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RESEARCH EXTENSION NOTE
NO. 5 – OCT 2009

Propagation and Fungal Inoculation of Black Huckleberry and Velvet-Leaf Blueberry: How Can These Species be Used in Ecological Reclamation?

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The correct citation for this paper is:

McKechnie, I.M., Burton, P.J. and Massicotte, H.B. 2009. Propagation and fungal inoculation of black huckleberry and velvet-leaf blueberry: How can these species be used in ecological reclamation? Natural Resources and Environmental Studies Institute Research Extension Note No. 5, University of Northern British Columbia, Prince George, B.C., Canada.

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Abstract

There is great demand for the inclusion of berry-producing plants, such as wild blueberries or huckleberries (*Vaccinium* species), in forest restoration and post-industrial reclamation efforts, due to their value to wildlife and traditional users of the land. However, the biology and propagation requirements of northern *Vaccinium* species and their potential for use in reclamation, ecological restoration, and horticulture are largely unexplored. Propagation trials of black huckleberry (*V. membranaceum*) and velvet-leaf blueberry (*V. myrtilloides*) using seeds, hardwood cuttings, and rhizome cuttings were carried out to determine propagation protocols. Outdoor trials tested the influences of mycorrhizal inoculation and soil amendment on seedling survival. Seeds and rhizome cuttings were found to be the most effective propagation techniques for *Vaccinium*. Soil amendment increased outdoor seedling survival; however, none of the seedlings showed mycorrhizal colonization, regardless of inoculation treatment. These *Vaccinium* species are dominant components of climax sub-boreal forests, well adapted to recovery after fire; however it is difficult for them to establish rapidly on degraded soils, making soil amendments important to their use in ecological restoration. Growers must have patience when growing northern *Vaccinium* plants, but successful establishment is possible with adequate care and planning.

Introduction

Ericaceous plants such as the many species of blueberry, huckleberry, and cranberry occur worldwide, often growing under harsh, nutrient-poor conditions. There is great demand for the inclusion of berry-producing plants, such as black huckleberry (*Vaccinium membranaceum* Douglas ex Torr.) and velvet-leaf blueberry (*V. myrtilloides* Michx.), in forest restoration and post-industrial reclamation efforts, due to their value to wildlife and traditional users of the land (Minore 1972). However, the biology and propagation requirements of northwestern *Vaccinium* species and their potential for use in reclamation, restoration, and horticulture remain largely unexplored.

Propagation by seeds is one of the oldest and most commonly used methods of shrub propagation. This method allows for the maintenance of genetic variability, and hence the potential for future genetic adaptations in lines of plant material (Rose *et al.* 1998). Seeds can be collected from many sources including seed orchards, nursery stock, and native populations. *Vaccinium* berries are collected in late summer or early fall. Both number and quality of berries produced by the plants can vary greatly year to year depending upon weather conditions during the growing season (Small and Catling 2005). Studies have suggested that *Vaccinium* seeds can be stored for up to 13 years without losing viability (Dirr and Heuser 1987). *Vaccinium* seedlings, however, grow very slowly, and this is a main reason that this technique is not often used for this genus.

Propagation from vegetative cuttings is one of the most economical techniques for shrub propagation. The resulting plants are genetic clones of the parent plant and will maintain all of its characteristics, but with none of the incompatibility issues noted when using grafting or budding techniques (Dirr and Heuser 1987). When propagating plants using cuttings, both stem cuttings and root cuttings can be used. Limited success has been reported using stem cuttings for black huckleberry, however, other *Vaccinium* species have been reported to root very successfully using stem cuttings (Kender 1967). Several *Vaccinium* species have been found to sprout from rhizome cuttings taken while the plant is dormant (Rose *et al.* 1998). The use of both stem and rhizome cuttings in the propagation of *Vaccinium* plants is an area that needs further research.

Ericoid mycorrhizal relationships benefit ericaceous plants, including *Vaccinium* spp., by allowing them to access nutrients that would otherwise be unavailable (Smith and Read 2008). The mycorrhizal fungi involved in these relationships appear to be ubiquitous in natural environments, but they are usually absent from potting mixes used in greenhouses. This is most likely because they can be killed by the water, fertilizers, pesticides, and fungicides used in most horticultural systems (Acree and Appleton 2000). Commercial inoculants are available for some mycorrhizal fungi; however, they are mostly developed for the more common arbuscular mycorrhizal species. The inoculation of several cultivated *Vaccinium* species with laboratory cultures of ericoid mycorrhizal fungi has been shown to increase fruit yield (Powell and Bates 1981) and plant dry weight (Scagel 2005); however, the influences of ericoid mycorrhizal inoculation on the growth and survival of *Vaccinium* species native to western North America has not yet been examined.

To address some of the gaps in native *Vaccinium* propagation knowledge, the research reported here consisted of testing and optimizing the propagation and culture of black huckleberry and velvet-leaf blueberry using seeds, hardwood cuttings (stem cuttings taken over the winter season), and rhizome cuttings. The latter half of this study was designed to look at factors influencing the performance of *Vaccinium* seedlings when they are moved from greenhouse to outdoor conditions, such as would occur if these species were used for reclamation purposes. Field trials were set up to determine the influence of mycorrhizal inoculation and soil amendments on the survival and growth of black huckleberry and velvet-leaf blueberry seedlings.

Methods

Initial propagation trials for *V. membranaceum* and *V. myrtilloides* were carried out using seeds, hardwood cuttings, and rhizome cuttings. In 2006, 2007, and 2008, seeds were extracted from multiple provenances of locally collected berries and surface sown on a 50:50 (by volume) peat: sand growing medium. Growing trays were kept under greenhouse conditions with a 21°/10°C day/night temperature and 15 hr photoperiod. Germination numbers were counted weekly after initial seedling emergence for a period of 9 weeks in 2006, 13 weeks in 2007, and 14 weeks in 2008.

Hardwood cuttings were collected from

Figure 1. Outdoor planting sites. a) One field site showing amended and control plots. b) Unamended plot showing placement of individual seedlings. c) Black huckleberry seedling in unamended plot. d) Velvet-leaf blueberry seedling in amended plot.

local plants in early February 2007. The bottom third of each cutting was submerged in an indole-3-butyric acid (IBA) solution, a synthetic growth hormone, and were planted in either rockwool or a 50:50 peat: perlite growing medium. Each treatment contained 60 cuttings. Growing trays were kept under the same greenhouse conditions as above with natural light conditions. Root production by the cuttings was evaluated 62 days after the cuttings were planted.

Rhizome cuttings were collected from local plants in October 2008. The cuttings were planted in either vermiculite or a 50:50 peat: perlite growing medium. Growing trays were kept under the above greenhouse conditions until a final evaluation of shoot sprouting was conducted 65 days after planting.

For the outdoor field trials, seeds were collected from north-central British Columbia during the 2007 growing season. These seeds were sown and kept under greenhouse conditions with a 21°/10°C day/night temperature and 15 hour photoperiod. A sub-sample of 500 seeds per species was monitored for germination. Six months after sowing (when in the 4-10 leaf stage), seedlings were inoculated, by pipetting fungal solutions on to the soil surface, with ericoid mycorrhizal fungi: either *Rhizoscyphus ericae* (D.J. Read) W.Y. Zhuang & Korf, *Oidiodendron maius* Barron, or *Meliniomyces* sp. in a potato dextrose agar (PDA) solution; control seedlings were inoculated with only the

Figure 2. *Vaccinium* seeds and seedlings. a) Germinating velvet-leaf blueberry seeds (2008). b) Black huckleberry seedlings 100 days from sowing (2008). c) Black huckleberry seedlings 121 days from sowing (2007). d) Velvet-leaf blueberry seedlings 148 days from sowing (2007).

sterile PDA solution. Approximately one month after inoculation, 1440 seedlings were transplanted to three field sites on the University of Northern British Columbia (UNBC) campus in Prince George, B.C. Sixty seedlings were planted into each of eight 1.4m x 1.4m plots that received randomly assigned species, soil amendment, and inoculation treatments at each of the three sites (Figure 1). Half of the plots in each site were amended with the equivalent of a 10 cm depth of local forest floor material (litter, fermentation, and humus layers, including some rotting wood), while the other half were left unamended. Survival, dry biomass, and mycorrhizal colonization status were assessed at the end of the growing season (110 days). A detailed discussion of the analytical methods used for all of the trials discussed in this note can be found in McKechnie (2009).

Results

Initially, black huckleberry and velvet-leaf blueberry seedlings were indistinguishable from one another. When the seedlings reached the 4-6 leaf stage, they began to show morphological differences between species (Figure 2). Germination levels for black huckleberry and velvet-leaf blueberry differed significantly over the three years of observation (p=3.48 x 10^{-8}). In 2006, 2007, and 2008, overall germination levels for black huckleberry seeds were 26.0 ± 4.9% (mean ± standard error of the mean), 4.70 ± 1.29%, and 66.0 ± 4.24%, respectively. The overall germination levels for velvet-leaf blueberry seeds were 14.7 ± 4.9%, 13.0 ± 1.68%, and 28.5 ± 5.48%, over the same three years (Figure 3).

None of the treatments tested impacted the development of roots in hardwood cuttings of black huckleberry and velvet-leaf blueberry (p=0.079). Both species had a very low success level of 2.5%, the equivalent of 6 out of 240 cuttings surviving (Figure 4, 5a-b).

The sprouting of rhizome cuttings of black huckleberry and velvet-leaf blueberry was not influenced by the growing medium in which they were planted (p=0.700).

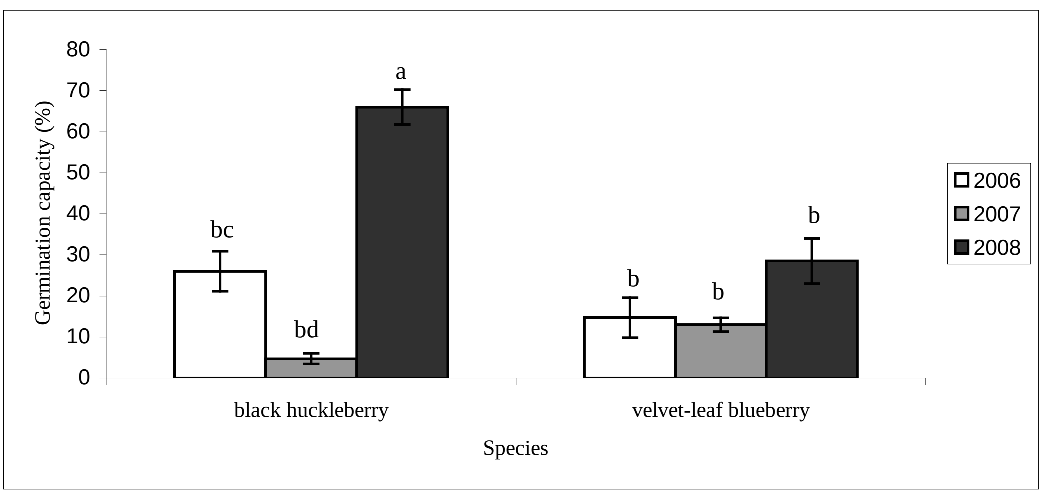

Figure 3. Total germination capacity (%) of black huckleberry and velvet-leaf blueberry seeds collected in 2006, 2007, and 2008. Error bars represent ± one standard error. Columns topped with the same lower case letter do not differ significantly at p<0.05.

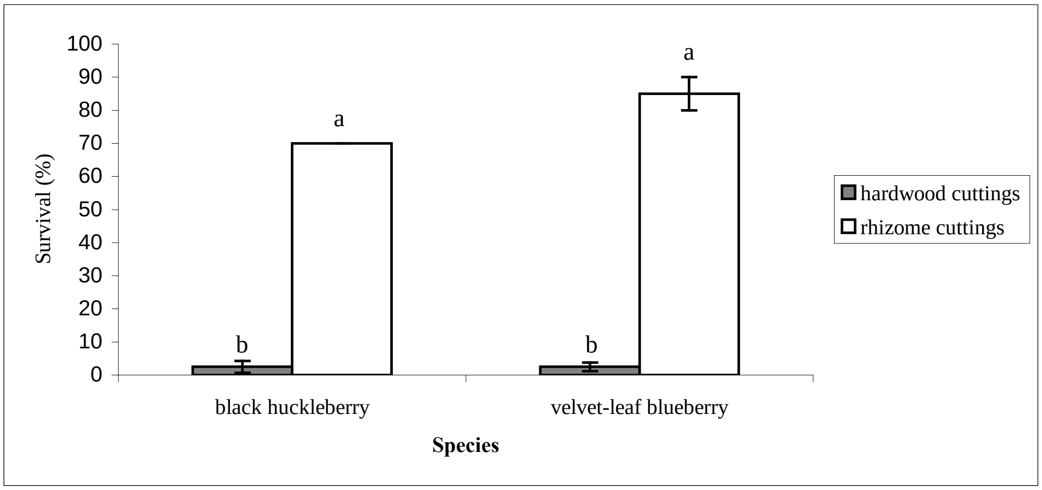

Figure 4. Total survival levels (%) of black huckleberry and velvet-leaf blueberry hardwood and rhizome cuttings. Error bars represent ± one standard error. Columns topped with the same lower case letter do not differ significantly at $p<0.05$.

Overall, black huckleberry rhizome cuttings sprouted at a rate of 70% and velvet-leaf blueberry rhizome cuttings sprouted at a rate of 85%, across all treatments (Figure 4, 5c-d).

Black huckleberry and velvet-leaf blueberry seedlings planted into field plots that had been amended with forest floor material had higher survival levels than those planted into unamended field plots (Figure 6). In the amended plots, black huckleberry seedlings had survival levels of 8.33 ± 3.86% and velvet-leaf blueberry seedlings had survival levels of 28.33 ± 4.58%. In the unamended plots, black huckleberry seedlings had survival levels of 4.17 ± 1.93% and velvet-leaf blueberry seedlings had survival levels of 15.93 ± 3.79%. None of the fungal inoculation treatments had any influence on the survival levels of the seedlings. When root samples were checked for mycorrhizal colonization, none were found to be colonized.

Discussion and Recommendations

The seed germination levels varied significantly among the different growing years. As growing conditions in the greenhouses did not vary among years, the significant differences in germination levels between the years can be attributed to natural variability in the seed lots. Given the germination levels reported earlier, we would recommend that growers over-sow black huckleberry seeds at a minimum ratio of 2:1 and velvet-leaf blueberry seeds at a minimum ratio of 3:1 to adequately produce the number of seedlings desired. Given our personal observations over three growing seasons, seed should be surface sown on a peat/sand medium, ideally with a pH level between 4.0 and 5.5. Seed trays should be kept with a 21°C/10°C day/night temperature regime and 12-15 hour of light/day. Covering the seed trays with clear plastic domes will decrease the amount of watering required. With adequate watering, and the occasional application of 20-20-20 N-P-K fertilizer, the seedlings should require very little maintenance until they are large enough to be transplanted into larger growing containers. *Vaccinium* seedlings grow slowly, and should ideally be left in the greenhouse for 2-3 years prior to being transplanted outdoors. During this time period, the seedlings should be transplanted

Figure 5. Hardwood cuttings and rhizome cuttings. a) Velvet-leaf blueberry hardwood cutting showing one root 62 days after planting. b) Black huckleberry hardwood cutting growing in rockwool 50 days after planting. c) Velvet-leaf blueberry rhizome cutting 153 days after planting. d) Black huckleberry rhizome cutting 153 days after planting.

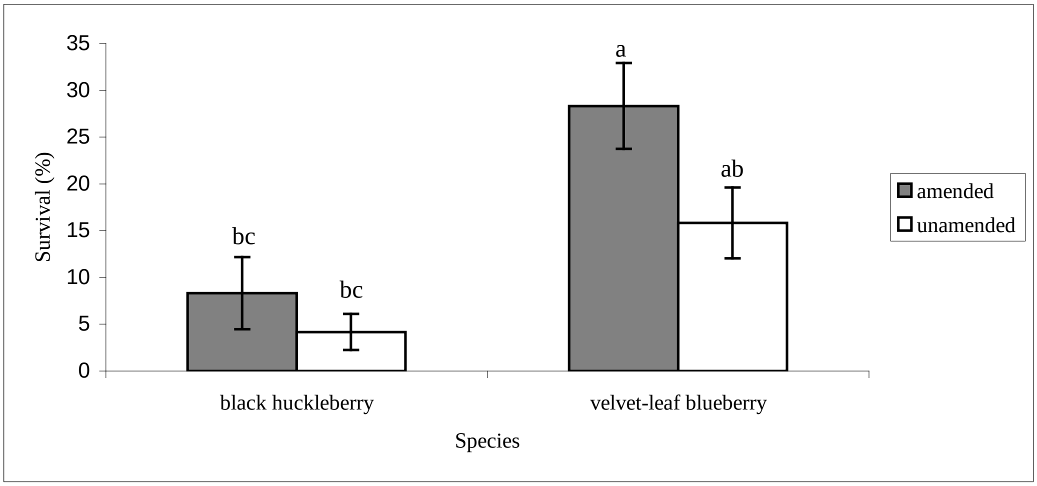

Figure 6. Total survival levels (%), after 110 days, of black huckleberry and velvet-leaf blueberry seedlings planted in amended and unamended plots. Error bars represent ± one standard error. Columns topped with the same lower case letter do not differ significantly at $p<0.05$.

into larger growing containers, as necessary, to promote the growth of the root system.

Rhizome cuttings are an especially attractive method of propagation where outplanting material is desired in a short period of time, and when plant salvage opportunities arise. Rhizome cuttings from northern *Vaccinium* species should be collected with care to avoid damaging the parent plant (unless conducted as a salvage operation prior to habitat destruction), while also ensuring that the correct species of rhizome is being collected. Rhizomes can be collected at any time during the fall and winter months, when the plant is dormant (Dirr and Heuser 1987). When bringing rhizomes into the greenhouse, it is important to either plant them immediately or store them in an environment where they will not dry out, mold, or start to sprout on their own. Cold storage at 4°C is usually sufficient for meeting these requirements. Rhizome cuttings of at least 10 cm, or containing 2-3 nodes, should be planted into a peat/perlite medium or vermiculite. When using vermiculite, care must be taken to ensure the cuttings do not dry out, as this will happen rapidly. If kept in a 21°C/10°C day/night temperature regime and 12-15 hour of light/day, the rhizome cuttings should sprout in 1-2 months. By a visual assessment, these cuttings grow more quickly than seedlings and can be expected to be ready to transplant outdoors after 6-12 months. Another alternative may be to plant unsprouted rhizome segments directly into new outdoor locations, although the efficacy of this method has not been tested. Given the poor success of the hardwood cuttings, the sheer number of cuttings that would have to be planted in order to result in a handful of viable plants makes this method of propagation completely impractical at this time.

Mycorrhizal inoculation of black huckleberry and velvet-leaf blueberry seedlings under one year old does not appear to be effective in producing mycorrhizal colonization. It is interesting to note that all of the rhizome cuttings were found to be colonized by ericoid fungi, despite not being inoculated in the greenhouse. Further research is required to determine the age at which these seedlings begin to form mycorrhizal associations and if the standard protocols used to grow fungi are adequate. Ideally, seedlings should be inoculated, with resulting colonization, before they are planted outside in order to take advantage of the benefits derived from ericoid mycorrhizas.

To the best of our knowledge, there are no existing published guidelines for the growth and transitioning of *V. membranaceum* or *V. myrtilloides* seedlings from indoor to outdoor growing conditions. Given the results of this study, it is recommended that if *Vaccinium* seedlings are to be taken from greenhouse conditions to outdoor areas, they should be at least two years old. Monitoring growth rates of seedlings over a longer period of time than was done in this study would allow a determination of the age when seedling growth rate begins to decrease. This time period might indicate an age where seedlings are less vulnerable to external conditions and would be better able to survive outdoors. Seedlings should be planted early in the growing season, but after the risk of frost, possibly in mid-May for central British Columbia. Outdoor planting areas composed mainly of mineral soil should be amended with local forest floor materials to improve soil structure, water retention, and nutrient availability. Soils should be tested for pH prior to planting and, if necessary, additional amendments should be applied to reduce pH to levels of less than 5 (Dirr and Heuser 1987)

Conclusions

Given the results of these studies, black huckleberry and velvet-leaf blueberry plants should be preferentially grown from seeds when time is not a limiting factor, but labour may be, and from rhizome cuttings when time is a limiting factor, but labour is not. While the influences of mycorrhizal inoculation remain unclear at this time, the application of inoculant may benefit growing plants, and certainly will not harm them. Northern *Vaccinium* species are not typically found as colonizers of severely disturbed environments. Consequently, their use in reclamation and ecological restoration may be most suitable where conditions of secondary succession (as characterized by a more or less intact forest floor), prevail, such as in clearcuts and under power lines. Without soil amendments and careful monitoring for moisture stress, it can be quite difficult to establish these species in areas dominated by bare mineral soil or subsoil, such as pipeline excavations, roadsides, or decommissioned roads and landings.

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Acknowledgements

The authors thank J. Orlowsky and S. Storch (UNBC greenhouse) for technical growing assistance and advice, M. Gorzelak for provision of fungal cultures, and C. Knudsen and S. Patton (UNBC Maintenance) for assistance in preparing field sites. We are grateful to NSERC for a Post-Graduate Scholarship to I. McKechnie and support to H.B. Massicotte in the form of a Discovery Grant.