out for several maples to be about 25% moisture.) These maples tend to have a seed coat that becomes more leathery upon drying. The leathery material of the seed coat is quite hard to re-wet so that water and oxygen will penetrate the seed coat and promote the biochemical changes that enhance germination. Drying the seed may also cause other unknown changes in woody seed biochemistry.

Examples in this group are *A. saccharum*, *A. circinatum*, *A. glabrum*, *A. pseudo-sieboldianum*, *A. palmatum* var. *atropurpureum*, and *A. campestre*. We find that to germinate these easily, we must collect them while the "nut" of the samara is still green, clean the fruit/fiber off the seed coat, and then store the seeds naked in polyethylene sacks in the cooler. Another, easier strategy is to just fall plant them in the field, with care taken to mulch the seedbeds well so that the germinating seeds do not succumb to frost in the spring.

Group 4. Among the most difficult to germinate maples are those that have a thick, bony seed coat. The trifoliate maples *A. griseum*, *A. maximowiczianum*, and *A. triflorum* are notable examples. Rare species such as *A. sterculiaceum* and *A. sutchuense* are also included; these are within the Lithocarpa section, meaning "fruit like a stone". Allowing this seed to dry below 25% moisture will usually result in at least a year delay in germination. Most nurserymen don't have the patience for germination more than 2 years after collection of the seed. Dried seeds in the *A. griseum* group can take two, sometimes even three cycles of warm/cold pretreatment to prompt them to germinate.

Setting Up a Small-Scale Micropropagation Lab

David Woodske
British Columbia Ministry of Agriculture & Food, 1767 Angus Campbell Road, Abbotsford, B.C., Canada, V3G 2M3

INTRODUCTION

During the past three decades, micropropagation has proven to be an economical and technically feasible method of propagation for some crops. However, individuals are often discouraged from setting up a lab because they believe it is very expensive. A large lab is expensive to set up. The cost to construct and equip a lab designed to produce 500,000 plants per year has been estimated to be $250,000 (Sluis and Walker, 1985). Establishing a small-scale lab to produce 100,000 plants per year should not be prohibitively expensive. In addition, it can provide an opportunity to train staff, develop culture protocols, and to evaluate the business with minimal capital inputs. If it shows promise, more expensive, specialized lab equipment can be purchased down the road to increase the lab's efficiency.

Before starting a lab, all propagation options should be considered, which includes hiring a lab to do contract micropropagation. Micropropagation is not an easy business to make successful. Many new labs do not survive more than a few years. One reason is the high operating costs the owner will have to cover in the early years while cultures are initiated and production protocols are perfected. It will take years

for the lab to have a good selection of varieties available. Other obstacles to success are the availability of skilled staff, maintaining healthy and vigorous cultures, and being able to market all of the product for a fair price.

ADVANTAGES OF MICROPROPAGATION

Micropropagation has several advantages relative to traditional methods of propagation:

- The ability to propagate crops that are difficult to propagate.
- The high rate of multiplication (Table 1). Changes in the multiplication rate have a dramatic effect on shoot production (Table 1). Therefore, the protocol must be reliable and production figures need to be closely monitored in order to meet production targets.
- The high quality of micropropagated plants. This is due to their freedom from diseases and enhanced basal branching (e.g. bushiness). Increased bushiness is a result of the plant's short internodes and the hormone treatments used in the lab to promote multiplication. Bushiness is not always a desirable trait. For instance, basal branches interfere with mechanical harvesting of blueberries.

Other advantages are the ability to produce plants year-round, the low land requirement, and the elimination of the need for stool beds.

Table 1. Influence of the multiplication rate on shoot production with a 6-week subculture period.

Multiplication Rate	Week 1	Week 17	Week 34	Week 52
2.0	1	8	64	512
2.5	1	16	244	3,815
3.0	1	27	729	19,683*

*Almost a million shoots could be produced from 50 shoots in a year.

POINTS TO CONSIDER WHEN SETTING UP A LAB

Staffing. Staff will have a profound impact on the success of the business. Hiring skilled staff is important, but often can be difficult. For a lab that plans to produce crops commonly micropropagated, staff with a basic knowledge of sterile technique and micropropagation may be adequate, since media recipes and production protocols for such crops are often available in the literature (e.g. books, the Internet, government and post-secondary education institutions, and research journals). Nursery crops that are commonly micropropagated include *Acer rubrum, Betula nigra, Clematis, Hemerocallis, Heuchera sanguinea, Hosta, Kalmia latifolia, Leucothoe fontanesiana, Rhododendron,* and *Syringa vulgaris.* Since developing and refining media recipes is always required for new crops, and is needed at times for a protocol from the literature or for a different variety of a crop, hiring an experienced micropropagator should be a priority.

For training in micropropagation, good courses are offered by community colleges and private businesses. Western Biologicals Ltd. in Aldergrove, B.C., provides a 2-day training course that covers the basics; media preparation, subculturing, and building a laminar-air-flow (LAF) cabinet.

Lab Size and Layout. A small-scale micropropagation lab does not require a tremendous amount of space. Two to three rooms with a total area of 50 m^2 is sufficient. A kitchen works well as a media preparation area, since basic kitchen appliances and facilities are used, such as a sink, dishwasher, powerful microwave oven (1000 W) to melt agar, refrigerator, freezer, and sufficient storage area for glassware. A chemical storage cabinet is required, as is a stove if a pressure sterilizer is used.

Labs generate considerable heat, especially the growth room, which can kill cultures. One way to reduce the heat load in the growth room is to wire the lights' ballasts into a closet or adjoining room. Air conditioning is often still required, since fluorescent tubes produce a lot of heat. The amount of cooling required will depend on the number of light fixtures used and the room's normal temperature. Light shelf requirements will depend on total production, and the number ofcrops harvested per year. Shoots are grown at a density of 1200 to 1700 m^{-2}. Locating the growth room in a cool basement may eliminate the need for air conditioning.

A major requirement of the lab is cleanliness. Culture contamination is the most serious threat to a lab and all steps possible should be taken to reduce contamination. Perhaps the most serious and damaging contamination occurs when thrips or mites enter the cultures. It usually is not identified until the situation has become too severe to avoid major culture losses. To reduce the incidence of contamination, the lab should:

- Have flooring that is easy to clean (not carpeting),
- Frequently mop the floors with a mild disinfectant,
- Individually seal all cultures (e.g. Parafilm®, plastic wrap) or entire trays of cultures,
- Keep traffic in the lab to a minimum,
- Consider filtering all air entering the lab with a HEPA filter, and
- Never use houseplants as part of the lab decor.

Equipment and Lab Supplies: The two major pieces of lab equipment required are a sterilizer and a LAF cabinet. The two types of sterilizers commonly used are pressure sterilizers and autoclaves. An autoclave is the preferred option, since it can sterilize considerably more medium per hour. This is a result of their larger capacity and of being fully automatic. However, a new autoclave can cost more than $5000 and may require 3-phase power. A pressure sterilizer, such as the All American™ brand, is a relatively inexpensive alternative ($300 to $500). However, even with two 25-litre sterilizers, the maximum rate of media preparation is 2 liter h^{-1} with baby food jars. With this system, media preparation will account for about 25% of a lab's direct labor costs.

Attempts to mechanize the subculture process have not been successful, yet. Labs still rely on workers to aseptically divide cultures in a LAF cabinet. Therefore, there is not a significant difference in the subculture rate between large and small labs, as long as the LAF cabinet is well-illuminated and is a comfortable size (i.e. at least 3 ft wide). LAF cabinets are easy and inexpensive to build. All that is required is a blower, HEPA filter, furnace pre-filter, and plywood to build the cabinet. HEPA

filters are sold by Western Biologicals Ltd. in Aldergrove, BC. A 4 ft cabinet can be built for $700, whereas a manufactured cabinet can cost $8000.

Other equipment required are forceps and scalpels ($150), a pH meter that has a resolution of at least 0.1($70 to $250), and a balance that can weigh to at least 0.01 grams ($450+). Two systems commonly used to sterilize the implements used during subculturing are dipping in ethanol and flaming, or electric heat units. Flaming works well and is relatively inexpensive, but it is hazardous due to the risk of fire. Electric units include glass bead sterilizers ($350 to $400), infra-red sterilizers ($450), or the Lee Precision Melter ($50). Melters are an inexpensive, variable-heat sterilizer. The chamber must be filled with a material to hold and distribute the heat, such as gravel, sand, or glass beads. They are available from Lee Precision Inc. (Tel: 414-673-3075).

A good supply of containers is also required. Container size is directly proportional to subculture rate and to the occurrence of culture contamination. Therefore, a tradeoff must be reached between subculture efficiency and culture losses through contamination. Test tubes are often used for the culture initiation stage to reduce the amount of material lost to contamination, which is often very high at initiation. Once a clean, proliferating culture is established, a larger container is used, such as baby food jars, canning jars, or polypropylene deli-tubs. Autoclavable lids for baby food jars are available from the Sigma Chemical Co. and cost $0.50 each.

A range of chemicals are used to make culture media. The lab can purchase *individual chemicals or prepared media powders. Individual chemicals provide the* lab with greater flexibility, and are essential to develop protocols in-house. The cost of media produced from individual chemicals is about $1.10 per liter, whereas prepared media cost over $5.00 per liter. One supplier of prepared media is the Sigma Chemical Co. Look in the Yellow Pages™ for chemical suppliers in your area. Purchasing chemicals is becoming more difficult and may restrict the operation of small labs. In Canada, chemical suppliers will only sell to businesses that have a chemical storage cabinet and that are located in a non-residential area.

Water is also required. For a small-scale lab, it is cheaper to purchase bottled water treated by reverse osmosis than to purchase a filter system. As a guide, about 250 liter of water is sufficient to produce 100,000 shoots.

CROP SELECTION

Micropropagation is not economically viable for most crops. It is labor intensive and, therefore, is a relatively expensive method of propagation. Labor can account for 40% to 50% of a lab's operating expenses. Costs of production vary between different varieties and labs. In general, the cost to produce an unrooted shoot is $0.15 to $0.25. Problems encountered during rooting can significantly increase production costs per plant.

The most appropriate plants to micropropagate are those that can demand a high price in the marketplace. Some micropropagated crops receive a high price as a result of their quality. However, labs usually need to select crops that are difficult to propagate or that are new introductions to the industry.

LITERATURE CITED

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