The Moss Machine in action: a preliminary test of a low-cost, indoor cultivation method for mosses

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Abstract

Mosses and lichens are not generally used in green architecture and gardens in Canada, despite being widely used elsewhere in the world. This may be partly due to a lack of products that promote or incorporate them as landscaping elements. We built a low-cost growth cart specifically for mosses and lichens, called the “Moss Machine”, and tested its ability to grow four types of moss over 4.5 months in an indoor setting. Moss growth ranged from 1.5-4.5 cm over the duration of the study, with differences primarily due to species identity. We discuss the limitations and benefits of this initial design and recommend improvements for the next stage of development.

Introduction

Mosses and lichens have many beneficial qualities that make them suitable for applications in landscape-architecture, but they are currently under-utilized in Canada. Examples of beneficial qualities include that many species can dry-out during hot weather and re-hydrate (like a sponge) when it rains, meaning their need for supplemental watering would be little to none after establishment. Such traits make them more suitable than vascular plants for applications where water conservation or runoff-moderation is a priority (Anderson et al. 2010). Another beneficial quality is the minimal structural support required for buildings with moss-dominated green roofs. Green roof opportunities in much of Canada are limited by the inability for older buildings to support the weight of soil-based green roofs. Mosses and lichens lack roots, and therefore do not require soil. This means they could be applied to rooftops with little to no structural enhancement (Anderson et al. 2010), and there is no risk of root-wedging leading to structural damage. The combination of a small shoot-size with a colonial, mat-forming growth habit makes them amenable to applications ranging from small-scale, indoor greenery to large-scale outdoor rehabilitation projects. Adding mosses and lichens to the suite of green architecture species expands the market to include residential, as well as commercial structures, thereby increasing access to green roof esthetics and ecological services for all Canadians.

Given these beneficial qualities, it is surprising that mosses and lichens have not already become more extensively used for landscaping applications in Canada. We speculate that this is because: (1) their small stature means they are more difficult to notice, and more difficult to identify; (2) there is a cultural bias, in that Canadian gardeners, planners, and botanical education programs have historically emphasized vascular flora over non-vascular flora and lichens to a greater extent than in some other countries (e.g., Japan); (3) there are limited resources (equipment, propagules, and expertise) available for those interested in growing mosses and lichens. Our goal in this paper is to fill the last of these three knowledge gaps, by building and tested a self-contained cart designed for rapid propagation of bryophytes and lichens.

Five features are important for rapid moss or lichen cultivation: (1) multiple layered substrata for high-volume production; (2) limiting temperatures to 21 C or less; (3) regular but intermittent water supply in dispersed form (i.e., misting), (4) a porous substrate for improved water retention during initial growth stages; and (5) an appropriate chemical composition of water. These features can easily be incorporated into a growth cart with relatively simple and low-cost equipment (e.g., Haughian and Frego, 2015; Katagiri et al., 2015), even with limited access to the amenities normally associated with large research institutions (e.g., de-chlorinated water, greenhouse space).

Materials and Methods

Study area and species

Source material was collected from Community Forests International's Rural Innovation Campus, otherwise known as Whaelginbran Farm, near Sussex, New Brunswick, Canada (45.735° N, -65.301° E). This area exhibits a northern temperate maritime climate, with cold, snowy winters and warm, wet summers. Mean daily temperatures range from -8.5°C in January to 19.2°C in August, and mean annual precipitation is 1170 mm, with approximately 20% falling as snow (Environment Canada 2017). Growth trials took place in a laboratory at the Port Hawkesbury Paper Mill in Port Hawkesbury, NS, Canada.

Mosses were chosen with the goal of representing dominant species in low- and high-light conditions. Pleurozium schreberi, a robust, laterally-growing (pleurocarpous) moss of the forest floor, and "Dicranum mix" (a 1:1 mix of Dicranum scoparium and Dicranum polysetum), consisting of robust, vertically-growing (acrocarpous) mosses of the forest floor, were collected from the shady habitat. This shady habitat was on a gentle, southeast-facing slope, in a mature forest dominated by Eastern White Pine, Eastern Hemlock, and White Spruce, with Balsam fir in the understory, where canopy cover ranged from 30-60%. In the high-light habitat, we collected Brachythecium salebrosum, a laterally-growing, medium-sized moss that is common on lawns and roadsides in the area, and an "Atrichum mix" (a 1:1 mix of Atrichum oesterdianum and Polytrichum juniperinum), which is common on roadsides, ditches, and other exposed or partially-exposed habitats. These high-light species were collected from road banks and a lawn approximately 300 m southeast of the forested area from which the shady species were collected. Approximately 8 L of each moss type was collected, stored in plastic bags, and kept in a refrigerator at 4°C overnight.

Growth cart design

Our growth chamber, which we call the "Moss Machine", is a multi-layered, low-cost (~$950) growth chamber that allows for rapid cultivation of mat-forming mosses and lichens over layers of organic fabric, resulting in blankets of live vegetation for landscaping applications. It combines the tray and misting systems used by many Japanese moss nurseries (e.g., **Error! Reference source not found.**c & d; Echigokokesho Corporation 2017, Japan Moss Technique Association 2017), with the space-saving requirements of stacked-tray hothouse production systems. It also takes advantage of recent advances in small-scale LED grow-lighting systems to limit the cost and stressful temperature increases associated with halogen or fluorescent lighting in growth carts (e.g., Floralight systems), and recent advances in small-scale activated carbon filtration systems to control water chemistry. All items used in the construction of this apparatus are readily available through internet-based stores or local department stores in much of North America.

The basic structure of the Moss Machine was a wire shelving unit in a pump-drained basin on wheels. The shelf was a 6-layer, stainless steel unit, supported by 2.5 × 200 cm stainless steel posts and holding six, 45 × 122 cm, wire trays. All stainless steel was coated with a white rubberizing spray (Plasti-Dip®) to reduce leaching of metal ions. A plywood tray (30 cm deep) was attached closely around the base of the scaffold, as a basin for water collection. The tray was fitted with a rugged plastic sheet (a backyard pond-liner), and attached to the shelf using the casters and shelf legs as a bolt and nut, thus securing the legs of the shelf to the basin, and ensuring that the entire unit could move freely within the laboratory space without leaking. The basin was fitted with a vertical float-switch connected to a submersible fountain pump (1280 L/hr) and garden hose (1.9 cm diameter), which drained into a nearby sink when water had accumulated in the basin. The entire apparatus was surrounded on the sides and top by clear plastic sheets, to prevent the water from impacting other laboratory operations, but ventilation gaps were left at the top corners to ensure that warm air could escape.

Figure 1: Photos of the Moss Machine, showing (a) the entire apparatus, uncovered, prior to operation, (b) the clear plastic covering and lighting in operation, and (c) the float-switch and pump in the drainage basin, under the lowest shelf.

The watering system was designed to provide light daily watering with dechlorinated water. A standard 0.95 cm plastic misting hose was woven through each of the long sides of each of the top 5 wire shelves, and nozzles were installed approximately every 16 inches (6 per shelf) and aimed towards the center of the shelf below. This ensured adequate coverage of water to the entire growing surface. The input to the watering system was municipally-treated tap-water, passed through a filter (Boogie Blue Plus+) for removal of fluoride, chlorine, chloramine, and other potentially harmful ions. The filter and misting system were fitted to a conventional laboratory faucet, with the cold water valve in the on position, with flow controlled by a single-zone digital water timer (Melnor Hydrologic system).

Lighting was a mix of ambient daylight and supplemental grow-lights, both to increase growth rate and to ensure comparable light penetration to different layers. Ambient daylight came through several south-facing windows in the laboratory. Water-resistant LED (blue and red) grow-light strips (5 m, 12 V) were woven through each of the top 5 shelves and aimed downwards.

Propagation & growing conditions

Clumps of mosses were sifted, fragmented, and layered over a nutrient jelly on burlap. Field-harvested material was broken up manually over a sieve under running water to wash off excess particles of soil or organic matter, and to remove visible fragments of non-target species. Once visibly clean, mosses were fragmented for approximately 10-15 seconds in a commercial blender with distilled water. The nutrient jelly was prepared from a solution (1.0 mL / L) of commercial hydroponics fertilizer (General Hydroponics Floragro) and distilled water, with (5 g / L) low-acyl Gellan gum (Special Ingredients Ltd., Chesterfield, UK) as a thickening agent. We chose this gelling agent because it is less susceptible to fungal contamination than traditional agar (Duckett et al. 2004), and this concentration of liquid fertilizer because it approximated the total N content of nutrient solutions used for bryophytes in previous studies (e.g., Sargent 1988). The nutrient medium was allowed to cool and set overnight, then stirred and evenly spread over a double-layer of burlap on each of four shelves (approximately 2 L per shelf). Moss fragments were then evenly scattered over the nutrient jelly.

Irrigation was scheduled to apply 1 minute of misting spray, every 20 minutes, 24 hours per day throughout the trial. Water usage was monitored with a digital electronic meter (P3 P0550) attached to the intake hose throughout this trial. Lights were set to a 12-hour on-off cycle, and ambient daylight ranged from 12-14 hours for this first growth trial. The machine and moss growth were inspected four times during the test period of 4.5 months (135 days).

Moss growth measurements

Moss growth was measured in two ways. Beginning with a mean height from the burlap surface of the propagules, the mean vertical elongation was recorded a total of four times throughout the initial 4-month growth period (May-September). While vertical elongation is not always an ideal measurement of growth for bryophytes, due to their varying growth strategies (i.e., predominantly lateral growth in some species), previous experience had suggested that all species would initially respond by exhibiting obvious vertical growth. Vertical growth was visually estimated as the maximum height on a ruler at which the gradations were obscured, when viewed in profile at the same height as the mosses. To account for horizontal variability within each layer, the mean of 5 haphazardly chosen measurement locations was used as the height for that layer.

The second growth measurement was only taken at the end of the experiment, due to logistical difficulties of applying it earlier on. The total cover of live material, and the relative greenness of each layer, were derived from digital photos (taken using the panoramic mode with a Samsung Galaxy S8$^+$), to provide a measure of horizontal spread and colony health. Total cover of live material was determined by manually applying a pixel classification of "live moss" (obviously green shoots) or "other" (bare burlap, blackened inoculum, or brown shoots), using the ‘threshold colour’, ‘threshold’, and ‘measure’ functions in ImageJ software (Rueden et al. 2017).

A third measurement, of overall moss “health”, was also inferred from the photos. The relative greenness of each layer was assessed using the ratio of mean G value to mean R or mean B values, across all pixels in the image, using the ‘RGB Measure’ plugin for ImageJ software (Rueden et al. 2017). While more sophisticated models exist for translating RGB image data into chlorophyll or Nitrogen content (Su et al. 2008; Yadav et al. 2010), none have been developed or tested for bryophytes, let alone for bryophytes raised under nursery conditions; developing such an index would be beyond the scope of this paper. Nevertheless, indices using mean G:R and G:B values may provide a reasonable index of the health and cover of photosynthetically active tissues in the photo until more sophisticated models can be developed and tested.

Results

Machine performance

The Moss Machine was sturdy and reasonably reliable throughout this initial test. The basin did not leak or fail, and the pump and float-switch drained accumulated water as needed.

Watering was consistent, with only 1,204 L being used over the duration. This is well within the filtration capacity of the activated carbon filter (180,000 L). The filter appeared to work effectively to limit harmful ions – although we did not measure the ionic content of water before and after filtering, the survival and growth of all moss species suggests low concentrations of harmful ions. In contrast, mosses watered with chlorinated tap-water rarely survive for more than two weeks (personal observation, SRH).

The distribution of water appeared to be uneven among layers: lower layers received more water, which was evident in the greater amount of pooling and overall wetness of the layers during monthly inspections).

The PlastiDip® coating limited rust development on the wire shelf, except where it had been abraded by shelf brackets and plastic ties, or at difficult-to-reach joints where the metal surface had not received sufficient coverage. However, this rust development was primarily limited to the periphery of the shelving unit.

Given the high rates of moss growth, the supplemental lighting seems to have had the desired effect. Moreover, no noticeable change in heat was present in the cart during measurement periods, confirming our prediction that LED lighting would cause significantly less heat accumulation than fluorescent lighting. Nevertheless, the specific lighting that was used was not completely reliable; in three out of four of the initial LED strip-lights, water seeped through the waterproofing layers, resulting in partial outages for unknown periods of time (outages were detected only upon finding them during monthly visits to the laboratory). Lighting was rearranged to compensate for outages immediately after being detected, by adding new strip-lights, or, when supplies ran low during the second-last visit, by redistributing lights among layers to ensure equal lengths of lighting in each layer. Nevertheless, the bottom layer (Brachythecium) may have been completely without supplemental lighting for upwards of 6-8 weeks, as both strips for the bottom layer had failed when the machine was inspected in August.

While power usage was not monitored, we estimate that, in the 3.5 months before growth plateaued, a maximum of 362.9 kilowatt-hours of electricity were consumed. This was calculated using the manufacturer's specifications for the light strips (12 V, 6 A), assuming all four were operating for 12 hours each day over the course of the study.

Figure 2: Short side of a shelf after 4.5 months, showing rust development at two joints in the wire shelf, and a location on the LED strip-lighting where water penetrated the waterproof coating.

Moss growth

For all species, newly grown shoots exhibited an irregular form, with small, uncharacteristically spreading leaves, long interleaf distances, and lighter than normal colours. In terms of vertical elongation, Pleurozium had the greatest growth, followed by the Dicranum mix, the Brachythecium, and the Atrichum mix (Figure 3). In terms of total area covered by live shoots, the Atrichum mix and Pleurozium showed the best growth, at 71% and 68%, respectively, followed by the Dicranum mix (59%) and Brachythecium (53%). Colour composition differed among moss types, with the highest mean G:R and G:B ratios recorded in the Atrichum mix, followed by Brachythecium (Figure 4).

Despite an apparently even distribution of fragmented mosses to each layer, some patchiness developed as mosses grew. This was most noticeable in Brachythecium (lowest) layer, where shoots were longer, growth was thicker, and fewer gaps in cover were present on one side of the cart than the other (Figure 6). This layer was the only one that sat completely below the height of the counters in the laboratory, thereby restricting the inputs of ambient light. The thicker side of this layer was also closest to the windows which allowed for natural light to enter the laboratory.

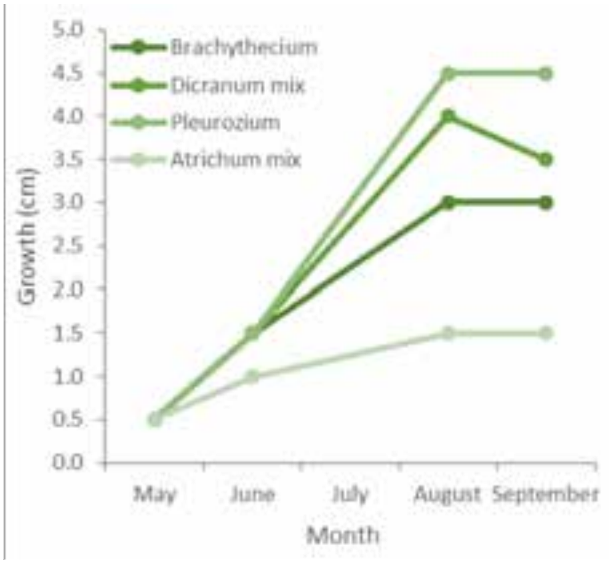

Figure 3: Vertical growth of different bryophyte types over 4 months, measured as mean vertical height of shoots from the substrate.

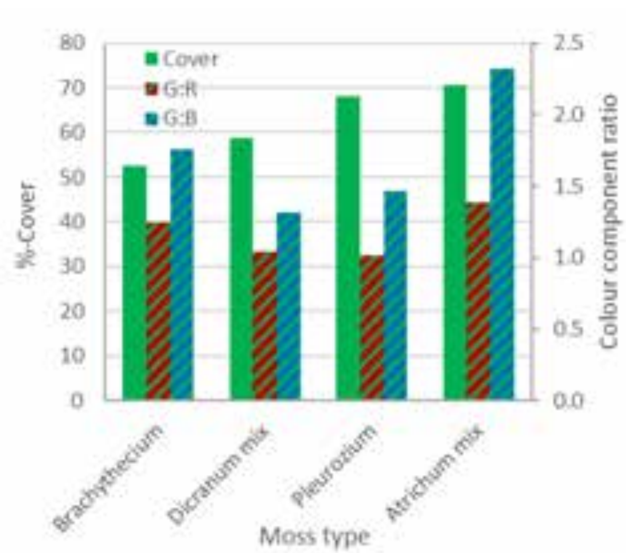

Figure 4: Cover (%) and RGB colour component ratios (G:R and G:B) of four moss types in the Moss Machine, after 4 months of growth.

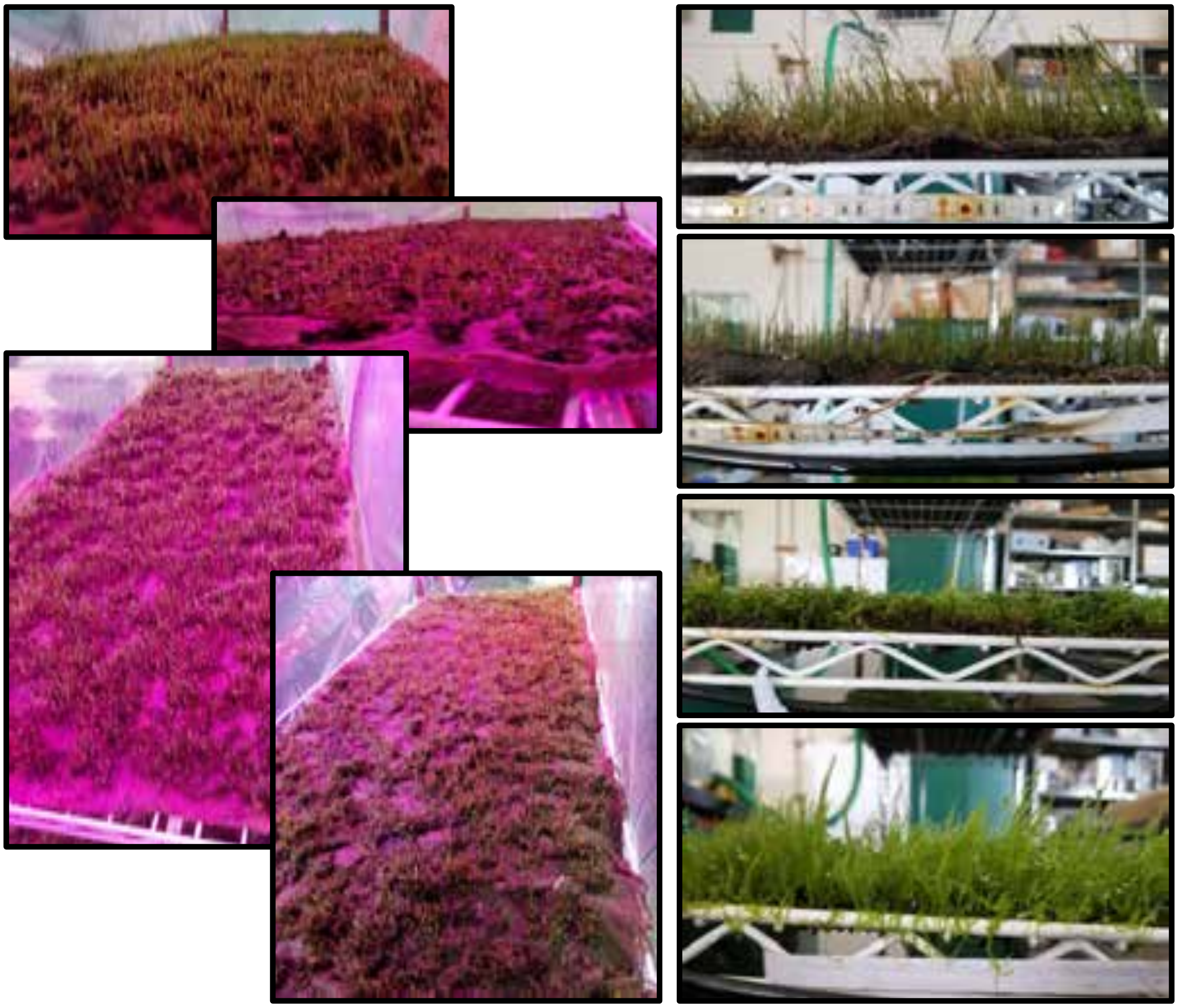

Figure 5: Moss layers from the narrow side of the shelf at 1 month (left) and 3.5 months (right); top to bottom are Pleurozium schreberi, Dicranum mix, Atrichum mix, and Brachythecium salebrosum.

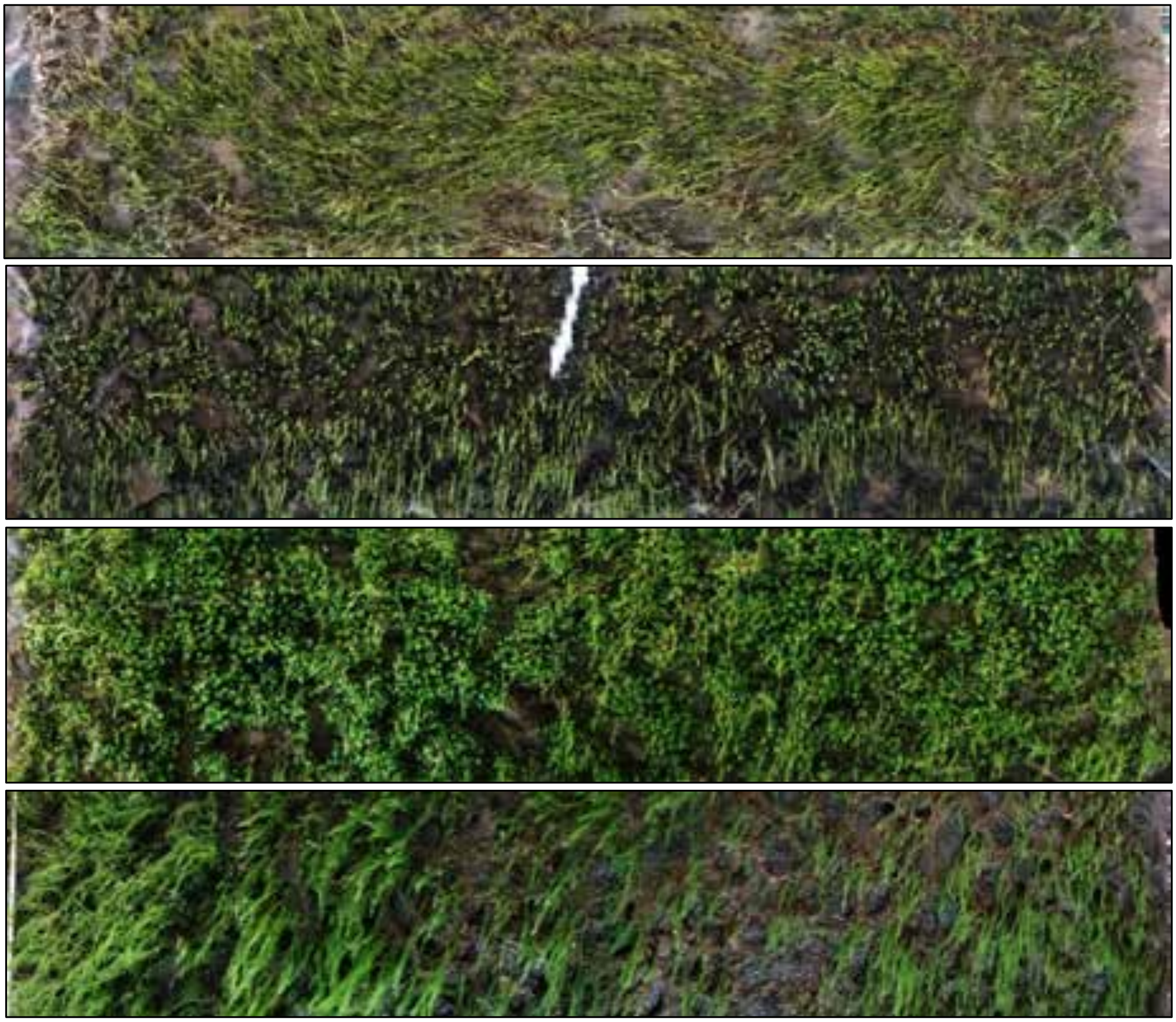

Figure 6: From top to bottom, Pleurozium schreberi, Dicranum mix, Atrichum mix, and Brachythecium salebrosum.

Discussion

Moss growth

All four moss types displayed measurable growth after 4.5 months in the Moss Machine, but apparently differed in how they allocated growth resources. Pleurozium, Brachythecium, and the Dicranum mix appeared to respond quickly to the conditions, exhibiting rapid and sustained, but irregular, shoot elongation. In contrast, the Atrichum mix exhibited the least vertical growth, but the greatest horizontal cover and G:B or G:R ratios at the end of the experiment. None of the moss species produced sporophytes.

The apparent stagnation of vertical height increase between the last two observation periods was likely because the weight-bearing capacity of the shoot base was exceeded once shoots reached 3-4 cm, resulting in shoots leaning towards the substrate (i.e., exhibiting decumbency), rather than because of a biological cessation of shoot-elongation. For the Atrichum mix, the reason for the apparent stagnation of growth was not obvious, as the shoots did not exhibit the same degree of rapid elongation and irregular growth or decumbency. It is possible that an additional application of nutrient solution would remedy this in the future.

The irregular growth forms seen here have been observed in other moss species under laboratory conditions, but this is the first study to document such growth in a relatively open misting chamber. In contrast, Haughian and Frego (2015) grew a Dicranum species with relatively normal morphology in a similar misting chamber; however, their mosses were watered on a different schedule (1 minute misting every 10 minutes for 6 hours each morning). Similarly, (Shaw 1986) used a misting system with misting delivered for shorter durations ("several seconds") and at longer intervals ("every 15-30 minutes"); however, the difference with his study is confounded by the fact that he grew bryophytes over soil in a greenhouse, rather than over sterile nutrient medium. The similarity with atypical growth often observed in in vitro growth trials, and dissimilarity with relatively normal growth observed by misting-chamber experiments (Shaw 1986; Haughian and Frego 2015), suggests that the irregular growth response is most likely due to constant, high humidity.

We speculate that both watering too frequently, and applying too much water over the course of the day, could limit gas exchange in mosses as it does in some lichens (e.g., Gauslaa 2014), since the diffusion resistance of CO_2 in water is significantly greater than that in air (Proctor 2009). Normally, many mosses have growth forms which are adapted to capture and hold water under mild vapour pressure deficits, and become desiccated under more severe ones (Proctor 2000). When forced to grow in an abnormally wet environment, developing exceptionally-long, thin, and sparsely-leaved shoots would reduce the volume of extra-capillary spaces, thereby enhancing the rate of drying and facilitating gas exchange between misting periods. Future studies should test whether the degree of irregularity in moss shoots is caused by the length of time in which the mosses are saturated with extra-capillary space water.

Moss Machine Design

Overall, the Moss Machine was an effective way to rapidly cultivate mosses under greenhouse conditions, without needing a greenhouse. Given the growth response curves that were observed, no more than 3.5 months should be necessary to achieve substantive vertical growth. Nevertheless, several improvements would likely enhance the consistency, vigour, and efficiency of moss growth in future trials.

While the lighting generated little heat, it was not as waterproof as was initially expected. In the future, more rigidly-housed, water-resistant lighting, or the addition of a layer of silicone to the back of lighting strips, would likely improve the longevity of supplemental lighting. In addition, using an entire strip in each layer, rather than bending and twisting the strip to illuminate part of another layer, would likely result in fewer breaks and opportunities for water intrusion, while also making replacement easier, because it would not require adjustment of all the other lights to compensate. Ambient light should also be controlled to a greater extent, either by regularly rotating which side of the Moss Machine is closest to any sources of natural light, or by adding additional supplemental lighting. The lack of even penetration of natural light, combined with the loss of supplemental lighting for unknown periods of time, was likely why the Brachythecium layer developed excessive patchiness.

By the second observation period, it was obvious that water was pooling to a greater extent in the lower shelves than in the upper ones. This was because they had the same misting spray as other layers, in addition to the water that seeped through upper layers. Reducing the number of misting nozzles in lower shelves might reduce this impact in the future, but it would be difficult to determine how many nozzles should be removed to match the water gained from leaching above. Instead, shelves should be given a shallow peaked shape (like a roof), and covered with a thin impermeable layer, such that excess water is still shed from the mosses, but drips down the sides of the machine rather than onto the layers below. The total amount of water can also be reduced; to achieve more regular growth forms, watering could be limited to 6-12 hours per day.

The development of rust spots would likely be reduced by the use of an anti-rust paint with an enamel finish. The PlastiDip® coating was easily abraded from the surface of the cart when other hard objects were rubbed against it. While the few rust spots that developed were not likely a problem for the mosses, given that these spots were largely on the periphery of the machine, limiting rust development from the start is preferable.

Future directions & follow-up

The market for these products appears to be growing in other countries and would likely be an attractive expansion for current nurseries and green-roof installers in Canada if the technology could be scaled-up. In the USA, Australia, and Japan, mosses are actively cultivated, sold, and used for gardens, green rooftops, living walls, and related architectural applications (Benner 2017; Echigokokesho Corporation 2017; Japan Moss Technique Association 2017; Martin 2017; Williams et al. 2017). Although one of these suppliers currently ships to Canada, they are unlikely to meet with success in the Canadian market, because the species they cultivate are adapted to a much warmer climate. Moreover, importing mosses from other countries risks spreading invasive species, and may therefore be restricted by international law pertaining to the movement of soils. For a Canadian market to thrive, locally-calibrated cultivation and reintroduction methods are likely required.

This technology would also be attractive for both conservation, demonstration, and scientific purposes. Several species of moss or lichen are currently classified as being of "special concern", "threatened", or "endangered" in Canada (e.g., Committee on the Status of Endangered Wildlife In Canada 2009, 2010). Nevertheless, because few mosses and lichens have been used in landscape architecture, ex-situ experiments, or public demonstrations, scientists lack many of the tools that have been used to increase awareness of plant conservation, augment native populations of animals and vascular plants, and understand the life-histories of such species. In contrast, seed-distribution programs and seed- and tissue preservation centres are comparatively well-established and successful for vascular plants (Donnell and Sharrock 2017; Mounce et al. 2017); while some programs for lichen and moss preservation have been recently initiated by European institutions (Rowntree et al. 2011; Sabovljević et al. 2014), conservation efforts are largely limited to monitoring and habitat protection in North America, and public awareness remains low. Among the few studies available, much of the published research on transplantation of mosses and lichens is by ecologists examining both the potential for reclamation of disturbed habitats (cf. Duncan 2015), and the impacts of forest disturbances on growth and sensitivity of different species (Palmqvist and Sundberg 2000; Muir et al. 2006). Developing and testing cultivation methods for common moss and lichen species would be an important step towards improving conservation, public awareness, and scientific access to rare or endangered mosses and lichens.

Currently, the greatest impediments to broad-scale application of mosses and lichens to green roof systems are the lack of efficient local production systems and the lack of awareness of their potential utility and attractiveness in landscape architectural applications. Ecologists have already successfully transplanted wild-harvested Cladonia species, and found that mosses and lichens have improved the properties of green infrastructure such as green roofs (Heim and Lundholm 2013, 2014; Heim et al. 2014). This study has demonstrated that mosses can be grown with ease in an indoor setting. The mosses from this first trial are now being field-tested on a green-roof, with survival and growth contrasted against wild-harvested material over several months. We look forward to additional testing of new watering regimens, lighting designs, and species combinations as we develop this valuable and under-utilized resource.

Literature Cited

Anderson, M., J. Lambrinos, and E. Schroll. 2010. The potential value of mosses for stormwater management in urban environments. Urban Ecosystems 13:319–332. 10.1007/s11252-010-

0121-z.

Benner, A. 2017. Moss Acres. Accessed April 13, 2017. http://mossacres.com/.

Committee on the Status of Endangered Wildlife In Canada. 2009. COSEWIC assessment and status report on the Vole Ears Erioderma mollissimum in Canada. Environment Canada, Ottawa, ON. 51 pp. 10.13140/RG.2.1.1039.4640.

Committee on the Status of Endangered Wildlife In Canada. 2010. COSEWIC assessment and status report on the Blue Felt Lichen Degelia plumbea in Canada. Environment Canada, Ottawa, ON. 42 pp.

Donnell, K. O., and S. Sharrock. 2017. Plant Diversity The contribution of botanic gardens to ex situ conservation through seed banking. Plant Diversity 39:373–378. Elsevier Ltd. 10.1016/j.pld.2017.11.005.

Duckett, J. G., J. Burch, P. W. Fletcher, H. W. Matcham, D. J. Read, A. J. Russell, and S. Pressel. 2004. In-vitro cultivation of bryophytes: a review of practicalities, problems, progress and promise. Journal of Bryology 26:3–20. 10.1179/037366803235001742.

Duncan, S. J. 2015. Woodland Caribou Alpine Range Restoration: An Application for Lichen Transplants. Ecological Restoration 33:22–29.

Echigokokesho Corporation. 2017. Echigokokesho moss nursery. Accessed April 13, 2017. http://www.echigokokesho.com/product/.

Environment Canada. 2017. Canadian Climate Normals 1981 - 2010. Ottawa, ON. 1-17 pp.

Gauslaa, Y. 2014. Rain, dew, and humid air as drivers of morphology, function and spatial distribution in epiphytic lichens. The Lichenologist 46:1–16. 10.1017/S0024282913000753.

Haughian, S. R., and K. A. Frego. 2015. Synthetic logs for controlled culture of epixylic bryophytes: log physical properties, microclimatic control, and Dicranum flagellare growth. Evansia 32:158–170.

Heim, A., and J. Lundholm. 2013. Cladonia lichens on extensive green roofs: evapotranspiration, substrate temperature, and albedo. F1000Research 2:274. 10.12688/f1000research.2-274.v2.

Heim, A., and J. Lundholm. 2014. Species interactions in green roof vegetation suggest complementary planting mixtures. Landscape and Urban Planning 130:125–133. 10.1016/j.landurbplan.2014.07.007.

Heim, A., J. Lundholm, and L. Philip. 2014. The impact of mosses on the growth of neighbouring vascular plants, substrate temperature and evapotranspiration on an extensive green roof. Urban Ecosystems 1119–1133. 10.1007/s11252-014-0367-y.

Japan Moss Technique Association. 2017. Japan Moss Technique Association Website. Accessed April 13, 2017. http://www.kokesin.com/plant/.

Katagiri, F., D. Canelon-Suarez, K. Griffin, J. Petersen, R. K. Meyer, M. Siegle, and K. Mase. 2015. Design and construction of an inexpensive homemade plant growth chamber. PLoS ONE 10:1–14. 10.1371/journal.pone.0126826.

Martin, A. 2017. Mountain Moss Enterprises. Accessed April 13, 2017. https://www.mountainmoss.com/.

Mounce, R., P. Smith, and S. Brockington. 2017. Ex situ conservation of plant diversity in the world's botanic gardens. Nature Plants 3:795–802. Springer US. 10.1038/s41477-017-0019-3.

Muir, P. S., T. R. Rambo, R. W. Kimmerer, and D. B. Keon. 2006. Influence of overstory removal on growth of epiphytic mosses and lichens in Western Oregon. Ecological Applications 16:1207–1221. 10.1890/1051-0761(2006)016[1207:IOOROG]2.0.CO;2.

Palmqvist, K., and B. Sundberg. 2000. Light use efficiency of dry matter gain in five macrolichens: Relative impact of microclimate conditions and species-specific traits. Plant, Cell and Environment 23:1–14. 10.1046/j.1365-3040.2000.00529.x.

Proctor, M. C. F. 2000. The bryophyte paradox: Tolerance of desiccation, evasion of drought. Plant Ecology 151:41–49. 10.1023/A:1026517920852.

Proctor, M. C. F. 2009. Physiological Ecology. P. 565, In B. Goffinet and A.J. Shaw (Eds.). Bryophyte Biology. Second edition. Cambridge University Press, New York.

Rowntree, J. K., S. Pressel, M. M. Ramsay, A. D. Sabovljević, and M. S. Sabovljević. 2011. In vitro conservation of European bryophytes. In Vitro Cellular and Developmental Biology - Plant 47:55–64. 10.1007/s11627-010-9326-3.

Rueden, C. T., J. Schindelin, M. C. Hiner, B. E. Dezonia, A. E. Walter, E. T. Arena, and K. W. Eliceiri. 2017. ImageJ2: ImageJ for the next generation of scientific image data. BMC Bioinformatics 18:1–26. BMC Bioinformatics. 10.1186/s12859-017-1934-z.

Sabovljević, M. S., M. Vujičić, J. Pantović, and A. D. Sabovljević. 2014. Bryophyte conservation biology: In vitro approach to the ex situ conservation of bryophytes from Europe. Plant Biosystems - An International Journal Dealing with all Aspects of Plant Biology 148:857–868. 10.1080/11263504.2014.949328.

Sargent, M. L. 1988. A guide to the axenic culturing of a spectrum of bryophytes. Pp. 17–24, In J.M. Glime (Ed.). Methods in Bryology: Proceedings of the Bryological Methods Workshop, Mainz. Hattori Botanical Laboratory, Nichinan, Japan.

Shaw, J. 1986. A New Approach to the Experimental Propagation of Bryophytes. Taxon 35:671–675.

Su, C., C. Fu, Y. Chang, G. R. Nair, J. Ye, I. Chu, and W. Wu. 2008. Simultaneous Estimation of Chlorophyll a and Lipid Contents in Microalgae by Three-Color Analysis. 99:1034–1039. 10.1002/bit.

Williams, L., S. Zappia, and R. Martin. 2017. Botánica Boutique. Accessed April 13, 2017. http://botanica.boutique/.

Yadav, S. P., Y. Ibaraki, and S. D. Gupta. 2010. Estimation of the chlorophyll content of micropropagated potato plants using RGB based image analysis. 183–188. 10.1007/s11240-009-9635-6.