

A review of lichen transplant studies and methods

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Background

Habitat loss and fragmentation due to human activities have been considered a significant threat to caribou (*Rangifer tarandus*) (Vors and Boyce 2009; Wittmer et al. 2010; Festa-Bianchet et al. 2011). Open spaces created by anthropogenic disturbances in caribou habitats attract competitive species such as moose (*Alces alces*) and deer (*Odocoileus virginianus*), and predators including wolves (*Canis lupus*) (Sorensen et al. 2008; Wittmer et al. 2010; Hervieux et al. 2013; Nagy-Reis et al. 2021). To promote species persistence and recovery, natural resource management professionals are continuously evaluating ways to develop alternative approaches to forest management that can promote caribou habitat within forests.

Caribou consume grasses, sedges, forbs, and shrubs' leaves in the summer, but a significant portion of their diet consists of lichens in the winter (Thomas et al. 1996). Important caribou forage includes terrestrial lichens, including species in genera *Cladonia*, *Stereocaulon* and *Cetraria* (Boertje 1994; Himmer and Power 1999). Caribou also consume a variety of arboreal lichens, including the species of *Bryoria* and *Alectoria* (Boertje 1994; Himmer and Power 1999). In west-central Alberta, *Cladonia* species have been shown to comprise 60–83% of the caribou diet in the winter (Thomas et al. 1996). *Cladonia* and *Cetraria* species have ~2 % protein content, while *Stereocaulon* has ~ 7 %, and *Peltigera* lichens have ~ 17–21% protein (Scotter 1965).

Following a disturbance, lichens have a long recovery time (Brodo 2001). For example, post-fire studies suggest lichen recovery times of 40 years in peatlands (Dunford et al. 2006) and 50–100 years in upland woodlands (Morneau and Payette 1989; Coxson and Marsh 2001). While the primary source of disturbance in the boreal and temperate regions is fire (Payette 1992), commercial forestry operations have increasingly replaced this natural disturbance in recent years (Cumming 1992). Lichens are poikilohydric organisms and cannot maintain or regulate water content (Gauslaa et al. 2017), making their growth and reproduction prone to natural and anthropogenic disturbances (Giordani 2006; Benítez et al. 2015; Benítez et al. 2018).

Regardless of the type of disturbance, lichens grow slowly and take many years to develop lichen mats that can serve as sufficient winter caribou forage. Thus, developing restoration strategies that promote caribou habitat will require techniques for restoring lichens and promoting their

growth (Holleman et al. 1979). Lichen fragment transplantation has been tested in various parts of the boreal for their potential to accelerate their reestablishment. However, the efficacy of these methods will need to be evaluated.

Our objective is to assess the feasibility of lichen transplantation for restoring harvested forest areas to usable caribou habitat. In this review, we first discuss lichen biology and restoration. We then cover the collection and storage of lichens, address the various methods used to transplant lichens, and assess the transplant of lichens onto the soil and trees. We also discuss the habitat considerations and environmental factors that influence the success of lichens. We review the methods that have been used to assess lichen health and growth and discuss the cost considerations for deploying lichen at operational scales. We discuss some knowledge gaps regarding lichen transplants, and we summarise important case studies in the appendix. Table 1 in the appendix provides information on various lichen studies, and Table 2 in the appendix summarizes operational costs. A comprehensive review will help ensure lichen growth and survival in future studies.

Lichens

Lichens are symbiotic organisms composed of lichen-forming fungi (mycobiont) and photoautotrophic partners (photobiont), which can include green algae and cyanobacteria (Nash III 1996). The mycobiont provides structure and a source of inorganic nutrients for photosynthesis (Nash III 1996). On the other hand, the photobiont provides carbohydrates through photosynthesis, and the cyanobacteria can also fix nitrogen and provide the fungus with a source of nitrogen (Nash III 1996).

Lichens are diverse and can be found on various substrates including living trees, dead wood, rocks and bare soil (Brodo 2001). Lichen growth forms (morphological types) are often categorized as fruticose, foliose, or crustose as they resemble shrubs, leaves or crusts, respectively. Lichens growing on the bark of trees are referred to as corticolous, and those growing on trees are considered arboreal. Lichens growing on the ground are known as terrestrial or terricolous lichens, which may have a higher abundance than arboreal lichens. There was an estimated 3120 kg/ha of terrestrial lichen and 3.74 kg/ha of arboreal lichen biomass in 51 forest stands in boreal Ontario (McMullin et al. 2011).

Lichens can undergo asexual and/or sexual reproduction. Asexual reproduction of lichens can occur through fragmentation, in which part of a lichen breaks off and grows into a new lichen (Heinken 1999). The other type of asexual reproduction is through the release of soredia, and small particles made up of multiple algae or cyanobacteria cells surrounded by fungal hyphae, or isidia, outgrowths of the upper thallus surface (Gauslaa 2006). These propagules and fragments contain components of both the fungus and algae/cyanobacteria (Gauslaa 2006). Sexual reproduction can occur by releasing fungal spores, which will have to find an algal or cyanobacteria partner in the environment (Munger 2008). The fungal hyphae will grow through the alga/cyanobacteria cell, and a new lichen will form (Munger 2008).

Most lichens have slow growth rates, though productivity may differ by species (Scheidegger et al. 1995; Sillett and McCune 1998; Campeau and Blahchard 2010). For instance, reindeer lichens in the genus *Cladonia* (e.g., *C. mitis*, *C. rangiferina*, *C. stellaris*, and *C. stygia*) are estimated to grow only 4–5 mm per year in Alberta (Duncan 2011). Hair lichens in North Carolina were observed to grow 1.8–3.5 cm per year (Allen 2017). *Rhizocarpon geographicum*, a crustose lichen, grew 0.6–0.9 mm per year, and lower growth rates were observed for older lichen thalli (Armstrong 1983). This variation in species growth was also noted by Benedict (2008); they suggested that growth is dependent on the age and size of the lichen.

Lichens are important components of an ecosystem. Lichen can have significant impacts on nutrient cycling, given that cyanobacteria can fix nitrogen and carbon, and the algae can fix carbon (Henry 2011). Lichens are also crucial for the formation and stabilization of soils (Leddy et al. 2019), and are an important source of food for many gastropods (Gauslaa et al. 2018) and caribou (Thomas et al. 1996). Humans have also used lichen to treat respiratory illnesses (Zarabska-Bozejewicz et al. 2015) and as an indicator for environmental pollution (Paoli et al. 2019).

Threats to Lichens and Restoration Efforts

Lichens face various physical and chemical threats in their natural habitats. These threats include forest management (Pykälä 2004; Richardson and Cameron 2004), climate change and acid deposition (COSEWIC 2002). Another equally important factor is fire, a significant source of disturbance in boreal and temperate regions, affecting forest structure, nutrient cycling, and

ecological succession (Minsley et al. 2016; Larjavaara et al. 2017). Various studies suggest that fire events greatly influence lichens (Reinhart and Menges 2004; Johansson and Reich 2005). However, the natural regeneration of forests in the boreal and temperate regions by fire has been increasingly replaced by commercial forestry operations. Forest management practices can have significantly different effects than fire on lichen growth and survival (Cumming 1992).

Harvesting practices may cause forest fragmentation, reducing lichen dispersal ability and the chance to reestablish in cleared areas (Rheault et al. 2003). Moreover, the adverse effects of silviculture and harvesting systems on lichen communities may occur when moving heavy harvesting equipment and machinery or when pulling cut trees out of a forest during large-scale clearcutting, which can directly harm the lichen thalli (Harris 1996). Indirect effects of forest management on lichen communities may also occur from a change in ground-level microclimate conditions (changes in surface air temperature, irradiance, and wind conditions) in response to canopy openings during harvesting (Harris 1996). These microclimate changes can increase desiccation at the ground level, affecting lichen growth.

Forestry operations can cause substantial damage to the ecosystem by removing large amounts of vegetation (Burton et al. 2006; Macdonald et al. 2015; Natural Resources Canada 2019). Trees, shrubs and forbs can often be planted to help restore these sites to achieve equivalent land capacity (Burton et al. 2006; Macdonald et al. 2015; Natural Resources Canada 2019). However, lichens are often neglected because of their slow growth rates (Duncan 2015). Hence, finding ways to restore lichens in harvested areas has become an important management consideration to reduce the impact of forestry operations on lichen communities and indirectly on caribou populations.

One area identified in lichen restoration efforts includes enhancing the spread of lichen and transplantation to facilitate the reestablishment of the previously occupied habitat. The dispersal ability of lichens severely limits the ability of new lichen populations to establish at a new site (Sillett and McCune 1998). For example, reindeer lichens in the genus *Cladonia* (e.g., *C. mitis*, *C. rangiferina*, *C. stellaris*, and *C. stygia*) do not often spread more than 70 cm from the parent material (Duncan 2015). Reindeer lichens rely on fragmentation for reproduction (Duncan 2011), and sexual reproduction in reindeer lichens is not often observed (Munger 2008). Therefore, the dispersal and arrival of lichen to a site will facilitate the amount of growth in disturbed areas

(e.g., cut blocks and mine sites). The addition of lichen propagules can increase lichen cover over time (Roturier et al. 2007; Campeau and Blanchard 2010). However, establishing long-term lichen populations has not been a significant focus of many lichen studies (Smith 2014), even though restoration would likely help prevent the decline of animals that rely on lichen for a food source.

Collection and Storage

Lichen must be handled with care to ensure the success of transplant studies. Lichen collection should not occur during dry summers or cold periods (Smith 2014). Ronalds and Grant (2018) collected reindeer lichens in June and September in British Columbia. Gauslaa et al. (2018) collected *Lobaria amplissima* and *Lobaria pulmonaria* in October, while Hazell and Gustafsson (1999) collected *Lobaria pulmonaria* in March and September in Sweden.

Researchers have manually collected lichens using gloves or bare hands (Rapai et al. 2017). Lichens can also be removed using razor blades (Allen 2017), knives (Schoenwetter 2010), and rakes (Rapai et al. 2017). Duncan et al. (2011) and Rapai et al. (2017) recommended that lichens be misted with water prior to collection to ensure that the lichens do not fragment. Duncan (2011) kept reindeer lichens in plastic bags filled with air in the shade while in the field. Ballesteros et al. (2017) collected *Diploschistes diacapsis* and placed them into paper bags to ensure sufficient air flow. It is recommended that no more than 20 % of lichens be collected from a site (Rapai et al. 2017), and that lichens should be cleaned prior to storage (Sillett 1994; Zarabska-Bożejewicz et al. 2015; Ronalds and Grant 2018).

Storage conditions of lichens vary significantly between studies. Reindeer lichen were stored in a dark, dry and cool area (Rapai et al. 2017). *Lobaria pulmonaria* and *Lobaria scrobiculata* (Hazell and Gustafsson 1999) and *Platismatia norvegica* (Hilmo 2002) were stored at -18 °C. Temperatures above freezing can also be used to store lichens, and Huss-Danell (1977) kept dry *Stereocaulon paschale* in the dark at 4 °C. Dry *Alectoria sarmentosa* and *Usnea dasopoga* and *Bryoria fuscescens* (fruticose hair lichens) from Norway and Sweden were stored in the dark at room temperature (Phinney et al. 2020). Likewise, *Lobaria pulmonaria* was kept at 15 °C at a relative humidity of 60 % (Coxson and Stevenson 2007).

The duration of storage also varies significantly between studies. A range of studies attempt to minimize the storage time of lichen, and Ballesteros et al. (2017) transplanted *Diploschistes diacapsis* thalli within 1 day, while Lidén (2009) transplanted *Platismatia norvegica* within 3 days during their experiments in Scandinavia. Schoenwetter (2010) collected and stored *Pseudocyphellaria* lichen for 3–4 days before the transplant, and Ronalds and Grant (2018) stored reindeer lichens for 7 days in their post-fire restoration experiment. Boreal hair lichens can be stored in the dark for 2–3 weeks, though they should be dried at room temperature beforehand (Phinney et al. 2020). *Lobaria amplissima* and *Lobaria pulmonaria* were air-dried and stored at room temperature with no light exposure for 2 weeks (Gauslaa et al. 2018). Similarly, *Lobaria pulmonaria* was stored for 2 weeks by Coxson and Stevenson (2007). Longer storage times have also been used in transplant studies with varying success. Storage of the *Lobaria oregana* and *Lobaria pulmonaria* lichen for longer than 90 days resulted in low growth rates, but storage at lower temperatures (< 12–24 °C) and humidity (< 50–60 %) may improve storage times (Denison 1988). *Lobaria scrobiculata* and *Platismatia norvegica* from central Norway were dried and kept in the dark for a total of 4 months for the analysis of chlorophyll content (Hilmo 2002). Dry *Stereocaulon paschale* and *Cetraria nivalis* were stored for 135 days in the dark (Glaholt et al. 1997). *Xanthoria parietina* can be stored up to 3 years at room temperature if sprayed with water occasionally (Honegger 2003).

The amount of lichen collected and removed from a region should be carefully considered, and this is especially important for more rare and endemic lichen species (Allen 2017). Lichen can be dry or moist when collected, and the use of bare hands does not appear to have any detrimental effects. In most of the studies, lichen was cleaned of debris; Ronalds and Grant (2018) indicate that debris can cause rotting in the lichen. However, lichen cleaning is time-consuming and may not be practical for large scale restoration purposes. Lichen can be stored in cool and dark places, but storage time should be limited. The study conducted by Denison (1998) clearly demonstrates an effect of storage length on lichen growth, and older lichens may have lower growth rates.

Transplantation Methods

Artificial lichen propagation has been used in various studies to measure air pollution levels (Hawksworth and Rose 1970; Galun 1988), growth requirements (Armstrong 1994), test genetic

variation among populations from different climates (Walser and Scheidegger 2002), and conserve endangered and threatened species (Gilbert 2002; Scheidegger and Werth 2009). A variety of transplantation methods can be applied, including using bark borers, removing sections of turf, using adhesives and wire to attach fragments to trees, rubbing thalli onto bark, and spreading thallus onto the substrate (Smith 2014). The transplantation method depends highly on the lichen form and whether it is naturally found on the ground or trees. We will discuss spreading lichen onto the ground and attaching lichen to tree branches and trunks.

1) *Spreading lichen*

Early attempts to transplant terricolous lichens were ineffective (Hale 1954), but recent transplant studies have shown that transplanting terricolous lichens can be successful (Gilbert 1991; Scheidegger et al. 1995). Roturier et al. (2007) simulated the recovery of *Cladonia mitis* after overgrazing and soil disturbance, and *C. mitis* fragments (i.e., 1 cm vs 3 cm) were marked with string and spread across 1 m × 1 m plots in a clearcut and pine forest in northern Sweden (Roturier et al. 2007). Larger lichen fragment sizes were more likely to stay within a plot than smaller fragments, resulting in greater lichen cover over time (Roturier et al. 2007). In another transplantation study aimed at evaluating and developing terrestrial lichen transplantation techniques, Rapai et al. (2017) used different fragment sizes (2 cm vs 7 cm) or lichen mats of *Cladonia mitis*, *Cladonia stellaris*, *Cladonia stygia*, and *Cladonia uncialis* and placed them into 100 m² plots.

Spreading fragments of other lichen genera has also been successful. Zarabska-Bożejewicz et al. (2015) dispersed fragments of *Cetraria islandica* and observed lichen cover over time in old pine stands. They found that lichen cover was greater when 7.88 g of lichen was spread onto plots compared to 3.85 g of material (Zarabska-Bożejewicz et al. 2015). However, the plots with lichen mats with clumps of *Cetraria islandica* weighing 12.62 g had higher cover until the very end of the study (Zarabska-Bożejewicz et al. 2015). In another study by Webster and Brown (1997), fragments of the foliose lichen *Peltigera canina* (2 × 3 cm and 3 × 4 cm) were planted directly into flowerpots and placed into a shady area to examine whether *Peltigera* would establish successfully, and samples from shady areas grew 6.4 cm per year. Sonesson (2007) collected patches of *Nephroma arcticum*, a foliose lichen, from various alpine and subalpine sites in Sweden and placed them into wooden frames covered with mesh. Rocks with crustose lichens

can also be moved from one area to another (Armstrong 1977). Thalli of the crustose lichen *Diploschistes diacapsis* have also been removed from rocks and placed directly onto the soil surface (Ballesteros et al. 2017).

Overall, spreading lichen fragments onto the ground can be an effective and cost-efficient way to transplant lichen (Roturier et al. 2007; Duncan 2011). The addition of larger lichen fragments and lichen mats appears to increase lichen cover faster. Spreading more significant amounts of lichen can improve lichen cover but can increase competition between fragments (Roturier et al. 2018). The addition of adhesives may enhance the success of lichen transplants. Water and glue helped *Diploschistes diacapsis* thalli attach to the soil surface (Ballesteros et al. 2017). Likewise, *Xanthoria parietina* and *Parmelia sulcata* were glued to ceramic tiles (Honegger 1993). Tackifiers, which are compounds used during hydroseeding to enhance attachment, have been used successfully with mosses (Blankenship et al. 2020). However, some tackifiers decreased the growth rates of the mosses at specific concentrations (Blankenship et al. 2020). Further assessments are required on using tackifiers for lichen attachment.

2) Attachment of lichen to tree branches and trunks

Lichens can be attached to trees using string or wire. The foliose lichens *Lobaria oregana* and *Lobaria pulmonaria* were divided into 2–3 cm segments that were combined and hung onto tree branches using string (Denison 1988). To examine the growth requirements of epiphytic cyanolichens, Sillett and McCune (1998) similarly transplanted *L. oregana* and *Pseudocyphellaria rainierensis* onto a branch by standardizing lichens into 5–10 cm fragments connected by string and attaching them to branches with or without moss. This technique was further developed using a single thallus fragment and nylon monofilament string to form a “pendant” (McCune et al. 1996). This pendant method was used for *L. oregana* and *L. pulmonaria* (Antoine and McCune 2004), *P. rainierensis* (Sillett 1994), and for the fruticose lichens *Letharia vulpina* and *Usnea scabrata* (Antoine and McCune 2004). Wire was used to attach branches with the foliose lichens *Lobaria scrobiculata* and *Platismatia norvegica* to trees in spruce forests in Norway (Hilmo 2002).

Lichens can also be transplanted by removing thallus lobes and stapling them directly onto tree bark (Scheidegger et al. 1995). Nylon mesh was stapled to trees with the foliose lichen

Parmotrema tinctorum in Japan (Kon et al. 2003), and *Lobaria amplissima* and *Lobaria pulmonaria* (foliose lichens) were cut and sewn onto the mesh in Sweden and attached directly to tree trunks using staples (Gauslaa et al. 2018). *Lobaria pulmonaria* thalli were stapled to tree bark with a net (Hazell and Gustafsson 1999). Sterile gauze pieces (5 × 5 cm) were placed on tree trunks, and small fragments of the crustose lichens *Graphis sterlingiana* and *Lepraria lanata*, and the foliose *Hypotrachyna virginica*, were placed onto them, though lichens were often lost on gauze (Allen 2017). *Lepraria finkii*, a crustose lichen, was placed onto gauze, burlap, and cheesecloth as well; burlap performed the best for this species (Allen 2017). Schoenwetter (2010) conducted lichen transplant trials with foliose *Pseudocyphellaria* species using a variety of methods (e.g., borers, glue, and the pendant method) in New Zealand. They found that tying thallus lichen to a mesh and placing it directly onto tree trunks was the most effective, and after one year, 94 % of thalli remained in the plots (Schoenwetter 2010).

Smaller lichen propagules can also be applied to tree trunks. Vegetative propagules using soredia of the foliose *Crocodia aurata* were placed onto surgical gauze using various adhesives and placed onto trees (Leddy et al. 2019). Applying diaspores on the tree back can be done using paint brushes, but thallus transplantation was more successful than soredia application for *Parmotrema crinitum*, *Lobaria pulmonaria*, and *Sticta sylvatica* (Scheidegger et al. 1995). Soredia of the foliose *Parmotrema clavuliferum* were collected and placed onto plates that were adhered to tree trunks, while thalli of the foliose *Ramalina yasudae* with soredia were attached using mesh, with both showing minor growth after 18 months (Kon and Ohmura 2010).

In some cases, methods of lichen transplant include blending nutrients (e.g., milk, fertilizer and jello) and mixing the solution with lichen thallus fragments. The mixture then can be applied directly onto trees. This technique has successfully promoted the growth of lichen fragments (www.blogs.evergreen.edu), and modelling efforts on this “painting technique” have shown that this method can potentially increase lichen area on rocks (Desbenoit et al. 2004). However, for Scheidegger et al. (1995), this painting method was not successful for the foliose lichens *Parmotrema crinitum* or *Lobaria pulmonaria*, perhaps due to weather conditions.

The addition of adhesives may also enhance the success of lichen transplants on trees. For example, gel adhesives improved the contact between gauze and *Crocodia aurata* soredia on tree trunks (Leddy et al. 2019). In another study, *Lobaria amplissima* was removed from trees in the

United Kingdom and glued onto the bark of other trees (Gilbert 2002). Growth was observed over 20 years, though only 6/15 lichens remained at the end of the experiment (Gilbert 2002). However, Schoenwetter (2010) found applying adhesives onto tree bark challenging and Ballesteros et al. (2017) suggested that the use of adhesives may affect lichen viability.

Habitat Considerations

Many fruticose lichens lack below-ground attachment systems and their establishment and growth depend on the ability of thalli to fasten to the substrate. Substrate type will influence the amount of lichen cover, but this appears more important in open areas (e.g., cutblocks) and younger forests. For instance, *Cladonia mitis* fragments marked with string were spread across 1 m × 1 m plots in a clearcut and pine forest in northern Sweden (Roturier et al. 2007). The plots were filled with 2 cm of mineral soil, moss, twigs or bark, and 30 individual fragments were spaced evenly across the plots (Roturier et al. 2007). Lichen growth was better on moss (than mineral soil, twigs and bark) in the clearcuts but was not influenced by the substrate in the pine forest (Roturier et al. 2007). This contrasts with Webb (1998), which reported no reindeer lichen colonizing on mineral soil. In general, reindeer lichens do not appear to grow well when placed directly onto mineral soil (Roturier 2009; Duncan 2015). In another study by Duncan (2011), growth rates of reindeer lichen in northern Alberta were higher on moss and litter relative to bare soil in 12-year-old forests, but no different in forests that were 24 years old. Sillett and McCune (1998) also observed low lichen growth on clearcuts, but no differences were observed between young and older forests.

Furthermore, Sillett (1994) found that lichen growth rates depended on their source and whether acclimation occurred. For instance, lichen collected from edge habitats were more drought tolerant than lichens collected from the forest (Sillett 1994). *Nephroma arcticum* (foliose lichen) acclimated when transplanted from a forest environment to a heath system, and changes in cortex thickness were observed (Sonesson 2007).

Growth Requirements and Environmental Factors

Lichens have evolved to disperse vegetatively by fragmentation and the production of isidia and soredia (Bailey 1976; Armstrong 1990). Most lichen-forming fungi also reproduce sexually,

which requires reestablishing a symbiotic relationship with the photobiont after each reproduction cycle. However, these processes are not necessary for most reindeer lichens of the genus *Cladonia*, as they mainly disperse by thallus fragmentation (McCune and Geiser 1997). Various studies have examined factors influencing the success of lichen transplant studies (Benedict 1990, 1991; Armstrong 1993a,b). Survival and growth of lichens are influenced by abiotic factors (e.g., the type of substrate, irradiance, and nutrient and moisture availability) and biotic factors (e.g., the presence of herbivores and competition).

Lichens can grow under broad ecological conditions (Klein and Shulski 2011). The optimal temperature for reindeer lichen growth is 15–25 °C (Coxson and Wilson 2004), but in some species, photosynthesis can continue down to -5 °C or even -10 °C (Skre 1975). In addition to temperature, lichen metabolism depends on moisture availability; thus, their growth rates rely heavily on moisture regimes. Kershaw and Rouse (1971) showed explicitly that the net assimilation rate of *Cladonia alpestris* in spruce–lichen woodland is associated with the moisture content of the thallus. Lichens are also known to go dormant under dry conditions, tolerating significant desiccation stress (Sillett 1994). Webster and Brown (1997) planted *Peltigera canina* into flowerpots and placed them into a shady area. Irregular watering decreased lichen growth initially, but growth rates increased at the end of the study (Webster and Brown 1997). On the other hand, continuous watering had no apparent detrimental effects on this species (Webster and Brown 1997). Irregular watering also appeared better for *Usnea lapponica*, *Usnea cavernosa*, and *Ramalina menziesii* growing in the greenhouse (Stewart 2019). Kärenlampi (1975) transplanted thallus fragments of *Cetraria nivalis* and *Cladonia alpestris* and found that increases in weight and length were positively correlated with mean daily rainfall in summer, but inversely related to temperature.

The relationship between irradiance and lichen growth was studied in foliose and fruticose lichens in Sweden (Palmqvist and Sundberg 2000). Thalli or lobes were transplanted at two geographically different sites in Sweden, where irradiance and growth were followed. They found a strong correlation between growth and light interception when the lichens were wet, and higher productivity in sites where the thallus retained higher moisture (Palmqvist and Sundberg 2000). Similarly, Gauslaa et al. (2006) examined biomass and area changes of transplanted thalli of *Lobaria pulmonaria* in response to contrasting light availability. Growth was higher under

greater irradiance and reduced in low light conditions (Gauslaa et al. 2006). Other studies have shown that interactions between irradiance, temperature and water availability are the major factors in determining lichen growth. In British Columbia, thalli of *Lobaria pulmonaria* were transplanted to two canopy structures to evaluate their growth responses to changes in microclimate (Coxson and Stevenson 2007). Coxson and Stevenson (2007) found that the growth rates of *Lobaria pulmonaria* had a strong correlation with canopy light transmission; < 5% vs ~ 20% annual mean dry matter gain under closed canopy even-aged stands and canopy gaps in old-growth stands, respectively. Timber harvesting can increase light availability, but this was not found to improve lichen abundance substantially after 19 years (Vitt et al. 2019).

In sites where light is the limiting factor, a larger fragment size could be favorable for artificial dispersal (Gaare and Wilmann 1998; Hammer 2000; Roturier et al. 2007). These larger fragments have greater access to light, and larger fragments are less vulnerable to wind, which is a major concern on clearcuts (Heinken 1999). High winds resulted in the loss of many transplants over time (Sillett and McCune 1998), and lichen pendants can be lost under windy conditions (Schoenwetter 2010). Moss sections can help retain transplants but may increase competition for the lichens (Sillett and McCune 1998). As previously mentioned, diaspores of *Parmotrema crinitum* and *Lobaria pulmonaria* were placed onto tree bark but were not successful due to weather conditions (Scheidegger et al. 1995). However, sufficient airflow may be required to prevent molding of lichens (Smith 2014).

Growth Assessments

The type of measurements used for lichen growth assessments depends on multiple factors, including the growth form of lichen (type of thallus) and the aim of the study (Armstrong 2015). While an increase in the linear size of lichen thalli is relatively easy to measure and can reflect biomass, interpretation of these parameters requires caution. For instance, a lichen's biomass accumulation may be confined to the inner layers and may not necessarily reflect changes in lobe length or thallus diameter (Armstrong 2015). The measurement of growth in foliose lichens (e.g., *Peltigera*) often involves measurements of lobe length (Armstrong 1995; Honegger 1995) or thallus diameter (Benedict 1990; Armstrong and Smith 1996). Digital photography and an image analysis system can then be used to measure growth increments (Armstrong 2015). While area

measures from photographs over time are complex for *Cladonia* species as compared to *Peltigera* species, data using this method have been reported. Hilmo (2002) outlined the edges of lichen thalli on photographs to determine area for *Lobaria scrobiculata* and *Platismatia norvegica*. Pictures were also used to estimate lichen cover by Roturier et al. (2007) and Lidén (2009). In some studies, photographs of lichen did not work well and were not recommended for assessment of lichen cover (Sonesson 2007) or growth (Duncan 2011). In addition to photographs, Duncan (2011) evaluated the growth of lichen by microscopically assessing hyphae growth and lichen branching.

Biomass is often used to assess lichen growth (McCune et al. 1996). The weight of lichens can be used to determine the health of lichens, and dead lichens are known to lose weight over time (Denison 1988). Lichens are often air-dried and weighed after a given amount of time to estimate biomass. Sacrificial lichens are often used to correct the biomass estimate and account for water content in the living lichen. To estimate biomass, McCune et al. (1996) dried samples of various lichen species for 12 h at a relative humidity of 20–40 %; this allowed them to reach equilibrium, and they used 5–20 transplants for the sacrificial method. Sillett (1994) oven-dried thalli of *Lobaria oregana* and *Pseudocyphellaria rainierensis* at 70 °C for 24 h for the sacrificial method. This destructive method is best when abundant lichen material is available (McCune et al. 1996). Zarabska-Bozejewicz et al. (2015) destructively harvested their lichens and stressed the importance of avoiding this method for rare species. McCune et al. (1996) recommend a reference method for less abundant lichen species, and suggested measuring weights over multiple time points to correct for water content. McMullin et al. (2011) non-destructively estimated the biomass of reindeer lichen in the field using a cover: biomass ratio. McMullin et al. (2011) associated lichen cover with dry lichen biomass, which was necessary as they could not harvest the entire plots during each measurement period.

Gas exchange measurements can also be used to assess lichen growth and health. Sonesson et al. (2007) assessed CO₂ exchange in lichen using an infra-red gas analyzer (IRGA). Ballesteros et al. (2017) evaluated the vitality of lichen using CO₂ exchange and fluorescence, which could be used as an indicator for photosynthetic performance and stress.

Lastly, the health of lichens can be assessed visually. Lidén (2009) used a vitality scale to assess lichen performance. This was based on a scale used by Sillett and McCune (1998); the scale

ranged from 0–5, with 0 representing no lichen cover and 5 representing cover over 50 %. Duncan (2011) used a scale to rate the health of lichens based on the amount of necrosis and bleaching, which are indicators of lichen mortality (Roturier et al. 2018; Stewart 2019).

Operational Scale

Ronalds and Grant (2018) conducted a restoration trial within the Chelaslie River region in British Columbia after a significant summer wildfire. Ronalds and Grant (2018) recommended restoring sites that were assessable by road to minimize travel costs. It was estimated that 200 kg of dry lichen could sufficiently cover a hectare of land, and spreading this amount of lichen over a week using aerial methods would cost an estimated \$125,000. Ronalds and Grant (2018) took an hour to collect 5 kg of lichen in the field. Rapai et al. (2017) took 30 min to collect 100 L of lichen by hand when there was abundant material, but an estimated 90 min when abundance was low. For Rapai (2015), two individuals collected 1100 L of lichen (*Cladonia mitis* and *Cladonia rangiferina*) in 4 hours. In addition, Rapai (2015) spread 80 L of lichen over 100 m², covering 50 % of the area in an estimated 15 mins. Reindeer lichen fragments were applied to burned sites by Roturier et al. (2018) in Sweden, and the cost was estimated to be USD 650 / ha at lower doses of 0.45 L/m² and USD 3,250 at higher doses of 2.25 L/m².

For the current Alberta Regional Caribou Knowledge Partnership (ARCKP) project, we collected lichen within 1 × 1 m quadrats. We identified the species present (e.g., lichens, mosses, and vascular plants) and estimated their percent cover. We also conducted an ecological assessment for each new site. With two individuals, we collected 10 quadrats of lichen within 2 days. In addition, two individuals collected lichen from eight 1 × 1 m quadrats in 1 day. An ecological assessment was not conducted for this collection, and the species were not identified. We placed the lichen bags into a truck, though Ronalds and Grant (2018) transported the lichen using a trailer that cost CAD 150 per day.

The costs associated with large-scale lichen transplantation projects will need to be investigated further. Given the sheer size of the lost caribou winter habitat that could benefit from restoration, the most effective methods will be required. Studies will need to examine the cost-effectiveness of various lichen transplantation technologies.

Knowledge Gaps and Future Research

Despite the challenges associated with growing lichens under controlled conditions or in the field, our literature review has shown that lichen fragments or mat transplants can be used to establish lichens. However, further information is required on growing lichens. First, the technical difficulty of transplanting lichens will be a significant challenge (Allen 2017).

Relatively few lichen transplantations studies have been carried out in the woodland caribou habitat of Alberta. In addition, a host of issues concerning transplantation studies, such as the ideal size of the transplanted thallus fragments on different substrates, will need to be addressed (Coxson and Stevenson 2007). This is essential for developing suitable lichen transplantation techniques to assist caribou habitat projects, as previous studies used whole-lichen transplants or large lichen fragments. Since lichen communities are slow-growing and sensitive to disturbance, making the most of harvested material is essential to large-scale lichen reclamation planning.

Second, we need to understand the factors that will promote the maximum amount of lichen growth. Growth chambers can be used to help determine the ideal growth conditions for lichens. For example, *Parmotrema tinctorum* lichen grew best at 100 % humidity, 20 °C, under 12 W/m² for 16 h (Bando and Sugino 1995). Bando and Sugino (1995) monitored lichen growth over 4 weeks in the chambers, and they were harvested and dried at the end of the study. However, there are very few studies on growing lichens in the greenhouse (Stewart 2019). Lichen growth in a greenhouse was first observed and documented by Culberson (1963). Stewart (2019) grew *Usnea* and *Ramalina* on sterilized branches in a greenhouse, watering them under two regimes: four times daily with tap water or occasional watering with deionized water. The lichens died within 2 weeks and 19 months, respectively, possibly due to the differences in water pH or overwatering (Stewart 2019). Henry (2011) grew *Parmelia Sulcate*, *Umbilicaria Hyperborean*, *Usnea lapponica*, and *Xanthoparmelia coloradoensis* in the greenhouse for 12 weeks. The lichens were rinsed with deionized water and placed into Styrofoam blocks (Henry 2011). Air temperatures followed a high elevation temperature site, and the lichen was watered 3 times per week (Henry 2011). No mortality was reported in this experiment, though the duration of the experiment was relatively short (Henry 2011).

Third, long-term monitoring studies need to be established to evaluate the best method for restoring lichen. Conducting a study for months to a few years to look at the survival and growth of lichen transplants is understandable. Nevertheless, long-term growth of the species onto the substrate is needed to evaluate the success of lichen transplants. Many studies have shown lichen growth over 2–3 years (Duncan 2011; Allen 2017). However, longer-term studies are rarer. The longest lichen monitoring studies have occurred over 20 years (Gilbert 2002) and 8 years (Sonesson et al. 2007). The duration of studies may influence whether lichen transplant studies are considered successful, and young lichen fragments can have faster growth rates than older lichen fragments (Zarabska-Bozejewicz et al. 2015).

Alternative methods to improve the dispersal and adherence of lichen fragments to substrates have not yet received the research attention needed. However, prior studies show promising results for transplanting lichens in the field. These methods may be used to help establish and increase lichen cover for caribou.

In most previous studies, reindeer lichens in the genus *Cladonia* have been studied to restore the caribou habitat. However, other ground lichens, including *Stereocaulon* spp. and *Cetraria* spp. are also fed on by caribou, and many other terrestrial lichen species are present within natural lichen communities that may be significant to their ecology and development. It is important to understand the potential positive and negative interactions these species may have and their different responses to transplantation techniques and substrates. Tree lichens in the genus *Bryoria* are also a food source for caribou and may be transplanted as well. These hair lichens have been transplanted previously and show high growth rates. Lichen transplants could be used for restoring caribou habitat, and both greenhouse and field trials could help initiate large-scale reclamation projects.

An additional and important area of study will be examining the impact of different lichen collection methods on existing lichen communities. This will be essential in preventing further harm to these communities in our attempts to restore them, particularly if being done on larger scales.

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Appendix

Case Studies

1) Tweedsmuir restoration trial (British Columbia)

Title: Tweedsmuir Lichen Restoration Trial Year 1 Report

Author: I. Ronalds, L. Grant (2018)

During the summer of 2014, wildfire spread along the Chelaslie River in British Columbia, destroying critical caribou habitat. This fire season had a significant impact on the habitat of the southern mountain caribou. The researchers explored various methods (different transplant techniques using either 40 or 80 L lichen / 100 m²) to deploy fragments at the burned sites. They also assessed the timing of lichen collection and transplant on lichen recovery. This is the first known study to use aerial vehicles to deploy lichens for restoration purposes.

Lichen was collected from a pine site at Fort St. James, British Columbia, during June 2017 and September 2017. First nation communities were consulted regarding lichen collection in Fort St. James. Individual lichen fragments were removed from the lichen mats collected in the field. The lichens were cleaned, but this required a substantial amount of time. Cleaning was considered necessary and helped prevent soil from clogging up the equipment used in the helicopters. Furthermore, this was assumed to protect the algal and cyanobacteria in the lichen from fungal infections. The major lichen species collected in this study included *Cladonia mitis*, *Cladonia uncialis*, *Cladonia rangiferina* and *Stereocaulon* spp.

The lichen material was either stored in burlap or nylon bags and fragmented by hand or weed trimmer. The latter was done by placing the lichen in a large container and using a trimmer to break apart the lichen. The weed trimmer did not affect the viability of the lichen and was faster than breaking apart the lichen by hand. The material collected in June was used for seeding lichen by hand or leaf blowers. The material collected in September was used for seeding lichen by helicopters. The authors recommended that lichen volume should be estimated consistently, and the lichen should be either dry or wet but not both.

The study occurred at Tetachuck Lake in British Columbia. Lichen was applied along a 100 m transect, and 1 × 1 m monitoring plots were set up to assess the growth of the lichen. The cover

of other vegetation was also assessed. Either 40 or 80 L lichen / 100 m² were applied to plots. In total, 8 transects were set up with a total of 98 monitoring plots. There were 3 control transects, 3 aerial transects, and 2 manual transects.

Lichen cover was greater in the transects where lichens were applied manually versus using helicopters. Lichen cover ranged between 1 to 4 % for the former and 1 to 1.7 % for the later. The controls had no lichen present.

The leaf blower did not work as well as spreading the lichen by hand. The leaf blower would get clogged with lichen and did not broadcast the lichen evenly. Aerial distribution in the study was considered efficient unless soil material clogged the equipment. The researchers recommend finding ways to develop a system to deploy lichen that was not cleaned as it would be more time-efficient and practical.

The aerial trial was considered successful, and 2400 L of lichen fragments were deployed in 5 min. However, the cost of the helicopter was expensive to rent, and unforeseen weather could increase the cost of restoration. Lichen cover in this study will be determined in the future. To assess the viability, the researchers will examine the color of lichen and conduct fluorescence tests. Yearly and bi-yearly measurements will be collected over the next decade. The authors suggested that lichen mats will need 40–70 years for recovery after fire, but lichen spread at 2.25 L / m² can help establish new mats within 10 years.

2) *Terrestrial lichen transplant in caribou habitat (British Columbia)*

Title: Examining the role of terrestrial lichen transplants in restoring woodland caribou winter habitat

Authors: S. Rapai, D. McColl, R. McMullin (2017)

Extensive wildfires burned large areas of land in northern British Columbia during the summer of 2014. This reduced the population of lichens, which are an important food source for woodland caribou in British Columbia. Caribou in British Columbia are listed as threatened or special concern, and they are known to feed on lichens from the genus *Cladonia* (sub-genus *Cladina*). The study's goal was to restore lichen and woodland caribou habitat in the burned area. It is currently unknown what the best methods are for lichen transplantation. The authors are currently collecting data from this lichen transplant study.

Lichen collection occurred outside the woodland caribou habitat in a pine forest about 200 km away from the study sites. Lichens were collected by hand, and less than 20 % of the lichen from the area was collected. These lichens were stored in a dark and cool area and monitored for signs of molding prior to transplanting. Reindeer lichens including *Cladonia mitis*, *Cladonia stellaris*, *Cladonia stygia*, and *Cladonia uncialis* were collected in the study; they made up an estimated 56 %, 0.001 %, 17 % and 26 %, respectively of the lichen material. Both lichen mats and fragments were collected in the study; the mats were the size of a hand, while the fragments were 2–7 cm in length.

The study consisted of 100 m² plots and 100 L of lichen was applied to each. For the control, no lichens were spread onto the plots. For the treatments, lichen mats or fragments were spread onto the plots. In addition, some plots received both lichen mats and fragments. Each of the four treatments were replicated 20 times for a total of 80 plots. The lichen fragments and mats were placed directly onto coarse and well-drained soil. The authors avoided pooling water, and plots had to be greater than 50 m away from both roads and existing lichens. The fragments were evenly applied to the plots. They ensured the lichen were in contact with the ground and not any debris or logs. The lichen mats were surrounded with the local substrate.

Spreading lichen fragments was observed to be faster than planting the lichen mats. To cover an area of 100 m², two workers were able to disperse fragments in 5 min. On the other hand, the mats took 10-15 min for the same area. The researchers used a total of 6000 L of lichen for their experiment.

3) Reindeer lichen transplant on oil sands (Alberta)

Title: Reindeer lichen transplant feasibility for reclamation of lichen ecosites on Alberta's Athabasca oil sand mines

Author: S. Duncan (2011)

The Athabasca oil sands spans an area of 602 km² and has disturbed the habitat of caribou in Alberta. Deploying lichen fragments in the field may help restore caribou habitat and increase their food availability during the winter. Lichen use the substrate for attachment and there may be a relationship between substrate type and lichen abundance. Prior studies have found that lichens do not grow well when placed directly on mineral soils. The study's goal was to test the

effect of substrate on lichen growth and determine the lichen species present in spruce and pine lichen forests in Alberta.

The study took place in northern Alberta at a Suncor mine at Fort McMurray, and *Cladonia mitis* was collected from Wood Creek. Cup lichens and *Cladonia mitis* were found in 24-year-old spruce and pine lichen forests, but they were not present in 12-year-old sites. *Cladonia stellaris*, *Cladonia rangiferina*, and *Cladonia stygia* were not found on the 24-year-old sites.

The top 2 cm of the lichen thallus was collected. In the field, distilled water was used to moisten the lichen prior to collection, and they were then placed into air-filled bags. The lichen was then placed into a truck in the shade and temporarily stored in a hotel room.

There were three study sites, and six blocks were set up at each site. There were three plots associated with the different soil substrates within each block. The substrate was either mineral soil, moss, or leaf litter, which was collected from the study area. In these plots, the vegetation was removed, and the substrate was placed on top of the sandy soil. In each plot, 25 lichen fragments that were 2 cm long were placed. Lichen's survival and condition was assessed after 2 years. The growth rate was determined by estimating the length of the lichen and the number of branches each year.

The fragments in the plots were observed to break apart over the study, and roughly 50 % of fragments were lost after 2 years. Image analysis was difficult because the fragments would break apart and be covered by the substrate. *Cladonia mitis* in this study had growth rates ranging from 3.8 to 4.7 mm per year, depending on the sites. Hyphal growth, apothecia formation and lateral branching was also determined in this study.

Substrate effects depended on the age of the forest. Moss and litter were a better substrate for lichen than bare mineral soil in the 12-year-old forest but not in the 24-year-old forest. In other words, there was no effect of substrate on lichen in the older forest. This was determined by assessing the number of fragments that remained at the site.

4) Transplant of lichen thalli for conservation (Poland)

Title: Transplantation of lichen thalli: A case study on *Cetraria islandica* for conservation and pharmaceutical purposes

Authors: D. Zarabska-Bozejewicz, E. Studzińska-Sroka, W. Fałtynowicz (2015)

Cetraria islandica is used for pharmaceutical purposes and can help treat respiratory problems. *Cetraria islandica* grows well in old-growth pine stands with little dead matter. However, lichen grows slowly, and lots of lands is required for pharmaceutical purposes. *Cetraria islandica* is also considered a protected species in Poland and has been declining due to pollution. The addition of lichen fragments may increase lichen cover within a given area. The study's goal was to transplant and assess the growth of *Cetraria islandica* and determine whether there were any changes in the chemical composition of the thallus.

This study took place in northern Poland for 3 years, which was enough time to observe changes in lichen growth. *Cetraria islandica* was collected from conifer stands and cut into 3 cm fragments. The authors set up circular sampling plots with a 0.5 m radius, and all the vegetation from the plots was removed. *Cetraria islandica* was evenly dispersed into the plots using either 3.85 g of fragments, 7.77 g of fragments, or lichen clumps weighing 12.62 g. There was also a control plot stripped of lichen and vegetation but received no lichen fragments or clumps. Each of the four treatments was replicated ten times for 40 plots in the experiment. Lichen cover was estimated eight times during the 3 year study. Measuring the length and surface area of lichen is rare in studies. As a result, the authors measured biomass by collecting the lichen from the plots and drying the material for 48 h at room temperature.

The lichens were well-attached to the substrate at the end of the 3 years, and there was little movement of lichen fragments in the plots. The authors observed that younger thallus fragments grew faster, but overall growth was low. Lichen cover was low likely due to competition with lichen that colonized the plots. However, dispersing thallus fragments will help increase cover. When no lichen was applied, there was an average lichen cover of 6 % from natural colonization. The 3.85 g plots had an average lichen cover of 15.1 %, while the 12.62 g plots had 25.5 %. The plots with lichen clumps had four times the amount of lichen cover as plots without lichen addition. The lichen transplant did not affect their chemical composition, but the amount of lichen produced may be too low for pharmaceutical purposes. However, lichen addition could have conservation benefits.

5) *Ground substrate effects on reindeer lichen (Sweden)*

Title: Influence of ground substrate on establishment of reindeer lichen after artificial dispersal

Authors: S. Roturier, S. Bäcklund, M. Sundén, U. Bergsten (2007)

Lichen is an important food source for reindeer, but overgrazing can reduce lichen biomass. In addition, forestry companies can cause soil disturbances (e.g., soil scarification) that influence lichen recovery. The lichen's substrate is essential and is used by the lichen to fasten to. The size of lichen fragments may also affect how well the thalli attach to the surface material. The authors set out to test the effects of substrate and fragment size on lichen establishment.

The study took place in a pine-heath system located in Sweden at the Vindeln Experimental Forests. Quadrats were set up in a clearcut or middle-aged pine forest. The vegetation from these 1 × 1 m quadrats was removed, and the plots were filled with 2 cm of mineral soil, moss, twigs or bark. The moss and twigs were separated using a compost mill. The moss and twig fragments were 3 cm in size. Both fine and coarse pine bark were collected and used as a substrate.

Cladonia mitis was collected at this site, and fragments 1 or 3 cm in size were spread across quadrats during October 2002. Within each quadrat, 30 individual fragments were carefully placed and tied with string. The researchers ensured that the fragments were spaced equally apart in the plots, and the position of each fragment was marked with sticks. Their plots were also marked with paint.

Lichen movement was determined after one year, and recolonization by lichen was observed over 3 years using photographs. They measured the number of fragments remaining in each quadrat. A Nikon Coolpix 4500 camera on a tripod was used, and photographs were taken at the beginning of the experiment and 1 and 3 years after the start. ImageJ was used to analyze the photographs.

In the clearcut, the best substrate was mineral soil and moss; the mineral soil was suitable for colonization by lichen outside the plots. Lichen colonisation mainly occurred outside the quadrats in the clearcut plots. In the pine forest, there were no differences in substrates. Colonization occurred throughout the quadrats in the forest plots (i.e., along the edges and inside the plots). Forest stands may favor moss growth over lichens because of potential differences in air ventilation. The researchers found that wind had the greatest effect on lichens in the clearcut

compared with the pine forest. Needles in the pine forest prevented lichen fragments from being lost in the wind, but the presence of needles could potentially reduce photosynthetic rates if the lichen are covered. Lichen cover was greater in the plots with 3 cm fragments, and the researchers hypothesize that these longer fragments have more access to light and water.

The authors saw an increase in lichen cover after spreading the lichen onto the plots. They suggest that artificial dispersal of lichen can help restore disturbed sites. They suggest that larger fragments in forest sites would be beneficial because the lichen would have more access to light and would be less impacted by wind. It is known that lichen fragments 2 mm in length can grow; in this study, growth was lower in the smaller fragments (1 cm) than, the larger fragments (3 cm). Colonization by lichen in the clearcut plots was more extensive than the pine plots. Fragment size was not important in the clearcut, but moss would help the lichen attach and establish.

6) *Eight-year transplant study (Sweden)*

Title: A comparison of the physiology, anatomy and ribosomal DNA in alpine and subalpine populations of the lichen *Nephroma arcticum*: The effects of an eight-year transplant experiment

Authors: M. Sonesson, B. Sveinbjörnsson, A. Tehler, B. Å. Carlsson (2007)

Prior studies have shown that *Nephroma arcticum* growing in different environments have different morphologies and photosynthetic potential. The authors previously investigated changes in *Nephroma arcticum* over 3 months and found substantial differences in populations. These differences can be a result of the environment and/or genetics. Lichen transplant studies have been conducted over short periods (between 1–2 years), but longer-term studies are not common. There is a need to assess lichen acclimation over longer durations. The goal of the study was to assess the acclimation of the lichen *Nephroma arcticum* over 8 years.

A long-term lichen transplant study was conducted in the Swedish Lapland. Two sites were selected, including a subalpine (380 m elevation) and alpine site (1100 m elevation). Ten clumps of *Nephroma arcticum* were collected from each site, each measuring 15 × 15 cm and containing portions of humus. The clumps were placed into wooden frames lined with nylon at the bottom to hold the lichen in place. Five frames were transplanted within site, and five were transplanted to the other site. For example, 5 subalpine frames were placed at the subalpine site, and 5 were

placed at the alpine site. There was a 5×5 m plot at each site, where the humus was removed. The ten frames were placed into these stripped plots, and the litter was removed from lichen in the frames.

The growth rates of the lichen were assessed using photographs. These measurements were considered unreliable, and this method was not used after the second year of the study. The authors measured the length of lichens on the sides of the frames instead.

Photosynthetic and respiratory measurements were also assessed. The authors sprayed the lichen with distilled water, and 10–15 mm by 3–4 mm thallus fragments were placed into 25 cm² nylon nets. They were then placed into cuvettes for gas measurements. The lichen was kept at photosynthetically active radiation (PAR) of 120 $\mu\text{mol m}^{-2} \text{s}^{-1}$ for 12 h at 5 °C and sprayed with distilled water. The lichen was measured at a CO₂ concentration of 400 ppm at a temperature of either 5 or 10 °C.

The lichen was found to acclimate to their environment. The total thallus thickness did not change, but transplanting lichen from the low alpine site to the high alpine site resulted in a thicker upper cortex and a thinner epicortical layer.

Photosynthetic rates increased as temperatures increased, and the optimum temperature for *Nephroma arcticum* is estimated between 10 and 20 °C. Light requirements are greater in high alpine sites than in low alpine sites. When transplanted, maximum photosynthesis and light compensation became similar to the controls, suggesting acclimation. Respiration rates decreased for the lichen transplanted to the low alpine site.

Acclimation may occur more quickly in the algae than the fungus. Both genetics and the environment have an impact on lichen traits. The authors suggest that an 8 year study may have been too short for full acclimation of the lichen.

7) *Adhesives for lichen translocation (Spain)*

Title: Successful lichen translocation on disturbed gypsum areas: A test with adhesives to promote the recovery of biological soil crusts

Authors: M. Ballesteros, J. Ayerbe, M. Casares, E. M. Cañadas, J. Lorite (2017)

Biological soil crusts are often composed of lichen, algae, cyanobacteria and moss. Mining and quarrying have destructive effects on the ecosystem and can damage biological soil crusts. Furthermore, lichen recovery is often neglected and not considered after mining disturbances. Transplanting lichen may help restore biological soil crusts, but lichen fragments may be lost to the wind. *Diploschistes diacapsis* is a crustose lichen commonly found in biocrusts in Spain. The study assessed the use of different adhesives to enhance the attachment of *Diploschistes diacapsis* to gypsum soil. The adhesives that were tested were glue, hydroseeding stabilizer, gum arabic, acacia tree extract, synthetic resin and water. Control plots had no additives applied to the thallus of the lichen.

Lichen was collected at a quarry site in Spain, soaked in tap water, and stored in paper bags. The lichen was separated into 15 mm disks and used within 24 h. Plastic trays (35 × 25 × 5 cm) were filled with gypsum soil. This experiment tested six adhesives (including the control treatment), and each was replicated 5 times. This resulted in a total of 30 plastic trays with 10 thallus fragments within each tray. The trays were placed into the greenhouse, and historic rainfall events were simulated over 2 weeks with a drop forming chamber. The amount of lichen attached and detached was assessed visually. The trays were placed outdoors from May to June, and they were assessed for viability. Specifically, CO₂ exchange and fluorescence were determined, and these measurements were conducted from 10:00 to 13:00 h. The lichen was moistened prior to any measurements and placed directly into the chamber.

There were 30 plots (0.5 × 0.5 m) in the field, and 35 lichen fragments were placed on each plot. The attachment was monitored for 15 months, and after 16 months, CO₂ and F_v/F_m (a measure of stress) was measured to assess lichen health. The latter was measured on dark-adapted lichens that were initially soaked with water. This was compared with dark-adapted lichens in the field.

Attachment in the field experiment differed depending on the additive used. In this case, water and glue were the best for lichen retention. Water would initially detach the lichen from the substrate, but it would attach to the substrate when the lichen dried. White glue, water, hydroseeder stabilizer, and gum arabic improved attachment to the soil. There were no differences in attachment amongst the additives in the greenhouse rainfall simulation. However, the authors predicted that differences might be observed for long-term studies; the greenhouse experiment was shorter in duration than the field experiment, where differences in adhesives

were observed. The field experiment also exposed lichen to wind and rain more often. When spreading lichen, the use of water may help promote the establishment and recovery of lichen. This is a very promising result for lichen transplants.

Reduced photosynthesis and necrosis were observed when the synthetic resin was applied. Overall, the adhesives did not affect the physiology of the lichen substantially. F_v/F_m values around 0.28 are typical of healthy *Diploschistes diacapsis* in the field. The values ranged between 0.21 and 0.31 for the adhesives in the study.

8) Informal study on greenhouse growth (United States)

Title: An informal study of growing fruticose lichens in greenhouses

Author: C. R. A. Stewart (2019)

Transplant (or translocation) studies can provide useful information for establishing methods to help restore disturbed areas. Lichen growing outdoor are exposed to grazing animals and potentially unfavorable weather conditions. These sites may also be far away and require time for travel and monitoring. Greenhouses can be used to help cultivate lichens for restoration purposes. However, very few studies assess how to grow lichens in the greenhouse, and there is a need to grow endemic or rare lichens in greenhouses. The goal of the study was to grow transplants in the greenhouse.

The study took place at the University of Colorado. *Usnea lapponica* and *Usnea cavernosa* were collected in Colorado, while *Ramalina menziesii* was collected in California. The lichens were collected from branches on the ground. In addition, branches that were 25–30 cm in length were sterilized in the oven for 2 h at 260 °C; this removed any bacteria and existing fungi. Silicon sealant was placed onto the branch and the lichens were held in place to assist their attachment. The lichens were spaced evenly apart on the branches.

Greenhouse A was watered with tap water, and the branches were misted four times a day (a total of 6 min per day). Greenhouse B was watered with deionized water once every 3 months. Each greenhouse received one branch with *Usnea* sp. and one branch with *Ramalina menziesii*. The transplants were visually monitored for survival. The lichens were considered dead when there was no color and bleaching occurred. No growth measurements were conducted because of

differences in humidity between the greenhouses. Differences in humidity will affect the amount of water stored in the lichen thallus.

Bleaching occurred within 2 weeks in the wet greenhouse for *Ramalina menziesii* and bleaching for the *Usnea* species occurred within 4 weeks. On the other hand, bleaching occurred within 19 months in the dry greenhouse. This would suggest that the lichens require a dry-down period between watering events. Consistently wet lichens may result in bleaching and death. In addition, the water supply may have a significant effect. The pH of the water should be considered, as the tap water used in the wet greenhouse may have been too basic for the lichens. Therefore, deionized water may be better for growing lichens in the greenhouse.

9) *Storage of lichens*

Title: Culturing the lichens *Lobaria oregana* and *Lobaria pulmonaria* on nylon monofilament

Author: W. C. Denison (1998)

Transplanting and establishing lichens in the greenhouse is difficult. Lichens are often observed in the field using photographs. The goal of the study was to establish transplants of *Lobaria oregana* and *Lobaria pulmonaria* and assess their growth. The author developed a method to transplant lichens. These *Lobaria* species are important nitrogen fixers in the environment, and they can obtain majority of their nutrients from the atmosphere through gases and precipitation.

Dry lichen was collected from western Oregon and stored at 12–24 °C at 50–60 % humidity. The lichens were cleaned of bark, twigs and moss. Lichens with a minimum of three lobes were selected, and holes that were 2–5 cm in size were taken. Cuttings that were 1–2 cm in size were also taken but not used in the experiment. The lichen fragments were strung onto a nylon monofilament string that was 42 cm long, and rubber tubing was placed at the end. Each string consisted of 25 lichen transplants, and between each cutting there was a plastic bead. The air-dry weight for the cuttings ranged from 1–6 g for the 25 cuttings.

The transplants were hung on racks outdoors, and growth was measured by weighing the transplants. They were taken to the lab and dried at 18 °C at a relative humidity of 55 % for 4 h. The weight of the string and beads were considered when determining the weight of the lichen.

The authors found that there was little damage over the 3 years and the weather did not cause any transplants to be lost. However, microorganisms attacked some thali and necrosis was observed in some of the lichen transplants. There was no grazing by invertebrates.

The authors found that 90 days or more of lichen storage resulted in no growth and stress. However, storage at a lower temperature (<12–24 °C) and humidity (<50–60 %) may extend the storage potential of lichen. *Lobaria oregana* stored for 48 and 73 days had positive growth rates, while transplants stored for 104 days lost weight. *Lobaria pulmonaria* stored for 22 and 90 days had positive growth, while transplants stored for 170 and 345 days lost biomass. Over a few months, the dead lichens lost an estimated 35–40 % of their weight. Growth rates vary from cutting to cutting, and initial biomass should always be measured.

10) Transplant method trials (United States)

Title: Testing lichen transplant methods for conservation applications in the southern Appalachian Mountains, North Carolina, U.S.A.

Author: J. L. Allen (2017)

Transplanting lichens could help prevent threatened and endangered species from going extinct. However, transplanting lichens is difficult, and it may take 10–30 years before one can assess the success of transplants because they have slow growth rates. In addition, crustose lichens are not often transplanted because they are difficult to remove from the substrate. The study's goal was to develop methods to transplant crustose, foliose, and fruticose lichens.

The author collected *Graphis sterlingiana* (crustose lichen), *Hypotrachyna virginica* (foliose lichen), and *Lepraria lanata* (crustose lichen) from the Pisgah National Forest in North Carolina. This region often has a 100 % humidity and receives precipitation daily. The transplants were set up May 2015 on Roan Mountain in the Pisgah National Forest. The thalli were cut using a razor blade, and small fragments were placed onto 2 × 2 in sterile gauze using forceps. *Graphis sterlingiana* was placed on *Betula alleghaniensis* (birch), while *Hypotrachyna virginica* was placed on *Abies fraseri* (fir) or *Picea rubens* (spruce). *Lepraria lanata* was placed near sheltered boulders and rock outcrops. These transplants were monitored in November 2015, May 2016, November 2016 and March 2017 using a hand lens and photographs. The gauze did not work, and the transplants were lost in the weather conditions. The success of gauze transplants may

depend on the environment and site conditions. The majority of the gauze placed on the trees fell off within 6 months of the study. Only one *Graphis sterlingiana* transplant remained at the end of the study.

Alternative transplant substrates were tested with *Lepraria finkii* (crustose lichen). On November 2015, lichen was placed on burlap, cheesecloth, gauze, and Honeywell filter stapled to *Betula alleghaniensis*. They were monitored during May 2016, November 2016, and March 2017. In addition, *Usnea angulata* (fruticose lichen) were collected from a fallen log. It was cut into 19.5–63.5 cm long pieces and attached to a monofilament. The loops were attached to a long dowel on November 2016 and 2017, and they were monitored for changes in length. Burlap performed the best, and it may be best to use burlap for crustose lichens. This material can hold moisture well and offers attachment points for the lichen. It also degrades more slowly. The *Usnea angulata* transplants grew well on the wooden dowels, and fast growth rates were observed in this study. The growth of *Usnea* exceeded the rates of other studies.

11) Transplant after fire (Sweden)

Title: Restoration of reindeer lichen pastures after forest fire in northern Sweden: Seven years of results

Author: S. Roturier, O. Sébastien, L.-E. Nutti, U. Bergsten, H. Winsa (2018)

Forest lichens have been declining in Sweden due to climate change, logging, reindeer grazing and fire suppression. The latter can increase the abundance of mosses, grasses and shrubs, which can outcompete lichens. Prescribed fires have now been allowed in Sweden, but the recovery of lichens is generally slow. Reindeer lichens, which rely on asexual reproduction through fragmentation, can be transplanted as fragments or mats to accelerate lichen recovery after fire. The goal of the study was to assess reindeer lichen transplants on burned sites and the effects of the season of transplant.

The study took place in Northern Sweden at a site where an accidental fire occurred in August 2006. The lichen was collected from Finland and fragmented using vacuum shredder. Before shredding, the lichen was not cleared of debris (e.g., pine needles). The lichens were transplanted onto three sites with varying fire severities and post-fire treatments (e.g., clearcut or untouched) in September 2008 or March 2009, and were applied at 0.45 L / m² or 2.25 L / m². The lichens

were stored for 2 days for the September transplant and 5 months for the March transplant. They were assessed for colour and substrate attachment in September 2010, August 2013 and September 2015.

Lichen survival and fragment establishment was observed. Forests that were not clearcut facilitated lichen attachment, and canopy closure (38 %) provided *Cladonia stellaris* with optimum light levels. The other sites were drier, and fragments were loosely attached to the substrate. Lichen establishment was higher when more fragments were applied to the plots, but higher mortality resulted from greater competition. Summer transplants had higher survival and establishment than ones transplanted in the winter on the snow. The storage of lichens and lower temperatures could have influenced the lower survival rates for the winter transplants. The storage of lichen may significantly influence the success of lichen transplants, and they should be stored in well-vented bags. Lichen transplants can be applied 2 years after fire, and partial tree cover was better for lichen than open sites.

12) Reindeer lichen regeneration in forests (Sweden)

Title: Managing reindeer lichen during forest regeneration procedures: Linking Sámi Herders' knowledge and forestry

Author: S. Roturier (2009)

The indigenous people of Sweden, the Sámi people, herd reindeer as a source of food. Terricolous and epiphytic lichens are part of the reindeer diet, and they forage through the snow for lichens in the winter. Soil scarification, which is a technique used for forest regeneration, can decrease the growth of lichens. In addition, fertilization can increase shrub and tree growth and increase competition for lichen. This study examined the recovery of lichen using fragments using different scarification methods. This study aimed to minimize damage to the soil to help restore lichen in forests while allowing for tree regeneration at the same time.

A series of studies took place in Sweden, and visual cover estimates using 50 × 50 cm frames with 25 individual squares. The height of the lichen in the plots was determined, and photographs and biomass measurements were conducted.

Cladonia mitis fragments (1 or 3 cm) were collected and spread onto plots. The bare mineral treatments did not facilitate lichen growth, but mosses, twigs and pine bark assisted the

attachment of lichen to the ground. *Cladonia* lichens were found to establish well from fragments, and the use of organic material can assist growth. Closed canopies also decreased wind effects and reduced the movement of fragments in the plots.

Patches of lichen were subjected to heavy reindeer grazing but recovered over time. The transplant of fragments over patches of lichens was recommended. In addition, more gentle scarification methods are recommended to reduce damage to lichens.

Tables

Table 1 Lichen studies using transplants in the field or greenhouse. The location, species, objectives, measurements and results for these studies are summarized. The studies have been organized by the year of publication. Refer to the main text for the full bibliography.

Author(s)	Year	Location	Species	Objective(s)	Environment	Measurement(s)	Result(s)
Phinney et al.	2020	Scandinavia	<ul style="list-style-type: none"> • <i>A. sarmentosa</i> • <i>U. dasopoga</i> • <i>B. fuscescens</i> 	<ul style="list-style-type: none"> • Testing the effect of precipitation 	Field	<ul style="list-style-type: none"> • Biomass • Relative growth rates 	<ul style="list-style-type: none"> • Rainfall improves growth in pale lichens
Leddy et al.	2019	Auckland	<ul style="list-style-type: none"> • <i>C. aurata</i> 	<ul style="list-style-type: none"> • Testing the success of lichen propagule dispersal 	Field	<ul style="list-style-type: none"> • Lobe formation 	<ul style="list-style-type: none"> • Use of gel enhances contact with gauze • Lobe formation observed
Paoli et al.	2019	Slovakia	<ul style="list-style-type: none"> • <i>E. prunastri</i> 	<ul style="list-style-type: none"> • Using lichen for biomonitoring 	Indoor and outdoor	<ul style="list-style-type: none"> • Elemental analysis • Vitality (fluorescence) 	<ul style="list-style-type: none"> • Heavy metals increased over time • Indoor lichens remained healthy
Pasiche-Lisboa et al.	2019	Manitoba, Canada	<ul style="list-style-type: none"> • 32-38 species 	<ul style="list-style-type: none"> • Assess lichen diversity in different stand types 	Field	<ul style="list-style-type: none"> • Microscopy used to assess propagules 	<ul style="list-style-type: none"> • Moss and lichen more similar in conifer stands compared to poplar stands
Stewart	2019	Colorado, United States	<ul style="list-style-type: none"> • <i>U. lapponica</i> • <i>U. cavernosa</i> • <i>R. menziesii</i> 	<ul style="list-style-type: none"> • Establish lichens in the greenhouse 	Greenhouse	<ul style="list-style-type: none"> • Color / bleaching 	<ul style="list-style-type: none"> • Low survival • Irregular watering better than overwatering
Vitt et al.	2019	Alberta, Canada	<ul style="list-style-type: none"> • 12 <i>Cladonia</i> spp. • <i>C. islandica</i> • <i>C. ericetorum</i> • <i>Bryoria</i> sp. • <i>Alectoria</i> sp. 	<ul style="list-style-type: none"> • Assess effects of canopy thinning on the ground layer after 19 years 	Field	<ul style="list-style-type: none"> • Abundance • Species richness 	<ul style="list-style-type: none"> • No significant changes in species diversity • Abundance of reindeer lichens increased marginally

Gauslaa et al.	2018	Norway	<ul style="list-style-type: none"> • <i>L. amplissima</i> • <i>L. pulmonaria</i> 	• Transplant lichen and test phosphorus fertilization	Field	<ul style="list-style-type: none"> • Biomass • Relative growth rates 	<ul style="list-style-type: none"> • Phosphorous fertilization had no effect • Gastropod grazing reduced the success of transplants
Ronalds and Grant	2018	British Columbia, Canada	<ul style="list-style-type: none"> • <i>C. mitis</i> • <i>C. uncialis</i> • <i>C. rangiferina</i> • <i>Stereocaulon</i> sp. 	• Transplant lichen to post fire sites	Field	• Lichen cover	<ul style="list-style-type: none"> • Addition of lichens improved lichen cover • Aerial deployment was effective but expensive
Roturier et al.	2018	Sweden	• <i>Cladonia</i> sp.	• Assess lichen transplants after fire, and the effects of season of transplant	Field	<ul style="list-style-type: none"> • Viability based on color • Attachment • Abundance 	<ul style="list-style-type: none"> • Transplanting was successful and enhanced recovery after a fire • Summer transplanting better than winter transplanting on snow cover
Allen	2017	North Carolina, United States	<ul style="list-style-type: none"> • <i>G. sterlingiana</i> • <i>H. virginica</i> • <i>L. lanata</i> 	• Test different substrates for lichen attachment	Field	• Amount of lichen on substrate	<ul style="list-style-type: none"> • Gauze is not a suitable substrate • Burlap is suitable for crustose lichens
Ballesteros et al.	2017	Spain	• <i>D. diacapsis</i>	• Test different adhesives for transplanting lichens	Field	<ul style="list-style-type: none"> • CO₂ exchange • Fluorescence 	• Water and glue enhanced thallus attachment
Rapai et al.	2017	British Columbia, Canada	<ul style="list-style-type: none"> • <i>C. mitis</i> • <i>C. stellaris</i> • <i>C. stygia</i> • <i>C. uncialis</i> 	• Assess lichen cover when fragments and mats are applied	Field	• Lichen cover	• Results TBD

Legebokow	2016	British Columbia, Canada	<ul style="list-style-type: none"> • <i>Bryoria</i> sp. • <i>Alectoria</i> sp. 	• Collect lichen for caribou feed	Field	• Weight	• N/A
Rapai et al.	2015	British Columbia, Canada	<ul style="list-style-type: none"> • <i>C. mitis</i> • <i>C. rangiferina</i> 	• Assess conditions needed for lichen transplant	Field	• Cover	<ul style="list-style-type: none"> • The addition of fragments or mats increased lichen cover • Forest litter did not influence lichen cover
Zarabska-Bozejewicz et al.	2015	Poland	• <i>C. islandica</i>	• Examine whether lichen addition improves cover	Field	• Lichen cover	<ul style="list-style-type: none"> • Lichen clumps had the highest cover • Greater amounts of lichen fragments produced higher cover
Smith	2013	United Kingdom	• <i>O. lithyrga</i>	• Transplant bricks with lichen	Field	• Survival	• Lichen survival over 1 year
Duncan	2011	Alberta, Canada	• <i>C. mitis</i>	• Substrate effects and species determination in forests	Field	<ul style="list-style-type: none"> • Photographs • Length • Microscopic 	• Moss and litter better than bare soil in younger forests
Campeau and Blahchard	2010	Labrador, Canada	<ul style="list-style-type: none"> • <i>Cladonia</i> sp. • <i>Stereocaulon</i> sp. • <i>Peltigera</i> sp. 	• Revegetate mineral disturbances	Field	• Visual inspection of lichen cover	• Increase in mosses, lichens, shrubs, and woody species after 5 years since propagule addition
Kon and Ohmura	2010	Japan	<ul style="list-style-type: none"> • <i>P. clavuliferum</i> • <i>R. yasudae</i> 	• Regenerate lichen on trees from soredia	Field	<ul style="list-style-type: none"> • Scanning electron microscope • Lobe formation 	<ul style="list-style-type: none"> • After 12 months, lobes formed on <i>P. clavuliferum</i> • After 18 months, lobes formed on <i>R. yasudae</i> • Fruticose lichen growth

Schoenwetter	2010	New Zealand	<ul style="list-style-type: none"> • <i>P. colensoi</i> • <i>P. faveolata</i> 	<ul style="list-style-type: none"> • Examine effects of tree stands on lichen growth 	Field	<ul style="list-style-type: none"> • Survival • Growth 	<ul style="list-style-type: none"> • Lichens influenced by tree level factors • No difference between stands of different age
Lidén	2009	Scandinavia	<ul style="list-style-type: none"> • <i>E. divaricata</i> • <i>R. dilacerata</i> • <i>P. norvegia</i> 	<ul style="list-style-type: none"> • Transplant lichen and assess their habitats and performance 	Field	<ul style="list-style-type: none"> • Photosynthetic (PSII) activation 	<ul style="list-style-type: none"> • Fragments and soredia can be used for restoration
Roturier	2009	Sweden	<ul style="list-style-type: none"> • <i>Cladina</i> sp. 	<ul style="list-style-type: none"> • Restore forest plots and examine effects of substrate 	Field	<ul style="list-style-type: none"> • Lichen cover • Photographs • Biomass 	<ul style="list-style-type: none"> • HuMinMix technique is best suited for scarification of soil • Ground litter helps lichen attach and limit losses by wind • The use of fragments is better than lichen mats, which may be grazed
Coxson and Stevenson	2007	British Columbia, Canada	<ul style="list-style-type: none"> • <i>L. pulmonaria</i> 	<ul style="list-style-type: none"> • Assess lichen growth in even and uneven age stands 	Field	<ul style="list-style-type: none"> • Biomass 	<ul style="list-style-type: none"> • Lichen in uneven age stands had greater growth than even age stands
Roturier et al.	2007	Sweden	<ul style="list-style-type: none"> • <i>C. mitis</i> 	<ul style="list-style-type: none"> • Test different substrates and fragment sizes 	Field	<ul style="list-style-type: none"> • Lichen cover 	<ul style="list-style-type: none"> • Moss better on clearcut, but not in the forest • Cover greater with larger fragments
Sonesson	2007	Sweden	<ul style="list-style-type: none"> • <i>N. arcticum</i> 	<ul style="list-style-type: none"> • Assess acclimation potential in lichen 	Field	<ul style="list-style-type: none"> • Photographs • Length • Photosynthesis and respiration 	<ul style="list-style-type: none"> • Lichen can acclimate to their environment • No change in thallus thickness • Changes in upper cortex

Antoine and McCune	2004	Washington, United States	<ul style="list-style-type: none"> • <i>L. oregana</i> • <i>L. pulmonaria</i> • <i>L. vulpina</i> • <i>U. scabrata</i> 	• Determine canopy position effects	Field	• Biomass changes in 1 year	<ul style="list-style-type: none"> • Lichen competition and microclimate may reduce <i>L. oregana</i> growth in the top canopy • Abundance related to growth rates for <i>L. vulpina</i> • <i>U. scabrata</i> grew at top of canopy but had higher growth rates at lower level
Kon et al.	2003	Japan	• <i>P. tinctorum</i>	• Transplant lichen	Field	<ul style="list-style-type: none"> • Surface area change • Morphology 	• Growth observed over 15 months
Gilbert	2002	United Kingdom	• <i>L. amplissima</i>	• Observe lichen over 20 years	Field	<ul style="list-style-type: none"> • Attachment • Tracing outline 	<ul style="list-style-type: none"> • Increasing size of lichen for 15 years • Last 5 years grazing became worse
Hilmo	2002	Norway	<ul style="list-style-type: none"> • <i>L. scrobiculata</i> • <i>P. norvegica</i> • <i>P. glauca</i> 	• Lichen growth in young and old spruce forests	Field	<ul style="list-style-type: none"> • Thallus surface area • Photographs 	<ul style="list-style-type: none"> • <i>L. scrobiculata</i> and <i>P. norvegica</i> growth did not differ between habitats • Indications of high adjustments in lichens
Hilmo and Ott	2002	Norway	<ul style="list-style-type: none"> • <i>L. scrobiculata</i> • <i>P. glauca</i> • <i>P. norvegica</i> 	• Observe development of lichen diaspores	Field	<ul style="list-style-type: none"> • Scanning electron microscope • Lobe length 	<ul style="list-style-type: none"> • All three species developed thalli • Slow process • 29 months for thalli to develop in <i>L. scrobiculata</i> (longest recorded)

Callaway et al.	2001	Georgia, United States	<ul style="list-style-type: none"> • <i>Parmotrema</i> sp. 	<ul style="list-style-type: none"> • Assess the growth of Spanish moss on branches with lichens 	Greenhouse	<ul style="list-style-type: none"> • Spanish moss length 	<ul style="list-style-type: none"> • 19.8 % lower growth when the lichen was not present on branches
Hazell and Gustafsson	1999	Sweden	<ul style="list-style-type: none"> • <i>L. pulmonaria</i> 	<ul style="list-style-type: none"> • Assess lichen growth on aspens in clearcuts 	Field	<ul style="list-style-type: none"> • Vitality class 	<ul style="list-style-type: none"> • Transplants dying slowly • Some new growth zones • Vitality higher on north • Mollusk grazing
Sillett and McCune	1998	Oregon, United States	<ul style="list-style-type: none"> • <i>L. oregana</i> • <i>P. rainierensis</i> 	<ul style="list-style-type: none"> • Evaluate growth of lichen in a Douglas-fir forest 	Field	<ul style="list-style-type: none"> • Assessed for physical condition • Mortality • Growth rate based on dry weights 	<ul style="list-style-type: none"> • Thick moss mats improve lichen activity • Lichen growth high in young and old forests • Growth rates and survival significantly lower on clearcuts
Webster and Brown	1997	United Kingdom	<ul style="list-style-type: none"> • <i>P. canina</i> 	<ul style="list-style-type: none"> • Establish lichen under different water regimes 	Garden	<ul style="list-style-type: none"> • Photographs • Length measurements 	<ul style="list-style-type: none"> • Observed growth in both the non-watering and continuous hydration treatments • Rhizinae growth greatest in wet pots
McCune et al.	1996	Oregon, United States	<ul style="list-style-type: none"> • <i>A. sarmentosa</i> • <i>E. prunastri</i> • <i>L. pulmonaria</i> • <i>U. longissima</i> • <i>P. rainierensis</i> • <i>L. oregana</i> 	<ul style="list-style-type: none"> • Develop lichen transplant techniques • Attempt to eliminate the need of punching a hole into the thallus 	Field	<ul style="list-style-type: none"> • Weight 	<ul style="list-style-type: none"> • Growth ranged from 5 % to 30 % • Need to correct weights from wet to dry estimates using reference or sacrificial methods • Reference sample better when using rare species • Sacrificial method better when lichen is abundant

Bando and Sugino	1995	Japan	<ul style="list-style-type: none"> • <i>P. tinctorum</i> 	<ul style="list-style-type: none"> • Test various environmental conditions for growing lichen in solution 	Growth cabinets	<ul style="list-style-type: none"> • Thallus area 	<ul style="list-style-type: none"> • 20 % lichen growth observed when grown at 100 % relative humidity at 20 °C with 16 hour lighting
Scheidegger et al.	1995	Switzerland	<ul style="list-style-type: none"> • <i>P. crinitum</i> • <i>L. pulmonaria</i> • <i>S. sylvatica</i> 	<ul style="list-style-type: none"> • Transplantation of adult lobes and vegetation diaspores 	Field	<ul style="list-style-type: none"> • Diaspore counts • Lobe growth 	<ul style="list-style-type: none"> • Substantial losses • Failure of diaspores likely due to weather conditions • Browsing of thallus
Sillett	1994	Oregon, United States	<ul style="list-style-type: none"> • <i>L. oregana</i> 	<ul style="list-style-type: none"> • Transplantation of interior and edge lichens 	Field	<ul style="list-style-type: none"> • Weight of air dried lichens 	<ul style="list-style-type: none"> • Lichens grew less along edge habitats • Interior lichen placed into an edge environment did not grow
Honegger	1993	Switzerland	<ul style="list-style-type: none"> • <i>P. sulcata</i> • <i>X. parietina</i> 	<ul style="list-style-type: none"> • Culture lichen in gardens 	Field	<ul style="list-style-type: none"> • Photographs • Dissecting microscope 	<ul style="list-style-type: none"> • Lichen fragments glued to ceramic tiles and thallus fixed themselves using rhizines • Transplants grew well outside in the city
Denison	1988	Oregon, United States	<ul style="list-style-type: none"> • <i>L. oregana</i> • <i>L. pulmonaria</i> 	<ul style="list-style-type: none"> • Suspending lichen transplants in the air to promote growth 	Field	<ul style="list-style-type: none"> • Air dried weights 	<ul style="list-style-type: none"> • Minimal weather damage over 3 years • Some damage from micoorganisms, but no grazing by invertebrates • 90 days or more of storage resulted in no growth and stress • Growth rates between 17 and 67%

Armstrong	1977	United Kingdom	<ul style="list-style-type: none"> • <i>P. orbicularis</i> • <i>P. conspersa</i> • <i>P. glabratula</i> • <i>P. saxatilis</i> 	• Transplant lichen onto rocks with different aspects	Field	• Radial growth	• Light, temperature and water on rocks of different aspect will determine growth
Culberson	1963	France	Unknown	• Observation of lichen growing in the greenhouse	Greenhouse	• Visual	<ul style="list-style-type: none"> • Crustose lichen • White and green • Resemble <i>L. aeruginosa</i>
Brodo	1961	New York, United States	<ul style="list-style-type: none"> • <i>C. chlorophaea</i> • <i>L. leptyroides</i> • <i>P. caperata</i> 	• Transplant lichen onto the bark of other trees	Field	<ul style="list-style-type: none"> • Survival • Color changes (decolorization) 	<ul style="list-style-type: none"> • Assessed 4 months after transplant • Some thalli were healthy while others were damaged • Lichens became pale and shriveled

Table 2 Summary of operational scale costs by study. The time required to collect and transplant the lichens, the application method and density, and costs are presented.

Author(s)	Year	Time to collect (per person)	Application method	Density of application	Time to apply	Cost
Ronalds and Grant	2018	1-1.5 bags / hr 100-150 L / hr	Aerial	800 L over 2000 m ² transect	15 mins for 2 people	\$125,000 / ha / wk, including crew accommodations and rentals
		1-1.5 bags / hr 100-150 L / hr	Hand	800 L over 2000 m ² transect	2 hr for 2 people	~\$350 / ha / person
Roturier et al.	2018		Hand	0.45 L / m ² = 45 L / ha		\$650 USD / ha
			Hand	2.25 L / m ² = 225 L / ha		\$3,250 USD / ha
Rapai et al.	2017	67-200 L / hr	Hand	100 L / 100 m ²	For two people: ~ 5 min for fragments ~ 10–15 min for mats	
Rapai et al.	2015	138 L / hr	Hand	80 L / 100 m ² = 8,000 L / ha	25 hr / ha	