Tree Planters' Notes

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Dear TPN Reader

In these pages you will find a variety of topics including propagation protocols, outplanting treatments, nursery strategies during the pandemic, and disease management. These articles span the globe, from the United States, Canada, U.S. Virgin Islands, Morocco, and the Federated States of Micronesia. This diversity is what I enjoy most as editor of this journal and one of the reasons it is so appreciated by its readers and authors.

Warm Regards ~

Diane

Diane L. Haase

A society grows great when old men plant trees whose shade they know they shall never sit in.

— Greek Proverb

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

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Vahl's Boxwood, (*Buxus vahlii* Baill.): A Federally Endangered Tree of St. Croix and Puerto Rico

Michael Morgan and Thomas W. Zimmerman

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Abstract

Vahl's boxwood (*Buxus vahlii* Baill.) is a federally endangered tree that occurs on four sites in St Croix. It is related to the European ornamental bush common boxwood (*Buxus sempervirens* L.), which is often trimmed to make elaborate hedges or topiaries in temperate climates around the world. The University of the Virgin Islands produces containerized seedlings of Vahl's boxwood so they can be planted in protected areas on the island of St. Croix. This nursery stock, once planted in permanent sites, will augment the number of plants growing in the wild, thus reducing the possibility of this rare plant species going extinct. This article describes the species' characteristics and our techniques for growing it from seed and from cuttings.

Introduction

Vahl's boxwood (*Buxus vahlii* Baill.) is an evergreen shrub that is found in four sites on the island of St. Croix in the U.S. Virgin Islands and two sites in Puerto Rico. This tropical species has been federally endangered since 1985 (USFWS 1987). Vahl's boxwood is related to the common boxwood (*Buxus sempervirens* L.) of Europe. Many people will recognize the common boxwood from its scent and because it is a popular ornamental planted in temperate climates around the world for hedges. Sometimes it is trimmed into fanciful shapes called topiaries.

Vahl's boxwood grows on limestone-derived soils within tropical dry forest vegetation. Of the four sites on St. Croix, one population is within the Sandy Point National Wildlife Refuge, two populations are on the hills south and east of the town of Christiansted, and a single individual is located in a former industrial site close to the Henry Rohlsen airport. With the exception of the former industrial site with a single plant, the three other sites support populations with fewer than 500 individuals. There are also two known sites in Puerto Rico that support small populations of Vahl's boxwood (Carrera-Rivera 2001, Daley and Ray 2014, Daley and Valiulis 2013).

Vahl's boxwood is threated by urban development resulting in habitat fragmentation and destruction, competition with exotic plant species such as snake plant (*Sansevieria trifasciata* hort. ex Prain.) and coral vine (*Antigonon leptopus* Hook. & Arn.), as well as devastating human-caused wildfires. Moreover, the species is threatened by its own reproductive biology. Seed dispersion occurs when the seed capsules dry out and split. The tiny seeds contained inside simply drop to the ground. As a result, the seeds do not travel far from the parent plant.

Description

Vahl's boxwood is a small tree or bush that has a maximum height of 15 ft (5 m). The bark is gray and finely fissured. The leaves are dark green, leathery, and stiff and are oppositely arranged on the branches (figure 1).

Figure 1. Foliage of Vahl's boxwood occur in an opposite arrangement. (Photo by Michael Morgan 2018)

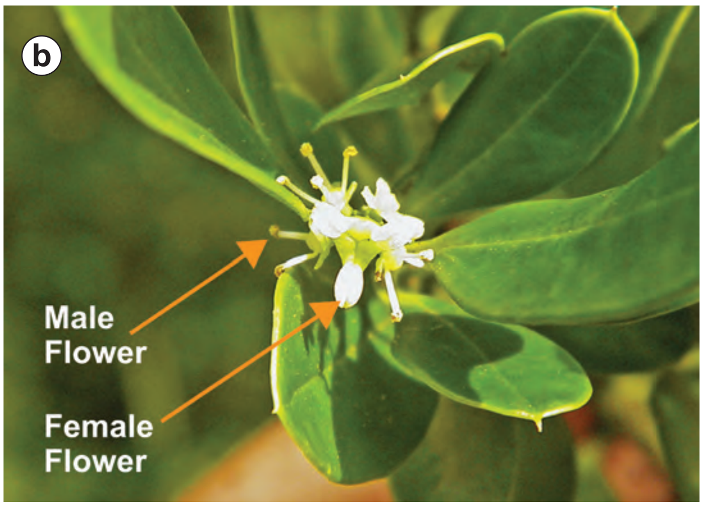

Figure 2. Male and female flowers of Vahl's boxwood. (a) The stigmas and ovary of the female flower develop into the seed capsule. (b) Note also the spike at the tip of the leaves. (Photos by Michael Morgan 2018)

The mid vein of each leaf is sunken and two slight side veins parallel the curve of the leaf edges (Little and Wadsworth 1964). Each leaf has a little spine at the tip (figure 2) which helps distinguish Vahl's boxwood from box-leaf stopper (*Eugenia foetida* Pers. formerly *E. buxifolia*), an unrelated, but similar-looking species that Vahl's boxwood grows in association with (personal observation, Morgan).

Vahl's boxwood is monecious, meaning its flowers are either male or female and both flower types are on the same plant. The flowers are greenish yellow with white anthers and occur in clusters at the leaf base (figure 2). Bees and other insects pollinate the flowers. The fruit are woody, green capsules about 0.25 in long (0.6 cm), with three "horns" on top (figure 3). When mature, the seed capsules turn brown then black and split open into three parts, ejecting the seeds.

The species is slow growing in height and diameter. Plants take approximately 2 years in a nursery setting to reach 12 in (30 cm) in height until they are ready to leave the nursery. Once planted outside the nursery, plants grow less than 1 ft (30 cm) per year. Two trees planted in the UVI Agroforestry plot are currently 6 years old and 66 in (165 cm) tall. They were 24 in (60 cm) tall when planted in 2014. This growth rate is an average of 7 in (17.5 cm) per year (Morgan, personal observation). Architecture of Vahl's boxwood can be influenced by site (Castellanos et al. 2011).

Figure 3. Seed capsules of Vahl's boxwood have 3 "horns" at the top of the capsule. (Photo by Michael Morgan 2018)

Flowering and fruiting of this species is precocious. Flowering and fruiting can begin the second or third year after being planted in the field. Containerized plants in a greenhouse setting can flower once they have reached a sufficient size of about 12 in (30 cm). Unlike plants growing in the wild, however, plants growing in a tree nursery get watered 2 or 3 times per week (personal observation, Morgan).

Propagation

The following section is based upon the experiences of the primary author growing this species at the University of the Virgin Islands Agricultural Experiment Station (UVI-AES).

Seed Propagation

Seeds can be collected year-round. In the U.S. Virgin Islands, flowering and fruiting of plants depends on local rainfall conditions. While there are "dry" and "wet" seasons in the Virgin Islands, the difference between the two seasons is not noticeable except during exceptionally dry or wet years. A little bit of rain will initiate flowering in Vahl's boxwood. However, flowering does not always lead to the production of seed capsules or viable seeds.

The woody seed capsules should be collected before they split open and eject their seeds. The best time is when capsules are turning from green to brown. Put the seed capsules in a dry, sunny place on a wire screen that is small enough to support the capsules, but big enough to allow seeds to fall through into a container below. Capsules take about 1 week to split and release their seeds. Seeds are extremely small (300,000 seeds per lb [660,000 per kg]) (figure 4). Once dried, seeds can be stored in a cool, dry place and will retain viability for at least 1 year (personal observation, Morgan).

It is sometimes recommended to rinse seeds in a weak bleach and water solution (1:10 ratio) to disinfect the seed surface of any harmful fungi or bacteria. Since Vahl's boxwood seeds are so rare and small, there is concern that the bleach rinse could damage the seeds. Sunlight is also an effective sterilizer, so we recommend exposing the seeds to direct sunlight for a day instead of rinsing with a bleach solution. The tiny size of the seeds also precludes physical scarification.

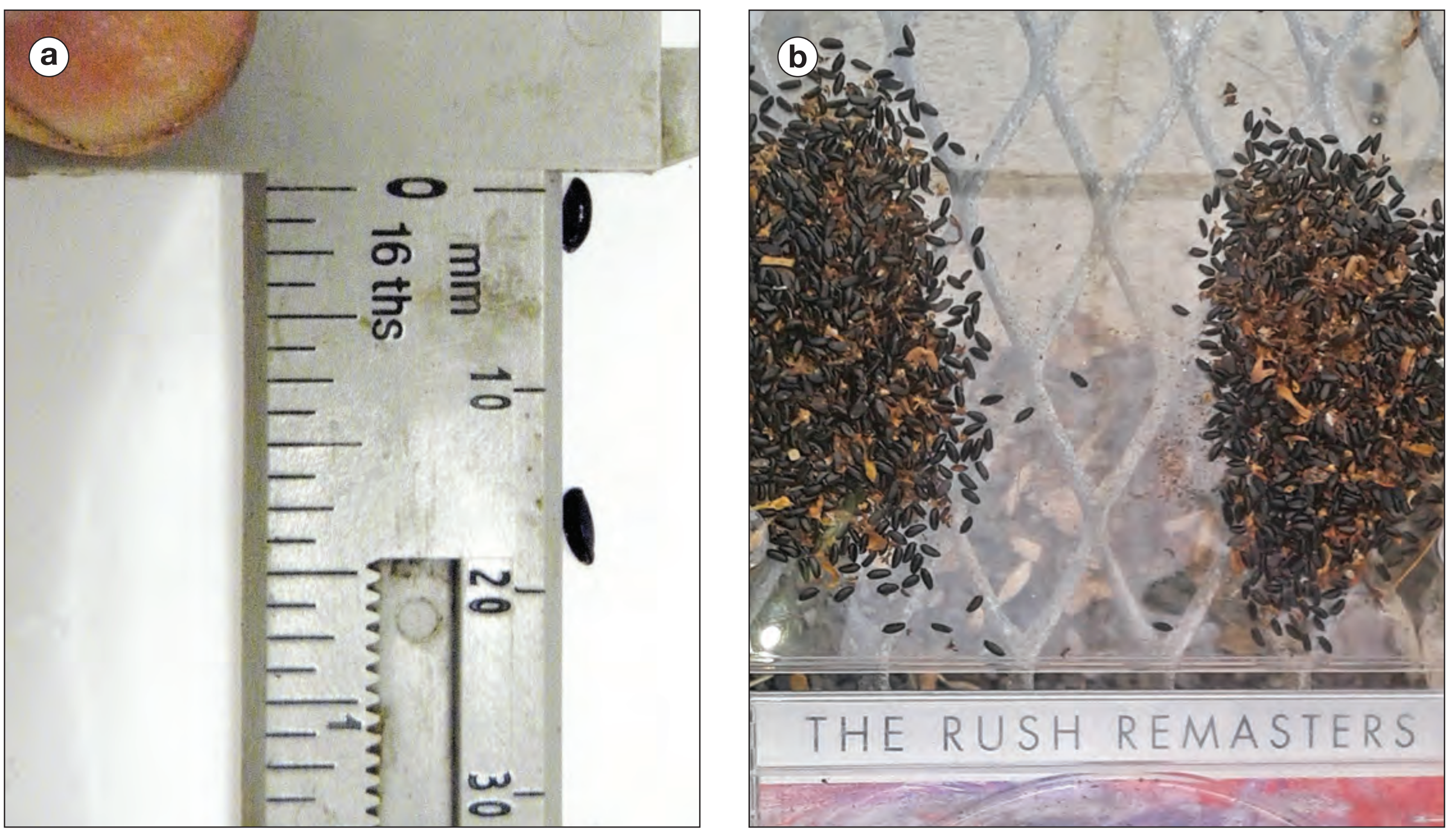

Figure 4. (a) Vahl's boxwood seeds are very small. (b) Each of these two piles contain approximately 900 seeds and are placed on a CD case for scale. (Photo by Michael Morgan 2018)

Vahl's boxwood seeds have an exceptionally low and slow germination rate (figure 5). Germination rate seems to be related to when and where the seeds were collected. Our best germination has been 10 percent and our worst was when only 3 seeds out of 5,000 germinated after 7 months. Germination of 5 or 6 percent is typical. It appears that this species compensates for its low germination rate by producing an abundance of seed.

The cause of low germination rate in Vahl's boxwood is unknown. We performed a tetrazolium test on seeds from three of the four populations on St. Croix to determine viability. Living seeds turn pink when exposed to tetrazolium. We compared the seed samples with seeds of roselle (*Hibiscus sabdariffa* L.) which has a high rate of germination. Roselle, locally called sorrel, is an agricultural crop in the U.S. Virgin Islands. The swollen sepals of the roselle flower are used for making juices and teas. The viability percentages of Vahl's boxwood seeds from the three populations were 37, 23, and 14 percent, respectively. Most of the roselle seeds were viable. Notably, the roselle seeds were a bright pink compared with a pale pink observed in Vahl's boxwood seeds, hinting at a certain lack of vigor. Seeds germinated after 39, 60, and even 114 days after sowing. On occasion, lost and forgotten seeds will germinate years after planting in containers with other plants when old planting substrate was recycled, or even on the gravel floor of the UVI-AES greenhouse (figure 5c).

Figure 5. (a) and (b) germination progression of Vahl's boxwood can be slow and uneven. In fact, germination can be delayed significantly as shown by (c) seedlings that germinate over time on the greenhouse floor. (Photos by Michael Morgan 2018)

Figure 6. In a trial of vegetative propagation of Vahl's boxwood, cuttings were regularly sprayed by misting nozzles. (Photo by Michael Morgan 2018)

Vegetative Propagation

We tried to propagate Vahl's boxwood via cuttings but only 14 percent of the cuttings put out new leaves and roots. Unfortunately, these plants remained stunted. During the following year, they never increased growth in height or diameter, so we disposed of the plants.

We took 6-in (15-cm) cuttings and tested them with and without dipping the cut end in rooting hormone. The rooting powder we tested had a concentration of 0.01 percent of the auxin indole-3-butyric acid (IBA). For planting substrate, we used a 50:50 mix of sand and Promix™, an amended peat moss. After placing in the substrate, cuttings were kept moist for a month by using a humidity tent or a mist bench. A humidity tent is simply a transparent plastic bag over the cutting and its container. The plastic bag recycles the water that evaporates from the moist planting substrate or is produced as a by-product of photosynthesis. The vapor condenses on the plastic and falls back into the planting substrate. If the planting substrate dries out, it must be rewatered. A mist bench sprays the cuttings with mist 2 times daily using a timer and misting spray heads (figure 6). We set our mist bench to spray for 4 minutes, at 4 p.m. and 4 a.m.

In our experience, it appears that there is no difference in propagation success between using hardwood or softwood cuttings, nor does there appear to be an effect of using rooting hormone.

We found that both the mist benches and the humidity tents gave the same results. Cuttings may put out new leaves (figure 7), but then die if they fail to initiate new

Figure 7. Only 14 percent of cuttings in the propagation trial put out new leaves. (Photo by Michael Morgan 2018)

Figure 8. (a) Successfully propagated planting stock was (b) planted at Sandy Point National Wildlife Refuge. (Photo by Michael Morgan 2018)

roots. In our experiment, the cuttings that survived remained stunted, even if they developed roots and new leaves, and did not reach sufficient size to be planted outside the nursery. Thus, refinement of vegetative propagation techniques is still needed for Vahl's boxwood. A stronger concentration of IBA may be worth testing.

Uses

The main reason for growing Vahl's boxwood is for the conservation of biodiversity (Lindsay et al. 2015) and restoration ecology. To that end, 40 plants were planted in the Sandy Point National Wildlife Refuge: 28 individuals at the end of 2015 and 12 more at the beginning of 2018 (figure 8). Additionally, there is potential to use this species in a landscape setting. The dense, dark green foliage of Vahl's boxwood is attractive and could therefore be desirable for use in hedges, and possibly for topiary like its temperate-zone relative, the common boxwood. Experiments are necessary, however, to determine how the species responds to pruning.

Prior to the discovery of quinine in the Americas, leaves of the European common boxwood were used as a fever reducer (Rushworth 1999). Perhaps the Tainos and Caribs, the indigenous peoples of the Caribbean, had a similar use for the leaves of Vahl's boxwood. Also, because the wood of common boxwood is light-coloured, hard, and dense, yet carves well, it is used for specialty products such as chess pieces, flutes, and oboes. The rarity of Vahl's boxwood, however, precludes such consumptive uses.

The Endangered Species Act forbids the destruction of Vahl's boxwood trees, and collection of botanical samples and seeds are regulated by permit. Overall, the most important role of the species is to provide intangible environmental ecosystem services such as biodiversity and the conservation of soil, pollinators, and habitat.

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Nursery Strategies to Maintain Production and Protect Human Health During the Coronavirus Pandemic

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Abstract

The coronavirus COVID-19 pandemic of 2020 caused a dramatic shift in operating procedures at forest and conservation nurseries throughout North America. Even though nurseries were deemed essential businesses, State and Provincial guidelines required nurseries to implement protocols to minimize the spread of the virus, including social distancing, mask requirements in closed spaces, frequent disinfecting, and monitoring symptoms of workers across all activities within nurseries. In August 2020, a panel of nursery managers from western Canada, western United States, and southern United States participated in a webinar to discuss individual strategies taken to keep workers safe while also ensuring seedlings were lifted and shipped and new seedlings were sown. All nurseries made substantial changes to limit contact between workers and developed contingency plans in case the virus spread within their facilities. Luckily, as of August 2020 no spread was recorded in any participating nurseries. The lessons learned and the steps taken to protect workers during the pandemic are described in this article. Although not without short-term impacts on added costs and decreased efficiencies, the practices implemented should help nurseries be more resilient to future events that may cause similar disruptions to operations.

Introduction

The COVID-19 pandemic that began in early 2020 caused a shift in all facets of life around the world, including rapid responses from forest and conservation nurseries to increase employee safety while minimizing operational disruption. All aspects of the seasonal nursery production cycle are busy, but the shutdowns and stay-at-home orders started at a critical time when nurseries were shipping the previous crop for spring planting, sowing the next crop, and, in some cases, lifting bareroot seedlings. Thus, it was imperative for nurseries to quickly assess how to keep employees safe and healthy and then implement new policies and practices within a few days.

The agricultural industry in the United States was deemed essential early in the pandemic. For example, the Federal Cybersecurity and Infrastructure Security Agency (CISA) in their August 18, 2020, "Guidance on the Essential Critical Infrastructure Workforce" report specifically listed workers supporting greenhouses as essential employees (CISA 2020). Some States issued guidelines for protecting workers, while other States, such as Oregon, issued temporary rules that all businesses must follow. Oregon's "Temporary Rule Addressing COVID-19 Workplace Risks" required physical distancing, face coverings provided by the employer, regular cleaning and sanitation of work and common areas, optimizing ventilation, training employees, and performing risk assessments, among other requirements (Oregon OSHA 2020). These rules and guidelines are continually updated with the best available information from the respective governmental agencies such as the U.S. Center from Disease Control and WorkSafeBC, requiring nurseries to constantly stay up-to-date on changing requirements and adjusting operational practices accordingly.

Very little research has been published on the impacts of the COVID-19 pandemic on the forest and conservation nursery industry, risk of disease transmission in nurseries, or changes to worker safety standards. One study from Europe examined risk of COVID-19 infection for different types of agricultural employees using the U.S.'s Standard Occupational Classification System (SOC 2018). The study found that graders and sorters of agricultural products perform the highest risk activities with 80 percent of their work tasks considered high risk for infection during normal operation (Bochtis et al. 2020). The next most at-risk group were nursery workers, where 43 percent of tasks were considered high risk for infection, followed by front-line supervisors at 29 percent and nursery managers at 20 percent (Bochtis et al. 2020). Given the high risk of infection across many nursery duties, nurseries in North America and around the world have made significant adjustments to protect worker health and safety. Some tasks, such as working in outdoor nursery beds, required minimal changes other than increasing social distancing, mask requirements, and supplying hand sanitizer and decontamination supplies for equipment. Indoor tasks required much greater changes. Kipp (2020) highlighted strategies used by the nursery industry in Oregon, including the use of barriers between employees when social distancing is difficult. Nurseries have also adjusted their seedling shipping operations, including reducing staff so that they can maintain social distancing (Goloski 2020).

To understand how tree seedling nurseries have adjusted to the COVID-19 pandemic, a virtual panel discussion was hosted by the Western Forestry and Conservation Association on August 12, 2020. The panel discussion was part of the 2020 North American Forest and Conservation Nursery Technology Webinar Series, a partnership between the Western Forest and Conservation Nursery Association, Intertribal Nursery Council, Joint Southern and Northeastern Forest and Conservation Nursery Associations, Intermountain Container Seedling Growers' Association, and the Forest Nursery Association of British Columbia. Panelists were geographically dispersed across North America and included bareroot and container operations. The following sections summarize practices and experiences discussed during the webinar.

General Strategies to Protect Employee Health

Each nursery represented on the panel established several practices and policies and procured supplies necessary to minimize potential transmission of the virus among employees. There were many commonalities among the nurseries, including providing all employees with instructions regarding the new expectations and daily communication with employees to ensure they fully understand and comply with all policies.

Nurseries set up handwashing and sanitation stations around the facility (figure 1) with anti-viral sprays, hand sanitizer, and/or hand soap. One nursery noted the importance of a label to identify the contents of each bottle, provide instructions for its use, and warn that it is not safe for consumption. Additionally, employees can be supplied safety data sheets from the product manufacturer.

Work areas and equipment are disinfected multiple times per day including before and after each use (in case an employee neglects to do it), at each break, and at the end of each shift so it is clean for the next worker. One nursery places an anti-viral spray bottle at each workstation and requires everyone to spray down their area when they leave. One nursery has the policy: "if you touch it, you clean it." Sharing supplies, even pens and paper, has been discontinued.

Nurseries regularly perform health checks on all employees. These checks include administering health questions via phone 24 hours before work commences and/or before entry on site and taking temperatures daily or if a person appears sick. One nursery informs employees upon hiring or first reporting to work that they should not come to work until tested if exhibiting potential COVID-19 symptoms per government health guidelines. The same nursery asks all employees how they are feeling upon arrival for work each day. If an employee develops symptoms at work, they take the government screening questionnaire and, if appropriate, leave work to get tested before returning.

All employees are provided with personal protective equipment (PPE) such as masks, face shields, gloves, and sanitizers. PPE is issued to each employee just like safety vests. For most nurseries,

Figure 1. To facilitate social distancing and reduce the risk of virus transmission, nurseries set up (a) outdoor and (b) indoor wash stations, (c) outdoor break areas (note tape on table to designate social distancing), and (d) portable breakrooms. (Photos by Jacky Friedman 2021)

masks are required inside but not outside if they can keep 6-ft (2 m) social distancing. If employees can maintain minimum social distance in the greenhouse, masks are often not required. Nurseries arranged for fabric masks to be made and/or bought surgical masks and face shields online. Availability of N-95 masks has been very limited, so nurseries have had few, if any, to use. One nursery noted that the best approach is to maintain adequate distance or use physical barriers whenever possible because masks can be uncomfortable, especially when the weather is warm.

To facilitate social distancing, mobile bathrooms and break rooms were brought to some nurseries (figure 1). Also, outdoor break areas were established at one nursery with tape placed on the table where employees could sit and maintain a 6 ft (2 m) distance between each other (figure 1), and breaks have been allowed in private vehicles. Additionally, one nursery removed their vending machines and water dispenser and keeps sanitation supplies by the microwave to sanitize the microwave and other surface after each use.

Documentation is an important aspect of daily COVID-19 policies. Nurseries keep records of wellness checks (figure 2), inspection of sanitation stations, etc. Documentation will help with contact tracing should it be necessary. Adding this documentation to the seedling-growing documentation already required is onerous but is important and necessary for managing the situation.


Question: Are you expericencing cold,flu or Covid-19 like symptoms, even mild ones? Symptons include: fever,chills,shortness of breath,sore throat or painful swallowing, stuffy or runny nose,loss of smell,muscle aches, fatigue or loss of appetite.

Employee	Date: 9.19	Date: 9.20	Date: 9.21	Date: 9.22	Date: 9.23
Nathan					
Sean					DO
Ron					
Pat					
Steven	V-6				
Bill					DO
Paul					
Regan					
Carmen					
Javier					
Nicolas					
Paul					
Claud					
Neil					
Carlos					
Cathy					
Pamela					
Dennis				DO	DO
Marina				DO	DO
Gerald	A-C	A-C		DO	DO
Joyce					
Helene					
Cyndi	A-C			DO	DO
Alex		A-C		DO	DO
Mac		A-C		DO	DO
Pablo					
Heriberto					
Alfredo					
Jorge					
Ignacio					
Agnes		L-C			
Anna					
Karen					
Thomas					

Figure 2. Daily documentation of wellness checks and sanitation practices are part of the nursery COVID-19 strategy. (Photo by Sean Webb 2020)

Strategies to Safely Accomplish Work

Developing a risk assessment for all aspects of daily nursery operations aids in efforts to keep staff healthy. One nursery designated each task as high, medium, or low risk; the closer the working proximity among workers, the higher the risk, such as the example shown in table 1. Using such a risk assessment enables managers to develop safe working procedures for each nursery task. For some nurseries, administrative staff have been able to telework to limit the number of people inside buildings.

As much as possible, work and training activities are conducted outdoors, where employees can maintain social distancing. This is relatively simple for bareroot nurseries because nursery beds are usually 6 ft (2 m) apart and workers can spread out for weeding and other tasks (figure 3). For indoor activities, most nurseries have facilitated extra spacing and created physical barriers between workers. For example, conveyor lines for harvesting, grading, or sowing have been reconfigured to space out the workers. When possible, additional conveyors have been added or have been set up outdoors. For some tasks, productivity is overly compromised with spacing, so barriers have been added when workers must work closer than 6 ft (2 m) from each other (figure 4). Some barriers are stationary but others are mobile so they can be oriented in various ways or relocated as needed to accommodate different tasks and allow people to get in and around them. Mobile barriers allow for maximum flexibility and protection for staff. Barriers have been constructed from 6-mil (0.15 mm) greenhouse poly attached to frames made from PVC irrigation pipe or wood (figure 4). The barriers can be weighted down for increased stability and safety. Other barriers can be constructed to hang between workstations (figure 4).

Establishing work groups (“pods” or “bubbles”) is another strategy during the pandemic. Employees within each pod work together throughout the day and stay separate from employees in other pods. Thus, if one person is infected, the number of others who have been exposed is limited. Similarly, some nurseries are using contract crews (limited to a specific number of people) who are trained to work only in their station area and are expected to stay separate from full-time staff as much as possible. Since the webinar, one nursery shifted employment

Table 1. Sample risk assessment and protocols for minimizing the spread of COVID-19 for different nursery activities.

Activity	Level of Risk	Control Protocols
Management	Low	• Implement and manage safety policies • Encourage safe practices outside of work • Consider contract workers instead of temporary workers • Establish work groups ("pods") • Stagger work schedules • Conduct daily wellness checks • Inspect sanitation stations frequently • Institute paperless timekeeping • Document safety and wellness checks • Post signs on all buildings for hygiene and distancing
Office Administration	Low	• Telework when possible • Maintain 6 ft (2 m distance) • Wear PPE • Install plexiglass barriers between workstations and at front desk • Sanitize commonly touched surfaces
Weeding/ Thinning	Low	• Position workers 6 ft (2 m) apart • Sanitize tools after each use. • Assign tools to individual staff • Provide disinfectant at all job sites • Provide PPE when distancing is difficult
Growing and culturing	Low	• Maintain physical distance • Provide PPE when distancing is difficult • Sanitize surfaces after each use
Delivery pickups and drop offs	Low/ Moderate	• Drop-off and pick-up parcels outside • Eliminate need for signatures or disinfect pens between use • Maintain physical distance when loading a truck • Provide PPE if interaction is necessary
Sowing	Moderate	• Position workers 6 ft (2 m) apart • Install hanging screens between individuals • Provide disinfectant at all job sites • Sanitize workstations between shifts • Provide PPE when distancing is difficult • Slow equipment speed to accommodate lower staffing
Lifting/grading	Moderate	• Install hanging screens between individuals • Use turntable stations • Provide disinfectant at each station • Disinfect all equipment between shifts • Provide PPE when distancing is difficult • Slow equipment speed to accommodate lower staffing
First Aid	High	• Designate a first aid attendant • Wear PPE while treating • Conduct evaluations/treatments outdoors when possible

Figure 3. Social distancing outdoors at a bareroot nursery is relatively easy because beds are 6 ft (2 m) apart and there is ample space within a row. (Photo by Gina Sowders 2020)

Figure 4. Some nurseries have constructed barriers as part of conveyor lines to separate workers from one another. Individual sanitation bottles are often provided at each station for frequent sanitation. (Photos by Thomas Stevens and Sean Webb 2020)

from temporary to contract employees on the packing lines and other places throughout the nursery. The justification for contract employees was to have a consistent workforce and known individuals working at the facility. Some nurseries have brought in porta-potties and installed separate wash stations to minimize interactions among pods, contract crews, and other staff. Additionally, work schedules have been adjusted with staggered start times and break times. To accommodate changing schedules, seasonal activities, such as packing, may need to commence earlier and finish later. When vehicles are used at the nursery, some nurseries have established a policy of one person per vehicle.

Strategies to Limit Outside Contact During Shipping and Delivery

Nurseries now have strict limitations regarding who can enter the nursery site. The nurseries are closed to the public and do not allow any visitors. If outside people do come on site, interactions are kept to a minimum and sanitation practices are followed after they leave. Before the pandemic, one nursery kept their gates open all day during work hours but have since reprogrammed the gates so they are only open in the morning when the crew arrives and again at the end of the day when the crew departs. A sign is posted at the gate that an appointment is

Figure 5. Most nurseries have closed to the public by keeping gates closed and instructing any visitors that appointments are required. (Photos Thomas Stevens 2020)

Figure 7. Signs are posted throughout nursery facilities to remind employees to follow new protocols. (Photo by Siriol Paquet 2020)

Figure 6. Screening people through questionnaires and temperature checks when arriving at the nursery are often done before they enter nursery facilities. (Photo by Thomas Stevens 2020)

required to enter the nursery (figure 5). Recurring vendors have been given the gate code but must be screened when they come into the facility.

Some nurseries require all outside people, such as contractors, vendors, and delivery drivers, to respond to a brief COVID-19 questionnaire and to have their temperature taken upon arrival (figure 6). One nursery sends the nursery COVID-19 policies via email to outside people before their arrival if possible, which is then signed upon arrival. To avoid interaction with nursery employees, delivery drivers are expected to call when they arrive, stay in their vehicle, and/or not enter any buildings. Nursery employees will load or unload the vehicle for them when possible. If there is a night pickup or delivery with no nursery staff present, drivers are required to spray anything that they touch with a sanitizing solution. Additionally, some nurseries require contractors and others to have their own COVID-19 policy so they know that the contractor has safe practices in place.

Figure 8. Outdoor areas are a great way to social distance employees during trainings and daily briefings. (Photo by Gina Sowders 2020)

Contingency Plans for Non-Conforming or COVID-19 Positive Employees

At the time of the webinar, none of the nurseries represented on the panel had had anyone at the nursery test positive for COVID-19. A few had been tested but all were negative. One nursery had someone who may have been in contact and sent that person home until their test results were available. An allied nursery did have a positive test and the subsequent contact tracing took out the whole staff. Nursery managers regularly remind their employees to be extremely careful with fellow employees, especially if they have any hint of symptoms.

Enforcing policies to wear masks, practice social distancing, and sanitize are needed constantly. Signs are posted liberally in work areas (figure 7). Managers frequently give reminders to keep distance; one even brought pool noodles to the nursery to help people understand exactly what a 6 ft (2 m) distance is. Management personnel also constantly remind each other to make sure they are setting the right example for the crew. It can be challenging. Some people complain that the mask is uncomfortable or say that they cannot hear what others are saying because of the mask.

When possible, management and crew meetings are held outside so people can take their masks off (figure 8). The new safety policies have slowly become a cultural practice to which people are getting accustomed. Employees are also expected to take precautions outside of work hours, although that cannot be enforced. One nursery provides all employees a washable cloth mask for use at work and outside of work.

For the most part, employees are taking the pandemic policies and practices seriously. Nurseries are aware, however, some people do not take it as seriously as others and pay extra attention to those individuals in the workplace. These people are reminded more frequently about distancing, PPE, and sanitation requirements and are kept a bit more separated from other employees if possible. At the time of the webinar, none of the nurseries had to do any disciplinary actions for noncompliance with the new health policies other than verbal warnings. One employee was told, “If you’re not part of the COVID team, then you should not be at work.” That statement was effective in changing the employee’s attitude. In general, the policy for most nurseries is to first give a verbal warning, then give a written warning, then send the employee home if they continue to not comply with safe practices.

If an employee is exposed to COVID-19, nurseries require that person to be tested before returning to work. Some nurseries require a 2-week quarantine following exposure; others do not require a quarantine if the test is negative. Contact tracing is done for those who are exposed to the virus or have tested positive. If there is a positive case at the nursery, the expectation would be that it is limited to a specific "pod" or "bubble," so productivity is not entirely halted. Extra sanitation is carried out immediately upon an employee leaving work, prior to test results.

If several employees are unable to work because of illness or quarantine measures, some nurseries have trained inmate and fire crews to assist with nursery tasks. Contract crews are also an option, although visa issues are a major concern. This alternative help will not cover management activities, however, and productivity may decline.

Figure 9. Nurseries use a variety of PPE supplies to keep employees safe. (Photo by Gina Sowders 2020)

Expected Impacts on Current and Future Production, Costs, and Sales

Implementing new safety protocols and potential interruptions in workforce numbers are expected to have an economic impact, although the extent of that is not yet known. All managers on the webinar panel emphasized that health and safety are the highest importance, but new practices will likely reduce productivity. The general approach is to be flexible, lower expectations, stay up-to-date with national and local guidance, and to maintain calm while navigating through this situation as carefully as possible.

Purchasing PPE and extra cleaning supplies is an added expense (figure 9). Labor expenses are also likely to increase. Nurseries plan to hire enough people to accommodate longer hours, staggered schedules, and weekend work to achieve production goals, while keeping people distant and/or separate. Some tasks will take more time than normal. For example, loading and unloading trucks takes longer. Also, keeping orders separate in the cooler at some nurseries could reduce available storage space. Another concern is potential weather effects on seedling quality and phenology as a result of extended (both early and late) sowing and lifting to accommodate modified work schedules or reduced workforce numbers. As a result of these expenses and concerns, some nurseries may have to increase their seedling prices next season. In mid-December 2020, the British Columbia provincial government announced the Forest Sector Safety Measures Fund Program, where small and medium-sized businesses, including forest nurseries, can apply for funds to help offset safety-related costs due to the COVID-19 pandemic. This program should help minimize increasing seedling prices from British Columbia nurseries.

On a positive note: nursery managers have noticed that some tasks now take less time because employees are not working next to each other and being distracted with conversation, a common occurrence before the pandemic that delayed completion of tasks. Another positive is that no one has been sick this year from other illnesses. Ordinarily, people would come to work with the flu and shortly thereafter, there would be several out sick. Also, there is an overall increased attention to cleaning.

Effects of the pandemic on future sales are unknown. Some nurseries expect sales to stay strong, while others are concerned about reduced demand. Logging and tree-planting activities have also had to modify standard operating procedures and have experienced cutbacks and delays. Still, increased demand for wood for home renovation projects and single-family home construction, plus demand for post-wildfire restoration, are increasing seedling demands in western regions.

Conclusion

The tree seedling nurseries who participated in this panel discussion had many similarities in their approach to maintaining productivity during the COVID-19 pandemic, which is likely similar at most nurseries across North America. All are taking the pandemic seriously over fears of complete shutdown or loss of productivity, and so far, none have experienced COVID-19 spreading among workers. This does not mean they have let their guard down. Quite the opposite; most nurseries have ramped up safety precautions using the best guidance from Federal, State, and Provincial governments and are learning new ways to keep their operations functioning. Demand for seedlings was near an all-time high in 2020, which is expected to continue despite some temporary reduction. The herculean effort nurseries put into growing, packing, storing, and shipping seedlings while protecting workers during the pandemic should be commended. The short-term setbacks from loss of productivity and extra costs associated with PPE and sanitation supplies and changes to workforce structure will hopefully result in changes to the nursery industry that will enhance resiliency into the future.

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Effectiveness of Fungicides and Biopesticides in Controlling *Botrytis* Gray Mold on Western Hemlock Nursery Stock

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Abstract

Botrytis gray mold is a disease that impacts conifer seedling production and causes postharvest losses during storage. This disease is difficult to control and often persists after common fungicide applications. Many new products exist with the potential to control *Botrytis* diseases, but little research has been conducted to determine their efficacy on conifers. Through support provided by the Washington State Department of Agriculture Specialty Crop Block Grant, USDA IR-4 Environmental Horticulture programs, and the USDA NIFA McIntire-Stennis program, research was conducted to evaluate the effectiveness of 31 fungicide and biopesticide products to control gray mold caused by *Botrytis cinerea* on 2-year-old container-grown western hemlock (*Tsuga heterophylla* [Raf.] Sarg.) seedlings. Although several effective products were identified for controlling gray mold on hemlock seedlings, additional research is needed to determine the optimal application rates and timing of these products to maximize disease control on a broad range of conifer hosts under nursery production conditions.

Introduction

It is estimated that nearly 62 million conifer seedlings are produced annually in Washington nurseries (Haase et al. 2020). These nurseries fall into two broad groups: those that produce bareroot seedlings and those that produce container stock (and some that grow both). Container production, especially of species that are difficult to germinate and grow, has been increasing over the past two decades and now accounts for about 26 million trees grown annually in Washington (Haase et al. 2020, Trobaugh 2012).

Botrytis gray mold is a chronic disease of conifer nursery stock, particularly in container-grown production systems (Haase and Taylor 2012, Lanthier and Watts 2020, Mittal et al. 1987). Although most *Botrytis* species are host-specific pathogens, *Botrytis cinerea* causes gray mold on many crops, including conifer nursery stock (Elad et al. 2007). In conifers, *Botrytis* primarily infects needle tissues (Haase and Taylor 2012). Symptoms appear as browning of needles, followed by development of masses of gray spores and mycelium (figure 1). The airborne spores are easily dislodged and can rapidly spread the disease, particularly in greenhouses. Conditions with high humidity and poor air circulation are especially conducive to disease development. Under these conditions, *Botrytis* can spread into stem tissues and kill seedlings (Haase and Taylor 2012). Additional losses can occur when seedlings are lifted, packed in boxes or bags, and then held in cold storage for several months prior to shipping and transplanting to the field. *Botrytis* can also cause a shoot blight of conifers in Christmas tree plantations and landscape plantings (Chastagner and Talgo 2018).

While most conifer seedlings are susceptible to gray mold, Douglas-fir (*Pseudotsuga menziesii* [Mirb.] Franco), spruce (*Picea* spp.), western hemlock (*Tsuga heterophylla* [Raf.] Sarg.), coast redwood (*Sequoia sempervirens* [D. Don] Endl.), and giant sequoia (*Sequoiadendron giganteum* [Lindl.] Buchholz) are among the species that are very susceptible to attack by *Botrytis* (Haase and Taylor 2012, Lanthier and Watts 2020, Mittal et al. 1987).

A recent survey of Canadian forest seedling nurseries indicated that *Botrytis* gray mold was the major disease of concern in forest seedling nurseries across

Figure 1. *Botrytis* gray mold on Douglas-fir seedlings. Under high humidity, grayish-colored mycelium and fruiting structures often appear on the foliage at the base of seedlings. Infection leads to discoloration and death of the needles. (USDA photo)

Canada (Lanthier and Watts 2020). Prior to the start of this project, we solicited input from growers in Washington conifer bareroot and container nurseries where *Botrytis* is an ongoing challenge. Some growers with new greenhouses and improved environmental control systems indicated that they could limit crop losses to 5 to 10 percent. Other growers, however, indicated that even with attempts to implement recommended best management practices, gray mold continues to elude reliable control, often resulting in crop losses of 10 to 50 percent, particularly on highly susceptible species grown in containers.

Management of *Botrytis* on conifer nursery stock relies on a combination of cultural practices, such as sanitation and the management of irrigation and ventilation to reduce periods of high relative humidity that favor infection (Dumroese and Haase 2018). Growers also typically make one or more application of fungicides, particularly in the fall when the weather is more conducive for disease development (Haase and Taylor 2012, Lanthier and Watts 2020). *Botrytis* can rapidly develop resistance to some classes of fungicide, however, which reduces the effectiveness of nursery disease management programs (Elad et al. 2007, James et al. 1982, Leroux 2007, Ogawa, et al. 1976, Stremeng et al. 2015).

Recently, several new reduced-risk fungicides and biopesticides that are potentially very effective against *Botrytis* have become available. Many of these products have been tested on a number of horticultural crops (Vea and Palmer 2017 and 2020). The identification of new fungicides and biopesticides that are effective in controlling *Botrytis* on conifer nursery stock would enable growers to integrate new classes of products into their disease management program to potentially improve the control of gray mold and reduce the buildup of fungicide-resistant strains of the pathogen in conifer seedling production systems. The objective of our study was to conduct an initial screening of fungicides and biopesticides for their ability to control gray mold on western hemlock seedlings.

Table 1. Products included in the *Botrytis* western hemlock trial (biopesticides are highlighted in bold).

Product and formulation	Percent active ingredient and common name	FRAC Code[1]
Affirm™ WDG	11.3% Polyoxin D zinc salt	19
Astun® (IKF-5411)	36% isofentamid	7
Botector®	*Aureobasidium pullulans* strains DSM 14940 + DSM 14941	NC
Broadform™ SC500	25% fluopyram + 25% trifloxystrobin	7 + 11
BW165N	*Ulocladium oudemansii* strain U3	NC
Chipco® 26019 F	50.0% iprodione	2
Cleary's 3336®	41.25% thiophanate-methyl	1
Daconil WeatherStik® SC	54% chlorothalonil	M5
Decree® 50WDG	50% fenhexamid	17
EcoSwing®	82% Extract of *Swinglea glutinosa*	P05
Empress®	23.3% pyraclostrobin	11
Fame™ SC	40.3% fluoxastrobin	11
Fore® 80WP	80% mancozeb	M3
Heritage®	50% azoxystrobin	11
MBI 110	96.4% *Bacillus amyloliquefaciens* strain F727	44
Medallion® WDG	50% fludioxonil	12
Mural® WDG	15% benzovindiflupyr + 30% azoxystrobin	7+11
Orkestra® Intrinsic	21.3% fluxapyroxad + 21.3% pyraclostrobin	7+11
OxiPhos®	17.7% phosphorus acid + 14% hydrogen peroxide	P07
Pageant® 38WG	25.2% boscalid + 12.8% pyraclostrobin	7 + 11
Palladium® 62.5WG	37.5% cyprodinil + 25% fludioxonil	9+12
Picatina™ Gold (A20808C)	7% pydiflumetofen + 9.3% azoxystrobin + 11.6% propiconazole	7+3+11
Proud 3®	5.6% Thyme oil	NC
Regalia®	5% Extract of *Reynoutria sachalinensis* (giant knotweed)	P05
Regime™ (F9110)	Extract of *Lupinus*	P
SP2480	Experimental	-
Spectro® 90WDG	78% chlorothalonil + 12% thiophane-methyl	M5+1
Triathlon®	98.85% *Bacillus amyloliquefaciens* strain D747	P
Trinity®	19.2% triticonazole	3
Tourney®	50% metconazole	3
ZeroTol® 2.0	27.1% hydrogen peroxide + 2.0% peroxyacetic acid	NC

[1] Fungicide Resistance Action Committee Code List 2019. http://www.frac.info/ (accessed May 2019)

NOTE: Some of the pesticides discussed in this paper were tested under an experimental use permit granted by WSDA. Application of a pesticide to a crop or site that is not on the label is a violation of pesticide law and may subject the applicator to civil penalties. It is your responsibility to check the label before using products to ensure lawful use and obtain all necessary permits in advance.

Methods

The study evaluated the effectiveness of 31 fungicide and biopesticide products (table 1) in controlling gray mold on 2-year-old western hemlock seedlings. Seedlings were initially grown in Superblock™ 112/95 Styroblocks® at a commercial container nursery for one year and then transplanted into D16H Deepot™ cells in Deepot™ D50 trays and maintained at Washington State University, Research and Extension Center (Puyallup, WA).

Product Applications

On October 1, 2019, each product was applied at a single rate except for BW 165N and SP2480 which were applied at two rates (table 2). Each treatment was applied to 10 seedlings with a handheld sprayer. The foliage on each seedling was sprayed until wet. Two sets of 10 seedlings were designated as untreated positive (inoculated) and negative (non-inoculated) controls and were sprayed with an equivalent amount of water alone.

Fungal Inoculation and Incubation

One day after fungicide application, all 10 seedlings from each treatment group and 10 untreated positive control seedlings were placed in separate 5-gal (19 L) buckets and the foliage was sprayed until wet with a suspension of *Botrytis cinerea* conidia (14.8 x 10^6 conidia per oz [5.0 x 10^5 conidia per ml]). The negative controls were sprayed with an equivalent amount of water alone. To ensure the foliage on the seedlings remained wet, 2 L of hot (125 to 136 °F [52 to 58 °C]) water was poured below the bases of the D16H Deepot™ cells in the bottom of each bucket and the inside of another bucket was sprayed with water, inverted over the top of the bottom bucket, and sealed with tape to create a moist incubation chamber. Seedlings were incubated in these chambers for 5 days at 68 to 72 °F (20 to 22 °C). After 5 days, the seedlings were removed and placed in Deepot™ D50 trays on benches in a greenhouse that was maintained at 59 to 68 °F (15 to 20 °C). Seedlings were overhead irrigated twice daily.

Disease Assessments

Disease symptoms on the shoot tips (figure 2) were assessed upon removal from the incubation chambers, which was 5 days post inoculation (5 dpi) and again

Table 2. Rates of products tested.

No.	Treatment	Product rate/100 gal
1	Non-inoculated check	-
2	Inoculated check	-
3	Affirm™	8 oz
4	Astun®	13.5 fl oz
5	Botector®	10 oz
6	Broadform™	8 fl oz
7	BW165N (3 lbs)	3 lbs
8	BW165N (4 lbs)	4 lbs
9	Chipco® 26019	16 fl oz
10	Cleary's 3336®	16 fl oz
11	Daconil WeatherStik®	2 3/4 pts
12	Decree®	1.5 lbs
13	Fore®	2 lbs
14	EcoSwing®	2 pts
15	Empress®	6 fl oz
16	Fame™ SC	8 oz
17	Heritage®	4 oz
18	MBI 110	6 qts
19	Medallion®	4 oz
20	Mural®	7 oz
21	Orkestra®	8 fl oz
22	OxiPhos®	1 gal
23	Pageant®	14 oz
24	Palladium®	6 oz
25	Picatina™ Gold	13.7 fl oz
26	Proud 3®	1 gal
27	Regalia®	1 gal
28	Regime™	45.7 fl oz
29	SP2480 (20 fl. oz.)	20 fl oz
30	SP2480 (30 fl. oz.)	30 fl oz
31	Spectro® 90	5.7 lbs
32	Triathlon®	6 qts
33	Trinity®	12 fl oz
34	Tourney®	4 oz
35	ZeroTol® 2.0	2 gal

Metric conversions: 1 oz. = 28.4 g; 1 lb = 453.6 g; 1 fl oz. = 29.57 ml; 1 gal = 3.8 L.

Figure 2. *Botrytis* shoot tip blight symptoms on western hemlock due to infection of the needles by Botrytis spores. (Photo by Gary Chastagner 2019)

29 dpi. On these dates, the incidence of diseased shoot tips was rated on a scale of 0 to 10 based on the percentage of tips that were blighted (0=none; 1=1 to 10 percent; 2=11 to 20 percent; 3=21 to 30 percent;… and, 10=91 to 100 percent). The severity of disease was also assessed 29 dpi to determine the percentage of shoots where disease had spread from the blighted tips down into the shoots while the plants were in the greenhouse (figure 3). Disease severity was rated on a scale of 0 to 10 (0=no spread, symptoms restricted to shoot tips; 1= spread into 1 to 10 percent; 2=spread into 11 to 20 percent; 3= spread into 21 to 30 percent;… and, 10=spread into 91 to 100 percent of the shoots) An overall disease level on seedlings at 29 dpi was calculated by multiplying the incidence rating by the severity rating, resulting in a disease index that ranged from 0 to 100.

Experimental Design and Statistical Analysis

Following incubation, seedlings were placed on greenhouse benches in a completely randomized design with one seedling per treatment in each of 10 blocks. Differences among treatment groups were analyzed with one-way ANOVA followed by Tukey's HSD test if results were significantly different at $p = 0.05$. All data analysis was done in R v. 4.0.01 (R Core Team 2020).

Results

Free moisture was observed on all seedlings when removed from the incubation chambers at 5 dpi,

Figure 3. *Botrytis* disease symptoms worsen after progression down the shoots of the branch. (Photo by Gary Chastagner 2019)

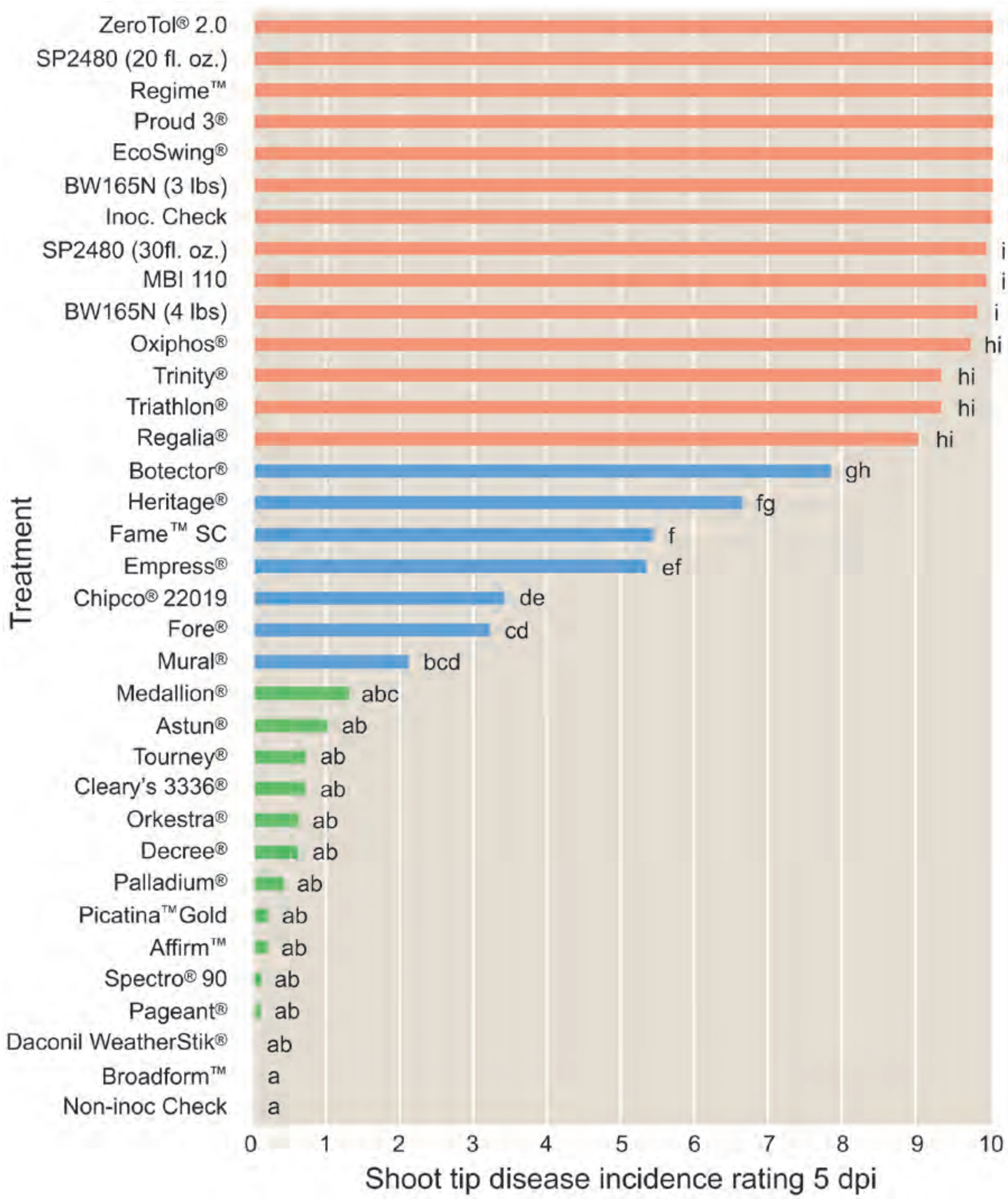

Figure 4. Shoot tip *Botrytis* disease incidence rating 5 days after inoculation. Columns followed by the same letter are not significantly different (p=0.05, ANOVA, Tukey test).

indicating that conditions were optimal for infection. Upon removal of the seedlings from the buckets, symptoms were restricted to blighted shoot tips. The inoculated positive controls had an average disease incidence rating of 10, while no disease was evident on the non-inoculated negative controls. Statistical analysis of the 5 and 29 dpi disease incidence ratings indicated that the treatment ratings fell into three groups (figures 4 and 5): those that were not significantly different from the inoculated check (red bars), those that were not significantly different than the non-inoculated checks (green bars), and those that had intermediate disease ratings (blue bars).

Overall, there was very little increase in the incidence of blighted shoot tips during the time seedlings were maintained in the greenhouse. The overall average disease incidence rating only increased from 5.0 at 5 dpi to 5.4 at 29 dpi. Blight severity,

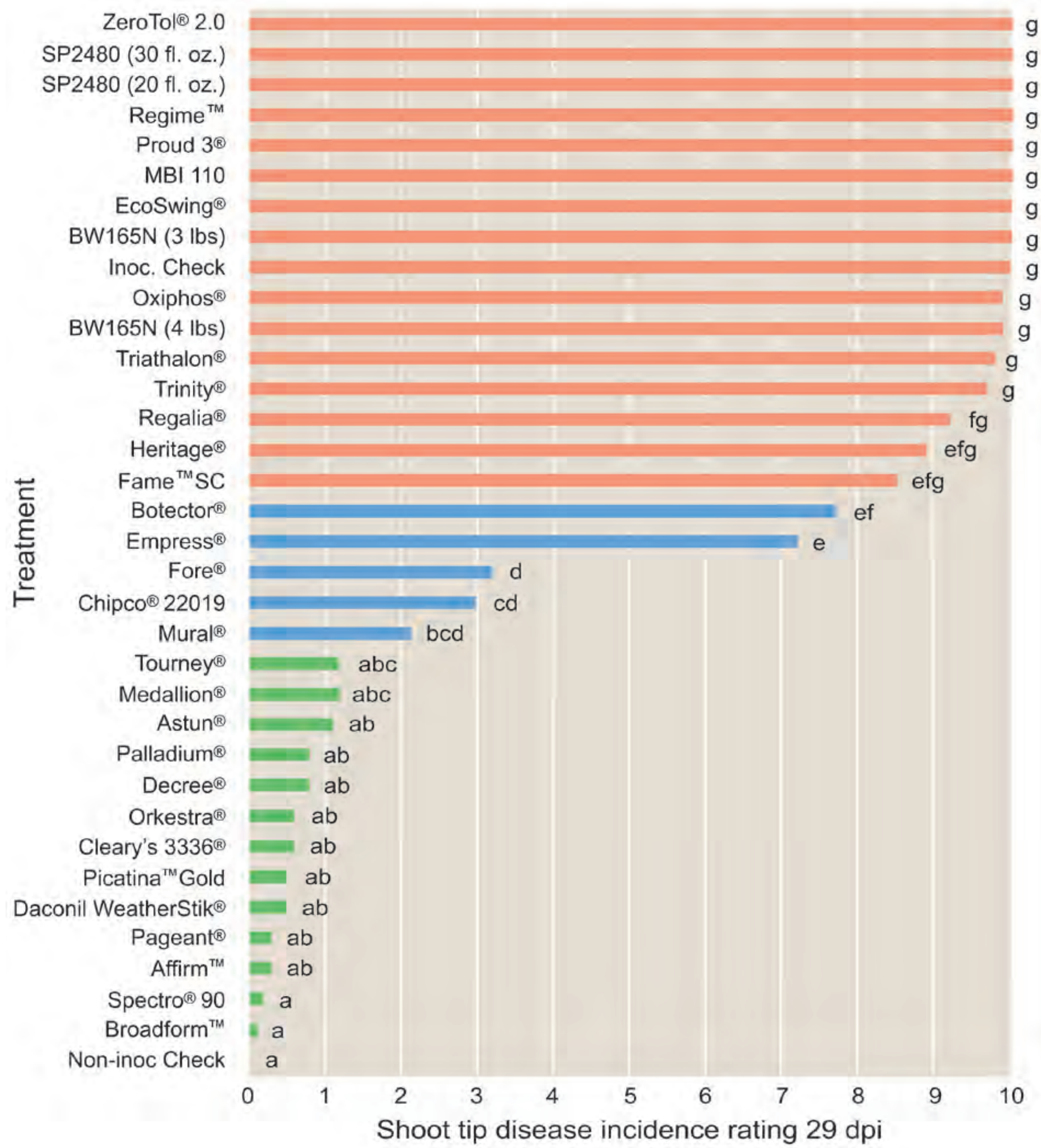

Figure 5. *Botrytis* shoot tip disease incidence rating 29 days after inoculation. Columns followed by the same letter are not significantly different (p=0.05, ANOVA, Tukey test).

however, increased on some seedlings as the disease spread down the shoots. Analysis of disease severity and disease index data at 29 dpi indicated that treatments fell into two groups (figures 6 and 7): those that were not significantly different from the inoculated checks (red bars) and those that were not significantly different from the non-inoculated checks (green bars), which continued to have no disease symptoms after nearly 1 month.

Discussion

Thirteen products (Broadform™, Spectro® 90, Affirm™, Pageant®, Daconil WeatherStik®, Picatina™ Gold, Cleary's 3336®, Orkestra®, Decree®, Palladium®, Astun®, Medallion®, and Tourney®) were very effective in reducing the incidence of blighted shoot tips. These products, along with Chipco® 26019, Botector®, Empress®, Fore® and Mural® also were

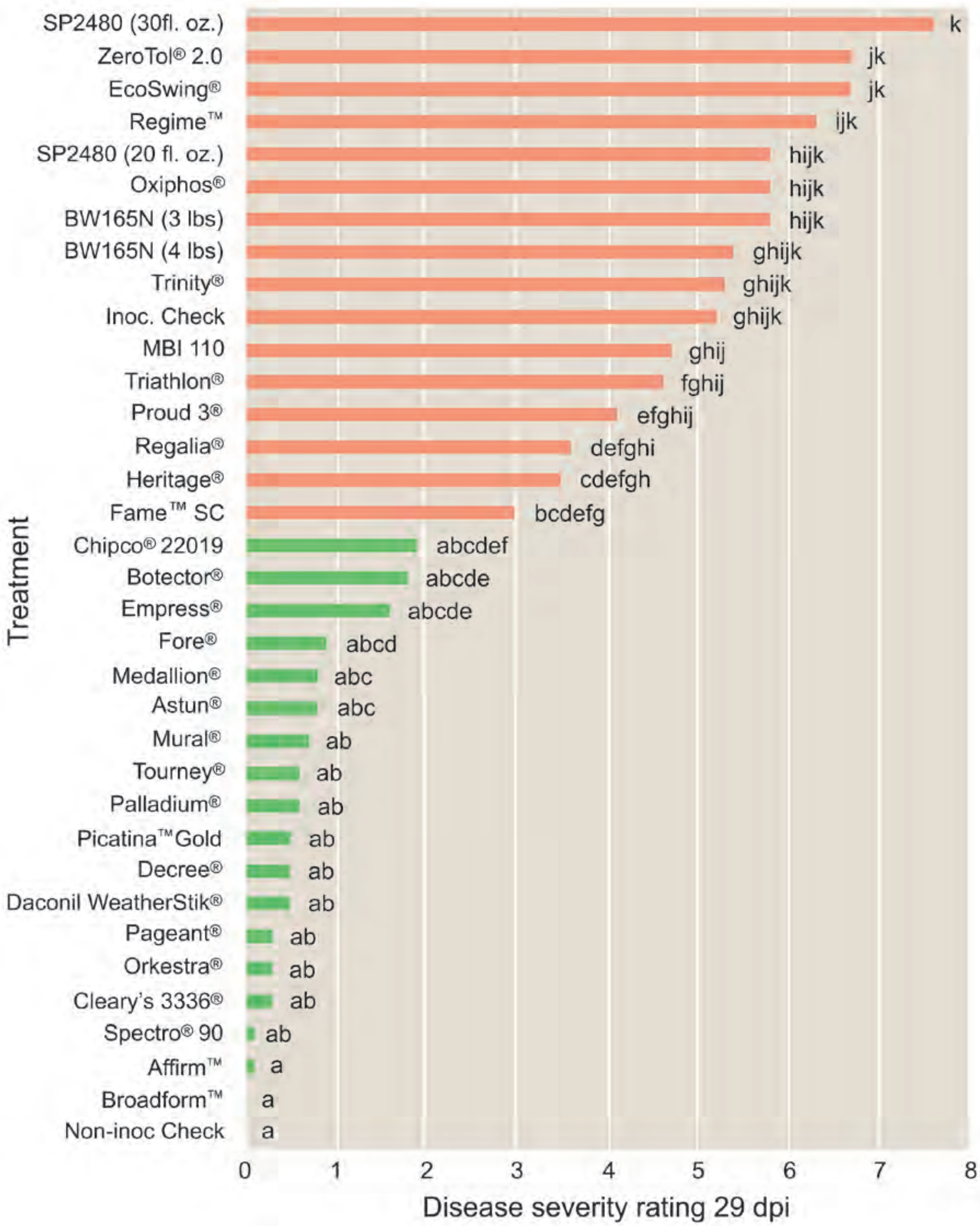

Figure 6. *Botrytis* disease severity rating 29 days after inoculation. Columns followed by the same letter are not significantly different (p=0.05, ANOVA, Tukey test).

the most effective products in reducing the disease severity and disease index ratings at 29 dpi. These products consist of a mix of standard fungicides used to control *Botrytis* on conifers and several newer products shown to provide good control of *Botrytis* on a number of ornamental crops (James et al. 1982, James and Woo 1984, Lanthier and Watts 2020, McCain and Smith 1978, Vea and Palmer 2017 and 2020). Although Capieau et al. (2004) demonstrated that applications of *Streptomyces*, *Trichoderma*, and *Gliocladium*-based biopesticides were potentially as effective as standard fungicides in controlling *Botrytis* on Scots pine (*Pinus sylvestris* L.) seedlings, none of the biopesticides and oxidizers (ZeroTol® and

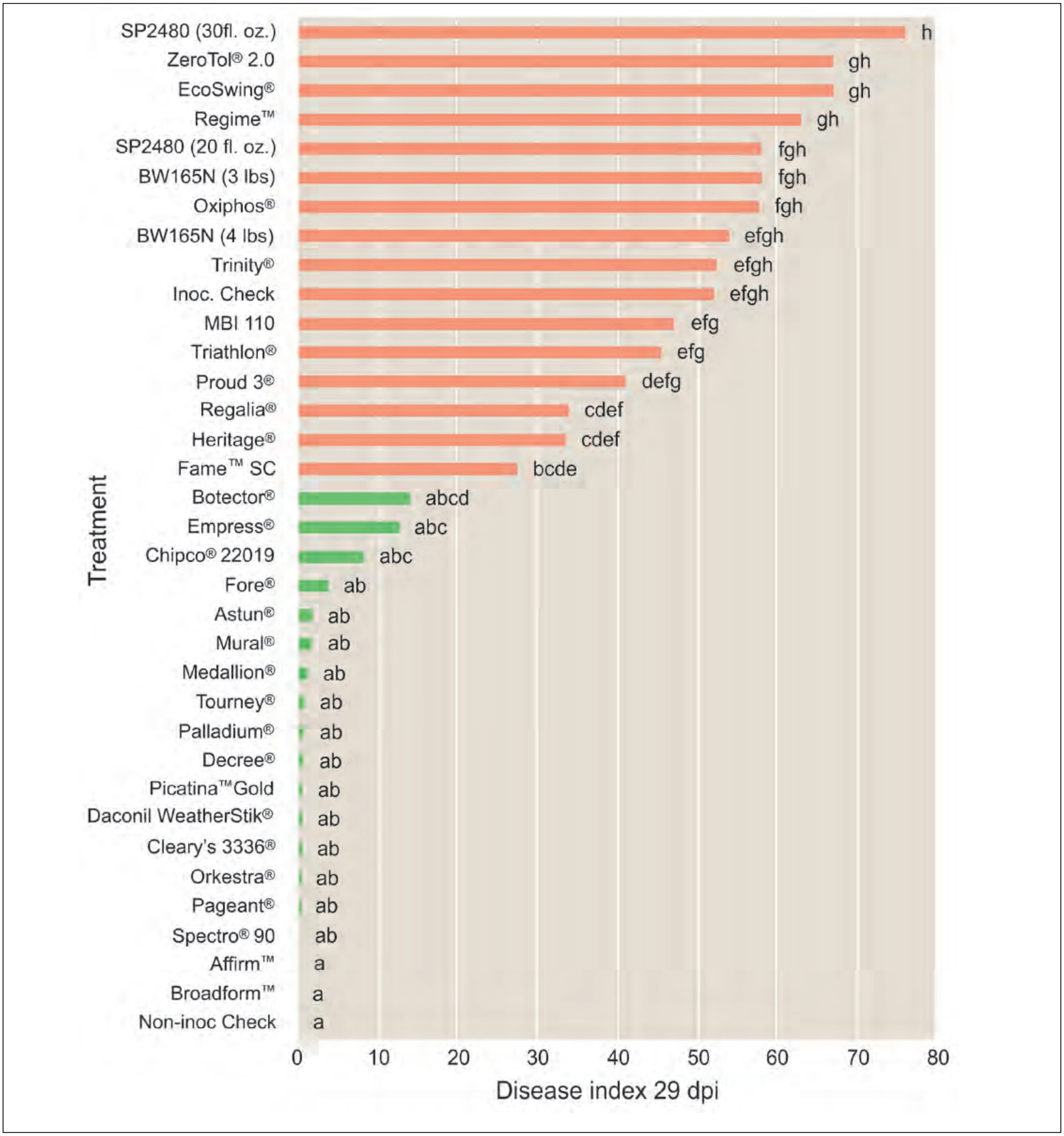

Figure 7. *Botrytis* disease index rating 29 days after inoculation. Columns followed by the same letter are not significantly different (p=0.05, ANOVA, Tukey test).

OxiPhos®) tested except Botector® were effective in reducing the incidence and severity of disease under our test conditions. Many of these products were also ineffective in controlling *Botrytis* development on a number of other ornamental hosts (Vea and Palmer 2017 and 2020).

The methods used in this trial were designed to efficiently screen a large number of products for their potential efficacy in controlling gray mold. Although several potentially effective products in controlling gray mold on western hemlock seedlings were identified, additional research is needed to determine optimal application rates and application timing for these products to maximize disease control on a broad range of conifer hosts under nursery production conditions. The effectiveness of these products under production conditions is likely to be affected by disease pressure; application methods, coverage, and timing; residual activity; and mix of products used. The potential adverse effects of products on seedlings also needs to be determined (James and Woo 1984).

Ultimately, an integrated approach that includes cultural practices, such as sanitation, promoting good air circulation, and reducing humidity in addition to the application of fungicides will increase the effectiveness of gray mold disease management programs (Dumroese and Haase 2018, Haase and Taylor 2012, Lilja et al. 2010, Pscheidt and Ocamb 2020). Fungicide resistance is a major problem in *Botrytis* disease management programs, particularly when products within high-risk FRAC (Fungicide Resistance Action Committee; https://www.frac.info/) groups, such as benzimidazoles and thiophanates (FRAC group 1), dicarboximides (FRAC group 2) and strobilurins (FRAC group 11), are applied multiple times during a growing season. Resistance to some high-risk fungicides such as benomyl has been detected in nurseries within a few years of introduction into nursery disease management programs (Gillman and James 1980). Rotating products with different FRAC codes or using products that contain a mixture of active ingredients with multiple FRAC codes is an important strategy to minimize the risk of fungicide resistance problems (Elad et al. 2007, Leroux 2007, Ogawa, et al. 1976). The highly effective products identified in our tests belong to nine unique FRAC groups or were mixtures of active ingredients with more than one FRAC code. If registered, these products would provide growers with multiple options to reduce the risk of fungicide resistance limiting the effectiveness of their gray mold disease management programs.

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Can Treatments at Planting Improve Noble Fir Seedling Survival?

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Abstract

Noble fir (*Abies procera* Rehd.) plantings for Christmas tree production have experienced poor to variable survival over the past decade. With little ability to provide supplemental irrigation, Christmas tree growers investigated first-year seedling survival results in response to a variety of pre- and post-planting treatments, root dips, and foliar sprays. In three separate trials, wood chip mulch or shade screens increased survival (though not consistently) in moderately dry summers in Oregon. None of the dips, sprays, or amendments, however, resulted in significant survival improvements.

Introduction

Over the past decade, Christmas tree growers have experienced significant seedling mortality in noble fir (*Abies procera* Rehd.) plantings due to prolonged summer droughts in the Pacific Northwest (PNW). Most Christmas tree plantings, like forest plantings, receive no supplemental watering. Of the commonly planted Christmas tree species in the PNW, noble fir is the species most planted and most affected by summer drought.

Because of the increased mortality, Christmas tree growers have experimented with providing supplemental water to boost seedling survival, especially in the year of planting. Since irrigation systems are absent at most sites, however, the distribution and application of water is expensive and near impossible on remote sites.

In prior studies (Landgren 2012) ectomycorrhizae and GeoHumus® additions at planting provided no growth improvement of planted noble fir seedlings. In the same study, Rootex™ root dip provided a modest growth improvement and shade produced the largest benefit. Cregg et al. (2009) showed improved growth and survival in Christmas tree plantings in Michigan using wood chips as mulch.

The objective of this 2-year project was to investigate a range of products and treatments that were deemed promising by growers for improving first-year seedling survival.

Materials and Methods

Seedlings and Sites

Two-year-old noble fir container seedlings (15 in^3 [245 cm^3]) were machine planted in May 2018 and 2019. All noble fir sources were from Oregon coastal mountain collections.

Studies were established on two successful second-rotation noble fir commercial Christmas tree plantations near Molalla, OR. In 2018, seedlings were planted on the Kirk site (figure 1) and in 2019, seedlings were planted on both the Kirk site and the Christmas Tree Business Mexico (CBM) (figure 2) site. At both sites, seedlings were planted in bare, weed-free soil. At both sites, post-planting weed control consisted of one application of Atrazine applied 2 days after planting. The soil type at the Kirk site is a Jory silty clay loam and at the CBM site, the soil is a Molalla cobbly loam.

Treatments and Measurements

With the exception of shade and wood mulch, all treatments were selected by the grower at each site and the study was established to meet their desire for an objective review of results. Treatments included a variety of root dips, foliar sprays, and planting amendments, in addition to a nontreated control,

Figure 1. The 2018 Kirk site trial on noble fir seedling with shade screens installed. (Photo by Judith Kowalski 2018)

shade, and mulch (figure 3). Treatment details and application methodologies for each site are summarized in tables 1, 2, and 3.

Initial planting survival at both sites in both years was above 99 percent as evaluated 2 weeks following planting. At the end of the growing season (October), survival was recorded for seedlings in all treatments. Seedlings were considered dead if all needles were red and all buds desiccated. Evaluations were conducted after the fall rains had completely saturated the rooting profile.

Experimental Design and Statistics

The project was conducted as a series of three trials: Kirk 2018, CBM 2019, and Kirk 2019.

Figure 2. The CBM site during planting of noble fir trees for the 2019 trial. (Photo by Judith Kowalski 2019)

Table 1. Treatments applied to noble tree seedlings in the 2018 trials at the Kirk site. Treatments were applied in a factorial combination of 2 shade treatments (with or without), 3 pre-planting dip treatments (Rootex™, water, or none), and 3 foliar treatments (Moisture-Loc™ applied once, twice, or none).

Treatment	Product	Application methodology
Shade	Mesh shade of non-toxic polyolefin and wire wicket support, (Terra Tech, LLC, Eugene, OR)	Placed on SW side of tree Installed at time of planting
Root dip: Water	n/a	Pre-planting dip
Root dip: Rootex™	7:47:6 (NH_4: P_2O_5:K_2O) with 40 percent inerts (Redox Ag, West Burley, ID)	1 lb/5 gal water (0.45 kg/18.9 L water) pre-planting dip
Foliar spray: Moisture-Loc™	Vinyl acetate polymer antitranspirant (Zorro Technology, Clackamas, OR)	10-percent solution applied once at budbreak and a second 6 weeks post budbreak application given to a sub-set

Table 2. Treatments applied to noble tree seedlings in the 2019 trials at the Kirk site. Treatments were applied in a factorial combination of 2 shade treatments (with or without), 2 mulch treatments (with or without), and 2 root dip treatments.

Treatment	Product	Application methodology
Shade	Non-toxic polyolefin mesh with wire wicket support (Terra Tech, LLC, Eugene, OR)	Placed on SW side of tree
Mulch	Mixed wood chips from local tree service	Applied to a depth of 2 in (5 cm) with a 12-in (30-cm) radius
Root dip: Stomaboost™ Supreme 7-17-4 + Dynahume™ SW 0-0-1	7:17:4 (N: P_2O_5:K_2O) with 0.003 microbial inoculum extracts (Schaeffer's Crop Enhancements, St. Louis, MO) + 1 percent K_2O, 10 percent humic acid, and seaweed extracts (Schaeffer's Crop Enhancements, St. Louis, MO)	Pre-planting dip 14-percent solution Stomaboost™ + 23-percent solution Dynahume™

Table 3. Treatments applied to noble tree seedlings in 2019 at the CBM site. Treatments were applied in a factorial combination of 2 shade treatments (with or without), 2 mulch treatments (with or without), and 2 root dip treatments (with or without), and 2 amendment treatments (with or without).

Treatment	Product	Application methodology
Shade	Non-toxic polyolefin mesh with wire wicket support (Terra Tech, LLC, Eugene, OR)	Placed on SW side of tree
Mulch	Mixed wood chips from local tree service	Applied to a depth of 2 in (5 cm) with a 12-in (30-cm) radius
Root dip: Vitamin B-1 Transplant Solution 0-2-0	Two percent K_2O with 0.1 percent iron (Fe) and 0.1 percent thiamine mononitrate (B-1) (Liquinox Company, Orange, CA)	14-percent solution Pre-planting dip
Amendment: Solid Rain®	Potassium polyacrylate (Lluvia Sólida, Santiago de Querétaro, Mexico)	1.5 to 2.0 g (0.05 to 0.07 oz) into planting hole

In the Kirk 2018 trial, the experimental design was a 3 x 3 x 2 factorial combination of pre-planting root dips (Rootex™, water, or none), antitranspirant foliar spray (Moisture-Loc™ applied once [July 18; 1X], twice [June 6 and July 18; 2X], or none), and shade (with or without) (table 1). A total of 1,264 trees were evaluated in the Kirk 2018 trial. Due to logistical limitations Moisture-Loc™ treatments (1X or 2X) were applied to 250 trees each and 300 trees were shaded. Thus, treatment assignment resulted in a variable number of seedlings in each treatment combinations with a minimum of 20 per combination.

For the Kirk 2019 trial, the study design was a 2 x 2 x 2 factorial combination of root dip (with or without Stomaboost™ + Dynahume™), shade (with or without), and mulch (with or without) (table 2). A total of 1,120 seedlings were evaluated in the Kirk 2019 trial. Seedlings were evenly divided between root dip treatments; 280 seedlings were shaded, and 130 seedlings were mulched with a minimum of 30 seedlings per treatment combination.

At the CBM 2019 trial, the experimental design was a 2 x 2 x 2 x 2 factorial combination of Vitamin B-1 root dip (with or without), Solid Rain® amendment (with or without), shade (with or without), and mulch (with or without) (table 3). A total of 840 seedlings were evaluated in the CBM 2019 trial. Seedlings were evenly divided among Vitamin B-1 root dip and Solid Rain® combinations, 300 seedlings were shaded, and 175 seedlings were mulched, with at least 20 seedlings in each treatment combination (n>=20).

Data for each experiment were analyzed by analysis of variance as a 3-way or 4-way factorial in a completely randomized design. Analyses were conducted using PROC GLM procedure in SAS (SAS Institute, Inc., Cary, NC) assuming all independent

Figure 3. This noble fir seedling at the Kirk site was treated with a combination of shade and mulch (Photo by Judith Kowalski 2019)

variables as fixed factors. For each experiment, usually only one or two main effects or two-way interactions effects were significant. Therefore, we focus our graphical summaries on those significant in each experiment.

Results

In the 2018 trial at the Kirk site, the shade treatment significantly increased overall survival ($P<0.0001$) from 64 percent without shade to 88 percent with shade (figure 4). Root dip and foliar spray treatments did not have a consistent effect on seedling survival. Mulch was not included in 2018.

In the 2019 trial at the Kirk site, average survival was 80.4 percent for seedlings in the no mulch + no shade treatments. For seedlings that were not shaded, mulch increased ($p \leq 0.05$) survival to 97.3 percent. Mulch provided less benefit when seedlings were shaded as survival increased from 88.4 to 94.7 percent (figure 5). The addition of Dynahume™ and Stomaboost™ did not affect survival ($p > 0.05$).

In the 2019 trial at the CBM site, application of Solid Rain® increased seedling survival from 91.0 percent to 98.6 percent in the absence of B-1 ($p \leq 0.05$; figure 6). Solid Rain® did not affect survival, however, when B-1 was applied ($p > 0.05$). Neither shade nor mulch affected seedling survival at the CBM site.

Discussion

The 2018 and 2019 years generally had more summer rainfall and better tree survival than was experienced in PNW Christmas tree plantations from 2015 to 2017. For example, average survival of noble fir in 2016 was only 30 to 50 percent, depending on site.

Despite testing numerous combinations of root dips, foliar treatments, and planting amendments, none of those used in these trials consistently improved survival. These finding are generally consistent with other trials. Landis (2006) showed variable and negative results with a range of root dips on a variety of bare root species. Bates et al. (2004) showed no improved survival when comparing root dips with water alone on four species of Christmas trees. Starkey et al. (2012) found that seedling survival was highly variable depending on the particle size and composition of the polymer dip, and, in some cases, actually increased seedling mortality. New products claiming the ability to improve survival regularly show up in the market. Our advice is to always test before investing at an operational scale. Often, products do not live up to their claims.

Our results show that wood mulch and, to a lesser extent, shade screens can improve noble fir seedling survival. Improvements in tree survival and growth have frequently been associated with mulch (Adams 1997, Johansson et al. 2005). Mulch can benefit newly planted seedlings by controlling weeds (Bartley et al. 2017, Saha et al. 2020) and improving soil moisture availability (Pardos et al. 2015). Mulching around seedlings can also moderate soil temperatures (Cregg et al. 2009) and increase soil organic matter (Flint 1987). Shade screens have been used

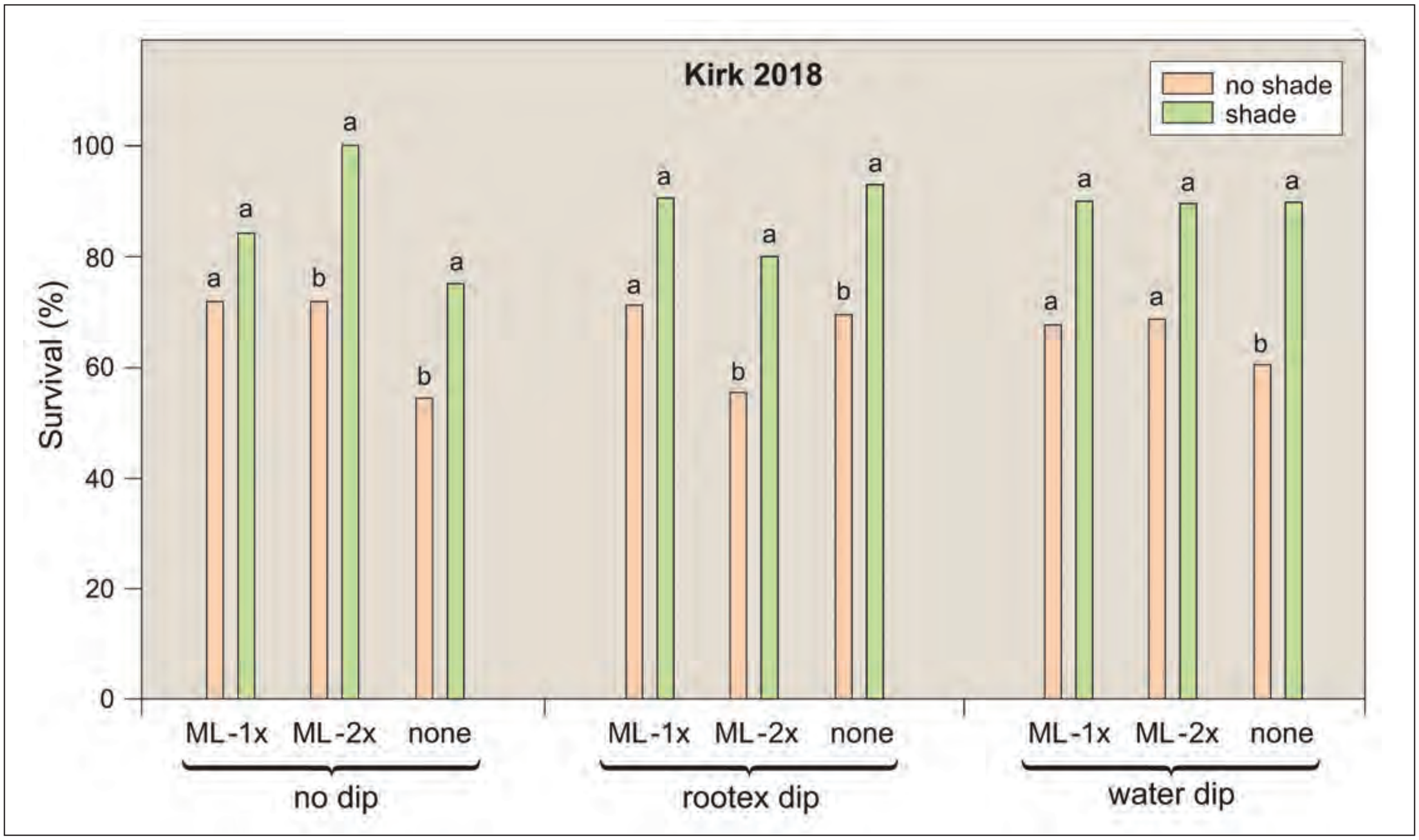

Figure 4. Mean survival of noble fir seedlings at the Kirk 2018 trial in response to root dip treatments (Rootex™, water, or no dip), applications of moisture-Loc™ (ML), and shade screens. Means within a pair of bars with the same letter are not different at P<0.05.

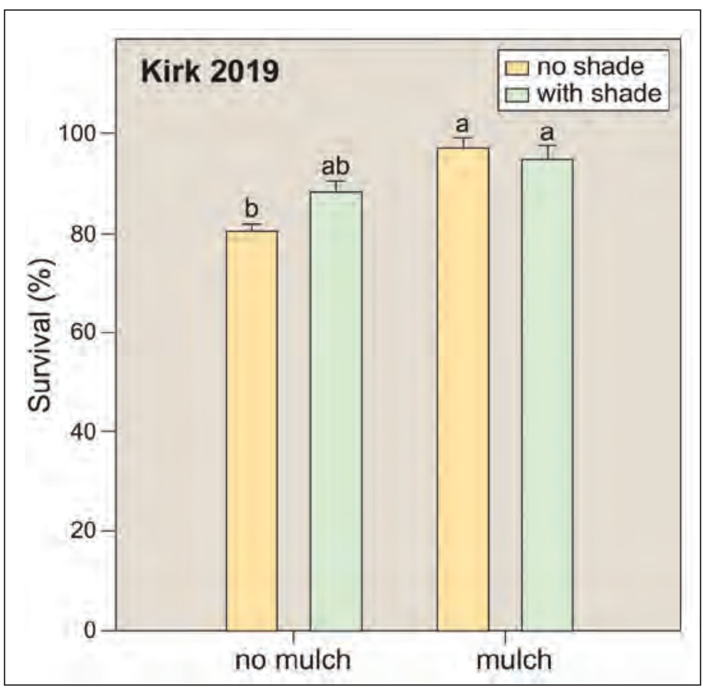

Figure 5. Mean survival of noble fir seedlings at the Kirk 2019 trial in response to mulch application and shade screens. Bars with the same letter are not different at P<0.05.

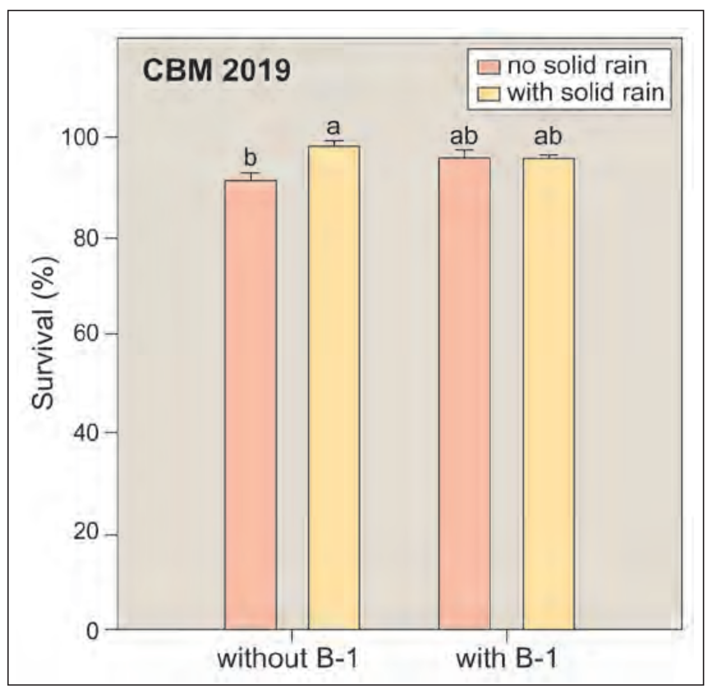

Figure 6. Mean survival of noble fir seedlings at the CBM 2019 trial in response to root treatment (dip with Vitamin B-1) and application of potassium polyacrylate gel (Solid Rain®). Bars with the same letter are not different at P <0.05.

Table 4. Cost benefit analysis for application of shade or mulch treatments based on 1,200 trees per acre.

Treatment	Material cost ($USD)	Installation cost ($USD)	Approx. total cost ($USD)	Potential savings ($USD)[2]
Shade	126[1]	30	156	180
Mulch	0-311[3]	30	30-341	0-306

[1]Unit Cost = $0.42 each (4-year useful life)
[2]Assumed 14-percent increased survival for each treatment (168 trees at $2 per tree= $336)
[3] Assumes 23 yd^3 (17.6 m^3)

to improve seedling survival on harsh sites in forest plantings for decades (Peterson 1982, Adams et al. 1966, Helgerson 1989). Shade can lower temperature and reduce moisture loss. Neither mulch nor shade, however, are substitutes for adequate soil moisture, especially in coarse soils.

Cost Analysis

Both mulch and shade incur installation and material costs. The shade screens and associated wire wickets cost approximately $0.42 each (TerraTech 2020) and should last at least 4 years. The mulch requires about 23 yd^3/ac (44 m^3/ha) for application of 2 in [5 cm] of mulch in a 12-in [30-cm] radius around 1,200 trees (Cregg 2020). Mulch cost varies significantly. In some cases, it can be very low cost (or even free) from local arborists, but in other cases, it may need to be purchased.

A cursory financial analysis of these treatments summarizes the costs and potential savings benefits (table 4). These rough estimates are subject to many unknowns. One never knows the fate of planted trees in advance. In a year with favorable conditions and excellent seedling quality, shade or mulch treatments would be a waste of money. In other years, a 14-percent increase in survival, which is achievable with shade or low-cost wood chips, would be "worth" about $336/ac ($830/ha). With even higher increases in survival such as the 24-percent improvement on the Kirk site, the cost benefits would be even more attractive.

It's important to note that the fields used in these experiments were weed free at planting and weed competition was partially mitigated by post-planting herbicide applications. These weed control measures likely contributed to seedling survival and moisture preservation as noted by Cregg et al. (2009).

This study only reviewed first-year survival. Future studies, particularly of the mulch/wood chip option, could include an assessments of seedling growth during a rotation and subsequent weed control benefits (or not) from mulch.

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Forest Restoration on Degraded Soils in Yap, Federated States of Micronesia

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Abstract

Reforestation of sites with acidic, highly leached soils has been a problem in the tropics worldwide, and results of fertilization have been varied. We applied both fertilizer and lime to a new plantation of *Acacia auriculiformis* A. Cunn. ex Benth. on an Oxisol on the island of Yap in the Federated States of Micronesia. Fertilized trees had 46 percent more height increment than control trees over a 2.4-year period, but there was no effect of lime application. A. auriculiformis is well known for being tolerant of soil acidity. Our results emphasize the value of fertilization during establishment and early growth for trees planted on leached, acidic tropical soils.

Introduction

Restoration of forest cover on deforested sites in the wet tropics can be challenging. Once native forest is cleared, the litter layer and topsoil may be lost due to the exceptionally fast oxidation rates that reduce organic matter at the surface of exposed tropical soils. Frequent fires in abandoned pastures or agricultural lands cause additional reduction of organic matter and can lead to erosion and further soil degradation (figure 1). Highly weathered Oxisols and Ultisols, typically found in tropical areas, are usually acidic and low in nutrients. These soils have high levels of phosphorus (P) fixation and may have high levels of toxic aluminum (Al) or manganese

Figure 1. Badly degraded, acidic soils pose a challenge for reforestation in Yap, Federated States of Micronesia. (Photo by James B. Friday 2015)

(Mn), which is limiting to tree growth (Marcar and Khanna 1997, Scowcroft and Silva 2005). Fertilization of tree plantations is a standard practice in some tropical countries, especially where soils are Oxisols or Ultisols (Gonçalves et al. 1997) or Andisols (Cannon 1983). Unless foresters fertilize trees properly at planting, reforestation projects may never develop well.

We report here on a fertilizer trial for a reforestation project on the island of Yap in the Federated States of Micronesia. Significant areas of Yap have been deforested but are no longer used for agriculture (Falanruw 1992, Mueller-Dombois and Fosberg 1998). These areas burn frequently and harbor invasive plants. Restoration of these deforested areas has long been a priority for Pacific Island foresters (DeBell and Whitesell 1993). Yap Forestry is working to reforest these areas for small-scale wood production, reduce soil erosion, stabilize watersheds, establish fuel breaks, reduce the frequency of grass fires, and shade out invasive grasses, particularly *Imperata cylindrica* (L.) P. Beauv. (figure 2).

Several tree species have been planted in trials in Yap and some of the best performing have been the Australian natives *Acacia auriculiformis* A. Cunn. ex Benth. and *A. mangium* Willd. (DeBell and Whitesell 1993). Foresters have favored these trees because of their ability to survive on poor sites, including on moderately acid soils, and their rapid growth on good sites (figure 3). The thick canopies formed by these species can shade out invasive plants in the understory. These species are not native to the Pacific islands and can be considered invasive in favorable environments. In some locations on Yap, however, growth of these trees has been slow, likely hampered by low pH, Al toxicity, and a lack of nutrients (figure 4). Exceptionally slow tree establishment is risky because their ability to recycle nutrients is impaired and they have low resistance to fungi, insects, and fire. As a consequence of these failings, the benefits associated with established trees are not fully realized.

Growth rate of trees depends greatly on soil fertility. As such, fertilizer experiments are important to determine tree responses to varying types and rates of supplemental nutrients applied to the soil. Cole et al. (1996) studied growth of seedlings of 12 species of Acacia, including *A. auriculiformis*, *A. mangium*, and *A. koa* A. Gray on an acidic Ultisol in Hawaii. They found that 8 of the 12 species responded to post-planting applications of P and potassium (K) but the other species did not. Earnshaw et al. (2016) studied fertilization of plantations of *A. koa* on two acidic Andisols in Hawaii and found that fertilization increased seedling height from 31 percent to 49 percent on one soil, which was on a cooler, rockier site, but not on the other. They also found similar gains in growth with seedlings receiving lower amounts of nutrients applied as slow-release fertilizer as seedlings receiving higher amounts of nutrients applied as soluble fertilizers. Amelioration of soil acidity by application of agricultural lime can increase cation exchange capacity and nutrient supply and decrease Al and Mn soil toxicities (Uchida and Hue 2000). Liming also supplies calcium (Ca) (and magnesium [Mg] if dolomite lime is

Figure 2. A 3-year-old *Acacia auriculiformis* survived one of many brush fires in Yap, Federated States of Micronesia. (Photo by James B. Friday 2014)

Figure 3. Good growth of 14-year-old *Acacia auriculiformis* trees planted on a site with relatively good soil conditions in Yap, Federated States of Micronesia. (Photo by James B. Friday 2014)

Figure 4. Poor growth of 4-year-old *Acacia auriculiformis* trees planted on a site with acidic soils on Yap, Federated States of Micronesia. (Photo by James B. Friday 2014)

used), both of which can be limiting nutrients (Fisher and Binkley 2000). However, liming is seldom used for forestry projects because of the expense of application, which is usually tons per acre, and the difficulty of tilling the lime into the soil.

The objective of our study was to determine whether application of slow-release fertilizer or lime at rates commonly applied during afforestation could help increase *Acacia auriculiformis* seedling growth.

Material and Methods

Site

The study was conducted on a recently planted site with homogeneous topography. The plantation had just one planted species (*Acacia auriculiformis*), planted one month earlier (February 2017) and was located near Graveyard 47 (close to the Yap International Airport, N 9° 29' 38.29 E 138° 5' 2.9). The soil is in the Gagil series, a fine, sesquic, isohyperthermic typic haploperox, typically with a strongly acid pH of 5.2 in the surface 4 in (10 cm) (NRCS 2020). The parent material is a breccia and topography is quite level.

The site had previously been cleared for agriculture but had since been abandoned. Annual precipitation averaged 117 in (2,970 mm) during the experiment (2017 through 2019; NOAA 2020). Yap has a short, but not very distinct dry season from February through April, although rainfall still exceeded evapotranspiration rates for each month during the experiment. The understory of the plantation consists mainly of the sprawling native fern *Dicranopteris linearis* (Burm.) Underw., which is generally regarded as an indicator of poor-quality, acid soils. Understory vegetation was manually controlled at planting and annually ahead of measurements.

Treatments

Trees were fertilized with a fertilizer and quicklime (CaO), both of which were purchased at the local hardware store. The fertilizer was a coated, slow-release formulation (Osmocote® 13-13-13; $N:P_2O_5:K_2O$). Actual composition of the fertilizer was 13 percent N (nitrogen), 5.7 percent P, and 10.8 percent K. The fertilizer was formulated for a gradual 7-month release, but in the warm, tropical environment of Yap, nutrient release was likely faster.

Four blocks (replications) were designated in the plantation test site, each consisting of 3 rows of 8 *Acacia auriculiformis* seedlings that had been planted 1 month previously. Each row in each block was randomly assigned to receive one of the following treatments:

1) Control (no fertilizer or lime applied)

2) Fertilizer-only (1.8 oz [50 g] per seedling)

3) Fertilizer plus lime (1.8 oz [50 g] of fertilizer and 3.5 oz [100 g] of lime)

Treatments were applied on March 23, 2017. Fertilizer was applied by splitting the dosage between two shallow holes 6 in (15 cm) from each seedling designated for fertilizer. After placement in the hole, the fertilizer was covered with 2 in (5 cm) of soil to prevent N volatilization. Lime was applied by spreading the dose evenly on the soil surface within a 24-in (60-cm) radius of the seedling stem.

Measurements and Statistical Analyses

All seedlings in the experiment were measured for height at the time of planting and again on September 29, 2017; February 1, 2018; August 13, 2018; and August 8, 2019. Any dead or missing seedlings were noted.

Data were subjected to a Shapiro-Wilke normality test and a Levene test for homogeneity of variance—both confirming that the data satisfied necessary assumptions for analysis of variance (ANOVA). ANOVA was then used to analyze differences in height increase among the three treatments over time. Eta-squared statistics were calculated from the ANOVA model to evaluate the size of any treatment effects. A t-test was subsequently used to assess whether the fertilizer and the fertilizer-plus-lime treatments differed non-randomly in their effects. The analyses were conducted using R (R Core Team 2020).

Results

Survival was high, with 95 percent of the trees surviving after 2.4 years. Tree height increased roughly linearly during the experiment for all treatments (figure 5). There was strong evidence of a treatment effect ($p < 0.001$) on height increase, with seedlings that received the fertilizer or fertilizer-plus-lime treatments growing 64 in (153 cm) more on average than unfertilized seedlings, a 46-percent increase (figures 6 and 7). The effect was not especially strong (treatment eta-squared = 0.26), indicating that only about a quarter of the variability in height was due to the treatments. Height increment did not differ significantly between the fertilizer and fertilizer-plus-lime treatments ($p=0.49$).

Discussion

Fertilizer Response

This simple experiment set up on one site in Yap clearly showed a growth response of *Acacia auriculiformis* to fertilizer application. An effect of this magnitude would typically be considered meaningful in forestry, but the plantation purpose and other factors need to be considered to justify the expense and effort of applying fertilizer. For the purposes of just having forest cover, it might not be worth it. If it is important to enable trees to grow faster to avoid the need for weed control, to provide an effective shaded fuel break, or to grow forest products, then this additional growth may be quite important. Other forest plantations on Yap have been burnt by wildfires before canopy closure could shade out understory grasses.

We did not investigate the effect of individual nutrients on tree growth; some future investigation might be useful. Although *Acacia* spp. have the ability to fix atmospheric N, this ability is strongly influenced by the supply of available P (Binkley et al. 2003)—an important factor to account for when considering this species. Leguminous trees growing in acid soils are often observed to nodulate poorly, with growth of the nitrogen-fixing rhizobium severely limited by the acidity (Marcar and Khanna 1997). We suspect that the fertilized trees in our study benefited from both the N and P in the fertilizer. Manubag et al. (1995) found responses to both N and P fertilization with the closely related species *Acacia mangium* in a strongly acid soil in the Philippines. Earnshaw et al. (2016) and Idol et al. (2017) found increases in height growth of *A. koa* with P fertilization on slightly acid to strongly acid soils in Hawaii. Working on similar soils to the Yap site on the neighboring island of Palau, Dendy et al. (2015) found that fertilization with a complete NPK fertilizer increased the rate of expansion of native forest patches into degraded savanna and increased fruit, flower, and leaf production of several native tree species. While N and K are likely leached quickly from the site if not taken up by plants, P fertilizers can have longer term impacts. Meason et al. (2009) found elevated levels of P 3 years after fertilization of an *A. koa* stand on an Andisol in Hawaii. Future tests of fertilizers containing differing combinations of N, P, and K and at varying application rates are warranted.

Lime Response

The lack of response to applying limestone in conjunction with the fertilizer is somewhat puzzling. Typically, lime is incorporated into the soil rather than simply topdressed as we did in this study. Incorporating lime into

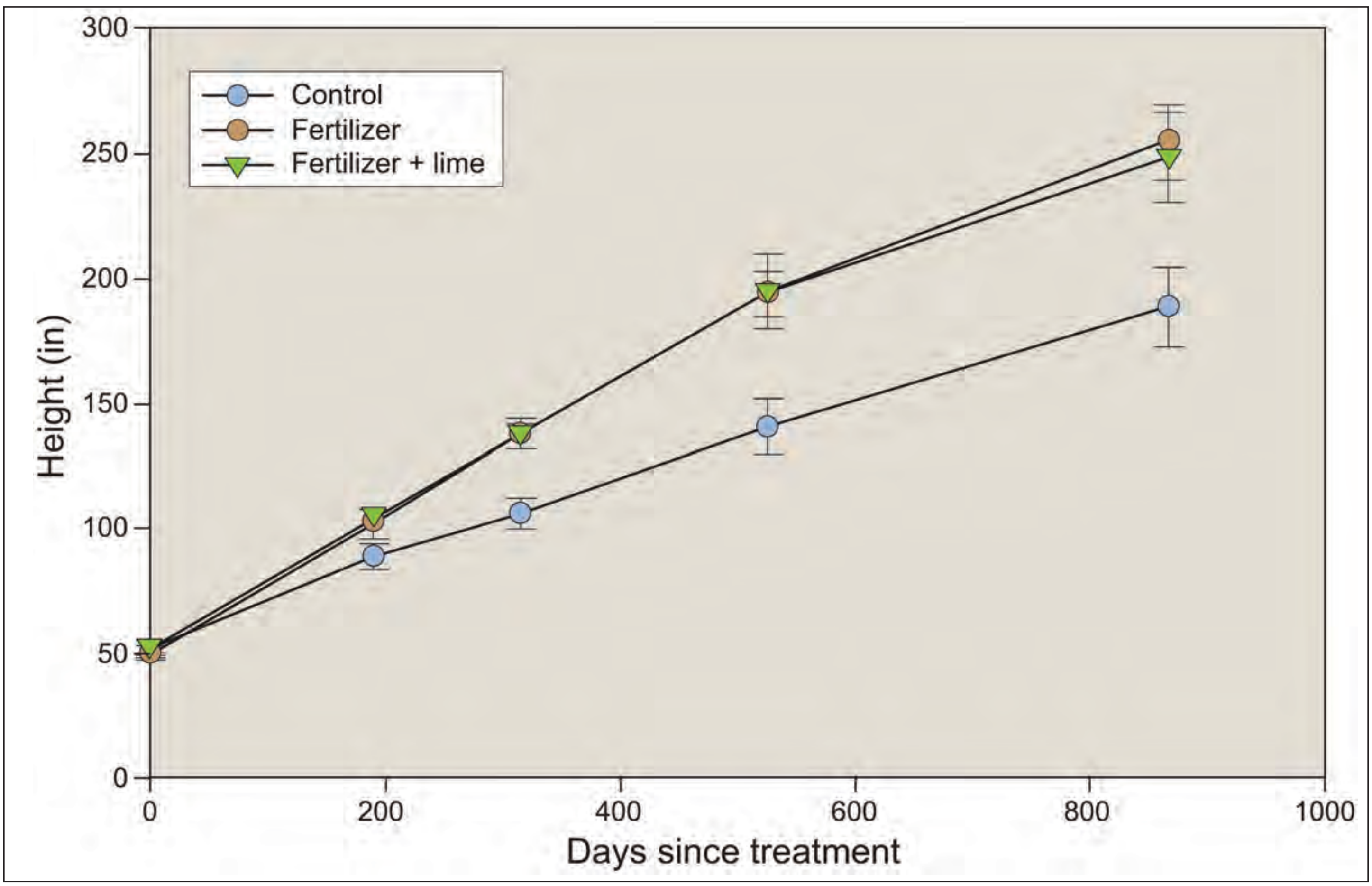

Figure 5. The effect of three fertilizer treatments on height growth of *Acacia auriculiformis* in Yap Federated States of Micronesia. Error bars represent standard errors.

Figure 6. Two-and-a-half-year-old *Acacia auriculiformis* seedling treated with fertilization plus lime 1 month after planting on Yap, Federated States of Micronesia. (Photo by James B. Friday 2019)

Figure 8. Satisfactory growth of an unfertilized 10-month-old *Acacia auriculiformis* seedling on Yap, Federated States of Micronesia. (Photo by James B. Friday 2018)

Figure 7. Growth of fertilized (left row) vs. unfertilized (right row) 19-month-old *Acacia auriculiformis* seedlings on Yap, Federated States of Micronesia. (Photo by James B. Friday 2018)

the soil before the trees were planted might have had a greater effect. *Acacia auriculiformis* is also known for tolerating acid soils (Marcar and Khanna 1997, Powell 1995), as opposed to many other legume trees such as *Leucaena leucocephala* (Lam.) de Wit (National Research Council 1984). In an experiment with *A. koa* on strongly acid soils in Hawaii, Scowcroft and Silva (2005) found differences between seed sources in tolerance to soil acidity. In our study, growth of the unfertilized, unlimed trees was still considerable, averaging 80 in (2 m) per year for the duration of the experiment (figure 8). On an extremely acid soil (pH 4.4) in a somewhat drier location in Hawaii, Cole et al. (1996) found that unfertilized young *A. auriculiformis* grew an average of 83 in (2.1 m)/yr in height but grew 106 in (2.7 m)/yr when limed and fertilized with additional P and K. Diameter growth (and hence volume) increased significantly with liming but not height.

Plants growing in soils with pH less than 5.5 typically show Al toxicity (Cole et al. 1996). The most toxic form of aluminum, Al^{3+}, predominates at pH values below 5.0, whereas the less toxic forms, $AlOH_2+$ and $Al(OH)^{2+}$, predominate at pH values of 5 to 6 (Bojórquez-Quintal et al. 2017). No measurements of soil pH were taken at our study site, but pH values taken on soils of the same series on Yap have ranged from 4.9 to 5.8 (Friday, unpublished data), and so this site may be marginal for showing effects of soil acidity. This experiment should be repeated on a soil type that clearly has Al toxicity issues (as can be evidenced by bauxite nodules lying on the soil surface) and should also include a full factorial such that the effect of lime without fertilizer can also be evaluated. Other research in Yap is currently investigating lime and mulch effects on seedling growth. Another approach to explore is evaluation of native trees with known tolerance to acid soils such as *Rhus taitensis* Guill., C*ommersonia bartramia* (L.) Merr., *Trichospermum ledermannii* Burret, or *Hedyotis* spp.

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Seed Preparation Techniques to Maximize Germination of Pacific Northwest Conifers

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Abstract

Stratification, the historical name for moist chilling seeds to mimic natural processes, is the primary means of releasing seed dormancy in most Pacific Northwest conifer species. This treatment removes internal, or physiological, dormancy, the main dormancy mechanism, but can also address external, or seed coat-imposed, dormancy by leaching chemical inhibitors and weakening mechanical restraints of the seed coat. Nursery personnel intentionally move seeds through the three stages of germination: hydration, activation, and emergence. To fully release dormancy, technicians target specific moisture contents during the activation phase. Maintaining seed in a surface dry condition for part, or all, of the activation phase allows for removal of dormancy while minimizing risks such as excess respiration, mold development, and premature germination. Several "advanced" techniques to maximize germination parameters include: extended stratification, delayed dryback during stratification, mid-stratification grading, and thermal priming (seed warming). These techniques do not involve specialized equipment but require close attention to detail to realize their potential. This paper is based on a webinar presentation given September 16, 2020 as part of the 2020 North American Forest and Conservation Nursery Technology Webinar Series, which can be viewed at https://vimeo.com/458771879. An accompanying online bulletin board is at https://padlet.com/nabilkhadduri/seedgerm-webinar.

Introduction

Reforestation nurseries strive to produce a uniform crop that meets target specifications (Landis et al. 2010). This effort starts with complete, fast, and uniform germination followed by adequate time to complete the rapid growth and hardening stages of seedling development. Nature takes a different approach, preferring in many cases to delay and spread out germination, even in ideal conditions, through some form of seed dormancy (Kildisheva et al. 2020). Spreading out germination over weeks, months, or even years helps ensure that some percentage of seedlings survive the gauntlet of environmental challenges to eventually reach reproductive maturity (Baskin and Baskin 2014).

In natural regeneration for most Pacific Northwest (PNW) conifers, seeds ripen, cones flare, and seeds fall to the forest floor in late summer or early fall. These seed banks are often exposed to ideal germination conditions in mid-fall, where regular rains return while temperatures are still relatively warm. Most PNW conifer seed lots have a range of dormancy, including some non-dormant seeds that will readily germinate whenever warm, moist conditions are present. The challenge for those early germinants is to establish rapidly enough to be able to endure harsh winter conditions. Due to a chilling requirement, however, most seeds hydrated by fall rains will not release dormancy for several weeks to months, generally when temperatures are too cold for germination. Only when warmer spring temperatures arrive will germination commence. To further reduce the risk of germinating into adverse environmental conditions, many seed lots include seeds that will not germinate for weeks, months, or even years (Landis et al. 1999).

Stratification

Nurseries release seed dormancy in most PNW conifers through a cool, moist chilling treatment, commonly known as stratification. This treatment has been in widespread use with many reforestation species for hundreds of years (Bewley and Black 1994). Traditionally, layers of seed were alternated between a medium such as peat moss or sand. Today, nurseries commonly use "naked" stratification where no media are layered or mixed with seed during moist chilling to allow for the close control of moisture content, which is crucial for maximizing stratification benefits. Seeds are typically hydrated in an initial water soak or rinse, then drained and chilled for several weeks or months in plastic bags that allow air exchange (Bonner and Karrfalt 2008).

Benefits of Stratification

By fully releasing dormancy during stratification, we can improve germination capacity, defined as the total germination potential. Seed vigor associated with dormancy release means we can also increase germination speed, especially in cool germination conditions found in bareroot soils, outdoor container compounds or unheated (or insufficiently heated) greenhouses. Finally, we can increase uniformity, where a crop germinates not only quickly, but close together in time.

Successful stratification shortens the "germination window," thereby reducing time spent misting new germinants, a practice which cools the growing environment and slows germination. A shorter germination window also decreases susceptibility to pest attack, as emerging seedlings are particularly vulnerable to animal predation and disease proliferation. Heating greenhouses can be expensive and a shorter germination window decreases these costs. Ultimately, seeds that have had their dormancy fully removed and germinate quickly, uniformly, and completely result in seedlings that are easier to cultivate and less costly to handle in the nursery.

Seed Dormancy Types in PNW Conifers

The *Woody Plant Seed Manual* (Bonner and Karrfalt 2008) defines dormancy as, "…a state in which a seed disposed to germinate does not, even in the presence of favorable environmental conditions." Seed dormancy in PNW conifers falls into two main categories: seed coat-imposed dormancy (physical and chemical) and, most commonly, embryo-imposed (physiological) dormancy (figure 1).

Physical Seed Coat Dormancy

Physical seed coat dormancy is often referred to as "hardseededness"—impermeable seed coats that do

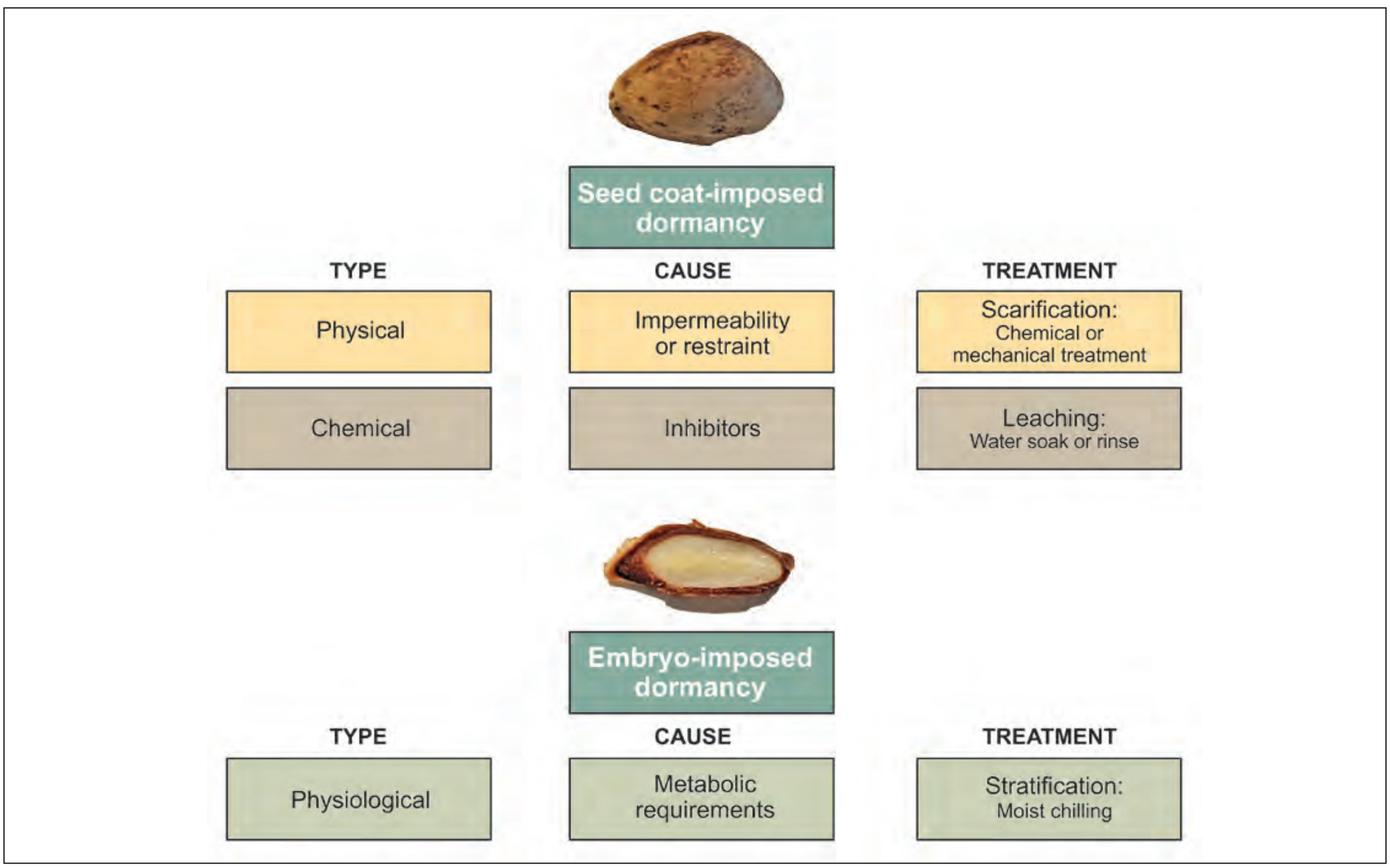

Figure 1. PNW conifer seeds can have physical or chemical seed coat imposed-dormancy along with the typical embryo-imposed dormancy. Except for impermeable seeds, the stratification process is a useful tool to break dormancy. (Photos by Nabil Khadduri 2020)

not allow gases or liquids to pass through. Many trees in the Leguminoseae family exhibit this kind of dormancy, such as locust (*Robinia* spp.). Hard seed coats can be broken down with sulfuric acid, hot water, or mechanical abrasion treatments (Khadduri et al. 2003). The only PNW conifer species thought to contain an impermeable seed coat is whitebark pine (*Pinus albicaulis* Engelm.) (Leadem 1996).

Some PNW conifers exhibit a physical dormancy where mechanical restraints keep the embryo from expanding. Water and air can pass through to the embryo, allowing it to enlarge, but growth and full imbibition are mechanically restricted by woody structures of the seed coat. This is a typical form of dormancy in southern pines (e.g., loblolly pine [*Pinus taeda* L.]) and is believed to be one form of dormancy in western white pine (*Pinus monticola* Douglas ex D. Don) (Bonner 1991).

Chemical Seed Coat Dormancy

A poorly understood mechanism of physical dormancy in PNW conifers involves the presence of chemical inhibitors in the seed coat. Some phenolic substances in the seed coat could be germination inhibitors, though their main role may be to limit growth of pathogenic organisms (Mohammed-Yaseen et al. 1994). In stratification, an initial running water rinse or simple water soak may leach chemical inhibitors (Leadem 1997).

Embryo Dormancy

Embryo-imposed dormancy is a physiological barrier to germination where metabolic blocks need to be removed and growth-promoting enzymes activated. This is the most common type of dormancy in PNW conifers and is treated with stratification. For example, one of the main internal impediments to germination of conifer seeds is high levels of abscisic acid (ABA). Feurtado et al. (2004) showed that embryo ABA levels rapidly drop during moist chilling of western white pine, greatly reducing this germination impediment.

Stratification: Nurseries Intentionally Move Seeds Through Three Stages of Germination

The three stages of germination that seeds must eventually pass through are: (1) hydration, (2) activation, and (3) emergence (figure 2a-d) (Bewley and Black 1994). In artificial regeneration, nurseries mimic natural dormancy release and stimulate germination through a series of intentional practices, with careful control of moisture content. We collect seeds either in the woods (woods-run or wild) or

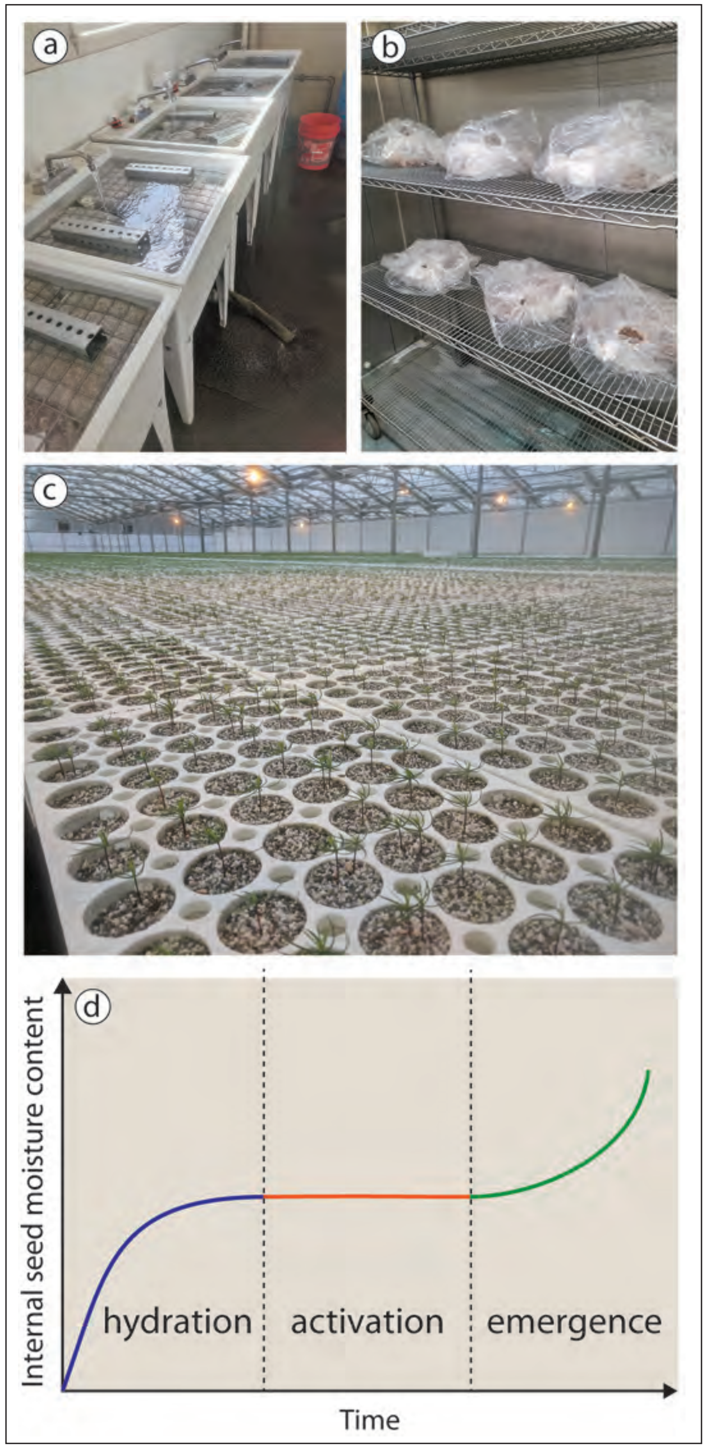

Figure 2. The three stages of seed germination are: (a) hydration, (b) activation, and (c) emergence. During hydration and emergence, (d) internal seed moisture increases. (Photos a, b, c by Nabil Khadduri 2020; d adapted from Bewley and Black 1994)

from managed seed orchards. Our seed banks are controlled storage facilities where temperatures are maintained at -18 °C (0 °F) and between 5 to 10 percent moisture content.

For most PNW conifer seeds, a stratification treatment acts as a panacea by addressing several dormancy mechanisms. Chemical inhibitors are assumed to be leached by the running water rinse or water soak during the hydration phase of the stratification treatment. The length of time spent in moist, cool conditions not only releases metabolic blocks and cues enzymatic growth processes, but may also allow mechanical restraints in the seed coat to break down. Thus, stratification may relieve chemical, physical, and physiological dormancy in one treatment (Leadem 1997).

Hydration stage

At Webster Nursery (Washington Department of Natural Resources, Olympia, WA), we initiate the hydration stage of germination with an aerated water soak or running water rinse. Seeds respire upon introduction to water and the aeration of the water soak (using a fish tank bubbler, for example) or a running water rinse introduces supplemental oxygen into the process (figure 2a). A rinse may more effectively remove chemical inhibitors (as well as pathogens on the seed coat) than a soak. After imbibition is complete, generally 24 to 48 hours, seeds are drained of excess moisture.

Activation Stage and the Importance of Surface Drying Seeds

For the activation stage of germination, we place seeds in plastic bags no thicker than 0.102 mm (4 ml) and filled with no more than 50 to 75 percent of the bag's volume (figure 2b). We use U-line 1 ml plastic bags (Pleasant Prairie, WI) at Webster Nursery. The thin plastic and partial filling ensure sufficient oxygen and carbon dioxide exchange (Landis et al. 1999). Partial filling also facilitates "massaging" of seed during stratification, typically carried out once or twice per week, to encourage further aeration and avoid heat build-up from excess respiration. Typical stratification temperatures are 1 to 5 °C (34 to 41 °F). At Webster Nursery, we maintain temperatures at 2 °C (35 °F). This relatively low temperature still releases dormancy, but also reduces premature germination or mold build-up during stratification that may accelerate on the higher end of the temperature range.

A major advancement in stratification protocols in recent years has been the careful monitoring and drying back of seed surface moisture content prior to, or in some cases during, the chilling period (Jones and Gosling 1994). The presence of a film of moisture on seed coats during stratification can lead to excess respiration, which depletes seed reserves (figure 3a). Also, gas exchange is reduced when seeds are in a surface wet condition, and massaging to break up clumps and increase air flow is more difficult. Observable problems include rapid mold development (figure 3b) and premature germination. To reduce these risks, chilling duration may be shortened, but this could result in failure to fully remove dormancy. The goal, then, is to safely remove dormancy by surface drying seed while maintaining internal seed moisture content.

Surface drying can be considered a form of "hydropriming" where we target specific seed moisture contents (weights) to prolong the activation phase and

Figure 3. (a) Surface dry (left) and surface wet (right) seeds. After the initial soak or rinse, seeds should be surface dried for part or all of the chilling period. Surface drying reduces (b) mold, excess respiration, and premature germination during stratification. (Photos by Nabil Khadduri 2020)

delay the start of stage 3 emergence (Kolotelo 2020b). By limiting surface moisture and prolonging the activation phase, we allow all seeds to release dormancy, even the more dormant seeds, thereby improving germination. Some species are best surface-dried immediately after hydration and draining, while others benefit from several weeks of "wet stratification" prior to surface drying (see section on Delayed Dryback).

Emergence Stage

The emergence stage ideally begins after we sow seeds in a greenhouse or field and visible germination takes place. A favorable warm and wet environment encourages germination, but only seeds whose dormancy has been completely removed will germinate with full vigor. Importantly, complete dormancy removal helps seeds cope with any suboptimal conditions they might experience during emergence.

Useful Tools to Aid in Surface Drying

Using Storage Seed Lot Moisture Content to Accurately Surface Dry Seeds

Seed plant operations use ovens or meters to accurately determine moisture content for long-term seed lot storage. The most common practice is to weigh several representative seed samples, oven dry at a set temperature for 24 hours, then re-weigh to determine current moisture content based on total weight loss. Seeds are only placed into long-term storage at 5- to 10-percent moisture content, a range that optimizes seed longevity.

In stratification, one can use this previously determined storage moisture content to monitor and manipulate current moisture content throughout the process. Jones and Gosling (1994) first described this non-destructive and rapid moisture content monitoring concept. At Webster Nursery, we have adapted the operational practices used at the BC Ministry of Forests Tree Seed Centre (Kolotelo et al. 2001).

The first step is to determine the oven dry weight (0 percent moisture content) of the seed lot intended for stratification. This information can be determined indirectly from the storage moisture content of the seed lot, a number that is generally available and can be requested from the seed vendor. If this is not available, you will need to determine the oven dry weight yourself through direct oven drying, but will only need to do this one time.

In Equation 1, we determine the oven dry weight of a seed lot withdrawal by plugging in the storage moisture content (in decimal form) along with the withdrawal weight.

Equation 1: *Oven dry weight = withdrawal weight * (1 – storage moisture content)*

For example, if we withdraw 980 g (2.16 lb) of coastal Douglas-fir seed from a seed orchard that was stored at 8.0 percent moisture content, the oven dry weight would be:

Oven dry weight = 980 g * (1 - .08) = 980 g x 0.92 = 901.6 g (1.99 lb)

Once we know the oven dry weight, we can determine the current moisture content at any point in the stratification process by weighing the fresh weight and plugging that into Equation 2.

Equation 2: *Current moisture content = (fresh weight – oven dry weight)/ fresh weight*

Using our example above, we know from Equation 1 that our oven dry weight for our withdrawn seed lot is 901.6 g (1.99 lb). If the current weight of our seed lot is 1,610 g (3.55 lb), after we have imbibed the seed but before surface drying, the current moisture content would be:

Current moisture content = (1,610 g – 901.6 g)/1,610 g = 0.44, or 44.0 percent

We can use Equation 3 to determine the target weight for a target moisture content.

Equation 3: *Target fresh weight = (oven dry weight)/ (1 – target moisture content)*

Continuing with our example, we know from experience that coastal Douglas-fir from a seed orchard surface dries to about 33 percent. So, the targeted fresh weight for this target moisture content would be:

Target fresh weight = 901.6 g / (1 – 0.33) = 901.6 g/ 0.67 = 1345.7 g (2.97 lb)

For additional examples, see Kolotelo (2018), as well as a typical spreadsheet from our nursery operations with equations plugged in at: https://padlet.com/nabilkhadduri/seedgermwebinar.

Kolotelo notes that one of the benefits of using this process to non-destructively monitor seed, as opposed to destructive tests, is that sampling error is reduced by weighing an entire bag of seed as opposed to small destructive samples to determine moisture content. Most PNW conifers surface dry to between 25 and 35 percent moisture, with relatively narrow ranges based on species. See Kolotelo et al. (2021) for a list of typical surface dry weights of PNW conifers based on their origin (woods-run vs. orchard grown).

Using a Laundry Spinner to Expedite Surface Drying

Following the hydration phase, we drain excess water from the seeds, but a film of water often remains. We generally surface dry seeds using some combination of indirect heat, forced air, and regular hand-mixing so that excess moisture is uniformly removed. Drying can take time, but there is a convenient tool to expedite the process for some species: a laundry spinner. Gosling et al. (1994) demonstrated that a laundry spinner can serve quite well as a seed spinner by quickly and consistently removing free moisture from a seed lot after the soak/rinse hydration phase.

For the past 15 years, we have used a Spin-X laundry spinner for this purpose (figure 4), the same brand used by Gosling et al. (1994). This spinner costs $495 USD, and a recent internet search (summer 2020) found several alternatives priced $200 lower than the Spin-X. We have been pleased with the durability of the Spin-X and cannot vouch for the longevity of other models. We developed a seed spinner guide for our nursery species, available at https://padlet.com/nabilkhadduri/seedgermwebinar. Seeds of some species, such as those with resin-vesicles (pitch sacks in the seed coat) are not recommended for spinning since damaged resin vesicles can release extracts that inhibit germination (Keeling et al. 2018). As with any new process, we advise trialing small lots before using at operational scale.

Figure 4. Excess water can remain following imbibition and draining. A laundry spinner repurposed as a seed spinner can be used for some species to efficiently remove excess water by placing seeds in the drum, balancing the weight of the seeds, and removing free water through centrifugal force during a preset (approximately 2-minute) spin cycle. (Photo by Nabil Khadduri 2020)

Advanced Techniques to Improve Germination

The "advanced" techniques detailed here require little additional equipment to carry out. What they do require is close attention to detail, persistence, and patience. Most utilize surface drying at some point in the process to be successful. As with all new techniques, try these on a small scale first, then gradually scale up as experience and confidence grows. A successful germination treatment for one species may harm performance in another, and even within species each seed lot can, and often will, respond differently to the same treatment. Try to develop treatments that are conservative enough to be applied to a broad range of seed lots within a species, and continue to evaluate to make sure they do not harm certain seed lots.

Extended Stratification

Why should growers consider extending stratification longer than what might be suggested in lab germination tests or the literature? Unlike field, or even greenhouse, conditions, lab tests are conducted in ideal situations with warm, controlled temperatures. By extending stratification one is more likely to completely remove dormancy from all seeds within a lot. Thus, operational tests in nursery conditions are important since extended stratification benefits may not be realized in ideal lab conditions (Edwards and El-Kassaby 1995).

In our experience at Webster Nursery, most PNW conifers almost always require stratification lengths longer than standard lab recommendations. Exceptions to this rule may include seed lots that are improperly stored or otherwise deteriorated. Think of lab stratification lengths, such as those from the Association of Official Seed Analysts, as the minimum, and extend from there for greenhouse and especially field sowing. For example, if a 4-week stratification is recommended in lab testing, compare germination capacity and speed for 4 weeks with 6 or 8 weeks. Kolotelo (2020a) and Lei (2021) note a consistent pattern of increased dormancy in orchard-grown seed compared with woods-run seed across several PNW species, regardless of seed size. This pattern concurs with common grower observations that orchard-grown PNW species seem to benefit from longer stratification than woods-run seed from a similar zone and elevation.

One of the biggest arguments for extending stratification is increasing germination capacity across a range of temperatures. Jones and Gosling (1994) stratified coastal Douglas-fir (Pseudotsuga menziesii Mirb. Franco) seeds for 0, 3, 6, or 18 weeks. They found that the lots they tested were only shallowly dormant, with 65-percent germination at ideal temperatures between 20 and 30 °C (68 and 86 °F) after 42 days for those with 0 weeks (i.e., no) stratification. For seeds with 3 weeks stratification, however, germination increased across a range of temperatures. For seeds stratified for 6 or 18 weeks, germination was further enhanced in the 10 to 20 °C range (figure 5).

Extended Stratification Trial for Douglas-fir: Lab and Greenhouse Comparison Trial

In 2010, we compared 4-, 8- and 12-week stratification durations in both lab (figure 6a) and greenhouse (figure 6c) settings on four orchard-grown coastal Douglas-fir lots. In the greenhouse, stratification lengths were 10 days longer than lab lengths due to sowing delays. Lab temperatures followed a standard 20 °C, 8-hr light and 30 °C, 16-hr dark protocol (AOSA 2007). While there was no significant difference in final total germination, 12 weeks of stratification resulted in seeds with higher germination speed compared with shorter stratification durations (figure 6b). In the greenhouse, three of the four lots showed incremental increases in germination speed and total germination with increasing stratification lengths (figure 6d). One lot, however, germinated faster at 66 vs. 94 days, illustrating that not all seed lots will benefit from extended stratification durations. It is critically important to monitor seed condition and the presence of excess moisture or seed drying when extending seed stratification.

Extended Stratification Trial for Western Hemlock: Lab and Greenhouse Comparison Trial

Seeds of western hemlock (*Tsuga heterophylla* [Raf.] Sarg.) surface dry (seed weight of imbibed seed after external moisture is dried off) to below 30 percent on average (Kolotelo 2018, Kolotelo et al. 2021). Previously, we maintained western hemlock at higher moisture values, with occasional pre-germination and/or mold during the chilling period as a result. By surface drying to lower levels, we hypothesized that

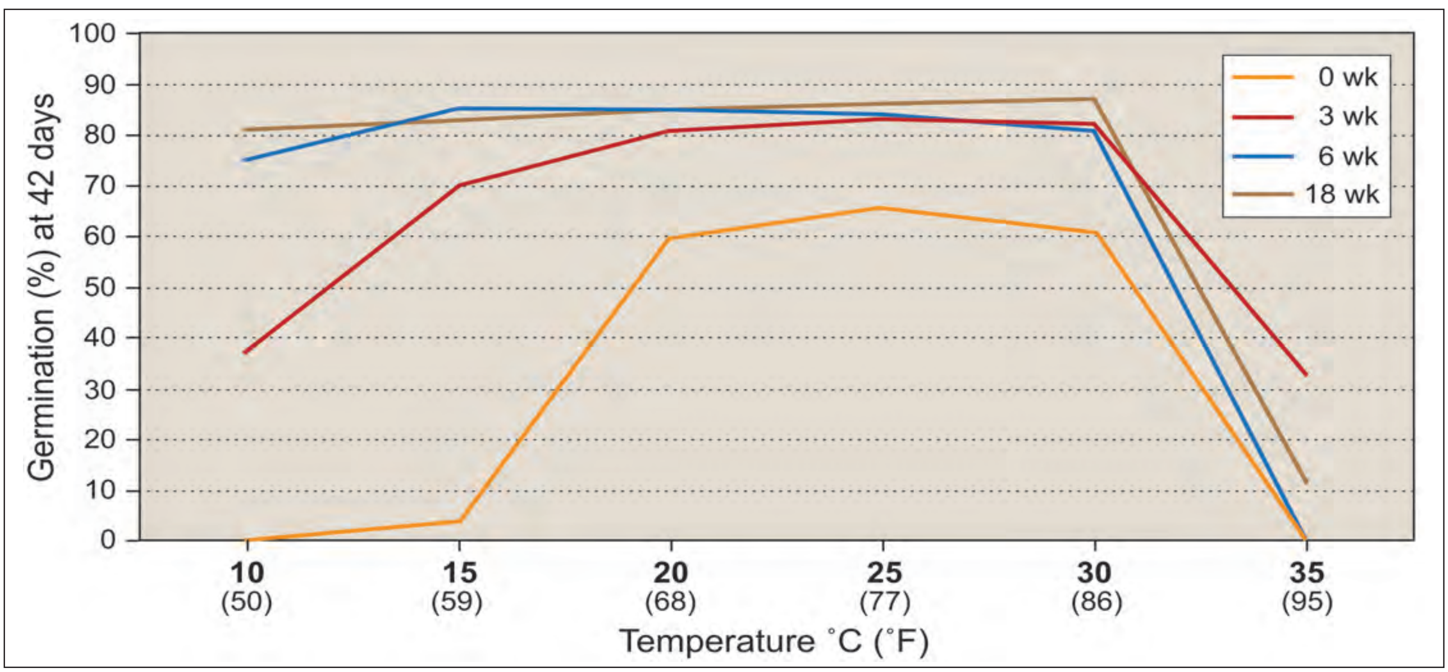

Figure 5. Extending stratification up to 18 weeks increased coastal Douglas-fir germination capacity across a range of temperatures, particularly temperatures below 20 °C (68 °F). (Adapted from Jones et al. 1994)

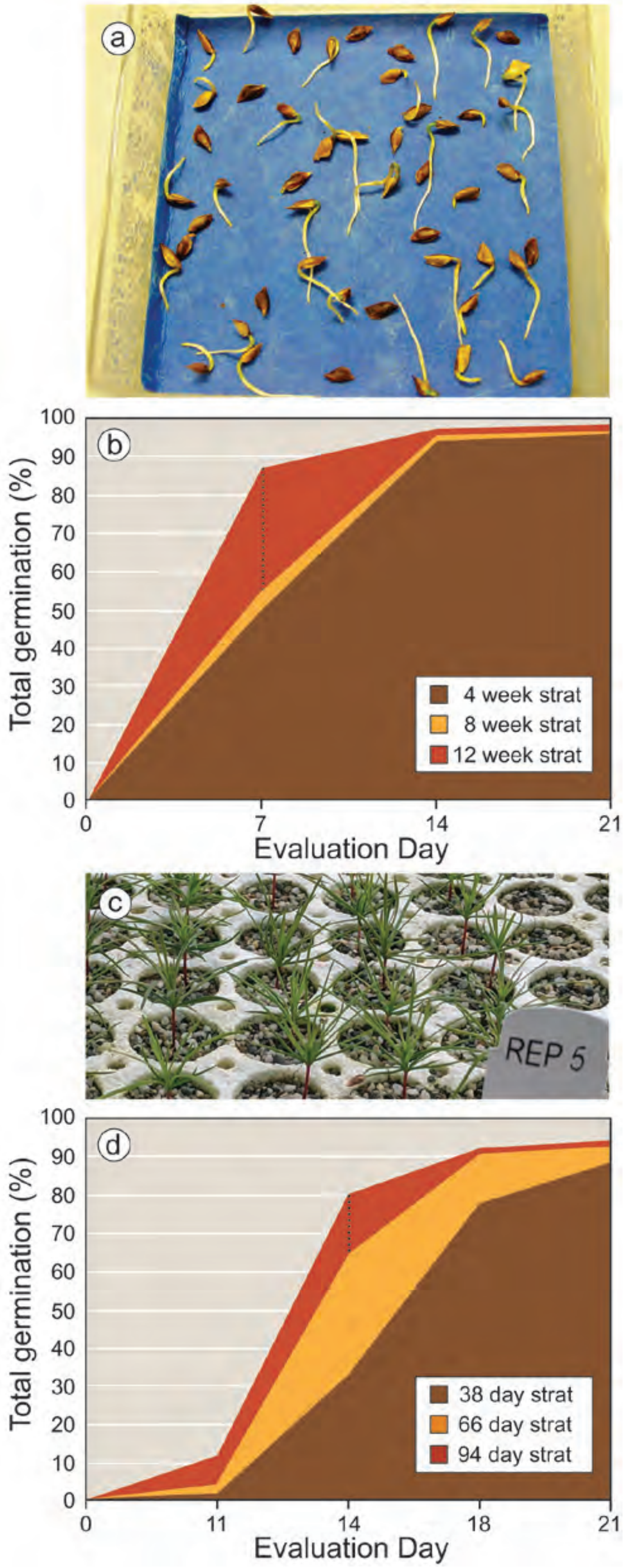

Figure 6. In a lab test under (a) controlled conditions, (b) 12 weeks of stratification increased germination by day 7 of four coastal orchard Douglas-fir lots but with no significant increase in total germination by the end of the test. In a (c) greenhouse test with the same four lots, (d) three of the lots showed incremental increases in germination speed from increasing stratification lengths. For one seed lot in the greenhouse (not shown), germination speed was fastest with the 66-day stratification but did not differ in total germination from other stratification durations. (Photos by Nabil Khadduri 2010)

extended stratification times beyond our 30-day operational treatment would expedite notoriously slow western hemlock germination.

In 2020, we ran western hemlock lab (figure 7a) and greenhouse (figure 7c) stratification trials. In the lab trial, we found that longer stratification times for two woods-run lots incrementally improved germination speed, even under warm lab conditions (23.3 °C [73.9 °F] average), though there were no differences in final germination (figure 7b). Normally, we stagger stratification dates so that all seed in a trial is sown on the same day. For logistical reasons, however, we started all stratification treatments for the greenhouse trial on the same day, so that 30-, 45-, and 60-day treatments were sown 15 days apart. While not an issue in standardized lab conditions, average greenhouse temperatures slowly increased through the trial from 19.8 °C to 20.5 °C. For this reason, we plotted greenhouse germination against growing degree days for each treatment to account for those temperature variations (figure 7d). Results paralleled the lab trial in terms of germination speed, with longer stratification treatments emerging faster. Unlike the lab trial, longer stratification durations also significantly improved final germination over the 30-day treatment.

Seed Sanitation During Extended Stratification

Occasionally seed lots, even with surface dryback precautions, will build up some level of mold during extended stratification. In addition to a presoak bleach treatment with some species, we may also use a 3.0 percent active ingredient hydrogen peroxide soak for 2 to 4 hours either in the middle or at the end of stratification, followed by a rinse of 2 to 4 hours. Some nurseries do not rinse after the hydrogen peroxide treatment, while others simply do a clear water rinse during stratification in lieu of a chemical treatment.

We continue to assess and update our seed sanitation program. For example, the use of sodium hypochlorite is both supported (Wenny and Dumroese 1987; Dumroese et al. 1988) and discouraged (Trotter 1990) in the literature. We apply sodium hypochlorite to certain species at varying concentrations, with an emphasis on seeds of species that are slow to take up water. In general, a post-imbibition hydrogen peroxide treatment should be safer to seed than an initial sodium hypochlorite treatment (Neumann et al. 1997). In the case of species with

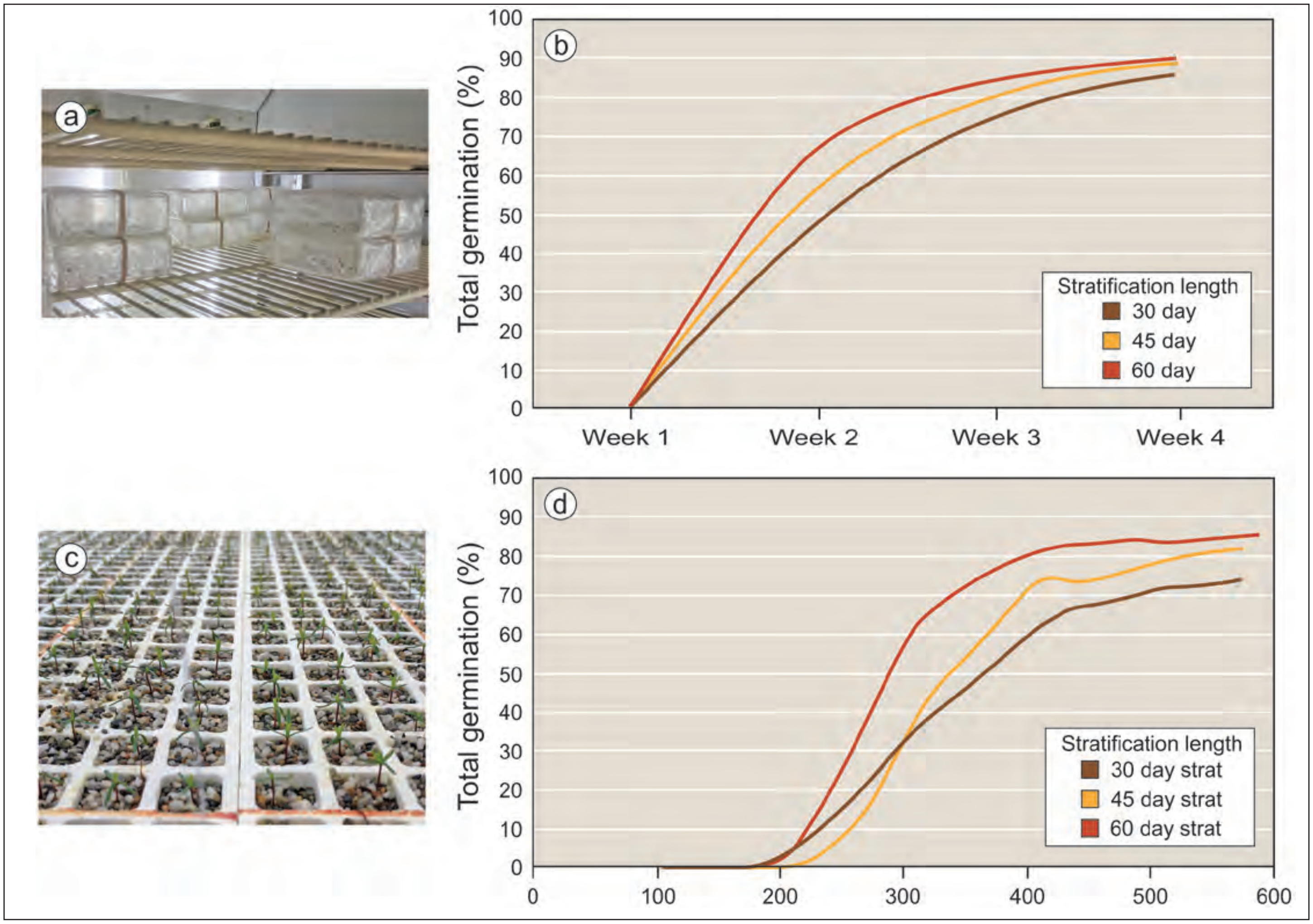

Figure 7. A western hemlock (a) lab trial demonstrated that (b) longer stratification times for two unimproved seed lots significantly improved germination speed over a 30-day treatment, even under warm lab conditions. In a corresponding (c) greenhouse trial, (d) germination speed significantly increased with increased stratification length and improved final germination over the 30-day treatment. (Photos by Nabil Khadduri 2015)

resin vesicles, neither chemical treatment is recommended, especially if resin vesicles have been damaged. In the PNW, these include true fir species as well as western hemlock. See https://padlet.com/nabilkhadduri/seedgermwebinar for an overview of our current nursery seed sanitation guidelines.

Delayed Dryback

As mentioned previously, surface drying (dryback) of seeds immediately following imbibition and prior to stratification reduces mold, excess respiration, and premature germination. Some species, however, benefit from a period of "wet stratification" for at least the first few weeks of the chilling process. True firs (*Abies* spp.) in particular (Edwards 1996), as well as western white pine (*Pinus monticola* Douglas ex D. Don) (deGraan et al. 2013), should not be surface dried for 4 weeks after imbibition (figure 8). It is not clear why this works. Surface moisture may be desirable for the first few weeks if additional imbibition is needed or if seed coats need to be additionally degraded in the presence of excess moisture. Some true firs such as noble fir (*Abies procera* Rehder) benefit from a chilling period of 84 days total with surface drying after the first 28 days (Edwards 1996).

Delayed Dryback Case Study: Western White Pine

Western white pine can benefit from an extremely long imbibition period, specifically a running water rinse of up to 2 weeks (figure 9). The USDA Forest Service Coeur d'Alene Nursery tested several lots of western white pine and found that some did not fully imbibe until 6 days of soak (Rhoades 2020). At Webster Nursery, we have found the 2-week hydration phase prescribed by the BC Ministry of Forests (Kolotelo 1993a, Kolotelo et al. 2001) to be successful. It is not clear why this lengthy running

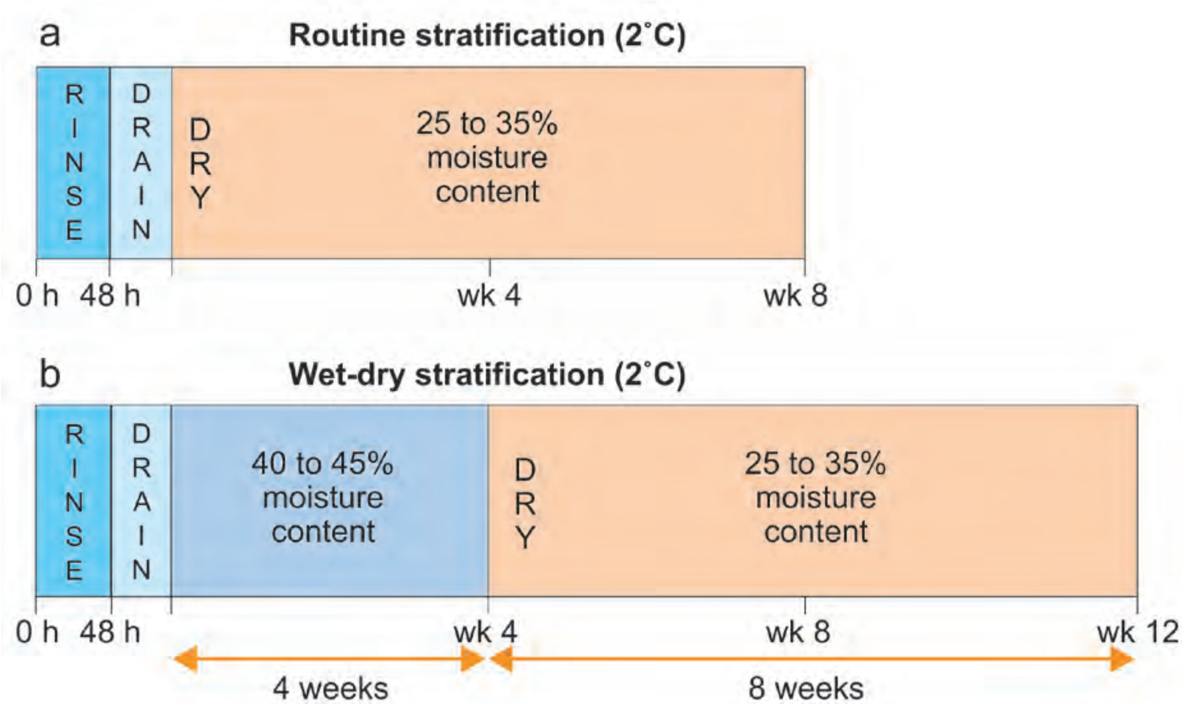

Figure 8. For (a) routine stratification, seeds are surface dried immediately following water uptake and draining. For (b) delayed dryback stratification, seeds are allowed to remain in a surface wet condition for about 4 weeks, then surface dried for the remainder of the chilling period. Delayed dryback generally allows for a longer overall chilling period than routine stratification.

water rinse works, but perhaps stubborn chemical germination inhibitors take time to leach out. Another possible explanation is that physical structures that impede embryo expansion continue to break down during the extended water treatment.

Modifying the BC protocol, we found that western white pine benefits from 4 weeks of surface wet stratification in the 42- to 44-percent range, followed by surface drying to 34 to 36 percent for an additional 14 to 15 weeks.

Western white pine is at risk for *Fusarium* fungal disease on the seed coat (Cram and Fraedrich 2009), and an aggressive seed sanitation protocol may be warranted, particularly due to the extreme length of time in chilling. We treat western white pine seed with an initial soak in 2.1 percent active ingredient bleach (sodium hypochlorite) for 10 minutes, followed by a 2-minute clear water flush. The intent is to kill fungal spores on the seed coat before active water uptake commences. The 14-day rinse presumably also helps remove bulk pathogens on the seed coat, though some *Fusarium* spp. can remain after running water rinse and/or a bleach or hydrogen peroxide treatment (Littke 1996).

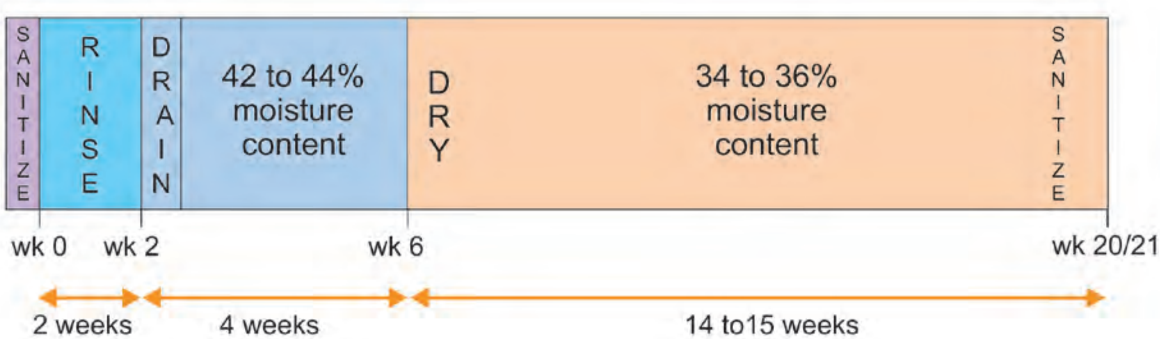

Figure 9. A series of stratification steps, including delayed dryback, can maximize germination parameters in western white pine. These steps include a pre-imbibition bleach dip (aggressively flushed), a 14-day running water rinse, a 4-week “wet” stratification, followed by an additional 14 to 15 weeks of surface dry stratification. While surface drying the seed helps prevent mold build-up, fungal development can be addressed with hydrogen peroxide and/or a short running water rinse late in the stratification period.

Grading Seed During Stratification Using Float Separation

Seed processing facilities use gravity tables, aspirators, and other tools to remove unfilled, partially filled, damaged, or insect-ridden seeds based on seed density. Some species, for example true firs, can present challenges to optimal cleaning due to thickened seed coats of hollow seeds, resin-filled seeds, or insect-damaged seeds. In all these instances, dead seed may have similar densities to live, filled seed that allows them to make it through the typical cleaning process.

The stratification process can facilitate seed grading because filled, live seed are more likely to bind to water and to increase in density due to biological growth in the activation stage of germination. While there are more involved forms of water-based seed separation prior to stratification such as pressure/vacuum treatment (PREVAC) or incubation drydown separation (IDS) (Karrfalt 2013, Karrfalt 1996, Simak 1984), one relatively straightforward technique is to simply float-separate seeds at some point during the stratification period. The greatest success with this technique is with lower-germination lots that have a relatively high proportion of dead, unfilled seeds (Kolotelo 1993b).

In float separation, surface dry seeds are placed in a tank (figure 10a), stirred to break surface tension, and allowed to separate based on density for several minutes or even hours. Ideally, unfilled or damaged seeds rise to the surface (figure 10b) and filled seeds sink to the bottom (figure 10c), though some seeds stubbornly remain in a suspended intermediate state. Cut tests should be used to determine proportions of viable seeds from each fraction (see Kolotelo 1997 for an excellent visual guide on cut tests of PNW conifers). Sylvan Vale Nursery (Black Creek, BC) reports that aeration may speed up the separation process. Also, float separation near the end of the stratification process can sometimes be successful when separation just after wet stratification fails to distinguish seed quality (Paquet 2020).

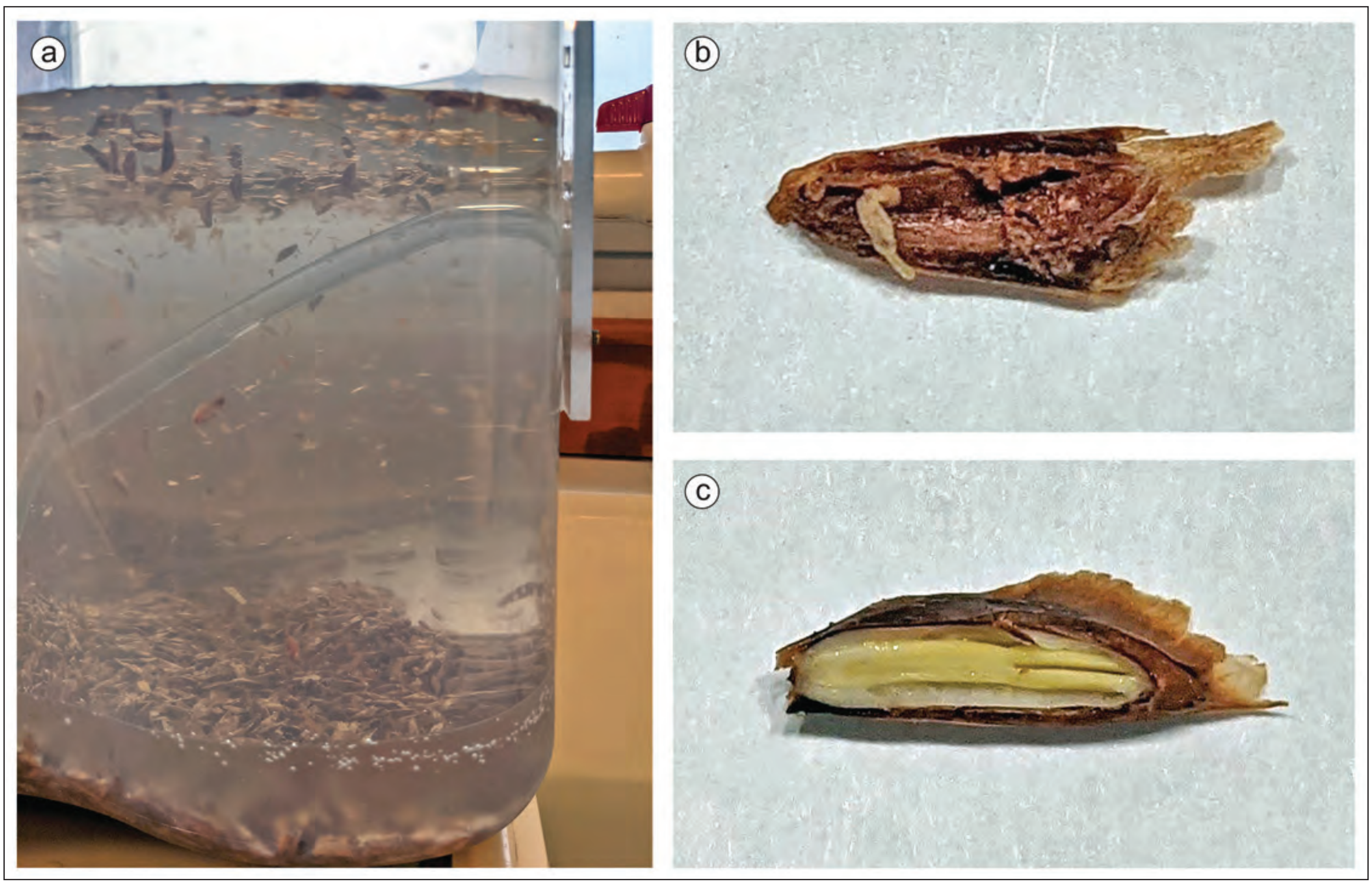

Figure 10. In float separation, surface dry seeds are placed in a (a) tank, stirred to break surface tension, and allowed to separate based on density for several minutes or hours. Cut tests can determine percentage of (b) empty or damaged seed and (c) filled seed in the floater and sinker fractions. This process is most successful for grading low germination lots with a high proportion of unfilled seeds. (Photos by Nabil Khadduri 2020).

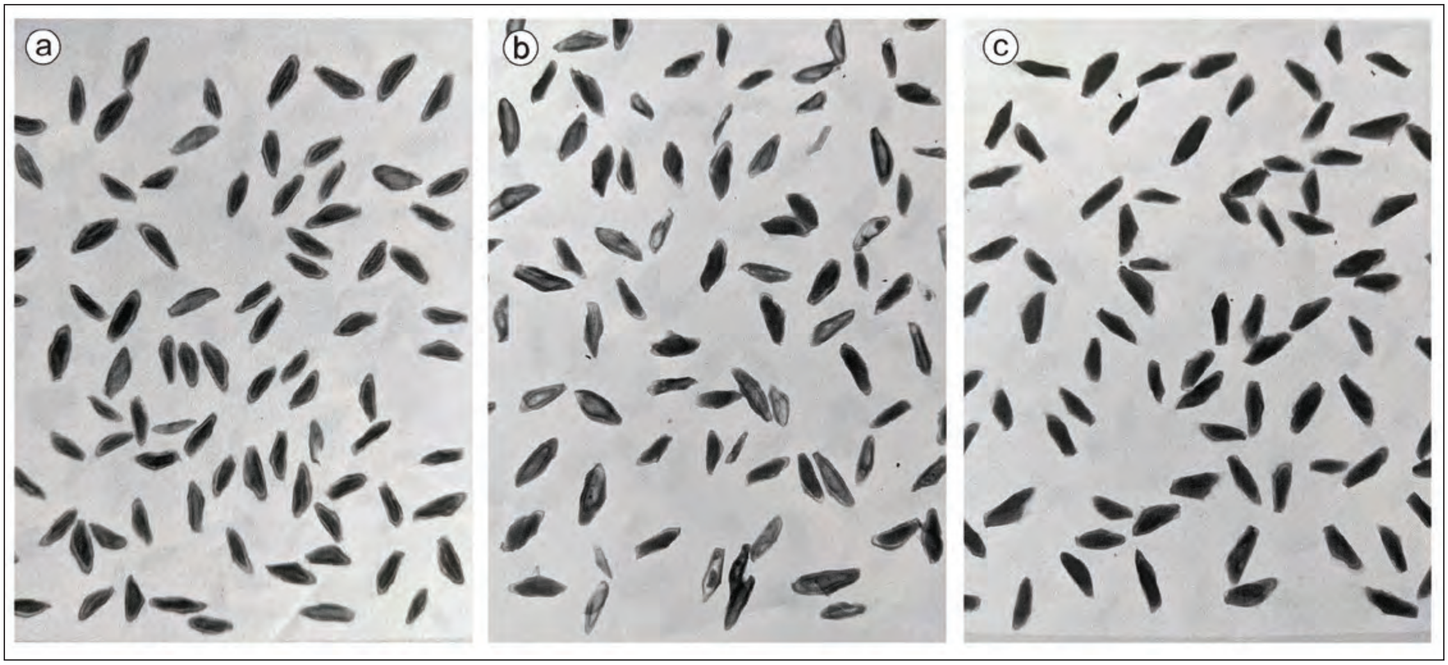

Figure 11. Seeds of Pacific silver fir seed lot 1386 were x-rayed (a) pre-stratification. Seeds were surface dried after "wet" stratification, then water separated into (b) floaters and (c) sinkers. Cut tests can also quickly help determine filled seed percentages. (Photos by Nabil Khadduri 2020)

In 2015, we separated several seed lots in a one-step separation process immediately following dryback after initial wet stratification. Figure 11 shows a Pacific silver fir seed lot (a) pre-stratification, (b) floater fraction with empty and insect-damaged seeds, and (c) sinker fraction with filled seeds. Results varied by species and by seed lot, with some floater fractions still containing significant quantities of viable seed. Nevertheless, greenhouse germination of graded seeds improved to 76 percent over a 69 percent lab test baseline (not shown) when averaged across all lots in this trial (figure 12). An important step when grading is to weigh the separations to make new sowing calculations of high-graded seed.

Thermal Priming (aka Seed Warming) to Jump Start Germination

Thermal priming refers to pre-warming seeds at the end of stratification prior to mechanical sowing. Because heat units needed for germination are unpredictable in a bareroot setting and expensive to supply in a greenhouse setting, intentionally heating seeds in a small, controlled environment prior to sowing can speed germination.

Careful attention and precautions must be followed in the seed warming process. K&C Nursery (Oliver, BC) recommends splitting lots into smaller bags and regularly rotating bags to facilitate even warming (Yang 2020). Dividing lots also allows extra air space for gas exchange during increased biological activity. Along with visual inspection, Kolotelo (2020) recommends weighing bags to make sure moisture loss is not taking place. Smaller-seeded species may benefit

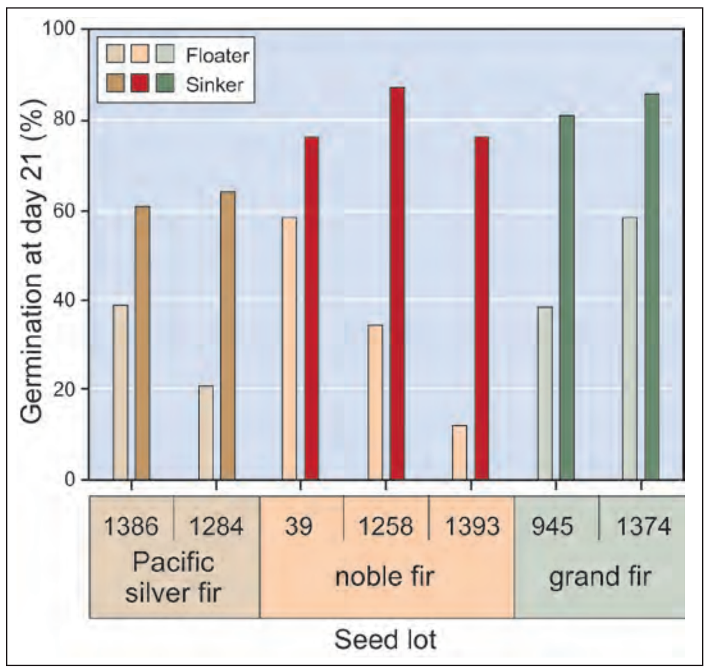

Figure 12. Seed lots of three *Abies species* (Pacific silver fir, noble fir and grand fir) were graded into floaters and sinkers mid-stratification. Greenhouse germination varied by species and seed lot and some floater fractions still contained significant quantities of viable seed. Still, overall greenhouse germination of graded seed averaged 76 percent compared with 69 percent for lab germination (lab results not shown).

from supplemental moisture. Another risk during thermal priming is stimulation of fungal growth. Fungi usually grow faster than seeds during the warming period (Dawes 2008). A third, and perhaps greatest, risk is premature radicle emergence such that most forms of mechanical sowing cannot be used.

To avoid the above risks, Yang (2020) recommends against thermal priming if abnormal fungal growth is observed during stratification, or the seed lot has a fermented odor (suggesting seed degradation), or if any radicles are observed to be emerging during stratification.

Provided stratification has proceeded normally with no complications, Kolotelo (2020b) suggests some useful guidelines for determining how much heat to supply in pre-warming without inducing germination before sowing. Radicles first emerge in lab germination tests between Day 2 and Day 5 for many species. In standard lab temperatures, this comes out to (25 °C [77 °F) x 8 hr) + (15 °C [59 °F) x 16 hr) = 440 growing degree-hours per day (based on a 5 °C [41 °F] baseline for accumulating heat). Thus, 880 degree-hours can be used as an estimate for seeds that may germinate as early as 2 days in test conditions, with 800 degree-hours a conservative starting point. Operationally, one can probably add additional heat units without risk of germination, but it is worthwhile to build up experience and comfort level. For example, K&C nursery pre-warms seeds up to 72 hours at 25 to 30 °C (77 to 86 °F), but this varies by species.

Kolotelo (2020b) points out that, for PNW conifers, how one accumulates heat units (steady or alternating diurnally) and the rate at which units are accumulated may not matter. It is just the total energy received that determines germination. In a study on white spruce (Picea glauca [Moench] Voss), Liu et al. (2013) found excellent germination characteristics using a combination of moist chilling with 72 hours of thermal priming at 15 °C (59 °F), and especially 20 °C (68 °F).

We plan to test thermal priming on a small scale at an average room temperature of 20 °C (68 °F). At a suggested conservative total of 800 degree-hours, that comes out to 2.2 days. As an added layer of insurance, we plan to evaluate our seed lots beforehand by calculating how many degree-hours are required to first see radicle emergence on fully stratified seeds. See Kolotelo (2020b) for additional information and references on the topic.

Conclusions

Several stratification strategies exist to increase germination uniformity, speed, and percentage. A dedicated, passionate, and experienced seed technician is key to successfully implementing the techniques described in this article. Through trial and error and attention to detail, a technician can develop techniques that are tailored to species and seed lots in operational sowing conditions. The goal in an operational program is to develop strategies that are aggressive enough to enhance germination performance, but conservative enough to be applied as a standard practice with minimal risk. Always remember to refine techniques by revisiting "tried and true" practices as experience dictates and time allows.

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Mycorrhizal Status and Mycorrhizal Colonization Potential of Rhizospheric Soils Around Introduced and Natural Argan Trees in Northwest Morocco

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Abstract

Soil and root samples were collected from the rhizosphere of planted and natural argan trees growing in the Bounaga and Smimou sites, respectively, in northwest Morocco. Frequency and intensity of mycorrhization and arbuscular content of the roots of argan trees varied between sites. Spore morphotypes on the two sites belonged to 14 species and 6 genera (*Acaulospora, Gigaspora, Glomus, Entrophospora, Scutellospora* and *Pacispora*). The number of infectious propagules of arbuscular mycorrhizal fungi (AMF) in the rhizospheric soils of argan trees at the two sites was estimated using the most probable number method (MPN). These results demonstrate that introduced argan trees form symbiotic AMF. Mycorrhizal inoculation of argan plants at the nursery stage may be beneficial, especially for harsh sites.

Introduction

The argan tree (*Argania spinosa* [L.] Skeels) is a fruit tree endemic to southwest Morocco. This species is biologically, phytogenetically, ecologically, economically, and socially important for the country (Aït Hammouda et al. 2013). Alarmingly, this fruit tree is declining rapidly due to the arid climate, poor soils, and anthropozoogenic action of its distribution area (Bousselmame et al. 2002, Reda Tazi et al. 2003). The natural regeneration of argan forests is totally absent, due to over-exploitation and the fact that the natural environment no longer has satisfactory conditions for seed germination (El Aïch et al. 2007). Furthermore, germination in the nursery does not exceed 27 percent because of embryonic dormancy, poor seed viability, and pre- and post-emergent damping-off diseases (Bani-Aameur and Alouani 1999).

The Water and Forestry services have made many efforts to plant argan trees in Algeria, Egypt, and Tunisia (Baumer and Zeraïa 1999), as well as in different areas of Morocco. In Morocco, the first introduction of the argan tree outside its natural area was in the early 1930s when reforestation work was launched on the banks of the Oued Cherrate River, 38 km south of Rabat; argan trees are still present on this site. These efforts have met with varying success, including some failures (Harrouni et al. 1999) and some with only a few surviving trees.

The argan tree is a mycotrophic species capable of developing a symbiotic association with endomycorrhizae (AMF) (Nouaïm and Chaussod 1996), an association that improves plant nutrition (mainly phosphorus), especially in arid and semi-arid environments, improves soil aggregation and stability (Rillig and Mummey 2006), and protects plants against phytopathogens (Newsham et al. 1995). AMF also helps plants in arid and semi-arid areas by reducing water stress (Honrubia 2009) and other environmental stresses (Martínez-García 2010) and improving physio-chemical and biological soil properties (Schmid et al. 2008). The production of good-quality plants is a necessary step to improve the survival and growth of plants in reforestation sites (Duryea 1985). Controlled mycorrhization of plants in nurseries (Nouaïm and Chaussod 1994), for example, could significantly increase growth (Ouallal et al. 2018, Sellal et al. 2017) and survival after outplanting (Echairi et al. 2008).

Sellal et al. (2016) described an indigenous endomycorrhizal complex encountered in 15 argan groves in southwest Morocco. The Water and Forest services have tried to introduce argan trees by planting them in these areas, but success has been mixed (Harrouni et al. 1999); the mycorrhizal status of introduced argan trees in these areas is unknown. Thus far, no research has been done on establishment of argan trees in northern Morocco. The aim of this work was to study AMF levels and colonization potential of the rhizospheric soils of introduced and natural argan trees in the northwest regions of Rhamna and Essaouira.

Materials and Methods

Study Sites

The study was carried out in two sites in northwest Morocco: Smimou (province of Essaouira; 31° 29′ 40″ N, 9° 28 57″ W) and Bounaga (Sidi Bou Othmane province of Rhamna; 31° 54′ 12″ N, 7° 56′ 32″ W). The Smimou site is located at an altitude of 665.5 m and has a dry climate with average annual precipitation of 251.1 mm, intense summer heat reaching 45 °C, and winter lows of 5 °C. The Bounaga site is located at an altitude of 450 m, has a warm Mediterranean climate with dry summers, and is characterized by an average annual rainfall of 250.9 mm, summer heat reaching 37 °C, and winter lows of 5 °C.

Soil and Root Sampling

Soil samples were collected in May 2017 at the base of five introduced argan trees (2 kg per tree) at the Bounaga site (figure 1) and five natural argan trees from the Smimou site (figure 2). Soil samples from each site were then composited. Additionally, samples of very fine roots, likely to be mycorrhizal and easily observable under the microscope, were collected from each tree at the same time as soil collection.

AMF Spore Extraction and Evaluation of AMF in Soil Samples

AMF spores were extracted from the soil samples according to the wet sieving method described by Gerdemann and Nicolson (1963). In a 1 L beaker, 100 g of each composite soil sample was submerged in 0.5 L of running water and stirred for 1 minute with a spatula. After 10 to 30 seconds of decantation, the supernatant was passed through four superimposed sieves with decreasing mesh sizes (500, 200, 80, and 50 µm). This procedure was repeated twice.

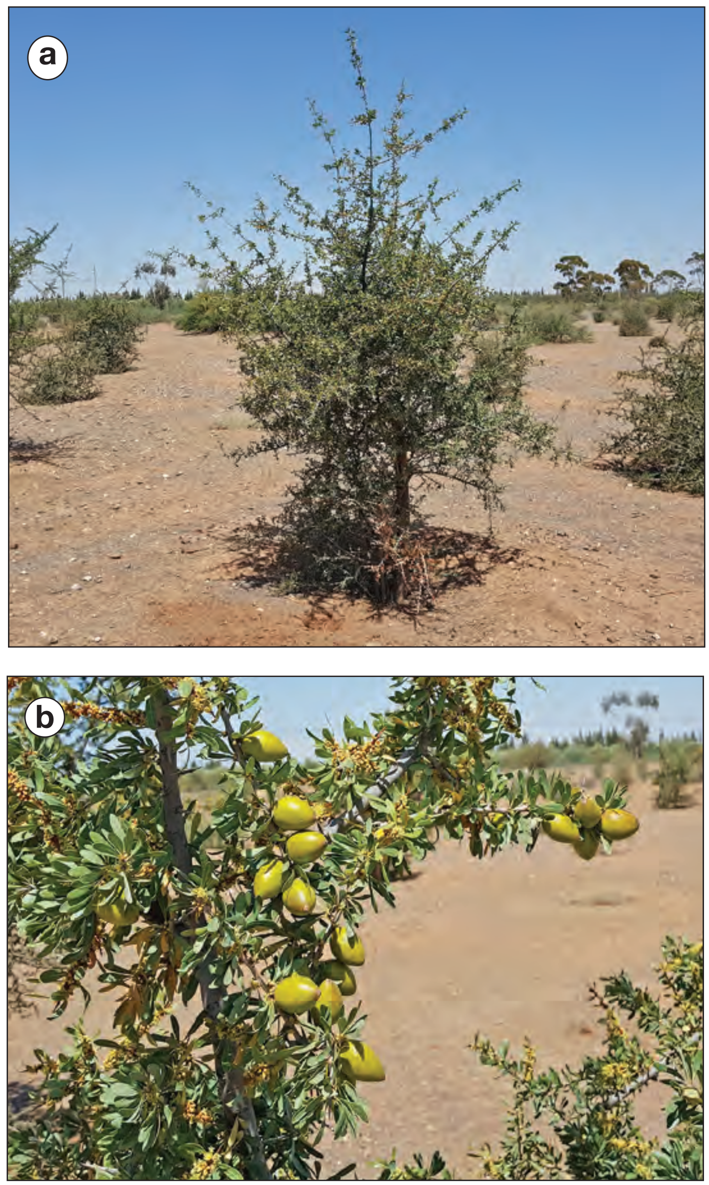

Figure 1. Soil and root samples were collected from argan trees introduced to the Bounaga site (Rhamna province) of northwestern Morocco. (Photos by M. Ouajdi 2017)

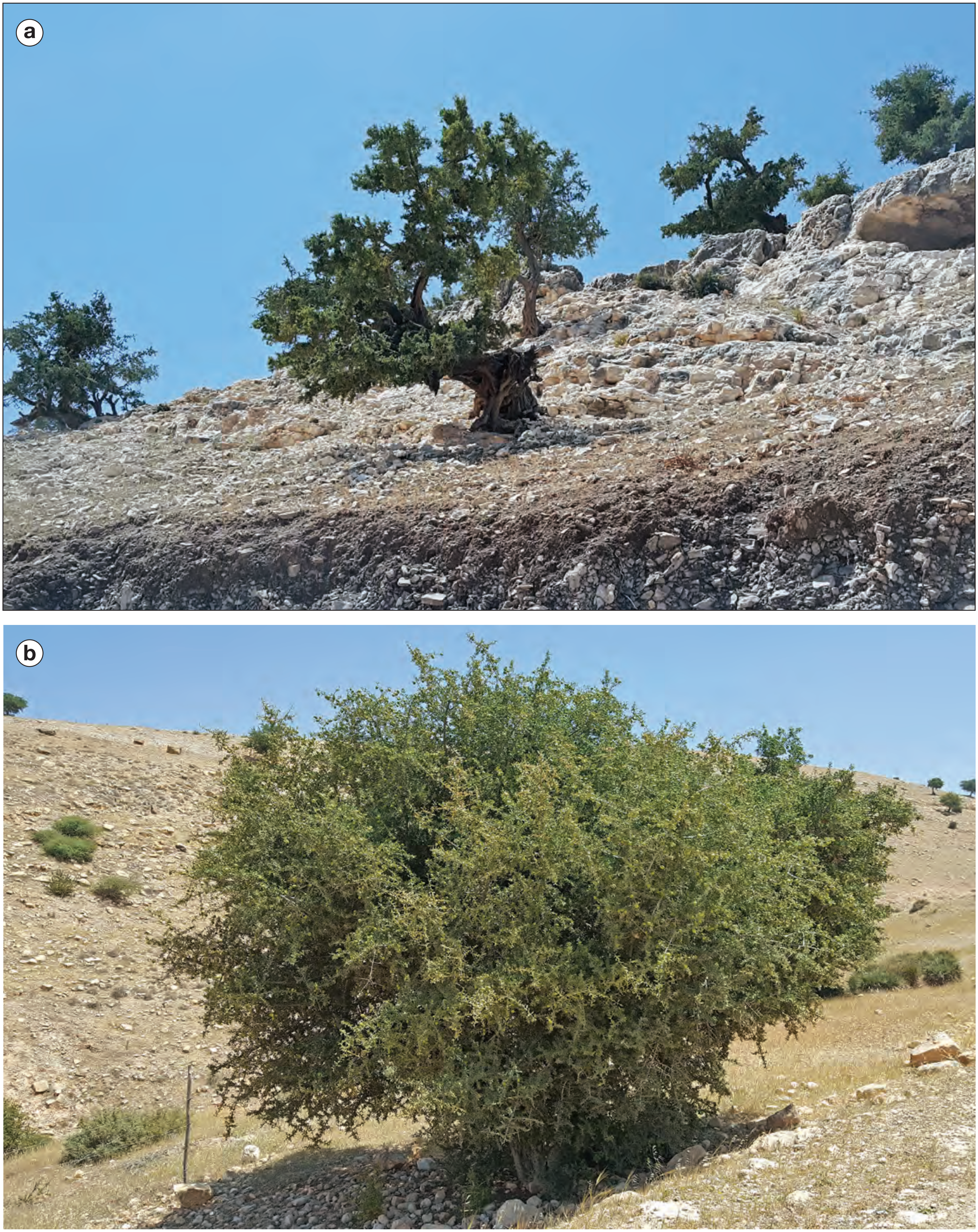

Figure 2. Soil and root samples were collected from argan trees introduced to the Smimou site (Essaouira province) of northwestern Morocco. (Photos by M. Ouajdi 2017)

Table 1. To estimate infectious propagules of AMF in the rhizospheric soils of argan trees at the two sites using the most probable number method (MPN) substrates were prepared with nine dilutions (n=3).

Dilution	Proportion of non-sterile soil	Quantity of non-sterile soil (g)	Quantity of sterile soil (g)
1	1\1	100.000	0.000
2	1\2	50.000	50.000
3	1\4	25.000	75.000
4	1\8	12.500	87.500
5	1\16	6.250	93.750
6	1\32	3.125	96.875
7	1\64	1.562	98.438
8	1\128	0.781	99.219
9	1\256	0.390	99.600
Control	0	0.000	100.000

The content retained by the 200, 80, and 50 µm sieves was distributed 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 and Sanders 1982). The mixture was quickly stirred, and the tube returned to the centrifuge for 1 min at 9000 rpm. The supernatant was then poured over a 50 µm mesh screen. The substrate obtained was rinsed with distilled water to remove the sucrose, then disinfected with an antibiotic solution (Streptomycin). The spores were then recovered with a little distilled water in an Erlenmeyer flask. Species richness was determined by the total number of species observed from each sampling site. The spores were observed using an optical microscope and identified morphologically according to several criteria including spore color, shape, size, and surface ornamentation. Spore identification was performed according to descriptions provided by the International Collection of Arbuscular Vesicular Mycorrhizal Fungi (INVAM 2017).

The number of infectious propagules of AMF in the rhizospheric soils of argan trees at the two sites was estimated using the most probable number method (MPN) based on Declerck et al. (1999) method. Sorghum seedlings were used as a mycotrophic plant. This plant is highly sensitive to colonization by AMF and exhibits rapid root development (Utobo et al. 2011). A dilution factor of 2 was used with 9 dilutions (table 1). Three replicate pots of each dilution were prepared for 100 g soil sampled from each of the two sites. In addition, a control pot containing only sterile soil was included. A sorghum plant was transplanted and grown for 6 weeks in each pot. Sorghum seeds were disinfected with sodium hypochlorite at 10 percent concentration for 15 min and rinsed 3 times with distilled water before being germinated. A 6-day-old plant was transplanted into each pot. Pots were placed in a greenhouse and watered with distilled water as needed. After 6 weeks, plants were removed from pots and assessed for AMF colonization and MPN calculations were made using the formula from Fisher and Yates (1949):

$$\text{Log MPN} = (x.\log a) - k(y,S)$$

Where:

x = the average number of mycorrhizal plants (total divided by number of replications)

S= the number of dilution levels

a = the dilution factor

y = the average of nonmycorrhizae plants (S-x).

K is given by the tables of Fisher and Yates (1949) as a function of y and S.

Evaluation of AMF Root Colonization

Fine roots collected from each argan tree, as well as roots collected from sorghum plants grown in rhizospheric soils, were prepared according to the method of Koské and Gemma (1989). They were first washed with water, cut in 1- to 2-cm lengths, immersed in a 10 percent KOH solution, then placed in an oven at 90 °C for 1 hour to remove intracellular constituents. The roots were then rinsed and transferred to a hydrogen peroxide solution (a few drops of hydrogen peroxide in 100 ml of distilled water) for 20 min at 90 °C until they whitened. The roots were then stained by submersion in 0.05 percent brilliant cresyl blue (modified from Philips and Hayman 1970) at 90 °C for 15 min.

After a final rinsing, 30 colored argan root fragments from both Smimou and Bounaga sites were randomly chosen and mounted in groups of 10 to 15 in glycerin between blade and cover slip (Kormanik and McGraw 1982). The remaining roots were kept in water or glycerol acid. Under a microscope, each fragment was carefully examined over its entire length, at magnifications of 100x and 400x to observe and record any mycorrhizal structures: arbuscules, partitions of hyphae, vesicles, intra- and intercellular hyphae, extramatric hyphae, and endophytes.

The presence of AMF arbuscules and vesicles were assigned a mycorrhization index (Derkowska et al. 2008): 0=absent; 1=trace; 2=less than 10 percent; 3=11 to 50 percent; 4=51 to 90 percent; 5=more than 91 percent.

Mycorrhization frequency (MF), estimates the proportion of the host plant's fine roots colonized by AMF.

$$MF = 100 (N - N0) / N$$

Where:

N = total number of mycorrhizal root fragments observed

N0 = number of non-mycorrhizalroot fragments

Mycorrhization intensity (MI) estimates the overall concentration of AMF colonization in the entire fine rootsystem:

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

Where:

n5, n4, n3, n2, and n1 indicate the number of fragments denoted 5, 4, 3, 2, and 1 on the mycorrhization index, respectively.

Arbuscule abundance (A) is calculated as follows:

$$A= (100mA3 + 50mA2 + 10mA1) /100$$

Where:

A1: 1 to 10 percent, A2: 11 to 50 percent, A3: 51 to 100 percent

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

Vesicle abundance (V) is calculated in the same way as that of the arbuscular abundance.

$$V = (100mV3 + 50mV2 + 10mV1) / 100$$

Statistical Analyses

The statistical processing of the data focused on the analysis of variance with a single classification criterion (ANOVA1). IBM SPSS 21.0 software was used for these statistical analyses. Each site was analyzed separately. Although the two sites could not be compared due to variations in environment and lack of both natural and planted trees at each site, observational similarities and differences are noted.

Results and Discussion

AMF Spore Extraction

The concentration of spores in the rhizosphere of introduced argan trees was approximately 22 spores per 100 g of soil and include 11 morphotypes, the most dominant of which are *Acaulospora gedanensis* and *Claroideoglomus etunicatum*. On the Smimou site, rhizosphere spores around natural argan trees averaged 45 spores per 100 g soil and include 6 species, with an abundance of *Acaulospora bireticulata*, *Dentiscutata nigra*, and *Gigaspora margarita*. The two sites have two species in common: *Endogone versiformis* and *Rhizophagus intraradices* (figure 3 and table 2). The duration of mycorrhization depends on the host, the infectious power of the mycorrhizogenic fungus, and the growing medium (Plenchette and Fardeau 1988). In the Ait-Baha region, Elmaati et al. (2015) noted 1127.66 spores per 100 g soil indicating that spore density in the northwestern Rhamna region studied is low compared with that of southern Morocco.

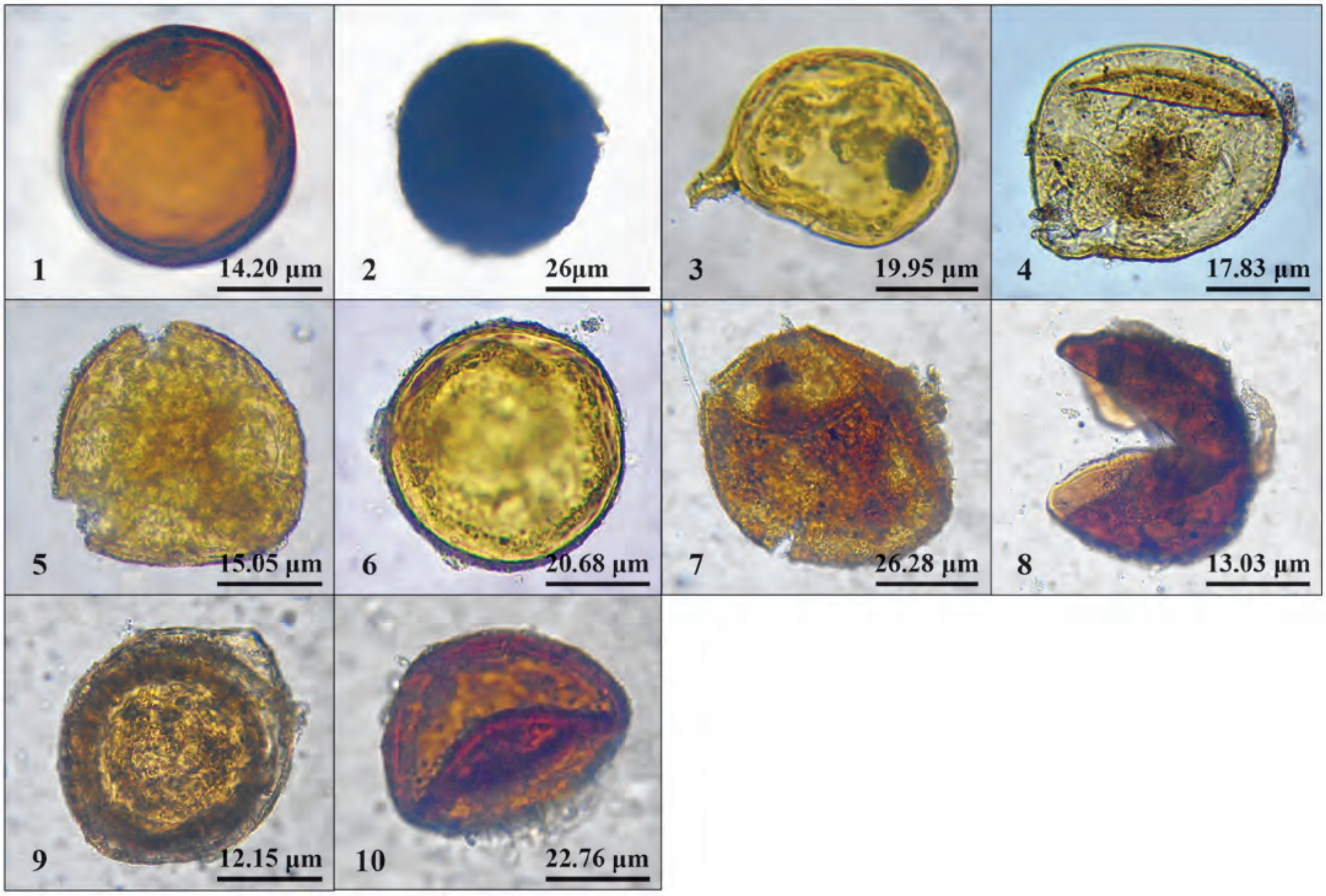

Figure 3. Some morphotypes of endomycorrhizal fungi isolated from the rhizosphere of argan trees on two sites in northwest Morocco (see also table 2). (Photos by S. Maazouzi 2018)

Table 2. Morphological characteristics of some species of endomycorrhizal fungi isolated from the rhizosphere of argan trees (see also figure 3).

Number	Name	Form	Color	Average height	Wall surface	Hypha length	Number of walls
1	*Claroideoglomus etunicatum*	Globular	Orange	83.3	smooth	-	2
2	*Dentiscutata nigra*	Globular	Brown	99.9	granular	-	2
3	*Rhizophagus fasciculatus*	Oval	Yellow	84.5	granular	17.4	2
4	*Glomus intraradices*	Globular	Yellow	99.9	granular	-	2
5	*Gigaspora margarita*	Globular	Yellow	89.6	granular	-	2
6	*Funneliformis geosporum*	Globular	Yellow	86.8	smooth	-	2
7	*Glomus intraradices*	Oval	Light brown	109.6	granular	-	2
8	*Funneliformis verruculosum*	Oval	Dark brown	67.9	granular	10	2
9	*Glomus aggregatum*	Globular	Light brown	68.9	granular	-	2
10	*Endogone macrocarpa*	Dark brown	Dark brown	133.2	granular	-	2

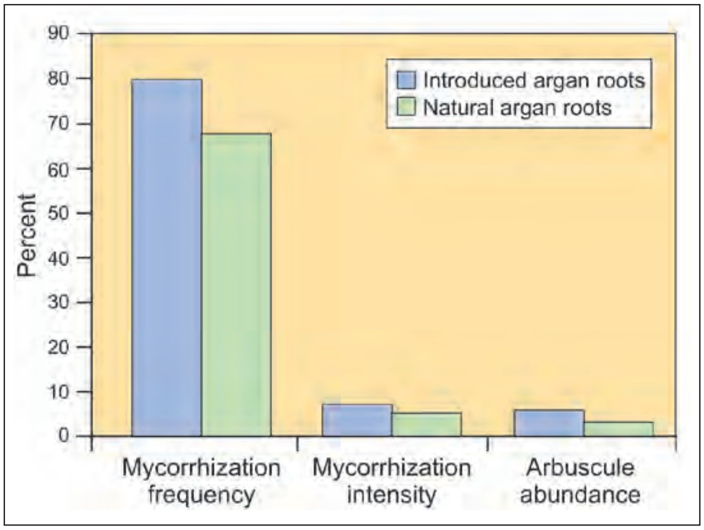

Figure 4. Mycorrhizae frequency (MF), Intensity (MI), and arbuscular content (A) of argan tree roots for introduced trees (Bounaga site) and natural trees (Smimou site).

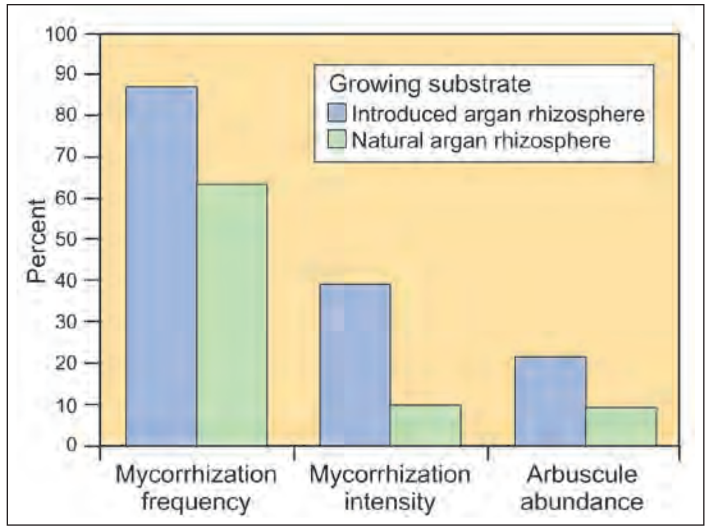

Figure 6. Frequency (MF), intensity of mycorrhization (MI), and arbuscular contents (A) of sorghum roots grown in rhizoshperic soils of argan trees.

AMF Root Colonization

The argan trees introduced into the Bounaga site seem to have adapted to the ecological conditions of the region, as confirmed by the AMF diversity and frequency found in the rhizosphere around sampled trees (figure 4). In other studies, mycorrhization frequencies were 100 percent for natural argan trees from the Taroudante and Toufalazte sites (Sellal et al. 2016).

Sorghum roots grown in rhizospheric soils collected around natural and introduced argan trees were mycorrhizal with characteristic AMF structures (figure 5). MF, MI, and A tended to be higher in sorghum roots growing in the rhizospheric soils of the introduced argan trees, compared with those grown in the soil of natural argan trees (figure 6). The number of spores isolated from the rhizosphere of sorghum plants also varied by dilution factor (table 3). The substrate from the soils of natural argan trees (1/1 dilution) included 6 different morphotypes: *Dentiscutata nigra* (7 spores), *Rhizophagus intraradices* (5 spores), *Endogone versiformis* (8 spores), *Glomus aggregatum* (3 spores),

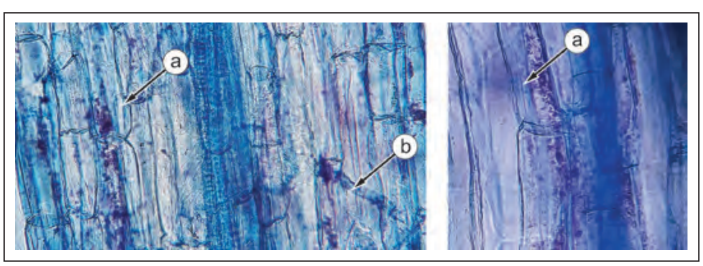

Figure 5. Different structures of arbuscular mycorrhizal fungi observed in the roots of sorghum plants: (a) arbuscules and (b) extracellular hyphae (× 400). (Photos by S. Maazouzi 2018)

Endogone macrocarpa (2 spores), *Funneliformis verruculosum* (3 spores). In the substrate of sorghum plants from introduced argan trees at the same dilution, 5 morphotypes were found: *Dentiscutata nigra* (6 spores), *Glomus* sp. (4 spores), *Gigaspora* sp. (3 spores), *Pacispora* sp. (2 spores), *Endogone versiformis* (5 spores).

MPN Soil Propagules

The MPN of the rhizospheric soil of introduced argan trees was 7.14 propagules per 100 g soil and that of the rhizospheric soil of natural argan trees was 1.78 propagules per 100 g of soil. Other studies have reported varying propagule concentrations in rhizospheric soil around other species, including 100 propagules per g around olive trees (*Olea europaea* L.) (Mekahalia 2013), 360 propagules per g around onion (*Allium cepa* L.) (Sow et al. 2008), and 353 propagules per 100 g around palmier (*Phoenix dactylifera* L.) (Meddich et al. 2015). According to Requena et al. (1996), the number of propagules encountered in a soil type depends on the diversity of plant species and on the region's dominant ecological factors (Sanon et al. 2006). Increasing plant cover also causes a decrease in the number of infectious propagules (Richter et al. 2002).

According to Adelman and Morton (1986), MPN is a very interesting technique for estimating the mycorrhizogenic potential of a given soil, and the experimental conditions must reflect the conditions on the

Table 3. The number of spores of arbuscular mycorrhizal fungi varied by site and dilution.

Dilutions	Natural argan trees	Introduced argan trees
1/1	28	20
1/2	15	10
1/4	11	8
1/8	9	5
1/16	6	4
1/32	3	2
1/64	2	1
1/128	0	0
1/256	0	0

ground. Thus, the higher the substrate dilution, the greater number of spores present. According to Neffar (2012), the MPN is variable during the year and the number of propagules depends on plant species diversity (Sanon 2006). The same result was noted by El Gabardi et al. (2019a, 2019b, 2019c), who found that phosphate washing sludges colonized by different plant species had a large number of spores of endomycorrhizal fungi and a number of infectious propagules estimated by PIM and MPN techniques.

In Morocco, the use of AMF on argan plants in the nursery may become common practice. Mycorrhizal plants produced in nurseries tend to have very developed root systems and are therefore able to tolerate drought conditions after planting (Nouaïm and Chaussod 1997). According to Sellal et al. (2017) and Ouallal et al. (2018), argan plants inoculated with AMF are more vigorous and can adapt to different soil and climatic conditions once replanted.

Conclusion

The results of the present study showed a diversity of endomycorrhizal fungal species in the rhizosphere of argan trees introduced into the Rhamna region. This diversity is significant compared to that encountered in the rhizosphere of natural argan trees at the Smimou site. These results demonstrate that introduced argan trees form functional and beneficial symbiotic associations with endomycorrhizae over time. Mycorrhization of argan plants at the nursery stage is likely to increase plant resistance to the harsh conditions they may encounter after being outplanted to the field.

Acknowledgments

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