Joint Meeting of the Intertribal Nursery Council, Western Forest and Conservation Nursery Association, and Intermountain Container Seedling Growers' Association

Bend, Oregon

September 11 to 13, 2012



<u>Joint Meeting of the Intertribal Nursery Council, Western Forest and Conservation</u> r Seedling Growers' Associatior <u>rsery Association, and Intermount</u>

Ponderosa pine drawing by Lorraine Ashland, College of Natural Resources, University of Idaho

The Importance of Good Seed

Robert P. Karrfalt

Robert P. Karrfalt is Director, National Seed Laboratory, USDA Forest Service, Dry Branch, GA 31020; email: rkarrfalt@fs.fed.us

Karrfalt RP. 2013. The importance of good seed. In: Haase DL, Pinto JR, Wilkinson KM, technical coordinators. National Proceedings: Forest and Conservation Nursery Associations—2012. Fort Collins (CO): USDA Forest Service, Rocky Mountain Research Station. Proceedings RMRS-P-69. 49-52. Available at: http://www.fs.fed.us/rm/pubs/rmrs_p069.html

Abstract: The importance of seed to human culture and conservation of the natural world is briefly discussed. The effect of seed on seedling quality and cost is described through several examples and illustrations.

Keywords: seed germination, seed vigor, seedling cost

Introduction

What is a seed? Biologically, it is an embryo that is often accompanied by some nutritive tissue for the embryo (endosperm or gametophyte) and enclosed in a seed coat. In most cases, seeds are the source of plants for regenerating native plant communities. From a philosophical and spiritual point of view, seeds are our past, present, and future. They provide cultural connections to past generations of people through foods and other plant materials. Natural selection worked in plant populations over the centuries to produce well adapted plants, and this adaptation is delivered to us in the seeds of today. Therefore, seeds sustain us in our present. Climates are changing. The strongest and most basic response to mitigating the risks of climate change is to preserve a maximum of genetic diversity among and within species of plants. Therefore, our plants determine what we can become in the future. The quality and abundance of our plants depends on high quality seeds.

Good Seed Defined

Good seed will have high germination, high vigor, and produce genetically adapted plants.

Germination, expressed as a percentage, is the ratio of the number of seeds that produce a normal seedling to the number of seeds that were sown. What is considered high germination will be relative to species, year, and nursery protocols. Pine and spruce will generally have higher germination than will true firs. In a bareroot nursery, germination as low as 90% might be considered high, but in a container nursery, germination below 90% begins to significantly affect cost of seedlings.

A *high vigor* seed is one that will germinate rapidly and perform better under suboptimal conditions. A second definition for high vigor seed is that it will maintain high germination over many years in storage.

The final characteristic of good seed is being *genetically adapted*. This is important because regardless of germination or seed vigor, if the plants are not adapted well to the growing conditions, they (and the new plant community they form) will fail to thrive. Only well-adapted plants or plants with the capacity (i.e. high genetic variability) to adapt to new conditions will survive.

Germination %	Seeds/cell	% Filled cells	% double seedlings	Some Consequences
100	1	100	0	Life is good
98	1	98	0	Life is still pretty good
95	1	95	0	5% space lost/cost per seedling up
90	1	90	0	10% space lost/cost per seedling up
90 + Thinning	2	99	81	Thinning required, higher seed costs
85	1	85	0	15 % space lost/cost per seedling up
85 + Thinning	2	98	72	Thinning required, higher seed costs

Table 1. The consequences of decreasing seed germination in a container seedling nursery.

The Economic Case for High Quality Seeds

Table 1 gives an overview of the effect of changing seed quality on container seedling production. As the table shows, 100% germination is ideal as all the cells are filled and no expense is incurred that does not return a seedling. As germination drops, even to 90%, significant losses begin to occur. While 90% might seem high, the cost to maintain the 10% of the cells that are empty has to be added to the cost of seedlings, and our production is decreased by 10%. One solution is to put two seeds in each cell. In this case the number of empty cells drops to 1%.

The number of filled cells is computed in this manner: at 90% germination, the probability of a seed not germinating is 10% (100 – 90). So the chance that the two seeds in any one cell both fail to germinate is 0.10 x 0.10 or 0.01. 100 cells – 1 cell that is empty makes for 99 full cells. However, seed costs had to be doubled because we used twice as many seeds. In addition, 81 cells have two seedlings and one seedling must be thinned out, which increases labor costs. There are 81 cells with two seedlings because the probability or chance that both seeds in a cell germinate is the product of the likelihood that each one geminates which is 0.90 x 0.90 or .81 (81% or 81 cells in 100 cells have two seedlings).

Table 2 illustrates more specifically how seed quality affects seedling costs. On the first line in this table 100% seed germination and a cost of \$200 per thousand is taken as the baseline for all other comparisons. With 100% germination then a seedling would cost \$0.20. The second line of Table 2 shows that if germination is 98%, and one seed is sown per container cell, then 980 seedlings are produced. This is 20 seedlings less per 1000 seeds sown than

if germination was 100%. Costs of production remain the same so now the \$200 per 1000 seedlings has to be spread over 980 seedlings. \$200/980 seedlings gives a cost of \$0.204 per plant, an increase of \$0.004 per plant (0.204 - 0.200). This amounts to a 2% increase cost per plant (0.004/0.20 = .02). In line 3 of Table 2, germination is further reduced to 95% while still sowing just one seed per cell. Repeating the calculations used in line 2 with this 95% germination shows that cost have increased 5.5%. Dropping germination to 90% with single seed sowing raises costs up 11%. An additional drop in germination to 85% raises seedling costs 17.5%. Cost increases are almost directly proportional to drops in germination.

Sowing two or more seeds per cell is one strategy to compensate for lower seed germination. In our example, double sowing reduces cost increases by half (line 6 of Table 2). However, to achieve this we had to waste 720 seeds for every 980 plants produced. That is 73% increase in the amount of seeds required. This strategy requires an abundant supply of seeds, and could lead to seed shortage if certain sources are harder to acquire. The thinning to remove the double seedlings also requires good timing to avoid major disturbance of the seedling that is kept.

Transplanting the thinned seedlings can recover some of the seed loss. In our example, line 7 of Table 2, seedling production costs are comparable to costs from double sowing and throwing away the extra seedlings. This operation is very time sensitive as germinates have a narrow window during which they can be transplanted without stunting or death occurring. This is not a very common practice because it is difficult to do successfully.

Detailed calculations for Table 2 are presented at the end of this paper. These calculations are only for illustrating general trends

Germination %	Seeds/cell	% Filled cells	% double seedlings	Cost per 1000 plants/ cost per plant	% Cost increase over 100% germination
100	1	100	0	\$200/\$0.20	0
98	1	98	0	\$200/\$0.204	2
95	1	95	0	\$200/\$0.211	5.5
90	1	90	0	\$200/\$0.222	11
85	1	85	0	\$235/\$0.235	17.5
85 + Thinning	2	98	72	\$213.50/\$0.214	8.75
Transplanted Seedlings	red - 100		0	\$214/\$0.214	7

Table 2. The cost of seedlings increases in a container nursery with decreasing seed germination.



Figure 1. Relative seedling costs and quality vs. seed quality.

and each nursery would need to make these calculations in accordance with the local conditions and financial constraints.

Figure 1 graphically illustrates the general relationship between seed quality and the cost and quality of seedlings. Better quality seed results in lower seedling costs and higher seedling quality.

The Role of Seed Vigor

Vigor is the ability of a seed to germinate under adverse conditions and/or produce vigorous seedlings. High vigor seeds also will store better than lower vigor seeds. Therefore, high vigor seeds are needed for routine seed banking and especially for genetic conservation through long term seed storage. High viability usually means high vigor, but not always. This can be illustrated as in Figure 2.



Figure 2. Fractions of viability and vigor of a seed lot.

All seed lots are made up of three portions: live high vigor seeds, live low vigor seeds, and dead seeds. Vigor tends to decline faster than germination. Therefore, germination in the nursery can take a sudden drop. This can be predicted with a current germination test. A significant drop in germination, usually more than 5%, would indicate vigor has probably changed to a greater degree. Although in tree seeds there is not an official test for vigor, paired tests have often been useful in detecting seed lots of low or declining vigor. A paired test, sometimes called a double test, is where an unstratified and stratified test are both conducted on the same sample submitted for testing. Alternatively, two stratified tests can be conducted but of different stratification lengths (e.g. one test

with 30 days stratification and one with 45 days stratification.) If both tests are equal in viability or the one with longer stratification is higher, the seed lot is of good vigor. If the test with longer stratification is inferior, then the seed lot is likely to be declining in vigor. It should either be used as soon as possible or not at all. Which alternative to choose will depend on the circumstances.

Summary

Good seeds are the foundation of native plant work. Good seeds enable significant benefits in cost control and higher quality plants. Even in a noncommercial environment, poor seeds will consume more resources than good seeds, resources that could and likely should go to furthering the main objectives. Good seeds ensure that plant production targets are met and that restoration projects will be completed and successful. For orthodox seeds, good seeds cost less to store and store for longer periods of time than poorer quality seeds. This better storability is very important for routine seed banking and especially important for long term seed storage for genetic conservation. Good seeds ensure our future survival and prosperity, and that of generations of people yet unborn.

Detailed Calculations for Table 2

Germination = 95%, 1 seed sown per cell, production costs of \$200 per 1000 cells.

Price: 950 plants produced, \$200/950 = \$0.211/plant Price increase: \$0.211 - \$0.200 = \$0.011 per plant, \$0.011/\$0.20 = 5.5%

Germination = 90%, 1 seed sown per cell, production costs of \$200 per 1000 cells.

Price: 900 plants produced, \$200/900 = \$0.222/plant Price increase: \$0.222 - \$0.200 = \$0.022 per plant, \$0.022/\$0.20 = 11%

Germination = 85%, 1 seed sown per cell, production costs of \$200 per 1000 cells.

Price: 850 plants produced, \$200/850 = \$0.235/plant Price increase: \$0.235 - \$0.200 = \$0.035 per plant, \$0.035/\$0.20 = 17.5%

Germination = 85%, 2 seed sown per cell, production costs of \$213.50 per 1000 cells.

Price: 980 plants produced per 1000 cells sown, \$200/980 = \$0.204/plant

Seed costs double: 2000 seeds are needed. At \$300/pound and 50,000 seeds per pound one seed costs 0.006/seed, or 1000 additional seeds x 0.006 = 6.00. This 6.00 of additional seed produces 980 plants. Therefore, per seedling cost of additional seed is 6.00/980 seedlings = 0.0061/seedling. ($100 - (.15 \times .15) = 100 - .02 = .98$ chance of filled cell. 1000 cells x .98 = 980 seedlings.)

Thinning costs: Minimum wage of \$7.25 per hour/3600 seconds in an hour = 0.002/sec. Thinning rate of 5 seconds per cell, 720 cells to thin = 5 x 720 = 3600 seconds, 3600 seconds x 0.002/ sec = 7.25. 720 cells to thin is the number of double seedlings which from Figure 1 is $0.85 \times 0.85 = .72$ or 72%. The chance that one seed germinates is .85 and the chance the second seed in the double sow germinates is .85. Chance that both germinate is the product. These thinning costs are shared over the 980 seedlings produced. Cost per seedling for thinning is 7.25/980 = 0.0074price increase per seedling.

Total cost increase for extra seeds and thinning is: 0.0061 + 0.0074 = 0.0135 per seedling or 13.50 per thousand seedlings. From above, the base cost of 980 seedlings was 0.204 per seedling or an increase of 0.004 per seedling in base cost from what they were

if germination was 100%. Total cost increase from all sources is, therefore, \$0.0175.

Price increase: \$0.2175 - \$0.200 = \$0.0175 per plant, \$0.0175/\$0.20 = 8.75%

Germination = 85%, transplant the extra seedlings.

Here, there is the attempt to save the thinned seedlings and recoup the extra seed cost. If it takes 10 seconds to transplant a seedling and labor is the minimum of \$7.25 per hour or \$0.002per second (\$7.25/hour divided by 3600 seconds/hour), then transplanting 1000 seedlings would cost \$20. Add this to the base cost of \$200 per thousand to produce seedlings and subtract the \$6.00 in seed cost which was part of the last example and we arrive at \$214 per thousand transplanted seedlings. These seedling are 7% more expensive than seedlings had it been possible to have 100% germination and produce seedlings for \$200 per thousand (14/200 = .07). Timing the transplanting of seedlings is very critical and would require an adequate labor supply to get the job done quickly. These costs do not also take into account the number of seedlings that might become stunted or die because of transplanting.

Low-Cost, High-Tech Seed Cleaning

Robert P Karrfalt

Robert P Karrfalt is Director, National Seed Laboratory, USDA Forest Service, Dry Branch, GA 31020; email: rkarrfalt@fs.fed.us

Karrfalt RP. 2013. Low-cost, high-tech seed cleaning. In: Haase DL, Pinto JR, Wilkinson KM, technical coordinators. National Proceedings: Forest and Conservation Nursery Associations—2012. Fort Collins (CO): USDA Forest Service, Rocky Mountain Research Station. Proceedings RMRS-P-69. 53-57. Available at: http://www.fs.fed.us/rm/pubs/rmrs_p069.html

Abstract: Clean seeds are a great asset in native plant restoration. However, seed cleaning equipment is often too costly for many small operations. This paper introduces how several tools and materials intended for other purposes can be used directly or made into simple machines to clean seeds.

Keywords: seed cleaning, seed drying, seed storage

Introduction

Clean seeds are important in restoration for many reasons. Germination is more accurately estimated on cleaned seed lots, and until seed tests can predict how many plants to expect, it cannot be known how well establishment protocols work. Cleaner seed lots can be examined for weeds more easily, helping ensure that weeds are not brought onto the restoration site. Cleaned seeds also require less storage space.

Often, however, equipment to clean seeds is too costly for small operations. With a good understanding of the principles of seed cleaning and an average mechanical ability, a person can construct relatively inexpensive devices that in many cases are comparable to high priced equipment. Principles of seed handling will be briefly presented here. More detail is provided in the introductory chapters of the Woody Plant Seed Manual (Bonner and Karrfalt 2008). All procedures and apparatuses discussed here have actually been tried by the author or the author observed them being used by others.

Seed Drying

Texts on seed management universally emphasize controlling seed moisture as the single most important action in preserving seed quality in storage. Therefore, it is necessary to be able to dry seeds rapidly and efficiently. For millennia people dried seed by spreading them out in the sun. This works well if you have enough sun (when you need it) and have the space to spread out the seed. Also, sun drying requires protection from predation, contamination, and wind. Even when done indoors this process is often inadequate. Therefore, effective seed drying often involves pressurized dryers. In a pressurized dryer air is forced through mesh bottom containers of seeds giving uniform and rapid drying with minimal labor because there is no turning of seeds.

Figures 1 and 2 show dryers made using stacks of paint strainers for the dryer trays. Paint strainers come in bucket size and barrel size with a variety of mesh sizes for the bottoms. Using the most open mesh size possible is best to minimize air resistance. Less air resistance allows more trays and more seed on the dryer in one charge. Very small seeds of course require using a very fine mesh. As long as a gentle air flow is felt coming from the top tray of seeds, then the dryer is properly loaded.





using barrel sized paint strainer.

Figure 1. Pressurized seed dryer Figure 2. Pressurized seed dryer using bucket sized paint strainer.

The trays need to be sealed tightly together within the stacks. Otherwise, the air will come out the sides of the tray stack since air flow follows the path of least resistance. The small travs must be separated with a collar made from a pail lid. Figure 3 shows how this collar is constructed. The opening in the lid is cut with a sharp utility knife. A piece of quarter inch (0.64 cm) foam weather stripping seals the edges.



Figure 3. Collar for sealing bucket sized paint strainers when stacked on the pressurized dryer.

The dryer fans must operate against the high resistance of the seeds and trays. High resistance would be 1.5 to 2.0 inches (3.8 to 5 cm) of water column. An induced draft blower motor is a relatively inexpensive (under \$150) motor that will do this. The blower motor is mounted in the dryer plenum (bucket, barrel, or box at the base; Figure 4) so it draws air from outside the plenum and forces it up through the stack of trays. The name "pressurized dryer "comes from this pressurizing of the stack of trays.

The final step in making the dryer work is to supply it with dry air; air that is ideally at 30% relative humidity. Some environments naturally are very dry, while in others it is necessary to use supplemental heat, a dehumidifier, or a dehumidifier and an air conditioner



Figure 4. Induced draft blower installed in the pressurized seed dryer.

in combination. When using one of supplemental methods to dry air, the dryer must be placed in a closed room. It is important to measure the relative humidity of the air entering the dryer. This is done with either a hygrometer or a psyc hrometer, instruments that can be purchased or that you can make using instructions from the internet.

Extracting Seeds

Extraction is one of the first steps in producing native plant seed. Drying is often part of this because drying makes the fruits or cones open up or become brittle enough to break apart. A shop vacuum works very well to extract aspen or milkweed seeds from the fluff. Starting with a clean vacuum, simply vacuum up the seed with fluff attached (Figure 5). Then open the vacuum canister and lift out the fluff which collects at the top and the extracted seed will be in the bottom of the canister, ready for cleaning.



Figure 5. Vacuuming milkweed seeds from pods separates the fluff from the seeds.

Rubber palmed gloves or rubber faced blocks and boards work well to rub appendages off of seeds. Kitchen blenders with the blades covered with rubber or plastic tubing make good cleaners for fleshy fruits.



Figure 6. A small hand operated tumbler for extracting seeds from dried open fruits or cones.

The seed dryers will be needed to dry the finished seeds. Tumbling is another way to separate seeds from the fruit or cone. Figure 6 shows a small homemade tumbler.

Dimensional Separations

Once extracted, the seeds are usually full of leaves, stems, and other trash. Separating seeds and trash by dimension is one important method. Most dimensional separation is done with screens. There are two basic screening operations, scalping and sieving. Scalping is when the seeds pass through the screen and the trash stays on top. Sieving is when the seeds remain on top of the screen and the trash passes through. Different sizes of hardware cloth, window screens, or kitchen sieves can help. For small seeds and sieving out very fine trash, the paint strainers used with the seed dryers (as described above in the section about drying) are useful and come in 4 different mesh sizes. Ready-made inexpensive screens offer only a limited range of opening sizes. The patient person might use a set of twist drills and perforate some sort of rigid sheet material to make more screen sizes. Hand screens can be purchased for about \$50 each and come in over 100 sizes. These are much more expensive than home kitchen utensils but will last for decades if taken care of.

Weight Separation

Much trash is lighter than the seeds and is taken away when the seed passes into a carefully regulated column of flowing air. There are two ways to move the air, push it or pull it. Blowers push air and aspirators pull air. Aspirators have been relatively simpler to construct compared to blowers so two types of aspirators will be discussed here: the pipe aspirator and the box aspirator.



Figure 7. A pipe aspirator made from a shop vacuum and 2 inch (5.1 cm) diameter pvc drain pipe.

Pipe Aspirator

A very simple and easy-to-build aspirator can be assembled from pvc DWV (Polyvinyl chloride, drain waste vent) pipe, a small shop vac, and long neck funnel, and a tray (Figure 7). The neck of the funnel needs to extend to just below the bottom of the pipe fittings. The vacuum cost \$20 and the pipe about the same, making the total cost \$40. The air gate detail is shown in Figure 8. Use pipe the same diameter as the inlet opening for the vacuum cleaner. Smaller diameter pipe might stress the motor and larger diameter would cause too great a pressure drop to maintain sufficient vacuum. The machine works by turning on the vacuum cleaner, gently feeding seeds down the funnel on the top, and closing the air gate until only trash is lifted and clean seed (more or less) falls out the bottom into the tray. You can then increase the feed rate to as fast as the machine can handle and still deliver the clean seeds to the tray at the bottom. Feeding in too many seeds at once will result in trash caught under the seeds and falling



Figure 8. Detail of the sliding doors on the pipe aspirator's air gate.

down the chute rather than being lifted up into the vacuum. If you find too many seeds in the vacuum, then too much air was used and the air gate needs to be opened up enough to prevent the seeds from being lifted with the trash. One problem that can occur with this aspirator is the buildup of static electricity along the pvc. It may need regular wiping with an antistatic cleaner or window cleaner. Be sure to have the parts thoroughly dried before putting seeds back into the cleaner.

Box Aspirator

A little more sophisticated, but possibly cheaper is a box aspirator (Figure 9). This machine has a wide seed chute that appears to be better suited to smaller seeds that have lots of fluffy trash. Because the chute is wide the seeds can be spread out well for good separation as they enter the air column rather than tangling together. The box aspirator can be made from rigid cardboard, and this might be a good way to start, ensuring the design is correct before investing in a more permanent material such as plywood. The sheets of cardboard are held together with packaging tape. The tape makes a tight

seal at all joints which is necessary to maintain the vacuum in the system. The main parts of the box aspirator are the seed chute, the settling chamber, the vacuum control gates, and the vacuum source (Figure 10). The settling chamber is the foundation of the box aspirator to which all other parts are attached. It can be any conveniently sized cardboard box.



Figure 9. Front view of the cardboard box aspirator.



Figure 10. Diagram of the box aspirator showing the major sections and the flow of seeds and trash particles.

Seed Chute

The first step in building the seed chute (Figure 11) is to compute its cross sectional area (width × depth) which will equal the area of the vacuum source inlet. For the vacuum with a 2 inch (5 cm) round inlet, the seed chute will have a cross sectional area of about 3 square inches (area of circle =). With a 6 inch (15.15 cm) wide chute, the depth can be $\frac{1}{2}$ inch (1.25 cm). Step 2 is to cut the top opening into one side of the settling chamber. Make it 4 to 6 times larger than the area of the chute. In our example, that would be about 2 to 3 inches (5.1 to 7.6 cm) high and 6 inches (15.2 cm) wide. The top opening needs to have the same width as the chute. Step 3 is to attach $\frac{1}{2}$ inch (1.3 cm) thick strips to the outside of the settling chamber to form the top and sides of the chute. The thickness of the strips must match the



Figure 11. View of the bottom or outlet of the seed chute on the box aspirator.

width of the chute. A deeper chute would need thicker strips to match. In our cardboard box aspirator, the strips were made of two layers of $\frac{1}{4}$ inch (0.6 cm) cardboard attached to settling chamber with white glue. The final step is to close the chute by attaching a face sheet over the strips that is wide enough to reach completely across the chute and cover the side and top strips. The length of the face sheet should extend at least 4 inches (10.1 cm) below the bottom of the top opening and about 2 to 4 inches (5.1 to 10.1 cm) above the bottom of the chute. Attach another shorter sheet over the chute extending from the very bottom to about $\frac{1}{2}$ inch (1.25 cm) from the bottom of the top sheet. This leaves a $\frac{1}{2}$ inch (1.3 cm) slot in the chute through which seeds are fed into the air column.

Vacuum Source Connection

Cut a hole for the vacuum source into the settling chamber on the side opposite the seed chute. Cut it near the top of the chamber (Figure 12). The connection to the vacuum must be as tight as possible to maintain good vacuum on the settling chamber.



Figure 12. Vacuum source connection for 2 inch (5.1 cm) pipe on the box aspirator.

Vacuum Control Gates

These gates (Figure 13) determine the strength of the vacuum in the seed chute and consequently the weight of particles that can be lifted. The vacuum cleaner runs at a constant speed pulling air out of the settling chamber. When the control gates are fully open, there is little to no vacuum in the seed chute. This is because the gates have about twice as much area of opening as the chute. Air draft will take the path of least resistance which will be through the control gates when they are open. The gates are gradually closed, gradually in-



Figure 13. View of the vacuum control gates on the box aspirator.

creasing the vacuum in the seed chute until it is strong enough to lift all or most of the light trash and let the seeds fall to the bottom. These gates are rectangular and narrow (1 inch [2.5 cm] wide) and long. This shape allows for more gradual and even changes in seed chute pressure compared to a wider or round opening. The gate doors are made of strips of material (cardboard in this case) 25% wider than the gate width (1 ½ inch [3.8 cm]). Track strips, the same thickness as the doors, are attached parallel to and ¼ inch (0.6 cm) back from the side of the gate opening. These track strips provide a track for the door strips to travel in. The total gate construction is finished by putting strips on top of the track strips that will overlap the track strip by ¼ inch (0.6 cm) to hold the door strip against the side of the settling chamber. Vacuum control gates are located on either side adjacent to the seed chute side of the settling chamber.

Access to the Settling Chamber

Access to the chamber is necessary to remove the light trash that has collected there. This access is provided by a door on one side. In this version of the aspirator, a sheet of cardboard is used as the door and packaging tape serves as the door hinge. The vacuum in the chamber will

keep the door closed during operation (Figure 14).

Adjusting the Aspirators

Both the pipe and box aspirators must be precisely adjusted to obtain the best seeds possible and to keep the yield as high as possible. The accuracy of separated seeds from trash is determined by the air pressure in the seed chute. A manometer (Figure 15) is a liquid filled tube that can be attached to the chute to measure the changes in pressure as the air gate is opened or closed. It will quickly help find the best



Figure 14. View of the access door into the settling chamber of the box aspirator.

setting and to find it again on future seed lots. An inexpensive manometer can be purchased for about \$60 or one can be constructed from plans found on the internet.



Figure 15. Manometer used to measure the strength of the vacuum in the seed chute of the aspirator.

Seeds Into the Aspirators

A seed scoop made from a 12 oz (355 ml) drink bottle can be used to feed seeds into the aspirators. It is especially well suited to the pipe aspirator and seeds that do not clump together with the trash. Short quick back and forth motions will scatter the seeds into the funnel and into the air column.

An aluminum pie pan (Figure 16) can be formed into a wide seed feeder for the box aspirator. This works better for seeds that clump with the trash because the uncleaned seeds can be sprinkled in a thin layer across the pan. The pan is then gently tapped to vibrate the seed into the seed chute.



Figure 16. Aluminum pie pan cut to form a hand held feeder to place seeds into the seed chute of the box aspirator.

References

Bonner FT, Karrfalt RP, editors. 2008. The woody plant seed manual. Washington (DC): USDA Forest Service Agriculture Handbook 727. 1223 p.

The content of this paper reflects the views of the authors, who are responsible for the facts and accuracy of the information presented within.

Designing Propagation Environments in Forest and Native Plant Nurseries

Thomas D Landis

Thomas D Landis is Retired Nursery Specialist, USDA Forest Service, and Consultant, Native Plant Nursery Consulting, 3248 Sycamore Way, Medford, OR 97504; Tel: 541.858.6166; email: nurseries@aol.com.

Landis TD. 2013. Designing propagation environments in forest and native plant nurseries. In: Haase DL, Pinto JR, Wilkinson KM, technical coordinators. National Proceedings: Forest and Conservation Nursery Associations—2012. Fort Collins (CO): USDA Forest Service, Rocky Mountain Research Station. Proceedings RMRS-P-69. 58-63. Available at: http://www.fs.fed.us/rm/pubs/rmrs_p069.html

Abstract: Propagation environments are areas that have been modified for plant growth, and can be designed using the law of limiting factors. Identifying critical factors that are most limiting to optimal plant growth is helpful when developing both bareroot and container nurseries. Propagation environments can be categorized into minimally-controlled, semi-controlled, and fully-controlled. The defining characteristics of each are discussed. When planning a nursery, three things should be considered: the type of crop, local soil and climate conditions, and available budget.

Keywords: limiting factors, nursery design, seedling, greenhouse, bareroot

Using Limiting Factors to Design Propagation Environments

A propagation environment can be defined as any area that has been modified to promote faster and better plant growth. Although most people immediately think of a greenhouse, a propagation environment may or may not involve a structure of some sort. When designing or managing a nursery, I like to think in terms of limiting factors. Liebig originally developed the concept of limiting factors and his law of the minimum for mineral nutrients (Wikipedia 2012). It was often depicted as a wooden barrel where the amount of water that the barrel could contain is limited by the shortest stave (Figure 1A). In actual practice, limiting factors are not independent but act sequentially (Figure 1B). Water is almost always the most limiting factor to plant growth and development, but when that factor has been culturally resolved, another will become limiting, and so on. The ideal environment where all potentially limiting factors have been resolved is a growth chamber, but the energy costs are prohibitive except for specific functions such as seed germination chambers. Once all the growth limiting factors have been resolved, this demonstrates the true genetic growth potential of the plants (Figure 1B).





Figure 1. The concept of limiting factors is often depicted as a wooden barrel where the total amount of water that can be contained is determined by the shortest stave (A). Limiting factors act in a sequential fashion; when one factor has been culturally overcome, another then becomes limiting (B).

So, for our purposes, an ideal propagation environment is one where all environmental factors that are potentially limiting to plant growth (Table 1) are kept at optimum levels. Although water is almost always limiting to plant growth (Figure 1), this factor is relatively easy and cheap to control with irrigation in a propagation environment. Some factors, such as light intensity, cannot be economically supplied because photosynthetic lighting requires large amounts of electricity.

In addition to physical and chemical factors, the propagation environment also contains a biological component - other organisms that often limit plant growth. Pathogenic fungi and insect pests can injure or even kill succulent nursery plants and, because of the lack of natural biological controls in nurseries, pests can build up to damaging levels very quickly. One of the primary attractions of container nursery culture is that growers have more control over these biological factors and can design propagation environments to exclude most pests. On the other hand, beneficial microorganisms such as nitrogen-fixing bacteria and mycorrhizal fungi can greatly affect plant growth and development so their absence can be considered a limiting factor (Figure 1; Table 1).

Types of Propagation Environments

The limiting factors concept can be applied to design and management of both bareroot and container nurseries; although, greenhouses and other specialized propagation structures offer many more options for managing limiting factors.

Minimally Controlled

All bareroot nurseries fall into this category because they can only manage two potentially limiting environment factors: water and mineral nutrients. All bareroot facilities feature some sort of irrigation system to supply water during dry periods. Even nurseries in the mild climates, where rain occurs at regular intervals throughout the growing season, have to supply irrigation at some point to prevent moisture stress. Irrigation can also be used to cool seedbeds during germination or to provide some degree of frost protection during periods of freezing weather in the fall or spring.

Smaller scale nurseries can use raised beds filled with special growing media to overcome cool soil temperatures in the spring. The wooden frames can also be fitted with hoops of wire or plastic pipes,

Table 1. Potentially limiting factors that can be controlled in a propagation environment.

Environmental Factors	Supplied in Nurseries By:
1. Water	Irrigation
2. Mineral nutrients	Fertilization
3. Light	Type of covering, photoperiod lighting, photosynthetic lighting, blackout curtains
4. Temperature	Heaters, ventilation, shadecloth
5. Humidity	Irrigation, ventilation
6. Carbon dioxide	Ventilation, carbon dioxide generators
7. Beneficial microorganisms	Inoculation with nitrogen-fixing bacteria or mycorrhizal fungi
8. Pests	Exclusion screens, pesticides, biocontrol agents

Limiting Factors	Minimally-Controlled	Fully-Controlled							
1. Water	All High – Irrigation can be performed any number of ways for each								
2. Mineral Nutrients	All High - fertigation or incorporation of c	ontrolled-release fertilizers into growing me	dia						
3. Light	Generally none, some use of photoperiod lights & blackout curtains Generally none, some use of photope- riod lights & blackout curtains Medium - coverings, pl lights, blackout curtains								
4. Temperature	Generally none, some use of irrigation & fabric covering for frost protection	Portable heaters, and some use of irriga- tion & fabric covering for frost protection	High - heaters, fans & vents						
5. Humidity	No	Low	High - irrigation, heat & vents						
6. Carbon Dioxide	No	No	Medium - CO2 generators						
7. Beneficial microorganisms	All High - apply inoculum as seed coating, top dressing, or incorporate into growing media								
8. Pests	Low – fencing to exclude deer	Moveable sidewalls prevent bird preda- tion of seed	High - permanent walls exclude insects and birds						

Table 2. Amour	t of Control of	Limiting Factors	in 3 Types of	Propagation	Environments
----------------	-----------------	------------------	---------------	-------------	--------------

which are then covered with clear plastic sheeting. These minimal structures capture the heat of the sun to allow earlier sowing during the spring or prevent frost damage during the fall (Table 2). Some larger bareroot nurseries have also used hoop structures over their seedbeds to support coverings with frost fabric to prevent cold injury (Moench 1994).

Minimally-controlled container nurseries are known as open growing compounds, and were developed to produce an inexpensive container seedling that was well acclimated to the environment. Although they have been most popular for growing southern pines in the southeastern US (Barnett and others 2002), open compounds have been the standard propagation environment in tropical and subtropical nurseries. In the Maritime provinces of Canada and coastal British Columbia, open compounds are used to produce a 2-year container stocktype. Typical compounds are graded for good drainage and either covered with weed barrier fabric and gravel or paved with asphalt. Semipermanent irrigation lines supply water and can also be used to supply mineral nutrients through fertigation (Table 2). Some open growing compounds are equipped with photoperiodic lighting to extend the growing season, and blackout curtains to induce dormancy and cold hardiness. Although open growing compounds are the least expensive way to produce container stock, growth rates are slow and, depending on the climate, it may take 1 to 2 years to produce a shippable seedling. Weather damage, such as a killing frost or torrential rain, is also a constant concern and so the risk of crop loss is the highest of all types of propagation environments (Landis and others 1995).

Semi-controlled

The only instance of semi-controlled propagation environments being used in bareroot nurseries were the bedhouses used at Wind River Nursery in the early 1980's (Hansen 1983). These bedhouses consisted of moveable high tunnels that protected the seedbeds early in the growing season and then were physically removed when ambient growing conditions became favorable. Although these bedhouses did improve seedling growth rates, and shorten the growing season for some conifer species, their use was eventually discontinued. Bedhouses of clear poly sheeting over metal hoops (Figure 2) were also tested in northern British Columbia for growing white spruce and lodgepole pine bareroot stock (Simpson 1990). These trials demonstrated increases in seed germination due to warmer soil temperatures and increased growth rates that produced larger 1+0 seedlings that were less prone to frost heaving. Although the bedhouse technology showed some advantages, it has not been widely adopted.

A wide variety of semi-controlled propagation environments have been used in container nurseries (Landis and others 1995). Crops can be grown in semicontrolled structures in all but the most severe climates. Some types of semicontrolled structures, especially shadehouses and tunnels, are also used for hardening and intermittent seedling storage. From an economic standpoint, semicontrolled environments are cheaper to build and operate than fully controlled environments, although there are considerable variations between the different types of structures.

Shelterhouses are a modification of the traditional greenhouse with a permanent transparent roof with movable walls that can be rolled up after the danger of frost has past (Hahn 1982). These structures can be outfitted with environmental control equipment that allow control of many of the potentially growth-limiting factors (Table 2). In the spring or in unusually cold weather at any time during the growing season, the sidewalls are kept down and portable heaters can used to raise temperatures. As soon as ambient temperatures become favorable, the sides can be raised to permit natural ventilation, eliminating the need for forced air cooling.

Semi-controlled environments also include hoop houses and tunnels that are used much more for ornamental or food crops than for



Figure 2. Bedhouses of clear poly sheeting over metal hoops are a type of semi-controlled propagation environment.

forest or native plant propagation. Hoop houses, also known as row tunnels or low tunnels, are low-profile metal bow-arch structures covered with poly sheeting that only retrain solar heat early in the growing season (Wells 1996). High tunnels can be equipped with portable heaters to prevent frost damage and accelerate seed germination (Kleinhenz 2011). During warm weather, the ends or even the sides of the tunnels can be rolled up to provide ventilation. They can also be covered with shadecloth. Hoop houses and tunnels are also equipped with ground-based irrigation lines to provide water and mineral nutrients through fertilizer injection (Table 2).

Shadehouses or lathhouses are semi-controlled propagation environments that have been widely used for growing forest seedlings and other native plants (Landis and others 1995). Traditionally, shadehouses were constructed of a wood frame covered with snowfence or wooden slats, but metal frames covered with shadecloth are also common. Shadehouses are usually equipped with basal or overhead irrigation so fertigation is also possible. Although traditionally used as hardening or holding areas, shadehouses can also be used to propagate many forest and native plants. In colder climates, shadehouses are also used for overwinter storage. Shadehouses with a permanent roof and open mesh sides have found considerable acceptance in the Tropics and the Subtropics, where sunlight can be too intense for young seedlings and torrential rains and wind can damage crops.

Fully Controlled

Greenhouses are the traditional propagation structure for producing container plants, and they can be equipped to fully control the propagation environment (Table 2). Greenhouses use natural sunlight that is trapped inside the transparent structure and converted to heat (the "greenhouse effect"). The drawback of the transparent covering is that greenhouses are inherently poorly insulated and require both high-capacity heating and cooling equipment for good temperature control. Depending on whether the climate is arid or humid, the greenhouse environment may need humidification or dehumidification. Many forest and native plant conservation species are sensitive to changes in daylength, and so photoperiodic lighting is often installed to prevent dormancy. Blackout curtains can be used to shorten the daylength and induced hardiness. Although carbon dioxide generators can be used to promote faster growth rates, they can only be run when the structure is completely closed. Irrigation systems with fertilizer injectors supply ideal levels of water and all the essential mineral nutrients. Computer-controlled equipment can keep potentially growth-limiting factors at optimal levels as well as provide a permanent computer record for growth comparisons and trouble shooting.

Greenhouses with retractable roofs and sides are the most recent innovation, and have useful applications for forest and native plant crops (Svenson 1996). Early in the growing season, the roof and sides are closed and the structure functions like a traditional greenhouse. Later, when ambient conditions improve, the roof and/or sides can be opened to allow for full air exchange. Retractable roof greenhouses are equipped with sophisticated computer controls that can open and close as needed to minimize the use of energy intensive heating and cooling equipment. The roofs and sides can be left closed early in the season and during cool weather, but opened later to allow natural hardening to under ambient conditions. This feature is ideal for forest and native plant crops which can be gradually hardened yet still be protected from climatic extremes (Landis and others 1995).

Things to Consider When Planning a Nursery

As you can see, there are a wide variety of possible propagation environments; the trick is to design one that is appropriate for your own situation. Here are three things to consider (Figure 3):



Figure 3. Several varied factors must be considered when designing a propagation environment, but one of the most important is to make certain that structures are appropriate for local conditions.

1. Type of Crop

Although this may seem obvious, all too often people wanting to start a nursery just assume "plants are plants" and can be grown in some average propagation environment. So, they spend most of their time on the economics of the situation or get caught up in design specifics. There's no such thing as an average propagation environment. The type of crop that you want to grow will have an enormous impact on your choice. The first decision is whether to grow crops as bareroot or container plants (Landis and others 1995).

Bareroot seedlings are grown in open fields in native soil, and consequently, the soil, water supply, and climate of the nursery site must be suitable for tree growing. The rate of seedling growth and length of the growing season are largely controlled by the climate at the nursery site. Quality sites are often difficult to find in convenient locations, and good agricultural land is almost always expensive. A considerable capital investment is usually required to develop a bareroot nursery of any size. Bareroot nurseries are also sensitive to the economies of scale. Once a nursery is established and operations have begun, it is important to function at near-capacity levels to have reasonable unit production costs. Compared to container nurseries, energy requirements and associated expenses are relatively low.

Container nurseries can be constructed on land with low agricultural value that would be unsuitable for bareroot seedling production. The amount of capital investment varies with the type of facility. Fully controlled greenhouses require expensive structures and en-



Figure 4. Growing schedules are useful during nursery planning to illustrate the time required for each phase of the nursery cycle from seed procurement to outplanting (Landis 2008).

vironmental controls, but open growing compounds are much less costly. Because container seedlings are grown at high densities, less land is required compared to a bareroot nursery. Container nurseries are less sensitive to economies of scale and, in extreme situations, part or all of the nursery can be shut down to reduce operating costs. Container seedlings have high growth rates, especially in fully controlled environments, and so crops can be produced in one growing season. From a business standpoint, this means that container nursery managers can respond quickly to changes in the market.

Once you've decided on either a bareroot or container nursery, the next things to consider are what plants you want to grow and how best to grow them. Let's say that you want to grow woody native shrubs in containers. Do you really have an idea of how long it will take to grow a saleable plant? Luckily, a source of "recipes" is available. In addition to information on seed collection and processing, the Woody Plant Seed Manual (Bonner and Karrfalt 2008) contains a section on Nursery and Field Practices for each plant genera. This book also has a chapter on the basics of plant propagation including growing schedules which show how long typical native plant crops take to produce (Figure 4). More detailed information can be found online at the native plant network (www.nativeplantnetwork.org) where several thousand propagation protocols can be found (Landis and Dumroese 2000).

2. Local Conditions

62

As we've already discussed, there is no ideal propagation environment; instead, each must be appropriate for the local situation. Irrigation water quality is of paramount importance for both container and bareroot nurseries, and there is no economical treatment for poor water quality. For bareroot nurseries, the next most important site quality factor is the soil. The ideal soil for a bareroot nursery is a sandy loam, primarily due to the ease of root culture and harvesting during the wet winter weather (Landis 1995). An excellent discussion of all the factors that must be considered during bareroot nursery site selection can be found in Morby (1984).

Container nurseries can be located on sites that would be totally inappropriate for a bareroot nursery because seedlings are grown in artificial growing media and with structures and equipment to modify the physical environment. Container nursery developers should allow adequate time to analyze potential sites because many biological and operational problems that develop later in nurseries can be traced back to site problems. The things to look for in a potential container nursery site can be divided into essential factors and desirable factors (Table 3). Essential site selection criteria consist of factors that are essential to a successful nursery operation. By comparison, desirable site factors are not absolutely necessary but will increase the economy and efficiency of the nursery operation. More detailed discussion on container nursery site selection is provided in Landis and others (1995).

3. Budget

Of course, economic considerations will always be paramount when planning a nursery operation. Unfortunately, it is impossible to provide detailed economic data because the size and objectives of for-

 Table 3. Site selection criteria for container nurseries (Landis and others 1995).

Essential Factors	Desirable Factors
Good solar access	Protected microclimate
High quality water	Gentle topography
Inexpensive and reliable energy	Seasonal labor supply
Adequate land area including room for expansion	Year round accessibility
Ecopolitical concerns, especially zoning	Distance to markets

est and native plant nurseries is so varied. The economic constraints for a small mom-and-pop native plant nursery have little in common with that of a large commercial forest nursery. Still, nursery developers must give careful consideration to the economic aspects of starting and operating a nursery through a comprehensive business plan.

References

- Barnett JP, Dumroese RK, Moorhead DJ. 2002. Growing longleaf pine in containers - proceedings of workshops 1999 and 2001.Washington (DC): USDA Forest Service, Southern Research Station, General Technical Report SRS-56. 63 p.
- Bonner FT, Karrfalt RP. 2008. The Woody Plant Seed Manual. Washington(DC): USDA Forest Service. Agriculture Handbook 727. 1223 p.
- Hahn PF. 1982. Practical guidelines for developing containerized nursery programs. In: Guldin, RW, Barnett JP, editors. Proceedings of the Southern Containerized Forest Tree Seedling Conference. New Orleans (LA): USDA Forest Service, Southern Forest Experiment Station. General Technical Report SO37. p 97-100.
- Hansen DC. 1983. Bedhouse seedling production. In: Sawyer, RA. compiler. Proceedings of the 1982 Western Nurserymen's Conference. URL: http://www.rngr.net/publications/proceedings (accessed 7 Nov 2012)
- Kleinhenz MD. 2011. High tunnels. Greenhouse Management 31(7):66-69.
- Landis TD. 2008. Chapter 7 Nursery practices. In: Bonner FT, Karrfalt RP, editors. The Woody Plant Seed Manual. Washington (DC): USDA Forest Service. Agriculture Handbook 727. p 125-145.

- Landis TD, Dumroese RK. 2000. Propagation protocols on the Native Plant Network. Native Plants Journal 1(2): 112-114.
- Landis TD, Tinus RW, Barnett JP. 1999. Seedling propagation. Vol 6. The container tree nursery manual. Washington (DC): USDA Forest Service, Agricultural Handbook 674. 167 p.
- Landis TD. 1995. What is a soil management plan and why would you want one? Forest Nursery Notes. USDA Forest Service, State and Private Forestry. URL: http://www.rngr.net/publications/fnn (accessed 7 Nov 2012).
- Landis TD, Tinus RW, McDonald SE, Barnett JP. 1995. Nursery planning, development, and management, Vol 1. The container tree nursery manual. Washington (DC): USDA Forest Service, Agricultural Handbook 674. 188 p.
- Moench RD. 1994. Use of frost fabric as a seedbed mulch and frost protection method. In: Landis TD, Dumroese RK, technical coordinators. Proceedings, Forest and Conservation Nursery Associations. Fort Collins (CO): USDA Forest Service, Rocky Mountain Forest and Range Experiment Station.: General Technical Report RM-GTR-257. p 166-168. Available at: http://www.fcnanet.org/ proceedings/1994/moench.pdf
- Morby FE. 1984. Nursery site selection, layout, and development. In: Duryea ML, Landis TD, editors. Forest nursery manual: [roduction of bareroot seedlings. Hingham (MA): Kluwer Academic Publishers p 9-15.
- Simpson DG. 1990. Seedbed coverings affect germination, growth, and frost heaving in bareroot nurseries. Tree Planters' Notes 41(4):13-16.

The content of this paper reflects the views of the authors, who are responsible for the facts and accuracy of the information presented within.

Advanced Techniques to Prepare Seed to Sow

Robert P Karrfalt

Robert P Karrfalt is Director, National Seed Laboratory, USDA Forest Service, Dry Branch, GA 31020; email: rkarrfalt@fs.fed.us

Karrfalt RP. 2013. Advanced techniques to prepare seed to sow. In: Haase DL, Pinto JR, Wilkinson KM, technical coordinators. National Proceedings: Forest and Conservation Nursery Associations—2012. Fort Collins (CO): USDA Forest Service, Rocky Mountain Research Station. Proceedings RMRS-P-69. 64-66. Available at: http://www.fs.fed.us/rm/pubs/rmrs_p069.html

Abstract: This paper reviews research on improving the basic technique of cold stratification for tree and shrub seeds. Advanced stratification techniques include long stratification, stratification re-dry, or multiple cycles of warm-cold stratification. Research demonstrates that careful regulation of moisture levels and lengthening the stratification period have produced a more vigorous response in several species. Advanced stratification techniques have also produced more uniform germination in species that, when treated in the basic manner, have failed to germinate or have germinated erratically. Nursery managers can improve seed germination at their nurseries by carefully and gradually adopting one of the advanced stratification techniques reviewed in this paper.

Keywords: cold stratification, warm-cold stratification, germination temperature, seed moisture

Introduction

For at least the last 60 years, cold stratification has been a primary method for preparing tree and shrub seeds for sowing. Cold stratification was necessary to overcome dormancy factors that prevented seeds from germinating in the fall when they were first shed from the mother tree. The basic technique was to soak seeds overnight, drain off the excess water, bag the seeds in a polythene bag ("polybag"), and place the seeds at temperatures just above freezing for 30 to 60 days. Several researchers explored variations on this basic technique, and there is now enough evidence to support refined practices at production nurseries. These advanced techniques involve:

- long stratification periods
- · stratification re-dry, or
- multiple cycles of warm-cold stratification.

The one common factor among all these more advanced approaches is a more precise control of moisture levels than was attempted under the basic stratification procedures. The 1974 edition of the Woody Plant Seed Manual (Schopmeyer 1974) stated simply that, "Full imbibition is essential for stratification..." In contrast, all of the advanced techniques make use of specific targeted moisture content.

Long Stratification and the Stratification of Non-dormant Species

Basic stratification approaches generally work well for moderately dormant species such as most spruce and pine. "Long stratification" means extending the stratification period beyond the basic stratification length. The length of long stratification varies depending on the dormancy of the species. Basic stratification periods for dormant species are mostly 30 to 45 days, extended to 60 days for more dormant species. Long stratification for dormant species therefore involves extending stratification periods beyond the basic 30 to 45 days. Non-dormant species are species that do not require stratification to germinate under favorable conditions. Therefore a general definition of long stratification for non-dormant species is the use of 14 to 28 days or longer of stratification than would be used with basic seed treatments.

Sitka spruce (*Picea sitchensis* (Bong.) Carrière) is a non-dormant species. Gosling and Rigg (1990) showed that unstratified Sitka spruce seeds germinated best at 20 °C and that germination dropped dramatically when the temperature was increased to 25 °C or decreased to 15 °C. Following 21 days of stratification, the seeds were able to germinate equally well at all three temperatures (Figure 1). Stratification also increased germination percentages and speed of germination for longleaf pine (Pinus palustris Mill) another non-dormant species (Karrfalt, 1988).



Figure 1. Stratification makes Sitka spruce seeds able to germinate well over a range of temperatures (Gosling 1990).

Douglas-fir (Pseudotsuga menziesii (Mirb.) Franco) is a moderately dormant species. Allen (1962) tested germination on 26 seed lots using 40, 80, and 120 days of stratification and germination temperatures of 25, 15, and 10 °C. Germination was best at 25 °C following all stratification periods, but the longer stratification time sharply improved the germination at the lower germination temperatures (Figure 2) just as occurred with the non-dormant Sitka spruce.

In basic stratification, there usually is a film of capillary water on at least some of the seeds. This is excess water that can lead to sprouting as the stratification period is extended. This is formally demonstrated by Gosling and Riggs (1990) with Sitka spruce. Seeds at 30% moisture content and no water film never sprouted even when the stratification period was extended to 140 days (20 weeks). Seeds that had a water film did begin sprouting in stratification (Figure 3). Therefore, removal of the water film is a mandatory requirement for long stratification because protruding radicles are usually damaged during sowing resulting in lost or stunted seedlings.



Figure 2. Long stratification makes Douglas fir seeds germinate better at cooler temperatures (Allen 1962).

Removal of the water film can be accomplished in several ways after draining the soaked seeds. The first is to simply spread the seeds out in thin layers for air drying. This takes a large flat area and continuous monitoring and turning of the seeds until the film of water is gone. A quicker way to do this is to use a pressurized drier (Karrfalt 2012). After draining the seeds, the seeds are placed in the drying tray and gently stirred continuously until the surface water is removed. Stirring is necessary or else seeds on the bottom of the tray might get too dry. A third way is to place bags of soaked seeds in a laundry spinner (Gosling and others 1994). When properly surfaced dried, the seeds will look damp but no longer have the shiny film of water.

Identifying the best length of stratification is done by simply testing different periods to determine which ones give the best germination responses. A qualified seed laboratory can run a series of tests using varying stratification lengths and even possibly germination temperatures to assist in identifying optimal stratification periods.

As with any new procedure at a nursery, a careful transition should be made when adopting longer stratification periods. The right balance must be found between removing the excess film of moisture that can cause sprouting and keeping the seed moist enough for effective stratification.



Figure 3. A film of water is not needed for effective stratification of Sitka spruce but can eventually lead to sprouted seeds in stratification (Gosling 1990).

Stratification-redry

Karrfalt

The stratification-redry used with true firs (Abies spp.) (Edwards 1981; Leadem 1986) is similar to the long stratifications already discussed with two differences. First the initial 30 days of stratification is conducted in the basic manner with some surplus water with the seeds. This initial 30 days gives more complete imbibition of water. Following the initial 30 days, the seed is surface dried as described in long stratification above. Secondly, the length of the stratification is measured for each lot by pulling small samples from the stratification bag and running a small germination test. Once a good germination is obtained the stratification is terminated. To determine when the best germination has been obtained it would be useful to have had an estimate of viability made with a tetrazolium test. In a tetrazolium test, the chemical tetrazolium chloride stains the viable seeds a light pink so they can be distinguished from the non-viable seeds. When the tetrazolium estimate is close to the germination then a good germination in the nursery would be expected.

Multiple Cycles of Warm and Cold Stratification

This approach holds promise for species of deep and variable dormancy such as rocky mountain juniper (*Juniperus scopulorum*) and cherries (*Prunus* spp.). Suszka and others (1996) describe methods employing multiple cycles of warm and cold stratification to achieve complete and uniform germination. These multiple cycles presumably mimic several cycles of growing and dormant seasons. Those species requiring this treatment would be ones that use a regeneration strategy of being able to germinate seeds from one seed crop over a period of years.

In the Suszka procedure, seeds are first imbibed to a moisture content of 35%, slightly less than full imbibition. Next the seeds are generally put through cycles of approximately 30 days at 20 °C and 60 days at 2 or 3 °C. How many such cycles depends on the species. The length of the final cold cycle is determined similarly to the stratification-redry. A small sample of seeds is placed in germination and when a good germination is obtained, the cold stratificationredry procedure, a tetrazolium test is useful to estimate the viability of the seed for comparison to the germination.

Typically species of deep and variable dormancy, when kept completely in cold stratification with a little excess moisture, will germinate in stratification over an extended period of time. In the nursery bed they can germinate over two, sometimes three growing seasons. Reducing the moisture content to 35% prevents germination but allows the dormancy breaking process to continue until all seeds are non-dormant.

Storing Non-dormant Seeds

With careful drying of treated seeds, some authors stated that storing seeds in a stratified condition without re-inducing dormancy appears to be possible. Drying stratified seeds generally puts seeds back into dormancy requiring a second stratification. Suszka and others (1996) reported that by slowly drying the seeds they could be stored in a non-dormant condition. This would be a great help to nurseries planning seedling production and responding to late orders. Allen (1962) reported a similar response in Douglas-fir. More research on how to conduct this procedure of bringing seed to low enough moisture status for storage and yet not inducing dormancy needs to be conducted before recommendations can be made for nurseries.

Summary

All of the more advanced approaches of preparing seed to sow involve a more precise control of moisture levels than the basic stratification procedures. The main key is to control the moisture content of the seeds by surface drying the seeds after they are fully imbibed. This allows for the stratification process to proceed and apparently reach an optimal state of preparation without starting germination.

References

- Allen GS. 1962. Factors affecting the viability and germination behavior of coniferous seed VI. Stratification and subsequent treatment, Pseudotsuga Menziesii (Mirb.) Franco. Forestry Chronicle, December, 38(4):485-496.
- Edwards DGW. 1981. A new prechilling method for true fir seeds. In: Proceedings, Joint Meeting of the Intermountain Nurseryman's Association and Western Forest Nursery Association. Boise (ID): USDA Forest Service, Intermountain Forest and Range Experiment Station. General Technical Report INT-109. p 58-66.
- Gosling PG, Rigg P. 1990. The effect of moisture content and prechill duration on the efficiency of dormancy breakage in Sitka spruce (*Picea sitchensis*). Seed Science and Technology 18:337-343.
- Gosling PG, Jones SK, Gardiner AS. 1994. Spin drying soaked tree seed before prechilling improves seed handling. Tree Planters' Notes 45(1):32-35.
- Karrfalt RP. 1988. Stratification of longleaf pine. In: National Nursery Proceedings – 1988. Southern Forest Nursery Association Charleston, SC July 25, 1988 – July 28, 1988.
- Karrfalt RP. 2013. Low cost high tech seed cleaning. In: Haase DL, Pinto JR, Wilkinson KM, technical coordinators. National Proceedings: Forest and Conservation Nursery Associations—2012. Fort Collins (CO): USDA Forest Service, Rocky Mountain Research Station. Proceedings RMRS-P-69. p 53-57.
- Leadem CL. 1986. Stratification of *Abies amabilis* seeds. Canadian Journal of Forest Research 16:755-760.
- Schopmeyer CS. technical coordinator. 1974. Seeds of woody plants in the United States. Washington, (DC): USDA. Agricultural Handbook 450. 642 p.
- Suszka B, Muller C, Bonnet-Masimbert M. 1996. Gordon A. translator. Seeds of forest broadleaves: from harvest to sowing. Paris, INRA. 1–294.

The content of this paper reflects the views of the authors, who are responsible for the facts and accuracy of the information presented within.

Calculating Optimum Sowing Factor: A Tool to Evaluate Sowing Strategies and Minimize Seedling Production Cost

Eric van Steenis

Eric van Steenis is Registered Professional Forester, Terralink Horticulture Inc. 464 Riverside Road, Abbotsford, BC, Canada V2S 7M1; email: eric@tlhort.com

van Steenis E. 2013. Calculating optimum sowing factor: a tool to evaluate sowing strategies and minimize seedling production cost. In: Haase DL, Pinto JR, Wilkinson KM, technical coordinators. National Proceedings: Forest and Conservation Nursery Associations—2012. Fort Collins (CO): USDA Forest Service, Rocky Mountain Research Station. Proceedings RMRS-P-69. 67-71. Available at: http://www.fs.fed.us/rm/pubs/ rmrs_p069.html

Abstract: This paper illustrates how to use an excel spreadsheet as a decision-making tool to determine optimum sowing factor to minimize seedling production cost. Factors incorporated into the spreadsheet calculations include germination percentage, seeder accuracy, cost per seed, cavities per block, costs of handling, thinning, and transplanting labor, and more. In addition to numerical outputs, the spreadsheet generates a graphical representation of results to present and evaluate options. Example scenarios demonstrate how the tool can be used. A copy of the spreadsheet tool is available by emailing the author: eric@tlhort.com or eric.vansteenis@gmail.com.

Keywords: seed, seedlot, sowing, cost, efficiency

Introduction

Seed is fundamental to the nursery industry. This paper illustrates how to use an excel spreadsheet to calculate optimum sowing factor (number of seeds per cell) and over-sowing factor (number of extra seeds to sow per cell). Some of the math involved is covered, and a graphical method for presenting and evaluating options is given. The objective is to evaluate different options for sowing and determine the best sowing strategy to minimize seedling production cost.

Selection Criteria Included in the Excel Spreadsheet Tool

The objective is for the nursery to produce 100% of the seedlings ordered by clients, all meeting contract specifications. The nursery also needs to be competitive and remain profitable. Hence the desire is to conserve seed, limit thinning, minimize extra non-salable green stems, and thereby arrive at the Minimum Seedling Production Cost. Employing the criteria listed below, the spreadsheet tool allows comparison of specific sowing factor strategies and alternatives.

Seedlot Germination Percentage

This is the number one selection criteria, and what sowing guidelines and seed allotments are largely based on. Sowing factor is calculated to limit empty cavity count. This varies with container type and nursery specific goals and costs, but the general goal is to have no more than a few percent

of cells empty after sowing. An example is a seedlot with 80% germination. Sowing 2 seeds per cell gives a probability of >1 viable seed in 96% of the cells, leaving 4% empties and a requirement for thinning. A minimum over-sow factor of 104.67% accounts for these empty cavities. Over-sow factors in excess of this minimum are designed to increase the "green stem count".

Seeder Accuracy

Inaccuracy in seed placement and/or pickup is mathematically similar to a reduction in seedlot germination percentage. In my calculations, I apply 50% of the inaccuracy to generation of empties, and the other 50% to generation of a multiple sown cell. In other words, 50% of the inaccuracy is expressed through lowering seedlot germination percentage, resulting in the "Adjusted Germination Capacity."

Cost per Seed

If calculated, cost per seed is a huge incentive to conserve seed. If borne by the nursery it becomes part of seedling production cost. It then weighs against thinning cost, and as a percentage of seedling production cost can be seen to carry less weight when producing larger and more valuable seedlings. This relates to the cost of carrying an individual empty cavity, which is higher for larger stock-types.

Cavities per Block

This is the # of cells (cavities) per seedling growing tray (styroblock), and in the calculations determines seedling growing density. i.e. the potential number of seedlings/unit growing area depending on whether or not every cell (cavity) in each block (seedling tray = styroblock) contains a seedling. The majority of the industry centers around growing seedlings in "styroblocks" with a standard outside dimension (format 600), and varying numbers of cells/cavities http://www.bpgrower.com/styroblock.html

Cost to Grow/Produce a Block

This relates to the per square meter growing space production cost. A 160 cavity block takes as much space as a 77 cavity block, hence costs the same to produce. However, the number of seedlings produced per unit area changes (from 756 to 364 per square meter), thereby affecting seedling production price, and how other input costs weigh against it.

Cost to Thin

Extra germinants per cell has a cost. There is a basic cost per block to enter the crop, which is spread over the number of cavities per block. In addition, there is the per cavity cost depending on the number of extra seedlings that require thinning in each cavity.

Desired/Required Nursery Handling Factor

This factor is instated to account for the fact that seeding machines are not 100% "tidy". It allows for spillage, damage and other "wasted" seed. This is normally a very low number such as 0.15 seeds per cavity sown.

Desired/Required Green Stem Count

This refers to the total number of seedlings required from which to choose 100% of requested seedlings. It must account for culls generated by seedling specifications, pests, diseases, and so on.

125% is usually adequate, but many growers strive to reduce this < 110%. Previous nursery proceedings papers have focused on seedling specifications and how they interact with chosen container type, seedling growing density, nursery growing context, species, genetics, and so on. This is nursery specific information based on experience.

Transplant Thinned Seedlings into Empty Cells

Transplanting thinned seedlings into empty cells has a cost, carries a risk of failure, but if done well, can be worthwhile. This option can be used to reduce both the sowing factor as well as the over-sow factor. It only takes a small increase in sowing factor to generate enough "thinnings" to allow transplanting to 100% cavity fill. Far less seed is required than merely multiple sowing and discarding the thinned seedlings. If 100% cavity fill is achieved this way, then the desired green stem count can be minimized. The risk relates to the ability for transplanted seedlings to achieve contract specifications, given that the shock sets them back.

Assumptions in the Spreadsheet Tool

A basic assumption throughout is that seedling customers are not interested in receiving overruns, and the nursery is not interested in producing overruns. The desire is to have all nursery space allotted to growing on contract. The assumptions are basic and simplified. Incorporation of more detailed, more accurate, and additional nursery costs and biological/physical concepts will increase the spreadsheet's predictive value as a decision making tool.

Example Scenarios Using the Spreadsheet Tool to Calculate Optimum Sowing Factor

Several situational examples are provided below to illustrate how the tool can be used.

Example Scenario 1 (Figure 1)

Scenario 1 assumes seed is free, empty cavities are carried (no transplanting), and nursery space is available to allow oversow factor adjustment as needed to provide for required green stems. Seeder accuracy is assumed to be 100% so we can easily see relationships between seedlot germination %, thinning cost, and seedling growing density when seed has no cost. The chosen seedlot quality of 96% shows the benefits of high quality seed, which encourages single sowing, and all the benefits that go along with that (minimum seed use, reduced disease transfer, maximum retention of genetic variability). The production cost of \$20 per Styroblock is arbitrarily chosen, but should reflect reality for many in 2012. Dividing this cost by the number of seedlings in a block, provides the minimum seedling production cost for a single sown, 100% germination capacity seed, where all seedlings meet contract specification by end of season. One moves up from this production cost by requiring extra green stems to select from, employing less than perfect seed and equipment, and so on.

Figure 1 shows that if sowing multiple seeds and thinning is chosen, there is a basic minimum jump in seedling production cost just to enter the crop for this purpose. Single sowing and carrying the empty cavities is the best scenario here (lowest seedling production cost).



Figure 1. Optimum sowing factor calculation, seed cost borne by the nursery (no transplanting).

As sowing factor increases, required oversow factor decreases until we reach 2 seeds per cavity. For a 96% germination seed lot, the probability of an empty cavity remaining beyond 2 seeds/cavity is essentially zero. At greater than 2 seeds/cavity, thinning extra seeds/cavity drives increasing cost. In this case we cannot beat the production cost of single sowing. Please keep "2 seeds per cavity" in mind as we examine other scenarios below.

The only reason to sow multiple seed is in the event nursery space is limited and will result in having to turn away additional contracts. By going from 1 to 2 seeds per cavity, we reduce oversow factor from 130 to 125%.

Example Scenario 2 (Figure 2)

In Scenario 2, seed is also free, but a drastic reduction in seed quality to 85% is imposed, along with some seeder inaccuracy. The seedling growing density is the same as Scenario 1, so we can observe the cost influence of empty growing space due to seedlot germination capacity weighed against benefits of increasing sowing factor.

Thinning the crop increases seedling production cost, but due to the large number of empty cavities, as soon as we move beyond a sowing factor of 1.1 seeds/cavity we start reducing seedling production cost relative to single sowing. Note the fastest reduction in empty cavity count and subsequent seedling production cost occurs between 1 and 2 seeds per cavity. Increasing sowing factor beyond 2 seeds per cavity reduces seedling production at a much decreased rate. Thinning costs weigh more heavily since extra seed expended does little in the way of eliminating empty cavities to offset additional labor costs.

Note that low germination seedlots require a larger commitment of nursery growing space at low sowing factors. Best approach in this case is to sow 2 seeds per cavity.

Example Scenario 3 (Figure 3)

Scenario 3 introduces a seed cost of 1 cent each. This might seem high/low depending on your situation, but gives an interesting result. We have raised germination percent to 94, but left seedling growing density and thinning costs the same as Scenarios 1 and 2.

Note the immediate impact on seedling production cost. Single sowing raises minimum cost per seedling by 1 cent/seedling. With a 94% seedlot in a relatively high density block (112), any increase in sowing factor adds proportionally significant (seed and thinning) costs to the price of each seedling.

Note that costs increase gradually up to 2 seeds per cavity, but then the law of diminishing returns takes effect in earnest. In this case, the lowest seedling production cost is at 1 seed per cavity. However, if nursery growing space is limiting and extra contracts are available, one could consider sowing up to 2 seeds/cavity. This



Figure 2. Optimum sowing factor calculation, seed cost borne by the nursery (no transplanting).



Figure 3. Optimum sowing factor calculation, seed cost borne by the nursery (no transplanting).

will reduce growing space for this contract by 8%. However, beyond 2 seeds per cavity it gets pricey, and we are not saving any more nursery growing space either. As seed price increases, single sowing becomes "economically" viable at lower seed-lot germination capacities, especially in small cavity blocks. This is in a 112 cavity block; results would be different for other sizes, such as a 240 cavity block.

Example Scenario 4 (Figure 4)

With seed cost at 1 cent each, in Scenario 4 we introduce a lower seedling growing density with a 77 cavity block. Notice that one can afford to throw extra 1 cent seeds at bigger cells (more valuable seedlings due to occupation of more growing space per seedling) to help eliminate empties, thereby reducing seedling production cost.

Again, sowing beyond 2 seeds per cavity does not make economic sense. At that point the minimum 1.20 oversow is realized, more seeds do not garner additional nursery space, and costs rise. In larger cavity blocks it is often economical to multiple seed even at higher germination capacities and incorporated seed costs. Basically, as the ratio of seedling production cost to seed cost increases, the more the economics favors multiple sowing.

Example Scenario 5 (Figure 5)

With seed cost remaining at 1 cent each, we introduce transplanting thinned seedlings. Note that at a sowing factor of \sim 1.4 you have enough thinned seedlings to transplant to 100% cavity fill. After that, the seed cost effects increasing seedling production cost, and there is no further gain in nursery growing space.

You can use the spreadsheet to insert your nursery's cost and success rate for thinned transplants. These are very important numbers to have. If in doubt, stay conservative. Even the 50% success rate we have assumed in this scenario can be optimistic depending on your nursery context.

Example Scenario 6 (Figure 6)

Scenario 6 compares carrying empties to transplanting thinned seedlings into empties as they become available with increasing sowing factor. Carrying empties has a \sim 1.7 cent/seedling higher "minimum seedling production cost". This is perhaps the most interesting spreadsheet in the workbook.

The spreadsheet assumes thinning costs are linear as sowing factor increases, which may not be correct. Note that in many cases a sow-



Figure 4. Optimum sowing factor calculation, seed cost borne by the nursery (no transplanting).



Figure 5. Optimum sowing factor calculation, seed cost borne by the nursery (thinnings transplanted).



Figure 6. Optimum sowing factor calculation, where the nursery bears ALL costs, including seed.

ing factor of 2 seeds per cavity gives an inflection point in the graph. Thinning (and seed) costs start to assume a larger role in seedling production cost than the reduction in empties generated by incremental increases in sowing factors. An interesting discussion ensues if we introduce the genetic value of seed instead of merely its production cost. This can be a fun brainstorm.

Summary

This spreadsheet is a useful tool to evaluate options for sowing factors incorporating multiple nursery costs. Constructive criticism and ideas for improvements to this tool are welcome.

The content of this paper reflects the views of the authors, who are responsible for the facts and accuracy of the information presented within.

Lengthened Cold Stratification Improves Bulk Whitebark Pine Germination

Nathan Robertson, Kent Eggleston, Emily Overton, and Marie McLaughlin

Nathan Robertson is Horticulturist, USDA FS Coeur d'Alene Nursery, Coeur D' Alene, ID 83815; email ndrobertson@fs.fed.us.

Kent Eggleston is Horticulturist, USDA FS Coeur d'Alene Nursery, Coeur D' Alene, ID 83815; email keggleston@fs.fed.us.

Emily Overton is SCEP Trainee, USDA FS Coeur d'Alene Nursery, Coeur D' Alene, ID 83815; email overtonec@gmail.com.

Marie McLaughlin is Seed Orchard Manager, USDA FS Sandpoint Ranger District, Sandpoint, ID 83864; email mmclaughlin01@fs.fed.us.

Robertson N, Eggleston K, Overton E, McLaughlin M. 2013. Lengthened cold stratification improves bulk whitebark pine germination. In: Haase DL, Pinto JR, Wilkinson KM, technical coordinators. National Proceedings: Forest and Conservation Nursery Associations—2012. Fort Collins (CO): USDA Forest Service, Rocky Mountain Research Station. Proceedings RMRS-P-69. 72-76. Available at: http://www.fs.fed.us/rm/pubs/rmrs_p069.html

Abstract: Crucial to the restoration of whitebark pine (Pinus albicaulis) ecosystems is the ability of forest managers to locate, propagate, and reintroduce viable, disease-resistant populations to these jeopardized systems. Currently, one of the most limiting steps in this process is the slow, labor-intensive, and expensive process of producing whitebark seedlings at forest nurseries. From a nursery production standpoint, whitebark seed dormancy is complex and more problematic that other western conifers. Although seedling culture has evolved and become more streamlined, overcoming seed dormancy is still a major challenge to efficient seed use and large-scale seedling production. Releasing seed dormancy through scarification and stratification needs to result in adequate and consistent germination percentages, and also needs to be practical and efficient at a restoration-production scale. This paper describes trials comparing germination percentages of whitebark seedlots grown under operational conditions at the Forest Service Coeur d'Alene Nursery to determine the relative influence of seed source elevation and location, seedlot (collection) age, and 60 or 90-days of cold stratification. The results of these studies indicate that, given proper seed collection, handling, cleaning, and storage: 1) 90-day cold stratification results in significantly increased germination over the 60-day treatment; 2) within the first decade of storage, seedlot age may not play as crucial a role in reducing germinative capacity as was previously thought; and 3) seedlot source geography may not have a strong enough influence on germinative capacity to merit altering seed use calculations or culture regimes for greenhouse production.

Keywords: Pinus albicaulis, scarification, nursery, seedling, restoration, seedcoat, dormancy

Introduction

Whitebark pine (Pinus albicaulis), the sole North American member of the Cembrae pine subsection, faces restoration challenges unique among the western conifers. Chief among these challenges is matching the pace of recruitment with an unnaturally accelerated mortality rate. The species faces the triple-pronged threat of introduced disease (*Cronartium ribicola*), native pine beetle (*Dendroctonus ponderosae*) epidemics, and historically uncharacteristic fire regimes (Mahalovich and others 2006). In the past several decades, much effort has been dedicated to identifying and securing seed from apparently disease resistant forest trees for the purposes of understanding disease resistance genetics and establishing seed banks for future seedling propagation and restoration efforts.

The success of such efforts hinges on the ability of tree nurseries to reliably germinate seed from various collections. The germination strategy of whitebark differs considerably from other western pine species. Most notably, in addition to a thick, hard seedcoat, which hampers imbibition, whitebark seeds have complex physiological dormancy release mechanisms (Riley and others 2007; Tillman-Sutela and others 2007). Because whitebark cone crops vary widely from year to year, and predation claims many of the nutrient-rich seeds, these mechanisms are presumably an adaptive strategy to delay the germination of dispersed seeds over the course of several years (Tomback and others 2001). This seed banking strategy may help offset periodic cone crop and seedling establishment failures, and balance recruitment through time. At the same time, these seed dormancy and germination characteristics make uniform artificial germination of whitebark seeds very difficult to achieve.

Past practices for producing whitebark seedlings in nurseries evolved out of germination and growth strategies developed for western white pine (Pinus monticola) (Burr and others 2001). However, stratification protocols for this species proved to be inadequate for whitebark pine, with its additionally complex dormancy mechanisms. Because of the difficulty of protecting, collecting, and cleaning whitebark seed, investment in collections is significant compared to other western conifers (Keane and others 2012). Due to this value, and the historically limited volume of seed being processed and seedlings being grown, very labor-intensive scarification and growing techniques have been used in an attempt to maximize the productivity of a given seed collection. These processes have included various chemical scarification regimes, hand- or machine-knicking individual seeds to disrupt the seed-coat integrity, germinating seeds in artificial germinators, hand-transplanting individual germinants when radicals appeared, and re-stratifying un-germinated seeds to produce additional germinant flushes (Gasvoda and others 2002; Pitel and Wang 1990; Wick and others 2008).

It is possible, through a combination of stratification, scarification, and highly controlled environmental parameters, to achieve nearly 100% germination of mature, viable seeds (Riley and others 2007; McCaughney 1992). However, replicating such conditions on the scale needed for mass seedling production for the purpose of restoration plantings has continued to be very problematic for nurseries, and at times prohibitively expensive (Eggleston 2012). These germination challenges, along with the slow growth of whitebark seedlings compared to other western conifers, has resulted in prices double or triple that of comparable products for other species (Eggleston 2012). Efforts to streamline seedling culture have increased mass production efficiency tremendously (Eggleston 2012). Still, overcoming low, erratic, and latent germination in whitebark seed at a large scale continues to be problematic.

At the USDA Forest Service, Coeur d'Alene Nursery (CDA Nursery, Coeur d'Alene, Idaho), whitebark seedling production has increased steadily in the past decade, and production levels are now near 200,000 seedlings per year (Eggleston, 2012). At this level, hand-knicking and hand-transplanting are logistically and economically impractical, so new methods must be developed to ensure adequate germination with a minimal investment of time and labor. Prior to the 2012 growing season, the CDA Nursery whitebark seed treatment protocols consisted of a 30-day warm strat, followed by a 60-day cold strat, after which seeds were scarified in a rotary drum sander (Missoula Technology & Development Center [MTDC]) (Gasvota and others 2002) for three hours. (Past trials at CDA Nursery have indicated that sanding beyond three hours does not increase germination [unpublished data]). This dormancy release treatment, in combination with direct sowing, has allowed the CDA Nursery to produce large numbers of restoration seedlings, while avoiding the costs of hand-knicking and hand-transplanting. Unfortunately, germination percentages using this method have historically ranged from 13-75% (Eggleston 2012). This wide range, coupled with the regular incidence of significant numbers of latent germinants, indicates that seed dormancy mechanisms were not entirely overcome, and/or that other factors influencing germination were at play.

If whitebark pine seedlings are to be produced in large quantities for the purpose of restoration plantings, nursery practices must be aimed at overcoming the complications associated with seed dormancy. Seed waste must be minimized by more fully realizing germinative capacity, while simultaneously avoiding laborious and expensive processes such as altering growing regimes to accommodate significant latent germination. In an effort to avoid seed and labor waste in future whitebark crops, we conducted a study comparing the current CDA Nursery stratification regime to one with an extended cold stratification treatment. We also considered potentially influencing factors, such as seed source and growing schedule. These studies were conducted in the 2011 and 2012 growing seasons at the CDA Nursery.

Materials, Methods, and Treatments

Studies were performed in conjunction with operational whitebark seedling production for National Forest and National Park System clients at the CDA Nursery. In total, 25 operational seedlots were sown, representing all six of the USFS Northern Rockies whitebark seed zones (Table 1). Four of these seedlots had comparable sowings in 2011 and 2012; the others were sown in the 2012 growing season only. Two studies were conducted using germination data collected from these operational (client-requested, large-volume nursery stock order) whitebark sowings.

The first study was designed to compare germination under a single stratification regime using variations in seed source and growing schedule as factors. Namely, seedlot age, source elevation, seed zone, and sowing date were considered as potentially influencing germination rate. Only lots sown during the 2012 growing season were considered in this trial. The second study compared germination rates of four seedlots using differing stratification protocols (60 or 90 days cold stratification) sown in 2011 and 2012.

For both studies, operational whitebark seedlots collected from various locations and years were cleaned, stored, scarified, and sown under standard operational conditions for whitebark seedling production at the CDA Nursery. Seed was cleaned to 98% or better purity, and 90% or better seeds filled. All seed was warm stratified at 18 °C (65 °F) for 30 days, then cold stratified at 0.5-1.7 °C (33-35 °F) for either 60 (2011 sowings) or 90 (2012 sowings) days. Following stratification, seeds were surface dried and scarified using a rotary drum sander by sanding 0.25 lbs (0.11 kg) of seed at a time for three hours, then washing to remove dust. Seeds were immediately sown into containers for operational seedling production by inserting the seed below the media horizon so as to be completely covered with moist media, but no more than 0.25 in (0.64 cm) deep, and covered with no more than 0.25 in (0.64 cm) of inert top-dressing. Containers were placed in greenhouses heated to 18 °C (65 °F), with upward daytime temperature fluctuated minimally, with a cooling set-point at 24 °C (75 °F). Due to the similar and controlled climate parameters in the greenhouses, differences in germination conditions between the 2011 and 2012 growing seasons were assumed to be non-significant for the purposes of this trial. Media was kept moist throughout the germination and growing process.

Seeds were sown at various dates ranging from 19 January to 7June of 2011 and 2012. Final germination counts were taken in early July 2011 and late August 2012. Containers were randomly selected from within a large seedlot block, and seeds (germinated or not germinated) counted as individual replicates. Seedlot sample sizes ranged from 7%-100% of seeds sown, with no less than 392 seeds being sampled for any one lot.

Germination data was compiled and statistical analyses were performed to assess the influences of potential variables on germination performance. Only data from seedlings grown in 2012 were used to assess potential influences of seedlot age, sowing date, and elevation. Least squares regression analyses were used to determine the relative influence and importance of each variable on germination performance in each grouping. Germination percentages for each lot were used as data points and tested for seed zone significance using lot germination averages in an analysis of variance. For the four seedlots sown in both 2011 and 2012, the Pearson's chi-square test was used to compare germinative performance and determine significance. Each seed lot was analyzed using a separate test.

 Table 1. Whitebark pine seedlots sown in 2011 and 2012 for cold stratification length trial, with associated sowing dates, collection geography, seed zones, and germination percentages.

Seedlot	Sow Year	Sow Date	Seed Zone	Collection Year	Elevation	Cold Strat Days	Germ %
GROUSEMTN09	2011	4-May	GYGT	2009	6.2	60	36.5%
UNIONPASS09	2011	25-Jan	GYGT	2009	6.5	60	36.1%
SAWTELL09	2011	2-Feb	GYGT	2009	6.7	60	23.5%
WB02091081	2011	18-Mar	BTIP	2009	8.1	60	21.0%
WBP2066	2012	7-Jun	GYGT	2006	6.4	90	86.2%
WBP2067	2012	7-Jun	GYGT	2006	6.0	90	90.6%
WBP2068	2012	7-Jun	GYGT	2006	6.4	90	86.0%
NUMA	2012	7-Jun	MSGP	2010	6.5	90	59.9%
WBP1262-09	2012	4-Apr	GYGT	2009	8.4	90	73.5%
PRESTONPARK07	2012	20-Mar	MSGP	2007	8.7	90	57.9%
SURPRISE10	2012	20-Mar	GYGT	2010	9.0	90	60.3%
BURKE09	2012	20-Mar	SKCS	2009	9.3	90	58.0%
WHITECALF	2012	20-Mar	MSGP	2010	8.9	90	88.8%
WB14091093	2012	28-Feb	BTIP	2009	8.1	90	63.0%
GROUSEMTN09	2012	26-Jan	GYGT	2009	6.2	90	59.9%
RISINGWOLF	2012	26-Jan	MSGP	2010	9.0	90	71.4%
OLDMAN	2012	24-Jan	MSGP	2010	9.1	90	78.6%
BIGMTN11	2012	8-Feb	MSGP	2011	6.9	90	89.3%
BETA_DESERT_NI	2012	7-Jun	MSGP	2011	7.0	90	73.0%
NAPA_SUNSET11	2012	9-May	MSGP	2011	9.1	90	82.7%
HORNET11	2012	12-Apr	MSGP	2011	6.2	90	70.6%
WB02091081	2012	23-Feb	BTIP	2009	8.1	90	80.6%
DEADLINE11	2012	19-Jan	GYGT	2011	10.0	90	66.6%
LITTLEJOE10	2012	20-Mar	SKCS	2010	9.5	90	66.9%
VIPONDPARK10	2012	27-Feb	CLMT	2010	8.6	90	67.3%
FREEZEOUT10	2012	4-Feb	CFLP	2010	9.2	90	75.8%
WB03030092	2012	24-Feb	GYGT	2003	6.3	90	77.3%
SAWTELL09	2012	23-Feb	GYGT	2009	6.7	90	74.5%
UNIONPASS09	2012	1-Feb	GYGT	2009	6.5	90	62.8%

Results and Discussion

In the first study, regression analyses were used to determine the effect of seed source variables and sowing date, rather than traditional significance tests, due to large sample sizes (n>391 for each lot tested). Regression results for seedlot age (R^2 =0.046; Figure 1), sowing date (R^2 =0.053), and elevation (R^2 =0.10) indicated that these factors held relatively little influence on germinative performance in this trial. Seed zone did not show a significant influence on germination either (p=0.73). In the second study, the results of the a Pearson's chi-square test indicated significant differences in germination between those seeds cold stratified for 60 days and those cold stratified for 90 days. Each of the four seed lots tested showed significantly increased germination when subjected to the longer stratification regime, with germination more than doubling in two of the lots (Figure 2).

It is to be expected that differences in seed collections result in differing germination rates among seedlots. Seed cleaning, handling, storage condition, and storage longevity also influence germinative capacity post-collection (Tomback and others 2001). To what degree variations in collections and seedlot age influence germination in whitebark pine seed, has yet to be determined. From a nursery operational standpoint, these factors are only important if they significantly influence germination rates at the time of seedling production. For the seedlots used in the first study of this trial, none of the source related variables proved to have a strong determining influence on germination. Based on this observation, seed-to-seedling ratios for whitebark pine are not likely be adjusted to accommodate small influences on germination arising from differences in source elevation or seed zone.

Germination is directly correlated to sowing date in an outdoor growing facility due to changing environmental conditions. However, in a greenhouse environment with artificially controlled light, moisture, and temperature, sowing date should not influence germination. This proved to be true in our trial. Of more importance is considering the time required to produce a containerized whitebark seedling, even under optimal growing conditions, and adjusting the planting date accordingly.

All conifer seed has a limited shelf-life; although, it varies considerably from species to species. Historically, much of the whitebark seed at this facility has been sown for seedling production within sev-



Figure 1. Percent germination by seedlot collection year (age) for lots sown in 2012, with 90-day cold stratification.

eral years after collection for two reasons. First, whitebark seed collections have barely kept pace with seed use for seedling production and banking, which resulted in minimal long-term storage. Second, it has been the experience of personnel at this facility (Burr and others 2001) that whitebark has very limited storage longevity, despite its considerable size and nutrient content. Most clients were encouraged to have their seed sown within 1-5 years of collection, to avoid viability losses in cold-storage. However, this assumption arose under older stratification, scarification, and germination protocols (Burr and others 2001). Our results and others (Berdeen and others 2007; McCaughney and others 1990) indicate that seedlot age plays a much less significant role in reducing germinative capacity than was previously thought. Granted, seedlots held in cold-storage necessarily begin to lose viability at some point; and our sample represents primarily young seedlots (<4 years in storage; Table 1). However, the strong germinative capacity of older seedlots in this trial (up to 9 years in storage; Figure 1), leads us to reconsider the role of seedlot age in reducing germination. When cleaned to a high purity and percentage of filled seeds, whitebark seed may have considerable longevity, likely exceeding a decade in cold storage without considerable loss of germinative capacity.



Figure 2. Effect of length of seed stratification on germination percentage. A chi-square test was performed individually on each seedlot. Different letters denote significance at α =0.05.

USDA Forest Service Proceedings, RMRS-P-69. 2013

Furthermore, empirical observations of seedlots being grown at the CDA Nursery suggests an improvement in germination rates for seedlots held in cold storage for at least a year post-collection.

The second study in this trial revealed the importance of stratification length in obtaining high germination percentages in whitebark. Although similar trials have been conducted on a relatively small scale (Riley and others 2007; Wick and others 2008), these works were research-oriented and used scarification and germination techniques not practical at an operational restoration scale. This combination of 90-day cold stratification and mass scarification resulted in higher and more consistent germination than has been seen before at this facility for operational whitebark crops (unpublished data). Because the two stratification groups were grown in separate years, it is possible that factors beyond those considered here influenced germination. However, as already discussed, greenhouse conditions and scarification regimes were unchanged between the two growing seasons. Theoretically, an additional year in cold-storage would have had no effect or a small negative effect on germination, and because all four of the lots used for the second study were collected in 2009, any storage influence would be shared equally amongst them. These factors fail to explain the significant increase in germination apparent in all four seedlots, which indicates that a 90-day cold stratification is instrumental in obtaining strong germination for direct-sown, restoration-level whitebark pine seedling production.

Summary

Whitebark pine seedling production levels continue to rise at the CDA Nursery, as forest managers are increasingly in a position to bolster recruitment in compromised stands using disease-resistant seedlings. At the production scale being seen now, original scarification and stratification methods are no longer economically viable, and waste of hard-won whitebark seed is not acceptable at these levels. In an effort to maximize germinative capacity without sacrificing production efficiency, dormancy release factors must be understood and overcome. Given proper seed collection, handling, cleaning, and storage, the results of these two studies indicate that: 1) 90-day cold stratification results in significantly increased germination over the 60-day treatment; 2) within the first decade of storage, seedlot age may not play as crucial a role in reducing germinative capacity as was previously thought; and 3) seedlot source geography may not have a strong enough influence on germinative capacity to merit altering seed use calculations or culture regimes for greenhouse production.

Although cold stratification lengths in excess of 90 days become logistically burdensome at this facility, further study should be conducted to see if longer cold stratification periods result in higher germination rates. Additionally, more research will be needed to better understand true whitebark seed longevity in cold-storage, especially with regards to variant embryo maturity (Tillman-Sutela and others 2008), and the potential positive effect of storing seed for at least a year post-collection. Seed managers and horticulturists at the CDA Nursery will use this data and future studies to increase whitebark seed use and production efficiency, in an effort to better contribute to the restoration of this high-elevation cornerstone species.

References

- Berdeen J, Riley L, Sniezko R. 2007. Whitebark pine seed storage and germination: a follow-up look at seedlots from Oregon and Washington. In: Goheen E, and Sniezko R, technical coordinators. Proceedings – Whitebark Pine: A Pacific Coast Perspective. Portland (OR): USDA Forest Service, Pacific Northwest Region R6–NR–FHP–2007–01. p 113-121.
- Burr K, Eramian A, Eggleston K. 2001. Growing whitebark pine seedlings for restoration. In: Tomback D, Arno S, and Keane R,

editors. Whitebark Pine Communities, Ecology and Restoration. Washington (DC): Island Press. p 325-345.

- Eggleston K. 2012. Personal communication. Coeur D Alene (ID): Greenhouse Horticulturist, USDA Forest Service, Coeur D Alene Nursery.
- Gasvota D, Trent A, Harding C, Burr K. 2002. Whitebark pine seed scarifier. In: Timber Tech Tips, Nov. 2002. USDA Forest Service, Technology & Development Program. 0221-2332-MTDC.
- Keane R, Tomback D, Aubry C, Bower A, Campbell E, Cripps C, Jenkins M, Mahalovich M, McKinney S, Murray M, Perkins D, Reinhart D, Ryan C, Schoettle A, Smith C. 2012. A range-wide restoration strategy for whitebark pine (*Pinus albicaulis*). Fort Collins (CO): USDA Forest Service, Rocky Mountain Research Station. General Technical Report RMRS-GTR-279. 108 p.
- Mahalovich M, Burr K, Foushee D. 2006. Whitebark pine germination, rust resistance, and cold hardiness among seed sources in the Inland Northwest: planting strategies for restoration. In: Riley L, Dumroese RK, LandisTD, technical coordinators. Proceedings – Forest and Conservation Nursery Associations – 2005. Fort Collins (CO): USDA Forest Service, Rocky Mountain Research Station. RMRS-P-43. p 91-101.
- McCaughney W, Schmidt W. 1990. Autoecology of whitebark pine. In: Schmidt W, McDonald K, compilers. Proceedings – Symposium on whitebark pine ecosystems: Ecology and management of a high-mountain resource. Ogden (UT): USDA Forest Service, Intermountain Research Station. General Technical Report INT-GTR-270. p 85-96.
- McCaughney W. 1992. The regeneration process of whitebark pine. In: Schmidt W, Holtmeier F, compilers. Proceedings – International workshop on subalpine stone pines and their environment: The status of our knowledge. Ogden (UT): USDA Forest Service, Intermountain Research Station. General Technical Report INT-GTR-309.

- Pitel J, Wang B. 1990. Physical and chemical treatments to improve germination of whitebark pine seeds. In: Schmidt W, McDonald K, compilers. Proceedings – Symposium on whitebark pine ecosystems: Ecology and management of a high-mountain resource. Ogden (UT): USDA Forest Service, Intermountain Research Station. General Technical Report INT-GTR-270. p 130-133.
- Riley L, Coumas C, Danielson J, Berdeen J. 2007. Seedling nursery culture of whitebark pine at Dorena Genetic Resource Center: headaches, successes, and growing pains. In: Goheen E, Sniezko R, technical coordinators. Proceedings – Whitebark Pine: A Pacific Coast Perspective. Portland (OR): USDA Forest Service, Pacific Northwest Region. Report R6–NR–FHP–2007–01. p 122-131.
- Tillman-Sutela E, Kauppi A, Karppinen K, Tomback D. 2008. Variant maturity in seed structures of Pinus albicaulis (Engelm.) and Pinus sibirica (Du Tour): key to a soil seed bank, unusual among conifers? Trees-Structure and Function 22(2):225-236.
- Tomback D, Anderies A, Carsey K, Powekk M, Mellmann-Brown S. 2001. Delayed seed germination in whitebark pine and regeneration patterns following the Yellowstone fires. Ecology 82:2585-2600.
- Tomback D, Arno S, Keane R. 2001. Whitebark pine communities: ecology and restoration. Island Press, Washington, DC, USA. 440 p.
- Waring K, Goodrich B. 2012. Artificial regeneration of five-needle pines of western North America: a survey of current practices and future needs. Tree Planters Notes 55(2):55-71.
- Wick D, Luna T, Evans J, Hosokawa J. 2008. Propagation protocol for production of container Pinus albicaulis Engelm. Plants (172 ml conetainers); USDI NPS – Glacier National Park, West Glacier, MT. In: Native Plants Network. URL: http://www.native plantnetwork.org (accessed 7 August 2012).

The Importance of Bees in Natural and Agricultural Ecosystems

Paul Rhoades

Paul Rhoades is Graduate Research Fellow, Department of Plant, Soils and Entomological Sciences, University of Idaho, Moscow, ID 83843; email: paul.r.rhoades@gmail.com

Rhodes P. 2013. The importance of bees in natural and agricultural ecosystems. In: Haase DL, Pinto JR, Wilkinson KM, technical coordinators. National Proceedings: Forest and Conservation Nursery Associations—2012. Fort Collins (CO): USDA Forest Service, Rocky Mountain Research Station. Proceedings RMRS-P-69. 77-79. Available at: http://www.fs.fed.us/rm/pubs/rmrs_p069.html

Abstract: As the world's most important group of pollinators, bees are a crucial part of agricultural production and natural ecosystem function. Bees and the pollination they provide are relevant to the nursery industry because of their role in the performance of seed increase plots as well as the importance of pollination in supporting persistent plant communities in restored areas. Agricultural producers can increase seed or fruit production with colonies of European honey bees, managed native bees, or by managing land to increase populations of native bees. By meeting requirements for food and nesting resources restored areas can support similar levels of species richness and abundance of native bees. Although the specific species of bees present may differ between restored and remnant sites, pollination function and community resilience can be restored.

Keywords: pollination, seed production, seed increase, bees, restoration

The Importance of Bees in Natural and Agricultural Ecosystems

Pollination services provided by insects are an indispensable component to both natural and agricultural ecosystems (Kremen and others 2002, Pauw 2007, Klein and others 2007). Estimates of the value of insect pollination of agricultural crops in the United States range from \$150 million paid for pollination services to \$3.07 billion in total crop value (Morse and Calderone 2000; Losey and Vaughan 2006). While agricultural intensification and urbanization may be reducing the numbers of wild pollinators (Kremen and others 2002), native (as opposed to managed) bees can still provide abundant crop visitation in a variety of landscape types (Winfree and others 2008). Although many taxa, including moths, flies, beetles, hummingbirds, and bats, function as pollinators, bees are most effective in many cases (Steffan-Dewenter and Tscharntke 1999). This is true for a few reasons. Bee larvae and adults both rely almost entirely on pollen and nectar for sustenance. Therefore the number of visits bees make to flowers, as well as the distance moved between flowers, are greater than in other pollinator taxa (Willmer 2011). As well, bees as a group have a wide range of sizes and morphological adaptations that enable mutual relationships with a wide range of host plants (Thorp 1979, 2000; Michener 2000). Conversely, wasps, the most similar group of organisms, feed on nectar only as adults, hunting other arthropods to provide the nitrogen needed for larval development. Other groups of animal pollinators important in temperate areas (butterflies, flies and beetles) also only feed on pollen and nectar as adults.

The mutual relationship between bees and plants is complex. The vigor of a bee community is determined by plant species richness and diversity at both small and large scales (Steffan-Dewenter and Tscharntke 1999; Hendrix and others 2010). Similarly, seed production and, potentially, eventual plant recruitment is dependent on a species rich and diverse bee community (Steffan-Dewenter and Tscharntke 1999; Slagle and Hendrix 2009). A variety of floral visitors is important as different bees have different habits and interact with the flower in different ways, improving pollination; a variety of accessible plants provides greater niche space (Potts and others 2003).

Providing Supplemental Pollination Using Honey Bees

Ensuring high levels of pollination can produce larger yields and higher quality fruit. Supplemental crop pollination is most commonly provided with managed colonies of the European honey bee. Convenience and predictable efficacy has made the honey bee an integral part of modern agriculture. About 150,000 honey bee colonies are rented to U.S. growers for pollination annually, with almonds, apples, and cherries comprising about half of all colony rentals (Burgett and others 2010). The European honey bee is very well suited to commercial pollination. Colony strength can be visually inspected and extremely high numbers of pollinators can be placed anywhere within the agroecosystem at a time that suits the grower. These traits allow growers certainty there will be sufficient pollination service when and where it is needed.

However, relying on pollination by managed honey bees has drawbacks. Pollination efficiency varies by crop, and some pants (such as alfalfa) are not effectively pollinated by honey bees. Additionally, Colony Collapse Disorder and honey bee colony death due to mites and disease continue to be problems. Although there has been much research into honey bee health recently (Figure 1), rentals of honey bee colonies are becoming increasingly expensive (about \$90/colony in the western U.S.) and may be unavailable in certain areas (Burgett and others 2010).

Encouraging Native Bee Pollination

Because of these drawbacks, growers in some systems have chosen to actively manage native bees for pollination. A robust population of native bees can provide pollination service throughout the growing season, which is important if the grower has a variety of crops requiring pollination that may flower at different times. In agricultural systems, diverse and species rich plant communities near pollinator dependent crops can increase pollination and greatly add to crop value (Kremen and others 2004).

The amount of land hosting diverse floral resources in the few hundred meters surrounding a flowering crop is directly related to pollen deposition (Kremen and others 2004). Additionally both



Figure 1. Bees feeding on a special diet formulated by the USDA-ARS. Honey bee die off may be caused by a combination of poor nutrition, pesticide exposure, pests, or pathogens. Honey bee colony rental is becoming increasingly expensive despite recent progress in understanding and mitigating honey bee stressors. (Photo: Stephen Ausmus USDA-ARS).

small and large alterations to the farmscape, such as leaving areas fallow, planting strips of wildflowers, or reducing tillage can increase local presence of pollinating insects (Vaughan and others 2007). Limiting pollinator exposure to pesticides cannot be stressed enough. Avoiding treatment of flowering crops or spraying in the evening with a low residual insecticide is the most important single thing a grower can do to limit pollinator mortality (Johansen and Mayer 1990). For more information see 'Farming for Bees' published by the Xerces society.

Effect of Restoration on Bee Populations

In natural settings a robust plant-pollinator community fosters ecosystem resilience because each supports the other (Fontaine and others 2005). A strong bee community requires a diverse selection of plants to provide places for nesting as well as sustenance throughout the season (Potts and others 2005; Franzén and Nilsson 2010). The diversity of a flowering plant community is closely linked to the functional diversity of a complementary community of pollinating insects (Fontaine and others 2005). Seed production in most plants is, in some years, limited by inadequate pollen deposition (Burd 1994), and many native forbs greatly increase seed production with pollinator visitation (Figure 2) (Cane 2008). Pollen limitation may be the most significant cause of reproductive failure in fragmented habitats (Aguilar and others 2006). For all these reasons, a robust community of native bees is necessary for thriving plant communities in restored areas (Handel 1997).

Although there may be structural differences in bee communities between remnant and restored areas, bee species richness and pollination function can be restored to levels similar to remnant native habitat (Exeler and others 2009; Williams 2011) as long as necessary food and nesting resources are available within foraging range (Potts and others 2005; Winfree 2010). Nesting resources can take the form of bare soil, dead woody substrate, pithy or hollow stems, or rodent burrows depending on the bee species. Food for bees is entirely composed of pollen and nectar produced by flowers. The timing and variety of floral resources available are important mediators of the bee community at a site. Restored areas should have plant species that provide pollen and nectar for the duration of the growing season. These plants have been referred to as 'framework' and 'bridging' plants depending on their role in the support of the pollinator community (Dixon 2009).



Figure 2. Pollinator visitation can greatly increase plant seed production. Increased seed production may increase resilience of plant communities.

Framework plants provide copious amounts of pollen and nectar to a wide range of bee species. This will ideally create a large and diverse bee community that then provides pollination to a number of less attractive plant species (Ghazoul 2006). However this strategy may fail if the framework plants chosen compete with other plants through pollination. It is difficult to predict if plant species sharing pollinators will compete for or mutually facilitate pollination (Menz and others 2011).

Bridging plants provide nectar and pollen in resource-limited times of year. The necessity of bridging plants depends on the length of the growing season and the pollinators involved (Dixon 2009). If the growing season is short enough bridging plants may not be needed because floral resources may be very common during the short time bees are actively foraging (Menz and others 2011).

Summary

Pollination is a crucial part of seed or fruit production; many native forbs and annuals produce much more seed when pollinators are present (Cane 2008; Slagle and Hendrix 2009). Pollination can be increased or ensured through introduction of European honey bee colonies or by managing the agro-ecosystem for native bee habitat. Honey bees may be an attractive choice for some growers because of their simplicity and general efficacy. However the cost of importing colonies may be high and honey bees are ineffective pollinators for some crops. By providing food and nesting resources near crops requiring pollination a species rich community of native bees can be maintained providing quality pollination service throughout the growing season.

An abundance of floral and nesting resources will also foster a strong community of native bees in restored areas. Replanting shrubs or woody plants, if appropriate, will complement existing soil nesting sites to provide nesting opportunities for a wide variety of bees. Restoring a site with a mixture of plants that offer nectar and pollen throughout the growing season will support a variety of bee species with different periods of activity. A diverse bee community can increase the likelihood restored plantings will persist by ensuring ecosystem function is restored along with the plant community.

References

- Aguilar R, Ashworth L, Galetto L, Aizen MA. 2006. Plant reproductive susceptibility to habitat fragmentation: review and synthesis through a meta-analysis. Ecology Letters 9:968–980.
- Burd M. 1994. Bateman's principle and plant reproduction: The role of pollen limitation in fruit and seed set. The Botanical Review 60:83-139.
- Burgett M, Daberkow S, Rucker R, Thurman W. 2010. US pollination markets: Recent changes and historical perspective. American Bee Journal 150:35-41.
- Cane JH. 2008. 4. Pollinating Bees Crucial to Farming Wildflower Seed for U.S. Habitat Restoration. In: James R, Pitts-Singer TL, editors. Bee Pollination in Agricultural Eco-systems, 1st edition. Oxford University Press, Oxford, England. p 48-65.
- Dixon KW. 2009. Pollination and restoration. Science 325:571.
- Exeler N, Kratochwil A, Hochkirch A. 2009. Restoration of riverine inland sand dune complexes: implications for the conservation of wild bees. Journal of Applied Ecology 46:1097-1105.
- Fontaine C, Dajoz I, Meriguet J, Loreau M. 2005. Functional diversity of plant-pollinator interaction webs enhances the persistence of plant communities. PLoS Biology 4:e1.
- Franzén M, Nilsson SG. 2010. Both population size and patch quality affect local extinctions and colonizations. Proceedings of the Royal Society B: Biological Sciences 277:79 -85.

- Ghazoul J. 2006. Floral diversity and the facilitation of pollination. Journal of Ecology 94:295-304.
- Hendrix SD, Kwaiser KS, Heard SB. 2010. Bee communities (Hymenoptera: Apoidea) of small Iowa hill prairies are as diverse and rich as those of large prairie preserves. Biodiversity and Conservation 19:1699-1709.
- Johansen CA, Mayer DF. 1990. Pollinator protection: a bee & pesticide handbook.
- Klein, AM, Vaissière BE, Cane JH, Steffan-Dewenter I, Cunningham SA, Kremen C, Tscharntke T. 2007. Importance of pollinators in changing landscapes for world crops. Proceedings of the Royal Society B: Biological Sciences 274:303-313.
- Kremen C, Williams NM, Bugg RL, Fay JP, Thorp RW. 2004. The area requirements of an ecosystem service: crop pollination by native bee communities in California. Ecology Letters 7:1109-1119.
- Kremen C, Williams NM, Thorp RW. 2002. Crop pollination from native bees at risk from agricultural intensification. Proceedings of the National Academy of Sciences 99:16812-16816.
- Losey JE, Vaughan M. 2006. The Economic Value of Ecological Services Provided by Insects. BioScience 56:311-323.
- Menz MHM, Phillips RD, Winfree R, Kremen C, Aizen MA, Johnson SD, Dixon KW. 2011. Reconnecting plants and pollinators: challenges in the restoration of pollination mutualisms. Trends in Plant Science 16:4-12.
- Michener CD. 2000. The bees of the world. JHU Press. 913 p.
- Morse RA, Calderone NW. 2000. The value of honey bee pollination in the United States. Bee Culture 128:1-15.
- Pauw A. 2007. Collapse of a pollination web in small conservation areas. Ecology 88:1759-1769.
- Potts SG, Vulliamy B, Dafni A, Ne'eman G, O'Toole C, Roberts S, Willmer P. 2003. Response of plant-pollinator communities to fire: changes in diversity, abundance and floral reward structure. Oikos 101:103-112.
- Potts SG, Vulliamy B, Roberts S, O'Toole C, Dafni A, Ne'eman G, Willmer P. 2005. Role of nesting resources in organising diverse bee communities in a Mediterranean landscape. Ecological Entomology 30:78-85.
- Slagle MW, Hendrix SD. 2009. Reproduction of Amorpha canescens (Fabaceae) and diversity of its bee community in a fragmented landscape. Oecologia 161:813-823.
- Steffan-Dewenter I, Tscharntke T. 1999. Effects of habitat isolation on pollinator communities and seed set. Oecologia 121:432-440.
- Thorp RW. 1979. Structural, behavioral, and physiological adaptations of bees (Apoidea) for collecting pollen. Annals of the Missouri Botanical Garden 66:788-812.
- Thorp RW. 2000. The collection of pollen by bees. Plant systematics and evolution 222:211-223.
- Vaughan M, Shepherd M, Kremen C, Black SH. 2007. Farming for bees. The Xerces Society for Invertebrate Conservation, Portland, Oregon 34 p.
- Williams NM. 2011. Restoration of nontarget species: bee communities and pollination function in riparian forests. Restoration Ecology 19:450-459.
- Willmer P. 2011. Pollination and Floral Ecology. Princeton University Press. 828 p.
- Winfree R. 2010. The conservation and restoration of wild bees. Annals of the New York Academy of Sciences 1195:169-197.
- Winfree R, Williams NM, Gaines H, Ascher JS, C. Kremen C. 2008. Wild bee pollinators provide the majority of crop visitation across land-use gradients in New Jersey and Pennsylvania, USA. Journal of Applied Ecology 45:793-802.

The content of this paper reflects the views of the authors, who are responsible for the facts and accuracy of the information presented within.

Current Seed Orchard Techniques and Innovations

Lawrence K Miller and Jeffrey DeBell

Larry Miller is Forest Geneticist, Oregon Department of Forestry, 2600 State St., Salem, OR 97310; email: Larry.K.Miller@state.or.us

Jeff DeBell is Silviculturist/Geneticist, Genetic Resources Program, Washington Department of Natural Resources, PO Box 47017, Olympia, WA 98504; email: jeff.debell@ dnr.wa.gov

Miller LK, DeBell J. 2013. Current seed orchard techniques and innovations. In: Haase DL, Pinto JR, Wilkinson KM, technical coordinators. National Proceedings: Forest and Conservation Nursery Associations—2012. Fort Collins (CO): USDA Forest Service, Rocky Mountain Research Station. Proceedings RMRS-P-69. 80-86. Available at: http:// www.fs.fed.us/rm/pubs/rmrs_p069.html

Abstract: As applied forest tree improvement programs in the US Northwest move forward into the third cycle, seed orchards remain as the primary source of genetically improved forest tree seed used for reforestation. The vast majority of seed orchards in this region are coastal Douglas-fir (Pseudotsuga menziesii (Mirb.) Franco), consistent with the high economic importance of this species. However, productive seed orchards are also in place for western hemlock (Tsuga heterophylla (Raf.) Sarg.), noble fir (Abies procera Rehd.), western redcedar (Thuja plicata Donn), ponderosa pine (Pinus ponderosa Laws.), lodgepole pine (Pinus contorta Dougl.), western larch (Larix occidentalis Nutt.), western white pine (Pinus monticola Dougl.), sugar pine (Pinus lambertiana Dougl.), and Port-Orford-Cedar (Chamaecyparis lawsoniana (A. Murr.) Parl.). To be successful, seed orchards must be managed intensively, including: control of weeds, mammals, and cone and seed insects; graft and crown maintenance; strict identity control; irrigation; fertilization; crop stimulation; and ultimately, harvest of high guantities of genetically improved seed. Over the past 40+ years, seed orchard management practices have been developed to improve the reliability and size of cone and seed crops and reduce damage and loss from cone and seed insects, thereby increasing the efficiency of orchard operations. In this paper, we discuss the current state of the art in seed orchard management in the Northwest, with particular emphasis on Douglas-fir.

Keywords: graft compatible rootstock, flower stimulation, cone and seed insect control, irrigation, weed control, Douglas-fir

Introduction

Although people have harvested seeds and fruit from trees for centuries, only after World War II did the science and theories of forest genetics begin to be seriously applied to and practiced in operational forestry around the world. Early tree breeders and geneticists showed that traits important to the production of timber and pulpwood were inherited and, as such, could be improved to increase forest productivity and wood quality. At some point however, these genetic improvements, or gains, need to be captured, packaged, and mass produced so that these gains could be deployed in operational forestry systems. Since the mid-to-late 1950's through to today, the most common approach is the use of seed orchards.

A seed orchard may be defined as:

"...a plantation of selected clones or progenies which is isolated or managed to avoid or reduce pollination from outside sources, and managed to produce frequent, abundant, and easily harvested crops of seed." (Feilburg and Soegaard 1975).

Today in the Northwestern US, much of the coastal Douglas-fir (*Peudotsuga menziesii* (Mirb.) Franco) and western hemlock (*Tsuga hetero-phylla* (Raf.) Sarg.) seed used for reforestation on industrial and some public forest lands is produced in seed orchards (Figure 1). Many of these seed orchards comprise second generation (or cycle) selected material from progeny tests, and either are, or soon will be, producing seed with

genetic gain estimates in the range of 20-30% for volume. Seed for a number of "minor" species is also being produced in Northwest US seed orchards, including:

- 1. Noble fir (Abies procera Rehd.)
- 2. Western redcedar (Thuja plicata Donn)
- 3. Ponderosa pine (Pinus ponderosa Laws.)
- 4. Lodgepole pine (*Pinus contorta* Dougl.)
- 5. Western larch (Larix occidentalis Nutt.)
- 6. Western white pine (Pinus monticola Dougl.)
- 7. Sugar pine (Pinus lambertiana Dougl.)
- 8. Port-Orford-Cedar (*Chamaecyparis lawsoniana* (A. Murr.) (Parl.)
- 9. Sitka spruce (*Picea sitchensis* (Bong.) Carr.)



Figure 1. The production of genetically improved seed in orchards is robust, mature technology.

Much of the current management practices in Northwest US seed orchards have been adapted from those first developed in southern pine orchards. In proportion to its economic importance, most of seed orchard research and application in this region has been devoted to Douglas-fir. Many of the techniques and practices used on Douglas-fir are also used successfully with other Northwest species, but there are a number of distinct differences. In this paper, we will discuss current advancements in seed orchard management in the Northwest, with particular emphasis on Douglas-fir. We will outline key practices that are important in successful seed orchards and briefly discuss areas of ongoing operational research.

Graft Compatible Rootstock

When forest geneticists identify phenotypically superior selections, elite (proven superior) material, or both, we want to make multiple copies of each selection for inclusion in the seed orchard. By far the most common method of vegetative propagation used in seed orchards is grafting. Grafting is a time-honored, very successful process of vegetative propagation used broadly in fruit and nut orchards and in forest tree seed orchards. It works well, is relatively simple, and very quickly can produce multiple copies of elite selections. That is of course, in almost all tree species except Douglas-fir.

Douglas-fir stands alone as the only major forest tree species that suffers from serious graft incompatibility (Figure 2). Graft incompatibility is analogous to tissue or organ rejection in human



Figure 2. Graft incompatibility in Douglas-fir.

transplant cases. In the late 1960's to early 1970's, early tree improvement workers hopes for success were cruelly dashed when, one by one, they watched grafted Douglas-fir in first generation seed orchards begin to die from graft incompatibility. In some of these early orchards, mortality from graft incompatibility ran 80% or more, leaving an uneconomical enterprise in its wake. Graft incompatibility was so bad in early Northwest seed orchards that many landowners switched to seedling seed orchards. This compromised genetic gain, but at least the orchard trees survived to produce seed!

However, one researcher at the PNW Research Station in Corvallis didn't throw in the towel. Over the course of more than 20 years, Don Copes conducted research designed to identify specific genotypes that were highly graft compatible. By the late 1970's, he identified 16 clones that were highly graft compatible. These were propagated in the thousands as rooted cuttings, and used as rootstock for new orchard establishment (Copes 1981). Later, controlled crosses were made between the compatible selections to produce highly graft compatible seed. Use of this seed for the production of Douglas-fir rootstock is standard practice, and has allowed the rapid development of new seed orchards. There is probably no other Douglas-fir seed orchard practice that has had more positive effect on program success than has the Copes graft compatible rootstock.

Flower Stimulation

Although it is taxonomically imprecise, tree breeders commonly use the term, "flowers" when referring to floral structures in trees. Thus, female flowers produce cones, and male flowers produce pollen. To efficiently produce seed, the only important goal of the seed orchard, trees must flower at an early age, reliably, and heavily. In natural stands, flowering occurs periodically and erratically, 3-7 or more years apart. In a seed orchard, such performance is unacceptable.

Thus, early researchers began looking into practices that might improve both the regularity and the abundance of flowering. Flower stimulation includes practices applied at key developmental points in year 1, which lead to significantly increased flowering in the spring of year 2. Looking to practices used in the US South, fertilization was tried with some success. However, the results weren't consistent across orchards, and applying the large amount of fertilizer needed for larger orchard trees has gotten quite expensive. Eventually, two practices were developed that consistently resulted in regular and abundant flowering – partial girdling and application of gibberellic acid (GA).

Partial Girdling

In many lower elevation coastal Douglas-fir seed orchards, partial girdling is a very safe, low cost, very predictable technique for stimulating consistent and reliable flower crops. It can be applied when the trees are at least 2 inches (5 cm) in diameter at the base, although in practice, most orchard managers wait until orchard trees are 6-7 years old. There are varying recommendations regarding optimal dates: at the time of vegetative bud swell (Ross and Bower 1989; Ross and Bower 1991), 6 weeks before vegetative bud break (Clemo, personal communication), 1-3 weeks before vegetative bud flush (Woods 1989), or at the beginning of pollen bud swell (Reno 2008). Orchard managers typically test different application dates, then settle on the time that works best for their orchard.

Girdling involves the application of two overlapping half-circumferential saw cuts that go through the bark and cambium just until the saw teeth reach the xylem (wood). The first girdle should be placed at a comfortable working height, with the second cut being placed roughly 1.5 times the stem diameter above or below the first cut (Figure 3). For large trees, cuts are spaced no more than a foot apart. Each year, girdles are made in fresh wood, not in old scars. Practiced with care on otherwise healthy trees, girdling can be applied every other year, producing very predictable flowering and cone crops. In orchard complexes containing multiple blocks of different material, undesirable cross pollination between blocks can be managed by the schedule of girdling. If we do not want Orchard A pollinating the trees in Orchard B, we simply girdle these orchards in alternating years. The flowering response occurs the following year, thereby minimizing pollen contamination.



Figure 3. Double, overlapping, partial girdling is a very successful means of flower stimulation in low elevation west side orchards.

Partial girdling works very well in Douglas-fir, but has been less predictable in other species. For some of these species, the application of GA has been shown to work well.

Application of Gibberrellic Acid (GA)

Much of the research on flowering and GA was done with Douglas-fir in British Columbia in the 1970's and 80's (Ross and Bower 1989). This research showed that proper timing, technique, and choice of GA are the keys to success. There are many GAs, usually designated by number. The combination of GA4 and GA7 has been shown to be very effective in stimulating flower production in Douglas-fir. GA 4/7 is available in either crystalline, or in liquid, ready-to-use formulations. Crystalline GA must be dissolved in ethyl alcohol before use. The liquid form is known by the trade name, ProConeTM (Valent Corporation), and is considered the industry standard. As with all chemicals, consult the label for safe and proper use.

Application is accomplished by drilling holes in each tree, and injecting a pre-measured amount of ProCone in each hole (Figure 4). The application rate is based on the cross-sectional area at the point of injection. First treatments can usually start when ramets are three or four years from grafting. A recent study indicated that some treatments applied 2 years after grafting could be effective (Cherry and others 2007). There are different opinions on best timing: two weeks before vegetative budbreak (Reno 2008), about the time of vegetative budbreak (Cherry and others 2007), when 50% of the trees have flushed (Ross and Bower 1991), when most of the trees have flushed (Ross and Bower 1989), and up to the time of that 50 - 90% of the year's vegetative growth has occurred (Pro-Cone label). Best results are usually obtained when using freshly purchased ProCone.



Figure 4. Gibberellic acid is also used to stimulate flowering.

In low elevation coastal Douglas-fir, GA 4/7 is used more in breeding orchards, where trees are smaller and younger, and could be damaged by girdling. However, GA gives excellent results in western hemlock, and has shown promise in ponderosa pine and noble fir.

GA is also used to stimulate flowering in western redcedar and Port-Orford-Cedar, but both the formulation and the method of application is different. For these species, research shows that GA 3 applied as a foliar spray in mid-summer stimulates significant flowering the following spring (Russell and Hak 2007).

Control of Cone and Seed Insects

A thorough discussion of seed and cone insect control is beyond the scope of this paper, so only a brief outline of key management practices will be presented. Successful seed orchards are intensively managed plantations, where maximum seed production per acre is the goal. Thus, anything that reduces seed yield negatively affects economic rate of return. Because orchards flower and produce cones much more frequently and more heavily than natural stands, damaging insect populations can build up and cause significant damage to developing crops. Seed orchard managers must become experts on the detection and control of a wide variety of insect pests. With time and experience, managers learn which insect pests are most important, and usually focus their control efforts accordingly.

The first step in any control program is to know which insect is causing damage to cones and loss of seed. An excellent reference for identifying cone and seed insects is "Cone and Seed Insects of North American Conifers" (Hedlin and others 1980). In the Northwest, several of the state forestry and natural resource departments have entomologists on staff that can assist with identification and control options. The USDA Forest Service also has trained staff, which may be able to help.

Over the years, control strategies for the most important cone and seed insects have been developed. Forestry is a small business compared to agriculture, so the available list of insecticides labeled for use in seed orchards is limited, and tends to change with time. No attempt will be made in this paper to list individual insecticides – it is better to work with other seed orchard managers and entomologists to determine which are labeled for use, and known to be effective. Rather, we will focus on application practices commonly in use in Northwest US seed orchards. As well, there are some technologies relatively new to forestry that may well become established as common use in the not-too-distant future.

Application Practices

Aerial Application

Where the surrounding landscape permits, aerial application is very effective and clearly the most cost effective means of applying insecticides to seed orchards in the US Northwest. Helicopters are used most often, effective in treating trees in blocks with widely different ages and thus, tree heights. Excluding ferry time, aerial application is very fast, which is helpful when the window for wind speed is open for only a short period of time (Figure 5).



Figure 5. Aerial spraying provides excellent, low cost control of cone and seed insects.

Ground Application

Where neighbor issues make aerial spraying problematic, ground systems using high pressure mist blowers are an effective alternative. Such equipment makes efficient use of chemical, but small droplet size means that drift must be carefully monitored. Maximum tree height is a limitation, so orchards should be managed for height with due consideration to performance of the available mist blower. Ground application is much slower than aerial, so spraying mat be stretched out over several days because of wind speed limitations. Hydraulic sprayers may also be used to apply insecticides in seed orchards, but these high volume systems are slow and have a higher risk of worker exposure.

Individual Tree Application

In cases where either non-farm land use begins to encroach on seed orchard sites, or internal organization policies prohibit aerial and ground spraying, some technologies relatively new in forestry are being considered, and used in some cases. Individual tree treatments typically involve stem injections of systemic insecticides, and come to forestry from the landscape and horticulture industry. When Dutch elm disease was killing American elm trees across much of the Eastern US, some towns and cities were able to keep large boulevard trees alive longer with stem injections to control the disease vector, the European elm bark beetle. Individual tree treatment is slow and expensive, but given the high value of trees in cities, the cost could often be justified.

Today, individual tree treatment is still practiced on town and city trees, but targets now include emerald ash borer, hemlock woolly adelgid, and mountain pine beetle (Doccola and others 2012). Fortunately for seed orchard use, techniques and equipment have been significantly improved, making application much faster per tree than in the past (Figure 6). Individual tree treatment is still quite slow, when compared to aerial and ground application, but in some orchard locations it may be the only viable method available to the seed orchard manager. Current technologies still rely upon drilling several holes in each tree, but the injectors used are considerably faster than previous versions. This technology holds considerable promise because some



Figure 6. Where aerial spraying cannot be practiced, individual injection provides another option.

of the research suggests that the control effect may last for several years (McCollough and others 2009). If multiple year control is achieved, the cost effectiveness of individual tree treatment will improve significantly.

Vegetation Management

When seed orchards are established, it is important to control vegetation within the newly planted tree rows so that the trees grow as free as possible from competition. The date of first flowering and cone production is correlated with tree size, so the sooner trees get bigger, the sooner genetically improved seed will be produced. Seed orchard managers typically use readily available forestry herbicides to maintain a weed free strip within the tree row. This weed free strip also reduces habitat for voles, which can cause significant damage to newly planted grafts.

Between rows, it is important to maintain a good running surface to lessen compaction and possible rutting from the regular equipment travel necessary in everyday orchard management. For many years, orchard managers would accept whatever grass came up between rows, spending much of each summer mowing to maintain access and reduce fire danger. Mowing is a necessary and often costly expense. Some orchards have established cover crops that are low growing to reduce mowing costs. More recently, short growing grass varieties classically bred to be tolerant of glyphosate have become available, and some managers are establishing new cover crops with this seed (Figure 7). Orchards containing this grass need to be mown once, rarely twice, and the grass never gets any taller than about knee high. Weeds between rows are easily cleaned up with glyphosate. In orchards where this grass variety has been established, mowing costs have dropped significantly.



Figure 7. Low growing grass varieties, classically bred to be tolerant of glyphosate, create an excellent orchard cover crop.

Crown Management

In the US Northwest, the tree species established in seed orchards have the inherent ability to grow quite tall. Harvesting cones from trees as they grow taller increases in cost as man lifts with greater reach must be either rented or acquired. The equipment needed to harvest trees that are 15-20 feet (4.5-6 m) tall is much less costly than that needed for trees 50-60 feet (15-18 m) tall. Thus some managers are testing the feasibility of regular tree topping to manage the height of their trees. One orchard is using a sickle bar mounted on a loader to accomplish automated topping of Douglas-fir (Figure 8). In this system, the orchard tree height is being managed so that all cone harvesting may be done from the ground or from short ladders. No man



Figure 8. Sickle bar mower for automated tree topping in a Douglas-fir seed orchard.

lift equipment will be needed. However, because cone production is correlated to tree size, shorter trees produce fewer cones. Thus, to increase cone production in such topped orchards, planting density must be increased. Compared to more traditional spaced orchard that may have 50-100 trees per acre (tpa; 125-250 trees per hectare), a topped orchard may have up to 500 tpa (1250 tph).

Other Douglas-fir orchards are being managed with lower density, and allow the trees to get proportionally taller. As these trees grow, man lifts are needed for cone harvest. However, large trees produce more cones than small trees, so the added cost of mechanized lifts may be justified (Figure 2).

For other species, topping has been shown to actually enhance cone and seed production. An example of this is western larch. While western larch is a regionally important reforestation species in the Interior West, until recently there were very few seed orchards, and none were producing. Again in an attempt to manage tree height to control cone harvesting costs, western larch orchard trees were topped to maintain a maximum height of 15 feet (4.5 m). In doing so, the orchard trees were stimulated to produce a higher proportion of so-called "hanger" branches, from which most larch flowers arise (Figure 5). With this result, topping is now standard practice in western larch seed orchards that are actively managed.

Topping has also been shown to work quite well in western hemlock orchards (Ross 1989), and is used on occasion with Sitka spruce, western redcedar, and lodgepole pine.

Controlled Mass Pollination (CMP)

In the US West, seed orchards not uncommonly comprise 30 clones, sometimes as many as 60-80. The genetic gain estimates for the individual clones in the orchard often vary widely, from very high to modest. Most orchards rely upon open, wind pollination amongst the orchard trees, resulting in an averaging effect on overall genetic gain. Recently, some orchards have started to practice controlled mass pollination (CMP) on an operational scale to increase genetic gain.

With operational CMP, controlled crosses are made between only the very highest gain clones in the orchard, producing targeted amounts of very high genetic gain seed. Ahead of CMP, large volumes of pollen must be collected from high genetic gain clones, and stored for future use. In the spring, orchard workers apply hundreds to sometimes thousands of pollination bags to branches bearing female cone buds. When the flowers become receptive, pollen is applied to each bag. To achieve good seed set, it is often necessary to visit and pollinate each bag two times (Figure 9). CMP is



Figure 9. Controlled mass pollination (CMP) is employed to significantly increase genetic gain.

expensive, and the amount of seed produced to date has been modest, especially compared to open pollinated production. However, techniques have improved over the past several years, so that yields per bushel of cones often equal, and sometimes exceed that of open pollinated cones.

Irrigation

Many west side, low elevation seed orchards are located in areas with significant summer drought. Irrigation can be critical to early orchard survival and establishment, and later to producing large trees that bear more cones in a shorter time frame. Some orchards are establishing drip irrigation systems, either temporary or permanent, to meet these needs. Once an orchard becomes well established, temporary systems are designed to be rolled up and used in a new orchard (Figure 10).



Figure 10. Temporary drip irrigation may be used until newly planted trees are established, then moved to a new orchard.

More conventional overhead irrigation is also used in some seed orchards, primarily for bloom delay and frost protection in the spring. Overhead sprinkling of seed orchards on temperate days in the spring has a cooling effect, affecting flower phenology. Development of clones that tend to flower early in the spring is retarded, resulting in more clones in the orchard flowering synchronously. This produces a more diverse orchard pollen cloud and thus, reduces pollen contamination from outside sources and creates a broader range of open-pollinated combinations from the desired orchard trees (Fashler and Devitt 1980). Overhead sprinkling has been used for many years in fruit and nut orchards to protect flowers from freezing temperatures. This treatment has been used in forest tree seed orchards, but care must be taken to avoid breakage of tops and branches from ice loading.

DNA Fingerprinting

Since the 1960's, forestry organizations and forest industry have invested, collectively, many tens of millions of dollars in applied forest tree improvement in the US Northwest. Considerable progress has been made through two cycles of tree improvement, and several cooperative Douglas-fir and western hemlock programs are entering their third cycle of breeding, testing, and selection. These tree improvement programs are the source of new selections that are propagated and established in new seed orchards. It is critical to long term, sustainable genetic improvement that parents and selections are accurately identified, and that pedigrees contain no errors. Unfortunately, despite the best efforts of tree breeders and orchard managers, identification errors occasionally occur.

A detailed discussion of various DNA fingerprinting techniques is beyond the scope of this paper. Suffice it to say that protocols and procedures have been developed that are effective in confirming the genetic identities of parents, and their putative offspring. In other words, we have the ability to determine whether or not a given selection could be the progeny of the two parents listed in the pedigree. Furthermore, we can test multiple ramets of a clone, to determine if any have been mislabeled. This is key to long term improvement programs, because it is critical to know that a tagged tree is actually what we think it is. With DNA fingerprinting, when we apply pollen or use a tree as a female, we have confidence that the cross pedigree is 100% correct.

Summary

The production of genetically improved forest tree seed in seed orchards is a mature, robust technology. Starting with selections arising from a linked tree improvement program, the establishment and management of successful seed orchards requires intensive culture and close attention to detail. Over the past 40+ years, applied research and development programs have helped develop the orchard management regimes in widespread use today. Key practices in seed orchards include: the use of graft compatible rootstock for Douglas-fir; flower stimulation for regular and abundant cone and seed production; effective control of cone and seed insects; vegetation management; crown shaping techniques to assist cone production and harvesting; controlled mass pollination to increase genetic gain; irrigation to improve orchard establishment, increase individual tree growth, and manage orchard phenology; and DNA fingerprinting to ensure accurate identification of selections and maintain pedigree control. Using these practices and techniques, plus many more mundane daily tasks, seed orchard managers in the US Northwest are very successfully producing predictably large, abundant, and easily harvested crops of genetically improved seed for reforestation programs.

References

Clemo L. 2012. Personal communication. St. Paul, OR. Assistant Orchard Manager. Oregon Department of Forestry, JE Schroeder Seed Orchard.

- Copes DL. 1981. Selection and propagation of highly graft-compatible Douglas-fir rootstocks – a case history. USDA Forest Service Research Note PNW-376. 8 p.
- Doccola JJ, Hascher W, Aiken JJ, Wild PM. 2012. Treatment strategies using imidicloprid in hemlock woolly adelgid (*Adelges tsugae* Annand) infested eastern hemlock (*Tsuga canadensis* Carriere) trees. Aboriculture & Urban Forestry. 38(2):41-49.
- Fashler A, Devitt WJB. 1980. A practical solution to Douglas-fir seed orchard pollen contamination. For. Chron. 56:237-240.
- Feilburg, L, Soegaard B. 1975. Historical review of seed orchards. In: Seed Orchards. Her Majesty's Stationary Office. London Forestry Comm. Bull. No. 54. p 1-8.
- Hedlin AF, Yates III HO, Tovar DC, Ebel BH, Koerber TW, Merkel EP. 1980. Cone and seed insects of North American conifers.Canadian Forestry Service, United States Forest Service, and Secretaria de Agriculture y Recursos Hidraulicos, Mexico. 122 p.
- McCullough DG, Poland TM, Anulewicz AC, Lewis P, Molongoski J. 2009. Evaluation of emamectin benzoate and neonicotinoid insecticides: two year control of EAB? In: Lance D, Buck J, Binion D, Reardon R, Mastro V, compilers. Proceedings Emerald Ash Borer Research and Technology Development Meeting. Morgantown (WV): Forest Health Technology Enterprise Team. FHTET-2010-01. p 69-71.

- Reno J. 2008. Flower induction techniques for outdoor breeding & production orchards for Douglas-fir, western hemlock & Noble fir. Originally presented at 1992 Winter Workshop, Northwest Seed Orchard Managers Association, Wilsonville, OR, January 28-29 1992. P. 27-29 and updated in 2000, 2007 and 2008.
- Ross SD. 1989. Long term cone production and growth response to crown management and gibberellin A4/7 in a young western hemlock seed orchard. New Forest. 3:235-45.
- Ross SD, Bower RC. 1989. Cost-effective promotion of flowering in a Douglas-fir seed orchard by girdling and pulsed stem injection of Gibberellin A 4/7. Silvae Genetica 38:189-195.
- Ross SD, Bower RC. 1991. Promotion of seed production in Douglas-fir grafts by girdling + gibberellin A 4/7, stem injection, and effect of retreatment. New Forests 5:23-34.
- Russell JH, Hak O. 2007. Increasing quality seed production in western redcedar seed orchards: A synthesis of a multi-year foliar applied gibberellins A3 study. Forest Genetics Council Extension Note 09. 10 p.
- Woods JH. 1989. Stem girdling to increase seed and pollen production of coast Douglas-fir. B.C. Ministry of Forests Research Branch, Research Note No. 103. www.for.gov.bc.ca/hfd/pubs/ docs/mr/Rn/Rn103.pdf

Climate Change and the Future of Seed Zones

Francis Kilkenny, Brad St. Clair, and Matt Horning

Francis Kilkenny is Post-Doctoral Research Geneticist, Pacific Northwest Research Station, USDA Forest Service, Corvallis OR 97331; email: ffkilkenny@fs.fed.us

Brad St. Clair is Research Geneticist, Pacific Northwest Research Station, USDA Forest Service, Corvallis OR 97331, email: bstclair@fs.fed.us

Matt Horning is Forest Geneticist, Region 6, USDA Forest Service, Bend, OR 97701; email: mhorning@fs.fed.us

Kilkenny F, St. Claire B, Horning M. 2013. Climate change and the future of seed zones. In: Haase DL, Pinto JR, Wilkinson KM, technical coordinators. National Proceedings: Forest and Conservation Nursery Associations—2012. Fort Collins (CO): USDA Forest Service, Rocky Mountain Research Station. Proceedings RMRS-P-69. 87-89. Available at: http:// www.fs.fed.us/rm/pubs/rmrs_p069.html

Abstract: The use of native plants in wildland restoration is critical to the recovery and health of ecosystems. Information from genecological and reciprocal transplant common garden studies can be used to develop seed transfer guidelines and to predict how plants will respond to future climate change. Tools developed from these data, such as universal response functions and trait shift maps, can help managers make informed decisions regarding restoration strategies, such as assisted migration, in the face of climate change.

Keywords: seed transfer guidelines, seed zones, assisted migration, common garden studies

Introduction

The use of native plants in wildland restoration is critical to the recovery and health of ecosystems and to ecosystem resilience in the face of climate change. Seed zones and seed transfer guidelines help ensure that adapted plant material is reintroduced after disturbances, such as fire or grazing, and may be particularly important for the long-term resilience of re-established native plant populations (Ying and Yanchuk 2006; see also Table 1 for a definition of adaptation). Seed transfer guidelines can also help land managers select plant material that is most likely to be adapted to future climates (Thomson and others 2010). Thus, the development of seed transfer guidelines forms an integral piece of the native plant restoration infrastructure.

Table 1. Definitions.

Adaptation: An adaptation is a trait with a functional role in the life history of an organism that is maintained and evolved by means of natural selection. An adaptation refers to both the current state of being adapted and to the dynamic evolutionary process that leads to the adaptation. Adaptations contribute to the fitness and survival of individuals.

Universal Response Function: Combines individual response functions - the expected change in a trait value due to climate differences across a set of seed sources - and individual transfer functions - the expected change in a trait value for a given seed source due to climate differences across a set of possible transfer locations - into a single equation. Universal response functions can be used to model expected changes in trait values for any given seed source due to transfers to new locations or due to changes in climate.

Development of Seed Transfer Guidelines

Information from genecological studies, in which multiple populations are grown in one or a few common gardens, has been successfully used to develop seed zones for a number of tree species (Sorenson 1992, St. Clair and others 2005). Work is now being performed to develop seed zones for native grasses and forbs (Horning and others 2010; Johnson and others 2010). Genecological studies have both benefits and limitations in developing seed transfer guidelines. The primary benefit of genecological studies is that a large number of populations are sampled, such that adaptive differences can be determined across large areas of a species' range. However, because gardens represent only a small portion of the climatic variation experienced by the study populations, interpretation of genecological data must assume that plant populations are adapted to local conditions at their source and that demonstrated differences are due to those adaptations. This is generally a safe assumption for native plant species; however, it may not always be the case.

Seed Transfer Guidelines and Climate Change

Reciprocal transplant studies, where plants from several populations are planted in a set of sites that represent local and non-local climates, are effective at testing whether and how plants from specific populations are adapted to their local environments (Kawecki and Ebert 2004). When sites represent extreme environments, these studies have been used effectively to predict how plants will respond to future climate change as climates shift towards new extremes, particularly in cases with a large number of both populations and garden sites. For example, a long term study on lodgepole pine (*Pinus contorta*) in British Columbia, which tested 140 provenances at 60 sites, used a universal response function (defined in Table 1) to determine expected growth rates under future climate conditions (Wang and others 2010). This study found that growth rates of lodgepole pine were likely to increase in much of the northern range, primarily because marginal habitats in that region would become more hospitable due to warming.

When large reciprocal transplant studies are not feasible, data from genecological studies can be used to estimate the impact of future climate change on seed transfer guidelines. For example, a study on white spruce (*Picea glauca*) in Ontario determined future seed zones under three different climate change scenarios (Thomson and others 2010). Interestingly, this study found that two out of the three climate change scenarios predicted little change from current seed zones, but the third scenario predicted substantial shifts from current seed zones, indicating inherent uncertainty.

We performed a genecological study to determine seed zones for bluebunch wheatgrass (*Pseudoroegneria spicata*) throughout the intermountain west (St. Clair and others in press). As a follow-up study we are developing models to determine how optimal trait values will shift under future climate change scenarios. To do this we use regression models to link population level differences in adaptive traits, determined from common garden data, with the local climates at each source population. These regression models are then used within a geographical information system (GIS) to map expected optimal trait values across the landscape for both current and future climates. For example, we found that optimal heading date values for bluebunch will shift toward both earlier and later dates in 2050, depending on location (Figure 1).

Assisted Migration

Assisted migration is a strategy for helping ecosystems to adapt to climate change by moving species from locations with suboptimal climates to more optimal climates. While this strategy is controversial, due mostly to the possibility of introducing species which subsequently



Figure 1. Map of predicted trait values for heading date of bluebunch wheatgrass (*Pseudoroegneria spicata*) in the intermountain west under current and future climate scenarios, and the difference between the maps indicating the expected shift in optimal heading date values under climate change.

become invasive, it may become necessary if climate change continues to accelerate. Estimates of natural species migration rates during past periods of climate change range from 100-400 m (328-1312 ft) per year (Davis and Shaw 2001; Aitken and others 2008). However, current rates of climate change may require migration rates of 3000-5000 m (9843-16404 ft) per year, well beyond the movement capacity of many native species. Species that are long-lived, have low dispersal potential, and/or have low genetic variation will be particularly threatened by this rate of climate change.

Genecological and reciprocal transplant studies can help inform both the necessity and expected efficacy of management decisions related to assisted migration. Trait shift maps such as the one presented in Figure 1 can show areas where current trait values of a species are out of synch with predicted future optimums, which will help determine areas where populations may be at risk of local extinction. Universal response functions can also help determine the expected outcomes of proposed transfers. For example, the British Columbia lodgepole pine study found that using population specific transfer guidelines to move seed to locations with optimal future climates could increase lodgepole pine growth rates beyond the predicted rates if no transfers occurred (Wang and others 2010).

Conclusion

Genecological and reciprocal transplant common garden studies are critical to the development of seed zones and seed transfer guidelines. Current modeling techniques using data from these studies can help determine how seed transfer guidelines will shift due to future climate change and will be particularly useful in making decisions regarding assisted migration. Design of future common garden studies, and the models developed from them, will need to take into account the inherent uncertainty of climate models predicting future change in order to help managers determine the best strategies for future native plant restoration.

References

Aitken SN, Yeaman S, Holliday JA, Wang T, Curtis-McLane S. 2008. Adaptation, migration or extirpation: climate change outcomes for tree populations. Evolutionary Applications 1:95-111.

- Davis MB, Shaw RG. 2001. Range shifts and adaptive responses to quaternary climate change. Science 292:673-679.
- Horning ME, McGovern TR, Darris DC, Mandel NL, Johnson R. 2010. Genecology of *Holodiscus* discolor (Rosaceae) in the Pacific Northwest, USA. Restoration Ecology 18:235-243.
- Johnson RC, Erickson VJ, Mandel NL, St Clair JB, Vance-Borland KW. 2010. Mapping genetic variation and seed zones for *Bromus carinatus* in the Blue Mountains of eastern Oregon, USA. Botany 88:725-736.
- Kawecki TJ, Ebert D. 2004. Coneptual issues in local adaptation. Ecology Letters 7:1225-1241.
- Sorenson FC. 1992. Genetic variation and seed transfer guidelines for lodgepole pine in central Oregon. Portland (OR): USDA Forest Service, Pacific Northwest Research Station. Research Paper PNW-RP-453. 30 p.
- St Clair JB, Kilkenny FF, Johnson RC, Shaw NL, Weaver G. Genetic variation in adaptive traits and seed transfer zones for *Pseudoroegneria spicata* (bluebunch wheatgrass) in the northwestern United States. Evolutionary Applications (in press).
- St Clair JB, Mandel NL, Vance-Borland KW. 2005. Genecology of Douglas fir in western Oregon and Washington. Annals of Botany 96:1199-1214.
- Thomson AM, Crowe KA, Parker WH. 2010. Optimal white spruce breeding zones for Ontario under current and future climates. Canadian Journal of Forest Research 40:1576-1587.
- Wang T, O'Neil GA, Aitken SN. 2010. Integrating environmental and genetic effects to predict responses of tree populations to climate. Ecological Applications 20:153-163.
- Ying CC, Yanchuk AD. 2006. The development of British Columbia's tree seed transfer guidelines: purpose, concept, methodology, and implementation. Forest Ecology and Management 227:1-13.

The content of this paper reflects the views of the authors, who are responsible for the facts and accuracy of the information presented within.

Growing Assisted Migration: Synthesis of a Climate Change Adaptation Strategy

Mary I Williams and R Kasten Dumroese

Mary I Williams is Post-Doctoral Research Ecologist, Michigan Technological University, 1400 Townsend Drive, Houghton, MI 49931 and stationed at the USDA Forest Service, Rocky Mountain Research Station, 1221 S Main St, Moscow, ID 83843; email: miwilliams@fs.fed.us

R Kasten Dumroese is Research Plant Physiologist, USDA Forest Service, Rocky Mountain Research Station, 1221 S Main St, Moscow, ID 83843; email: kdumroese@fs.fed.us

Williams MI, Dumroese RK. 2013. Growing assisted migration: synthesis of a climate change adaptation strategy. In: Haase DL, Pinto JR, Wilkinson KM, technical coordinators. National Proceedings: Forest and Conservation Nursery Associations—2012. Fort Collins (CO): USDA Forest Service, Rocky Mountain Research Station. Proceedings RMRS-P-69. 90-96. Available at: http://www.fs.fed. us/rm/pubs/rmrs_p069.html

Abstract: Assisted migration may be necessary as a climate change adaptation strategy for native plant species that are less adaptive or mobile. Moving plants has been practiced a long time in human history, but movement of species in response to climate change is a new context. First proposed in 1985, assisted migration has gained attention since 2007 as a strategy to prevent species extinction, minimize economic loss, and sustain ecosystem services. We present a synthesis of proposed assisted migration guidelines and provide resources for nurseries, landowners, and researchers.

Keywords: climate change, decision framework, implementation, managed relocation, native plant transfer guidelines, seed transfer zones

Introduction

Climate change adaptation strategies may not be at the forefront of everyone's mind, but within the context of seed technology for forest and conservation nurseries they have significant merit. If temperature and precipitation predictions are correct, plant populations in their native settings will have to adapt or move to avoid maladaptation and/or extinction (Peters and Darling 1985). Current climate predictions would require plants to migrate 3000 to 5000 m (9842 to 16404 ft) per year far exceeds their observed maximum rates of less than 500 m (1640 ft) per year (Davis and Shaw 2001; Aitken and others 2008; Lempriere and others 2008).

Assisted migration of plants, that is, human-assisted movement, may be necessary for species that are less mobile or adaptive (Peters and Darling 1985; Hoegh-Guldberg and others 2008; Vitt and others 2010). Short-lived and annual species will likely adapt faster to changes in climate than long-lived species (Jump and Penuelas 2005; Vitt and others 2010). Despite disparity in rates between climate change and observed plant migration, survival may be more determined by available geophysical connections among landscapes needed for plants to move (Hannah 2008) and whether or not suitable recipient ecosystems exist (Aubin and others 2011). Furthermore, impacts from climate change can be so abrupt, for example, the mountain pine beetle outbreak on populations of lodgepole pine (*Pinus contorta*) (Regniere and Bentz 2008) that management options will be limited.

Moving plants has been practiced for a long time in human history, but the movement of species in response to climate change is a relatively new concept (Aubin and others 2011). First proposed in 1985 (Peters and Darling), assisted migration has gained attention since 2007 as a climate-change adaptation strategy (Hewitt and others 2011). Preventing species extinction, minimizing economic loss (for example timber production), and sustaining ecosystem services (for example wildlife habitat, recreation, and water and air quality) are three reasons for assisted migration (Aubin and others 2011). The only known assisted migration program in the U.S. is a grassroots effort to save Torreya taxifolia (*Florida torreya*), a southeast-

ern evergreen conifer, from extinction (McLachlan and others 2007; Barlow 2011). Since 2008, Florida torreya has been planted on private lands in five southern states (Torreya Guardians 2012). To prevent economic loss in the timber industry, some Canadian provinces have adjusted their planting guidelines. (Pedlar and others 2011). Using assisted migration to sustain ecosystem services has been addressed, but is not well-studied (Jones and Monaco 2009; Aubin and others 2011). If ecosystem function and structure become a main focus in assisted migration plans, it will prompt ecologists to consider moving assemblages of species rather than moving a single species (Harris and others 2006; Park and Talbot 2012).

Risks such as establishment failure and negative effects on the recipient and donor ecosystem are associated with assisted migration (Aubin and others 2011). Establishment failure can result from moving the species before the donor site is suitable and from any number of factors familiar to traditional planting efforts (Vitt and others 2010). The species could have negative effects on the recipient ecosystem, such as genetic pollution, hybridization, function/structure impairment, pathogens, and invasion. The risk of invasion, however, is subject to debate in regards to assisted migration and climate change because the definition itself depends upon human perception (Mueller and Hellman 2008). Some degree of "invasiveness" in an assisted-migratory might be necessary for establishment. Effects on the donor ecosystem are less definitive. Over-harvesting a population at risk of decline or extinction is a concern (Pedlar and others 2011). Removing seeds or plant materials from a donor ecosystem could hinder natural adaptation and migration (Vitt and others 2010; Aubin and others 2011).

Whether or not assisted migration is implemented or even possible, management and conservation plans need to incorporate climate change research as soon as it becomes available (Peters and Darling 1985). Unfortunately, since 1985, only a handful of assisted migration guidelines have been proposed (Hoegh-Guldberg and others 2008; Vitt and others 2010; Lawler and Olden 2011; Pedlar and others 2011; Schwartz and others 2012), largely born out of conservation biology, restoration ecology, and forestry. We present a synthesis of these guidelines and include examples of current efforts and available resources for nursery managers, land managers, and restorationists.

Informed Decisions

An overwhelming conundrum for assisted migration lies in the matching of existing plant materials (that is, seed, nursery stock, or genetic material) with ecosystems of the future that have different climate conditions (Potter and Hargrove 2012). To alleviate the challenge, a few tools are available to make informed decisions about assisted migration (Lawler and Olden 2011; Schwartz and others 2012). Bioclimatic models coupled with species genetic information in a GIS can be used to identify current and projected distribution (for example Rehfeldt and Jaquish 2010, McLane and Aitken 2012, and Notaro and others 2012). These forecasts can assist land managers in their long-term management plans, such as, where to collect seeds and plants. In Rehfeldt and Jaquish (2010), western larch (*Larix occidentalis*) distribution and seed zones are mapped under a combination of climate change scenarios for 2030 and 2060. Although the modeled projections have some uncertainty, they provide some indication of how seed zones will change over time.

We can gain much information from past reintroductions given our long history of moving and re-establishing species, not only from forestry, agriculture, and horticulture, but from restoration ecology (for example coal mine reclamation). Experiments such as the Assisted Migration Adaptation Trial (Marris 2009) in Canada and the Florida torreya project in the southeastern U.S. can inform us of how species respond to migration and warming. Further, we can use pollen and fossil records to understand how species responded to past climate changes. Of the published frameworks, Hoegh-Guldberg and others (2008) present a decision matrix to help identify species risk and feasibility of migration under climate change (Figure 1). Addressing ethical, legal and policy, and ecological questions such as "What are the priority taxa, ecosystem functions, and human benefits for which to consider assisted migration?" and "Do existing laws and policies enable assisted migration actions?" (Aubin and others 2011; Schwarz and others 2012) are central to species selection and navigating through the matrix. Maintaining or improving conservation plans would be sufficient for species at low risk, whereas species at moderate or high risk require more involved actions (Figure 1).





Figure 1. An assisted migration decision matrix can be used to determine adaption strategies for a plant species that has conservation, economic, or social value. Genetic information, bioclimatic models, historical records, and current assisted migration experiments should be consulted in navigating through the matrix. In order to implement assisted migration the species must be at high risk of decline or extinction, establish well, and provide more biological, economic, and social benefits than costs. (From Hoegh-Guldberg and others 2008).

Assisted migration may be warranted if: 1) a species is at high risk of extinction or if loss of the species would create economic or ecosystem loss, 2) can be established, and 3) provides more benefit than cost. In the event that establishment is not possible or costs constrain assisted migration, alternative options to facilitate migration or conservation would be considered. For example, reducing fragmentation, increasing landscape connections, collecting and storing seed, and creating suitable habitats could facilitate "natural" migration. Risk status will change over time. Existing programs (see Beardmore and Winder 2011) such as the Forest Tree Genetic Risk Assessment System (ForGRAS, Devine et al. 2012), NatureServe Climate Change Vulnerability Index (NatureServe 2011), System for Assessing Species Vulnerability (SAVS, Bagne and others 2011), and Seeds of Success program (Byrne and Olwell 2008) are available to determine a species' risk to climate change. Species most vulnerable to climate change are rare, long-lived, locally adapted, geographic and genetically isolated, and threatened by fragmentation and pathogens (Erickson and

others 2012). Suitable candidates are those that may decline in growth and productivity under climate change. Listing species as candidates for assisted migration is a practical first step (Vitt and others 2010; Pedlar and others 2011), but requires a substantial amount of knowledge about the species and their current and projected habitat conditions. Provenance data exist for several commercial tree species and should be used to estimate their response to climate scenarios (for example Rehfeldt and Jaquish 2010). In the U.S. we know a lot about conservation and commercial species because of their social and economic value. Regardless, the decision matrix is a proactive starting point that can be tailored over time, and not just to plants.

Implementation

In the following sections, we outline guidelines, including issues to consider, in an assisted migration plan (Figure 2). Largely from Pedlar and others (2011) and Vitt and others (2010), the guidelines are not unlike conventional reforestation and restoration approaches. We illustrate each component from an assisted migration and climate change perspective. We do not detail conventional guidelines. The Nursery Manual for Native Plants (Dumroese and others 2009), Raising Native Plants in Nurseries: Basic Concepts (Dumroese and others 2012), Seedling Nutrition and Irrigation (Landis and others 1989), Seedling Processing, Storage, and Outplanting (Landis and others 2010), Seedling Propagation (Landis and others 1998), The Society for Ecological Restoration International Primer on Ecological Restoration (SER 2004) and the Woody Plant Seed Manual (Bonner and others 2008) are appropriate resources to consult for seed and plant collection, propagating, site selection and preparation, outplanting, and maintenance.



Figure 2. A guide for implementing assisted migration which can be adapted to address a single species or an assemblage of species. Although species selection (1) and migration distance (2) are principle components in an assisted migration program, cost, location, and public support will determine implementation. (From Pedlar and others 2011; Vitt and others 2011).

Select Species

Whether the species is of commercial and/or conservation value, the decision matrix (Figure 1) can help identify a candidate species for assisted migration. Species selection will dictate migration distance, collection, propagation, planting site, outplanting method, and maintenance. Species may be selected on the basis of their risk of decline or extinction, importance to economic services, or contribution to ecosystem sustainability. For example, assisted migration could target commercial tree species that are predicted to decline in productivity under climate change (O'Neill and others 2008). Suitability of assisted migration for conservation species could be determined by a number of indicators such as available habitat, endangered status, and migration potential (Vitt and others 2010).

Determine Suitable Migration Distance

Distance is the safest geographic and/or climatic distance that populations can be moved to avoid maladaptation (reduction in fitness, health, or productivity as a result of growing in an unsuitable environment). Seed transfer zones and guidelines developed using species-specific genetic and climatic information can be used to determine distances. Guidelines and zones are available for many commercial tree species and some conservation species (Table 1). Empirical guidelines and zones created from common garden studies are available for a few grasses and shrubs, such as blue wildrye (*Elymus glaucus*) (Kitzmiller and Hanson 2011) and sagebrush (*Artemisia* spp.) (Mahalovich and McArthur 2004).

The paucity of transfer zones and guidelines established for shrubs, grasses, and forbs is a major limitation in making informed decisions about assisted migration. At best, we can rely on provisional seed zones (for example Seed Zone Mapper - Table 1) developed from temperature and precipitation data and Omernick level III and IV ecoregion boundaries (Omernik 1987) to evaluate candidates for assisted migration where species provenance data and bioclimatic data are lacking. Another option is to match the seed source climate with projected climate at the outplanting site with the assumption that the intended site is within the projected habitat of the species. This option requires knowing when the migration or outplanting will occur (Pedlar and others 2011).

Seed transfer functions can be used to calculate migration distances under climate change (Thomson and others 2010; Ukrainetz and others 2011). These functions relate performance of provenances at given test sites to climatic distance between the test site and outplanting site (Raymond and Lindgren 1990). Online tools are available to assist forest managers and researchers in making decisions about matching seedlots with outplanting sites and seed transfer (Table 1). The Seedlot Selection Tool (Howe and others 2009) is a mapping tool that matches seedlots with planting sites based on current or future climates and Seedwhere (McKenney and others 1999) can map out potential seed collection or outplanting sites based on climatic similarity of chosen sites to a region of interest. Rehfeldt and Jaquish (2010) employed bioclimatic models to map current and projected seed transfer zones for western larch. Others have performed similar assessments for aspen (Populus tremuloides) (Gray and others 2011), longleaf pine (Pinus palustris) and dogwood (Cornus florida) (Potter and Hargrove 2012), and whitebark pine (Pinus albicaulis) (McLane and Aitken 2012).

Identify Collection Sites, Collect Seeds, and Propagate Plants

Seed collection sites and collection and propagation methods will depend on the target species and purpose of assisted migration (that

Table 1	. Resources rela	ated to native	plant transfer	guidelines,	climate ch	ange, an	d assisted	migration	for the U.S.	. and C	Canada.	Most program
are easi	ly located by se	arching their	names in com	imon web b	orowsers. A	All URLs v	vere valid	as of 15 C	October 201	2.		

Resource or Program	Description	Authorship
Center for Forest Provenance Data http://cenforgen.forestry.oregonstate.edu/index.php	Database for tree provenance and genecological data that allows public access. Users are able to submit and retrieve data.	USDA Forest Service and Oregon State University
Centre for Forest Conservation Genetics http://www.genetics.forestry.ubc.ca/cfcg/	Portal for forest genetics and climate change research conducted in British Columbia, Canada.	Ministry of Forest and Range, BC
Climate Change Resource Center http://www.fs.fed.us/ccrc/	Information and tools about climate change for land man- agers and decision-makers.	USDA Forest Service
Climate Change Tree Atlas http://www.nrs.fs.fed.us/atlas/tree/tree_atlas.html	An interactive database that maps current (2000) and potential status (2100) of eastern US tree species under different climate change scenarios.	USDA Forest Service
Forest Seedling Network http://www.forestseedlingnetwork.com	Interactive website connecting forest landowners with seedling providers and forest management services and contractors	Forest Seedling Network
MaxEnt (Maximum Entropy) http://www.cs.cmu.edu/~aberger/maxent.html	Software that uses species occurrences and environmen- tal and climate data to map potential habitat. It can be used to develop seed collection areas.	Carnegie Mellon University
Native Seed Network http://www.nativeseednetwork.org/	Interactive database of native plant and seed information and planting guidelines for restoration, native plant propa- gation, and native seed procurement by ecoregion.	Institute for Applied Ecology
Seed Zone Mapper http://www.fs.fed.us/wwetac/threat_map/Seed- Zones_Intro.html	An interactive seed zone map of western North America. User selects areas to identify provisional and empirical seed zones for grasses, forbs, shrubs, and conifers. Map displays political and agency boundaries, topography, re- lief, streets, threats, and resource layers.	USDA Forest Service
Seedlot Selection Tool http://sst.forestry.oregonstate.edu/index.html	An interactive mapping tool to help forest managers match seedlots with planting sites based on current climate or future climate change scenarios. Can also be used to map present or future climates defined by temperature and precipitation.	USDA Forest Service and Oregon State University
Seedwhere https://glfc.cfsnet.nfis.org/mapserver/seedwhere/ seedwhere-about.php?lang=e	GIS tool to assist nursery stock and seed transfer deci- sions for forest restoration projects in Canada and the Great Lakes region. It can identify geographic similarities between seed sources and planting sites.	Natural Resources Canada, Canadian Forest Service
System for Assessing Species Vulnerability (SAVS) http://www.fs.fed.us/rm/grassland-shrubland- desert/products/species-vulnerability/savs-climate- change-tool/	Software that identifies the relative vulnerability or resil- ience of vertebrate species to climate change. It provides a framework for integrating new information into climate change assessments.	USDA Forest Service

is, commercial or conservation). Seed collection areas, zones, and orchards exist for most commercial tree species. Species of concern are not regularly collected or propagated at the same scale as commercial species making assisted migration a challenge, but provisional seed zones can be used to select collection areas (Table 1).

Guidelines that maximize genetic diversity within outplanted materials provide some long-term insurance that would counter against uncertainty in climate predictions and species reactions to climate change (Ledig and Kitzmiller 1992; Vitt and others 2010). Seed collection guidelines to increase genetic diversity with assisted migration in mind are synthesized by Vitt and others (2010). Selecting a few extreme variants within seed collections or allowing for physiological or morphological variation in nursery stock might serve to facilitate migration (Pedlar and others 2011). For example, drought tolerance in nursery stock would be a desirable trait for planting sites projected to experience warmer and drier conditions. Establishing seed orchards and collecting seed from low elevations or southern latitudes so that the resulting material is adapted to these conditions are other options (Pedlar and others 2011).

Select Outplanting Sites

Creating suitable outplanting sites might be necessary for species at moderate or high risk of decline or extinction (Hoegh-Guldberg and others 2008; Aubin and others 2011). The target species and its habitat requirements will dictate outplanting site selection. Some species have well-defined habitat conditions that can help with site selection. Soil surveys and ecological site descriptions provide additional support for site selection (Herrick and others 2006) as well as current and projected seed transfer zones and guidelines (Table 1). Site selection for commercial tree species, which have a long history of human-assisted propagation, is largely determined by harvest and reforestation operations, which by their very nature produce planting sites (Pedlar and others 2011). Conversely, species of conservation value have a short history of human-assisted propagation and outplanting sites are not routinely created through commercial activities. However, using disturbed areas as outplanting sites to test assisted migration has been suggested (Jones and Monaco 2009; Aubin and others 2011).

Outplanting

Volume 7 of the Container Tree Nursery Manual - Seedling Processing, Storage and Outplanting - provides thorough outplanting guidelines for trees including outplanting window, or, best time to plant (Landis and others 2010). Outplanting window can vary year to year even within current climate conditions, therefore the "window" will be difficult to determine for assisted migration. In other words, when and where do you plant a long-lived species in a rapidly changing climate? Maladaptation may occur if a species is introduced too soon to its "new" environment or it may competitively interact with other species causing loss of ecosystem function or structure (Aubin and others 2011). Assisted migration experiments coupled with projected climate change may help determine the best time to deploy plant materials (Lawler and Olden 2011).

Monitoring and Maintenance

Adaptive monitoring and management is imperative to any natural resource program, especially in an assisted migration program given the uncertainty in climate change projections and adaptation to changes in climate. Programs need to encourage feedback and learning which can be used to change and/or create management actions. Short-(months to years) and long-term (several years) monitoring of survival and growth will provide valuable feedback about plant performance and measures of success to nursery and land managers (Landis et al. 2010). Postestablishment maintenance such as watering, herbicide application, and pest/predation control can be employed post-planting to help the species establish (Pedlar and others 2011). Questions such as "Which reference ecosystem should be used to evaluate an assisted migration effort?" and "What measures do we use to determine success?" will help determine what characteristics to monitor in the species and receiving ecosystem (Aubin and others 2011). Growth measurements, reproduction, ecosystem health (structure and function), and degree of invasiveness are indicators to consider (Herrick and others 2006; Pedlar and others 2011).

Assisted Migration Examples

Other than the Florida torreya assisted migration project in the southeastern U.S., only a few assisted migration efforts are underway in North America, and all of them are in Canada. In response to a changing climate, seed transfer guidelines for Alberta have been revised to extend current zones northward by 2° latitude and upslope by 200 m (656 ft). Alberta is also considering the evaluation of ponderosa pine (*Pinus ponderosa*) and Douglas-fir (*Pseudotsuga menziesii*) as replacements for lodgepole pine because it is predicted to decline in productivity or suffer from extinction under climate change (Pedlar and others 2011). In British Columbia, a large, long-term experiment called the Assisted Migration Adaptation Trial (AMAT), a

collaborative effort between B.C. Ministry of Forests and several agencies and stakeholders, tests both assisted migration and climate warming (Marris 2009). The program evaluates the adaptive performance of 16 tree species collected from a range of sources in B.C., Washington, Oregon, and Idaho and planted in several sites in the same areas. Two components of the trial are to test how sources planted in northern latitudes perform as the climate changes and evaluate endurance of northern latitude sources to warmer conditions in southern latitudes.

Limitations

We cannot reliably predict future climates so it is difficult to know which or how ecosystems will be affected. We have a long history of moving plants, but limited knowledge about establishing native plant materials outside their range in anticipation of different climate conditions. To further complicate matters, we know little about the long-term ecological effects of assisted migration, such as, invasiveness, maladaptation, and site stability (Aubin and others 2011). One way to address uncertainty is to maximize genetic and geographic diversity in plant materials (Ledig and Kitzmiller 1992), but seed collection efforts will need to factor this into their budgets (Vitt and others 2010).

Research Needs

To make informed decisions about implementation, we need a central, standardized database of species-specific genetic, ecological, and geographic information. Databases listed in Table 1 can serve as templates for non-commercial species, but we need to solicit and organize existing data in order to identify gaps. Discussion and evaluation of complementary actions, such as ecosystem engineering (for example using drastically disturbed areas as sites to test assisted migration) and increasing landscape connectivity (for example reduce fragmentation) are also warranted (Jones and Monaco 2009; Lawler and Olden 2011).

Dynamic seed transfer zones and guidelines are also needed. Transfer guidelines based on geographic boundaries and provisional zones may not be suitable, especially in regions without supporting genetic and climatic information (Mahalovich 1995). This was demonstrated, for example, by blue wildrye, where supporting common garden information showed that seed zones based solely on ecoregions mapped the species' adaptive variation poorly (Erickson and others 2004). Climate-based seed transfer guidelines should overcome these restrictions (Rehfeldt 2004), but the guidelines need to factor in future climate conditions - a major challenge for nursery and land managers given uncertainty about which climate to prepare for (Park and Talbot 2012; Potter and Hargrove 2012). This is especially true for long-lived species and populations that take several decades to reach reproductive maturity and become adapted through evolution to a new climate (Potter and Hargrove 2012). Park and Talbot (2012) suggest that managers prepare for all future climate scenarios. This might entail small-scale experiments, such as, planting fast-growing trees adapted to projected climate in the next 15 to 30 years (Park and Talbot 2012) or randomly planting a variety of seed sources in one area and monitoring their adaptive response (similar to provenance testing) (Pedlar and others 2011).

Not only must one factor in performance of delineating seed zones and transfer guidelines but also cost. Cost increases with an increase in the number of seed zones in terms of seed and nursery productions (stock, storage, and delivery), administrative regulations, and record keeping (Lindgren and Ying 2000). The biological, operational, and administrative tradeoffs are vital considerations in transfer guideline development for future climate scenarios.

94

Conclusion

Regardless of the debate on assisted migration, we have little time to act given current climate change predictions and restricted ability of plants to adapt or migrate rapidly on their own. Framing the discussion to identify objectives and produce frameworks that lead to strategies is pertinent (McLachlan and others 2007; Lawler and Olden 2011; Park and Talbot 2012). Ultimately our capacity to implement projects will be limited by cost, location, and time (Park and Talbot 2012), but recognizing and synthesizing what we already know about plant adaptation and climate change is a necessary start.

Acknowledgements

This collaborative work between Michigan Technology University, School of Forest Resources and environmental Science and the USDA Forest Service, Rocky Mountain Research Station, Grassland, Shrubland, and Desert Ecosystems Program is accomplished through Joint Venture Agreement 11-JV- 11221632-130.

References

- Aitken SN, Yeaman S, Holliday JA, Wang T, Curtis-McLane S. 2008. Adaptation, migration or extirpation: climate change outcomes for trees. Evolutionary Applications 1:95-111.
- Aubin I, Garbe CM, Colombo S, Drever CR, McKenney DW, Messier C, Pedlar J, Sander MA, Venier L, Wellstead AM, Winder R, Witten E, Ste-Marie C. 2011. Why we disagree about assisted migration: Ethical implications of a key debate regarding the future of Canada's forests. The Forestry Chronicle 87:755-765.
- Bagne KE, Friggens MM, Finch DM. 2011. A system for assessing vulnerability of species (SAVS) to climate change. Fort Collins (CO): USDA Forest Service, Rocky Mountain Research Station. 28 p.
- Barlow C. 2011. Paleoecology and the assisted migration debate: why a deep-time perspective is vital. URL: http://www.torreyaguardians.org/assisted-migration.html (accessed 10 October 2012).
- Beardmore T, Winder R. 2011. Review of science-based assessments of species vulnerability: Contributions to decision-making for assisted migration. The Forestry Chronicle 87:745-754.
- Bonner FT, Karrfalt RP, Nisley RG, editors. 2008. The Woody Plant Seed Manual. Washington (DC): USDA Forest Service. Agriculture Handbook 727. 1223 p.
- Byrne MK, Olwell P. 2008. Seeds of success: the national native seed collection program in the United States. Public Gardens 23: 3-4.
- Davis MB, Shaw RG. 2001. Range shifts and adaptive responses to Quaternary climate change. Science 292:673-679.
- Devine W, Aubry C, Miller J, Potter KM, Bower A. 2012. Climate change and forest trees in the Pacific Northwest: Guide to vulnerability assessment methodology. Olympia (WA): USDA Forest Service, Pacific Northwest Region. 49 p. URL: http://ecoshare. info/wp-content/uploads/2012/04/CCFT_Methodology.pdf (accessed 3 May 2013).
- Dumroese RK, Luna T, Landis TD. 2009. Nursery manual for native plants: A guide for tribal nurseries. Washington (DC): USDA Forest Service. Agriculture Handbook 730. 302 p.
- Dumroese RK, Landis TD, Luna T. 2012. Raising native plants in nurseries: basic concepts. Fort Collins (CO): USDA Forest Service, Rocky Mountain Research Station. 92 p.

- Erickson VJ, Aubry C, Berrang PC, Blush T, Bower AD, Crane BS, DeSpain T, Gwaze D, Hamlin J, Horning ME, Johnson R, Mahalovich MF, Maldonado M, Sniezko R, St Clair JB. 2012. Genetic resource management and climate change: genetic options for adapting national forests to climate change. Washington (DC): USDA Forest Service. 24 p.
- Gray LK, Gylander T, Mbogga MS, Chen P, Hamann A. 2011. Assisted migration to address climate change: recommendations for aspen reforestation in western Canada. Ecological Applications 21:1591-1603.
- Hannah L. 2008. Protected areas and climate change. Annals of the New York Academy of Sciences 1134:201-212.
- Harris JA, Hobbs RJ, Higgs E, Aronson J. 2006. Ecological restoration and global climate change. Restoration Ecology 14:170-176.
- Herrick JE, Schuman GE, Rangoa A. 2006. Monitoring ecological processes for restoration projects. Journal for Nature Conservation 14:161-171.
- Hewitt N, Klenk N, Smith AL, Bazely DR, Yan N, Wood S, MacLellan JI, Lipsig-Mumme C, Henriques I. 2011. Taking stock of the assisted migration debate. Biological Conservation 144:2560-2572.
- Hoegh-Guldberg O, Hughes L, McIntyre S, Lindenmayer DB, Parmesan C, Possingham HP, Thomas CD. 2008. Assisted colonization and rapid climate change. Science 321:345-346.
- Jones TA, Monaco TA. 2009. A role for assisted evolution in designing native plant materials for domesticated landscapes. Frontiers in Ecology and the Environment 7:541-547.
- Jump AS, Penuelas J. 2005. Running to stand still: adaptation and the response of plants to rapid climate change. Ecology Letters 8:1010-1020.
- Kitzmiller JH, Hanson L. 2011. Patterns of adaptation in three native grasses in northern California. Native Plants Journal 12:45-61.
- Landis TD, Dumroese RK, Haase D. 2010. Seedling processing, storage, and outplanting, Vol. 7, The container tree nursery manual. Washington (DC): USDA Forest Service. Agriculture Handbook 674. 192 p.
- Landis TC, Tinus RW, Barnett JP. 1998. Seedling propagation, Vol.6, The container tree nursery manual. Washington (DC): USDA Forest Service. Agriculture Handbook 674. 166 p.
- Landis TD, Tinus RW, McDonald SE, Barnett JP. 1989. Seedling nutrition and irrigation, Vol. 4, The container tree nursery manual Washington (DC): USDA Forest Service. Agriculture Handbook 674. 119 p.
- Lawler JJ, Olden JD. 2011. Reframing the debate over assisted colonization. Frontiers in Ecology and the Environment 9:569-574.
- Ledig FT, Kitzmiller JH. 1992. Genetic strategies for reforestation in the face of global climate change. Forest Ecology and Management 50:153-169.
- Lempriere TC, Bernier PY, Carroll AL, Flannigan MD, Gilsenan RP, McKenney DW, Hogg EH, Pedlar J, Blain D. 2008. The importance of forest sector adaptation to climate change. Edmonton (AB): Natural Resources Canada, Canadian Forest Service, Northern Forestry Centre. Information Report NOR-X-416E. 78 p.
- Lindgren D, Ying CC. 2000. A model integrating seed source adaptation and seed use. New Forests 20:87-104.
- Mahalovich MF. 1995. The role of genetics in improving forest health. National silviculture workshop: Forest health through silviculture. USDA Forest Service, Rocky Mountain Forest and Range Experiment Station. General Technical Report RM-GTR-267. p 200-207.

Mahalovich MF, McArthur ED. 2004. Sagebrush (*Artemisia* spp.): seed and plant transfer guidelines. Native Plants Journal 141-148. Marris E. 2009. Planting the forest for the future. Nature 459:906-908.

McKenney DW, Mackey BG, Joyce D. 1999. Seedwhere: a computer tool to support seed transfer and ecological restoration decisions. Environmental Modelling 14:589-595.

McLachlan JS, Hellmann JJ, Schwartz MW. 2007. A framework for debate of assisted migration in an era of climate change. Conservation Biology 21:297-302.

McLane SC, Aitken SN. 2012. Whitebark pine (*Pinus albicaulis*) assisted migration potential: testing establishment north of the species range. Ecological Applications 22:142-153.

Mueller JM, Hellman JJ. 2008. An assessment of invasion risk from assisted migration. Conservation Biology 22:562-567.

NatureServe. 2011. NatureServe Mission. URL http://www.natureserve.org/aboutUs/index.jsp (accessed 15 September 2012).

Notaro MA, Mauss A, Williams JW. 2012. Projected vegetation changes for the American Southwest: combined dynamic modeling and bioclimatic-envelope approach. Ecological Applications 22:1365-1388.

Omernik JM.1987. Ecoregions of the conterminous. Annals of the Association of American Geographers 77:118-125.

O'Neill GA, Ukrainetz N, Carlson M, Cartwright C, Jacquish B, King J, Krakowski J, Russell JH, Stoehr M, Xie CY, Yanchuk A. 2008. Assisted migration to address climate change in British Columbia—recommendations for interim seed transfer standards. Victoria (BC): British Columbia Ministry of Forest and Range, Research Branch. Technical Report 048.

Park A, Talbot C. 2012. Assisted migration: uncertainty, risk and opportunity. The Forestry Chronicle 88:412-419.

Pedlar J, McKenney DW, Beaulieu J, Colombo S, McLachlan JS, O'Neill GA. 2011. The implementation of assisted migration in Canadian forests. The Forestry Chronicle 87:766-777.

Peters RL, Darling JDS. 1985. The greenhouse-effect and nature reserves. BioScience 35:707-717.

Potter KM, Hargrove WW. 2012. Determining suitable locations for seed transfer under climate change: a global quantitative method. New Forests 43:581-599.

Raymond CA, Lindgren D. 1990. Genetic flexibility - a model for determining the range of suitable environments for a seed source. Silvae Genetics 39:112-120.

Regniere J, Bentz B. 2008. Mountain pine beetle and climate change. USDA Forest Service. Research Forum on Invasive Species: 63-64.

Rehfeldt GE. 2004. Interspecific and intraspecific variation in *Picea* engelmannii and its congeneric cohorts: Biosystematics, genecology, and climate change. Fort Collins (CO): USDA Forest Service, Rockdy Mountain Research Station. General Technical Report RMRS-GTR-134. 22 p.

Rehfeldt GE, Jaquish BC. 2010. Ecological impacts and management strategies for western larch in the face of climate-change. Mitigation and Adaptation Strategies for Global Change 15:283-306.

Schwartz MW, Hellmann JJ, McLachlan JM, Sax DF, Borevitz JO, Brennan J, Camacho AE, Ceballos G, Clark JR, Doremus H, Early R, Etterson JR, Fielder D, Gill JL, Gonzalez P, Green N, Hannah L, Jamieson DW, Javeline D, Minteer BA, Odenbaugh J, Polasky S, Richardson DM, Root TL, Safford HD, Sala O, Schneider SH, Thompson AR, Williams JW, Vellend M, Vitt P, Zellmer S. 2012. Managed Relocation: Integrating the Scientific, Regulatory, and Ethical Challenges. BioScience 62:732-743.

tion. Tucson, AZ. 15 p.

Thomson AM, Crow KA, Parker WH. 2010. Optimal white spruce breeding zones for Ontario under current and future climates. Canadian Journal of Forest Research 40:1576-1587.

Torreya Guardians. 2012. URL: http://www.torreyaguardians.org/ assisted-migration.html (accessed 10 October 2012).

Ukrainetz NK, O'Neill GA, Jaquish BC. 2011. Comparison of fixed and focal point seed transfer systems for reforestation and assisted migration: a case study for interior spruce in British Columbia. Canadian Journal of Forest Research 41:1452-1464.

Vitt P, Havens K, Kramer AT, Sollenberger D, Yates E. 2010. Assisted migration of plants: changes in latitudes, changes in attitudes. Biological Conservation 143:18-27.

The content of this paper reflects the views of the authors, who are responsible for the facts and accuracy of the information presented within.

Phytosanitation: A Systematic Approach to Disease Prevention

Thomas D Landis

Thomas D Landis is Retired Nursery Specialist, USDA Forest Service, and Consultant, Native Plant Nursery Consulting, 3248 Sycamore Way, Medford, OR 97504; Tel: 541.858.6166; email: nurseries@aol.com.

Landis TD. 2013. Pytosanitation: a systematic approach to disease prevention. In: Haase DL, Pinto JR, Wilkinson KM, technical coordinators. National Proceedings: Forest and Conservation Nursery Associations—2012. Fort Collins (CO): USDA Forest Service, Rocky Mountain Research Station. Proceedings RMRS-P-69. 97-101. Available at: http://www.fs.fed.us/rm/pubs/rmrs_p069.html

Abstract: Phytosanitation is not a new concept but has received renewed attention due to the increasing threat of nursery spread *Phytophthora ramorum* (PRAM), the fungus-like pathogen that causes Sudden Oak Death. This disease has the potental to become the most serious forest pest since white pine blister rust and chestnut blight. Phytosanitation can help prevent the spread of this and other pathogens to or from nursery operations. Phytosanitation can most simply be viewed as an input-output model: prevent pests from entering your nursery and make certain that your plants are not carrying pests when they leave your nursery for sale or outplanting. Two major approaches to phytosanitation can be employed. The systems approach is based on a Hazard Analysis of Critical Control Points and comprehensive programs that have been developed for ornamental nurseries can easily be modified for forest, conservation, and native plant facilities. A second approach based on target pests might be easier for smaller nurseries with limited funds and manpower. Here, the idea is to learn as much as possible about pests that are found in your nursery or ones, like *Phytophthora ramorum*, that could threaten it. By focusing on the type of pest and its methods of spread, nurseries can adapt their scouting and cultural practices to minimize adverse affects. Because their stock is outplanted directly into forests and other wildland plant communities, nursery managers should be especially vigilant to make sure that PRAM isn't spread to or from their operation.

Keywords: nursery, forest, native plant, seedling, Phytophthora ramorum (PRAM)

Introduction

A good working definition of phytosanitation is "concerning the health of plants; especially the freedom from pests requiring quarantine" (Wiktionary 2012). So, phytosanitation is similar to integrated pest management but is especially concerned with nursery pests that are subject to quarantine regulations. A nursery pest can be defined as any biological stress factor that interferes with healthy seedling development and causes a sustained departure from the normal physiological or morphological condition that characterizes a healthy plant (Dumroese 2012). The most common nursery pests are microorganisms such as fungi and bacteria but insects and weeds also fit this definition (Landis and others 1990). So, a working definition of phytosanitation means that you work to prevent pests from entering your nursery, as well as ensure that your nursery stock isn't infected or infested when it leaves your nursery (Figure 1).

Phytosanitation is not a new concept, but has been discussed for over 50 years in ornamental nurseries. The subtitle of The U.C.



Definition: Concerning the health of plants, especially avoiding pests requiring quarantine

Figure 1. Phytosanitation means that you prevent pests from entering your nursery as well as make certain that your plants are not carrying pests when they leave your nursery for sale or outplanting.

System for Healthy Container-Grown Plants (Baker 1957) was "Through the Use of Clean Soil, Clean Stock, and Sanitation." This classic nursery manual introduced steam sterilization of soils and the development of artificial growing media as a way to prevent the introduction of damping-off and other root diseases in container nurseries.

In the United States, phytosanitary inspections are regulated by the Plant Protection and Quarantine (PPQ) program of the Animal and Plant Health Inspection Service (APHIS), US Department of Agriculture (USDA 2012), but individual states can also impose phytosanitary restrictions. The current interest in phytosanitation was stimulated by the sudden oak death (SOD) disease which is caused by the fungus-like pathogen *Phytophthora ramorum* (PRAM).

The Systems Approach to Phytosanitation

The PRAM situation in California, Oregon and Washington has focused renewed interest in developing phytosanitation programs for ornamental nurseries (Parke and Grunwald 2012; Griesbach and others 2011). Because of problems with contaminated food back in the 1970's, the US Food and Drug Administration developed a systematic approach called Hazard Analysis of Critical Control Points (HACCP). A control point is any step in a production system that can be measured, monitored, controlled, and corrected, and a critical control point is the best step at which significant hazards can be prevented or reduced. The HACCP system consists of a series of logical steps to identify, evaluate, and correct sources of hazards (USFDA 2012).

The HAACP approach has been developed to prevent the spread of pests and diseases in ornamental nurseries in Oregon (Parke and Grunwald 2012). The Oregon Association of Nurseries has recently published the "Safe Procurement and Production Manual: a Systems Approach for the Production of Healthy Nursery Stock" (Griebasch and others 2011). This comprehensive guide integrates HAACP principles into system approach and, although some of the production systems are different, the same basic concepts can be applied to forest and native plant nurseries. A free PDF version is available online at website: (http://www.science.oregonstate.edu/bpp/labs/grunwald/publications/ SafeProduction.pdf), or a print version is available from the OAN office, 503-682-5089 or 800-342-6401.

The first step is to view your nursery in terms of production systems, and then to identify the control points and critical control points (CCP) in each system. For example in a container nursery, the sowing operation consists of a series of consecutive steps which can be analyzed for their potential to spread diseases and pests. The steps at which hazards can be reduced or eliminated are your critical control points for the sowing operation (Figure 2A). For example, we know that fungal spores or weed seeds can be introduced in growing media, so the components should be tested and then pasteurized if necessary. Likewise, fungal spores can be introduced from soil or root fragments so used containers should be washed and sterilized. The last CCP in this operation are the seeds. The spores of pathogenic fungi, such a Fusarium spp., have been proven to be carried into nurseries on seedcoats of conifer seeds (Figure 2B). Large and rough textured seeds are particularly susceptible so seed samples should be tested and cleansed before sowing. A "running water rinse" has been shown to be very effective in this regard, a quick soak in a dilute bleach solution also works (Figure 2C).

A good bareroot nursery example where the HAACP process can be applied is the transplanting operation, where there are 2 CCPs (Figure 3A). Many nurseries either purchase seedlings for transplanting from other nurseries or obtain them from a customer. The introduction of transplants has been shown to be a significant risk for introducing pests, especially root rot fungi, into the transplant nursery (Figure 3B). The major problem is when bareroot seedlings are transplanted into another nursery (Cram and Hansen 2012); the risk of spreading root disease on container transplants is much less. Root rot fungi and nematodes can also be introduced into a bareroot nursery on cultivation or transplanting equipment. For this reason, savvy nursery managers insist that operators clean and sterilize their equipment (Figure 3C) when it is moved from one field to another, and especially when equipment is leased or borrowed from other nurseries.

Once you have analyzed all your production systems then the final step is to develop Best Management Practices that address the potential problems at each CCP.





Figure 2. A hazard analysis of each step in a container sowing operation identifies critical control points (boxes with bold text and dark shading) where pests can enter your nursery (A). For example, fungal spores carried on seed-coats (B) can be eliminated by quick soak in a dilute (1 bleach:10 water) bleach solution (C).









Landis

Figure 3. Root rots can easily be introduced into your nursery during transplanting so a hazard analysis should examine each step in the operation (A). The critical control points are when seedlings are purchased from another nursery (B), or when equipment carries infected soil from another location (C).

Phytosanitation Techniques for Specific Target Pests

A simpler yet still effective approach to phytosanitation is to make a list of your most significant nursery pests, and do some research into how they spread. This approach is probably more practical for small nurseries that don't have the funding or manpower to implement the systematic approach. There is a wealth of good information published on nursery pests. For example, Forest Nursery Pests (Cram and others 2012) has just been published and contains excellent information on the most common pest problems that you might encounter in your nursery, and well as other useful information on diagnosis and integrated pest management.

1. Damping-Off

This is one of the oldest diseases of forest nursery plants, and affects germinating seeds or just-emerged seedlings before their stems become lignified. Most conifer and broadleaved plants can be affected (James 2012).

Type of Pest:

Several genera of fungi, such as *Fusarium* and Rhizoctonia, or Oomycetes including *Pythium*, and *Phytophthora*.

Method of Spread:

Spores on seeds, in soil or growing media, or in water.

Critical Control Points:

Spores can be transmitted in nursery soil or can be introduced on seedcoats. Certain damping-off fungi have motile zoospores which can move in water or wet soil.

Phytosanitary Risk to Your Nursery:

Damping-off is a nursery disease that's been around forever, and nursery managers have to be constantly vigilant. It can easily be controlled, however, by learning how it spreads and taking preventative measures that are well documented (James 2012, Landis and others 1990).

Phytosanitary Risk to Your Customers:

Because damping-off that is only seen during germination and early growth, it would not be carried on healthy nursery stock that are shipped to the field.

2. Grey Mold or Botrytis Blight

Like damping-off, this is a very common nursery disease and can affect most plants grown in nurseries (Haase and Taylor 2012).

Type of Pest:

Botrytis cinerea, a fungus.

Method of Spread:

Aerial spores, or from seedling to seedling.

Critical Control Points:

Botrytis is one of those diseases that seem to come out of nowhere, so it's hard to identify specific control points.

Phytosanitary Risk to Your Nursery:

It would be almost impossible to prevent Botrytis but both research and practical experience have shown that constant vigilance to catch infections early and continuous rogueing of diseased plants are effective.

Phytosanitary Risk to Your Customers:

Botrytis is often identified during packing on the senescent foliage of plants that have been grown close together. Because this fungus can spread at temperature above freezing, many nurseries have converted to freezer storage. Otherwise healthy plants with minor infections will not spread the fungus after outplanting as this disease will not survive under drier conditions.

3. Sudden Oak Death

Althought this is a relatively new disease that has caused serious damage in forests in the US and Europe, *Phytophthora ramorum* (PRAM) also infects nursery plants as a shoot or leaf blight.

Type of Pest:

PRAM a fungus-like pathogen that produce relatively minor symptoms in nursery stock, but research has shown that it can persist on plant material or even organic matter.

Method of Spread:

This pest produces 3 types of spores: motile zoospores, which can actively disperse in water; chlamydospores, which can survive long periods in plant tissue or even organic matter (Figure 4a); and thick walled oospores that are sexually produced by the combination of two mating types (Chastagner and others 2012).

Critical Control Points:

Due to its many spore types, PRAM has multiple modes of transmission. It is most commonly spread through any type of plant material shared between nurseries including cuttings and transplants. Seed transmission has not been proven so far. Zoospores can spread through any form of water such as rain splash and surface runoff, and has been shown to persist in waterways around nurseries (Chastagner and others 2012).

Phytosanitary Risk to Your Nursery:

The disease potential for this pathogen is extreme. Because over 100 species of trees and shrubs from 36 different families are susceptible (Chastagner and others 2012), PRAM has the potential to



become the most serious forest pest since white pine blister rust and chestnut blight. Although PRAM has only been positively identified on ornamental nursery stock as of the current date, it is only a matter of time until infections are discovered on forest, conservation, and native plant species. Although PRAM has not proven to be a serious nursery disease, it can still have serious economic impacts due to plant quarantine regulations. At one ornamental nursery in Southern California, over 1 million camellias worth \$9 million had to be destroyed because of a PRAM infestation (Alexander 2006). Therefore, nursery managers must become familiar with disease symptoms and keep up-to-date on the latest developments.

Phytosanitary Risk to Your Customers:

Disease symptoms on nursery stock are relatively minor. What's most worrisome is that many infected plants show no visible symptoms at all (Vercauteren & others 2012). Genetic testing has proven that long-range spread can be attributed to the shipping of infected nursery stock, and that PRAM can then be transmitted to surrounding forests (Mascheretti and others 2008). Because they ship their plants directly into forests and other natural settings, forest and native plant nurseries represent a serious transmission threat. Unfortunately, this has already happened in the United Kingdom. In this case, nursery stock has been shown to be the cause of a devastating forest disease outbreak in Japanese larch plantation where 3 million trees have been killed (Brasier 2012).

The PRAM epidemic has resulted in both state and federal quarantine restrictions (Kliejunas 2010). The state of Oregon instituted a quarantine on nursery stock coming from California in 2001, and APHIS issued a federal regulation in 2004 to regulate interstate transportation for PRAM host materials, including nursery stock, from the states of California, Oregon, and Washington. By 2009, more than 68 countries had some quarantine regulations concerning PRAM nursery stock (Sansford and others 2009).

Although little has been published on the effects of PRAM in forest, conservation, and native plant nurseries, a comprehensive article is being written for the Winter 2013 issue of Forest Nursery Notes. The most current information on PRAM can be found on-line at the following websites:



Figure 4. Phytophthora ramorum is a new insidious nursery pest because, although nursery symptoms are very minor, it can persist in root systems and leaf litter (A). This fungus-like pest has motile zoospores and can spread in water; this map shows PRAM detected in waterways around nurseries (B). (A from Elliott 2012; B from Chastagner and others 2010)

1. <www.suddenoakdeath.org> - This website contains a section on *Phytophthora ramorum* in nurseries including diagnostic guides. It also has contact information for your local state.

2. <http://www.aphis.usda.gov/plant_health/plant_pest_info/pram/ index.shtml> - This APHIS website has a section on *Phytophthora ramorum*/Sudden oak death, which includes the most current host lists and legal information on quarantine restrictions

Summary

Phytosanitation should become a part of your overall nursery management. Due to the increased concern about PRAM, a wealth of recent information on phytosanitary concerns in nurseries is available. Either the systems approach based on Hazard Analysis of Critical Control Points or, for smaller nurseries, a targeted approach based on pests of greatest concern can be effective. Phytosanitation is an essential practice to help prevent the spread of PRAM and other pathogens to or from your nursery operations.

Acknowledgements

Special Thanks to: Gary Chastagner and Marianne Elliott, WA State University; Susan Frankel & Ellen Goheen, USDA Forest Service; Prakash Hebbar & Scott Pfister, USDA, APHIS; Jennifer Parke, OR State University, and Jane Alexander, University of California.

References

- Alexander J. 2006. Review of *Phytophthora ramorum* in European and North American Nurseries. In: Frankel SJ, Shea PJ, Haverty MI, technical coordinators. Proceedings of the sudden oak death second science symposium: the state of our knowledge. Albany (CA): USDA Forest Service, Pacific Southwest Research Station. General Technical Report PSW-GTR-196. p 37-39.
- Brasier C. 2012. EU2, A fourth evolutionary lineage in *P. ramorum*. University of California: Sudden Oak Death 5th Science Symposium. Website: http://ucanr.org/sites/sod5/Agenda/. Accessed August 18 2012.
- Baker KF. 1957. The U.C. system for producing healthy containergrown plants through the use of clean soil, clean stock, and sanitation. Berkeley, CA: Univ. of California, Division of Agricultural Sciences, Manual 23. 332 p.
- Chastagner G, Elliott M, McKeever K. 2012. Sudden oak death. In: Cram MM, Frank MS,
- Mallams KM. technical coordinators. Forest nursery pests. Washington (DC): USDA Forest Service, Agriculture Handbook 680. p 135-137.
- Chastagner G, Oak S, Omdal D, Ramsey-Kroll A, Coats K, Valachovic Y, Lee C, Hwang J, Jeffers S, Elliott M. 2010. Spread of P. ramorum from nurseries into waterways—implications for pathogen establishment in new areas. In: Frankel, SJ, Kliejunas JT, Palmieri KM, technical coordinators. Proceedings of the sudden oak death fourth science symposium. Albany (CA): USDA Forest Service, Pacific Southwest Research Station. General Technical Report PSW-GTR-229. p 22-26.
- Cram MM, Hansen EM. 2012. Phytophthora root rot. In: Cram MM, Frank MS, Mallams KM. technical coordinators. Forest nursery pests. Washington (DC): USDA Forest Service, Agriculture Handbook 680. p 126-128.

- Cram MM, Frank MS, Mallams KM. technical coordinators. 2012. Forest nursery pests. Washington (DC): USDA Forest Service, Agriculture Handbook 680. 202 p.
- Dumroese RK. 2012. Integrated pest management. In: Cram MM, Frank MS, Mallams KM. technical coordinators. Forest nursery pests. Washington (DC): USDA Forest Service, Agriculture Handbook 680. p 5-12.
- Elliott M. 2012. Life Cycle of *Phytophthora ramorum* as it relates to soil and water. URL: http://www.forestphytophthoras.org/sites/ default/files/educational_materials/P.%2520ramorum%2520lifecyc le%2520ME.pdf1 (Accessed August 4 2012).
- Griesbach JA, Parke JL, Chastagner GA, Grunwald NJ, Aguirre J. 2011. Safe procurement and production manual: a systems approach for the production of healthy nursery stock. Wilsonville (OR): Oregon Association of Nurseries. 96 p. Website: http://www. science.oregonstate.edu/bpp/labs/grunwald/publications/SafeProduction.pdf (Accessed October 18, 2012).
- Haase DL, Taylor M. 2012. Gray mold. In: Cram MM, Frank MS, Mallams KM. technical coordinators. Forest nursery pests. Washington (DC): USDA Forest Service, Agriculture Handbook 680. p 121-122.
- James RL. 2012. Damping-off. In: Cram MM, Frank MS, Mallams KM. technical coordinators. Forest nursery pests. Washington (DC): USDA Forest Service, Agriculture Handbook 680. p 115-116.
- Kliejunas JT. 2010. Sudden oak death and *Phytophthora ramorum*: a summary of the literature. 2010 edition. Albany (CA): USDA Forest Service, Pacific Southwest Research
- Station. General Technical Report PSW-GTR-234. 181 p.
- Landis TD, Tinus RW, McDonald SE, Barnett JP. 1990. The biological component: nursery pests and mycorrhizae, Vol. 5, The container tree nursery manual. Washington (DC): USDA Forest Service, Agricultural Handbook 674. 171 p.
- Mascheretti S, Croucher P, Vettraino A, Prospero S, Garbelotto M. 2008. Reconstruction of the sudden oak death epidemic in California through microsatellite analysis of the pathogen *Phytophthora ramorum*. Molecular Ecology 17(11):2755-2768.
- Parke JL, Grünwald NJ. 2012. A systems approach for management of pests and pathogens of nursery crops. Plant Disease 96(9):1236-1244.
- Sansford,CE, Inman AJ, Baker R, Brasier C, Frankel S, de Gruyter J, Husson C, Kehlenbeck H, Kessel G, Moralejo E, Steeghs M, Webber, J, Werres S. 2009. Report on the risk of entry, establishment, spread and socio-economic loss and environmental impact and the appropriate level of management for *Phytophthora ramorum* for the EU. Sand Hutton, York, UK: Forest Research, Central Science Laboratory. EU Sixth Framework Project, RAPRA. Deliverable Report 28. 310 p. Website:http://rapra.csl.gov.uk/RAPRA-PRA 26feb09.pdf (Accessed October 18, 2012).
- USDA 2012. Plant Health. Website: http://www.aphis.usda.gov/ plant_health/index.shtml (Accessed October 15 2012).
- USFDA. 2012. Hazard Analysis & Critical Control Points (HACCP). United States Food and Drug Administration. Website: http://www. fda.gov/Food/FoodSafety/HazardAnalysisCriticalControlPointsHACCP/default.htm. (Accessed October 18, 2012).
- Vercauteren A, Riedel M, Maes M, Werres S, Heungens K. 2012. Survival of *Phytophthora ramorum* in rhododendron root balls and in rootless substrates. Plant Pathology. Website: http://onlinelibrary.wiley.com/doi/10.1111/j.1365-3059.2012.02627.x/abstract (Accessed October 24, 2012)
- Wiktionary (2012). Phytosanitary. Website: http://en.wiktionary.org/ wiki/phytosanitary (Accessed October 15 2012)

The content of this paper reflects the views of the authors, who are responsible for the facts and accuracy of the information presented within.