Germination Responses to GA_3 and Stratification of Threatened *Festuca* L. Species from Eastern Mediterranean

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Z. Naturforsch. **61 c**, 372–376 (2006); received November 29/December 30, 2005

The seed germination characteristics of three threatened *Festuca* sp. [*F. punctoria* Sm., *F. cyllenica* Boiss. et Heldr. subsp. *uluana* Markgr.-Dannenb., *F. paphlagonica* (St.-Yves) Markgr.-Dannenb. subsp. *paphlagonica*] were investigated. These species are endemic and spread on alpine belt. The study was carried out with wet-cold and dry-cold stratification throughout 15 days, different doses of GA_3 (50, 100 and 150 ppm) and hormone-stratification combined treatments, and non-treatment series. We found that the germination rates of three fescue seeds for various treatment series were different. The mean germination percentage of *F. cyllenica* was higher (80%) than that of *F. punctoria* and *F. paphlagonica* which were fairly low (50–60%). Germination rates increased by wet-stratification treatment in *F. punctoria* and also increased with 100 ppm GA_3 application to the seeds of *F. paphlagonica.* When taken into consideration the germination percentages of all fescue species, the seeds of *F. punctoria* and *F. paphlagonica* can be dormant, but the seeds of *F. cyllenica* are non-dormant.

Key words: Festuca sp., Germination, Threatened Species

Introduction

Seed dispersal and germination are phases in the reproductive cycle that are of great importance for species fitness. Variations in seed dispersal efficacy or germination percentage are often interpreted as reflecting adaptations to specific ecological conditions (Grime *et al*., 1981; Nishitani and Masuzawa, 1996). Species that live in very specific habitats often produce seeds with highly specialized adaptations. Specialized seeds are produced by species living in wildfire-prone habitats (Grime, 1979), by coastal halophytes (Pickart, 1988) and species from alpine habitats with a very short vegetation period (Bliss, 1971; Ellenberg, 1988; Körner, 1999). Alpine and subalpine plants provide good opportunities to determine differences between germination responses of species. Investigations on germination ecology of alpine plants are rare, thus the factors and mechanisms regulating germination in alpine habitats are also little known (Baskin and Baskin, 1998). In addition, germination requirements for native species, particularly for rare and/or endemic species, are usually important for conservation biology (Cerabolini *et al*., 2004). Their investigation may show how the specific germination process is adapted to habitat conditions, how it is regulated by environmental factors (Van Assche *et al*., 2002), and how it influences a following seedling establishment in the particular habitats (Schütz and Rave, 1999).

There have been no previous studies of seed germination of these species. The aim of the present study was to determine the response of seed germination of the fescue species *Festuca punctoria*, *F. cyllenica* and *F. paphlagonica* to a dry-cold treatment and wet-cold stratification for 15 days at different doses of GA_3 application. Hormone treatments and stratification were combined and compared with germination of these fescues without these treatments.

Material and Methods

F. punctoria Sm., *F. cyllenica* Boiss. et Heldr. subsp. *uluana* Markgr.-Dannenb., *F. paphlagonica* (St.-Yves) Margr.-Dannenb. subsp. *paphlagonica* are threatened species (Güleryüz *et al*., 2005). They are scattered in Northern Turkey. *F. punctoria* is an endemic plant from Mount Uludağ (Mt Olympos Bythynus) and it is an Eastern Mediterranean mountain element. Besides the Mount Uludağ, the areas of occurrence of *F. cyllenica* and *F. paphlagonica* are at Mount Köroğlu-Bolu and Mount Küçük Ilgaz-Kastamonu, respectively. They are endemics from Turkey and euxine (the

0939–5075/2006/0500–0372 $ 06.00

floristic section in Black Sea region of the Euro-Siberian phytogeography region) elements of the high mountains. *F. punctoria* and *F. cyllenica* are densely caespitose perennial plants and they are dominant species of hard cushion *Festuca* communities in sub-alpine and alpine belts of Mount Uludağ (Rehder *et al.*, 1994; Güleryüz *et al.*, 1998). *F. paphlagonica* is a relatively slender caespitose form. Nomenclature follows Flora of Turkey and East Aegean Islands (Davis, 1985).

Seeds from different individuals of the three fescue species were randomly collected at the alpine belt of Mount Uludağ, Bursa, Turkey, during July 2002. Seeds were stored dry in paper bags at ambient laboratory conditions between collection and the initiation of each experiment. A minimum of 5 individuals with mature seeds for each *Festuca* species was used for the study. Seeds were surface-sterilized for 10 min with 5% sodium hypochlorite and then rinsed twice in sterilized distilled water. Our experiments were structured as a series of four treatments; controls without any treatments, three doses (50, 100 and 150 ppm) of hormone (GA_3) application, dry-cold treatment and wet-cold stratification throughout 15 d at +4 °C and a combined hormone-stratification treatment. In all treatments, 25 seeds per species germinated on two layers of sterilized filter paper in 9 cm Petri dishes, and the dishes were covered with stretch film. The study included three replications per treatment. The tests were carried out in an incubator at (22 ± 1) °C in continuous darkness during 21 d.

Germinated seeds were counted every day or every second day. Seeds were scored as germinated when the radicule and at least 1 mm of the coleoptiles were visible (Schütz and Rave, 1999). Finally, with the obtained data we calculated the final mean germination percentage. For all treatments, mean time of germination was also calculated (Ellis and Roberts, 1981).

Data were analyzed by one-way analyses of variance (ANOVA) to test for overall differences among treatments related to the final germination percentage and mean germination time. Significance of differences amongst treatment means was assessed using a Tukey comparison test (HSD test). All statistical analyses were based on a significance level of 0.05. The analyses were conducted using the Statistica for Windows version 6.0 (SAS Institute Inc., Cary, NC, USA).

Results

The control germination rates of seeds of each fescue species seeds are shown in Fig. 1. The final germination percentages were found (58.7 ± 8.3)% in *F. punctoria*, (53.3 ± 9.2)% in *F. paphlagonica* and (85.3 ± 2.3)% in *F. cyllenica* seeds. In each of the fescue species the germination rate did not increase further after the 9–10th days (Fig. 1). In Fig. 2 the germination dynamics are documented for seeds of *F. punctoria* and *F. paphlagonica*. The different germination rates and time requirements of these species are shown in the histograms of Fig. 3; different letters above the columns indicate significant differences ($p < 0.05$).

In *F. punctoria* the mean time of germination was (6.6 ± 0.79) days in the control group (Fig. 3). Treatments of 50, 100 and 150 ppm GA_3 decreased the germination percentage and also the mean time of germination at least if applied in higher content. Dry-cold treatment and wet-cold stratification for 15 days increased the germination percentage, and moist caryopses germinated quickly (Fig. 3). The combined hormone-stratification treatments did not positively affect the germination percentage. However, the mean time of germination became significantly shorter by these treatments vs. control ($p < 0.05$; Fig. 3).

The mean time of germination of *F. paphlagonica* was (5.1 ± 0.38) days in non-treatment series. The mean germination percentage with 50 and 100 ppm GA_3 applications was higher than for the control group. The germination percentage increased by wet-cold stratification for 15 days and

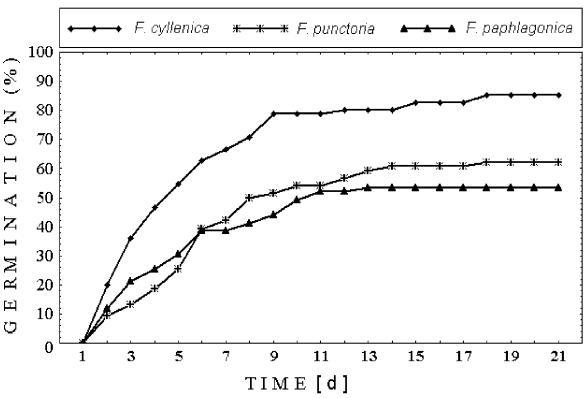

Fig. 1. Germination dynamics of three *Festuca* species (*F. cyllenica, F. punctoria, F. paphlagonica*) seeds in controlled environmental conditions (21 darkness days, at 22–23 °C).

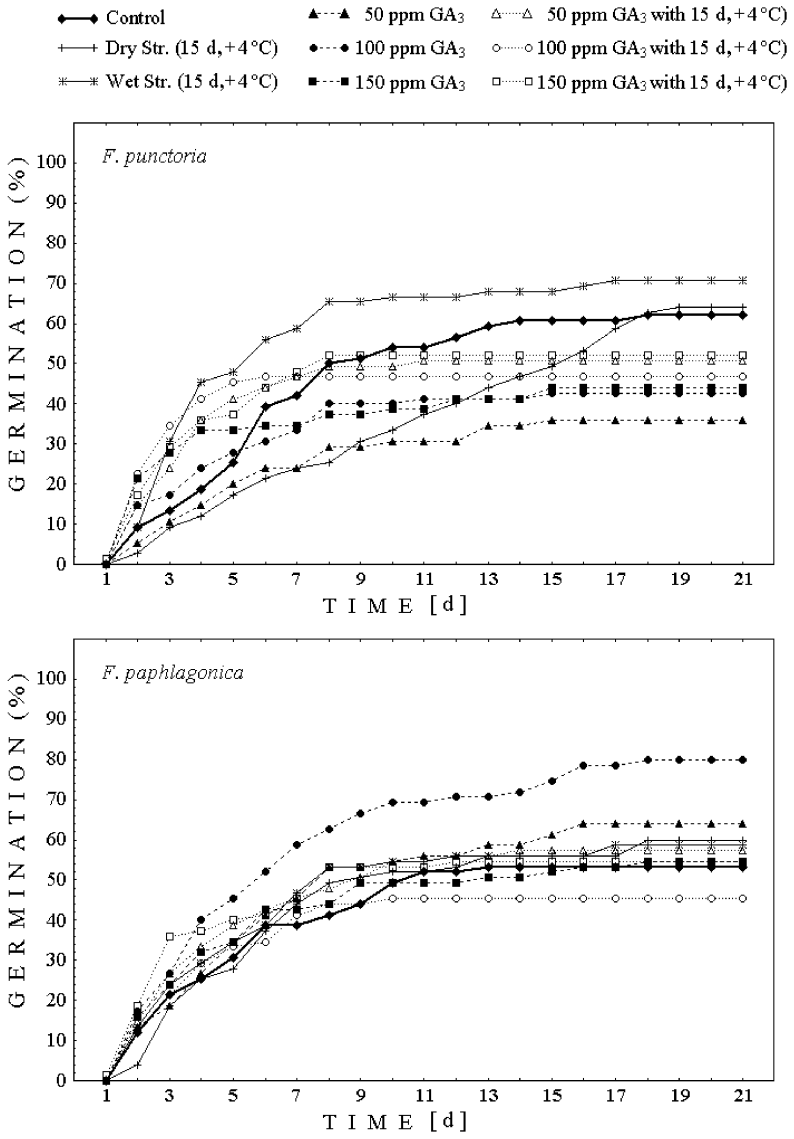

Fig. 2. Fluctuations of germination rates in seeds of two *Festuca* species (*F. punctoria, F. paphlagonica*) exposed to different treatments during a 21-days period.

by the hormone-stratification combination treatment. This increase was significantly different between the respective means ($p < 0.05$; Fig. 3). No significant differences between the treatments were found with respect to the mean time of germination ($p > 0.05$; Fig. 3). Because of the high germination success, hormone and stratification trials with F. *cyllenica* seeds were not tested.

Discussion and Conclusion

The importance of regeneration characteristics for species dynamics, coexistence and survival has been emphasized by both conservation biologists and plant ecologists (*e. g.* Fagerström, 1989). It has been observed in various studies that seed germination, an important regeneration characteristic, can be triggered (or dormancy can be induced) by a variety of environmental factors (Washitani and Masuda, 1990). Onsetof germination and subsequent fate of seedlings can influence drastically the local establishment of a species so that the probability of seedling survival is closely connected to the seasonal timing of germination (Harper, 1977). Seed dormancy and germination,

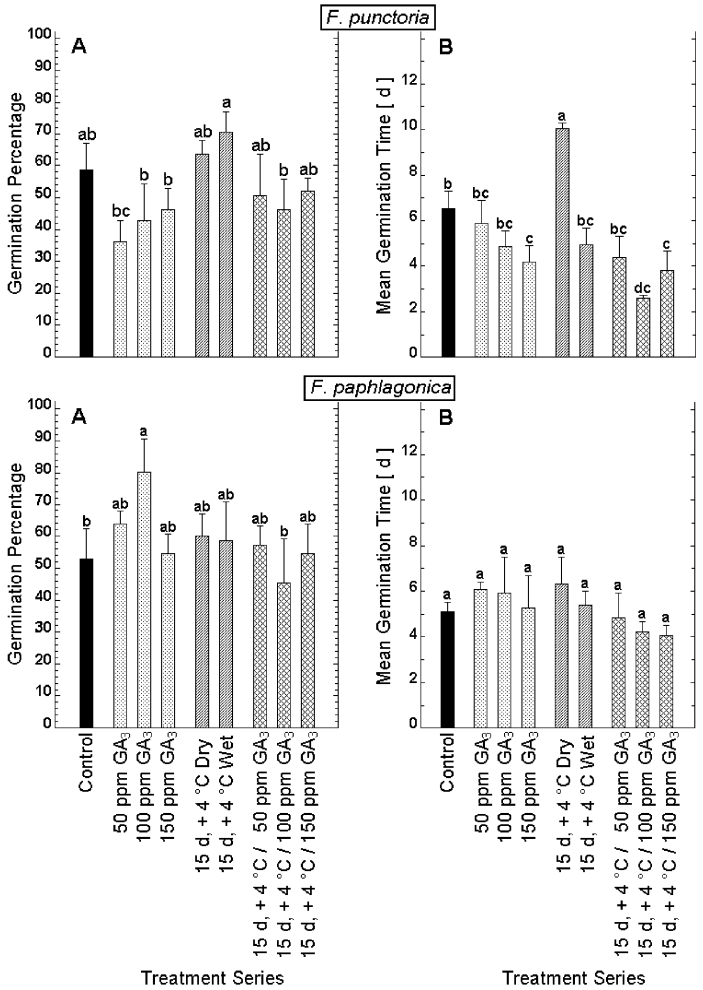

Fig. 3. Comparison of mean germination percentages (A) and mean germination times (B) in seeds of *F. punctoria* and *F. paphlagonica* among treatments series. Different letters indicate significant differences amongst the groups according to Tukey HSD test (rejection level 0.05). Error bars depict standard errors of means ($n = 3$).

especially of alpine species, have been studied by several researchers (*e.g.* Reynolds, 1984; Nishitani and Masuzawa, 1996; Schütz, 2002; Cerabolini *et al.*, 2004). Seed dormancy occurred in some of these species, very few required a cold treatment to germinate, and many species germinated best in the light (Amen, 1966; Bell and Amen, 1970; Rochow, 1970; Bliss, 1971).

The results of our experiments show that the three fescue species differ in their germination behaviour (Figs. 1, 2); *F. cyllenica* seeds are non-dormant showing the greatest germination rate in the control (> 80%; Fig. 1). On the contrary, *F. punctoria* and *F. paphlagonica* seeds can be dormant because the germination rates of both are low (50–60%, Fig. 1). The germination rates were increased with two different treatments in these species. These findings show that the germination rates of these species can be affected by various environmental factors. Furthermore, these results can also be related to the different altitudes of occurrence of these species. *F. punctoria* is confined to a higher altitude than *F. paphlagonica*. *F. punctoria* can be found up to 2543 m. However, the altitudinal limit of *F. paphlagonica* is at ca. 1700 m.

Consequently, although the three fescue species are in the same genus they show a different germination response. The reason of differences may be due to environmental differences of light and temperature climate present in the native habitats of the species.

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