Propagation of *Aronia* by seed, cuttings, tissue culture and grafting©

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INTRODUCTION: WHY CARE ABOUT PROPAGATING?

Aronia spp. are an important group of plants because they have great potential as ornamental landscape plants and as a novel fruit crop with nutraceutical properties. *Aronia* species are native to many parts of the eastern United States, especially the Northeast. They are deciduous shrubs that make outstanding landscape plants due to their multi-season interest in the form of white spring flowers, black or red fruit in summer and fall, and orange or red fall foliage color. They are also adaptable plants that tolerate a range of challenging environmental conditions and require little landscape care.

The dark-fruited species of *Aronia* have been promoted as a new fruit crop for the United States and appear to hold considerable potential in this capacity (Secher, 2008). *Aronia* berries have the highest levels of antioxidants of any fruit and contain high levels of anthocyanins and polyphenolics (Benvenuti et al., 2004; Wu et al., 2004). Studies indicate that *Aronia* juice and polyphenol-rich extracts possess a wide range of bioactivities, including modulation of endothelial function, blood cholesterol levels, inflammation, oxidative stress, and blood pressure (Jurgoński et al., 2008; Naruszewicz et al., 2007; Valcheva-Kuzmanova et al., 2007; Li et al., 2012). *Aronia* has been widely grown in Eastern Europe and Russia where the fruits are processed and used in beverages, wine, jelly, and baked goods (Kask, 1987). Production of *Aronia* berries in the United States has been increasing, with acreage in the Midwest exceeding 1000 hectares and two million plants (communication from Midwest Aronia Association).

THE GENUS CHOKEBERRY

Aronia, commonly known as chokeberry, has historically been considered to be comprised of three species that includes *A. arbutifolia* (red chokeberry), *A. melanocarpa* (black chokeberry) and *A.* × *prunifolia* (purple chokeberry) (Hardin, 1973). *Aronia* × *prunifolia* is considered by many to be an interspecific hybrid between *A. arbutifolia* and *A. melanocarpa* (Brand, 2010). Observations of the *Aronia* germplasm collection at the University of Connecticut (over 140 accessions) show that *A.* × *prunifolia* is a variable species that seems to exhibit a continuum of traits between *A. arbutifolia* and *A. melanocarpa*, providing support to the idea that it is an interspecific hybrid. Recently, *A. mitschurinii* has been identified as a fourth species of *Aronia* that is the large fruited type grown by orchardists (Leonard et al., 2013). This species is likely a hybrid between *A. melanocarpa* and *Sorbus aucuparia* that is approximately 75% *A. melanocarpa* and 25% *S. aucuparia*.

Flow cytometry has shown that *A. melanocarpa* exists as diploids and tetraploids in the wild, with diploids only existing in New England (Connolly, 2014). *Aronia arbutifolia*, *A.* × *prunifolia* and *A. mitschurinii* are all natural tetraploids and have not been found as diploids. AFLP analysis of the large germplasm collection at the University of Connecticut has been able to effectively group *Aronia* accessions into six taxonomic groups (Obae and Brand, 2014). These groupings are diploid *A. melanocarpa*, tetraploid *A. melanocarpa*, tetraploid *A. arbutifolia*, tetraploid *A. prunifolia*, tetraploid *A. mitschurinii* and a novel black fruited tetraploid *Aronia* that has only been found in the southwestern portions of the range of *A. melanocarpa*.

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PROPAGATION

Seed propagation

Fruits of different *Aronia* species ripen at different times of the year (unpublished data from M. Brand) and fruit harvest must be adjusted accordingly to maximize yield of viable seeds. *Aronia melanocarpa* in the Northeast ripens starting around the second week of July and continues until the end of the third week of August. Diploids ripen on the earlier end of this range and tetraploids on the later end of the range. *Aronia* × *prunifolia* ripens from mid-August to mid-September and *A. arbutifolia* ripens from late September through early November. *Aronia mitschurinii* fruits are typically ripe during the second, third, and fourth weeks of August. Fruits of *A. arbutifolia*, tetraploid *A. melanocarpa*, *A.* × *prunifolia,* and *A. mitschurinii* will often remain on plants in a shriveled up condition long after peak fruit ripe dates and can be effectively harvested for seed with reduced yields. Diploid *A. melanocarpa* fruits do not seem to persist on plants after peak ripe dates, so these should be collected during peak fruit ripeness to obtain maximum seed collection potential. In some years, especially when wet conditions exist during peak bloom time, a fungal disease (possibly *Fusicladium* or a powdery mildew), can infect fruits and cause premature fruit drop and poor seed availability. Also, drought conditions, especially during May and June, have caused nearly complete abortion of *Aronia* fruit crops.

Fruits are typically soaked for a few days to a week in shallow trays of water to soften the fruit and make seed extraction easier. Water should be changed daily. *Aronia arbutifolia* and *A.* × *prunifolia* fruits are drier, harder fruits and will take longer than *A. melanocarpa* or *A. mitschurinii* fruits to soften and be ready for seed extraction. Once fruits are softened, they are macerated to release the seeds. Small batches of fruit can be macerated by hand, but larger batches can be macerated with a hand held potato masher or a hand held puree mixer. The majority of fruit skins and pulp can be floated away from the seeds. Dry seeds and residual fruit skins and pulp can be rubbed gently to separate the seeds from the pulp and skins and the mixture can be placed in a fine sieve. A vertically oriented fan can be used to blow the lighter skins and pulp away from the seeds. Hand removal of any remaining debris will result in a relatively clean batch of seeds.

Aronia seeds are relatively small; about 2-3 mm long and 1-2 mm wide. *Aronia mitschurinii* seeds are larger than any of the other *Aronia* seeds and diploid *A. melanocarpa* seeds are among the smallest. *Aronia* seeds at maturity are comprised of an embryo axis, large cotyledons, little endosperm, and a brown seed coat. Seeds appear to have two types of dormancy. There is some dormancy associated with the seed coat. I have found that fresh *A. mitschurinii* seeds with their seed coats removed will germinate readily in vitro without any other treatment, such as chilling, while those with intact seed coats fail to germinate at all (Figure 1). With other species of *Aronia*, seed coat removal does not result in consistent and complete dormancy removal. All *Aronia* species (including *A. mitschurinii*) will have their dormancy relieved by appropriate durations of cold moist stratification. I have found that 60 days of cold moist stratification is sufficient to break dormancy of *A. melanocarpa* (both diploid and tetraploid) and *A. mitschurinii*. Best success with *A. arbutifolia* and *A.* × *prunifolia* can be achieved by extending cold moist stratification to 90-120 days. Once stratification has been completed, seeds germinate readily, usually within 10-20 days, and seedlings are easy to grow on using typical methods employed for many woody plants. In terms of relative ease of producing *Aronia* plants from seed, *A. mitschurinii* is very easy, *A. melanocarpa* is easy, *A.* × *prunifolia* is easy-moderate and *A. arbutifolia* is the most challenging, but still not difficult.

Figure 1. *Aronia mitschurinii* seed germination: left with coat on, right seed coat removed.

It is important to discuss that apomixis plays an important role in seed formation in *Aronia*, as in many other members of the Pyrinae. All tetraploid *Aronia* species appear to produce seed using apomixis (Brand, 2010). Preliminary AFLP and morphology data generated in the Brand lab suggests that diplospory, a form of gametophytic apomixis, may be at play in *Aronia*. With this type of apomixis, seed progeny will have a genotype very similar to the maternal parent and will appear virtually indistinguishable from the maternal parent. This means that seed propagation of *Aronia* can be used to produce plants without significant phenotypical variation, but provides an impediment for plant breeding. Diploid *A. melanocarpa* appears to produce completely sexual progeny, although partial apomixis cannot be ruled out at this point.

Rhizome division

Vegetative propagation by division or separation of rhizomes is an easy and highly successful method that can be used when relatively few plants are needed. *Aronia arbutifolia*, *A. melanocarpa,* and *A.* × *prunifolia* are all appropriate candidates for rhizome propagation because they are rhizomatous species, but *A. mitschurinii* is not rhizomatous due to its *Sorbus* parentage. I have used this method often to collect germplasm when out in the wild and have affectionately called the propagules “rip-ups”, because that is just what they are. Most plants will have rhizome sucker shoots emerging from the soil nearby the parent plant. These can often be crudely dug or pulled from the ground to yield a plant or plants. I have been successful using this method when the plants are winter dormant or summer dormant. I have even been successful with this method during active shoot expansion. In fact, I cannot recall an instance where this method has failed.

Ideally, dug rhizome divisions should be potted immediately, or healed in some moist peat moss in containers. However, on collecting trips I have frequently just put the “rip-ups” in a plastic bag with a splash of water and let the plants ride around in the car with me for a couple of days or more without any casualties. Potted “rip-ups” will do best if placed in intermittent mist or a humidity chamber for a couple of weeks. Even if the tops of the plants wilt down, shoots die back some, and the plants look dire above ground, I have found that they are establishing in the root zone area and are usually fine. Regular fertilization of acclimated “rip-ups” often results in vigorous growth from the plant base or below ground stems. If new above ground shoot growth doesn’t occur prior to the dormant season, plants usually break bud and grow very vigorously the following spring after a period of cold dormancy.

Plants produced by rhizome division will initially lack the uniformity required for commercial nursery production, but if cut back hard following dormancy they will fill in

nicely within a single growing season. Certainly rhizome division has its place in the arsenal of propagation methods that can be successfully used for *Aronia*, especially for research, breeding, and plant collection purposes or when only small numbers of plants are required.

Stem cuttings

As with many woody shrub species, stem cutting propagation represents the most efficient method for large scale clonal production of *Aronia* species. *Aronia* stem cuttings can be rooted as either hardwood or softwood stem cuttings, with softwood cuttings being the preferred and easier method (Figure 2). Frequently, *Aronia* is included in lists of shrub species that can be propagated by hardwood stem cuttings, but there is very little information in the literature about this method of propagation for *Aronia*.

Figure 2. Rooted cuttings of *Aronia mitschurinii, A. melanocarpa,* and *A. prunifolia*.

On occasion, I have used hardwood stem cuttings to propagate *Aronia*, but all of my experience has been with *A. mitschurinii*. I have had success with 6-8-in. long hardwood cuttings taken in December, given a basal wound and a 3,000 ppm indole-3-butyric acid (IBA) in talc treatment. Cuttings were bundled in groups of 20 and their bases were placed in 50:50 peat:perlite medium in deep flats. Cuttings were rooted in an unheated pit greenhouse, in a humidity chamber, and bottom heat was provided, but tops were allowed to remain cool/cold. Short roots were evident at the bases of cuttings after 8 to 12 weeks. At bud break in the spring, individual cuttings were potted into quart containers. Shoot growth was initially slow, but eventually strong growth occurred. I believe similar results can be expected with other *Aronia* species, but perhaps at a less satisfactory rate.

Softwood *Aronia* stem cuttings can be rooted relatively easily with high success rates. In my experience, *A. mitschurinii* can be expected to root at levels close to 100%. *Aronia arbutifolia*, *A. melanocarpa,* and *A.* × *prunifolia* will typically root at rates between 85% and 95%, although there are the occasional genotypes that are challenging and root at rates less than 50%. Ideally, softwood cuttings from field-grown plants should be collected from mid-June through July, but I have had reasonable success with cuttings collected even toward the end of August. For container nursery stock where shoot growth is advanced in the spring, cuttings can be taken earlier than mid-June, and a second round of cuttings can be made late in the summer from new lateral shoots stimulated from previous cutting collection or pruning. Basal suckers with indeterminate growth can also be a good source of a limited number of late season softwood cuttings.

Cuttings should be 4-6 in. long and can be both terminal and non-terminal when long shoots are available. One method that works well is to wound cuttings at the base, use 3,000 ppm IBA in talc, and stick cuttings individually in cells or small pots containing 50:50 peat moss:perlite medium. Stuck cuttings should be placed in intermittent mist with 30-50% shade, but humidity chambers will also work. Rooting of most genotypes will occur in 5 to 8

weeks. Rooted cuttings can be potted up and fertilized, which will typically result in some new shoot growth, especially for cuttings stuck during the earlier part of the cutting timeframe. Cuttings are easy to overwinter in minimal heated houses and plant losses are negligible. After the dormant period, rooted cuttings can be directly potted into 1- or 2-gal containers, given controlled release fertilizer and placed directly into production growing areas.

Tissue culture micropropagation

Aronia species are generally very easy to propagate in vitro using typical shoot multiplication methods followed by rooting and acclimation. Brand and Cullina (1992) found that the most appropriate medium to use for *Aronia* shoot multiplication was Murashige and Skoog (MS) medium (1962), although Woody Plant Medium (Lloyd and McCown, 1981) will also work, but not as well. Shoot multiplication medium should contain 2-3% sucrose, 0.8% agar, and 0.5 to 1.0 mg L^{-1} benzyladenine (BA), depending on the genotype. Light levels should be between 40 and 60 µM m^{-2} s^{-1} from cool white fluorescent bulbs with a 14-16-h photoperiod. Subcultures should be performed every 6 to 8 weeks and multiplication rates of between 5 and 15 usable shoots can be expected. Both shoot tips and nodal stem segments will produce good subsequent shoot multiplication. Typically all shoot development is from axillary buds and there is little if any adventitious shoot formation, so the risk of off-type plants is minimal.

Microcuttings are easily rooted under non-sterile conditions (Figure 3) in humidity chambers and take between 10 and 21 days to root fully. A simple, but very successful treatment is to stick microcutting bases in 1,000 ppm IBA in talc. *Aronia mitschurinii* is exceptionally easy to micropropagate and produces very robust liner plants. Rooting can also be accomplished in vitro on half-strength MS medium containing 1 mg L^{-1} IBA. In vitro rooting may provide some utility for research purposes and for very valuable germplasm, but for commercial propagation purposes non-sterile rooting is ideal.

Figure 3. Microcuttings rooting ex vitro nonsterile environment.

Acclimation of rooted microcuttings is easy using typical methods where light levels are gradually increased and humidity levels are gradually decreased over a period of 14 days or so (Figure 4). Young tissue culture plantlets develop quickly in the greenhouse, especially if they arrive there in late winter and early spring when day length and light levels are increasing.

Figure 4. Microcuttings 3 weeks after acclimation.

Grafting

There are several reasons why it may be desirable to graft *Aronia*. Grafting could be used to raise the canopy of *Aronia* berry orchards to facilitate more complete machine harvesting from branches that are too low to the ground to be picked up by the harvesters. Grafting may have effects on time to first fruit harvest in orchards, it may alter fruit ripening date, and it may change fruit biochemical composition, although the potential for this is still unknown at this point. For ornamental *Aronia* plants, grafting would allow for the creation of unique growth forms where prostrate genotypes could be grafted on upright standards to produce weeping plants. Similarly, upright *A. mitschurinii* cultivars could be grafted high on standards to produce fruiting forms of *Aronia* that would have a unique, formal growth form that may appeal to gardeners.

In the Brand lab, we have primarily worked with chip bud bench grafting conducted in March and April in the greenhouse. We have used bare-root seedling rootstocks and dormant budsticks held in a cooler at 35°F. Rootstocks were grafted bare root and then potted. Mostly we have used *A. mitschurinii* 'Viking' as the scion genotype. In addition to chip budding, we have also been successful with wedge grafting using dormant 3- to 4-node scions.

Aronia mitschurinii can be grafted onto *S. aucuparia* rootstocks at success rates of at least 85%, and this value likely can exceed 95%. Successful grafts can be made close to the ground or 24+ inches above the soil line. *Sorbus alnifolia* can also serve as a compatible rootstock, but it does not seem to work quite as well as *S. aucuparia*. Successful graft unions will be less likely with *S. alnifolia* than with *S. aucuparia*, and subsequent plant growth will also be less vigorous. *Pyrus communis* is another rootstock that can be used successfully with *Aronia*, but grafting success rates may be closer to 50% and 2-year graft survival is around 85%. *Crataegus laevigata* has also been used as a rootstock for *A. mitschurinii* scions with about a 33% success rate, but scion shoot growth is weak and 2-year graft survival is around 85%. *Aronia-Pyrus* graft combinations produced early fall foliage coloration in comparison to own root *Aronia* and *Aronia* grafted on *Sorbus* or *Crataegus*. Most of our grafting work to date has been with *A. mitschurinii* scions, but a limited number of grafting attempts have been made using other *Aronia* species as scions and it appears that *S. aucuparia* rootstocks can be highly successful with all *Aronia* species (Table 1).

Table 1. *Aronia mitschurinii* 'Viking' Scion bench grafted in April; chip-bud; bare root rootstocks.

Rootstock species	Graft height (in.)	Successful unions (%)	First season shoot growth (cm)	Two year survival (%)
Crataegus laevigata	6	33	9.8	85
Pyrus communis	6	53	22.4	86
Sorbus alnifolia	6	50	23.4	100
Sorbus aucuparia	6	84	47.2	100
Sorbus aucuparia	24	88	41.7	100
Control: own root	6 (cutting)	96	15.6	100

Micrografting is another option we have explored for creating combinations of *S. aucuparia* root systems with *A. mitschurinii* scions. *S. aucuparia* microshoots are pre-rooted in vitro and then *A. mitschurinii* scion microshoots are micrografted onto the *Sorbus* stem using a modified wedge graft. Rootstock microshoots are cut horizontally to remove the shoot top and then a longitudinal cut about 2-3 mm long is made down the middle of the stem top. Scion shoots have a single diagonal cut made at the microshoot base. The scion microshoot is wedged into the rootstock cut and then the micrograft is returned to a culture tube to allow the graft union to form without desiccation stress to the scion. The union forms on one side of the scion and rootstock only and this is sufficient to make a strong graft union. We found that making diagonal cuts on both sides of the scion reduced the micrografting success rate.

With chip-bud grafting there will be rootstock suckering during the first two growing seasons and these must be removed to allow for scion growth only. It appears that resuckering slows down after the first two growing seasons. Rootstock suckering also occurs on micrografted plants, but appears to stop much more quickly than on conventionally grafted plants.

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