Embryo Excision for Accelerated and Uniform Germination of Hard-to-Germinate Maple Species

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INTRODUCTION

Virtually all fall-maturing maple seeds exhibit some form of dormancy (Browse, 1979). In one respect, this is advantageous for the seeds as it prevents them from germinating at a time when climatic conditions are unfavorable for plant growth and survival. During dormancy, however, the seeds are vulnerable to a number of factors which can reduce their viability: desiccation, fungal infection, and insect and rodent damage. The longer the seeds are in this vulnerable condition, the greater the chance of loss to these factors. The extended period of time some maple species must be exposed to either natural conditions or various stratification treatments in order to overcome dormancy (as long as 3 years for some trifoliate maples; Fordham, 1969) contributes to an already low germination rate in those species. Finding alternate ways to overcome dormancy in difficult-to-germinate species can reduce frustration for hobbyists, and can make additional species feasible for researchers to study and growers to produce.

In the scientific literature there is information about seed dormancy and methods other than stratification that may be used to overcome it. As early as 1955, Heit realized that the germinability of the naked embryo was a truer measure of the viability of a seed lot than that of the intact fruit. Wilson, Hibbs, and Fisher (1979) developed this further and showed the importance of the seed coat, also known as the integuments or testae, in preventing germination. These reports, and others, show that seed dormancy in maples can be caused by two types of conditions that may occur separately or in concert. The physical structures enclosing the embryo may prevent its germination or the embryo itself may be dormant. Stimart (1981), in a very elegant study, investigated how best to overcome embryo dormancy using various concentrations of a number of growth regulators, in combination with light and dark treatments.

In 1993, in an attempt to expedite the process of tree breeding and provide uniform-sized seedlings for physiological studies, I incorporated a number of the techniques recorded in the literature in a seed treatment that was successful on a broad spectrum of maple species (Wiegrefe, 1994). In October of 1997 I received a number of high-value seed lots of maples from a plant exploration trip to China. In most cases, little or no information was available about the seed dormancy types of these species. The current paper reports the results of using the procedures developed earlier to: (1) hasten germination, (2) trigger more uniform germination, (3) maximize germination percentages, and (4) determine the type(s) of dormancy affecting each species.

Materials and Methods. From 29 to 64 seeds were available for use for each of six species (Table 1). The time required for the embryos excisions ranged from 6 to 12 min per seed. Thus, although all of one seedlot were treated in 1 day, the excisions spanned 2 weeks.

Intact fruits were surface-sterilized in 70% ethanol in water for 3 min, then soaked in filtered water for a minimum of 3 min. Very hard, dried seeds were soaked for as long as 2 days to make the tissues more pliable. The pericarp (hard, protective "nutlet" portion of the fruit) was then removed by either: prying open with a vise and small screwdriver (*Acer triflorum*); scoring the corner of the nutlet with a single-edged razor blade, then vertically compressing the nutlet with a needle-nosed pliers until the two halves begin to separate (*A. mandshuricum* and *A. pseudosieboldianum*); or cutting around the periphery of the embryo and peeling open with a jeweler's forceps (*A. tegmentosum*, *A. tschonoskii*, *A. mono*, and *A. ukurunduense*). In most treatments (Table 1), jeweler's forceps were then used to remove the integuments or testae (the thin membrane still covering the embryo). The naked embryos were soaked for 2 days in filtered water to leach out germination inhibitors and allow imbibition to occur. The embryos were then blotted on a paper towel and placed in 10-cm petri plates on filter paper that had been moistened with 2 ml of one of four solutions: filtered water (distilled water can be substituted), 10 ppm benzyladenine (B-3274, Sigma-Aldrich, Ltd., St. Louis, MO), 10 ppm gibberellic acid (ProGibb®4%, Abbott Laboratories, North Chicago, IL), or 1 ml each of the BA and GA solutions for concentrations of 5 ppm each. The plates were sealed with Parafilm laboratory film (American National Can, Chicago, IL) and placed either under florescent lights at 16-h photoperiod or in a dark drawer, both at room temperatures around 21C/70F. The embryos were determined to be germinated when the radicle had grown at least 2 mm and shown a distinct gravitropic curve. Embryos were observed until they deteriorated or germinated. After germination, the embryos were planted and plastic film was used to loosely cover the flat for desiccation protection. The film was removed for increasing durations as the seedlings adjusted to the lower humidities in the greenhouse.

RESULTS

Integument Effects. Only one species, *A. tschonoskii*, was tested for germination without having its testae first removed and the contrast in the results was dramatic (Table 1). No germination occurred for any of the embryos placed on water-or growth-regulator-soaked filter paper if the testae were intact. Once the testae of those same embryos were removed and the embryos were placed on BA-moistened paper on Day 12, 100% germination occurred in 1 week. Naked embryos placed on filter paper on Day 0 with either 5 or 10 ppm BA germinated within 1 week, but naked embryos in contact with water or 10 ppm GA did not germinate in less than 12 days.

Growth Regulator Effects. Benzyladenine, at a concentration of 10 ppm, was more effective than 10 ppm GA or water and equally effective at 5 ppm concentration when combined with 5 ppm GA in eliciting germination in *A. tschonoskii*. Gibberellic acid was only slightly less effective than BA in eliciting germination in *A. pseudosieboldianum* and *A. triflorum* (probably no statistically significant difference), but much less effective in *A. tschonoskii*, where no germination occurred in the 12 days of the 10 ppm GA treatment. In *A. triflorum* treatments, germination began in the water treatment in 3 days (data not shown) and was the only water treatment that was continued unchanged for the duration of the experiments.

Light Effects. Three species were treated to contrasting light treatments (*A. barbinerve*, first 8 days vs. subsequently; *A. mono*; and *A. tschonoskii*: Table 1). No

germination occurred in the 8 days the *A. barbinerve* were subjected to darkness. However, 2 days after being placed in light, germination began. *Acer mono* seeds that differed in the light conditions they experienced for the first 8 days after excision did not differ in their days to germinate. There was little difference in *A. tschonoskii* placed on BA paper in the light or darkness in percent germination, days to germinate, or uniformity of germination. Light in combination with GA or water did not result in germination.

DISCUSSION

Dormancy Observations. Multiple factors were found to contribute to seed dormancy in the species studied. In many of the cases, the impact of the testae on enforcing dormancy was not investigated, thus no conclusions can be drawn on its impact on the germination behavior. The findings for each species are discussed below.

Acer barbinerve. Light was found to promote germination in naked embryos of this species. This is similar to the response of *A. maximowiczianum* (syn. *A. nikkoense*) (Stimart, 1981). The lack of a difference between the water and BA treatments indicate that the growth regulator is not required to stimulate germination.

Acer mandshuricum. The conditions determined by Stimart (1981) to be necessary for successful germination (i.e., removal of all covering structures, low levels of BA, and light) were applied to this very small seed lot with great success — 100% germination in 4 days.

***Acer pictum* (syn. *A. mono*).** Although my previous work on *A. truncatum* had convinced me that this closely-related taxon would exhibit only a testae-imposed dormancy (Wiegrefe, 1994), this study indicated otherwise. Treatment with BA was required to stimulate germination in this seed lot indicating the presence of embryo dormancy as well. It is unclear to me whether this discrepancy in findings is due to taxonomic differences in the plant materials used or whether provenance differences, summer growing conditions, or post-harvest handling influenced the seed behavior. This seed lot was also found to have low vigor, with many embryos deteriorating in the petri plates.

Acer pseudosieboldianum. Growth regulators were found to be helpful in stimulating germination in this species. Gibberellic acid was almost as effective as BA in this respect. The fact that the germination occurred so quickly following transfer of the water-treated embryos to BA indicates that they may have germinated eventually even in the absence of exogenous growth regulators. The thick pericarp is presumed to play a major role in preventing germination of this species.

Acer tegmentosum. The lack of germination of naked embryos on water-moistened filter papers compared to the BA treatment indicates that there is an embryo dormancy present in this seed lot/species. Darkness was effective in promoting germination in this experiment, but previous experience (unpublished data) has taught me that this species is neutral in its light requirement for germination.

Acer triflorum. Naked embryos in all three solutions used (i.e., water, BA, and GA) germinated in less than 3 weeks under lights. The use of either growth regulator reduced the maximum number of days to germinate to 8 and significantly reduced

Table 1. Results of seed treatments given to seedlots of eight Chinese maple species. Abbreviations: DTG = Days to Germination, CBS# = Changbai Shan expedition collection number, X = excised (pericarp removed), TR = Testae removed, BA = 10 ppm benzyladenine, GA = 10 ppm gibberellic acid, BA/GA = 5 ppm each benzyladenine and gibberellic acid.

Acer species	Treatment	No. of Seeds	Germination (%)	Mean DTG	Std. Dev. DTG
barbinerve CBS#018	X + TR + H_2O + dark[1]	5	0/ 60[1]	14	0.0
	X + TR + BA + dark[1]	10	0/ 70[1]	13	1.8
mandshuricum CBS#135	X + TR + BA + light	5	100	4	0.0
pictum CBS#100	X + TR + H_2O + light[2]	11	0/ 82[2]	12	4.2
	X + TR + H_2O + dark[2]	11	0/ 55[2]	12	2.2
pseudosieboldianum CBS# 110	X + TR + H_20 + dark[2]	10	0/ 70[2]	10	3.1
	X + TR + BA + dark	9	100	4	0.7
	X + TR + GA + dark	10	90	8	3.3
tegmentosum CBS#016	X + TR + H_2O + dark[3]	7	0/ 100[3]	12	0.5
	X + TR + BA + dark	8	100	3	0.8
triflorum CBS#108	X + TR + H_2O + light	10	90	10	6.0
	X + TR + BA + light	8	100	8	0.5
	X + TR + GA + light	10	100	7	0.9

tschonoskii CBS#028	X + TR + H_2O + light[4]	7	0/ 86[4]	19	0.0
	X + TR + BA + light	7	100	7	2.6
	X + TR + GA + light[4]	7	0/ 100[4]	17	4.3
	X + TR + BA/GA + light	7	100	7	2.5
	X + TR + BA + dark	7	100	6	1.9
	X + H_2O + light[5]	7	0/ 100[5]	19	0.0
	X + BA + light[5]	7	0/ 100[5]	19	0.0
	X + GA + light[5]	7	0/ 100[5]	19	0.0
	X + BA/GA + light[5]	7	0/ 100[5]	19	0.0
ukurunduense CBS#071	X + TR + BA + light	24	71	5	0.2

[1] Indicates the treatment for the first 8 days, on Day 9 the treatment was changed to (X + TR + [same solution as previous] + light). The first germination percentage reflects the situation on Day 9.

[2] Changed to (X + TR + BA + light) on Day 8. First germination percentage reflects situation on Day 8.

[3] Changed to (X + TR + BA + light) on Day 4. First germination percentage reflects situation on Day 4.

[4] Changed to (X + TR + BA + light) on Day 12. First germination percentage reflects situation on Day 12.

[5] Changed to (X + TR + BA + light) on Day 12. First germination percentage reflects situation on Day 12.

the variation in germination dates. Thus, although the pericarp and/or testae-imposed dormancy (or correlative inhibition, Samish, 1954) is the major factor enforcing the prolonged seed dormancy, growth regulators can further stimulate the naked embryos to germinate.

Acer tschonoskii* subsp. *koreanum (syn. *A. tschonoskii* var. *rubripes* Komarov). With the luxury of many seed to work with, we were able to study this seedlot quite thoroughly. We determined that both a testae-imposed dormancy and an embryo dormancy are present in this species. Unlike in other instances (see *A. pseudosieboldianum* and *A. triflorum*), GA was not effective in stimulating germination. Benzyladenine at either 10 ppm or 5 ppm (the latter with 5 ppm GA) was required. This species was also found to be nonspecific in the light conditions needed for germination.

Acer ukurunduense (syn. *A. caudatum* subsp. *ukurunduense* (Maximowicz) Murray). As no alternative treatments were attempted, I can only state that treatment of the naked embryos with BA and light was successful in triggering rapid and uniform germination. Since growth regulators in the absence of the preferred light condition is ineffective in eliciting germination (see *A. barbinerve* above), we can assume that either *A. ukurunduense* prefers light or is neutral in its light requirements.

Treatment Recommendations. The treatment that was most effective in causing the greatest, fastest, and most uniform germination for all species tested was: the removal of the pericarp and testae followed by a 2-day water soak and placement of the naked embryos on filter paper moistened with 10 ppm BA. The optimal light condition depends upon the species, and some species may be equally satisfied with light or dark during germination provided other conditions are met.

In my previous work, I found that embryos without an embryo dormancy that are treated with BA can develop a club-shaped radical that is slow to elongate and develop root hairs. For this reason, I would recommend placing the naked embryos of a maple seedlot with unknown dormancy type(s) on water-moistened filter paper for 3 to 7 days and transfer to the BA treatment if no germination occurs within your chosen time limit.

SUMMARY

Embryo excision combined with leaching and with or without growth regulators can reliably result in fast and uniform germination of maple seeds. Due to the time involved, the procedure (unless modified) will not be feasible to be used in most commercial nursery situations. However, where high-value seeds or species are involved, it may be very useful. Some of the most valuable applications for the technique are to be found in plant breeding programs, where acceleration of germination and reduced time per generation is desired, and in physiological research, where the generation of uniform-aged and -sized plants are needed. The risk of damaging a few embryos in the extraction process is more than compensated for by the immediate and high percentage of germination.

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Perennial Propagation in the New Millennium

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INTRODUCTION

I chose this topic with a sense of excitement, panic, and frustration because in many ways the propagation and production of certain herbaceous perennials on a commercial scale has changed radically in the last 30 years, yet at the same time the methods used for the vast majority of taxa has not really changed much at all. As we approach this upcoming turn of the century mark, knowing the rate of technological change in the world today, one wonders just what we might be growing, say, 20 years from now. Gazing into the future is not an option for the vast majority of us here today, yet it is worth looking at where we have come from and pondering what may lie ahead in the propagation of such a diverse and exciting group of garden plants.

Without a doubt, one of the most revolutionary developments in commercial plant production in past decades was the invention of the plug flat. Hardly a nursery exists that does not make use of these handy things for the production of seedlings or for rooting cuttings of nearly any plant imaginable. Plug flats allow growers to minimize transplant shock by eliminating "pricking out", mechanization of potting is possible, as well as the ability to hold seedlings or cuttings for longer periods of time before they are moved up.

These are all advantages, particularly for easier-to-germinate perennial species. Production on an enormous scale is possible, such as at Raker's Acres in Michigan, so much so that it may be more economical for a nursery to purchase ready-to-transplant plugs of more common seed-grown types, thus utilizing one's limited space for cutting propagation or growing seedlings not available elsewhere.

The plug revolution has been an enormous boon to growers, without a doubt. In recent years, however, as the end consumer — the gardening public — has become more sophisticated, the items many growers used to consider to be "bread-and-butter" plants have taken some radical downshifts in popularity. In other words, gardeners are now wanting something more exciting than seed-grown carnations or shasta daisies, and they have been whipped into a frenzy by our friends, the garden