ontario, Canada.

measurement period. Each of these are discussed further in the following paragraphs.

First, the extensive genetic variation existing at the intra-population level in white spruce (Bousquet, 1984, Li et al. 1993) constitutes a major challenge when examining genetic differentiation among populations at fine geographical scale as the case herein. Also, the narrow geographical range (2.37° of latitude) and the small number of seed sources tested in the present study may lead to the observed contrasting results with previous range-wide scales studies. In fact, Bousquet (1984) used 91 provenances covering 11.5° of latitude and 62° of longitude, and Li et al. (1993) used 57 provenances from Québec and Ontario covering 6.5° of latitude and 20° of longitude.

Second, breeding programs for white spruce started in 1972 and resulted in the installation of a series of first-generation and second-generation seed orchards. This artificial selection substantially improved white spruce growth. The increase in growth traits by artificial selection may either result from an increased growth rate or from an indirect selection for increased growth duration. The effect of artificial selection on the level of local adaptation of adaptive traits still remains unquantified. For now, MacLachlan et al. (2018) found a similar level of local adaptation for phenological traits between natural and improved seed sources for interior spruce (*Picea glauca* × *P. engelmannii*) in Alberta, Canada. In a previous study (Benomar et al. 2015, 2016), it was found that height growth could be explained by the mean value of photosynthetic-related traits, but the small variation in growth existing between seed sources may suggest an unbalanced effect of selection on bud set timing among seed sources. Further investigations are necessary to confirm this last hypothesis.

Third, the warm conditions during the growing season when data were collected likely delayed bud set and particularly the last phase. Bud set for determinate species such as white spruce, however, is known to be controlled endogenously through preformed growth units related to number of primordia (formed during the previous growing season), and therefore, environmental cues may have little effect on the time course of bud formation.

In contrast with previous findings (Bousquet 1984, Jaramillo-Correa et al. 2001, Li et al. 1993), the timing of bud set in our study explained only a small part of the observed variation of height growth among seed sources. In our previous studies (Benomar et al. 2015, 2016), we found a significant contribution of the CO_2 assimilation rate to variation in height growth among the tested seed sources. Given that, we expect that the variation in growth performance among the seed sources tested in the present study is linked to photosynthetic rate.

Based on these results, a similar timing of dormancy induction and cold tolerance is likely among tested seed sources, and if so, risks of cold injury could be limited for southern seed sources transferred to the northern locations. Our results, however, were

obtained at the end of the first growing season on three plantation sites located in different bioclimatic domains in Quebec and revealed a high survival rate (> 98 percent) and complete absence of frost damage on the three sites (Villeneuve et al. 2016). Further investigations of the time course of dormancy and cold hardiness are recommended.

Perspectives

Phenotypic plasticity (i.e., the ability to change the phenotypic expression of a genotype in response to a change in environmental conditions) of bud set and cold hardiness for spruce species still deserves investigation. Better knowledge on bud set plasticity and its variation among seed sources, as well as its relation with frost hardiness, should help elucidate physiological and phenological influences on survival and performance of varying seed sources when transferred to sites that are currently colder but where temperature will increase over time due to climate change.

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Biochar Potential To Enhance Forest Resilience, Seedling Quality, and Nursery Efficiency

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Abstract

Land managers face a mounting variety of challenges, including how to efficiently dispose of excessive woody residues on forest sites (especially in the Western United States), maintain and improve soil productivity, improve forest resilience to changes in climate (especially as it pertains to drought and fire), and increase the effectiveness of reforestation activities. The use of biochar, a charcoal that is not readily degraded and is made specifically for land application, may have a role in meeting these challenges. Moreover, biochar may provide nursery managers with opportunities to produce seedlings for reforestation and restoration in a more sustainable way, particularly by reducing irrigation inputs, as evidenced through several trials summarized here.

Introduction

Many forests, especially those in the Western United States, face management challenges related to wildfire, insect and disease outbreaks, and invasive species because of overstocked or stressed stands (Weatherspoon and Skinner 2002). The nexus of these challenges facing land managers is what to do with the resulting excessive wood on forest sites that has little or no economic value. This wood comes from precommercial thinning of overstocked stands to reduce fire hazard, tremendous loss of standing timber to drought and bark beetle infestation (i.e., 29 million trees in the Sierra Nevada as of 2015), and conventional thinning and logging (Thibodeau et al. 2000, Fettig et al. 2019). Combined, these activities have created, literally, mountains of slash that are eventually burned (figure 1). Although slash pile burning is an economical method for disposing of undesired woody residues, burning can wreak havoc on the soils beneath piles, often rendering them unproductive for decades (figure 2). In addition, smoke and particulates contribute to air quality issues, and release of CO_2 adds to climate change. An alternative is to burn these residues under controlled conditions, thereby reducing emissions, generating bio-energy, and sequestering carbon (Jones et al. 2010, Page-Dumroese et al. 2017).

Figure 1. Burning large slash piles can cause long-term damage to soil. (Photo by USDA Forest Service 2012)

Burning residues under controlled conditions can create biochar (charcoal made for land application) and currently the interest in using woody forests residues, mill shavings, and invasive woody species to make biochar is increasing. Widespread use of this technique is limited, however, because transportation costs to bioenergy facilities where biochar can be made by pyrolysis (burning in the absence of oxygen) can be expensive. On-site production, however, is possible and encouraged (Page-Dumroese et al. 2017). In general, the carbon (C) concentration of the biochar is about double that of the original feedstock, but each type of feedstock and burn conditions creates unique biochar

Figure 2. Slash pile burn openings (non-green dots) created when large piles were burned 50 years ago. (Photo by USDA Forest Service 2010)

(table 1). Biochar derived from burning wood usually has a pH that is compatible with plant growth, whereas some feedstocks, such as poultry litter, yield biochar with very high (>8) pH (table 1). The benefits of adding biochar to soil are many, including an increase in water- and nutrient-holding capacity while sequestering C belowground (Page-Dumroese et al. 2016b). Although biochar is a form of organic matter, it persists much longer in the soil profile than litter or humus because it is charred. It has high cation exchange capacity and has been suggested for use in plant propagation (e.g., Dispenza et al. 2016, Dumroese et al. 2018).

Furthermore, many soils have lost appreciable soil organic matter from overgrazing, cultivation, forest harvesting, and erosion (Lal 2009). These soils could benefit from biochar additions during reforestation because it adds a highly recalcitrant form of C and promotes long-lasting effects (e.g., retention of cations, anions, and water; Thomas and Gale 2015). For example, biochar, wood ash, and biochar mixed with manure were applied on restoration sites in the Lake States and resulted in increased soil water-holding capacity and cation exchange capacity, and increased seedling growth (Richard et al. 2018).

Forestry Trials – On-site Creation and Use

Biochar is made by burning biomass under controlled conditions. Commonly, it is created in

Table 1. Examples of carbon and nitrogen concentrations, pH, and electrical conductivity (EC) in biochar created from woody residues from the Western United States (from Page-Dumroese et al. 2016b).

Tree species or species mix		Production method	Carbon (%)	Nitrogen (%)	pH	EC (µS/cm)
Mixed conifer	Primarily ponderosa pine (*Pinus ponderosa* Lawson & C. Lawson) and Douglas-fir (Mirb.) Franco	Improved slash pile	28	0.22	7.5	150
Mixed conifer	Primarily ponderosa pine and Douglas-fir	Gasifier	89	0.26	8.1	103
Fire-killed salvage	Mixed conifer but primarily ponderosa pine	Gasifier	94	0.34	7.4	258
Beetle-killed salvage	Mixed conifer but primarily ponderosa pine	Mobile pyrolysis	86	0.18	8.1	90
Oregon white oak	*Quercus garryana* (Douglas ex Hook.)	Mobile pyrolysis	87	0.62	7.9	180
Scotch broom	*Cytisus scoparius* (L.) Link	Mobile pyrolysis	80	0.51	7.5	235
Western redcedar	Thuja plicata (Donn ex D. Don)	Mobile pyrolysis	92	0.30	5.4	789
Twoneedle pinyon pine and common juniper	*Pinus edulis* (Englem.) and *Juniperus communis* (L.)	Metal kiln	76	0.50	6.5	330
Pacific madrone	*Arbutus menziesii* (Pursh)	Mobile pyrolysis	85	021	4.5	789
Ponderosa pine		Fast pyrolysis/byproduct of bioenergy	85	0.74	7.5	197
Russian-olive	*Elaeagnus angustifolia* (L.)	Rotary kiln	73	1.69	7.6	190

large-scale bioenergy facilities by using pyrolysis (low-oxygen conditions) or gasification (partial oxidation of biomass). Both of these methods create high C biochar that has a small particle-size (<4 mm; Anderson et al. 2013) making it an excellent soil amendment, but these methods are often not amenable for small landowners and nursery managers, or for processing low- or no-value woody biomass created from restoration harvest operations.

Numerous biochar trials have been installed in the West. These trials have shown that biochar added to soil on many forest, rangeland, and mine reclamation sites can decrease the number and amount of invasive species (Adams et al. 2013, Bueno et al. 2019), increase water-holding capacity (Basso et al. 2013, Page-Dumroese et al. 2016b), and decrease greenhouse gas emissions (Sarauer et al. 2019) while concurrently sequestering C belowground.

Biochar can be applied with a biochar spreader (Page-Dumroese et al. 2016a), manure spreader, tractor, or by hand. On forested sites, biochar is not incorporated into the soil, but moves into the mineral soil with rain or snow melt. During tree planting in the Lake States, biochar was applied in the planting hole (Richard et al. 2018) and was shown to increase water-holding capacity. On agricultural sites, biochar is incorporated using available equipment. Recommendations for how much biochar to apply, however, largely depend on the soil texture and organic matter content. For example, a loamy-textured soil with abundant organic matter may benefit from only a small amount of biochar (~1 ton/ac [2,242 kg/ha]), but a coarse-textured, low fertility, low organic matter soil could benefit from 10 tons/ac (22,417 kg/ha). Although we have experimented with applying greater quantities, we have observed that large amounts of biochar can be detrimental to water infiltration into the soil and immobilization of nitrogen (N) (Page-Dumroese et al. 2015, 2018).

To avoid the economic costs of transporting woody residues to bioenergy facilities, we have been examining other ways to create biochar for wildland soil use. Three that show promise are better-designed slash piles, kilns, and air-curtain burners.

Slash piles

Properly constructed slash piles can maximize the creation of biochar to be distributed on wildland sites. The best slash piles have logs with the largest diameters at the bottom of the pile (with some gaps between them to encourage air flow) and smaller material piled perpendicularly on top (figure 3).

Grapplers can be used to build the piles, which are then lit from the top, allowing the fire to burn downward. Once the flames have gone out, the pile is extinguished with either soil or water to maximize the amount of char, rather than ash. After the biochar has cooled, it can be raked around the site. This method has four advantages:

1. Potential for greater air flow to dry wood.
2. Limited moisture wicking up from the soil into the wood.
3. Construction time is similar to other pile-building methods.
4. Limited soil impacts.

As noted in table 1, the biochar created in slash pile burns can be low in C, but on sites low in organic matter, this biochar can still provide additional water-holding capacity.

Kilns

Kilns (e.g., figure 4) have been used for centuries to make charcoal. They can be earth-covered pits or mounds, or made from bricks, metal, or concrete. Kilns work in batch jobs in which the feedstock is added, burned, quenched, and the charcoal removed and spread on the soil. Some kilns can be highly portable to use for on-site biomass processing. Mini-kilns, such as those made by Wilson Biochar Associates (www.wilsonbiochar.com) are ideal for small landowners interested in conservation stewardship or soil enhancement projects. These small kilns can be operated by one or two people. Depending on the type and size of kiln, processing can take hours to days to complete. Newer rotary kilns (Utah Forest News 2015) can be used for large-scale operations, but wood must be chipped before adding it to the kiln (Page-Dumroese et al. 2017). This equipment is housed in a shipping container so it can be relatively portable.

Kilns produce relatively high C biochar (table 1). Unless the biomass is chipped before burning, however, the resultant biochar can be chunky and slow to incorporate into the soil.

Figure 3. Slash pile built to maximize biochar production. Note the larger diameter logs at the bottom; they help keep the heat away from the soil surface and provide maximum airflow. (Photo by USDA Forest Service 2011)

Air Curtain Burners

Air curtain burners are an alternative to burning wood in slash piles. These are usually used for large-scale projects, but the burners come in different sizes (https://airburners.com). A series of blowers push air across the top of the fire box to create an air curtain, recirculating gases and particulates back into the fire for secondary combustion. Similar to kilns, air burners work in batch jobs. In air curtain burners, wood is burned continually, forming some biochar, but the primary product is wood ash unless the fire is quenched. This equipment can be used to rapidly dispose of fresh or dried woody residues, but because the air burners are heavy, equipment is needed to dump the ash out. Although the ash has value as a fertilizer, it does not have the high water- and nutrient-holding capacity of biochar.

Figure 4. Kiln used to convert juniper slash into biochar. (Photo by Eric Roussel, Nevada Division of Forestry 2016)

Nursery Trials

One way to increase the conversion of forest woody residues into biochar is to expand markets for using

bioenergy and biochar. Biochar from woody biomass has been used to increase agricultural crop, grass, and urban tree growth (Jones et al. 2012, Scharenbroch et al. 2013). Because of these benefits, an obvious potential market for biochar is use in nurseries, especially if it could replace expensive, non-sustainable ingredients in growing substrates, such as Sphagnum peat moss, perlite, or vermiculite (Dumroese et al. 2011). Woody biomass can create high-quality biochar that is 70- to 90-percent C and, when used as a medium for plant production, can help sequester C belowground while improving soil properties. Using biochar can be an efficient way to sequester C because, once added to nursery growing media, biochar becomes part of the root plug already destined to be outplanted. Thus, the transportation and burial costs are already included *de facto* (Dumroese et al. 2011). We have conducted a series of trials to examine the potential of using biochar in container seedling substrates as summarized in the following sections.

Trial 1 – Pelletizing Biochar to Facilitate Handling

We examined the chemical and physical properties of biochar to determine its feasibility for growing seedlings. Because fine-granular biochar can be very dusty, we pelletize biochar with a wood flour binder, hypothesizing that the larger pellets may also provide benefit in the medium (Dumroese et al. 2011). We replaced Sphagnum peat moss with biochar from 25 to 75 percent by volume. At the 75-percent peat / 25-percent biochar level, we saw less shrinkage of the medium during the growing season (i.e., it was more stable than 100-percent peat), but rates of pelleted biochar > 25 percent yielded poor results because the pellets swelled excessively when irrigated (Dumroese et al. 2011).

Trial 2 – Pelletized versus Granular Biochar: Impacts on Seedling Growth

As a follow-up to the first trial, we looked at ponderosa pine growth with the pelleted biochar and the biochar in its original, fine-granular, non-pelleted form and added at the same rates described above (Dumroese et al. 2018). We were very strict with irrigation and N fertilization to avoid confounding the treatments. Irrigation occurred to all seedlings at the same dry-down percentages and we applied a discrete amount (mass) of N per week, at both a low (to achieve 20 mg N total for the experiment) and a high (i.e., normal, 80 mg N) rate; the low rate was used to see if biochar could improve fertilizer use efficiency. Because of expansion problems with pellets identified in the first trial, and very poor seedling growth observed with any pellet treatment (data not shown; see figure 6 in Dumroese et al. 2018), the likely scenario for nursery managers is just to use biochar in powder/fine granular form. In this form, medium pH ranged from 5.0 to 6.7 moving from 25- to 75-percent biochar in the medium. On the first irrigation, the volume of the 100-percent peat treatment shrank about 10 percent, but addition of biochar reduced that shrinkage to just 3 to 5 percent, suggesting that biochar helps maintain porosity. Adding 25- or 50-percent biochar reduced irrigation frequency 12 and 25 percent, respectively (Dumroese et al. 2018). At the low N rate, seedling growth was poorer with any addition of biochar (figure 5). At the high rate of N, adding 25-percent biochar had no effect on height, slightly increased root collar diameter (RCD), reduced shoot biomass, and increased root biomass (Dumroese et al. 2018).

Recalling that we held the fertilizer N rate constant (in terms of mass), we must add a caveat to our findings. Our data suggest that early in the crop cycle, biochar likely absorbs N on its cation exchange sites. Under a production scenario where nursery staff are monitoring growth against a target growth curve, a prudent manager could readily do some real-time nutrient manipulations to keep the crop growing on target.

In figure 6, for example, all seedlings were given the same mass of N and the same amount of water. If, however, they were grown operationally and the nursery manager regularly compared actual growth with target growth, and subsequently tailored the culturing regime to meet the target growth curve (by adding more N), we hypothesized that seedling quality could be maintained across a range of biochar additions.

Trial 3 – Using Granular Biochar in an Adaptive Way

To test the hypothesis framed at the conclusion of Trial 2, we added 25, 46, and 43 percent more N to the seedlings growing with 25-, 50-, and 75-percent granular biochar treatments at the 80 mg N rate to keep them on their target growth curves. Our results reveal that any short-term nutrient problems associated with the high cation exchange capacity (or some other factor) of the

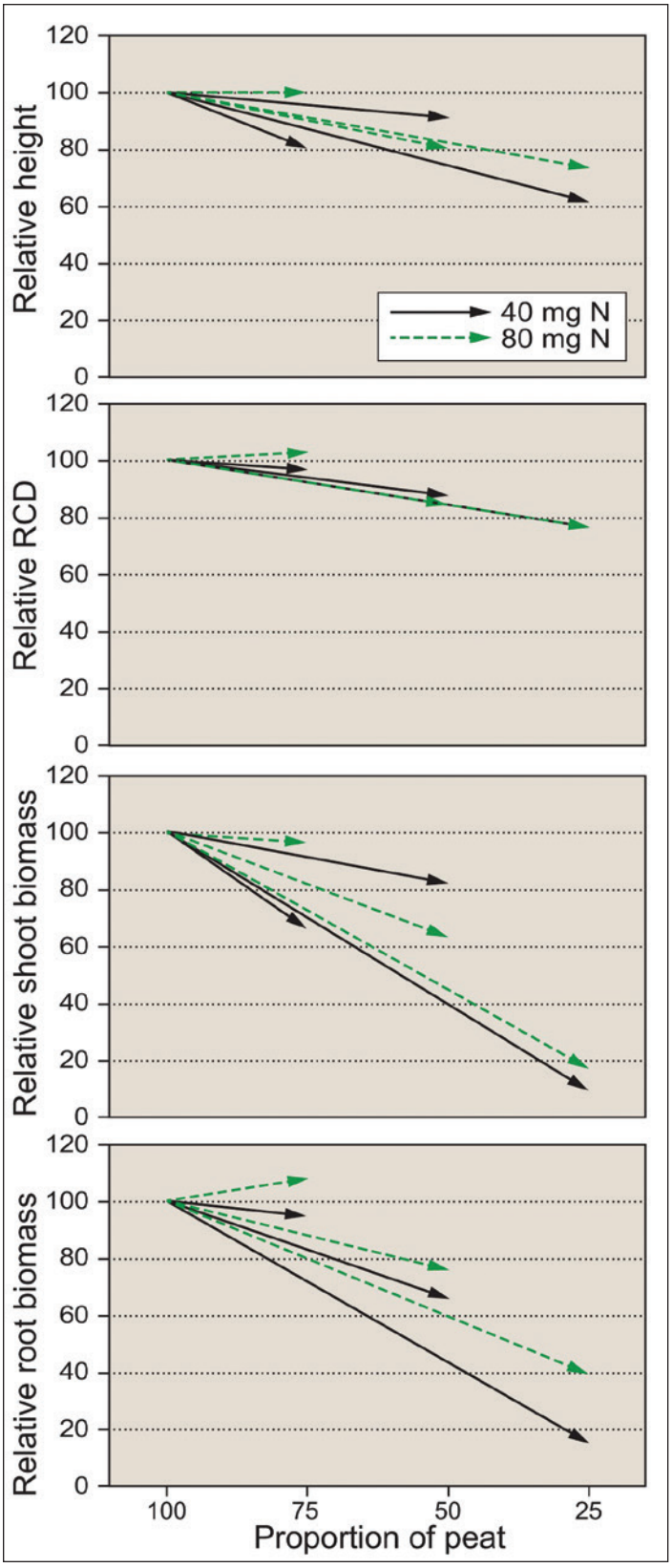

Figure 5. Vectors represent relative changes in seedling morphology of seedlings grown in a biochar-amended substrate compared to the control with 100-percent peat. X-axis reflects percentage of peat; Y-axis reflects relative value of the treatment to the control (i.e., 100-percent relative value is the value obtained with the 100-percent peat). In general, downward pointing arrows indicate morphologies smaller than the control. (Modified from Dumroese et al. 2018)

biochar can be overcome by manipulating the fertigation regime (figure 7). Remember that the seedlings shown in figures 6 and 7, grown with 25-percent addition of biochar by volume, yielded similar seedlings to those grown in the 100-percent peat control at the same high rate of N (which was really the "normal" rate of N we typically use to produce ponderosa pine) (Dumroese et al. 2018). Although we have not tested composting biochar, research indicates that mixing biochar with compost can initially charge the cation exchange sites of the biochar (Agegnehu et al. 2017). Pre-charging the biochar may avoid the lag in early growth we observed in Trial 2 and mitigate the need to manipulate N levels, as done in Trial 3.

Trial 4 – Testing More Species Than Pine

This trial also used biochar powder from a woody feedstock (Matt et al. 2018). Because of our results with 25-percent granular biochar in previous trials, we bracketed our rates in this experiment around that value, and replaced peat with biochar at rates of 0, 15, 30, and 45 percent (by volume). We also looked at three plant forms (i.e., tree, forb [an annual and a perennial], and grass). Ponderosa pine was the tree, pinkfairies (*Clarkia pulchella* Pursh) was the annual forb, blanketflower (*Gaillardia aristata* Pursh) was the perennial forb, and Idaho fescue (*Festuca idahoensis* Elmer) was the grass. We strictly controlled the N rate and irrigation to ensure all treatments were given the same amounts. In this trial, we found similar biomass (shoot, root, total) regardless of biochar rate for everything except the grass, which performed poorer than the control when any rate of biochar was added (Matt et al. 2018). A notable result from

Figure 6. Left to right: Seedlings grown with 0-, 25-, 50-, or 75-percent granular biochar at the 80-mg N rate. (Photo by R. Kasten Dumroese 2010)

Figure 7. Short-term nutrient problems can be overcome by manipulating the fertigation regime. Left to right: Seedlings grown with 0-, 25-, 50-, or 75-percent granular biochar and receiving 80, 100, 117, and 114 mg N, respectively, in order to achieve the same overall growth as the control (i.e., 80 mg N). (Photo by R. Kasten Dumroese 2010)

this trial was the reduced irrigation frequency afforded by the biochar (table 2; Matt et al. 2018).

Trial 5 – Biochar and Symbiotic Organisms

Our last trial examined whether biochar had any effects on the development, growth, and function of rhizobia during nursery production. Rhizobia are micro-organisms that form symbiotic relationships with legumes (Fabaceae), converting atmospheric N into a form useful to their host plants. In this trial, our host plant was black locust (*Robinia pseudoacacia* L.), and we amended *Sphagnum* peat moss with 5-percent (by volume) granular biochar. Our preliminary results revealed that biochar had no effect on the abundance of rhizobia or their ability to fix atmospheric N, but that biochar produced from gasification yielded larger seedlings than those grown with biochar from pyrolysis (unpublished data).

Table 2. Number of irrigation events, as triggered by a 75-percent container capacity threshold (from Matt et al. 2018, using Dumroese et al. 2015; scientist method; actual change in water mass).

	Biochar (% by volume)			
	0	15	30	45
Clarkia pulchella	38	36	32	27
Festuca idahoensis	31	26	21	20
Gaillardia aristata	53	51	44	41
Pinus ponderosa	49	44	39	37

Outplanting Seedlings with Biochar

An early study found benefits in the addition of biochar at planting (Richard et al. 2018). The benefits were hypothesized to include improved soil water-holding capacity dynamics, increased nutrient retention, and enhanced carbon sequestration. In a growth chamber study, we found no marked differences in growth of Norway spruce (*Picea abies* [L.] Karst.) seedlings transplanted into an alluvial silty soil amended up to 60 percent by volume with biochar (Heiskanen et al. 2013). This suggests that biochar may be added to mineral soils without detrimental effects to outplanted seedlings.

We outplanted, on a forest site in Alabama, longleaf pine (*Pinus palustris* Mill.) and loblolly pine (*Pinus taeda* L.) seedlings that were grown in containers with two sources of granular biochar (mixed conifer and proprietary) at rates from 0 to 20 percent by volume. After three growing seasons, we observed no differences in survival or growth (unpublished data).

Management Implications

Soil scientists, land managers, and nursery managers have an incredible opportunity to convert excess woody biomass that would normally be burned in slash piles into a high-carbon product and use it for soil restoration or as a component in growing media for native seedling production. Biochar can be a replacement for other forms of organic matter, but has the advantage of being highly recalcitrant, has a high cation-exchange capacity, can reduce leaching, and increases soil water-holding capacity. Furthermore, it sequesters C belowground, reduces the volume of woody residues and fire risk, and, because of the low N content, can limit invasive species. Most biochars have a relatively high pH and can also help remediate sites with a low pH by acting as a liming agent. Biochar, when used in combination with other soil restoration efforts (e.g., mycorrhizae inoculants, compost), should reduce recovery time and plant failure.

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