Aseptic Germination of *Trillium erectum* and *Trillium grandiflorum* Seed

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INTRODUCTION

Trillium species are a delightful group of spring-flowering woodland plants for the shade garden. The North American species are frequently more showy than the Himalayan and Northeast Asian forms. They are long-lived, easy-care plants when sited properly. Hence, they are much sought after by sophisticated temperate-zone gardeners in both the Northern and Southern hemispheres. *Trillium* species would be an excellent perennial nursery crop if they could be easily propagated in volume. And therein lies the problem. They are slow to flower from seed, taking up to 3 to 7 years to build a large rhizome (food storage organ) if one is able to germinate the seeds. Seeds of some species have been reported to exhibit double-dormancy. Division is also a slow, but not impossible, process. Several species are classified as threatened or endangered in the wild, which is the source of many plants marketed today.

Work was undertaken several years ago to develop a method for propagating native hardy forms of the genus *Trillium* using micropropagation techniques. I expected that this approach could generate a more rapid increase in clonal plant material than traditional nursery notching and division techniques, and would be more appropriate than collecting plants from the wild.

The initial question asked in this learning process was: What is the easiest method to obtain a large quantity of aseptic plant material to use as an experimental vehicle for further research? Pence and Soukup (1986) reported considerable difficulty with contamination when attempting to use buds from mature *Trillium* rhizomes excised from garden-grown plants for micropropagation. My own experience with using buds from rhizomes of the genera *Hosta, Sarracenia,* and *Astilbe* confirmed the reported decontamination difficulties. In response to the query, I selected aseptic germination of seed (the explant) as the course of action for testing.

MATERIALS AND METHODS

Freshly picked green (unopened) seed capsules were the targeted source of seed in order to reduce the amount of contamination from the natural environment. Seeds from friends, commercial sources, and plant society seed exchanges were used in subsequent experiments. A moderate quantity of *T. erectum* and *T. grandiflorum* seed was readily available in a local private garden where they were growing profusely, having been brought in from a farm in upstate New York many years earlier. Timing of seed collection became a major concern. Ants are an important means of seed distribution in some native *Trillium* species because they are believed to be attracted by the aril as a food source. An aril is a fleshy attachment to the seed. The ants quickly remove seed from split capsules to their nests.

Capsules were watched for ripeness and collected just as they were turning from green to red in late July. Within 24 h of being collected the capsules were washed in a 10% bleach/detergent solution in distilled water for 20 min, placed in a plastic bag

with a moist (not wet) paper towel, and stored at about 40F (5C) for 30 to 45 days before being started in culture.

Knutson's C medium, a nutrient-lean orchid medium, was chosen for the initial germination experiment because the extra nutrition of a Murashige and Skoog (MS) medium (Arditti and Ernst, 1984) was not deemed critical in the beginning cycle for seed germination. Pence and Soukup (1986) had also reported that reduced-strength MS medium was useful for some rhizome culture. The Knutson's C medium was augmented for the first transfer cycle in culture with zeatin at 1.0 mg $liter^{-1}$. Zeatin is a powerful natural phytohormone (plant growth regulator) in the cytokinin class, which influences several plant functions (Donnelly and Vidaver, 1988). When MS medium was used in later experiments for the first culture cycle, it was also augmented with zeatin at 1.0 mg $liter^{-1}$. Agar was added at 8 g $liter^{-1}$, while the medium was adjusted to pH 5.5.

After the initial 30- to 45-day cold/dark treatment, the seeds were recleaned with an alcohol dip for 1 min followed by two 20-min washes of 10% bleach/detergent. Seeds were then placed into plastic egg culture containers holding 16 ml of Knutson's C medium, 4 to 14 seeds per egg, depending on the experiment and seed quantity available. The plastic eggs are made of autoclavable polycarbonate divided in half horizontally with a flat bottom. Their advantages are that they are easy to access with forceps, easy to handle in the lab, and confine any contamination to small quantities of materials.

Culture containers received cold/dark treatment in a refrigerator—18 eggs to a Magenta tray placed into a plastic bag sealed to reduce dehydration. For subsequent warm/dark treatment, the trays of containers in bags were moved to a growing room, which was maintained at 70F (21C) to 84F (29C) with relative humidity held to between 60% and 80%. For warm/light treatment, the trays with eggs were removed from plastic bags and placed on a rack 4 in. under standard white fluorescent tubes on a 16 h light/8 h dark cycle.

When seed was obtained from sources where it was not freshly collected, i.e., received at least 3 months after harvest, it was dry. The seed had been treated to the vagaries of handling by plant society seed exchanges and commercial seed purveyors, and sitting in a desk drawer of another researcher and later refrigerated. In each case, seed of 11 species obtained in subsequent seasons was several months old and dry. It is fair to assume that all the seed received was neither stored in cool conditions nor kept in air-tight containers.

RESULTS

Freshly collected seed of both *T. erectum* and *T. grandiflorum* in the initial experiment germinated after the following treatment:

- Dark/cold 30 to 70 days cold storage
- Dark/cold 60 to 90 days in culture
- Warm/dark 90 to 120 days in culture

Containers were moved to warm/light as roots from germinating seed were observed. At the end of 10 months all seed that had germinated had extended at least one shoot, usually with the now broken seed coat still attached to the tip. This was followed by the extension of a second shoot within 45 to 120 days—a pattern typical for both species.

Final results at the end of this initial experiment:

	T. erectum	*T. grandiflorum*
Eggs per culture containers	2	18
Seeds per egg	9	5
Total seeds	18	90
Seeds germinated after 10 months	13	35
Germination rate (%)	72	41

Other variables tested in subsequent experiments were species, nutrient media, plant growth regulator, and length of cold/dark and warm/dark periods. A total of 750 seeds of nine other species from several sources were tried, using the plant growth regulator gibberellin, MS medium modified for hosta (Kyte, 1987), and Knutson's C medium. Because the seed was not fresh in every subsequent experiment, it was soaked for up to 42 h to hydrate it. None of the seed germinated.

DISCUSSION

Reasonable germination of *T. erectum* and *T. grandiflorum* seed was achieved in 10 months, providing aseptic cultures of rhizomatous plant material for further experiments on multiplication and transplanting plantlets from culture. Contamination only became a problem when the cleaning process was shortened or sterile technique failed.

The exact seed dormancy and germination process for all species in the genus *Trillium* may not yet be completely understood. One can proceed on the basis that, at least for *T. erectum* and *T. grandiflorum*, they are not doubly dormant and it is possible to obtain germination aseptically in one growing season. By ignoring conventional wisdom that dictates two seasons to germinate seed that is doubly dormant, a sequence can be used for *Trillium* similar to modern nursery practice with the native woody plant *Chionanthus virginicus*. One skilled researcher-propagator regularly produces *C. virginicus* seedlings during one winter season by accelerating the cold and warm periods.

The use of dry seed in the experiments was a risky approach, given the known reluctance of some *Trillium* to germinate, even when using fresh seed. Contributing to the failure of the dry seed could have been an overly long period (more than 3 to 6 h) of water soak, which may have caused the seed to over imbibe with subsequent damage to internal tissue (See Deno, 1993 for further thoughts on *Trillium* seed germination).

A cursory survey of the horticultural literature immediately available to the author failed to turn up definitive rules for handling *Trillium* seed. Botanic gardens, arboreta, commercial firms, plant society seed exchanges, and those who donate to them could benefit from standard procedures for handling and germinating *Trillium* seed. This could increase the rate of germination and make commercial propagation more attractive.

LITERATURE CITED

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Grafting on Roots

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INTRODUCTION

Root pieces have a number of advantages over regular understocks. They are among others things, readily available when harvesting nursery stock, can be dug from original plants, are compatible with plants from which they originate, and prevent abnormal growth habits that can occur with seedlings. The disadvantage is, root pieces that can be used as root cuttings will generally produce shoots if used, but are the only choice for grafting of taxa that can not be produced any other way.

Root pieces are harvested when plants have ripened sufficiently so that they could be dug bare-root. After harvesting, roots should be packed in moisture-retentive material and stored at 0C. They could be cut into pieces at this time to facilitate accurate counts. At no time should roots be permitted to dry.

Size of root pieces optimally should be from 6 to 15 mm thick, their length can be between 10 and 20 cm depending on the pot size if they need to be potted. Branched roots are best. Roots that need added temperature to heal are potted and placed into a grafting case 3 to 4 weeks before grafting is to commence—temperature should be 18 to 20C. This procedure promotes heavy rooting. Roots that do not need heat are taken directly from cold storage and grafted and waxed. Grafting for either method is by a side graft. Scions should be from one to five buds in length depending on the particular plant. Rubber grafting strips are used to tie the grafts. If heat is required the grafts are placed back into the grafting case with the unions covered by a moisture-retentive material. The case can stay closed until callusing commences—usually between 4 to 6 weeks. Top growth will also have started by this time and the plants need to be hardened gradually with the temperature not exceeding 20C. After the plants have fully hardened, the grafting strips are removed and the grafts are planted into larger pots or grown on in open ground. It is important that the root part of the graft is covered when planting.

OBSERVATIONS AND TRIALS

Observations and trials, that I have made with plants grafted on roots.

***Aralia elata* 'Variegata'**. This plant is a bud sport and in my experience it can not be rooted. Graft this cultivar on *A. elata* roots in winter, pot, and keep at 20C. In addition it is possible to wax the grafts and keep cool until field planting. There is less suckering if grafts are planted deep and in a heavy soil. A patch bud should be used.