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Transplantation experiments with *Neckera pennata* and *Lobaria pulmonaria* in nemoral woodland key habitat and managed forest

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Abstract: Transplantation experiments with *Neckera pennata* and *Lobaria pulmonaria* were conducted in nemoral Woodland Key Habitat and managed forest (in total 38 trees) in Latvia. Elastic cotton bandage was the best method for the present transplantation experiments. Dispersal and establishment limitation were found to be crucial for *N. pennata* and *L. pulmonaria* distribution between forest stands.

INTRODUCTION

Due to increased forestry impact on biodiversity worldwide, knowledge about species requirements for long-term existence in forest landscape is necessary. Epiphytic bryophytes and lichens are good indicators to evaluate the forest naturalness in Northern countries (Ikauniece et al., 2012). Among these indicators are red-listed species and ecology of these species are poorly known. Forest fragmentation increases the distance between species populations affecting also local microclimate (light intensity, humidity), providing difficulties for species survival (Baldwin & Bradfield, 2007; Svoboda et al., 2010). Therefore species dispersal ability (Jüriado et al., 2011) and establishment conditions in a new suitable patch are important drivers for species distribution (Sillett et al., 2000; Snäll et al., 2003). Transplantation experiments can help to identify dispersal or microclimatic conditions, which restrict the successful epiphyte distribution between forest stands on suitable substrates.

Transplantation experiments are needed to predict factors influencing the distribution of epiphytic species, comparing the growth success of model species between transplants in Woodland Key Habitat (WKH) and in managed forest. *Lobaria pulmonaria* (L.) Hoffm. and *Neckera pennata* Hedw. are characteristic species of old-growth nemoral forests (Lesica et al., 1991; Ikauniece et al., 2012) and also suitable model organisms for transplantation experiments (Ingerpuu et al., 2007; Bidussi et al., 2013).

Gauslaa et al. (2001) conducted an experiment with *L. pulmonaria* on wooden blocks, and found that the growth of lichen was correlated with rainfall during the studied time period and *L. pulmonaria* was also susceptible to light after transplantation. Edman et al. (2007) concluded that selective cutting affected negatively the abundance and frequency of *L. pulmonaria* fertility. Branches with transplanted *L. pulmonaria* grew comparatively better in old-growth forest with higher light than in managed forest (Coxson & Stevenson, 2007). In contradiction Hilmo (2002) did not find a difference in lichen *Lobaria scrobiculata* growth after transplantation experiments in old-growth forest and young planted forest, confirming the hypothesis, that dispersal limitation could be more important than microclimatic conditions in a particular forest stand for this lichen species distribution. Hazell and Gustafsson (1999) found, that highest survival of *L. pulmonaria* was found in clustered trees on the clear felled sites and the survival was similar between the scattered trees and in clear-cuts and in the forests. The vitality of *L. pulmonaria* was highest on clustered trees in the clear-cut and lowest in the forest (Hazell & Gustafsson, 1999). *Antitrichia curtipendula* (Hedw.) Brid. as well as *Neckera pennata* are old-growth forest bryophyte species. Hazell and Gustafsson (1999) found that survival and vitality of transplanted *A. curtipendula* was significantly higher in forest than in a clear-cut.

Different methods have been used for transplantation experiments. Rosso et al. (2001) used plastic net transplants in bags for biomass study with *A. curtipendula*. Hazell and Gustafsson (1999) used plastic nets with metal staples for

L. pulmonaria and *A. curtipendula* transplantation. Gauslaa et al. (2006) used frames for transplantation experiments with *L. pulmonaria* on *Picea abies* (L.) Karst. in Norway. Ingerpuu et al. (2007) made successful transplantation experiment with *Neckera pennata* in Estonian nemoral forests by pressing bryophyte into bark crevices.

In boreo-nemoral zone bryophyte transplantation experiments have rarely been done and results of these studies may help to give recommendations for nature protection and forestry planning. The aims of the present study are to find the most appropriate transplantation method for studied nemoral forests and to check if dispersal is more important for *L. pulmonaria* and *Neckera pennata* distribution than a change in forest management regime, comparing nemoral WKH versus managed forest stand.

MATERIALS AND METHODS

Study area

Transplantation experiments were conducted in Zīļu pļavas, Skrīveri district, Skrīveri village, Mid Latvian Geobotanical region in nemoral WKH (56°40'0''N 25°03'07''E) and in a nemoral managed forest stand (56°40'14''N 25°03'07''E). The WKH forest stand was 130 years old with *Fraxinus excelsior* L. dominating, but recent cuttings had been made in managed forest stand, where isolated *F. excelsior* in age between 50-100 years were left. Natural structures as coarse woody debris in different decay stages, living trees with different DBH were present in WKH. *N. pennata* was growing also naturally in the selected WKH. The distance between studied WKH and managed forest stand was around 300 m. Recently cut *F. excelsior* stumps were presented in managed forest stand and only solitary *F. excelsior* individuals were left. The territory in transplantation experiment was selected based on WKH inventory data.

Transplantation

Neckera pennata is autoicous epiphytic and epilithic lowland moss. It is distributed in Circumpolar boreal-montane zone. This moss is glossy yellowish green with undulate leaves, and up to 10 cm long secondary shoots growing off the substrate with pH between 3.8–6.1. The capsules are common, hidden by the leaves (Smith, 2004; Strazdiņa et al., 2011).

Lobaria pulmonaria lichen thallus is pale brown when dry and green when wet growing mainly as an epiphyte. Lobes are 8–30 mm wide and until 7 cm long with strongly ridged surface, soralia are present in lobe margins and along the thallus ridges. Isidia may be present among soredia, apothecia infrequent. Good indicator of rich, unpolluted, old forests (Brodo et al., 2001).

L. pulmonaria and *N. pennata* transplants were taken from five adjacent *Fraxinus excelsior* donor trees with diameter at breast height (DBH) of 1.00 m, located between the managed forest and the studied WKH. All recipient *F. excelsior* trees were selected randomly with similar DBH (>0.23 m). Tree DBH varied among these trees in WKH between 0.24–0.41 m and in the managed forest between 0.29–0.40 m. Bark crevice depth varied from 2.00 to 8.00 mm in WKH and from 2.00 to 5.00 mm in the managed forest. Tree bark pH varied between 4.59–6.21 in WKH and between 4.08–5.63 in the managed forest.

Transplants of *N. pennata* and *L. pulmonaria* were removed at 1.20 m height from the five *F. excelsior* donor trees. The length of *N. pennata* transplants varied from 6.00 to 8.30 cm and they were around 2 cm wide (2–3 secondary shoots). The size of *L. pulmonaria* transplants was around 9 cm^2. All *N. pennata* transplants were put at a height of 1.20 m on a tree in North direction, and *L. pulmonaria* at a height 1.30 m in North direction on receiving *F. excelsior* trees in a managed forest and in the WKH. The lichens and bryophytes growing on transplant contact zone were removed before transplanting.

Transplantation was started in November 2006 with 10 transplants in managed forest and 10 transplants in WKH. Transplants were attached with fishing line (tied two times around the tree) for *N. pennata* and with combined method (fishing line and fishing net) for *L. pulmonaria* to recipient trees. One transplant unit of *L. pulmonaria* and one of *N. pennata* were transplanted to each tree. All transplantations were conducted on the same day when they were collected and each transplant was pulverized with distilled water to decrease physiological stress.

Checking of transplants was made in April 2007. Two receiving trees in the WKH and one in the managed forest had fallen down. Also, two lichen transplants in WKH and three lichen transplants in the managed forest had fallen down. New additional trees were selected instead of dead trees and transplanted with the species. In addition 10 new trees in the WKH and in managed forest were selected in May 2007. Since several transplants had fallen down, now the other method, the cotton bandage was used for attaching transplants. Cotton bandage consists of cotton and elastic material used in medicine and being available in pharmacy.

In total transplantation experiment was made on 40 trees (20 in WKH and 20 in managed forest).

The next observations were made in August 2007, December 2007, March 2008, August 2008, December 2008, and November 2009.

A digital photo was made of each transplant at each inspection time. Transplants were photographed with a Powershot SX100 IS Canon digital photo camera with 8.0 mega pixels, Canon zoom lens 10xIS, and aperture 6.0-60.0 mm 1:2.8–4.3.

The vitality of transplants was evaluated subjectively on a four grade scale based on digital photographs: 1) high vitality; moist transplant is greenish, without damaged patches or margins, 2) medium vitality; transplant is still greenish, but some damaged patches occur, 3) low vitality; more than half of transplant area is damaged, remnant green patches left, 4) transplant is dead; transplant is brown, without living tissues.

Data analysis

Transplantation data were analyzed with Chi-square test in the R 2.7.2 package to test differences between WKH and managed transplant vitality. In total 19 *Neckera pennata* and 19 *Lobaria pulmonaria* transplants from managed and from WKH were analyzed from April 2007 and November 2009.

RESULTS

Based on preliminary study in 2006, fishing line was not the best method for transplantation experiments since in total 5 transplants from 20 were fallen down. Cotton bandage was more appropriate transplantation method in the present study – only six transplants from 40 were fallen down. Transplants fixed with cotton bandage were in the same position also after three years. Transplants tied with fishing line had in several cases (especially transplants of *Lobaria pulmonaria*) changed the angle or location on tree.

In 2009 the highest percentage of *Neckera pennata* transplants (52.63%) in the managed forest belonged to second vitality class, but in WKH to the third vitality class (63%; Fig. 1). The highest percentage of *Lobaria pulmonaria* transplants both in managed forest (44.44%) and in WKH (63.16%) belonged to the second vitality class. Completely dead transplants (fourth

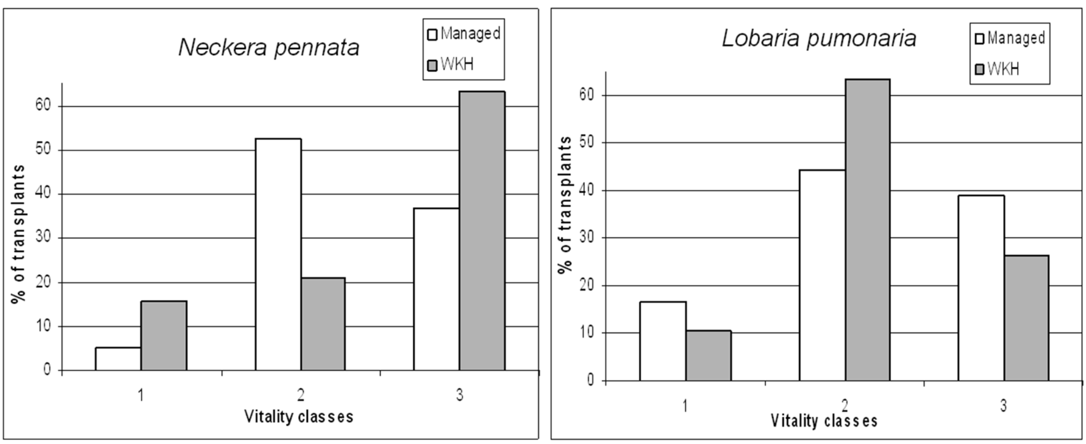

Fig. 1. Transplants of *Neckera pennata* and *Lobaria pulmonaria* divided into vitality classes (1–3) in managed forest and WKH at the end of the experiment.

vitality class) were found neither in managed forest nor in WKH. The highest percentage of transplants for first vitality class was found in *L. pulmonaria* (16.67%) in managed forest stand, while *N. pennata* showed 15.79% of transplants in first vitality class in WKH.

No significant differences were found in *Neckera pennata* and *Lobaria pulmonaria* transplants vitality between WKH and managed forest (Table 1).

Table 1. Chi-square test between transplant vitality classes in managed forest versus WKH.

Transplants	x-squared	df	p
Neckera pennata vitality	5.33	3	0.15
Lobaria pulmonaria vitality	1.31	3	0.52

DISCUSSION

Fishing line and fishing net are not flexible enough for fixing the transplants and also not strong enough and are probably damaged by winter frosts. During the experiment, the fishing line and fishing net became slack after one year (personal observation). Medical cotton bandage was much more elastic and may preserve its pressing property during harsh winter frosts better. Based on the results of the present study, the selected transplantation method with a cotton bandage appears promising for transplantation experiments in the future alongside with a plastic net with metal staples, applied for *Antitrichia curtipendula* and *Lobaria pulmonaria* transplantation in Sweden (Hazell, Gustafsson; 1999).

Transplant vitality of *Neckera pennata* in managed forest and WKH did not differ significantly in the present study. The present result partly agrees with Ingerpuu et al. (2007), who found that dispersal limitation is important for *Neckera pennata* distribution, but not microclimatic conditions. Wiklund and Rydin (2004) found that yearly precipitation was the most important factor as well as establishment properties for *Neckera pennata* colony growth, but not forest site. Werth et al. (2006) hypothesized that ecological conditions are crucial for establishment of *Lobaria pulmonaria* instead of dispersal limitation.

Significant difference was not observed also in vitality of *Lobaria pulmonaria* transplanted to a managed and WKH forest stand. The present study is in accordance with study by Öckinger et al. (2005), where dispersal limitation with mean dispersal distance 35 m was found for *Lobaria pulmonaria* in deciduous forest of Southern Sweden. The vegetative dispersal distance for *L. pulmonaria* was found even shorter in Estonian forests, 15–30 m between host trees (Jüriado et al., 2011). The present study is in contradiction with the study by Edman et al. (2007), who found that selective cutting affected negatively the abundance and frequency of *Lobaria pulmonaria. L. pulmonaria* was found to be sensitive to light after transplantation experiments by Gauslaa et al. (2001) and Gauslaa et al. (2006), suggesting, that *L. pulmonaria* might die in open habitats by high light during long dry periods. *Lobaria pulmonaria* transplants grew comparatively better in old-growth forest with higher light compared with managed forests (Coxson & Stevenson, 2007). Other studies support the results our study, e.g. limited dispersal was found important for *Lobaria oregana* (Sillett et al., 2000).

The present study shows, that dispersal and establishment limitation are significant variables shaping *Lobaria pulmonaria* and *Neckera pennata* distribution in nemoral forest stands since significant differences were not find in transplant vitality between managed forest and WKH. This should be taken into account planning nature protection and long term forestry in landscape and forest stand scale. Retaining potential substrate trees, especially *Fraxinus excelsior*, for epiphyte distribution in nemoral managed forests adjacent to nemoral old-growth forests with species with limited dispersal, transplantation and forest continuity could ensure continuous existence of epiphyte species, especially specialists with limited dispersal abilities in boreo-nemoral zone.

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