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Determining methods of propagation for the investigation of intraspecific variability of climate change responses of Appalachian plant species

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**Determining methods of propagation for the investigation of
intraspecific variability of climate change responses of Appalachian
plant species.**

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Departmental Thesis

University of Tennessee at Chattanooga

Department of Biology, Geology, and Environmental Sciences

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Examination Date: October 29, 2015


Examination Committee:

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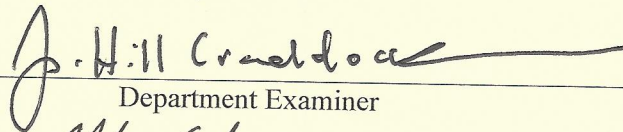
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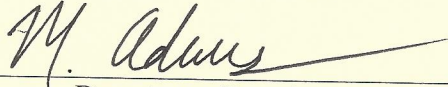
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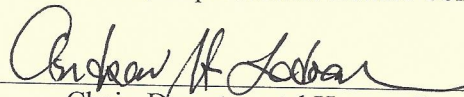
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Subdiscipline Symbiosis: Supporting climate change research by bridging a gap between ecology, taxonomy, and horticulture.

Ecology is the study of how organisms interact with their biotic and abiotic environment. Studying ecological relationships can help us better understand the world around us and improve critical aspects of our lives, including our environment, public health, and the management of natural resources (ESA Public Affairs Office 2010). The efforts of ecologists are particularly relevant today because of the numerous global-scale anthropogenic changes that have occurred during the past few centuries as a consequence of human population growth and economic development. The impacts of anthropogenic climate change, in particular, on natural systems has been called a contemporary ‘grand challenge’ in ecology (Thuiller 2007), and determining how species will respond to climate change is a related and fundamental ecological question of our time (Sutherland et al. 2013).

The impacts of climate change on plants are especially important to investigate and understand given the fundamental role that plants play in the biosphere. Plants form the base of trophic energy transfer, and thus are the direct or indirect energy source for nearly all other organisms (Daily et al. 1997). Plants also perform vital ecosystem services such as supplying oxygen to and removing carbon dioxide from the earth’s atmosphere, as well as driving the water cycle (Daily et al. 1997). Additionally, many of our most important resources – including most of our foods and pharmaceuticals – are completely or partially derived from plants (Daily et al. 1997). As rapid climate change continues, many plant species are predicted to become extinct, while others may

hybridize or migrate as warming temperatures shift historically suitable habitat poleward and to higher elevations (Walther et al. 2002). Ecological studies of how plants will respond to projected changes can help us better implement conservation initiatives that protect plants at the species, population, and community levels.

Importance of taxonomic and horticultural knowledge to plant ecologists

The nature of ecological problems often requires ecologists to take a multidisciplinary approach to their research (Novicic et al. 2012). A thorough understanding of various physical, life, and social sciences can provide researchers with a holistic view of what may be impacting species in an ecosystem (Perrings et al. 1992). Ideally, ecologists would have comprehensive understanding and expertise in related subfields.

Yet, despite the benefits of multidisciplinary expertise, it remains a limitation to ecological research even when the subfields involved are closely related. Plant ecological experts often have little knowledge or expertise in the subfields of plant taxonomy and horticulture (Gotelli 2004, Hardwick 2011), but both the ability to properly identify plant species (taxonomy) and knowledge of practical propagation and maintenance techniques (horticulture) are often involved in plant ecological research and vital to the success of research projects. For example, horticultural techniques developed by botanical gardens could greatly improve community design in restoration ecology (Hardwick 2011). Applying other aspects of plant science in their research could facilitate existing research foci and help ecologists to broaden the context of their research through better understanding of the basic biology and life history of their study species.

Case Study: Application of Taxonomic and Horticultural Techniques to Ongoing Plant Ecological Research at UTC

Species range migrations are a well-documented response to climate change. Palynology records reveal that as global temperatures increased during the end of the last ice age, boreal forest assemblages shifted poleward or to higher elevations, following envelopes of climate conditions to which they are adapted (Loarie et al. 2009). Scientists predict that similar range shifts are presently underway due to dramatic increases in global temperatures and greenhouse gases since the beginning of the Industrial Age (Walther et al. 2002). By 2100, global temperatures may increase by 4°C; atmospheric carbon dioxide levels may increase beyond 800 ppm, twice the current level (Pachauri et al. 2014, Thuiller 2007). Species unable to respond quickly enough to changing climate face greater risk of extinction.

Sessile life forms such as plants must rely on phenotypic plasticity and rapid adaptation in order to survive rapidly changing climate conditions. As range shifts occur, variation in phenotypic traits within plant species across climate gradients could affect their abilities to acclimate or adapt to a changing climate (Hooper et al. 2005, Souza et al. 2011). In 2014, Dr. Jennifer Boyd (BGES) initiated a research project to examine potential intraspecific phenotypic variation within wide-ranging Appalachian plant species of responses to projected future atmospheric CO₂ and temperature regimes. A potential list of study species included summer sedge (*Carex aestivalis*), Canada mayflower (*Maianthemum canadense*), pin cherry (*Prunus pensylvanica*), pink azalea (*Rhododendron periclymenoides*), and wreath goldenrod (*Solidago caesia*). These species were considered because collectively they represent a substantial component of plant

communities (graminoids, monocotyledonous forbs, dicotyledonous forbs, and woody species), and because they occur throughout, but are restricted to, the Appalachian region, which allows for capture of their full range limits. The ultimate goal of this study is to provide information about how physiological traits and intraspecific variability influence species climate change responses toward improvement of climate models that predict changes in future species distribution.

The role of taxonomy and horticulture in this study is directly linked to our experimental design. In order to compare treatment responses of propagules sourced from different sites, we must be certain that the same species are collected at each site.

Taxonomic tools and records such as dichotomous keys and herbarium samples enable us to identify study species accurately and consistently. Our methods are representative of a classic common garden approach (Reich et al. 1996, Turesson 1922, Vitasse et al. 2009), in which individuals from distinct populations of the same species are grown together under the same conditions to determine if there are genetic differences underlying their responses to environmental factors (in this case, temperature and CO₂). Environmental heterogeneity of our field sites could mask genetic differences in our study populations. Field-collected specimens are also likely to vary in age, which would also impact responses to treatments. In contrast, using offspring from distinct populations that begin their life cycle in common environmental conditions allows for assessment of genetic differences in those populations that would qualify them as ecotypes.

To utilize offspring in an ecological common garden experiment, we must correctly identify and cultivate propagules of study species to carry out this experiment; thus, we must use taxonomic and horticultural techniques to accomplish ecological

research. Since we plan to expand this project, we must know how to both propagate these species and correctly maintain successive generations for further experimental trials, particularly specimens collected from sites that are difficult to access. Familiarity with the taxonomy of each species is important for correct field identification and understanding the basic biology of each species.

My objectives

My main responsibility in this project was to determine best practices for successful field identification, propagation, and care of the plant species selected for study during the 2014-2015 academic year. This necessitated extensive research of available taxonomic and horticultural information for each of our study species, so that we could correctly identify, cultivate, and propagate them for use in our experiments. I compiled my research findings into a manual intended as a guide for future ecology students in Dr. Boyd's lab, so that they can be better prepared to contribute to this long-term project. This manual includes profiles on how to properly identify, propagate, and care for each species included in this project, as well as accompanying helpful resources. As additional species are added to the project, the profile outline I created may act as a template for incorporation of those species into the manual. Just as the larger project is a work in progress, so is this written work.

Research Methods for Experimental Preparation

I utilized a wide variety of sources to determine the most effective and appropriate methods for identification and propagation of the candidate study species. I

first familiarized myself with general research of the growth form, habitat, life history, and reproduction methods of each species. To prepare for field identification, I obtained specific identification information for each candidate species from taxonomic keys, floras, and identification manuals. Online floristic databases, such as the *Flora of North America*, were particularly helpful sources of botanical information. Our field team was fortunate enough to be able to confirm identification of our field-collected specimens with records at the Steere Herbarium at the New York Botanical Garden.

Determining the best methods of propagation required a more creative research approach. Wildtype specimens of native species, particularly those without showy blooms, are generally not as popular in landscaping and other areas of cultivation practice, and thus horticultural information on such specimens is few and far between. Thus, instead of relying on conventional horticultural sources like gardening forums, I extensively studied the environmental preferences and phenology of each species to understand their optimal reproductive conditions. Information sources I used included a handbook of propagating native southeastern North American plants (Midgley 1999), scientific articles in which the authors gave detailed descriptions of either field conditions or laboratory propagation techniques of our candidate species, and fact sheets assembled by various extension agencies along the Appalachian region. Methods of plant care were also deduced from environmental conditions and climate patterns of field sites where we collected study specimens.

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Determining the best methods of propagation required a more creative research approach. Wildtype specimens of native species, particularly those without showy blooms, are generally unpopular in landscaping and other areas of cultivation practice, and thus horticultural information on such specimens is very limited. Thus, instead of relying on conventional horticultural sources like gardening forums, I extensively studied the environmental preferences and phenology of each species to understand their optimal reproductive conditions. Information sources that I used included habitat descriptions in field guides, scientific articles in which the authors gave detailed descriptions of either field conditions or laboratory propagation techniques of our candidate species, and fact sheets assembled by various extension agencies along the Appalachian region. Methods of plant care were also deduced from environmental conditions and climate patterns of field sites where we collected study specimens.

To educate myself on general horticultural techniques such as potting, watering, and pest management, I consulted resources published by botanical gardens, extension agencies, and freelance botanical professionals. Information on pressing concerns, such as unexplained plant injuries, was obtained by consulting the Hamilton County extension agency and other horticultural professionals in Chattanooga.

Much of my research required pulling information from a variety of sources to formulate an answer to a single question. For example, ecological data on a given species' preferred habitat informed me of how to re-create optimal environmental conditions for that species in a horticultural setting. The mosaic nature of my research further illustrates how the various subfields of plant science intersect, and highlights the importance of better communication and collaboration between ecologists, horticulturalists, and taxonomists.

Summer Sedge

(*Carex aestivalis* M.A. Curtis ex A. Gray)

I. Field Identification

Carex aestivalis is a species of superorder Liliales, order Poales, family Cyperaceae (ITIS 2014). It occurs throughout the Appalachian region, from Orange County, Vermont to Jackson County, Alabama (Kartesz 2014, Figure 2.1). As a graminoid - a species of family Cyperaceae, Juncaceae, or Poaceae - *C. aestivalis* represents a major functional group of plant communities (UC ANR 2014, Wellendorf n.d.). *Carex aestivalis* was collected only at the mid-latitude and northern field sites; it is highly scarce in its southern range and we could not get confirmation of its occurrence in our southern field site (Kartesz 2014; Cherokee National Forest).

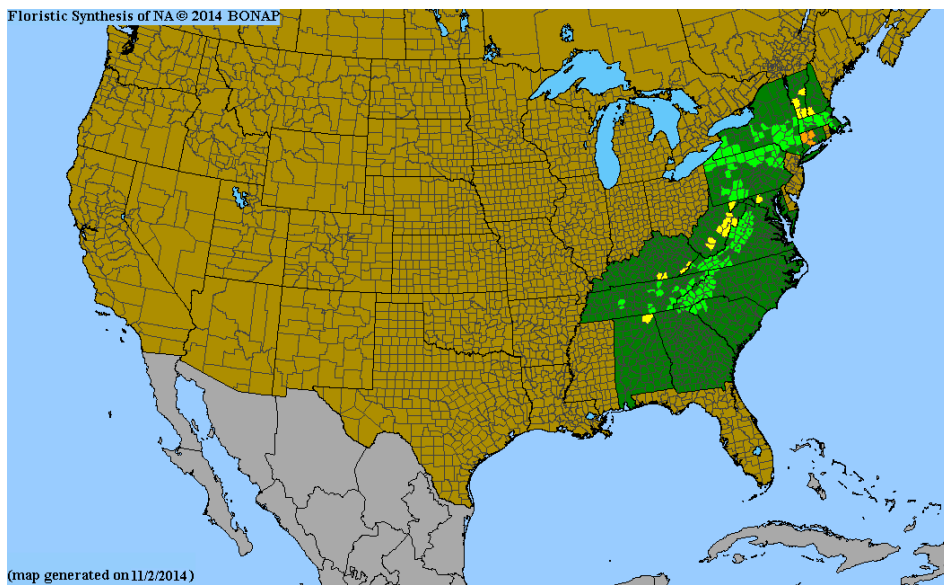


Figure 1. *C. aestivalis* range as of 11/2/2014. Color Key: dark green - species present in state and native; gold - species not present in state; bright green - species present in county and not rare; yellow - species present in county and rare; orange - historic range, species extirpated. Image from Bonap (Kartesz 2014).

Graminoids - species of the families Cyperaceae, Juncaceae, and Poaceae - are among the most difficult to identify. When attempting sedge identification, it is helpful to first recognize general characteristics of Cyperaceae. Like species in Poaceae, Cyperaceae flowers lack both petals and sepals. A single bract arises just below each flower, which are usually arranged in clusters of 3 - 4 (UC ANR 2014). The flowers are usually monoecious and located on separate spikes or different portions of the same spike (UC ANR 2014). The stems are triangular in cross-section and solid. The leaves are arranged on the stem in groups of three, and the leaf sheaths are closed (UC ANR 2014).

Carex is the largest genus of the plant world, containing over 2,000 species worldwide (ITIS 2014). Due to its vast size, *Carex* is divided into several sections; *C. aestivalis* and its immediate relatives are contained in section Hymenoclaenae, which is characterized by upland habitat, cespitose growth, and red-purple pigmentation of the basal sheaths (Waterway 1988, 1990, 1990b).

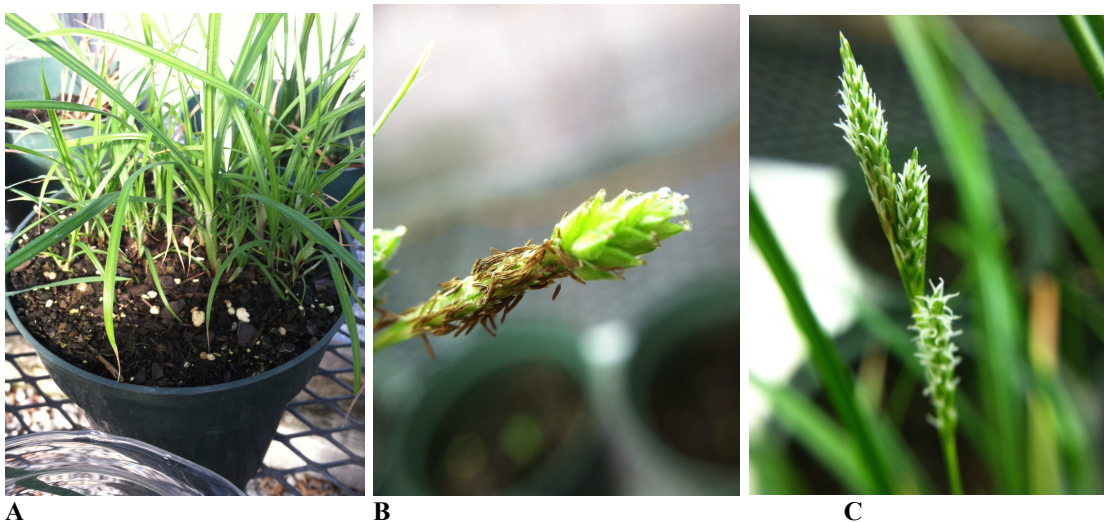


Figure 2. Potted shoots of *Carex aestivalis* (A), a glycandrous terminal shoot (upper portion pistillate, lower portion staminate; B), and a flowering stalk containing three spikes (one terminal glycandrous spike, two lateral pistillate spikes; C). Photos by Gayle Tyree.

Carex aestivalis grows as a bunch graminoid, 1 - 2 feet in height with densely caespitose shoots (Waterway 1988, 1990, 1990b; Figure 2). The root system is composed of short rhizomes, which grow together to form a shallow, fibrous mat (Waterway 1988, 1990, 1990b). The leaf sheaths reach 9 mm up the stalks, are maroon at the base, bladeless, and sparsely pubescent. Blades are flat, 1.5–3.0 mm wide, smooth or sparsely pubescent on both surfaces, especially near sheath, and finely scabrous on the leaf margins (Waterway 1988, 1990, 1990b). Flowering stems are 25–60 cm in length, glabrous or sparsely pubescent, and finely scabrous within the inflorescence (Waterway 1988, 1990, 1990b).

The flowers are monoecious, spike-shaped, and mounted on elongate, nodding, glabrous stalks that are 5–25 mm in length (Reznicek et al. 2011, Waterway 1988, 1990, 1990b; Figure 2). There is one elongate terminal spike and 2–4 erect lateral pistillate spikes per flowering shoot (Waterway 1988, 1990, 1990b). The terminal spike is gynecandrous, meaning that it has a staminate base and a pistillate tip (20–35 mm long, 1.5–3.5 mm wide; Figure 2). The lateral pistillate spikes support 15–30 fruit enclosed within ovary capsules called perigynia. The perigynia are green, red dotted, 2-ribbed, and glabrous, have 12 - 15 fine veins, and gradually taper to an acute beakless apex (Waterway 1988, 1990, 1990b; Figures 4 and 5). Pistillate scales subtend the perigynia; these are pale, transparent, and golden to reddish brown with a broad green midrib (Waterway 1988, 1990, 1990b; Figures 4 and 5).

Like the flowers, the seeds are tightly clumped in a single cylindrical seed head at the stalk tips (Reznicek et al. 2011). The seed heads are ½ - 1" long, with up to 4 heads on each stalk (Reznicek et al. 2011; Figure 2). The achenes are ovoid-ellipsoid, measure

2 mm × 1 mm, and have a distinct stipe measuring 0.5 mm (Waterway 1988, 1990, 1990b).

C. aestivalis must be carefully distinguished from the other species of its section, particularly Roan Mountain sedge (*Carex roanensis*) and graceful sedge (*Carex gracillima*; Figure 4). These species occur within the same range and are morphologically the most similar to *C. aestivalis* of the species in Hymenochlaenae. *Carex roanensis* is distinguishable from *C. aestivalis* by its pubescent rather than glabrous perigynia texture and its slightly longer achene length (3-4 mm rather than 2-3.2 mm) (Waterway 1988, 1990, 1990b). The leaf sheaths of *C. gracillima* are glabrous rather than pubescent, and its leaf blades grow to 9 mm in length, while the leaf blades of *C. aestivalis* do not grow wider than 4.5 mm (Waterway 1988, 1990, 1990b). *Carex aestivalis* is known to hybridize with *C. gracillima*, producing *C. aestimeliformis* (Waterway 1988, 1990, 1990b; Figure 5). The hybrid (*C. aestivaliformis*) has wider leaf blades, less elongate spikes, and a more elongate inflorescence.

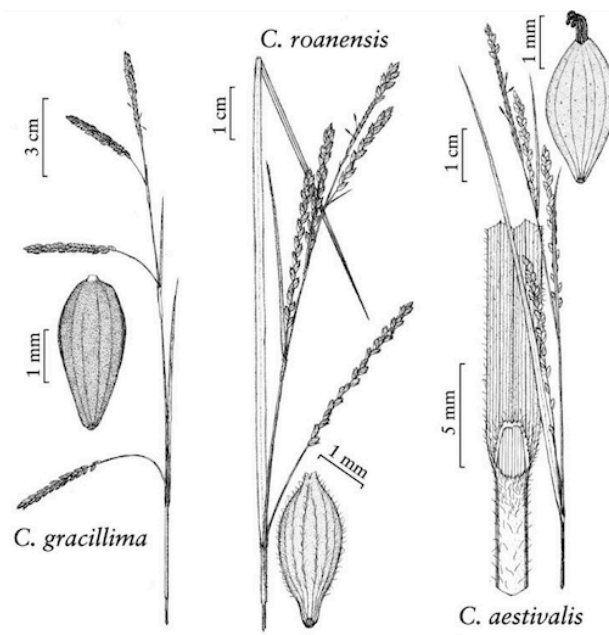


Figure 4. Morphological diagrams of *Carex aestivalis*, *C. gracillima*, and *C. roanensis*. Diagrams by Waterway (1999).

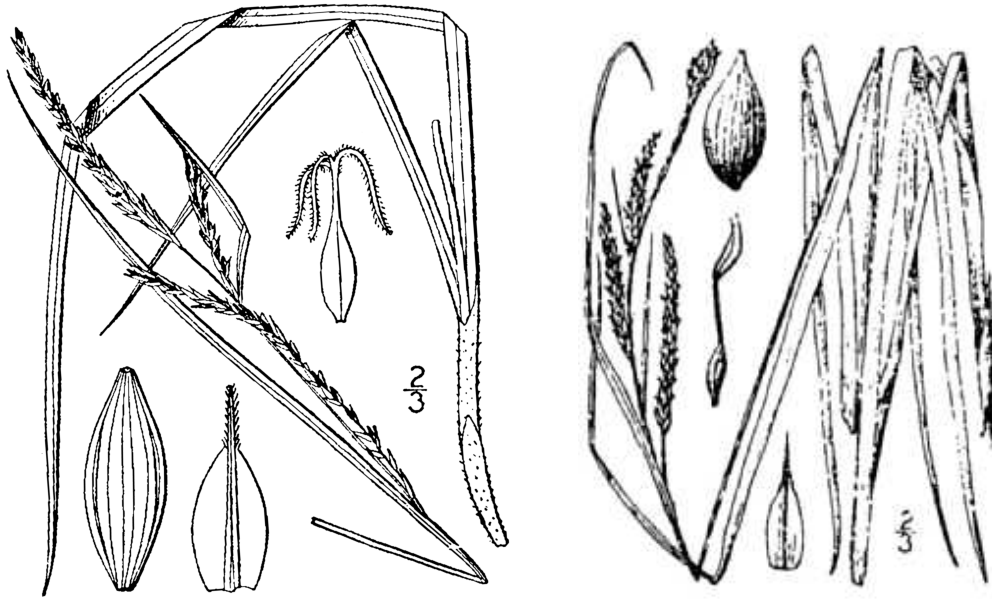


Figure 5. Morphological differences between *Carex aestivalis* (left) and *C. aestivaliformis* (right). Diagrams by Britton and Brown 1913.

C. aestivalis can be found in a wide variety of forest types, including rich mesic forests to grass/shrub balds, open oak forest, boulder fields and outcrops, and seepage slopes (The Nature Conservancy n.d., Reznicek et al. 2011). At the field sites in Shenandoah National Park, VA and Black Rock Forest, Cornwall, NY it was found in montane open oak woods at high elevations (3538 ft and 1019 ft, respectively). It has no known significant associated species.

II. Propagation

Due to difficulties with identification, it is best to collect wild specimens in late summer (late July or August) when the fruits are ripe (NY Natural Heritage Program 2013). Care should be taken to disturb the seed heads as little as possible during collection, especially if specimens are being collected later in the season (past August, or when seed head have begun to turn brown). Collect whole plants; each individual consists

of one defined root clump. Roots may need to be pried from rock crevices. Store collected specimens in inflated sealed bags or wrapped in moist paper towels at or near 4 °C until planting (Houseal and Smith 2010). Seeds should be cleaned and packaged properly for storage. See the seed cleaning protocols in Appendix B for instructions. Successful field identification of *Carex* species is greatly aided by using a hand lens and a dichotomous key (Houseal and Smith 2010). See Appendix D for a dichotomous key of *C. aestivalis*. If possible, use herbarium records to confirm identifications.

Carex aestivalis can be propagated both sexually by seed and vegetatively by whole plant division (Elliot 2003, Houseal and Smith 2010, Leif 2012; Appendix B). Houseal and Smith (2010) suggest separating the perigynia from seeds by hand-screening them with 1 - 2 mm soil screens after two or more weeks of drying to improve germination. Stratify seeds in cold-moist conditions (4°C) for 1 - 3 months to maximize germination success (Houseal and Smith 2010; Appendix B). For planting, sow seeds heavily (20 seeds per pot) on the surface of the potting medium and cover with a clear plastic bag or lid to hold in soil moisture (Houseal and Smith 2010, Midgley 1999, Appendix B). Remove the covering upon seed germination. Apply water with a spray bottle to avoid seed displacement. Germination should occur within 2 weeks (Houseal and Smith 2010).

Divide whole plants in winter after plant tissues have hardened and new growth has stopped (Midgley 1999). Release the root ball from the pot and untangle the root structure so that you are left with clumps of 5 - 6 shoots (Leif 2012). Replant these divisions in 50/50 peat-perlite soil (Midgley 1999). See Appendix B for more general information on seed treatment and plant division.

III. Plant Care

C. aestivalis is a highly hardy species that is tolerant of adverse conditions due to the nature of its natural habitat. This species is tolerant of dry environments and may grow in rocky, nutrient-poor soils (The Nature Conservancy n.d., 2014 field observation). *C. aestivalis* is adapted for partial shade, but can tolerate full sun (The Nature Conservancy n.d.). For this study, specimens of *C. aestivalis* were collected from Zone 6(a/b) (USDA Plant Hardiness Zone Map 2014) and Zone 4-5 (American Horticultural Society Plant Heat Zone Map 2014). Using this information, we can expect our specimens to be tolerant down to 0 to -10 °C and tolerant of temperatures at or above 30 °C about 8.2% of the time (30 days per year have temperatures at or above 30 °C). Maintain summer growth temperatures between 20 - 25 °C and winter dormancy temperatures between 10 - 15 °C (Houseal and Smith 2010, Midgley 1999). Once established, the plants require minimal care until seed harvest (Houseal and Smith 2010). For daily maintenance, water as needed and check regularly for pests and pathogens.

IV. Issues and Complications

Over time, specimens can become root-bound due to the cespitose nature of the root system. When a plant is root-bound, it is easier for it to become water and nutrient limited; plant division is useful for prevention of this condition (Krug 2012, Midgley 1999). Overwatering may contribute to common infections such as root rot (Krug 2012). Rodents can cause damage to root systems if plants are overwintered outdoors or in a greenhouse (personal observation). Place mechanical or zinc-phosphite bait traps among specimens if rodent damage is detected (Njue 2014).

Canada Mayflower

(*Maianthemum canadense* var. *canadense*, Densf.)

I. Field Identification

Maianthemum canadense is a perennial understory herb species of superorder Liliaceae, order Asparagales, family Asparagaceae (ITIS 2014). It occurs throughout the Appalachian region, from Canada to Oconee County, South Carolina and west across the great lake states to Crook County, Wyoming (Kartesz 2014, Figure 2.1). In this project, *M. canadense* represents the monocotyledonous forb plant functional group. Monocots, an early clade of angiosperms, are characterized by one cotyledon in the leaf embryo, parallel leaf veins, floral parts in multiples of three, and scattered vascular bundles (Harms 2009, Speer 1995). *Maianthemum canadense* specimens were collected at all three field sites (Cherokee National Forest, TN; Shenandoah National Park, VA; Black Rock Forest, NY).

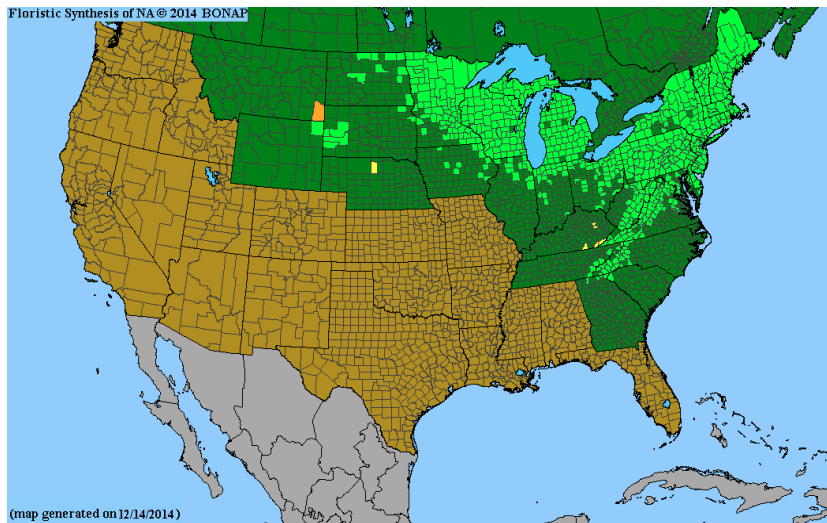


Figure 1. *Maianthemum canadense* range as of 11/2/2014. Color Key: dark green - species present in state and native; gold - species not present in state; bright green - species present in county and not rare; yellow - species present in county and rare; orange - historic range, species extirpated. Image from Bonap (Kartesz 2014).

Maianthemum canadense can be easily recognized by its clonal growth habit; where it occurs, colonies of small, cordate leaves carpet the forest floor (Taki et al. 2008, Wilson 2005b, Figure 5). These colonies typically are composed of one genet, with dozens of ramets, most of which are vegetative (Taki et al. 2008, Wilson 2005a, Figures 2 and 5). During May and June, the sexual shoots can be recognized by terminal inflorescences of 6 - 40 small, white, dimerous flowers (Barrett and Helenurm, 1987, Meng et al. 2008, Figure 2). From June to September, these stalks bear fleshy, purple-speckled fruits that gradually ripen to a deep red (Hemmerly 2000, Taki et al. 2008, Figure 3). The roots of *M. canadense* are thin, white, rhizomes with white, translucent sheaths that are located within the leaf litter or within 5 cm of the soil strata (Flinn and Pringle 1983, Figure 3).

Maianthemum species are adapted to cool, mesic habitats, and often can be found growing near spruces (*Picea* spp.) and hemlocks (*Tsuga* spp.; Salome et al. 1995, Shebitz 2003, Herbert 2008) as well as huckleberries (*Vaccinium* spp.; Shebitz 2003).



Figure 2. Flowering and vegetative shoots of *Maianthemum canadense*. Photo credit: Gayle Tyree 2014.



Figure 3. Left: Root mass of *Maianthemum canadense* with apical shoots in center. Right: Unripe fruit of *M. canadense*. Image credit: Gayle Tyree 2015.

Maianthemum canadense shares a high degree of morphological similarity with several of its congeners. Fortunately, the two that are the most morphologically similar to *M. canadense* - *Maianthemum bifolium* and *Maianthemum dilatatum* - are completely geographically distinct (BONAP, Kawano and Suzuki 1971, Kim and Lee 2007). Both *Maianthemum racemosum* (Feathery False Solomon's Seal) and *Maianthemum stellatum* (Starry False Solomon's Seal) occur throughout the Appalachian mountains, but can be easily distinguished from *M. canadense* (Meng et al. 2008). Specifically, *M. racemosum* grows up to three times the height of *M. canadense* (36 in/90 cm tall) and bears up to 18 foliage leaves, 6 - 9 times that of *M. canadense* (Kim and Lee 2007, Midgley 1999), while *M. stellatum* has an inflorescence similar to that of *M. canadense*, but like *M. racemosum*, it is much taller (12 - 24") and has compound leaves (Habeck 1992). *Maianthemum trifolium* (Three-leaved False Solomon's Seal) is a bog dweller of the northern Appalachians. It has a less-compact terminal inflorescence than *M. canadense* that is composed of flowers with six petals rather than four, and has lanceolate leaves that closely sheath its stem (Hemmerly 2000). Finally, *Convallaria majalis* (True Lily-of-the-

Valley) has lanceolate leaves and a similarly shaped inflorescence that hangs downward rather than upright (Hemmerly 2000).

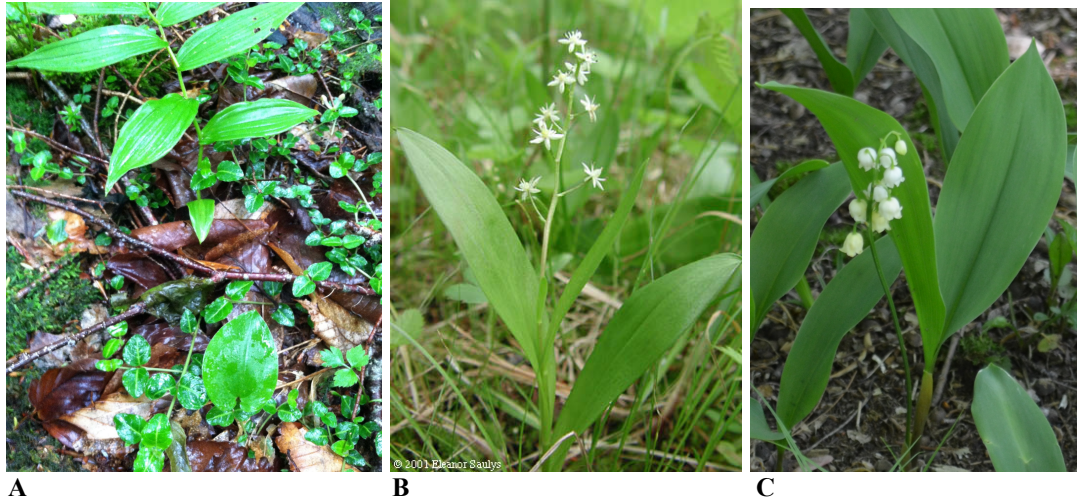


Figure 4. Co-occurring *Maianthemum racemosum* and *M. canadense* (Gayle Tyree, 2015; A); *M. trifolium* (Elenor Saulys, 2001; B); *Convallaria majalis* (Tom Todd 2015; C).

II. Propagation

Collect whole ramets (shoots and roots) of *M. canadense* during summer after flowering (July) (Lezberg et al. 2001, Figure 5). Take care to collect plenty of roots with healthy axillary buds, as these are what will give rise to new ramets (Figure 5). Replant specimens as quickly as possible after harvesting. The shoots still might die back after potting, but this is acceptable as long as the root systems remain healthy. Seeding individuals can be collected, but unless you plan to propagate *Maianthemum* from seed this is not necessary. Use a trowel to dig up the rhizomes and try to disturb these as little as possible. Store plants in inflated plastic bags on ice until planting (Lezberg et al. 2001).

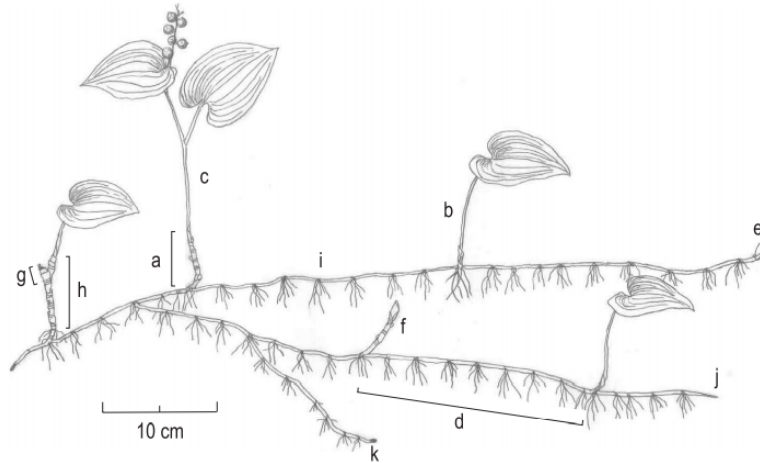


Figure 5. Anatomy of an *M. canadense* individual. Short shoots (a) give rise to either vegetative (b) or sexual shoots (c). Whole ramets (d) consist of a length of lateral growth that terminates in an upward-growing lateral shoot. Preformed shoots (e) may develop during fall and remain dormant until spring, when they either develop or remain dormant throughout the growth season (f). The raceme stalk of a sexual shoot abscises after flowering (g). Feeder roots sprout from nodes along the rhizomes (i), which continue to grow (j) or terminate (k). Diagram by Lezberg et al. 2001.

Reproduction of clonal understory herbs is characterized by occasional, disturbance-induced seedling recruitment followed by long periods of vegetative growth (Barrett and Helenurm 1987). Seed germination can take significant time – up to 18 months – and success rate is usually low (Barrett and Helenurm 1987, Bierzychudek 1982, Eriksson 1992, Kawano et al. 1968, Schebitz 2003). For this reason, it is logical to take advantage of the clonal nature of *M. canadense* and propagate it vegetatively by rhizome division. This should be done either in the spring, prior to flowering, or in the fall after the shoots have died back (Midgley 1999, Shebitz 2003). Divide rhizomes into 3-inch sections with at least one latent bud (Figure 5). Plant the cuttings, spaced well apart, within 5 cm of the potting medium surface (Flinn and Pringle, 1983). Cover pots with bags or a plastic lid to hold in moisture just as is done with surface-sown seeds. Refer to Appendix B for more general information on rhizome propagation.

Maianthemum canadense undergoes a stage of dormancy during the winter (Diboll 2008, Nivot, Olivier, and LaPointe 2008, Yoshie 2008). Some plants require a dormancy stage to fulfill certain metabolic functions; the result is that when such plants are given a “winter,” they experience more prolific growth the following spring. This pattern was witnessed with greenhouse-raised *M. canadense* during spring of 2015 (personal observation). If plants are collected during their dormant period (after summer leaves have senesced), then cold storage is required to break natural dormancy (Nivot, Olivier, and LaPointe 2008). Maintain rhizomes in moist conditions, either in soil or wrapped in dampened paper towels, at 4 °C for at least 35 days or until ready to plant (Nivot, Olivier, and LaPointe 2008, Yoshi 2008). Replant the rhizomes in room-temperature soil (~20 °C).

A key reason for the long germination period of *M. canadense* is that the seeds require “double-dormancy” to germinate (Diboll 2008). This means that the seeds require *two* winters to initiate the germination process. This time frame can be shortened by a long-term stratification strategy in which the seeds are cold stratified twice in the same year (Diboll 2008). After collecting seeds in the fall, cold treat them during the first winter either outside or in a refrigerator. Bring them out in dormant conditions in March (see potting protocol below). In June, place the seeded containers in a cooler at 4 - 10 °C for 1-2 months in order to mimic a second round of winter conditions. Remove the seeds from these conditions in August-September and store them in a greenhouse to germinate. Grow the seedlings through the fall and allow them to go dormant in winter. By simulating two winters within one year, the germination length of *M. canadense* can be shortened by up to eight months (Diboll 2008).

III. Plant Care

Analyzing the natural habitat of study species can help researchers reproduce their species' optimal growing conditions in the lab. Often, *M canadense* is found in hemlock and spruce forests, which are characterized by cool, dark, mesic conditions (2014 field observations). Thus, *M. canadense* has relatively high water requirements; potting substrate should be kept moist at all times. We also can deduce that *Maianthemum* species prefer acidic soil conditions with high organic matter content (Postma et al. 2007). Use a standard or moisture-control 50% peat-perlite-pine bark mix with a pH of < 6.8 as a growth medium. As a clonal species, *M. canadense* is highly responsive to environmental heterogeneity in its microhabitat, and if a specimen experiences poor soil conditions, the rhizomes can truncate and severely affect the overall size and health of the plant (D'Hertefeldt and Jónsdóttir 1994, Hutchings and Slade 1988, 1989). If you notice yellowing leaves or shoot loss before fall senescence, apply a standard liquid fertilizer weekly until shoot health improves (see Appendix C for information on fertilizers).

Although *M. canadense* has been reported to be tolerant of a wide variety of light levels (23 – 80% full sunlight; Shirely 1945), the species is best suited to shady conditions. Using a 30 – 50% shade cloth in the greenhouse and low light levels (<250 micromoles/meter²/second) in the growth chamber help protect the species from sun scorch (Hebert 2006, Yoshie 2008). However, one must not limit light so much that compensatory stalk growth occurs. This happens when light levels are too low and prompts plants to invest growth energy in their stalks to get their leaves closer to a light source. This kind of growth is a misappropriation of the plant's energy resources and can be harmful to reproductive efforts, particularly in the spring when the plants are

flowering. If plants must be kept indoors, use a timed vitamin-D lamp or growth chambers to regulate light resources.

Maianthemum canadense is also tolerant of a wide range of temperature tolerance (-23 to 60 °C), but grows well on a diel cycle at 25/20 °C (Flinn and Pringle, 1983, USDA 2012, Yoshie 2008). Be mindful that increased temperatures can lead to accelerated emergence and decline (Farnsworth et al. 1995). See Appendix E for temperature data for *Maianthemum* spp. If proper growth conditions are implemented, then daily maintenance for *M. canadense* should not extend beyond regular watering.

IV. Issues and Complications

Populations may “skip” their dormancy period if they are kept at warm temperatures during winter. This may cause less prolific growth the following spring. Year-to-year fluctuations in ramet density is normal for understory herbs (Brewer 1980, Davidson and Forman 1982). It is also common for *M. canadense* to experience ramet decline from one growing season to the next; however, this is not thought to be indicative of plant death, but rather of energy allocation to underground structures (Salome et al. 1995). Although *M. canadense* has no notable pests or diseases, its congener *M. racemosum* has been known to contract the foliar infection *Phytophthora ramorum* (Hadwen Arboretum Project 2014).

Bluestem Goldenrod

(Solidago caesia var. caesia A. Gray)

I. Field Identification

Solidago caesia is a perennial herb of superorder Asteranae, order Asterales, family Asteraceae (ITIS 2014). It occurs from Canada south to Franklin County, Florida, and west to Garvin County, Oklahoma (Kartesz 2014, Figure 1). It is a representative of dicotyledonous forbs, which are characterized by two cotyledons in the leaf embryo, reticulated leaf veins, floral parts in multiples of four or five, root development from a radicle, and a ringed arrangement of stem vascular bundles (Speer 1995). Specimens were collected at all three field sites (Cherokee National Forest, TN; Shenandoah National Park, VA; Black Rock Forest, NY).

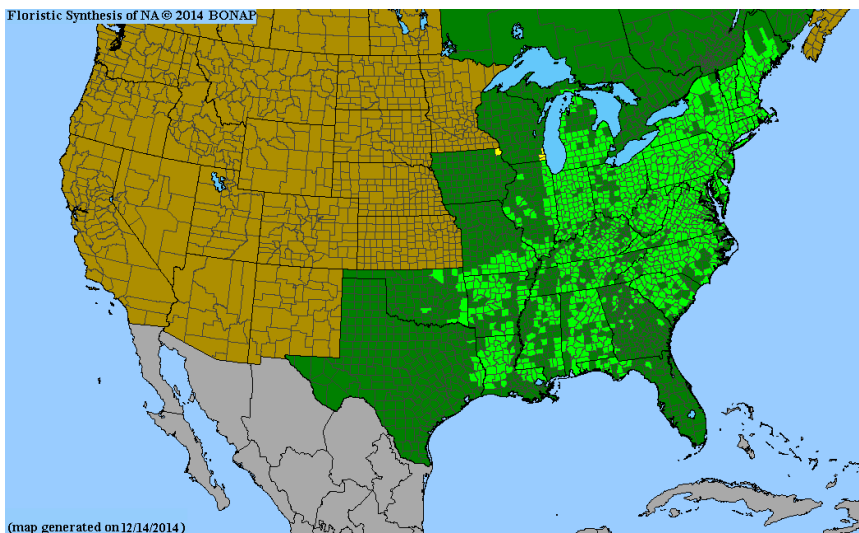


Figure 1. *S. caesia* range as of 11/2/2014. Color Key: dark green - species present in state and native; gold - species not present in state; bright green - species present in county and not rare; yellow - species present in county and rare; orange - historic range, species extirpated. Image from Bonap (Kartesz 2014).

The genus *Solidago* is highly morphologically diverse, and identification can be difficult not just between species, but even within populations (Fewless n.d.). Leaf characteristics and inflorescence shape are particularly important morphological features to study when identifying *Solidago* species (Fewless n.d.). The leaves of *S. caesia* are typical of many *Solidago* species: alternate, lanceolate, toothed, smooth or slightly pubescent, and sessile (having no peduncle) (Johnson 1995, Midgley 1999). Leaves vary in length from 1 - 3" with length generally three times the width, and leaf size increases down the stem (Midgley 1999).

The inflorescence, however, is highly distinctive. Racemes of perfect, bright yellow flowers, subtended by leafy bracts, sprout from the leaf axils on the top half of the stem (Johnson 1995, Midgley 1999; Figure 2). The seeds (achenes) have a single row of white pappus bristles, which dries and fluffs out in preparation for wind dispersal when the seeds mature (Johnson 1995, Midgley 1999; Figure 2). The shoots grow to be 1 - 3 ft tall and have few branches. The stems arch slightly and may zig-zag between leaf nodes (Midgley 1999). The stems are cylindrical with no ridges, hairless, and are usually covered with a waxy, bluish-white coating, which is easily rubbed away (Johnson 1995, Midgley 1999). The rhizomatous roots can be either short and compact or long and creeping (Midgley 1999).

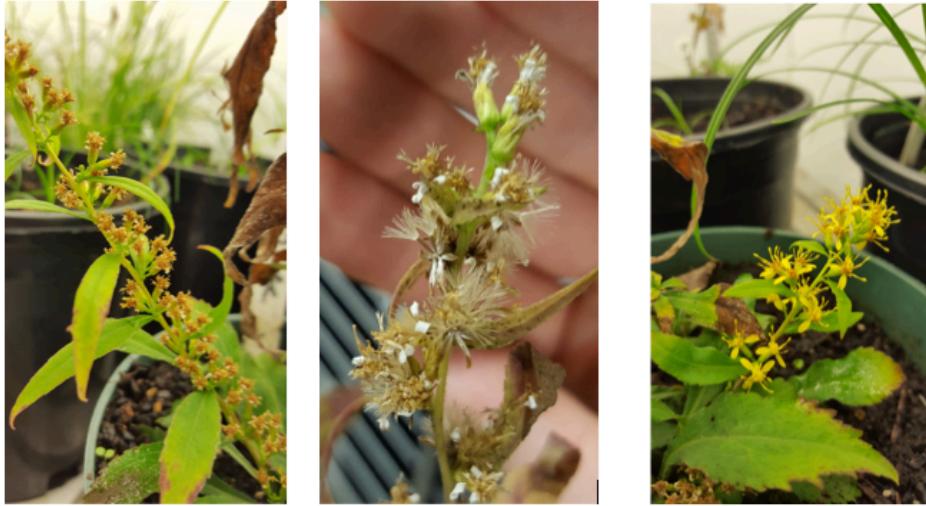


Figure 2. Left to right: Inflorescence shape, flower structure, and seed structure of *Solidago caesia*. Images by Gayle Tyree.

Mesic or dry-mesic open deciduous woods are the preferred habitats of *S. caesia* (Johnson 1995, Shenandoah National Park Staff 2014, WI DNR, field observations 2014). In its southern range, it is often found in oak-hickory-pine forests (Midgley 1999). Common associate tree assemblages include *Quercus* spp., *Carya ovata*, *Liriodendron tulipifera*, *Betula lenta*, *Magnolia macrophylla*, *Prunus serotina*, *Acer saccharum*, *Fagus grandifolia*, *Tilia americana*, and *Aesculus octandra* (Farmer et al. 1982). Common understory forb associates include yellow dogtooth violet (*Erythronium americanum*), mayapple (*Podophyllum peltatum*), false Solomon's Seal (*Maianthemum racemosum*), and wild geranium (*Geranium maculatum*) (WI DNR)

Due to the unusual morphology of *S. caesia*, there are few species that it can be mistaken with, if collections are done when it is flowering or seeding. However, there are two other goldenrod species that are similar enough with it to be problematic with identification. *Solidago flexicaulis* (Zigzag goldenrod) has similar flower morphology, with axillary racemes sprouting at leaf nodes along the stem (Midgley 1999). However, it

has a larger terminal inflorescence than *S. caesia* and very different leaf morphology (leaves are broad, less than 2.5 times as long as wide) (Midgley 1999). It occurs in the same range as *S. caesia*, but prefers soils that are more basic (pH > 6.0) and is more often found in lowlands rather than upland woodlands (Hilty 2014, Midgley 1999). One must also be wary of the sister variant of *S. caesia* var. *curtisii*. This variant is distinguishable from var. *caesia* by its grooved rather than terete stem (Johnson 1995).



Figure 3. Left: *Solidago flexicaulis* (Zigzag goldenrod). Note the similar inflorescence arrangement to *S. caesia*, but the highly dissimilar leaf morphology (Image credit: Donald Cameron 2015). Right: *S. caesia* var. *curtisii* (Diagram credit: Britton and Brown 1913).

II. Propagation

Solidago caesia reproduces both vegetatively by rhizomatic clones or herbaceous cuttings and sexually by seed dispersal (Midgley 1999). Optimal collection time depends on the type of propagules being collected. Herbaceous cuttings should be taken in early spring once the stems have become firm (Midgley 1999). Make your cutting 3 - 6 in/8 - 15 cm long and include at least four leaf nodes (Midgley 1999). Whole plants should be collected in early July, when the flower buds can be used for identification (WI DNR).

For whole plant collection, dig up specimens with a trowel or shovel. Contain the root balls in moist soil in inflated plastic bags until planting.

Seeds can be collected between late September and late November (Midgley 1999). Regardless of whether they are collected in the wild or from collected whole individuals, seeds should be allowed to dry on the flower head before harvesting. Upon collection, clean seeds of all debris, using a screen if necessary (Midgley, Appendix B), and store them in manila coin envelopes or ziplock plastic bags (Midgley 1999). If using envelopes, tape the corners so the seeds do not fall out. Since the seeds of *S. caesia* dry (non-fleshy), they can be stored at room temperature (20 °C), but germination rate is higher when seeds are stored near 4 °C (Midgley 1999). If stored properly, seeds can remain viable for at least three years (Midgley 1999). No seed stratification is required for germination of *S. caesia* (Leif, 2012). For planting, sow seeds heavily (20 seeds per pot) on the surface of the potting medium and cover with a clear plastic bag or lid to hold in soil moisture (Midgley 1999, Appendix B). Remove the covering upon seed germination. Apply water with a spray bottle to avoid seed displacement.

If propagating seed from whole plants or cuttings, one should keep pollination in mind. *Solidago caesia* is insect-pollinated, so plants should be kept outdoors during the flowering period (3 - 4 weeks between July and October) (Midgley 1999). In order to prevent cross-pollination, many miles should separate populations, preferably. For example, specimens from the first collection were separated so that the southern population was kept in Fort Oglethorpe, GA, the mid-latitude population at UTC, and the northern population on Signal Mountain.

III. Plant Care

Solidago caesia has been described as an aggressive pioneer species that requires very little care when cultivated (Farmer et al. 1982, Midgley 1999, Pavék 2011). The species can tolerate semi-dry conditions as long as it has adequate drainage (Midgley 1999). It prefers acidic soils (pH: 5.0 - 6.0), but is not confined to a narrow pH (Hilty 2014, Midgley 1999). It can grow on a variety of soil types (loam, clay-loam, or rocky soil), but humus-rich potting soil should be used for best results (Midgley 1999). Medium shade to partial sun are tolerable light levels for *S. caesia* (Hilty 2014). For this study, specimens of *S. caesia* were collected from Zone 6(a/b) (USDA Plant Hardiness Zone Map 2014) and Zone 4-5 (American Horticultural Society Plant Heat Zone Map 2014). Using this information, we can expect our specimens to be tolerant down to 0 to -10 °C and generally intolerant of temperatures at or above 30 °C. Summer growth temperatures of 20 - 25 °C work well for this species. During winter, temperatures should be lowered to 10 - 15 °C in order to induce plant dormancy (Midgley 1999). For daily maintenance, water as needed and check regularly for pests and pathogens.

IV. Issues and Complications

Goldenrods are susceptible to most common greenhouse diseases, such as powdery mildew, rust, and bacterial or fungal leaf spots (Moorman 2015). In most cases, these problems can be treated with commercial fungicides (Smith 2011), and can be prevented by using correct watering technique (Krug 2012) and providing plants with ample light, space, and air circulation. More specialized diseases like aster yellows (*Phytoplasma*), crown galls (*Agrobacterium tumefaciens*), lesion nematodes

(*Pratylenchus pratensis*), and tomato spotted wilt virus (*Tospovirus*) require more drastic measures: plants with these diseases should be immediately isolated and destroyed (Moorman 2015). Aster yellows and tomato spotted wilt virus are both spread by insects (leafhoppers and western flower thrips, respectively); if these diseases are contracted, non-infected plants should be treated with insecticide (Moorman 2015).

Cultivators of *Solidago* have reported that it is common for a significant proportion of a seed cohort to be infertile (Midgley 1999). However, this may simply be due to improper seed processing and storage. When preparing seeds for storage, one should make sure that the seeds are completely dry before packaging. If seeds are stored wet, they are prone to rot and fungal infection, particularly if they are packaged in ziplock bags and kept unrefrigerated (personal observation). Cleaning seeds of debris beforehand and storage in paper envelopes - in which they can dry further - can help avoid this problem (Midgley 1999). Germination success is, of course, heavily impacted if seeds are not stored properly.

During seed germination, overwatering and poor air circulation can attract fungal diseases and insect pests such as shore flies and fungal gnats. Plastic covers are used to retain moisture during seed germination, but can create suitable habitat for pests. If you detect pests, then these plastic coverings should be removed. See Appendix C for general information on pest identification and treatment. If seeds are stored at room temperature, fumigation can repel potential insect predators. Fumigate dry seeds by putting an anti-pest strip in a paper bag with cleaned seeds for two weeks; this should take care of arthropod pests like ants and insect larvae (Midgley 1999). If one wants to avoid treating seeds with chemicals, clean, dry seeds can also be frozen for 1 - 2 days (Midgley 1999).

Propagation Information for Manuscript Methods Sections

Field Collection of Parents

To obtain propagules for our experimental research we first located populations of three study species – *Carex aestivalis*, *Maianthemum canadense*, and *Solidago caesia* – in field locations at low, mid-, and high latitude in the Appalachian region of eastern North America from herbaria information and personal communications. These populations occurred near Chattanooga, TN, and in Cherokee National Forest, TN (low latitude), in Shenandoah National Park, VA (mid-latitude), and in the Black Rock Forest, Cornwall, NY (high latitude). Collection of propagules from these locations occurred in summer 2014. Specifically, we collected at least 18 mature, entire individuals of *C. aestivalis* each from mid-latitude (N 38.52373, W 078.44370, elevation 1078.4 m and high latitude (N 41.39589, W 073.99262, elevation 311 m) populations; *M. canadense* from low latitude (N 35.31089 W084.03902, elevation 1343 m), mid-latitude (38.519628, W 078.430817, elevation 1045 m), and high latitude (N 41.40744, W 074.01360, elevation 297 m) populations; and *S. caesia* from low latitude (N 34.892250, W 085.367709, elevation: 335 m), mid-latitude (N 38.28519, W 078.65878, elevation: 884 m), and high latitude (N 41.39603, W 074.03513, elevation 414 m; N 41.40174, W 074.02531, elevation: 385 m) populations.

Propagule Collection

All collected propagules were wrapped in moistened paper towels and stored in inflated ziplock bags on ice or in a refrigerator at 4°C until planting. Since there were often long periods of travel time between collection and replanting, particularly for the

mid-latitude and northern propagules (four to seven days), the inflated bags and moistened paper towels were necessary to give the propagules a CO₂ supply and maintain turgidity in the plant tissues.

Upon arrival in the laboratory, all collected individuals were potted in separate #1 size plastic nursery pots and housed in a greenhouse for propagule harvest. *Maianthemum canadense* specimens were potted in 0.95-liter pots, and *C. aestivalis* and *S. caesia* specimens were potted in 1.67-liter pots. Pots were filled with commercially available pre-fertilized pine bark-peat-perlite potting mix (Potting Mix 0.21 - 0.11 - 0.16, Miracle-Gro Lawn Products Inc., Maryville, Ohio) and all individuals were planted so root crowns were level with the soil surface. All individuals were watered to saturation as needed for the duration of the pre-experimental period.

During field collection, we observed that *C. aestivalis* individuals held immature seed; as seeds ripened, they were collected and stored separately for each individual in a cool, dry location. Based on planting trials prior to the experiment, we determined that *M. canadense* was most successfully propagated vegetatively, so individuals of this species were stored as live specimens for future rhizome harvest in accordance with the methods of Midgley 1999 (Appendix B). We observed that *S. caesia* individuals were not yet flowering at the time of field collection. To prevent cross-pollination, individuals of the three populations were separated when they began to flower in late summer 2014. Seeds of this species were collected when they ripened and stored separately for each individual in a cool, dry location.

Propagation

The first replicate of our experiment (to be blocked through time) designed to investigate intraspecific variability of responses of the study species to projected warming began in late winter 2015. For this replicate, we propagated 18 individuals each of the three study species, six individuals from each field site at which they occurred, in separate #2 size plastic nursery pots filled with the same commercially available potting soil used to pot the field-collected parent plants. Both *C. aestivalis* and *S. caesia* were propagated by tapping approximately 20 seeds per pot into the soil surface; *M. canadense* was propagated vegetatively from rhizomes following the protocol of Midgley 1999 (Appendix B). Six pots from each species were assigned randomly to each of three controlled-environment growth chambers (PGR-15, Conviron Inc., Winnipeg, MB, Canada). Following germination or emergence, seedlings were thinned to the single observably healthiest individual per pot. All individuals were watered to saturation as needed for the duration of the experimental period. Temperature was maintained at 20-28°C (day-night) to replicate current summer conditions in our high latitude collection location, 24-32°C (day-night) to replicate current summer conditions in our low latitude collection location and projected future conditions in the high latitude location, and 30-36°C to replicate projected future conditions in our low latitude collection location. In all three chambers, atmospheric CO₂ was maintained at 400 ppm, photosynthetic photon flux density (PPFD) at the leaf surface was maintained at 400 μmol photons m⁻² s⁻¹ with a 16-h photoperiod, and relative humidity was maintained at 50% throughout the diurnal period.

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Appendices

Appendix A

General horticultural information

Environmental preferences vary with every species, but among all plants, there exist common-ground resource requirements: water, temperature, growing medium, nutrients, and light. Horticulturalists have developed numerous ways of applying these crucial resources so that each can be applied to meet an individual plant's needs as precisely as possible.

Understanding horticultural basics is especially important when raising native plant species. Of all extant plant species, only a tiny fraction are used in the applied plant sciences (agriculture and landscaping), and understandably, very little propagation and cultivation research has been done on species that are not part of these select few. In this chapter are guideline for basic horticultural techniques and aspects that should be kept in mind when one cultivates plants of any type.

I. Watering

Watering is by far the most important part of plant propagation and is a difficult task to master (Getter 2014, Kessler n.d., Krug 2012). In fact, many growers describe it as more of an art form rather than a technical skill. No matter what irrigation method you use, the goal is always the same: adequately saturate the root media so that the plant remains sustained until its next irrigation (Krug 2012). To do this, water the medium to container capacity (the maximum amount of water that the substrate in a container can hold against gravity) each time (Krug 2012).

Environmental variation and variation between and within species causes some plants to be a little drier than others at any given time. Generally, plants will need more water on hot, sunny days than when it is overcast, and more water during spring and summer than during winter, when they are dormant (Krug 2012). Despite this variation, it is important to distribute water consistently among plant individuals; doing so will prevent the danger of causing further environmental heterogeneity within populations.

Maintaining consistent hydration among individuals in one's population requires consistency in one's technique. This involves several factors, including height of the nozzle above the substrate, the use of counts, and rhythm. A major challenge of hand watering is adequate watering of belowground tissue without dampening the aboveground tissue. Wet foliage is vulnerable to attack from pathogens, particularly fungal agents (Krug 2012, Premier Tech Horticulture Team 2012). It is good practice to water in the morning, before the surrounding environment becomes too hot. This allows plant roots to take up water before it is evaporated and gives plant foliage time to dry before nightfall. Foliage should never remain wet through the night.

Lower the nozzle within 6" of the potting medium and move from pot to pot, watering each plant individually. Hold the nozzle at each pot for a consistent count (more or less depending on the size of the pot) to control the amount of water that each pot receives. Do *not* hold the nozzle high above the pots; doing so results in three major problems. First, the foliage becomes wet, increasing the chances of disease (Krug 2014). Second, only the top ¼ - 12" of potting substrate will be saturated, so the plants will require more frequent waterings (daily or even multiple times a day) and can truncate the root systems (Krug 2014). Finally, it is difficult to maintain a steady pace and pattern

when watering plants with this method, so some pots will be over watered and some underwatered (Krug 2014).

When hand watering, it is crucial to check the plants daily to determine if water is needed. Do not rely on the color of the substrate to determine pot hydration. The top 1” or so of potting substrate often dries out over the course of the day, while the rest remains adequately hydrated. Horticultural experts recommend trusting one’s hands, rather than one’s eyes (Getter 2014; Krug 2012, 2014). Below are some tried-and-true methods of determining whether or not a plant needs water. NOTE: All of these tests are based on the judgment of the grower. One should perform at least two different tests to determine a plant’s hydration needs. Ask the advice of a more experienced grower if you are unsure.

- **Heft test:** Weigh each pot in your hand. If it feels light, it probably needs water; if it feels heavy, it likely does not (Getter 2014; Krug 2012, 2014).
- **Finger test:** Place your finger one inch into the potting medium. If it feels dry, water the plant. If it feels moist, then the plant does not need water (Pederson 2001).
- **Chopstick test:** Push a non-lacquered chopstick or a similar unfinished wooden object one inch into the potting medium, then pull it back out. If the chopstick is discolored, then the plant is well hydrated and does not need to be watered (Pederson 2001).
- **Squeeze test:** take a small handful of substrate from the pot and squeeze it. One should be able to squeeze out just enough water to lightly wet one’s hand. If no water can be squeezed out, then the plant needs water. If so much water is

squeezed out that several drops drip off your hand, then the plant is overwatered and should be left alone for another day or two.

- **Root ball test:** Occasional dismantlement of the root ball to further inspect soil conditions may be necessary (Krug 2012). Gently loosen the potting medium by tapping the sides of the pot and upturning the pot, being careful not to harm the shoots. This allows you to examine the state of the medium as well as the roots. Healthy roots will be white and have numerous root hairs (Getter 2014).

Unhealthy roots will be dark yellow or brown; this is a sign of root rot (personal observation, Getter 2014, Krug 2012). Absence of roots in the lower portion of the medium is a sign that the plant is receiving inadequate water doses (Kessler n.d.).

Infrequent watering predictably results in plant wilting. Similarly, Limited water doses (even when frequently applied) limits the available space for roots, making nutrients in the dry medium unavailable (Kessler n.d.). Plant symptoms of underwatering include wilting, reduction of internode and leaf size, brittle stems, premature leaf loss leaf discoloration and “scorching”, or browning at the margins and tips (Kessler n.d., Missouri Botanical Garden n.d.).

Overwatering - a problem that growers often don't consider - can be just as detrimental to plants as water limitation. Providing too much water at one time results in leaching. In moderation, this can help flush excess minerals from the substrate, but excessive leaching can reduce crucial nutrients present in the medium (Kessler n.d.). Providing water too frequently can result in growth that appears lush and healthy, but is more susceptible to sudden dryness and diseases, as well as the development of “water

roots” (long, thin roots with few root hairs), which are less effective at taking up nutrients than healthy roots are (Kessler n.d.). Signs of overwatering include root rot, algal growth on the potting medium, and blistering or yellowed of the leaves (Kessler n.d., Krug 2012, Missouri Botanical Garden n.d., Pederson 2001).

Choosing an appropriate water breaker (point at which the water leaves the vessel) is important to good watering procedure. Desirable water flow is a soft stream that delivers an adequate volume for the container. If using a hose, water pressure should be matched to your breaker. Many nozzle attachments are designed with a quarter-turn valve just behind the breaker or a squeeze attachment that allows the grower to adjust water pressure as needed. Pressure should be set at the lowest point at which water still flows from the breaker uniformly (Krug 2014).

Proper tools also need to be used to water indoor plants. For plants in the growth chambers, use a small, steel-galvanized watering can with a nozzle head attachment. The nozzle should be 3 – 5 inches across; this will allow you to control the direction of the flow but will not concentrate the flow so much that soil displacement occurs. Smaller nozzle holes result in a gentler flow from the nozzle, and are less likely to displace medium and cause damage to the plant.

Automated irrigation systems are another method of irrigating greenhouse plants. Such systems have their benefits; many feed water directly into the potting substrate, reducing the risk of fungal and bacterial diseases (Cox 2009, Kessler n.d.). Automated systems are often less wasteful, and provide more control over the amount of water applied (Cox 2009). However, most irrigation systems are highly expensive and only practical if one has a large number of specimens to be watered.

Propagation of species such as *Rhododendron* requires mist or fog systems unless they are grown in areas with optimal environmental conditions. Misters and foggers, like hand-watering, need to be run early in the day so that plants have time to absorb adequate moisture and then have their foliage dried by the sun. Wet leaves during the evening increases the risk of plants contracting diseases. High-pressure overhead systems generally perform better than low-pressure systems because they provide a more accurate simulation of humid conditions and can be used to cool the crop microenvironment (Bartok 2009). Misters are particularly useful when propagating cuttings, due to their ability to cool the propagation site and hydrate cuttings without soaking them. This maintains turgidity of the cuttings (Sommerville 2014).

II. Temperature and Air Circulation

All plants have specific heat and cold tolerance ranges that depend on the plant's biogeography and associated adaptations. Temperature stress can impact embryonic development and biochemical processes in plant tissues and, if extreme, results in plant death (Wahid et al. 2007). Temperature tolerance varies between different ontogeny stages, with germinating seeds and seedlings being more susceptible to temperature stress than mature plants (Wahid et al. 2007). Plant temperature tolerance varies both between and within species, and can be complicated by numerous environmental factors.

Plants begin to sustain physiological damage from heat at 30 degrees C (86 deg F) (American Horticultural Society 2015). All specimens used in this study were collected from Zone 6(a/b) on the USDA Plant Hardiness Zone Map and Zone 4-5 on the

American Horticultural Society Plant Heat Zone Map (AHS, USDA 2014). Generally, we can expect our specimens to be tolerant down to 0 to -10 degrees Celsius and tolerant of temperatures at or above 30 degrees Celsius about 8.2% of the time (30 days at ≥ 30 deg. C per year).

Controlled-environment growth chambers are a high-tech and extremely efficient method of controlling temperature. Plants stratified under alternating temperatures can be kept in growth chambers for ready temperature fluctuation. In greenhouses, cooling fans, high-pressure misting systems, and heating lamps and beds are tools commonly used to control temperature (Bartok 2013).

Scorching or sunburn of plant tissues, inhibited growth of above and belowground structures, premature leaf senescence and abscission, fruit damage, and reduced yield are signs of heat stress (Wahid et al. 2007). Chilling injury refers to cold-related damage that is sustained above freezing temperatures; chill-injured leaves may become red or purple-tinged and wilt (UCCE 2015). Freezing injury occurs when water in plant tissues turn to ice and damage plant cells; this often results in plant death (UCCE 2015).

III. Scheduling

Scheduling is intensively used in commercial horticulture to time flowering with plant sale. What makes this possible is detailed knowledge of a plant's **phenology** (reproduction cycle). Growers determine the desired week of flowering and then work backwards along a species' phenological cycle to determine when seeds need to be sown (UMass Extension 2015). This strategy can also be put to good use in plant ecology

research. For example, one species included in this manual, germination of *Carex aestivalis*, involves 1 - 3 months of cold stratification of seeds before they are sown. Thus, if we want to begin an experimental trial of *C. aestivalis* on June 1st, seeds must begin cold stratification between March 1st and May 1st. The take-away is that horticultural work does not begin when your experiment begins, so planning ahead is a must!

Many temperate perennial plants are adapted to benefit from winter chilling, so understanding how to induce dormancy is an important skill in horticultural work. The species used in this study are winter-chilled a few degrees above freezing (2 - 4 degrees Celsius). For plants kept in the growth chambers prior to chilling, temperatures should be decreased gradually over a period of 1 - 2 weeks. Similarly, plants kept outside during the fall should be covered or brought into an indoor chilling facility right before the first frost. Allowing plants to gradually acclimate to colder temperatures results in tissue hardening and minimizes the risk of chilling injury (Smith 2013a). Dormancy should be induced in indoor plants before premature fall shoot growth due to artificially prolonged light and temperature conditions can occur. Plants should be brought out of dormancy in early spring (March - April) (Smith 2013a).

Photoperiod (the hours of daylight in a 24-hour period) can be used in conjunction with temperature to control when plants flower. This is because many plants respond to the number of hours of darkness and use this measurement as an “alarm clock” with which to detect the onset of spring. Plants can either be short-day, long-day, or day neutral in response to photoperiod, and may be obligate or facultative for each of these categories. Short-day plants will flower when there are 11 or fewer hours of daylight in a

24-hour period, and long-day plants flower when there are 14 - 16 hours in the same period (Warner 2006). Each plant species has its own specific photoperiod. Photoperiod can be easily manipulated in controlled-environment growth chambers by changing the light settings. In a greenhouse or other facility without computer-controlled lighting, day length is shortened by covering seedlings with black cloth and lengthened with HPS lamps (≥ 10 foot candles are required for adequate lengthening during night time) (Warner 2006).

IV. Fertilizers

Particularly during the spring growth flush, application of fertilizer can be of great benefit for container plants. Some things to keep in mind when applying fertilizer are fertilizer type (chemical composition), rate (ppm), frequency, and volume of application, leaching fraction, plant growth rate, and environmental conditions of your growth site (Cox 1997). Leaching fraction refers to the proportion of water or fertilizer solution applied that is lost from the plant container by leaching (Cox 1997). Lower leaching fraction results in greater nutrient and salt retention in the growth medium. Plants should be fertilized according to growth rate to avoid excess soluble salts, nutrient deficiency, or too much growth (Cox 1997). Fertilization application should cease well before plants approach dormancy; doing so will encourage root rather than shoot growth and help the plants harden (Cox 1997). Spring fertilization should not begin until the danger of frost has past and new growth begins. Early application of fertilizer may make the plants susceptible to injury from spring frosts or cold spells (Cox 1997).

V. Pest Control

Pest and disease control is a constant problem when raising any living organism. Fungal and bacterial agents are common in horticultural work. Contaminated tools or medium, improper watering technique, and environmental conditions can all contribute to risk of plant infection. Unfortunately, accurate diagnosis of bacterial and fungal diseases can be devilishly difficult, so it is often difficult to determine what the cause of an infection may be. On-site identification requires unique symptoms (not a common thing among such diseases); accurate diagnosis often requires sending samples to a testing lab (Wick 2000). However, taking certain measures can greatly reduce the risk of contracting such diseases.

Diseases of this type thrive in warm, wet, and often-dark environments. Therefore, other than making sure that your original specimens are free from contamination, the two most important things you can do to prevent infection by fungi, bacteria, or viruses is to maintain a clean growing area with high light quality and good air circulation and practice proper watering technique. Wet foliage and water-saturated medium, coupled with stagnant air, poor lighting, and warm temperatures, creates very suitable conditions for disease growth (Krug 2012, Midgley 1999, Smith 2011, Wick 2000). If your plants contract a disease, identify it as specifically as you can (talk to your local extension agent if necessary) and remove as much affected debris as possible. See Appendix C for descriptions and treatment options of common horticultural diseases.

As with bacterial and fungal invaders, the risk of attracting arthropod pests can be reduced by some simple precautions. Thoroughly inspect all incoming plant material. As with fungal and bacterial problems, the likelihood of insect pests can be reduced by

practicing proper watering technique, and good sanitation (Smith 2011). Screens, insecticide sprays, and systemic insecticides are useful methods of controlling insect problems. Avoidance of excessive fertilization (which promotes new, soft plant tissue) can dissuade phloem feeders such as aphids. For more information on choosing insecticides, see the table in Appendix C.

Although they are easier in some ways to deal with than other pests, rodents can destroy greenhouse plants overnight. They feed on both young seedlings in springtime and dig up root structures during the winter. Plants that are overwintered in a greenhouse or outdoors are most vulnerable to rodent damage. Mice, voles, and other small mammals feed on root tissues in the winter and can wreak havoc on plants (Njue 2014). Risk of rodent damage can be reduced by removing brush and debris near the chilling site, treating plants with anti-pest chemicals, or setting mechanical or poison (zinc phosphide) traps around the site (Niue 2014). Poison traps should only be used where children, pets, and innocuous wildlife cannot reach them. Signs of digging or scratching at the soil surface and large pieces of missing plant material are key signs of rodent damage.

VI. Potting Medium

Proper choice of potting material, or media, is an important component of container propagation. Potting media is rarely composed of true “soil”, but mixes of materials whose physical and chemical properties meet plant health needs. Media must be sufficiently porous to allow root aeration and drainage, but capable of retaining water and

nutrients (Reddick 2013). The addition of slow-release fertilizers helps meet nutritional needs, but growers should keep in mind that this material degrades over time and eventually fertilizer must be added (Petinelli n.d., Reddick 2013).

Peat moss and Sphagnum moss are two popular types of potting material that are derived from the same plant, *Sphagnum*, which grows in peat bogs (Reddick 2013). *Peat* moss is the dead portion of the plant, which accumulates in bog water and eventually becomes peat while *Sphagnum* moss is the living portion of the plant (Reddick 2013). Peat moss has a tight, fibrous, highly absorbent structure, which provides good drainage but must be watered often (Reddick 2013). It is often sterilized, which decreases the risk of disease (Reddick 2013). Sphagnum moss is composed of long, fibrous, decomposition-resistant fibers and is good for plants that require moist medium with good aeration (Petinelli n.d.). Both are naturally acidic (pH: 3.5 - 4.5), and thus good for acid-loving species (Reddick 2013).

Many common potting materials are useful for mixing together custom substrates. Each of these have their own strengths and weaknesses. Pine bark, when ground fine, retains water well in media; larger grains can be used to increase aeration (Reddick 2013). Coarse sand is also useful for increasing media aeration and water drainage, but can be too heavy for germinating seeds (Reddick 2013). Vermiculite, or processed mica, is capable of holding large quantities of water and nutrients (Reddick 2013). It is pH neutral (6.5 - 7.2) and available in various particle sizes. Media aeration increased as particle size increases. Over time, vermiculite tends to break down into a heavy, sludgy material that offsets its aeration benefits (Midgley 1999). Replace the medium if you notice this decomposition. Perlite is light, ground volcanic rock that also aids medium

aeration. It does not hold water as well as vermiculite (Midgley 1999, Reddick 2013). Standard pH: 7.0 - 7.5. Styrofoam can be used as an inexpensive substitute for perlite (Petinelli n.d.). Calcined clay is good for keeping media loose and promoting drainage.

Choice of media depends primarily on the pH and soil drainage needs of the plant species in question. Peat media is a good choice for plants that prefer moist, acidic soils with high organic matter content (Midgley 1999). Plants that require more basic soil would prefer calcined clay or vermiculite mixes. For young plants or seed germination, use mixes of vermiculite, perlite, or other light material that will provide less resistance to root spread (Midgley 1999).

It goes without saying that proper cleaning and storage of supplies is important practice for any grower. These habits help reduce transmission of pathogens between specimens and also extend the life of your tools. Tools that directly contact individuals such as pruners, scalpels, and knives should be cleaned with soap and water before and after use. Effort should also be made to keep workspaces generally clean and free from dust and debris.

Appendix B

Propagation Protocols

In this appendix are general protocols for different methods of propagation. Species-specific modifications of these protocols can be found in the species profile chapters.

I. Vegetative Propagation Protocols

Standard Potting Procedure (Midgley 1999)

1. Select pots that are appropriately sized for the specimen. Try to use the same sized pots for all specimens in a population. Make sure all pots are free from dirt and debris, and wash dirty pots with soap and water.
2. Fill each pot about $\frac{3}{4}$ of the way full with the chosen medium.
3. Using the crown (the transition from root to shoot) of the specimen to be potted, add medium to the pot until the crown is $\frac{1}{2}$ " below the pot's top edge. The plant should be situated so that the crown is level with the medium's surface (i.e. none of the shoot portion is covered with medium, and no roots protrude above the medium).
4. Water the newly potted plant *gently*. The surface will likely sink as the water compacts the medium; add more medium if roots become exposed. **NOTE:** do not apply water *directly* at the crown! Doing this will almost certainly expose the roots! Apply water slowly, holding the watering vessel no more than 4" above the medium surface. Apply water in a circular fashion around the plant, starting at the pot's edge and working your way inward.

Sticking Herbaceous, Softwood, and Semi-Hardwood Cuttings (Evans and Blazich 1999; applicable for Prunus pensylvanica, Rhododendron periclymenoides, and Solidago caesia)

1. Remove the leaves from the lower one-third to one-half of the cutting. Cut large leaves in half to reduce water loss.
2. Wound the bottom ½” of the cutting with a clean, sharp knife.
3. Dip bottom ½ - 1” of cutting in rooting hormone. Tap off excess hormone powder. (Avoid dipping the cutting directly into the hormone powder container so that the substance is not contaminated).
4. If rooting herbaceous or softwood cuttings, prepare holes for the cuttings with a pencil, so that the shoots will not be bruised when pushed into the soil.
5. Insert the cuttings one-third to one-half their length into the medium, maintaining vertical orientation of the stem (point the buds upward). Space cuttings far enough apart to allow all leaves to receive sunlight (4 - 6”). If holes were pre-prepared as in step 4, gently firm the potting media around the shoots.
6. Cover the cuttings with clear plastic bags or coverings and place in indirect light. Avoid direct sun.
7. Keep the medium moist until the cuttings have rooted. Rooting will be improved if the cuttings are misted on a regular basis.
8. In 4 - 6 weeks, test for rooting by gently tugging on cuttings. If there is resistance, lift 2 - 3 cuttings with a plastic fork to see if roots are present. If there are no roots present, or a donut-shaped callus, re-insert the cuttings. If roots are present (even small ones) the plants are ready to be re-potted.

Containers: Make sure the containers for your cuttings are well-drained (with plenty of drainage holes). This is important for two reasons: 1) the cutting must be held upright by the medium in the container until roots are produced, and 2) waterlogged medium will cause your cuttings to rot and die.

Media: Cuttings should be stuck in very lightweight medium (example: 1 part vermiculite, 2 parts perlite). These types of medium are highly porous and thus drain well. Transplant the cuttings as soon as they are well-rooted (after ~8 weeks) into pots with regular soil (example: 1:1 peat-perlite mix) (Midgley 1999).

Protocol for Rhizome Division (Midgley 1999; Applicable for Maianthemum canadense)

1. Divide rhizomes into 3-inch (7.6 cm) sections. Each section should have at least one latent bud.*
2. Fill small pots $\frac{3}{4}$ of the way full with growth medium.
3. Lay 3 cuttings on the surface, spaced evenly apart, about 3 cm from the pot edge.
4. Cover cuttings with 3-5 cm of soil. Do not plant rhizomes deeper than 5 cm.
(Flinn and Pringle, 1983).
5. Water gently, making sure that the cuttings remain covered with soil.
6. Cover pots with bags or a plastic lid to hold in moisture and place in growth chamber.

*If desired, treat rhizomes with fungicide by soaking in a 1.2 g/L solution of systemic fungicide (such as benzimidazole fungicide (C₁₄H₁₈N₄O₃)) for 15 minutes before cutting the rhizomes into pieces and planting (Nivot, Olivier, and LaPointe 2008).

Alternative rhizome division procedure (Hebert 2006)

Plant 20-cm rhizome cuttings in a heated vermiculite propagation bed for 9-12 months.

The following spring, transplant new shoots into small (~800mL) pots. Individual plants rooted tight after 12 weeks.

Protocol for Whole Plant Division (Midgley 1999; applicable for Carex aestivalis and Solidago caesia)

1. Divide the shoots in late winter, after plants have hardened and new growth has stopped.
2. Remove the root ball from pot by massaging the pot's sides until the soil has loosened, then gently work the root ball out of the pot.
3. Gently shake off most of the soil, then rinse the root ball in a tray of lukewarm water to clean it further. Keep the naked roots moist with damp paper towels and out of the sun or other direct light.
4. Divide the shoots and root system by gently untangling the root structure.
 - a. Divide *C. aestivalis* into clumps of 5 - 6 shoots (Lief 2012).
 - b. Crown-type root structures must be cut apart. Using a 10 - 12 inch pointed, serrated knife, cut between buds on the crown, in the woody

material separating them. Do not slice through feeder roots. Cut only through thickened areas at natural division points.

5. Remove all dead and unhealthy plant material from the divisions.
6. Dust divisions with wettable sulfur powder if you have detected fungal growth on the mother plant (Appendix A).
7. Replant divisions in well-drained, size-appropriate containers with humus-rich medium. Water and apply liquid fertilizer if necessary.

II. Sexual Propagation Protocols

Seed Cleaning: Fleshy seeds (Maianthemum canadense, Prunus pensylvanica)

- When fruits are mature, clean flesh from seeds by maceration, using a Dybvig seed cleaner or by hand with a screen (Hebert 2006, Leif 2012). Separate seeds from inert material by floating the macerated material in water. Discard hulls and any seeds that float (these are not viable). Allow seeds to dry before placing in cold storage (4 deg Celsius at 40% humidity) (Fulton 1974).

Seed Cleaning: Dry seeds (Midgley 1999; Carex aestivalis, Solidago caesia)

1. Let seeds dry thoroughly before cleaning.
2. Work over a clean, white surface, such as a large piece of paper.
3. Separate seeds from all non-seed material, including flower parts (petals, sepals, capsules), stems, and leaves. Discard the debris.

- a. If desired, remove the **pappus** from *S. caesia* seeds by rubbing the seeds over a mesh screen and processing them with an air column separator (USDA-NRCS 2011).

Rhizome Stratification (Maianthemum canadense)

Maintain rhizomes in moist conditions (either in soil or wrapped in dampened paper towels) at 4 degrees Celsius for at least 35 days or until ready to plant (Nivot, Olivier, and LaPointe 2008, Yoshi 2008). Transplant rhizomes into room-temperature soil (~20 deg. C) after stratification.

Fleshy seed Stratification (Maianthemum canadense, Prunus pensylvanica)

Breaking Double Dormancy* (Diboll 2008)

1. After collecting seeds in the fall, cold treat them during the first winter either outside or in a refrigerator. Bring them out in of dormant conditions in March (see potting protocol below).
2. In June, place the seeded containers in a cooler [4 - 10 deg C] for 1-2 months in order to mimic a second round of winter conditions.
3. Remove the seeds from these conditions in August-September and store them in a greenhouse to germinate.
4. Grow the seedlings through the fall and allow them to go dormant in winter.

*This method should give seedlings an eight-month “head start” (Diboll 2008). The above method is primarily meant for *M. canadense*, in which double dormancy has been

confirmed (Diboll 2008, Yoshie 2008), but it may work to speed germination in *P. pennsylvanica* as well.

Stratification of *Prunus pennsylvanica* (Gayyad et al. 2010)

1. Clean seeds with a macerator.
2. Soak seeds with gibberellic acid (1250 ppm) for 24 hours at ambient temperature under dark conditions. Rinse seeds with DI water at the end of the acid treatment.
3. Package seeds with moist inert material and place in a 4° C refrigerator. Keep seeds under these conditions for 10 weeks.
4. Place seeds in a growth chamber set to 4 °C. After 4 weeks, increase the temperature to 23 °C for two weeks, then bring the temperature back down to 4 °C for four more weeks.
5. At the end of the final cold period, sow seeds according to the instructions below.

Dry seed Stratification (Midgley 1999; Carex aestivalis)

1. Place seeds in plastic ziplock bags with a small amount of inert material (oak/pine sawdust or peat moss).
2. Lightly moisten the material so that no excess water is expelled when squeezed.
3. Mix the seeds and inert material thoroughly and place in the fridge 30 - 90 days before scheduled planting (Diboll 2008).

Dry seeds (ASA 2009; Rhododendron periclymenoides)

1. Soak the peat moss separately until thoroughly wet before application. Surface sow seeds and mist it lightly (be careful not to scatter seeds out of the container).
2. Place a clear plastic covering over the container, and put it under a light or in partial sun. Germination should occur in 2 - 6 weeks.
3. When the seedlings have developed two sets of true leaves, carefully transplant them 2 - 3 inches apart in another container filled with a mixture of sand and peat, leaf mold, or perlite. Use a plastic fork or other fine instrument to transplant seedlings. Water the seedlings with a fine spray to settle the soil around the roots.
4. Cover with plastic, and place it under lights or on a north facing windowsill, or outdoors in the shade if the possibility of freezing is past.
5. Within a few weeks, the seedlings should be established and the plastic can be removed. Fertilize with very dilute liquid fertilizer. After one year, the seedlings can be repotted. Keep the young plants well hydrated and in the shade.

Sowing fleshy seeds (Maianthemum canadense, Prunus pensylvanica)

- *Maianthemum*: Sow seeds $\frac{1}{4}$ - $\frac{1}{2}$ " deep in a peat-perlite medium (Midgley 1999). Cover with 30 - 50% shade cloth. Water well. Apply $\frac{1}{2}$ - strength liquid fertilizer when leaves have unfurled.
- *Prunus*: Sow individual seeds in pots with peat-based medium at a depth of $\frac{1}{4}$ - $\frac{1}{8}$ in (0.3 - 0.6 cm). Keep sown seeds well-hydrated and under high light conditions (Auchmoody 1979). Transplant seedlings when roots fill the pot. Fertilize with $\frac{1}{2}$

- strength liquid fertilizer when seedlings have two sets of true leaves
(Auchmoody 1979).

Dry, surface-sown seeds (Midgley 1999; Carex aestivalis, Solidago caesia)

1. Fill pots with medium to 2" from the edge. Water medium before sowing seeds.
2. Sow seeds heavily (20 - 50 per pot) on the surface of the medium. Do not cover with excess medium.
3. Apply water gently, so that seeds do not blow out of the pot.
4. Cover pots with a transparent plastic bag or lid to retain microclimate moisture until germination occurs (~2 weeks). Remove the covering promptly after germination.
5. Once seedlings develop true leaves, fertilize them weekly with 1/3-diluted liquid fertilizer containing 1:1 proportions of N-P-K.
6. Seedlings can be transplanted (if necessary) when they have two sets of true leaves.

NOTE: Seed propagation guidelines for *R. periclymenoides* are included in the species profile chapter, as the techniques are more specialized.

Appendix C

Management of Pests and Diseases

Table 1. Selected fungicides labeled for greenhouses

Fungicide (common name, trade name, reentry interval, toxicity)	Application/Target Diseases	Crops and Comments
<i>Bacillus subtilis</i> QST713 (Cease) 4 hr. REI	Cease: Suppresses <i>Rhizoctonia</i> , <i>Pythium</i> , <i>Fusarium</i> , <i>Phytophthora</i> , <i>Alternaria</i> , <i>Botrytis</i> , powdery mildew, downy mildew, and leaf spot diseases.	Preventative biological fungicide. Also labeled for herbs and leafy vegetables.
<i>Bacillus subtilis</i> GB03 (Companion) 4 hr. REI	Companion: Suppresses <i>Rhizoctonia</i> , <i>Pythium</i> , <i>Fusarium</i> , <i>Phytophthora</i>	Preventative biological fungicide.
Chlorothalonil (Daconil Ultrex) 12 hr. REI,	Foliar application for many foliar diseases including blackspot, <i>Botrytis</i> , powdery mildew and rust.	Greenhouse ornamentals.
Fenhexamid (Decree WDG) 4 hr. REI,	Foliar application for <i>Botrytis</i> only.	Greenhouse ornamentals.
Fludioxonil (Medallion WP) 12 hr. REI, Caution	Foliar application for <i>Alternaria</i> , <i>Botrytis</i> , <i>Cercospora</i> and <i>Rhizoctonia</i> . soil application for <i>Rhizoctonia</i> and <i>Thielaviopsis</i> .	Greenhouse ornamentals.

Hydrogen dioxide (Oxidate, ZeroTol) 0 hr. REI 1 hr. REI (spray)	Foliar application for anthracnose, downy mildew, powdery mildew. Soil drench for <i>Pythium</i> root rot.	ZeroTol: Greenhouse ornamentals. Oxidate: Greenhouse vegetables, herbs. Contact oxidizing sanitizer.
Ipodione (Chipco 26019) 12 hr. REI,	Foliar application for <i>Alternaria</i> and <i>Botrytis</i> . Soil application for <i>Rhizoctonia</i> and others.	Greenhouse ornamentals.
Phosphorous acid (Alude) 4 hr. REI	<i>Pythium</i> , <i>Phytophthora</i> , Downy mildew and others.	Systemic fungicide, stimulates plants' natural defenses. Preventative.
Potassium bicarbonate (Milstop SP, Kaligreen) 4 hr. REI, Caution	Foliar application for powdery mildew and others.	Greenhouse ornamentals, herbs and vegetables. Contact eradicant fungicide. Uniform coverage important. High rates may burn some plants.
Thiophanate methyl (Cleary's 3336 F, FungoFlo F, OHP 6672 F) 12 hr. REI,	Foliar application for broad-spectrum of foliar diseases, anthracnose and soil application for <i>Rhizoctonia</i> and <i>Thielaviopsis</i> .	Greenhouse ornamentals. Systemic fungicide. <i>Botrytis</i> has shown widespread resistance.
Thiophanate methyl and chlorothalonil (Spectro 90 WDG) 12 hr. REI,	Foliar application for <i>Alternaria</i> , <i>Botrytis</i> , <i>Cercospora</i> , powdery mildew, <i>Rhizoctonia</i> and others.	Greenhouse ornamentals. Contains the active ingredients found in Daconil and Cleary's 3336.
Triadimefon (Strike WDG)	Foliar application for powdery mildew, rust, and black spot (Rosaceae).	Greenhouse ornamentals. Systemic fungicide.

12 hr. REI,		
Trichoderma harzianum Rifae strain KRL-AG2 (PlantShield HC)	<i>Pythium</i> , <i>Rhizoctonia</i> , <i>Fusarium</i> , <i>Cylindrocladium</i> , and <i>Thielaviopsis</i> . Suppression of botrytis and powdery mildew.	Preventative biological fungicide. Becomes active when soil temperatures are above 50F.
Triflumizole (Terraguard 50 W) 12 hr. REI,	Foliar application for <i>Alternaria</i> , <i>Rhizoctonia</i> , powdery mildews, rust diseases. Soil application for <i>Rhizoctonia</i> , <i>Thielaviopsis</i> .	Greenhouse ornamentals. See label precautions for use on impatiens.

Information from Smith 2011

Table 2. Selected insecticides labeled for greenhouses

Insecticide (common name, trade name, reentry interval, toxicity)	Application/Target Pests	Crops and Comments
Abamectin (Avid 0.15 EC) 12 hr. REI, Warning	Foliar application for leafminers, mites, thrips and whiteflies.	Greenhouse ornamentals except ferns and Shasta daisies. Repeat applications to newly developed tissue may be necessary.
Acetamiprid (TriStar 30 SG) 12 hr. REI	Foliar application for aphids, mealybugs, whiteflies, scale insects, caterpillars and others.	Greenhouse ornamentals and some vegetable transplants. Translaminar and systemic.
Azadirachtin (Azatin XL, Molt-X, Ornazin) 4 hr. REI, Caution	Foliar application for aphids, caterpillars, leafminers, thrips, whiteflies, fungus gnat and shorefly adults and soil application for larvae.	Greenhouse ornamentals, herbs and vegetables. Insect growth regulator for immature stages of insects. Repeat applications needed.

<p><i>Bacillus thuringiensis</i> Subsp.<i>kurstaki</i>(Dipel DF) 4 hr. REI, Caution</p>	<p>Foliar application for many caterpillars.</p>	<p>Greenhouse ornamentals, herbs and vegetables.</p>
<p><i>Beauveria bassiana</i> Strain GHA (Botanigard ES, Botanigard 22 WP, Mycotrol O)) 4 hr. REI, Caution</p>	<p>Foliar application for aphids, mealybugs, thrips and whiteflies.</p>	<p>Greenhouse ornamentals, herbs and vegetables. Contains a fungus that must contact the target pest. Do not tank mix with fungicides. Thorough spray coverage needed. Treat when insect populations are low. Do not apply through irrigation system.Note: The ES formulation has been shown to cause edema-like symptoms on tomato plants.</p>
<p>Bifenazate (Floramite SC) 4 hr. REI, Caution</p>	<p>Foliar application for mites.</p>	<p>Greenhouse ornamentals. For all life stages of two-spotted spider mite.</p>
<p>Pymetrozine (Endeavor 50 WDG) 12 hr. REI, Caution</p>	<p>Foliar application for aphids.</p>	<p>Greenhouse ornamentals. Feeding inhibitor, aphids stop feeding within hours. Translaminar, systemic. Controls aphids for up to two weeks.</p>
<p>Horticultural Oil (SuffOil-X, Ultra-Pure Oil) 4 hr. REI, Caution</p>	<p>Foliar application for aphids, fungus gnat adults, mealybugs, scale, mites, thrips and whiteflies. Also powdery mildew.</p>	<p>Most greenhouse ornamentals, herbs and vegetables. A two-week interval between treatments is recommended. May burn flowers. See label for plant safety.</p>
<p>Imidacloprid (Marathon II) 12 hr. REI, Caution</p>	<p>Foliar application for aphids, leafminers, mealybugs, thrips (suppression) and whiteflies.</p>	<p>Greenhouse ornamentals and vegetable bedding plants. Control for up to three weeks. Broad label including vegetable transplants.</p>

<p>Insecticidal Soap (M-Pede) 12 REI</p>	<p>Foliar application for aphids, leafminers, mites, mealybugs, root mealybug, shore flies, scale, thrips, whiteflies.</p>	<p>Greenhouse ornamentals, herbs and vegetable bedding plants. Good coverage is needed. Works on contact. Avoid treatment when plants are stressed.</p>
<p>Spinosad (Conserve SC) 4 hr. REI, Caution</p>	<p>Foliar applications for thrips, leafminers and caterpillars.</p>	<p>Greenhouse ornamentals. Contact and stomach poison. Thorough coverage important. Thrips resistance reported.</p>
<p>Pyriproxyfen (Distance EC) 12 hr. REI, Caution</p>	<p>Foliar application for whiteflies, scale and mealybug. Soil application for fungus gnats and shore fly larvae.</p>	<p>Most greenhouse ornamentals. See label for plant safety. For immature stages.</p>
<p>Parasitic nematodes (Scanmask, Nemasys, NemaShield) REI Exempt</p>	<p>Soil application for fungus gnat larvae.</p>	<p>Greenhouse ornamentals, herbs and vegetable bedding plants. Apply to moist growing media, temperatures between 50-85F.</p>

Information from Smith 2011

Appendix D

Dichotomous Key of *Carex* section Hymenochlaenae

1	Terminal spike staminate; lateral spikes staminate, androgynous, or pistillate; base of culms tan, brown, or ivory.	2
+	Terminal spike staminate or gynecandrous; lateral spikes pistillate, gynecandrous or rarely distal spike staminate; base of culms usually covered with dark maroon bladeless sheaths (often missing or very short in <i>C. prasina</i>).	5
2 (1)	Plants without rhizomes or with very short ones, densely cespitose.	3
+	Plants with short-creeping rhizomes, loosely cespitose or colonial.	4
3 (2)	Perigynia 5–8 × 1.3–2.7 mm, gradually tapering to beak, 12–20-veined and evenly short pubescent, loosely enveloping achenes; flowering culms more than 1.3 mm wide and bearing 5+ lateral pistillate spikes; California.	<i>Carex obispoensis</i>
+	Perigynia 4.5–6 × 1.4–1.8 mm, abruptly narrowed to elongate beak, 2-ribbed but otherwise veinless or nearly so, glabrous, tightly enveloping achenes; flowering culms less than 1.2 mm wide and bearing 2–4 lateral pistillate spikes; e North America.	<i>Carex sylvatica</i>
4 (2)	Perigynium abruptly narrowed to beak as long as body; basal sheath fibrous.	<i>Carex sprengelii</i>
+	Perigynium more gradually narrowed to beak about 1/2 as long as body; basal sheath not fibrous or not conspicuously so.	<i>Carex cherokeensis</i>
5 (1)	Perigynia 2-ribbed but otherwise veinless or nearly so, green to yellow at maturity.	6
+	Perigynia 2-ribbed and veined between ribs, often conspicuously so, green to olive-green at maturity, usually red dotted.	7

6 (5)	Pistillate spikes densely flowered with perigynia strongly overlapping, more than 10 per spike; perigynia green, membranous, tapered gradually to bent beak, surface smooth and glabrous.	<i>Carex prasina</i>
+	Pistillate spikes sparsely flowered with perigynia barely overlapping, fewer than 10 per spike; perigynia yellow at maturity, cartilaginous, tapered gradually to straight beak, surface pubescent and roughened distally.	<i>Carex assiniboinensis</i>
7 (5)	Perigynia fusiform to narrowly lance-ovoid, longer than 5 mm including elongate beak; leaves generally less than 5 mm wide; leaf sheaths usually glabrous, at least on back.	8
+	Perigynia ovoid-oblong to lance-ovoid, 2–6 mm (mostly 5 mm or less) tapering to beak shorter than body or beakless; leaves 2.5–12 mm wide; leaf sheaths glabrous or pubescent.	9
8 (7)	Lateral pistillate spikes linear, 25–80 × 2–3 mm; pistillate flowers attached 2–9 mm apart, each perigynium strongly overlapping only the 1 immediately above; perigynia 4.5–9.5 mm; pistillate scales white-hyaline with green midrib, less than 1/2 as long as mature perigynia.	<i>Carex debilis</i>
+	Lateral pistillate spikes cylindric, 10–50 × 4–5 mm; pistillate flowers usually attached 1–3 mm apart, each perigynium strongly overlapping at least 2 perigynia above; perigynia 4.6–9 mm; pistillate scales chestnut-hyaline with green midrib, at least 1/2 as long as mature perigynia.	<i>Carex venusta</i>
9 (7)	Terminal spike usually gynecandrous, rarely staminate.	10
+	Terminal spike usually staminate, rarely gynecandrous.	15
10 (9)	Perigynia 2–4 mm, beakless or nearly so; pistillate spikes linear, 10–70 × 2–3.5 mm, usually longer than 40 mm.	11
+	Perigynia at least 3.5 mm, apex tapering to abrupt beak; beak less than 0.7 mm; pistillate spikes cylindric, 10–50 × 3–6 mm.	13
11 (10)	Leaf sheaths glabrous or sparsely short-pubescent; leaf blades 3–9 mm wide.	<i>Carex gracillima</i>
+	Leaf sheaths pubescent, leaf blades less than 4.5 mm wide.	12

12 (11)	Perigynia glabrous; achenes 2–3.2 mm.	<i>Carex aestivalis</i>
+	Perigynia pubescent; achenes 3–4 mm.	<i>Carex roanensis</i>
13 (10)	Bract of proximal pistillate spike usually longer than entire inflorescence, 2–6 mm wide; pistillate scales cuspidate or with rough awns nearly as long as body of scale; perigynia 4.5–6 mm.	<i>Carex davisii</i>
+	Bract of proximal pistillate spike not usually exceeding inflorescence, 1.5–4.5 mm wide; pistillate scales acute to mucronate or with awns much shorter than body of scale; perigynia 3.5–5 mm.	14
14 (13)	All spikes gynecandrous, lateral spikes with at least 1 staminate flower at base; peduncles longer than pistillate spikes.	<i>Carex formosa</i>
+	Only terminal spike gynecandrous, lateral pistillate; peduncles shorter than pistillate spikes.	<i>Carex oxylepis</i>
15 (9)	Lateral pistillate spikes drooping at maturity, short cylindrical to linear, 8–80 × 3–5 mm, on slender arching peduncles.	16
+	Lateral pistillate spikes erect at maturity, narrowly oblong to cylindrical, to 25 × 2.5–9 mm, on stiff peduncles.	17
16 (15)	Pistillate spikes linear, 25–80 × 3–4 mm; proximal bract sheaths longer than 10 mm; leaf blades glabrous; perigynia distinctly stipitate.	<i>Carex arctata</i>
+	Pistillate spikes short cylindrical, 8–25 × 4–5 mm; proximal bract sheaths very short, often less than 2 mm; leaf blades pilose; perigynia acute at base, but not stipitate.	<i>Carex castanea</i>
17 (15)	Leaf blades 4 mm wide or less.	18
+	Leaf blades wider than 5 mm, the proximal often 9–10 mm wide.	19
18 (17)	Proximal inflorescence bracts with sheaths less than 5 mm; leaf blades less than 2 mm wide; leaf sheaths pubescent; perigynia pubescent distally; s Appalachian mountains.	<i>Carex misera</i>
+	Proximal inflorescence bracts with sheaths more than 8 mm; leaf blades 2–4 mm wide; leaf sheaths usually glabrous;	<i>Carex mendocinensis</i>

	perigynia glabrous or with a few stiff hairs on beak; California and Oregon.	
19 (17)	Pistillate spikes crowded toward tip, at least the distal 2 strongly overlapping each other and the terminal staminate (rarely gynecandrous) spike; pistillate scales maroon to chestnut with green midrib, broadly obovate, often short-cuspidate.	<i>Carex gynodynamis</i>
+	Pistillate spikes, at least proximal ones, usually remote from terminal staminate spike; only distal pistillate spike usually overlapping the terminal staminate spike, if any; pistillate scales green to golden brown, oblong, with green midrib extending to ciliate short-aristate tip.	<i>Carex hirtissima</i>

Key from Waterway 1988, 1990a, 1990b

Appendix E

Mean and max/min air temps (°C) of *M. dilatatum* native habitat from 1971 - 2000.

Month	Max Temperature	Min Temperature	Mean Temperature
May	13.2	6.0	9.4
June	16.4	10.9	13.4
July	20.4	15.7	17.7
August	23.0	17.8	20.3
Overall	18.3	12.6	15.2

Appendix F

Fire Cherry

(*Prunus pensylvanica* L. f.)

I. Identification

Prunus pensylvanica is a woody deciduous tree species of the superorder Rosaceae, order Rosales, Family Rosaceae (ITIS 2014). As a woody species, it represents a substantial plant functional group found in the heavily forested Appalachian region. Its range stretches from Canada south to North Carolina and west to Montana (Kartesz 2014).

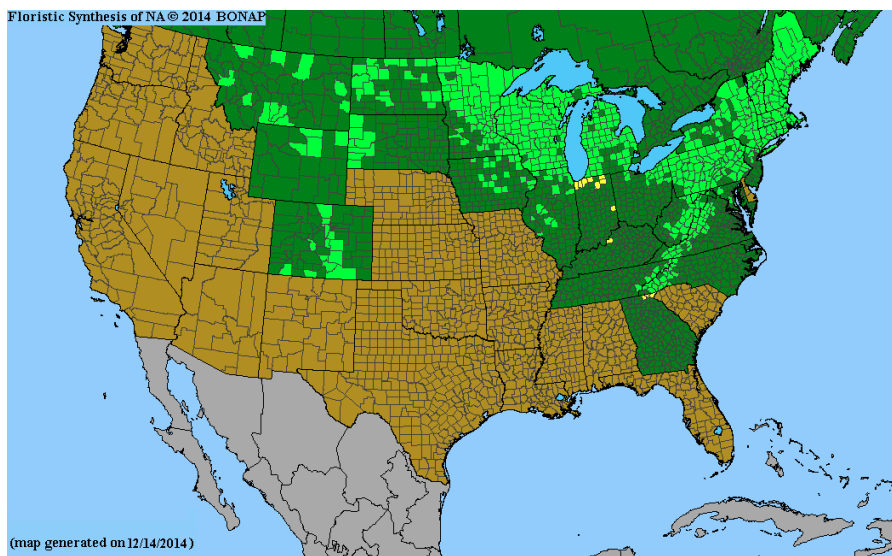


Figure 1. *P. pensylvanica* range as of 11/2/2014. Color Key: dark green - species present and native; gold - species not present; bright green - species present and not rare; yellow - species present and rare; orange - historic range, species extirpated. Image from Bonap (Kartesz 2014).

During both identification and propagation of this species, the most important trait of *P. pensylvanica* to consider is its life history. *Prunus pensylvanica* is an early successional tree that rapidly establishes in disturbed areas (Anderson 2004). Its maximum height is only about 30 feet (Leopold 2012). Its leaves are alternate, simple,

lanceolate, 3.5 - 5 inches long and 1 - 1.5 inches wide, finely serrated margins with slightly rounded teeth, bright green above and slightly paler green below (Eaton 2014, Leopold 2012, Figure 2). The bark is shiny, red-brown, and has long, horizontal, pinkish lenticels; bark may peel away horizontally (Leopold 2012, Figure 2). Twigs are slender, glabrous, reddish-brown, with small, ($\frac{1}{8}$ - inch) glabrous, red-brown buds clustered at twig ends (Leopold 2012, Figure 2). All *Prunus* species twigs also have a bitter almond smell (Eaton 2014). Flowers are arranged in terminal clusters of 12 - 16 white, perfect, $\frac{1}{2}$ - inch flowers with long pedicels in an umbel shape (Hall et al. 1981, Leopold 2012). Flowers expand with the leaves (Hall et al. 1981). Fruit are in the form of fire-engine red drupes, $\frac{1}{4}$ - inch in diameter, in clusters of 12 - 16 (Leopold 2012). Fruit are attached directly to the slender stem (Eaton 2014, Figure 2).

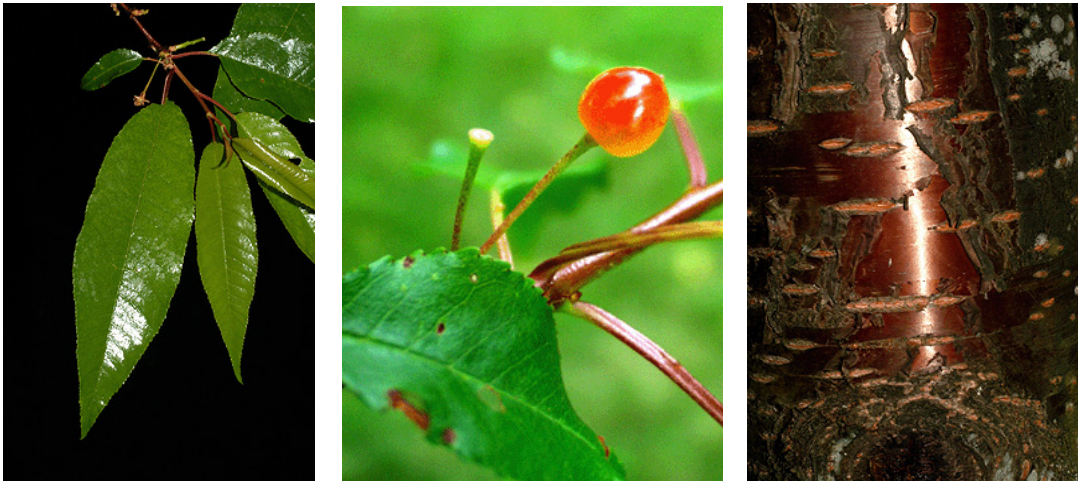


Figure 2. From left to right: *Prunus pensylvanica* leaves and leaf arrangement, drupe, and bark with horizontal lenticels. Image credit: Seiler et al. 2015.

As an early successional species, *P. pensylvanica* is common in edge habitat and other disturbed areas (Anderson 2004). It is a high-altitude species, occurring mainly at or above 3000 ft in its southern range. In its northern range, pin cherry can be found in nearly every forest type; In Appalachia, it is a characteristic early successional species in

oak-hickory or spruce-fir forest ecosystems (Anderson 2004, Graber 1980). The best way of locating pin cherry is to look in edge and other early-successional habitat in high elevations. Because of its wide range and generality among forest types, pin cherry has dozens of associated species (Anderson 2004, Graber 1980). As such, relying on association is not the most effective identification method of locating this species in the field.

Two other *Prunus* species occur in Appalachia that may be easily confused for *P. pensylvanica*: black cherry (*Prunus serotina*) and chokecherry (*Prunus virginiana*). *Prunus serotina* occurs at lower elevations (0 - 1520 m) than *P. pensylvanica* (Nelson 2003) and has a greater maximum height than (90 feet) (Eaton 2014). The leaf margins also have rounded teeth, but are more coarsely serrate (Leopold 2012, Figure 3). Its most important identifying characteristic is its distinctive bark texture, which resembles burnt corn flakes (Leopold 2012, Figure 3). The fruit of *P. serotina* mature later than that of *P. pensylvanica* - in August rather than July - and the fruit are attached to the stem with a broad, expanded cup and are arranged in a raceme rather than an umbel shape (Eaton 2014, Leopold 2012, Figure 3). The mature trees of *P. virginiana* are smaller than mature *P. pensylvanica* (16 ft max height) and the leaf teeth, while also finely serrate, are pointed, not round (Eaton 2014, Figure 4). Like *P. serotina*, the fruit are arranged in a raceme rather than an umbel shape, but, like *P. pensylvanica*, are attached directly to the slender stem (Eaton 2014, Figure 4). Fruit maturation also occurs later in *P. virginiana*, around the same time as *P. serotina* (Eaton 2014).



Figure 3. From left to right: *Prunus serotina* leaves, bark, and drupes. Image credit: Seiler et al. 2015.

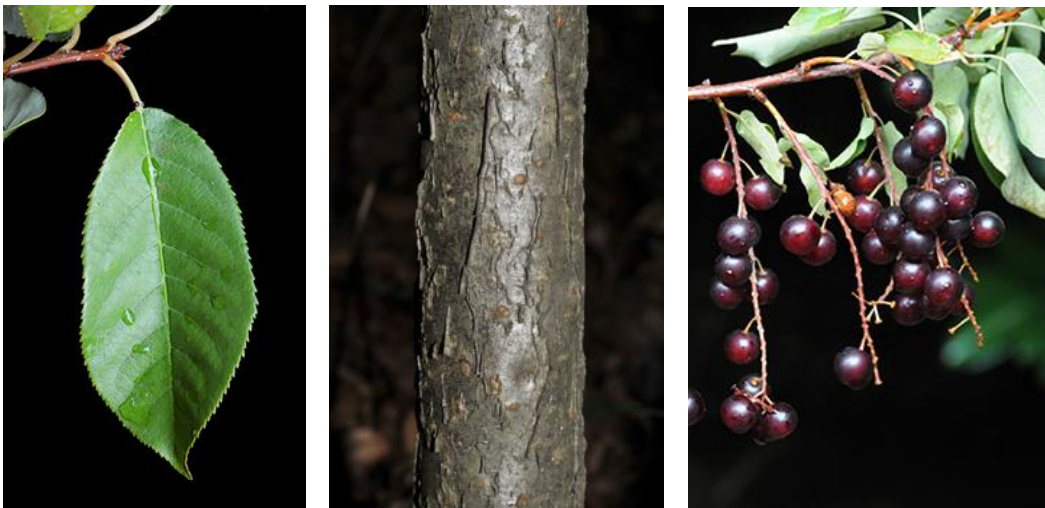


Figure 4. From left to right: *Prunus virginiana* leaf, bark, and drupes. Image credit: Seiler et al. 2015.

II. Propagation

Collect softwood cutting earlier in the year (May - June) as new growth begins to harden. Suitable shoots have a gradation of leaf size (oldest leaves are mature while newest leaves are still small) and can be easily snapped and bent. Care must be taken to

keep the shoots from drying out; if the cutting loses turgidity, success of rooting greatly decreases (Evans and Blazich 1999). Collect semi-hardwood cuttings later in the year (July - August) when the twig wood is firm and all leaves are mature (Evans and Blazich 1999). This type of cutting is more tolerant of dryness than softwood cuttings. Collect fruit during the summer when fruit turns red (Lief n.d.), at the same time as semi-hardwood cuttings. Collect lateral shoots only from young, healthy individuals that are not under noticeable stress. Both softwood and semi-hardwood cuttings should be collected as heel cuttings 4 - 6 inches in length (Bir 1996, Figure 5). Store propagules in cool, moist conditions until sticking or sowing.



Figure 5. Examples of mallet and heel type woody cuttings, as well as a cutting wounded in preparation for sticking. Image credit: Dennis Chuah.

Fire cherry reproduces primarily from seed. It takes at least four years for pin cherry to sexually mature, and several years longer to produce substantial quantities of fruit (Marks 1974). Seeds are mainly dispersed by animals, particularly birds, and after digestion, germinate on the forest floor (Marks 1974). Seed germination is highly stimulated by disturbances such as fire and forest clearing (Marks 1974). These adaptations make proper seed preparation important when attempting to grow pin cherry

from seed. *P. pensylvanica* can also be propagated vegetatively by several methods, but propagation by softwood or semi-hardwood cuttings is easiest (Evans and Blazich 1999). Seeds of pin cherry may also be double dormant, and germination may be accelerated by double dormancy treatments (Anderson 2004, Diboll 2008). For a detailed description of propagation by cuttings, see the protocol in Appendix B.

Pin cherry seeds require disturbance for germination. Laidlaw (1987) noted that removal of the forest overstory triggers germination by increasing light levels, and consequently, soil temperatures. Marks (1974) found that major disturbances such as heavy cutting or burning also stimulate pin cherry germination. As the seed endocarp ages, it becomes more permeable to water and oxygen, and more responsive to alterations in its microclimate (Marks 1971). Numerous methods of scarification and stratification have been tested on *Prunus* seeds. Leif (2012) proposed a method involving 60 days of continuous warm stratification (30° C) followed by 90 days of cold stratification (4° C); however, this method yielded a germination rate of only 15 - 20%. Grisez (1974) put forth a similar method in which the first stratification stage is composed of alternating cool-warm temperatures (20° to 30° C). Laidlaw (1987) found that mechanical removal of the endocarp and cool stratification (15° C), followed by seed treatment in 0.5 M hydroxylammonium chloride and a series of temperature fluctuation treatments increased seed germination rate over 75%. Similarly, researchers recently demonstrated that removal of the endocarp with gibberellic acid (1250 ppm), followed by an initial 10-week period of cold stratification (2 - 4° C) and a secondary period of alternating temperature stratification (2-4 °C and 20-25 °C) yielded a 58% germination rate in a shorter germination period (Gayyad et al. 2010). This final method has great success and

simplicity, as well as the added advantage of speeding time to germination; see Appendix B for a detailed description.

IV. Plant Care

Prunus pensylvanica grows on a wide range of soil drainage classes, but is generally absent from wet sites (Anderson 2004). Watering needs for mature plants are average. However, one should keep in mind that watering needs are higher for seedlings and stem cuttings. It will grow well in regular commercial potting soil. Its optimum pH is 5.0 - 6.0, but it can grow in more acidic soils (Anderson 2004, Graber 1980). As an early successional species, pin cherry has high light requirements (full sun) and is highly intolerant of shade (Anderson 2004). All propagation methods should be carried out in high light conditions. The species is cold-hardy down to -23 degrees C in its northern range and -4 in its southern range, and is heat-tolerant up to 29 degrees C throughout its range (Anderson 2004). Daily maintenance is minimal, other than regular watering.

IV. Issues and Complications

Pin cherry is susceptible to numerous pests and diseases. Maintaining a high-light environment with adequate airflow and using proper watering technique (Appendix A) can greatly reduce the chances of specimens contracting diseases (Wick 2000). See Appendix C for information on treating pests and diseases. Common fungal diseases of *P. pensylvanica* include black knot (*Apiosporina morbosa*); cherry leaf spot (*Coccomyces*

hiemalis) and other leaf spot diseases (*Cercospora circumscissa*, *Coryneum carpophyllum*, and *Phyllosticta* spp.); powdery mildew (*Podosphaera oxyacanthae* var. *tridactyla*); rusts, especially *Tranzschelia pruni-spinosae*; leaf curler (*Taphrina cerasi*); and eastern trunk rot (*Fomes pomaceus*; Wall 1986). Many insect pests also plague *P. pensylvanica* (Baker 1972, Waage and Bergelson 1985, Wall 1986). It is most often affected by leaf feeders, but is also preyed upon by the uglynest caterpillar (*Archips cerasivoranus*), the eastern tent caterpillar (*Malacosoma americanum*), the cherry leaf beetle, (*Pyrrhalta cavicollis*), Bruce spanworm (*Operophtera bruceata*), fall canker worm (*Alsophila pometaria*), and the web-spinning sawfly (*Neurotoma fasciata*).

Appendix G

Pinxterbloom Azalea

(*Rhododendron periclymenoides* Michx. Shinnery)

I. Identification

Rhododendron periclymenoides is a woody deciduous shrub species of the superorder Asteranae, order Ericales, Family Ericaceae (ITIS 2014). As a woody species, it represents a substantial plant functional group found in the heavily forested Appalachian region. Its range stretches from Vermont south to Alabama and west to Hardeman County, Tennessee (Kartesz 2014).

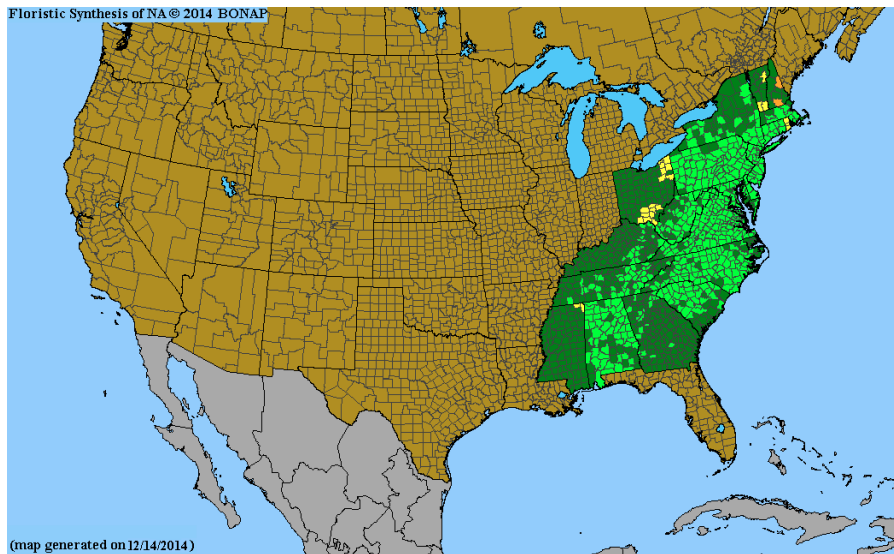


Figure 1. *R. periclymenoides* range as of 11/2/2014. Color Key: dark green - species present and native; gold - species not present; bright green - species present and not rare; yellow - species present and rare; orange - historic range, species extirpated. Image from Bonap (Kartesz 2014).

Like *Carex*, *Rhododendron* identification is highly difficult due to the degree of morphological similarity between congeners. Most *Rhododendron* species in the United

States occur along the Appalachian mountains, and many are indistinguishable apart from flower morphology (Kartesz 2014). Field identification should be done during the flowering period; if collection during this period is not possible, specimens should be flagged so that they can be identified later in the year.

The species grows to 5 meters in the form of a shrub with low branches, often with a crooked stem (Seiler et al. 2015, Shinnors 1962). The twigs are slender, red-brown to gray, and bristly-hairy; they have multiple terminal buds that are pointed and yellow-green to red-brown; flower buds are larger than leaf buds ($\frac{1}{2}$ " length) and are broadly ovate. (Seiler et al. 2015). Bark is gray to reddish-brown, and finely shredded. (Seiler et al. 2015, Shinnors 1962). The leaves are simple, deciduous, and ovate, 1 to 3 inches long, pinnately veined, dull green above and green below with a ciliate margin. Leaves have an alternate arrangement, but appear whorled due to tight clustering at the twig tips (Seiler et al. 2015). The flowers are monoecious; fragrant, very showy, light pink to violet, $1\frac{1}{2}$ " long and $1\frac{1}{2}$ " across at the opening. Flowers are arranged in large terminal clusters (6 - 15 flowers per cluster) appearing with or just before the leaves in mid-spring. (Coladonato 1992, VA Tech). Each flower is on a pedicel (4-19 mm), which can be either glabrous or pubescent (Shinnors 1962). The corolla is deep pink to white, with a pink tube and no blotches of color on the upper lobe (Shinnors 1962). The flower is funnelform (23-45 mm) and pubescent (Shinnors 1962). The petals are connate (fused together), with the flower tube (12-27 mm) gradually expanding into the petal lobes (12-27 mm), which are equal in length or shorter than the tube (Shinnors 1962). There are five protruding stamens that are unequal in length (32-68 mm) (Shinnors 1962). The fruits are oblong, dry, many-seeded woody capsules, $\frac{1}{4}$ to $\frac{1}{2}$ inch long, with ascending

hairs. These capsules split when ripe, releasing tiny winged seeds (Coladonato 1992, Seiler et al. 2015).



Figure 2. From left to right: *Rhododendron periclymenoides* flowers, shrub structure, and opened seed capsules. Image credit: Seiler et al. 2015.

Pink azalea commonly occurs in mixed deciduous forests on well-drained acid soils in cool, moist locations, such as stream bottoms, bogs, shaded mountain sides, and ravines (Coladonato 1992). Common tree associates include birches (*Betula* spp.), blackgum (*Nyssa sylvatica*), ironwood (*Ostrya virginiana*), pitch pine (*Pinus rigida*), oaks (*Quercus* spp.), flowering dogwood (*Cornus florida*), and eastern hemlock (*Tsuga canadensis*; Coladonato 1992). Common understory associates of *R. periclymenoides* include mountain laurel (*Kalmia latifolia*), rosebay rhododendron (*Rhododendron maximum*), highbush blueberry (*Vaccinium corymbosum*), and maple-leaved viburnum (*Viburnum acerifolium*; Coladonato 1992).

Although most species of North American *Rhododendron* occur in the same range as *R. periclymenoides*, the only species with extremely similar flower morphology to *R. periclymenoides* is *R. canescens* (Kartesz 2014, Hyatt 2001, Figure 3). This congener can

be distinguished from the target species by the presence of sticky glandular hairs on its flower tubes. The flower tubes of *R. periclymenoides* are often pubescent but they lack sticky hairs (Hyatt 2001).



Figure 3. Flowers of *Rhododendron canescens*. Image credit: Seiler et al. 2015.

II. Propagation

Rhododendron periclymenoides can be propagated asexually by softwood or semi-hardwood cuttings or sexually (Evans and Blazich 1999). Propagation of *Rhododendron* by seed takes over a year, and individuals require at least three years to reach full maturity. Instructions for seed propagation is included below, but for the purposes of this study, propagation by cuttings is the most practical method.

Because the flowers are so critical to correct identification, it is prudent to flag individuals for collection between April 22 - May 16 when the flowers are in bloom (Azalea Society of America 1999). This way, they can be correctly identified later in the year. Take actively-growing softwood cuttings between May 1st - 20th (Summerville 2014). Collect specimens early in the morning while it is cool and the plants are full of water (Bir 1996, Summerville 2014). The shoots must be highly turgid (full of water) when cut, so try to collect after the region has gotten some rain. Place cuttings in an

airtight plastic bag with a wet paper towel, and store in a cooler on ice. Store cuttings in their plastic bags in a refrigerator for 12 - 48 hours before sticking (Bir 1996).

The cuttings may be stuck according to regular protocol (Appendix B). After rooting, cuttings should be given half - strength liquid fertilizer and 3 - 4 hours of extra light to stimulate active growth (use 75-watt bulbs placed 6” or further away from plants) (ASA 2009). Terminate the extra lighting in late September so that the cuttings can harden off for the winter (ASA 2009). Do not re-pot the plants until active growth begins the following spring (ASA 2009). Cuttings stuck in May should begin to show new growth by July, and should have 6 - 8” of additional growth by October 1st (Summerville). Rooted cuttings can be transplanted in mid-October to a polyethylene-covered cold frame to overwinter, or can be overwintered in their rooting pots and transplanted when new growth appears in the spring (Bir 1996, Summerville). Seeds should be sown in early January, and do not require cold stratification (ASA 2009). For medium, use a mixture of sand and peat or leaf mold or perlite covered with a ½” of milled peat moss (ASA 2009). For more information on seed treatment, see the protocol in Appendix B.

III. Plant Care

Azalea, unlike the other plants featured in this manual, requires a specialized watering apparatus, the mister, in order to propagate successfully (Bir 1996). This apparatus keeps the growing environment cool and moist, akin the the species’ natural

habitat. Cuttings absolutely must not be allowed to dry out during rooting! See Appendix A for information on mist systems.

Stick cuttings in well-drained medium with a significant organic component. A mix consisting of equal parts pine bark, peat moss and Perlite works well (Bir 1996, Summerville). Fertilizer of choice is Peters 21-7-7 distributed in a Gewa injector with a 1-100 mixing valve. If watering by hand, mix one teaspoon of fertilizer per gallon. Fertilize cuttings beginning on the fifteenth day of June and every ten days thereafter until transplanted (Summerville).

Rhododendrons are late-successional species, and are thus highly shade tolerant (Coladonato 1992). Rooted cuttings in summertime require only 30 - 50% light penetration (Bir 1996). Keep newly rooted cuttings in a shaded area so that they can harden off for winter (Bir 1996). Likewise, high heat (over 25 - 30 deg C) is detrimental to *R. periclymenoides* cuttings when rooting, as it evaporates the crucial water vapor and overheats the plant (Bir 1996). If using plastic tents to hold in moisture, regularly measure the temperatures inside the tents. Vent tents if the temperature rises to near 100°F (Bir 1996). Providing shade (50 - 70%) helps to keep temperatures down, and reduces the need for venting and rewetting potting medium (Bir 1996). Maintain the potting medium around 75°F during rooting. Use shade cloth and misting to keep medium cool in a greenhouse (Bir 1996). Check cuttings regularly for signs of mildew, and remove any that die. Water as needed.

IV. Issues and Complications

Since *Rhododendron* cuttings require high-moisture environments, this increases the risk of fungal and bacterial infection. Quality air circulation should be maintained along with cool temperatures and high humidity when rooting cuttings (Bir 1996). See Appendix C for more information on fungal and bacterial diseases.

Appendix H

Glossary of Botanical Terms

Achene: a dry, 1-seeded, indehiscent fruit formed from a superior ovary of one carpel¹

Alternate: borne singly and spaced around and along the axis, applied to leaves or other¹ organs on an axis. Also used to describe the position of floral parts of different whorls on different radii, e.g. stamens with respect to petals¹

Axil: the angle between one part of a plant and another part, e.g. a branch and a leaf¹

Beak: a prominent terminal projection, especially of a carpel or fruit¹

Bract: a leaf-like structure, usually different in form from the foliage leaves, associated with an inflorescence or flower¹

Campanulate: bell shaped¹

Ciliate: fringed with hairs¹

Connate: fused to another organ of the same kind¹

Cordate: heart shaped in outline, i.e. having the base broad and distinctly notched¹

Corolla: the floral whorl inside the calyx, usually consisting of petals or a corolla tube and corolla lobes¹

Cusp: an elongated, usually rigid, acute point¹

Cuspidate: somewhat abruptly and sharply concave; constricted into a cusp¹

Drupes: a 1-celled fruit with one or two seeds enclosed by a stony layer (endocarp) which is embedded in succulent tissue (mesocarp) surrounded by a thin outer skin (epicarp)¹

Elliptic: oval in outline and with a length:breadth ratio between 3:2 and 2:1¹

Endocarp: the innermost layer of the fruit wall, derived from the innermost layer of the carpel wall¹

Genet: a group of genetically identical organisms (clones)¹

Glabrous: without hairs¹

Glaucous: blue-green in colour, with a whitish bloom¹

Gynecandrous: a stipitate inflorescence with staminate flowers at its base.

Inflorescence: the arrangement of flowers in relation to the axis and to each other¹

Internodes: the part of an axis between two successive nodes, joints or point of attachment of the leaves¹

Lanceolate: lance shaped, much longer than wide, the widest point below the middle¹

Lenticel: a lens shaped dot or pit on young bark, through which gaseous exchange may occur¹

Margin: the edge of a leaf¹

Monoecious: having both male and female unisexual flowers on the same individual plant.

Nodes: the point on a stem at which one or more leaves and associated axillary buds arise²

Oblong: rectangular with a length:breadth ratio between 3:2 and 2:1¹

Ovate: a 2-dimensional shape; resembling a section through the long axis of an egg, attached near the broader end and with a length:breadth ratio between 3:2 and 2:1¹

Pappus: a tuft (or ring) of hairs, bristles or scales borne above the ovary and outside the corolla in Asteraceae and possibly representing the calyx; often persisting as a tuft of hairs on a fruit¹

Perfect (flowers): a flower with functional stamens and pistils²

Perigynia: the part of the flower enveloping an ovary and formed by the fusion of the calyx tube, corolla tube and sometimes stamen filaments and receptacle¹

Petals: a free segment of the corolla¹

Pinnate: segments are arranged around an axis like a feather¹

Pistillate: a flower with functional pistils only²

Pubescent: covered with short, soft hairs¹

Ramet: an independent member of a clonal organism¹

Rhizomes: a creeping stem, usually below ground, consisting of a series of nodes and internodes with adventitious roots. *adj.* rhizomatous¹

Scabrous: rough to the touch¹

Scales: thin flap of epidermal tissue²

Sepal: free segment of the calyx¹

Serrate: toothed so as to resemble a saw; with regular, asymmetric teeth pointing forward¹

Sheath: lower part of a monocot leaf, closely and completely surrounding the stem¹

Simple: a leaf that has one part, not subdivided into leaflets¹

Spike: an unbranched inflorescence of sessile flowers¹

Staminate: flowers with functional stamens only²

Stipe: a support such as the petiole of a frond or the stalk of an ovary or fruit¹

Stipitate: having a stalk or stipe, usually of an ovary or fruit¹

Tepals: a segment of the perianth, which is not differentiated into calyx and corolla; a sepal or petal¹

Terminal: at the apex or distal end of a stem or branch¹

Umbel: an inflorescence in which the pedicels originate from one point on top of the peduncle and are usually of equal length¹

Whorl: a ring-like arrangement of similar parts arising from a common point or node¹

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Appendix A

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