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Estimating vegetative reproduction and population size of *Saussurea esthonica*

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Abstract

Population size and reproductive potential are factors that affect viability of species and populations. Wild perennial species often reproduce both sexually (generatively) and clonally (vegetatively). Population size of *Saussurea esthonica* was estimated by counting flowering individuals in two natural populations in Latvia. In addition, ability to propagate vegetatively was evaluated *ex situ*, and two types of vegetative shoot formation were observed. The proportion of vegetative and generative shoots, which can be affected by environmental factors, was evaluated.

Key words: reproduction, *Saussurea esthonica*, vegetative propagation.

Introduction

During the past decades increasing attention has been focussed on *ex situ* conservation of rare plant species, and in vitro, seed bank and field collections of endangered plants have been created (Dubova et al. 2010; Kaninski et al. 2012; Miranto et al. 2012; Cristea et al. 2013). However, in situ conservation remains to be one of the fundamental methods of species protection. The main indicator of species survival is population viability, which is affected by demographic, environmental and genetic factors and is strongly dependent on population size (Lande 1988; Luijten et al. 2000). Population size is one of the attributes used to characterize long-term survival of populations, and determination of population size is necessary for prediction of species and population viability. As wild perennial plants often reproduce both vegetatively and generatively, estimation of correct population size and number of individuals is complicated. Two main approaches have been described. The first method is by counting flowering stems (Schmidt, Jensen 2000; Jacquemyn et al. 2002; Hensen et al. 2005; Stöcklin et al. 2009). The second method is counting both vegetative shoots and flowering stems (Hensen, Oberprieler 2005; Hensen, Wesche 2006). Selection of the most appropriate approach depends on species biology and experimental limitations.

Saussurea esthonica Baer ex Rupr. syn. *Saussurea alpina* (L.) DC. subsp. *esthonica* (Baer ex Rupr.) Kupffer (Estonian saw-wort), is a perennial plant belonging to the genus *Saussurea*, which is one of the largest genera of the family Compositae. The species is found in Latvia, Estonia and the Leningrad (St. Petersburg) region of Russia (Ingelög et al. 1993). S. esthonica is an endangered species in Latvia, and is included in the Red Data Book of Latvia, in the European Commission Council Directive 92/43/EEC on the Conservation of natural habitats and of wild fauna and flora Annex II and in the National Biological Diversity Monitoring Programme (Kļaviņa et al. 2004).

After 1930 *S. esthonica* was considered to be extinct in Latvia. However, in 1991, a Latvian population first described in 1884 was rediscovered, along with another new population (Andrušaitis 2003). Currently two populations are known in Latvia, both of which are located in *Natura 2000* protected areas (micro-reserve *Dubļukrogs* (site code LV0830400) and nature reserve *Popes zāļu purvs* (site code LV0531900)) and in the text are referred to by the names of the closest settlements, Apšuciems and Pope. *S. esthonica* inhabits calcareous soils with fluctuating water regimes. The exact life span of individuals is not known. Non-flowering plants form leaf rosettes. Flowering plants are about 40 to 50 cm in height, with inflorescences containing bisexual disk flowers set in capitula (Andrušaitis 2003).

It is not clear how to best estimate the size of *S. esthonica* populations, as these populations contain many plants with vegetative rosettes, but the proportion of flowering plants is low. It is considered that large populations (with > 200 genets) have a higher probability of survival in comparison with smaller populations, and that populations smaller than 25 individuals are unable to maintain a high diversity of mating types (Luijten et al. 2000). In addition, seed survival in the soil may affect population persistence, but there are no published results about soil seed bank characteristics for *S. esthonica*.

Depending on species, clonal plants have the ability for generative and vegetative propagation, with clonal reproduction occurring more frequently under cold, wet and nutrient-poor conditions (van Groenendael et al. 1996). *S. esthonica* has been classified as a subspecies of *S. alpina* (Narits et al. 2000; Kell et al. 2005). *S. alpina* exhibits generative reproduction via seeds, and vegetative reproduction via new rosettes developing from stolons (Hill et al. 2004). *S. alpina* plants have sympodial branching rhizomes with epigeogenous or hypogeogenous stems (Klimešová, de Bello 2009). There is a lack of data about the proportion and type of *S. esthonica* vegetative propagation. Vegetative growth is strongly influenced by plant age, seasonal development and environmental factors, for example in arctic and alpine regions by moisture and altitude (Klimešová, Klimeš 2008, Klimešová et al. 2011). *S. esthonica* inhabits wetlands – fens and rich paludified grasslands (Narits et al. 2000; Pakalne 2008) with a changing water regime during the growing season, which could be one of the factors limiting reproduction.

The aim of this study was to study population size and vegetative reproduction of *S. esthonica* in conditions of Latvia, as these factors can affect its long-term survival. Population size was estimated by counting flowering individuals *in situ*, and reproduction potential was estimated *ex situ* by counting above ground shoots as well as flowering stems.

Materials and methods

The number of flowering individuals and capitula per individual were counted in each population in July in three successive years (2009 to 2011) to estimate population size of flowering plants.

Seeds were collected from both Latvian natural populations. Seeds were germinated in Petri dishes lined with a double layer of filter paper moistened with distilled water in a climate cabinet and incubated at 22 °C. Ten seedlings (genets) were planted in separate pots in peat substrate from the raised bog KKS-1 (Laflora, Latvia) in spring 2010, and cultivated and overwintered in open air conditions. Over a six year period (2011 to 2016), the number of shoots per genet was counted in spring when shoots started to grow, and in June the number of flowering stems was counted. To examine underground structures, in 2011 two individuals displaying differing placement of shoots in relation to the mother plant were removed from pots and morphological features were described.

Results

Estimation of flowering in situ

The Apšuciems population of *S. esthonica* was larger with more flowering individuals and capitula per individual (in average eight). The Pope population was smaller, with a smaller number of flowering individuals, with an average of six capitula per plant (Table 1). The average number of capitula per plant in both populations decreased in 2010. In 2011 a large decrease of flowering individuals in the Apšuciems population was observed, but the average number of capitula per individual remained the same.

The number of flowering individuals did not significantly differ between both populations, but a significant difference was found between the number of capitula per individual ($p = 0.02$).

Propagation in ex situ *conditions*

The number of vegetative shoots was determined at the beginning of the vegetation season. Over the course of the vegetation season, the number of shoots varied due to mortality of shoots and formation of new shoots (data not shown).

From ten genets, 50 vegetative shoots developed during the first vegetation season after overwintering (Table 2). Three genets did not produce new shoots, and remained with only one shoot. The other plants developed between two to 11 shoots per plant, and four genets had flowers. Two genets produced two inflorescences each and two other genets produced one inflorescence each. In 2012, the number of observed vegetative shoots increased and ten genets produced 66 shoots (two to 17 shoots per plant). However, the number of vegetative shoots and flowering stems did not significantly differ between years. Two genets did not produce flowering stems during the six-year period, but produced many vegetative shoots. Two genets flowered three times within six years. However no flowering stems were observed in 2014. In some cases new shoots developed close to the main shoot but in most cases the plants produced below-ground rhizomatic branches

Table 1. Number of flowering individuals and average number of capitula per individual of *Saussurea esthonica* in both Latvian populations in 2009 to 2011

Population	Year	Number of flowering individuals	Number of capitula per individual	Average number of capitula per individual
Apšuciems	2009	90	5 – 19	8.2 ± 0.55
	2010	94	4 – 11	7.8 ± 0.28
	2011	19	4 – 14	7.9 ± 0.92
Pope	2009	52	4 – 11	6.3 ± 0.28
	2010	43	3 – 9	5.0 ± 0.24
	2011	46	3 – 16	6.8 ± 0.50

Table 2. Number of vegetative and generative shoots observed on *ex situ* genets of *Saussurea esthonica* at the beginning of the vegetation period

Year	Vegetative shoots per genet	Flowering stems per genet
2011	5.00 ± 1.30	0.60 ± 0.27
2012	6.60 ± 1.33	0.50 ± 0.08
2013	4.80 ± 0.84	0.60 ± 0.37
2014	6.50 ± 1.88	0
2015	10.78 ± 4.79	0.44 ± 0.24
2016	9.50 ± 3.26	0.13 ± 0.13

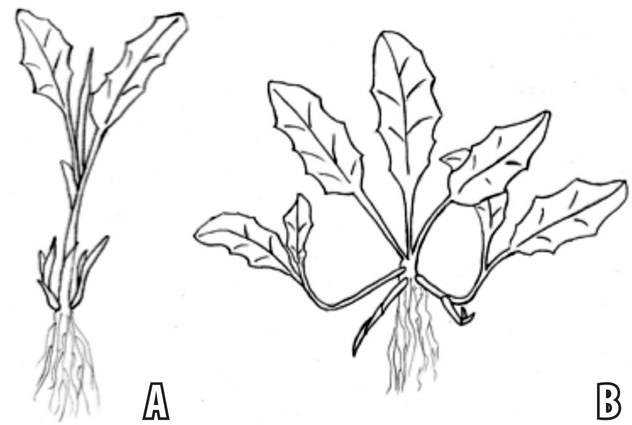

Fig. 1. Vegetative reproduction of *Saussurea esthonica* plants. A, vegetative shoots close to the main shoot; B, vegetative shoots spreading underground with rhizomatic stems.

without splitting from the mother plant (Fig. 1). Rhizomes had several nodes; fresh rhizomes were white and became dark brown with age. Shoots close to the main shoot did not produce roots, but shoots located further from the mother plant developed some roots.

Discussion

The method of counting only flowering individuals was used for population size estimation because it is complicated and experimentally unfeasible to count all individuals. While the three-year period of the study was too short for accurate estimation of population stability, nevertheless, the number of flowering individuals of *S. esthonica* in the Pope population remained stable, and the same was noticed in the Apšuciems population in 2009 and 2010 (Table 1). However, in 2011, a significant decrease in the number of flowering individuals in the Apšuciems population was observed. It is not clearly known which factors caused such a strong decrease in flowering individuals. In general, the Apšuciems population had more flowering individuals (except in 2011) and more capitula per inflorescence than the Pope population. Based on the average number of flowering stems over the three-year period, and the number of inflorescences per genet obtained in the experiments in *ex situ* conditions, the population size of the Pope population was estimated to be approximately 124 genets. Estimation of population size for the Apšuciems population was more complicated due to the large decrease of flowering stems in 2011. However, disregarding the data from 2011, and using the average of the remaining two years, population size can be estimated to be 240 genets.

These estimations are only approximate because the ability to produce generative shoots might be affected by plant age and environmental factors and since one genet is able to produce more than one inflorescence, as observed in the *ex situ* experiment. Tepedino (2012) suggested that stem counts be adjusted to include the positive effects of ramets and conclude that it is necessary to increase the accuracy of population size estimation of rare clonal plants. Small populations have less than 100 individuals (Ellstrand, Elam 1993), very small populations – less than 50 individuals (Oostermeijer et al. 2003). Our data show that the sizes of the *S. esthonica* populations are not critically small.

One of the main life history strategies for plants is persistence to survive periods of unfavourable conditions as well as demographic strategies involving regeneration (García, Zamora 2003). *S. esthonica* can propagate both vegetatively and generatively and this could be one of the factors ensuring the survival of these two populations. After the first overwintering, there were some genets that did not produce new shoots, but after the second overwintering all genets produced new shoots. The calculated proportion of inflorescences per genet ranged from 0.13 to 0.60. More than one inflorescence per genet was observed, but not on all of the genets which flowered. Some genets did not produce flowering stems over the course of the experiment, and only vegetative rosettes were observed. The reproductive strategies in natural populations may differ from these observations due to the experiment being carried out in *ex situ* conditions, as substrate and soil moisture content differed from that of natural populations. Sexual reproduction was positively correlated with soil composition, moisture content and pH level and negatively correlated with ramet density in *Carex brevicuspis* (Chen et al. 2015).

More detailed studies are needed because the ability of plants to produce generative or vegetative shoots could be affected by environmental factors and plant age. The factors explaining why in 2014 no generative shoots were formed in the *ex situ* experiment, and that in 2011 the number of flowering shoots was very low in the Apšuciems population, remain unexplained. In addition, it is not clearly known why some genets never produced flowering stems *ex situ*, as plants were grown in similar conditions in the same substrate, but it is known that water content and favourable soil nutrient content tend to promote clonal growth, while light quality is a factor influencing generative reproduction

(Yang, Kim 2016).

The number of new shoots was larger at the beginning of the season, and the number of shoots decreased over the growing season. In comparison, in North American grasslands, grass stem density was observed to be greatest in June and negatively correlated with below-ground bud density (Dalgleish, Hartnett 2006). This suggests that the number of new shoots can vary within the growing season. Nevertheless, vegetative reproduction may be one of the main factors ensuring the long-term survival of *S. esthonica* populations.

The present results indicated that it was possible to determine approximate population size by counting flowering individuals; however, these estimates need to be used with caution as they are based on *ex situ* experiments with a small number of individuals. Two types of vegetative shoot formation were observed. In addition, number of inflorescences were very variable between years. While flowering has been reported to be affected by environmental factors, the exact factors responsible for the variation observed in this study were not determined. Therefore, future studies about factors affecting the amount and ratio of vegetative and generative reproduction are needed, as well as long-term monitoring of these Latvian populations.

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