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Regeneration of a *Gentiana pneumonanthe* population in an oligotrophic wet meadow

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Abstract. The aim of this study was to evaluate the critical phases of the life cycle of *Gentiana pneumonanthe*, the marsh gentian, a threatened species of the Bohemian flora. The effect of various conditions on germination and seedling establishment and the possible effect of competition on the performance of the species were tested. Seeds were sown in plots which were subjected to four treatments in a randomized complete blocks experiment: unmanaged meadow, mown meadow, burned meadow, and meadow with cut sod. The significantly highest recruitment was found in plots with cut sod, the lowest in the unmanaged control plots. Seedling survival also differed among the treatments. In the following year surviving individuals were only found in plots with cut sod. The influence of neighbouring vegetation on target gentian individuals was evaluated by removing the surrounding vegetation and comparing the performance of these individuals with controls. The initial height of each individual was measured and used as a covariable. No significant effect of neighbouring vegetation on performance was detected. Thus, the establishment phase appears to be critical for population persistence and is also more influenced by the management regime than other stages of the life cycle.

Keywords: Conservation biology; Critical phase; Disturbance; Gap; Management; Seedling recruitment.

Nomenclature: Rothmaler (1976).

Introduction

Mechanisms for the maintenance of species diversity and the role of disturbance in this process are topical issues of contemporary community ecology (e.g. Grime 1979; van der Maarel 1993; Palmer 1994). Those issues are not only of academic interest, but also extremely important for management plans for plant communities in nature conservation. Data on the biology of rare species are crucial for successful management, together with the recognition of the critical stages of plant development necessary for proper management practices. The species of our concern, *Gentiana pneumonanthe*, has been declining throughout Europe (Hvatum 1993; Oostermeijer et al. 1992). Reduction of populations of *Gentiana pneumonanthe* has seriously influenced dependent insects as well, notably the rare butterfly *Maculinea alcon* which has disappeared from many localities (Elmes & Thomas 1992).

For many perennial species, the successful establishment of a new genet is the most critical step in their life history (see the concept of 'regeneration niche', Grubb 1977, and the carousel model of van der Maarel & Sykes 1993 for the community consequences). Transient gaps in the turf are instantly colonized and occupied, while new gaps are being created elsewhere (Rusch 1988; Rusch & van der Maarel 1992). Gap dynamics is an important mechanism for the maintenance of diversity in grasslands (Grubb 1977; Rusch 1988; van der Maarel & Sykes 1993). Tilman (1993) considered inhibited germination and/or survival of seedlings in productive grasslands to be a major cause of decrease in species richness along a productivity gradient.

In central Europe, meadows at lower altitudes are man-made and depend upon continuing human intervention. Traditional management practices, mainly grazing and cutting (Bakker 1989), have maintained these meadows for centuries, but recently the management practices have changed dramatically. Management was either intensified by fertilization, or the meadows were abandoned because they were no longer profitable. Both processes produce changes in species composition which are usually accompanied by extirpation of some species and overall loss of species diversity. At present, this trend can be exemplified with *Gentiana pneumonanthe* in most of its European localities. Regeneration in abandoned meadows is limited, as only adult plants survive, where the populations become senile (Oostermeijer et al. 1994b). However, it has been shown that new plants establish following disturbance (Chapman & Rose 1982; Chapman et al. 1989).

The aim of our study was to experimentally test the importance of various management and disturbance practices, including creation of artificial gaps in the turf for the regeneration of *Gentiana pneumonanthe* and to evaluate the effects of neighbouring vegetation on the performance of established plants. In this manner, the sensitivity of various stages of the life history of *Gentiana pneumonanthe* to influences of the neighbouring vegetation was compared and the critical phases of the plant life cycle determined.

Material and Methods

Study species

Gentiana pneumonanthe L., the marsh gentian, is a long-lived perennial species, which passes through the winter as a rosette of short shoots with very small leaves. The species occurs in wet heathlands and moist oligotrophic to mesotrophic hay meadows across temperate Europe and Asia, ranging in Europe from southern Scandinavia to the Balkans and northern Spain (Simmonds 1946). Reproductive adult plants can reach 50 cm in height. They usually have 1 - 10 stems, each stem bearing 1 - 25 flowers per year. The seeds (usually 300 - 700 per capsule) ripen during September and October. They are small and light (0.32 mg on average), and contain very little endosperm (Salisbury 1942; Petanidou et al. 1991; Petanidou et al. 1995; unpubl. data).

In the Czech Republic, *Gentiana pneumonanthe* occurs at the margins of peat bogs and in wet oligotrophic meadows, mainly in the piedmont. The species was previously quite common on traditionally managed meadows, but many populations decreased in size or became locally extinct during the last decades. At present, the majority of the populations survive in small isolated habitat islands, with low and declining population sizes. This imposes a further threat to their genetic diversity (Oostermeijer et al. 1994a; Raijmann et al. 1994; Lande 1988). A similar decline has been recorded for marsh gentian populations in Norway (Hvatum 1993), the United Kingdom (Chapman & Rose 1982) and the Netherlands (Oostermeijer et al. 1992a,b; Schaminée et al. 1995), with a likely similar pattern through all of central and western Europe.

Study site

All observations and field experiments were carried out at Ohrazení, near České Budějovice, southern Bohemia, Czech Republic. This site contains one of the largest remaining populations in Bohemia, with several hundreds of mature *Gentiana pneumonanthe* plants.

Here we are dealing with a wet oligotrophic meadow of ca. 1 ha in size, situated between a field and a pine-oak wood that was abandoned five years ago (some of the wetter parts even earlier). The unfertilized meadow community belongs to the *Molinion* alliance according to the Zürich-Montpellier phytosociological classification. It is characterized by the following relevé on a representative plot of 5 m × 5 m:

Cover of the herbaceous layer: 95 %. Species (first graminoids, then broadleaved herbs, both groups in alphabetical order) with their cover-abundance according to the Braun-Blanquet cover-abundance scale:

Anthoxanthum odoratum	2	*C. pulicaris*	+
Avenochloa pubescens	1	*Cynosurus cristatus*	1
Briza media	2	*Festuca rubra*	2
Carex flava s.l.	+	*Holcus lanatus*	1
C. hartmanii	1	*Juncus effusus*	1
C. leporina	+	*Luzula multiflora*	1
C. nigra	1	*Molinia caerulea*	2
C. pallescens	1	*Nardus stricta*	2
C. pilulifera	1		

Betonica officinalis	1	*Potentilla erecta*	1
Cirsum palustre	+	*Ranunculus acris*	+
Dactylorhiza majalis	+	*R. auricomus*	1
Galium boreale	2	*R. nemorosus*	+
Gentiana pneumonanthe	+	*Rhinanthus minor*	+
Leontodon hispidus	1	*Scorzonera humilis*	+
Leucanthemum vulgare s.l.	r	*Selinum carvifolia*	2
Lychnis flos-cuculi	1	*Serratula tinctoria*	+
Lysimachia vulgaris	+	*Succisa pratensis*	1
Myosotis palustris s.l.	+	*Valeriana dioica*	1
Plantago lanceolata	+	*Viola canina*	1

Seedling establishment

Seedling recruitment was evaluated under various conditions. Capsules of *Gentiana pneumonanthe* containing ripe seeds were collected in October 1992 and stored at 5 °C for three months. The experiment was arranged in five randomized complete blocks of 0.5 m × 0.5 m square plots. In January, aliquots of 0.6 g equivalent to 15000 - 20000 seeds, were sown in plots subject to the following four treatments:

(1) meadow after mowing: above-ground part of the biomass was cut off and the litter completely removed;

(2) burned meadow: the above-ground vegetation and the litter were burned, with the ash left in situ;

(3) meadow with cut sod: the sod was cut, about 10 cm deep, with the cut portion turned upside down;

(4) control: meadow was left in the original condition.

In all the treatments the seeds were sown by hand with extreme care to evenly cover the entire plot. However, edge density was slightly lower, but this should not have influenced the results as any effect was similar in all the treatments.

The number of emerging seedlings was counted only within the inner 0.25 m × 0.25 m plots to avoid edge effect. The first sampling in summer 1993 provided information about seedling emergence, with the following two (autumn 1993 and summer 1994) yielding data for evaluation of seedling survival. With regard to the biology of the species we expected that only a negligible fraction of seeds would germinate between the summer and autumn censuses. Our personal, non-quantified observations, showed that the majority of seedlings emerged in mid-spring. A similar behaviour was quantitatively described for *Gentianella campestris* by Milberg (1994).

A univariate repeated measures ANOVA (i.e. split-plot design applied to the repeated-measures data, von Ende 1993), reflecting the block structure of the experiment, was used to analyse the data. Generally, we can say that in the repeated measures analysis, the between-subject variation corresponds to differences between observed sampling units (block and treatment) and the within-subject variation corresponds to changes in time (see von Ende 1993 or any other appropriate statistical text for more detailed explanation). The numbers of seedlings were log-transformed – using log $(x + 1)$ to handle zeroes in the data – to achieve normality and homogeneity of variance. After log-transformation, the between-subject factor corresponds to differences in seedling establishment, and the interaction term between treatment and time corresponds to differences in seedling survival. Provided that the seedling survival is independent of the treatment, the number of seedlings in a plot after a time interval is $N_2 = p\,N_1$ in all plots, where N_1 and N_2 are numbers of seedlings in a plot in respective periods and p is the common probability of survival. Then $\log N_2 = \log p + \log N_1$, i.e. the factors treatment and time (i.e. survival) are additive, without any interaction. The SYSTAT package (Wilkinson 1988) was used for statistical tests.

Competitive ability of adult plants

To assess the influence of neighbouring vegetation on the gentian plants, 30 target individuals were chosen at random and the surrounding vegetation was removed by clipping at ground level to a distance of 0.3 m every two weeks during the growing season. Performance of these target plants was compared with 30 control individuals. The initial height of all individuals was measured and used as a covariable in the analysis of variance. Two types of influence of the neighbouring vegetation are considered here: (1) negative, due to suppression of growth by competition, and (2) positive, due to protec-

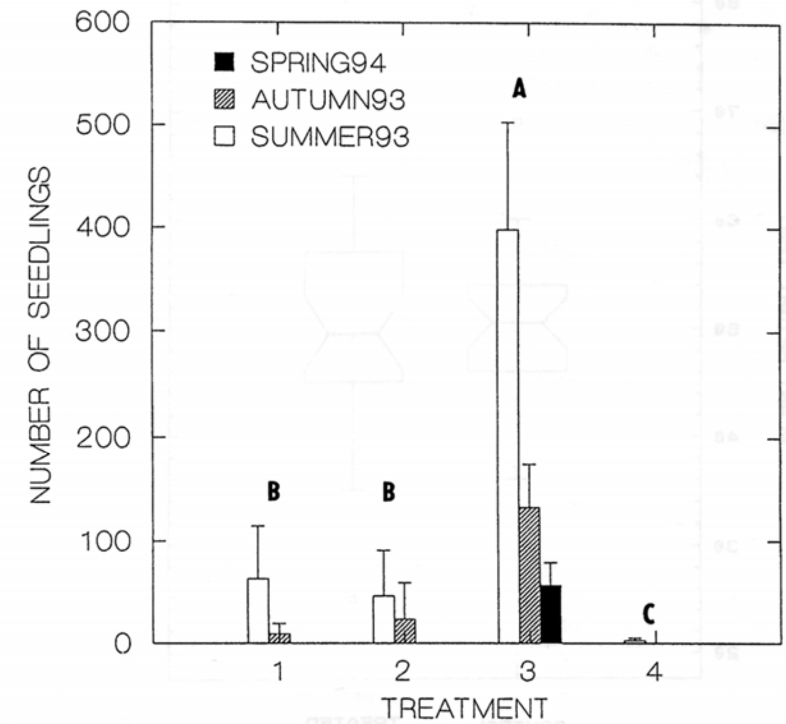

Fig. 1. Average number of seedlings in plots during various censuses. Treatments: 1 = mown; 2 = burned; 3 = cut sod; 4 = control. Error bars represent standard deviation values. Letters above bars indicate the results of the Tukey-Kramer HSD test for the summer and autumn of 1993; treatments labeled with the same letter do not differ significantly ($P > 0.05$). Identical results were obtained for both years.

Table 1. Repeated measures analysis of log-transformed counts of seedlings during the first season; the numbers of seedlings in June and September 1993 in each plot are the response variables.

Between Subjects					
Source	*Ss*	*Df*	*Ms*	*F*	*P*
Block	1.532	4	0.383	1.561	0.247
Treatment	22.988	3	7.663	31.233	0.000
Error	2.944	12	0.245		
Within Subjects					
Source	*Ss*	*Df*	*Ms*	*F*	*P*
Time	2.783	1	2.783	108.400	0.000
Time*Block	0.165	4	0.041	1.604	0.237
Time*Treatment	0.351	3	0.117	4.553	0.024
Error	0.308	12	0.026		

tion of individuals from grazing. Plant height and number of flowers were recorded at the end of the growing period in September 1993 and employed as the measure of plant performance. On a single day, 21 June, 1993, the photosynthetic activity of leaves of all the 60 individuals was measured in situ using a portable LCA2 analyzer.

Results

Seedling establishment

The highest germination rate was observed in plots with cut sod and the lowest in the unmown meadow. The difference between plots with burned vegetation

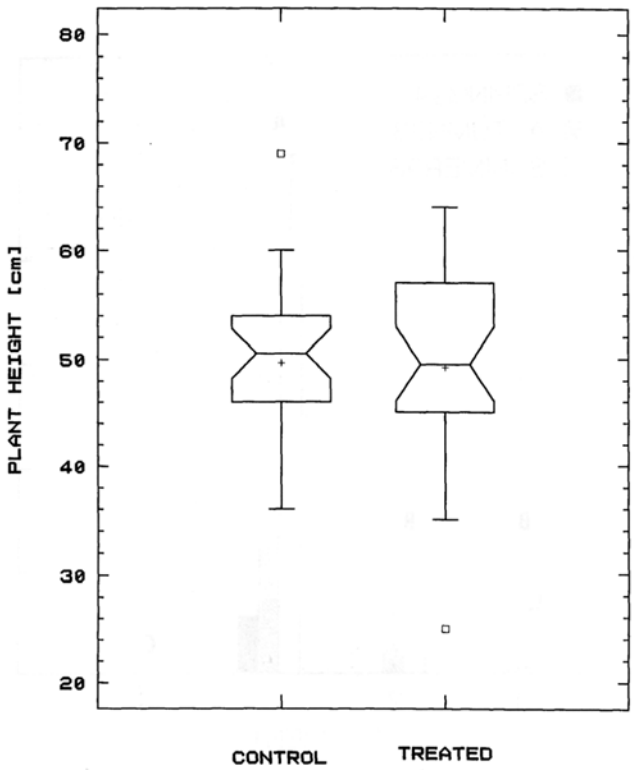

Fig. 2. Notched box and whisker plot of plant heights for control and treated plants (i.e. where the surrounding vegetation was cut). The box encloses the middle 50% of observations (i.e. between the upper and the lower quartile), with the median in the middle. The length of the notch represents an approximate 95% confidence interval for the median. Whiskers extend to the extreme points within 1.5 interquartile ranges from the quartile. Data points beyond this range are plotted individually.

and those with mown vegetation was not significant (Fig. 1, Table 1). At the end of the first growing period, the largest number of seedlings was found in plots with cut sod, whereas no seedlings were found in the control plots. The highest survival rate was observed in burned plots, 43 %, vs. 34 % in cut sod and 13 % in mown plots. Nevertheless, owing to low initial densities, absolute numbers of seedlings in plots with burned and mown vegetation were low. During the first month of the second growing period, seedlings were found only in plots in which the sod was cut and there they were surviving mainly in depressions among portions of the reversed sod.

Because individuals were found only in plots with cut sod in the second season, we only used data from the first year to test for differences in seedling germination and survival. Seedling establishment differed according to the treatment (significant between subjects Treatment, Table 1) and the same was true for the seedling survival (significant Time × Treatment interaction). Separate analyses of summer and autumn counts showed that during both periods, counts of seedlings were significantly highest in cut sod and significantly lowest in the controls (Tukey-Kramer HSD).

Competitive ability of adult plants

No differences in plant height, number of flowers per plant, or photosyntetic activity between treated and control plants were found ($P > 0.05$ for all variables (Fig. 2). Some individuals were damaged by browsing (probably deer), including both treated and control plants. Individuals which were browsed at the beginning of the vegetation period regenerated well and produced more stems and flowers than undamaged plants. Because of the low number of browsed plants the differences were not significant, neither the probability of damage by browsing nor for differences in stem and flower number between browsed and untouched plants.

Discussion

The results show that the recruitment of *Gentiana pneumonanthe* was only successful in plots where the sod was cut and turned upside down. These gaps are probably the only safe sites for the regeneration of the species. Although cutting or ploughing the sod has never been part of any conventional meadow management in Bohemia, the conditions imposed by our experimental treatment correspond approximately to those in which gaps may occur in the turf under natural disturbances, even though such natural gaps are usually smaller. Our finding is in agreement with other reports concluding that regenerating of *Gentiana pneumonanthe* occurs mainly in gaps (Oostermeijer et al. 1992a,b, 1994b; pers. observ.). Gaps were periodically created in traditionally mown meadows by horseshoe footprints, machinery tracks, missed strikes of the scythe while mowing by hand, etc. In contrast, gaps are rare or absent on abandoned meadows and in addition these meadows are covered with a thick layer or thatch of litter, which is another factor preventing seed germination. Consequently, the abandonment of meadows leads to the development of senile populations and eventually to local extinction. The remaining localities with this species should be monitored by counting the numbers of individuals at different life stages (Oostermeijer et al. 1994b). Thus, *Gentiana pneumonanthe* is an additional example of a rare species with disturbance-dependent persistence (Pavlovic 1994).

Our experiment did not demonstrate the competitive suppression of adult plants by neighbouring vegetation. However, these results cannot be generalized for other meadow types. In our oligotrophic study site, the vegetation was neither dense nor tall. In other localities where the marsh gentian has been declining the sod is usually more compact and the vegetation taller.

The results of both our experiments clearly show that the establishment phase is more critical for population persistence than later development stages, and in turn establishment is most influenced by management practice. This is probably true for many other meadow species as well, although the regeneration niche differs markedly among such species (Grubb 1977). This supports Tilman's (1993) hypothesis that the inhibition of seedling recruitment is the major cause of decreases in species diversity in productive grasslands and even though the regeneration requirements differ among species, the majority are negatively influenced by increased litter and decreased light penetration (Grime 1979; Carson & Peterson 1990). Any explanation of species richness of plant communities ignoring the mechanism of regeneration must necessarily be incomplete. Understanding the demography of particular species is of vital importance for the understanding of mechanisms that maintain species diversity.

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