7 Media for Cutting Propagation

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Propagation media include a wide variety of substances, from field soils to preformed synthetic foam blocks. The most appropriate medium is determined by a combination of factors including commercial availability, the species being propagated, the type of cutting, season, the type of propagation facility (e.g., intermittent mist, fog, polyethylene tunnels—Chapter 2), and environmental factors, such as light, air and medium temperature, and relative humidity. Traditionally, the most common commercial propagation media included combinations of *Sphagnum* sp. peat mixed with perlite, vermiculite, and/or sand. More recently, options have expanded to include a variety of regionally available composted wood products, processing by-products such as rice hulls, and synthetic foam flakes and blocks (Chapter 6). The best medium is one that physically supports the cutting and provides a moist, well-aerated, nontoxic environment for root initiation and development.

In addition to the basic medium, fertilizer is sometimes added either as a foliar mist application or incorporated into the medium to improve cutting quality following rooting. Fertilizer added to the medium is usually a slow-release formulation lasting eight months or more. These nutrients are especially beneficial during the hardening-off period following root initiation and development but prior to transplanting. In addition to fertilizers, mycorrhizal fungi may be incorporated into the propagation medium to improve rooting and root development. These fungi associate with plant roots and aid in nutrient and water uptake by increasing the surface-absorbing area of roots. Some also produce growth-promoting substances such as auxins, gibberellins, and vitamins. The symbiotic association (mutualism) of mycorrhizal fungi and plant roots has been shown to improve the growth of plants especially when incorporated into pasteurized media at a very early stage of development. With some plants, inoculation of the propagation or transplant medium with the fungus or a root extract may increase rooting percentages, root survival, root quantity, and plant quality following transfer to a potting mix.

The purpose of these laboratory exercises is to demonstrate methods of propagating cuttings with common commercial propagation mixes as well as synthetic blocks and cubes. The effects of fertilizer and mycorrhizal fungus media amendments on rooting, root development, and transplant quality also will be explored.

SAFETY CONSIDERATIONS FOR ALL EXPERIMENTS

Propagation knives and clippers are sharp. Care must be taken to avoid cuts. Some of the propagation media, especially dry *Sphagnum* peat, vermiculite, and perlite are very dusty and should be handled in a well-ventilated room. Use a face mask suitable for nuisance dust when mixing media for propagation flats. Eye irritation is possible from dust, so wear safety goggles. Moistening the medium prior to use helps minimize dust problems.

EXERCISES

Experiment 1. Rooting Cuttings in Various Media

The type of medium can influence rooting percentages, root quality and quantity, rooting time, the impact of diseases and insect pests on rooting, and the successful transplanting of rooted cuttings into growing media. This experiment will compare various rooting media and identify the best choice for a variety of plants and propagation systems. It will allow for a comparison of common media, such as perlite and vermiculite, as well as locally abundant resources such as rice hulls and other composted materials. It will illustrate the challenges facing propagators in the choices necessary in developing a rapid, consistent, and easy-to-use propagation system for a variety of plants.

Materials

The following materials will be required to complete this experiment:

- Propagation flats.
- Media—The choice of media depends on availability and may include one or a combination of: organic materials [shredded or milled *Sphagnum* or *Hypnum* peat, coconut fibers (coir), composted softwood bark (pine, fir, spruce), composted hardwood bark (oak, beech), rice hulls, composted sawdust, paper mill biosolids, composted wood fiber]; inorganic media: (horticultural grade perlite (3 to 4 mm size), horticultural

grade vermiculite (<3 mm size), water-absorbent and/or water-repellent rock wool granules, sand (washed, pasteurized, lime free, 0.5 to 2 mm size), pumice]; and synthetic media [expanded polystyrene flakes or beads, urea-formaldehyde foam resin flakes]. Peat-based media attract fungus gnats whose larvae will decimate new roots. Avoid these combinations in greenhouses with fungus gnat populations.

- Plant materials—Suggested greenhouse plants: Angel Wing Begonia (*Begonia corallina* Carriere and related species); Fibrous-rooted Begonia (*Begonia* Semperflorens-Cultorum Group); Rhizomatous Begonia (*Begonia* Rex-Cultorum hybrids); Christmas Cactus (*Schlumbergera Bridgesii* (Lem.) Lofgr.); Thanksgiving Cactus (*Schlumbergera truncata* (Haw.) Moran); Florists' Carnation (*Dianthus Caryophyllus* L.); Florists' Chrysanthemum (*Dendranthema* X *grandiflorum* Kitam.and cvs); Cigar plant (*Cuphea ignea* A. DC.); Coleus (*Coleus scutellarioides* (L). Benth); Crown of thorns (*Euphorbia Millii* Desmoul.); Fuchsia (*Fuchsia* X *hybrida* Hort. Ex Vilm.); Geranium (*Pelargonium* X *hortorum* L.H. Bailey); Scented Geranium (*Pelargonium* sp. L'Her. Ex Ait.); Heliotrope (*Heliotropium arborescens* L.); Hydrangea (*Hydrangea paniculata* Siebold); Impatiens (*Impatiens Walleriana* Hook.f); Canary Island Ivy (*Hedera canariensis* Willd.); English Ivy (*Hedera helix* L. and cvs); Jade plant (*Crassula argentea* Thunb. C. argentea 'Variegata'); Flowering Maple (*Abutilon* X *hybridum* Hort.); Orchid cactus (*Epiphyllum* spp. Haw.); Poinsettia (*Euphorbia pulcherrima* Willd ex Klotzsch) Polka-dot plant (*Hypoestes phyllostachya* Bak.); Purple Heart (*Tradescantia pallida* Rose); Spider-wort (*Tradescantia albiflora* Kunth.); String of pearls (*Senicio radicans* (L.f.) Schultz-Bip.); Swedish Ivy (*Plectranthus australis R. Br.*); Tradescantia (*Tradescantia fluminensis* Vell.); Wax Plant (*Hoya carnosa* (L.f.) R.Br.); Zebrina (*Zebrina pendula* Schnizl.).
- Suggested herbaceous and woody perennials: Achillea (*Achillea ptarminca* L. *A. millefolium* L. *A. filipendula* Lam.); Boxwood (*Buxus sempervirens* L.); Buddleia (*Buddleia Davidii* Franch.); Black currants (*Ribes nigrum* L. *R. hudsonianum* Richardson.); Red and white currants (*Ribes triste Pall. Ribes* sp. L.); Deutzia (*Deutzia gracilis* Siebold & Zucc.); Dianthus (*Dianthus superbus* L.); Redosier Dogwood (*Cornus sericea* L.); English Lavender (Lavandula angustifolia Mill.); Forsythia (*Forsythia* X *intermedia* Zab., *F. ovata* Nakai); Heather (*Calluna vulgaris* (L.) Hull); Hibiscus (*Hibiscus Rosa-sinensis* L.); Juniper (*Juniperus horizontalis,* Moench *J. chinensis* L.); Kinnikinnick (*Arctostaphylos uva-ursi* (L.) K. Spreng.); Mock Orange (*Philadelphus coronaries* L., *P. lewisii,* Pursh.); Cottage Pink (*Dianthus plumarius* L.); Potentilla, Cinquefoil (*Potentilla fruticosa* L.); Privet (*Ligustrum japonicum,* Thunb., *L. ovalifolium* Hassk.); Rosemary (*Rosemarinus officinalis* L.); Speedwell (*Veronica incana* L. , *V. spicata* L.); Bridal wreath spiraea (*Spiraea* X *Vanhouttei* (C. Briot) Zab.); False spiraea (*Sorbaria sorbifolia* (L.) A. Braun.); Pink spiraea (*Spiraea Douglasii* Hook.); Tatarian honeysuckle (*Lonicera tatarica* L.); , English Thyme (*Thymus vulgaris* L.); Vinca, Periwinkle (*Vinca minor* L., *V. major* L.); Weigela (*Weigela florida* (Bunge) A. DC.).
- Propagating knife or clippers.
- Dibble, stake, or pencil for making furrows.
- Plastic or wooden labels and marking pens.
- Rooting powder with paper towel or cup or rooting liquid.
- Intermittent mist propagation system, high-humidity propagating box, or other propagation facility.
- Ruler or calipers for measuring root length.
- Materials for measuring bulk density, total pore space, available water holding capacity, air-filled porosity, pH, and electrical conductivity (Chapter 6).

Follow the instructions outlined in Procedure 7.1 to complete the experiment.

The choice of medium is directly related to the type of propagation facilities, moisture availability, and environmental conditions. Experiment 1 may be repeated with additional treatments such as the following: (1) compare rooting in media on an intermittent mist propagation bench versus a closed polyethylene propagation box (Chapter 2), mist versus fog (Chapter 4), etc.; (2) compare media under two or more misting frequencies or watering regimes; and (3) compare media with and without bottom heat, overhead lights, and rooting compounds.

Additional interesting comparisons for group experiments include the following: (1) organic versus inorganic and synthetic media, (2) grades (particle size) of a product such as vermiculite, sand, peat, and coconut fiber, (3) types of peat: *Sphagnum, Hypnum,* and sedge, and (4) combinations of media commonly used by commercial greenhouses and nurseries, such as (1) *Sphagnum* peat, sand, peat/sand mix in one of the following 1:1, 3:1, or 4:1 ratios (by volume), (2) *Sphagnum* peat, perlite, peat/perlite mixes in 1:1, 2:1 or 3:1 ratios, (3) *Sphagnum* peat, vermiculite, peat/vermiculite mix in 1:1 or 2:1 ratios.

Procedure 7.1
Rooting Cuttings in Various Media

Step	Instructions and Comments
1	Fill four flats with one of the following media: (1) Sphagnum peat/sand 1:1 (by volume), (2) horticultural grade perlite, (3) horticultural grade vermiculite, (4) perlite/vermiculite 1:1 (by volume) or other combinations.
2	Measure and record for each medium: bulk density, total pore space, available water holding capacity, air-filled porosity, pH, and electrical conductivity (Chapter 6).
3	Make 20, 3- to 6-inch (7.5 to 15 cm) uniform stem tip cuttings each of at least four different greenhouse plants and four herbaceous or woody plant materials.
4	Make labels for each treatment with your last name, medium type, plant name, and date.
5	Remove any flowers from each cutting, and remove leaves from the proximal (lowest) 1 1⁄2 to 2 inches (4 to 5 cm) of each cutting.
6	Treat all cuttings with a rooting powder or quick dip solution appropriate for the type of cutting.
7	Beginning in the upper left hand corner of the flat, make lengthwise furrows in the medium with a dibble, stake or pencil. Furrows should be about 3/4 the depth of medium.
8	Insert the label at the top of the furrow, and insert five cuttings at 1- to 2-inch (2.5 to 5 cm) spacing, depending on the size of the cutting. Do not push the cuttings to the bottom of the flat. Repeat with a second species, inserting a label at the head of the furrow until the row is full.
9	Firm the medium around the base of each cutting, and remove any leaves that are partially buried in the medium. Repeat with additional furrows, moving from left to right in the flat until all cuttings are stuck.
10	Place flats into the propagation box, mist bench, etc. Check cuttings weekly until first root appearance. Gently tug on cuttings to dislodge them from the medium. Once roots are observed on one cutting in each treatment, do not disturb the others. Record days to first rooting. When reinserting cuttings into the medium, avoid damage to new roots by using a dibble or stake, opening a hole in the medium, and carefully returning cuttings to the propagation medium.
11	After 4 weeks for greenhouse plants and 6 weeks for herbaceous or woody perennial plants (or at the end of the semester or quarter), harvest all cuttings, keeping treatments separate with their labels.
12	Record root color and percentage of rooting for the five cuttings in each treatment.
13	Evaluate root abundance using the scale: 0 = no roots; 1 = few roots, easy to count, no secondary branching, little medium attached to roots; 2 = moderate amount of roots, difficult to count, some secondary branching; 3 = abundant roots, nearly impossible to count, obscured by the medium, abundant secondary branching. Calculate average root abundance per treatment.
14	Measure the longest root on each cutting from point of origin on the stem to root tip. Calculate average maximum root length per treatment.
15	Select the longest root and bend it in half onto itself. Evaluate root brittleness using the following scale: 0 = root is flexible, does not break, can be bent nearly 180 degrees without breaking; 1 = root is stiff but bendable, can be bent to 90 degrees before breaking; 2 = root is brittle and easily breaks when bent less than 90 degrees.
16	Construct at least 2 tables and/or bar graphs: (1) medium characteristics (from Step 2) and rooting characteristics. Table or figure 2 in your laboratory report should include a comparison of media and species in relation to root quality (color, brittleness, abundance), rooting success (percent rooting), and timing (days to first rooting).

Anticipated Results

Results will vary depending upon the treatments chosen as well as the formulations of the media tested. The tropical greenhouse and herbaceous species suggested generally root easily, but certain species may root better in specific media. For example, succulents, such as *Crassula argentea* (Jade Plant), may do better in well-drained media with a higher proportion of sand or perlite relative to the peat. Other species will root well in a variety of media, but the quantity and quality of the roots and the rooting pattern along the stem may differ among media. Since some of the more difficult woody plants take longer to root and thus are in the rooting medium a longer time, these species may reveal subtle differences among the rooting media that were chosen, especially the speed of rooting and the quality of roots.

Questions

- Compare and contrast media and the rooting percentages of all species. Is one medium superior to all others?
- How did root quality, quantity, and rooting speed differ among media and species?
- What physical characteristics of each medium might explain the rooting results?
- Does each medium fulfill the requirements of a good propagating medium (paragraph 1 in introduction)?

Experiment 2. Compressed Pellet or Solid Block Media and Rooting of Cuttings

Compressed pellets and solid block media are common in certain horticulture businesses, such as the mass production of chrysanthemum, geranium, and poinsettia rooted cuttings. The blocks are easy to handle and provide a uniform rooting environment, often with standard spacing. The pellet or block is transplanted with the rooted cutting into a growing medium, thus reducing transplant shock. This experiment will demonstrate the advantages and disadvantages of using pellets or blocks for mass production of rooted cuttings. It may be combined with Experiment 1 to show a variety of propagation systems available to growers.

Materials

The following materials will be needed to complete this exercise:

- Propagation flats.
- At least three of the following media: compressed peat pellets or blocks with or without added fertilizer, rock wool compressed cubes, urea-formaldehyde or polyurethane foam cubes (e.g., Oasis® Rootcubes®, Wedges®, Jiffy 7, and Jiffy 9, etc.). Media should be drenched, if necessary, to leach toxic substances from the blocks. Saturate and expand compressed cubes with water at least 24 h before class.
- Dowel, dibble, or pencil for making holes for stems if not premade.
- Propagating knife or clippers.
- Plastic or wooden labels and marking pens.
- Rooting powder or liquid (concentration for herbaceous stem cuttings).
- Florists' chrysanthemum (*Dendranthema x grandiflorum* (Ramat.) Kitam.), carnation (*Dianthus caryophyllus* L.), poinsettia (*Euphorbia pulcherrima* Willd. ex Klotzsch), geranium (*Pelargonium x hortorum* L.H. Bailey), or other easily rooting species.
- Washed quartz sand—many of the propagating blocks come with premade holes for stems. Choose stems that match the diameter of the holes or use sand to fill in the hole and support the cutting.
- Intermittent mist propagation system or propagating box.
- Ruler or calipers for measuring root length.
- Materials for measuring bulk density, total pore space, available water holding capacity, air-filled porosity, pH, and electrical conductivity (Chapter 6).

Follow the instructions described in Procedure 7.2 to complete this experiment.

Anticipated Results

The media chosen have been specially formulated for use in rooting plants, and thus, all of the media should perform well. There will be differences in ease of handling, which the students will experience as they examine the rooted cuttings and collect data. If a fertilizer-enhanced medium is used and compared to a control without fertilizer, marked differences in growth may not be apparent until the cuttings are well rooted and developing. The watering and misting regime for many pellets and blocks differs from cuttings in propagating flats. Pellets, especially solid peat pellets, can hold a lot of water and cause cuttings to rot. Comparisons between the two methods should use separate misting systems.

Questions

- Compare rooting and transplant growth among cuttings in different media. Is one medium superior to all others?

Procedure 7.2
Compressed Pellet or Solid Block Media and Rooting of Cuttings

Step	Instructions and Comments
1	Prepare a flat that contains at least 10 cells of three media (compressed peat, rock wool, foam propagation blocks or others). Make sure the flat has sufficient drainage so there is no standing water.
2	Measure and record for each medium or obtain from manufacturer's published information the following: total pore space, available water holding capacity, air-filled porosity, pH, and electrical conductivity (Chapter 6)
3	Make 30 cuttings of florists' chrysanthemum, geranium, poinsettia, carnation or other easy to root species.
4	Treat all cuttings with a rooting powder or quick dip liquid with a concentration appropriate for herbaceous cuttings.
5	Some blocks have preset holes, whereas others do not. If holes do not exist, make them with a dowel, dibble, or pencil the diameter of each cutting in the top of each saturated block or pellet. Insert one cutting per hole not more than 3/4 of the total block or pellet depth. If the hole is too large to fit the cutting snugly, make a second hole close to the original or add a small amount of washed quartz sand to the hole.
6	Label the flat with your last name, plant name, and date. Place the flat in the propagation box or beneath intermittent mist. Keep media and cuttings moist.
7	Check the sides of the pellets or blocks weekly for root emergence. Record the date of first root emergence for each treatment.
8	After 3 to- 4 weeks, separate all cubes or pellets and count the number of emerging roots tips. Do not try to remove the cuttings from the media. Record percent rooting for the cuttings in each type of medium, and describe the roots (color, presence of root hairs, general appearance compared to the other treatment media).
9	Rate each cutting based on the following scale: 0 = no roots; 1 = few roots visible, all less than 1 inch (2.5 cm) long, no secondary branching; 2 = moderate amount of roots visible, at least half are 1 inch (2.5 cm) long or longer, some secondary branching; 3 = abundant roots, most are 1 inch (2.5 cm) long or longer, abundant secondary roots. Calculate the average root abundance per treatment.
10	Measure the length of the longest root from the medium surface to root tip. Calculate average length of longest root for each treatment.
11	Construct a table and/or bar graph comparing media effects on root quality (color, abundance, root length), rooting success (percent rooting), and timing (days to first root appearance).
12	Plant the rooted cuttings including the propagation medium. Bury the pellet or block at least 1⁄4 inch (1⁄2 cm) into a standard greenhouse potting mix.
13	Measure the height of each cutting, and count the number of leaves immediately after planting and again 4 weeks later. Calculate the difference between plant height and leaf number between dates, and compare growth of rooted cuttings following transplanting
14	Construct a table or bar graph comparing plant growth following rooting among media types.

- How did root quality, quantity, and rooting speed differ among media?
- What physical characteristics of each medium might explain the rooting results?
- Does each medium fulfill the requirements of a good propagating medium (see introduction)?

Experiment 3. Rooting of Cuttings in Fertilizer-Amended Media

Growers are interested in producing high-quality rooted cuttings in the least amount of time. For some species, especially those that require long rooting times, the addition of fertilizer, either as a liquid mist or incorporated into the medium, produces a superior rooted plant, one that will justify adding fertilizer to production costs. This experiment will demonstrate the changes in rooting and plant growth with the addition of a slow-release fertilizer to the rooting medium.

Materials

The following supplies will be required to complete this experiment:

- Propagation flats

- Peat/perlite , perlite/vermiculite(1:1 by volume) propagating medium or other combinations
- Florists' chrysanthemum (*Dendranthema x grandiflorum* (Ramat.) Kitam.), carnation (*Dianthus caryophyllus* L.), poinsettia (*Euphorbia pulcherrima* Willd. ex Klotzsch), geranium (*Pelargonium x hortorum* L.H. Bailey), or other easily rooting species
- Propagating knife or clippers
- Dibble, stake, or pencil for making furrows in the medium
- Labels and marking pens
- Rooting powder or liquid (concentration for herbaceous cuttings)
- Osmocote® 18-6-12 fertilizer or similar slow release formulation
- Intermittent mist propagation system or propagating box
- Ruler or calipers for measuring cuttings
- Peat/lite commercial potting mix and containers appropriate for the size of the rooted cuttings

Additional interesting comparisons for group experiments include the following: (1) comparisons of a variety of organic, inorganic, and synthetic media with and without fertilizer, (2) intermittent mist versus propagation boxes using flats with and without fertilizer, and (3) comparison of soluble fertilizer applied in mist or fog with media-incorporated fertilizer.

Follow the instructions in Procedure 7.3 to complete this experiment.

Anticipated Results

Addition of a slow-release fertilizer may or may not show differences in overall rooting percentages, depending on the plant used, but root quantity, quality, especially total root length and branching patterns will show a response to fertilizer. The amount of time a cutting is in the propagation structure also may be shortened by addition of fertilizer. The quality of the shoots following rooting, especially changes in color, can be very different in fertilized versus nonfertilized cuttings. Following transplanting to a potting medium, differences may also be measurable in shoot growth. Rooted cuttings may also begin new shoot growth more rapidly in cuttings treated with fertilizer than untreated cuttings.

Questions

Compare and contrast fertilizer-amended media with the control and the rooting ability of all species.

- Is there an advantage to incorporating fertilizer in the mix? How do rooting percentages differ from an unfertilized control?
- How did root quality, quantity, rooting speed, and new growth differ among treatments and species?

Experiment 4. Rooting and Growth of Kinnikinnick, Arctostaphylos uva-ursi, Using Medium Amended with Mycorrhizal Fungi Root Extract.

Kinnikinnick is a common evergreen ornamental ground cover used in home and public landscapes. It is also one of the most studied species in terms of colonization by mycorrhizal fungi. Plant roots are easy to harvest, and those colonized by mycorrhizae are easy to distinguish from noncolonized roots with a hand lens or stereomicroscope by their thickened root tips. This experiment demonstrates a simple method of inoculating pasteurized or sterilized propagation and growing media with mycorrhizae that may significantly improve the growth and establishment of this nursery ground cover.

Materials

The following materials are needed to complete the experiment:

- Propagation flats
- *Sphagnum* peat, sand
- Kinnikinnick (*Arctostaphylos uva ursi* (L.) Spreng.) nursery stock or established landscape plants
- Rooted or unrooted kinnikinnick stem cuttings
- Dissecting microscope
- Blender, sieve, piece of window screen or kitchen wire strainer
- Large plastic buckets or storage containers for mixing extract
- Spray bottle or garden watering can with water breaker
- Intermittent mist propagation bench or propagation box
- Labels and marking pens

Alternative Experiments

Depending upon conditions, rooting and mycorrhizal colonization may require more time than allocated for a single semester or quarter. Use cuttings prerooted in a sterile medium and apply the mycorrhizal root drench to the transplant medium rather than the rooting medium to learn how mycorrhizae influence growth of rooted cuttings. Additional comparisons with groups include the following: treat half of cuttings in each with rooting powder or quick dip appropriate for cutting type. Compare mycorrhizal treatments among different rooting media.

Procedure 7.3
Rooting of Cuttings in Fertilizer-Amended Media

Step	Instructions and Comments
1	Fill three flats with Sphagnum peat/sand 2:1 (by volume) or other propagating medium. Incorporate into one flat, 3.0 g/L 18-6-12 (8 to 9 month) Osmocote® controlled-release fertilizer or similar product and into the second flat, 6.0 g/L Osmocote®. The third flat is the no-fertilizer control.
2	Make fifteen 3- to 6-inch (7.5 to 15 cm) long, stem tip cuttings each of at least three different greenhouse plants: florists' chrysanthemum, hydrangea, English Ivy, and three herbaceous or woody plant materials: potentilla, vinca, speedwell (see additional suggestions in Experiment 1).
3	Make labels for each treatment with your last name, medium type, plant name, and date.
4	Remove any flowers from each cutting, and remove leaves from the basal 1½ to 2 inches (4 to 5 cm) of each cutting.
5	Treat all cuttings with a rooting powder or quick dip solution appropriate for the type of cutting.
6	Beginning in the upper left hand corner of the flat, make lengthwise furrows in the medium with a dibble, stake, or pencil. Furrows should be about 3/4 the depth of the flat or container.
7	Insert the label at the top of the furrow, and insert five cuttings at 1- to 2-inch (2.5 to 5 cm) spacing, depending on the size of the cutting. Do not push the cuttings to the bottom of the flat. Repeat with a second species, inserting a label at the head of the furrow until the row is full.
8	Firm the medium around the base of each cutting, and remove any additional leaves that are partially buried in the medium. Repeat with additional furrows until all cuttings are stuck.
9	Place flats into the propagation box, mist bench, etc. Check cuttings weekly until first root appearance. Gently tug on cuttings to dislodge them from the medium. Once roots are observed on one cutting in each treatment, do not disturb the others. Record days to first rooting. When reinserting cuttings into the medium, avoid damage to new roots by using a dibble or stake, opening a hole in the medium, and carefully returning cuttings to the propagation medium.
10	After 4 weeks for greenhouse plants and 6 weeks for herbaceous or woody perennial plants (or at the end of the semester or quarter), harvest all cuttings, keeping treatments separate with their labels.
11	Record root color and percentage of rooting for the five cuttings in each treatment.
12	Evaluate root abundance using the scale: 0 = no roots; 1 = few roots, easy to count, no secondary branching, little medium attached to roots; 2 = moderate amount of roots, difficult to count, some secondary branching; 3 = abundant roots, nearly impossible to count, obscured by the medium, abundant secondary branching. Calculate average root abundance per treatment.
13	Measure the longest root on each cutting from point of origin on the stem to root tip. Calculate average maximum root length per treatment.
14	Measure new growth per cutting, including number of new stems and leaves. Calculate the average number of new shoots and leaves per cutting for each treatment.
15	Construct a table and/or bar graph including a comparison of fertilizer treatments and species in relation to root quality (color, abundance), rooting success (percent rooting), timing (days to first rooting), and amount of new growth (new stems and leaves).

Try other species such as birch (*Betula papyrifera* Marsh. and other species), cottonwood (*Populus balsamifera* L. and other species), and Arbutus (*Arbutus menziesii* Pursh). Plants with ectomycorrhizal associations are the easiest to study because fungal associations may be observed under a dissecting microscope. Other types of mycorrhizae require more complex microtechniques (cell clearing and staining) to detect presence of fungi.

Follow the instructions in Procedure 7.4a or 7.4b to complete this experiment.

Anticipated Results

The results of this experiment will be determined by the length of the experiment and the success in introducing mycorrhizae to the medium. Rooting percentages

Procedure 7.4a

Rooting of Kinnikinnick (*Arctostaphylos uva-ursi)* Using Media Amended with Micorrhizal Fungi Root Extract

Step	Instructions and Comments
1	Fill two propagation boxes or flats with a 2:1 (by volume) mixture of moistened Sphagnum peat/sand.
2	Collect branched stems of kinnikinnick from wild stands, ornamental plantings, or containers. Make 20 stem tip cuttings, 4 to 6 inches (10 to 15 cm) in length.
3	Remove all flowers and/or fruit from each cutting, and remove leaves from the proximal 1 to 1½ inches (2.5 to 4 cm) of the stem.
4	Stick cuttings using a dibble, pencil, or stake into the medium at 1-inch (2.5 cm) spacing.
5	Collect enough kinnikinnick roots to fill a 50-mL (¼ cup) container from wild stands or commercially propagated kinnikinnick. Remove most of the surrounding soil or potting mix, enough to locate the root tips. Examine the root tips using a stereo (dissecting) microscope to see evidence of mycorrhizal fungi associated with the roots. Mycorrhizal roots are thickened at the tips with a mantle of fungus. Roots covered with the mantle seem to be swollen, cigar-shaped, with stubby branches and whitish webbing (hyphae) surrounding the roots. Nonmycorrhizal roots are thin and hairlike, without the very obvious thickening mantle.
6	Add mycorrhizal roots to a blender half filled with water. Blend until roots are well chopped (approximately 3 min).
7	Filter the liquid through a screen (kitchen strainer, 16 mesh (1.0 mm, 0.04 inch) soil sieve, or window screen will work).
8	Mix filtrate with sufficient water to drench one-half of the propagating flats for the entire class, 500 mL per standard (20 (10 inch, 51 (25 cm) flat. Spray the extract over the cuttings and flat to thoroughly drench the medium.
9	Label each flat with your last name, treatment, and date.
10	Place flats in a propagating box. Check cuttings weekly until first root appearance (3 to 4 weeks). Gently tug on cuttings to dislodge them from the medium. Once roots are observed on one cutting in each treatment, do not disturb the others. Record days to first rooting. When reinserting cuttings into the medium, avoid damage to new roots by using a dibble or stake, opening a hole in the medium, and carefully returning cuttings to the propagation medium.
11	At the end of the semester or quarter, harvest all cuttings, keeping them separate by treatment.
12	Record root color and percentage of rooting for the 10 cuttings in each treatment.
13	Evaluate root abundance using the scale: 0 = no roots; 1 = 1 to 2 roots, easy to count, no secondary branching; 2 = 3 to 5 roots, some secondary branching; 3 = more than 5 roots, abundant secondary branching. Calculate average root abundance per treatment.
14	Evaluate micorrhizal associations in roots using the following scale 0 = no mycorrhizal fungi evident; 1 = half of root tips enlarged with a fungal mantle or hyphae; 2 = abundant evidence of mycorrhizal association in more than half of the root tips.
15	Measure the longest root on each cutting from point of origin on the stem to root tip. Calculate average maximum root length per treatment.
16	Construct a table or bar graph comparing treated and untreated media in relation to root quality (color, root abundance, fungal association scale), rooting success (percent rooting), and timing (days to first rooting).

Procedure 7.4b

Rooting of Kinnikinnick (*Arctostaphylos uva-ursi)* Using Rooting Compounds and Media Amended with Micorrhizal Fungi Root Extract

Step	Instructions and Comments
1	Fill one propagation box or flat with a 2:1 (by volume) mixture of moistened Sphagnum peat/sand.
2	Collect branched stems of kinnikinnick from wild stands, ornamental plantings, or containers. Make 20 stem tip cuttings, 4 to 6 inches (10 to 15 cm) in length.
3	Remove all flowers and/or fruit from each cutting, and remove leaves from the proximal 1 to 1½ inches (2.5 to 4 cm) of the stem. Treat each cutting with rooting powder or quick dip liquid for woody plants.
4	Stick cuttings using a dibble, pencil, or stake into the medium at 1-inch (2.5 cm) spacing. Label with the name of the plant and date. Place on an intermittent mist propagation bench until cuttings are well rooted (3 or more weeks).
5	When cuttings are well rooted, transplant into a sterile commercial peat-lite potting mix to which sterile sand has been added (2:1 by volume) in cell packs.
6	Collect enough kinnikinnick fine roots to fill a 50-mL (¼ cup) container from wild stands or commercially propagated kinnikinnick nursery stock. Remove most of the surrounding soil or potting mix, enough to locate the root tips. Examine the root tips using a stereo (dissecting) microscope to see evidence of mycorrhizal fungi associated with the roots. Mycorrhizal roots are thickened at the tips with a mantle of fungus. Roots covered with the mantle appear swollen, cigar-shaped, with stubby branches and whitish webbing (hyphae) surrounding the roots. Nonmycorrhizal roots are thin and hairlike, without the very obvious thickening mantle.
7	Add mycorrhizal roots to a blender two-thirds filled with water. Blend until roots are well chopped (approximately 3 min).
8	Filter the liquid through a screen (kitchen strainer, 16 mesh (1.0 mm, 0.04 inch) soil sieve, or window screen will work).
9	Add sufficient water to make 1 L of solution. Thoroughly drench half the transplants and medium with the liquid. One L will be sufficient for 48 cells in a standard flat
10	Label each flat with your last name, treatment, and date.
11	After 4 weeks, measure percent survival of rooted transplants and the number of new shoots and leaves per rooted transplant. Calculate the average number of new shoots and leaves per cutting for each treatment.
12	Wash the medium from the roots and evaluate root abundance using the scale: 0 = no roots (roots killed); 1 = 1 to 3 root tips; 2 = 4 to 6 root tips; 3 = more than 6 root tips. Calculate average root abundance per treatment.
13	Evaluate micorrhizal associations in roots using the following scale 0 = no mycorrhizal fungi evident; 1 = half of root tips enlarged with a fungal mantle or hyphae; 2 = abundant evidence of mycorrhizal association in more than half of the root tips.
14	Construct a table or bar graph comparing treated and untreated media in relation to shoot growth and root quality (root abundance and fungal association scale).

may be increased and root quality improved (increased root branching and root length) on treated plants, but the greatest differences occur after rooting. If the time is long enough, roots on the treated plants will show colonization by the mycorhizae (swelling of root tips), and the timing from rooting to removal from the propagation bench will be reduced. At least three months are needed if the experiment is started with unrooted cuttings and two months with rooted plants.

Questions

- How did mycorrhizal extract influence rooting percentages or rooted cutting growth?
- How did root quality, quantity, and rooting speed change with the treatment?
- What percentage of root tips showed colonization by mycorrhizae on rooted cuttings or transplants?
- If no evidence of mycorrhizae is present following rooting or transplant development, what environmental conditions may have impacted the results of this project?

SUGGESTED READINGS

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