Propagation of *Myrica gale* by Micropropagation and Traditional Propagation Techniques©

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INTRODUCTION

The genus *Myrica* consists of about 60 species of shrubs and small trees with worldwide distribution. *Myrica gale* is a deciduous, erect nitrogen-fixing shrub commonly found in open peat lands and along shores of sea, lakes, and streams in northern Europe and North America. Wet heath, upland moors, and swamps can support large communities. It can tolerate a wide range of pH (3.8 to 6.1) and grows at altitudes ranging from sea level to 400 m. The name *Myrica* is derived from Greek, probably connected with the word "myron", meaning perfume.

It can reach a height of 0.6 to 2 m. The main stem is brown, twigs are reddish-brown with alternate sub-sessile green leaves (2 to 5 cm long), and volatile oil glands are found on both surfaces. Flowers are borne on the bare wood of the previous year's growth and appear before leaves. The plant is usually dioecious, but monoecious plants and hermaphrodite flowers also occur. The male catkins are reddish brown, 10 mm long, and appear usually in May or June. Female catkins are smaller (6 to 7 mm), but thicker and closely set, with green bracts, 2 stigmas, 2 bracteoles, red protruding styles, and a single basal ovule. The fruit is a dry, flattened, nut with the exocarp secreting wax. Once flowering is completed the vegetative buds lower down the previous season's shoots start to open. A limited number of buds appear to grow into shoots, with the lower buds remaining dormant. New branches are produced from the dormant buds following pruning.

Seedlings are rare in the field and the plant commonly spreads by suckers, which arise from adventitious buds. These suckers become very long and woody and act as a major nutrient store over winter. The plant can be propagated by seeds, stem cuttings, root division, or sucker transplants in early autumn or spring.

Myrica gale, *Hippophae rhamnoides,* and *Alnus glutinosa* are the only species of actinorhizal plants native to the U.K. with nitrogen-fixing root nodules in symbiosis with the bacterial genus *Frankia*. All three species prefer damp habitats, but *M. gale* is the only one well adapted to truly flooded conditions.

Dried leaves and fruits have been used as a spice in soups and stews, as a flavouring for beer, and used to perfume linen. Roots and bark were a source of yellow dye and tannins. Catkins and fruits were a source of wax for candles and leaf and fruit infusions were used as an insecticide, for stomach and cardiac disorders, and also as an abortifacient.

Volatile oils from leaves and flowers have been analysed and the main monoterpene and sesquiterpene fractions have been identified. The main components were alfa-pinene, 1,8-cineole, germacrene, and cadinene. Leaf oil yield was between

0.05% to 0.4% (v/w) and flower yield 0.5% to 1.0% (v/w). Volatile oil is synthesised and stored in the glands on the leaf surfaces.

Recently there has been an increased interest from pharmaceutical and aromatherapic companies in volatile oils from Scottish *Myrica* plants gathered in the wild. Bog myrtle communities have been surveyed to determine the possibility of commercially viable wild harvesting, taking into account population density, cutting treatment, and regrowth.

Extensive harvesting could have a significant ecological impact on the species and this has prompted our investigation into propagation techniques, including tissue culture, as a means of producing large quantities of young plants that could be planted into existing and new areas with the aim of increasing leaf and oil production.

SEED PROPAGATION

We collected seed from wild sites in the Autumn of 2003. We cleaned and soaked them for 8 h then mixed them with sand and a small amount of moist peat before putting them into cold store at 0 to 2 °C in tied plastic bags. We started stratification treatment of between 1 and 3 months in December 2003. We stratified some batches in light to determine if this was a required pre-treatment.

On 1 June 2004 we sowed all the seed into trays filled with a peat-based substrate and covered them with medium grade vermiculite. We placed them in an unheated greenhouse to germinate. There was no difference in viability between stratification treatments and light was not required. We also determined that light was not required for germination. We anticipate that 100 saleable plants can be grown per gram of seed collected (Table 1).

CUTTINGS

Previous attempts to root leafy stem cuttings from plants growing in the wild have given poor rooting performance. Cuttings taken from plants grown on from micropropagated material have given excellent results. This is not unexpected since the process of micropropagation produces juvenile plants that have a greater propensity to propagate by traditional vegetative means.

We took 6-cm long cuttings, with the bottom two leaves removed, from stock material grown on from micropropagated propagules. We rooted them under low polythene with no rooting hormone or base heat. The most successful cuttings were taken in mid-July from semi-ripe shoots but cuttings can be taken from new spring growth in late April until August. The rooting percentage was between 60% and 70%. We found that the rooted cuttings were not good transplanters and so they were rooted in the final container using a proprietary peat-based ericaceous-growing medium.

SUCKERS

During July and August 2002 we collected root and sucker propagules from 14 sites in the Highlands. Seventy-five specimens were collected from plants at the late vegetative stage. We took care to include a small amount of soil around the roots and suckers, with an adjoining aboveground stem of approximately 20 to 40 cm. We transferred all propagules to 3-L pots containing a peat-based ericaceous-growing medium and kept them in a non-heated glasshouse, with a natural light regime, over winter.

Table 1. Seed quality from six collection sites, Autumn 2003.

Weight of seed collected (g)	Weight of clean seed (g)	Seed count (per kg)	Number of seed collected	Viability as % of seed collected	Number of viable seed	Number of germinable seed	Germinable seed as (%) of seed collected	Saleable plants at 80%
200	110	432,000	47,520	55	26,136	19,150	40	15,320
200	155	261,000	40,400	55	22,250	18,350	45	14,680
250	160	321,000	51,360	55	28,248	19,200	37	15,360
150	125	597,000	74,600	55	41,030	40,500	54	32,400
100	60	395,000	23,700	55	13,035	12,300	52	9,840
100	75	456,000	34,200	55	18,810	15,700	46	12,560
								100,160

Ninty-five percent of suckers rooted successfully and the first shoots were clearly visible at the end of March 2003. Even those cuttings that looked dead showed buds on close inspection within the root system and produced new shoots during the summer season. We monitored several plants closely for bud, leaf, and flower growth and development.

Following the success of transplanting the suckers we carried out a field trial to see if the method could be used to increase natural populations. In practice, the cost of large-scale lifting and transplanting was found to be as high as the production and transplanting of micropropagated plants.

MICROPROPAGATION

Materials and Methods. A literature search back to 1976 revealed only two references relating to in vitro propagation of this species, and both used, as the starting material, germinating seeds. Consequently we had to develop our own technique for micropropagating *M. gale* using young shoot tip explants.

We collected young shoot tips, cones, and side shoot tips from 14 sites throughout northern Scotland in Spring and Summer 2002. We chose randomly distributed plants at each site and tagged them as reference for future visits. We also repeated our micropropagation experiments on shoots from regenerated cuttings collected in Summer 2002. Young shoot tips were taken from pot grown cuttings between March and June 2003.

We cut the explants from branches immediately prior to surface sterilisation by immersing in 5% sodium hypochlorite containing 0.1% Tween 20, agitating for 5 min, and rinsing three times in sterile distilled water. Following sterilisation, we trimmed loose leaves and cut 1 to 2 mm off the lower extremity of the explant to remove any damaged tissue.

The medium was based on the mixture used by Tavares et al. (1998), with 2 $g \cdot L^{-1}$ of glycine, 30 $g \cdot L^{-1}$ of sucrose, 1.23 mM IBA (indole-3-butyric acid) as a growth regulator, and vitamins of Gamborg et al. (1968).

We incubated all cultures in a growth room with a 24 to 22 °C thermoperiod and a 17-h photoperiod. Subculturing into the same medium after 6 weeks resulted in successful production of plantlets within the next 3 weeks.

We split single shoots (15 to 35 mm in height) from the clumps of microshoots and placed them in rooting medium. After 3 to 4 weeks, thick pink roots (2 to 10 mm long) were formed in clumps on single microshoots. Then, after 1, 2, or 3 weeks, we transferred these to a peat and perlite mix, or to vermiculite, in P-180 plug trays which we incubated in the growth room in polythene bags for 1 week. After that we transferred them to a glasshouse.

In our first experiments we initially placed the trays of plants on a glasshouse bench inside a polythene tent covered with gauze and three layers of Lowbrene 50% shading to maintain humid conditions. We misted the plants three times a day and after 1 week of acclimatisation the polythene was removed. In subsequent experiments, were placed the plants on a damp sand bed under a polythene tunnel covered with gauze and shading. The polythene was removed after 3 days, the gauze after 1 week, and shading was kept in place.

We transferred the established plantlets to Alba Trees for further growth. Here we put them into In Plasnor 150 modular trays with a standard, peat-based, ericaceous-growing medium and kept them in the nonheated glasshouse for 1 year.

Results. Plant material taken in June and the beginning of July regenerated easily in contrast to material from the August harvest. The later explants were severely contaminated and the remaining sterile cultures usually died within 6 weeks. From 1300 explants only 18 were viable after that period. All cones were heavily contaminated and none produced viable shoots.

Explants originating from the 2003 cuttings survived well in the humid conditions of the polythene tent; all plants in peat and perlite were healthy and green. Plants in vermiculite developed brown leaf tips while early removal of polythene resulted in dried up, brown seedlings.

Established plants at Alba Trees had a survival rate of 80% and were suitable for planting out after 1 year of growth.

CONCLUSIONS

We established 20 clones representing 11 sites and produced successfully thousands of plants. However, only shoots taken from the plants at the beginning of the vegetative period were highly successful in micropropagation.

Division and multiplication can take place every 3 to 4 weeks. Three weeks in shooting medium and 3 weeks in rooting medium is optimal for healthy plant establishment. We estimate 3 weeks is sufficient time for explants from the rooting medium to be weaned in potting medium with peat and perlite producing the best results. The sand bed method of weaning provided the best weaning environment and resulted in the best plant survival rate of between 80% and 90%, depending on the viability of a given clone. Supplementary lighting is required for plants weaned in the glasshouse between September and March.

Fungal infection was the main identifiable cause of losses but there was also unexplained browning of tissue and unexplained shrivelling and death, and loss of vigour. These signs were not consistent within any given clone.

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