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Assessment of Rare Epiphytic Liverwort Transplantation Method in Populus Tremula Forest

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***Abstract*. Epiphytic bryophytes are important biodiversity elements in forest ecosystems globally. In addition, bryophytes take part in ecosystem functioning and are excellent environmental indicators. Almost half of the red-listed bryophyte distribution in Latvia is related to forest habitats. However, despite the increasing knowledge about epiphyte ecology, we are lacking information about individual rare species environmental demands. The present study aimed to evaluate the transplant disc method in rare liverwort transplantation success in aspen forest. As a result, we found that the transplant disc method can be used in epiphytic liverwort studies, but improvements are recommended in sealant selection for transplantation. Liverwort transplants were sensitive to changed substrate quality. Although transplant physiological stress during the transplantation experiment could be important. Epiphyte transplantation studies could help to test epiphyte sensitivity to global climate change in the future.**

Keywords*: *transplantation, liverworts, epiphytes, Populus tremula.

I. Introduction

Bryophytes are important biodiversity elements in forest ecosystems worldwide [1]. They have significant role in ecosystem functioning, providing shelter for other organisms and their role in human life (for instance, peat-forming) is huge [2]. Bryophytes are also much better environmental indicators than vascular plants [3]. Due to their indicator ability, bryophytes were used as excellent indicators in studies of air pollution and ecology [4].

Epiphytic bryophytes and lichens have been used in transplantation experiments using different methods for several decades [5], [6], [7], [8]. Pioneering work by Brodo [9] showed the practical use of epiphytic lichen transplants as indicators of air pollution. Similar method could be applicable also to epiphytic bryophytes. The results of transplantation experiments can reveal new knowledge about a wide range of ecological questions from local to worldwide scale [8].

Despite of many epiphyte transplantation studies around the world, epiphytic liverworts (one of three bryophyte groups) were rarely used as a model organism. A recent review indicated that only 4% of bryophyte and lichen transplantation studies until 2020 around the world used liverworts as model species [8]. However, liverworts are important biodiversity elements and are sensitive to changed environmental conditions. This shows that they could be important indicators of the changed environment. The last compilation counted 7486 liverwort species globally [10] contributing significant part of global biodiversity.

Almost half of red-listed bryophyte species distribution in Latvia is related to forest habitats [11]. The studies about ecology of most of these species are missing, especially about red-listed liverworts. The transplantation approach could contribute to knowledge about liverwort ecology.

The aim of this study was to evaluate the transplantation method potential of red-listed epiphytic liverwort *Lejeunea cavifolia* for ecological studies in aspen *Populus tremula* forest.

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II. Material and methods

We studied *Lejeunea cavifolia* transplant vitality in aspen *Populus tremula* dominating forest stand in Ābeļi Nature Reserve in the south-eastern part of Latvia (Fig. 1). This study was conducted with the permission of Nature Conservation Agency Republic of Latvia.

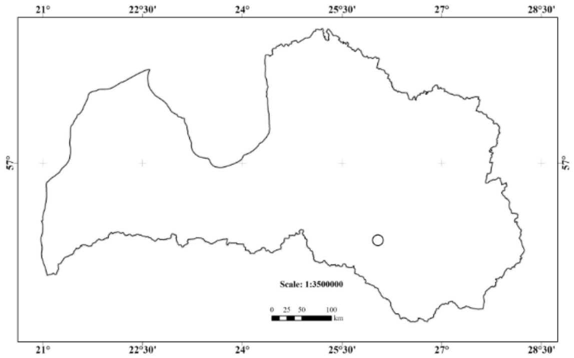

Fig. 1. Study site (circle) in Latvia. Base map author: Māris Nitcis.

Lejeunea cavifolia is red-listed liverwort species and is protected by Latvian government regulations [12], [13], [14]. This species is growing on deciduous trees in old-growth slope, deciduous and boreal forests [15] and is distributed in Europe, Asia, North America, South America and Macaronesian Islands [16]. *Lejeunea cavifolia* has a scattered distribution in Latvia [17] and reproduces sexually by spores [18].

We conducted transplantation experiment in aspen forest stand that was 92 years old with area of 4.6 ha (Latvian Forest Resource Database). We transplanted *L. cavifolia* transplants to a good substrate (control treatment) and a bad substrate treatment. As a good substrate we defined living aspen individuals with stems larger than 0.20 m at the diameter of breast height (DBH). Bad substrate aspen stem DBH was less than 0.20 m. Transplant consisted of a piece of the tree bark disc with living *L. cavifolia* plants. We took *L. cavifolia* transplants from several good substrate trees (donor trees) which we attached to recipient trees. One tree could serve as a donor for several transplants. In total, we selected 20 good substrate recipient trees and 20 bad substrate recipient trees. We applied the bark disc transplantation method [9]. Initially, transplants were removed from the donor tree bark (bark discs 4.5 cm in diameter) with a knife and electric drill. Each transplant was affixed to the recipient tree by silicon sealant (water-resistant, for outdoors) in a pre-prepared bark hole (part of the bark was removed before). We took digital photographs on a transplantation day: in the summer of 2020 and after a year in 2021. Each transplant photograph was analyzed and transplant vitality was evaluated based on four vitality classes: 1) high vitality: moist transplant is green or without damaged patches or spots; 2) medium vitality: transplant is green, but some damaged or dead patches or points occur (<50% damaged); 3) low vitality: more than half of the transplant area is dead, some remnant green patches left; >50% damaged; 4) transplant died; transplant is brown without living tissues.

To compare the transplant vitality within vitality classes between 2020 and 2021 we applied Chi-square test. Data analyses were performed in R programme [19].

III. Results and discussion

Most of *Lejeunea cavifolia* transplants were still in place after a year. However, one transplant in good substrate treatment and one transplant in bad substrate treatment were fallen. Several transplants were partly detached from the recipient tree bark. This could be related to insufficient silicon sealant supply during the initial transplantation in 2020. Transplantation was done in dry weather, however, rainy weather could follow after transplantation causing some transplant partial detachment and contamination. Some transplants were partly damaged by silicone sealant. Probably other silicon sealant or glue substance could be more successful in the future. Also, stochastic reasons may cause the falling of transplants.

Transplants of *Lejeunea cavifolia* showed significant differences in vitality between 2020 and 2021 in good substrate and bad substrate treatments (Fig. 2).

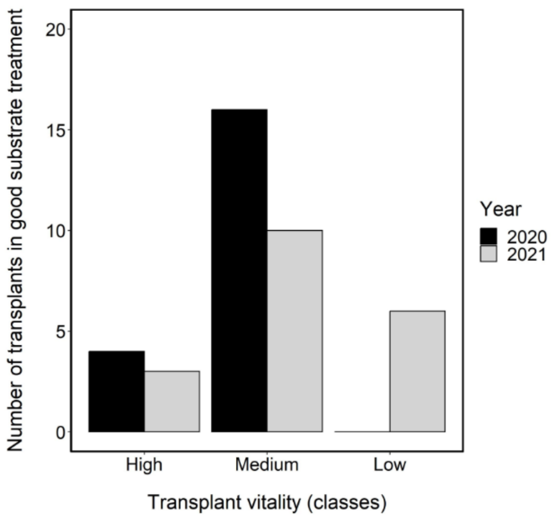

Fig. 2. Transplant vitality class (high, medium, low) in good substrate treatment within the study period. Significantly higher number of low vitality transplants ($p=0.01$) was found in 2021.

The number of transplants with high and medium vitality did not differ significantly, but the number of transplants with low vitality differed significantly between 2020 and 2021 in good substrate treatment (Fig. 2). This could be related to transplant physiological stress that could be caused by the microenvironmental differences between donor and recipient trees shortly after transplantation.

The number of *L. cavifolia* transplants with medium vitality decreased significantly in bad substrate treatment between 2020 and 2021 (Fig. 3). In addition, the number of low vitality transplants increased significantly in bad substrate treatment. Other transplantation studies showed that bryophytes are sensitive to microclimatic changes in transplantation experiments [8].

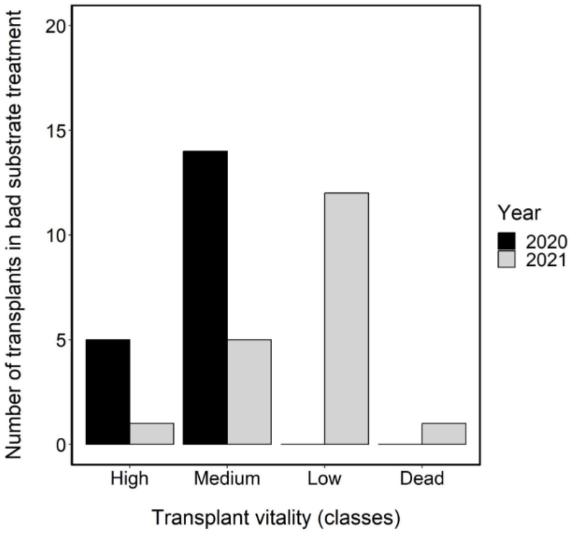

Fig. 3. Transplant vitality class (high, medium, low, dead) in bad substrate treatment within the study period. Significantly lower number of medium (p=0.04) and significantly higher number of low (p<0.01) vitality transplants were found in 2021.

The substrate is one of the most important variables in epiphytic bryophyte distribution. It is also known that tree bark pH differs among tree species [20]. Living aspen tree bark pH was around 4 in Sweden [21]. Mežaka and Znotiņa [22] found that average pH value of aspen is 5.2 in slope forests of Latvia. However, bark pH can change with age, when bark physical properties are changing and amount of environmental dust is increasing on a bark. Older bark obtains more crevices than younger and this could be suitable for epiphyte establishment.

Alexander et al. [23] highlighted the importance of transplantation experiments in ecological studies of community interactions in global change perspective. This could be useful approach also in epiphytic bryophyte community studies in a future.

IV. Conclusions

In general, the bark disc method was suitable for *Lejeunea cavifolia* transplantation in aspen forest. However, the method could be improved with different sealant or other gluing substances that would ensure bark disc attachment in a long-term, preferably several years. This method could be used in further ecological studies with careful selection of silicone sealant. *Lejeunea cavifolia* is dependent on substrate quality and this should be taken into account in forest conservation planning in aspen-dominating forest landscape.

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