The Effect of Cryopreservation on Germination of Dandelion Seeds

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Abstract

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Germination experiments frequently use seeds that had been stored frozen. We investigated whether short, 5 day freezing changes percentage and rate of germination of seeds of dandelion (*Taraxacum officinale* agg.). Seeds (*i*) collected at dispersal, (*ii*) dried at +50°C for 5 days, (*iii*) frozen at –20°C for 5 days, (*iv*) dried for 5 days and subsequently frozen for 5 days, and (*v*) frozen and subsequently dried, were then germinated at +10°C and a long-day photoperiod. None of the temperature pre-treatments affected the proportion of germinating seeds. By contrast, the time to germinate 50% of the seed (germination time) was shortened slightly (0.7 days) but significantly following the freezing treatment, regardless of whether it was applied without, before or after drying of the seed. Cryopreservation is therefore a convenient method of seed storage for comparative studies of seed germination because it causes no change in germination percentage and only a small and systematic change in germination rate.

Keywords: dandelion; *Taraxacum officinale*; weed; seed; freezing; drying; germination; storage

Dandelion (*Taraxacum officinale* agg.) is an important weed of pastures and ornamental grasslands and also a convenient subject for studies of seed predation and germination (Honek & Martinkova 2005; Honek *et al*. 2005). The species is particularly suitable for this type of investigation because its readily germinating seed is produced through the whole vegetative season and consequently exposed to a range of seed consumers and germination conditions. In studies of the seasonal variation in seed quality and its consequences for plant biology, dandelion seed is collected at successive dates. Design and purpose of the experiment then determines whether experiments will be done on different dates and thus keeping seed age constant, or seed of different dates is to be stored and experiments with seed of particular dates then done simultaneously. In our laboratory we used the latter alternative which makes the experimental conditions of all replicates identical. To avoid the possible effect of different length of afterripening, the seed samples were frozen 2 days after collection and preserved until the experiment. Freezing storage of dry seeds delays ageing and preserves germinability for a long time (Roberts 1973; Ellis & Roberts 1980; Dickie *et al*. 1990).

Frozen dandelion seeds maintain > 90% germination for more than 10 years (Hong *et al*. 1998) while at room temperature germination ceases within 3 years (Comes *et al*. 1978). On the other hand, exposing seed to different moisture treat-

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ments under moderate temperature conditions, although it did not affect germination percentage, significantly affected the germination rate (Martinková, Honěk and Pekár, unpubl.). This effect of moisture pre-treatment on subsequent germination, so-called "priming", is well established in many species (Hardegree *et al*. 2002; Chiu *et al*. 2002; Li *et al*. 2005). Little is known, however, about the effect of freezing itself on the germination rate, i.e. whether a fast cooling at –20°C and subsequent thawing (as used in our experiments) influence seed quality. This problem is relevant because the water content of seed frozen within a few days after dispersal is typically 7–9%. This moisture content is slightly above the range (2–6%) recommended for seed preservation (Ellis 1988) and may cause a change in seed characteristics.

In this study we investigated the effect on the proportion of germinating seeds and germination rate by short-time freezing at –20°C, applied soon after seed dispersal or following a period of drying. We tested the hypothesis that freezing does not change the characteristics of (*i*) the proportion of germinating seeds, and (*ii*) the germination rate (i.e. the time elapsed from the start of the germination experiment to germination of particular seeds).

MATERIAL AND METHODS

Seed collection. Experimental seed was collected at Prague-Ruzyně, on a trampled sward (50°05'13.9"N, 14°18'16.6"E, 340 m a.s.l., 3000 m^2 area). The seed was collected from 100 inflorescences, at the stage of seed dispersal, in the afternoon (14:00) of a sunny December 12, 2006. At the time of collection the seed was dry.

Temperature treatment. Two hours after collection the seed was divided into five lots, each of which was then subjected to a particular temperature treatment:

lot C, control, no temperature treatment;
lot D, 5 days of drying at +50°C (a temperature approaching that of insolated ground surface);
lot F, 5 days freezing at –20°C;
lot D/F, 5 days of drying at +50°C followed by 5 days freezing at –20°C;
lot F/D, 5 days freezing at –20°C followed by 5 days drying at +50°C.

The seed was dried or frozen in open Petri dishes (9 cm) on filter paper, either in an air-driven thermostat (+50°C) or a freezing box (–20°C).

Germination test. Seed was put up for germination 2 h after seed collection (control C) or after one of the four temperature treatments. Ten batches of 50 seeds were germinated of each seed lot. Each batch was placed into a Petri dish with dense filter paper (Filtrak®) moistened with 2 ml tap water. Petri dishes were kept at 10°C and a 17 h light:7 h dark photoperiod. These conditions approached the average temperatures in late spring when the majority of natural seeds germinate. Germinated seeds were counted and removed from Petri dishes at daily intervals. Germination extended over a 21 days period, sufficiently long to reveal differences in germination rate between treatments.

Data elaboration. Three characteristics of the seed lots were calculated:

(*i*) Proportion of germinated seeds was calculated for each dish. The differences between lots subjected to particular temperature treatments were tested by one-way ANOVA with arcsin transformed germination proportions of particular Petri dishes as response variable and temperature treatment as factor. As distribution of the germination time of particular seeds left-skewed and different from normality (Kolmogorov-Smirnov test: $P < 0.01$) the data were log transformed.

(*ii*) Differences in mean germination rate were tested using one-way ANOVA with log transformed data of average germination time as response variable and temperature treatment as factor.

(*iii*) To investigate the variation in distribution of germination rate, the germination time was converted to probits. The regression of probit transformed germination data on log germination time was calculated and the difference in slopes of regression lines compared using analysis of covariance ANCOVA with probability of germination on particular days as response variable, temperature treatment as factors and germination time (days) as covariate.

In this paper, means are accompanied by standard errors (± SE). All calculations were made using Statistica® (StatSoft 1994).

RESULTS

Germination percentage

The average proportion of germinating seeds was 54.2 ± 1.0%) and varied little between temperature treatments (53.4 ± 2.3–55.8 ± 1.8%). These differences were not significant (ANOVA: $F_{4.45} = 0.2053$, $P > 0.05$).

Table 1. Germination time (in days; mean ± SE, 95% confidence intervals) in the seed lots control (C), 5 days dried (D), 5 days frozen (F), 5 days dried and 5 days frozen (D/F), and 5 days frozen and 5 days dried (F/D)

Treatment	*N*	Mean ± SE[1]	± 95% confidence interval
C	268	10.2 ± 0.16a	9.84–10.49
D	279	10.6 ± 0.15b	10.28–10.88
F	267	9.6 ± 0.12c	9.33–9.81
D/F	273	9.7 ± 0.13c	9.40–9.92
F/D	267	9.6 ± 0.15c	9.29–9.89
Average		9.9 ± 0.07	9.79–10.05

[1]the difference between means followed by the same letter is not significant at $P < 0.05$; *N* – number of seeds that germinated following each of the temperature treatments

Germination rate

The average germination time varied significantly between treatments (ANOVA: $F_{4.60} = 4.225$, $P < 0.005$). The seed of lots C and D that were not frozen took slightly longer to germinate than seed of lot F frozen for 5 days and seed of lots D/F and F/D subjected to freezing and drying in different sequence of this temperature treatment (Table 1). However, the difference between mean germination time of pooled frozen (F, D/F and F/D) vs. pooled non-frozen (C and D) seed (Figure 1) was only 0.7 days. The regression of germination probability on germination time (Figure 2) revealed that the course of germination following each particular treatment was significantly different from that of the others (ANCOVA: $F_{4.60} = 15.63$, $P < 0.001$). The major difference between treatments were in the right tail of the distribution of germination time, among the slowly germinating part of the seed material.

DISCUSSION

The results confirmed the expectation that freezing *per se* does not change the proportion of germinating seeds (hypothesis *i*), but were wrong in predicting no effect on germination rate (hypothesis *ii*). As the manner of seed storage has no effect on percentage of germination, frozen seed may be used safely for experiments studying this quality of seed materials (Martinkova and Honek

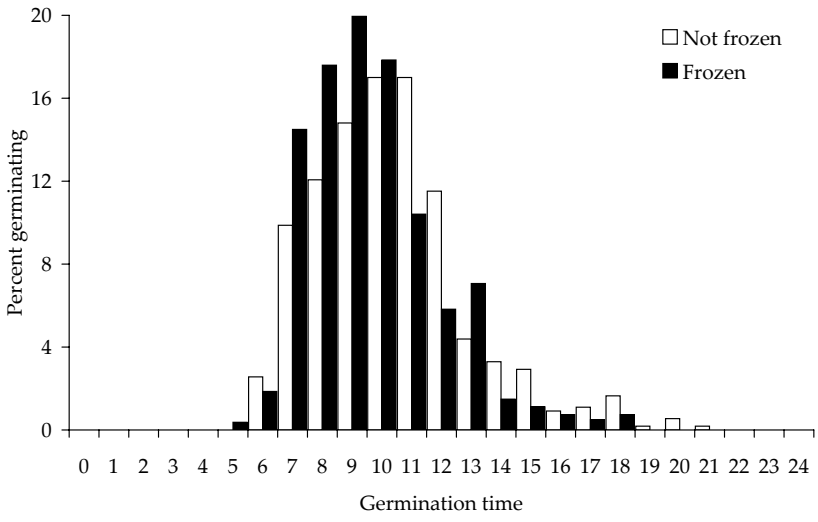

Figure 1. Histogram of the distribution of germination time (in days) in the pooled non-frozen lots (C and D) and pooled frozen lots (F, D/F and F/D)

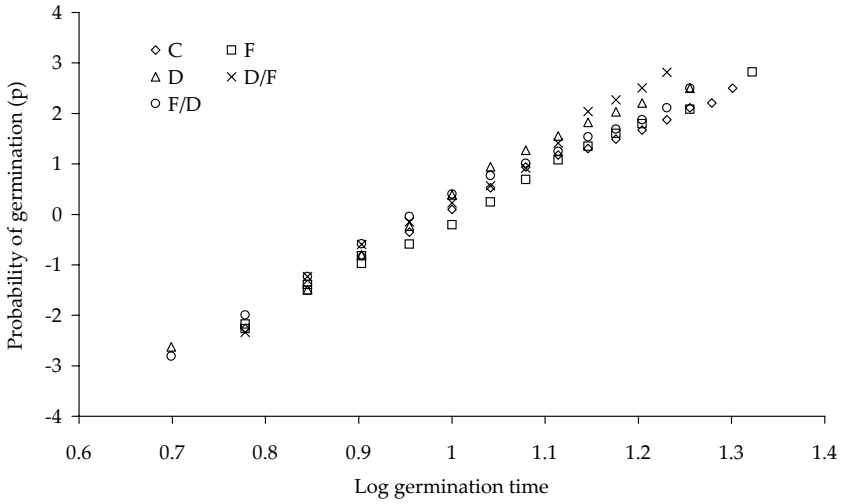

Figure 2. The regression of germination probability (probits p) on log germination time for particular temperature treatments

in prep.). By contrast, studies of germination rate are slightly affected by freezing preservation of seed materials. The treatments differed in some aspects besides freezing: seed of lot F was only frozen, seed of F/D was frozen before desiccation using high (+50°C) temperature, and seed of D/F was frozen after desiccation treatment. Nevertheless, freezing caused a uniform shift in germination rate, regardless of the time of its application. The difference of 0.7 days in average germination time between frozen and non-frozen seed was smaller than the differences caused by e.g. seed pre-conditioning using various combinations of moisture and temperature conditions (Martinková, Honěk and Pekár in prep). The small difference in germination rate caused by freezing is probably acceptable for most studies where frozen seed materials are used as the change in germination rate (decreasing the time to germination) is similar following different freezing treatments and small (≤ 1 day difference in average germination time).

It is still open how far the results may differ when using seed materials collected at different dates. In our material the proportion of germinating seed was 53–56%, which is lower than in seed collected in spring or summer when it is typically 85–95% (Martinková & Honěk 1997; Stewart-Wade *et al*. 2002). A decline of germination percentage of seed produced in late autumn is general, i.e. occurs in different years and at several localities (Martinková, Honěk and Pekár in prep). The difference in germination rate of frozen and non-frozen seed materials may be increased by moisture content of seed (8.7%). Although this moisture is above the limit recommended for seed storage in commercial banks (Hong *et al*. 1998) it is well below the limit of safe moisture content for cryopreservation of seeds of several species of wild herbs and crops (Stanwood & Roos 1979; Zewdie & Ellis 1991).

From this study, we may conclude that using frozen seed in germination experiments is safe for establishing germination percentage. It is also safe for establishing germination rate provided that all compared seed materials were frozen. Freezing may elicit a small difference in germination time compared to fresh seed. But even this difference is probably acceptable in most kinds of studies of seasonal variation of seed quality. The innate differences between seed accessions (Hardegree *et al*. 2002) and differences caused by "priming" of seed exposed to moisture and temperature treatment before germination are much greater than the differences caused by cryopreservation.

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References

Chiu K.Y., Chen C.L., Sung J.M. (2002): Effect of priming temperature on storability of primed sh-2 sweet corn seed. Crop Science, **42**: 1996–2003.

Comes R.D., Bruns V.F., Kelley A.D. (1978): Longevity of certain weed and crop seeds in fresh water. Weed Science, **26**: 336–344.

Dickie J.B., Ellis R.H., Kraak H.L., Ryder K., Tompsett P.B. (1990): Temperature and seed storage longevity. Annals of Botany, **65** :147–151.

Ellis R.H. (1988): The viability equation, seed viability nomographs, and practical advice on seed storage. Seed Science and Technology, **16**: 29–50.

Ellis R.H., Roberts E.H. (1980): Improved equations for the prediction of seed longevity. Annals of Botany, **45**: 13–30.

Hardegree S.P., Jones T.A., Van Vactor S.S. (2002): Variability in thermal response of primed and non-primed seeds of squirreltail [*Elymus elymoides* (Raf.) Sweezey and *Elymus multisetus* (J.G.Smith) M.E. Jones]. Annals of Botany, **89**: 311–319.

Honek A., Martinkova Z. (2005): Pre-dispersal predation of *Taraxacum officinale* (dandelion) seed. Journal of Ecology, **93**: 335–344.

Honek A., Martinkova Z., Saska P. (2005): Post-dispersal seed predation of *Taraxacum officinale* (dandelion) seed. Journal of Ecology, **93**: 345–352.

Hong T.D., Linington S., Ellis R.H. (1998): Compendium of Information on Seed Storage Behaviour. Royal Botanical Garden Kew, London.

Li W., McDonald M.B., Bennett M.A., Kwong F.Y. (2005): Hydropriming of differing sized impatiens 'Expo Wine' seeds. Seed Science and Technology, **33**: 639–646.

Martinková Z., Honěk A. (1997): Germination and seed viability in a dandelion, *Taraxacum officinale* agg. Ochrana Rostlin, **33**: 125–133.

Roberts E.H. (1973): Predicting the storage life of seeds. Seed Science and Technology, **1**: 499–514.

Stanwood P.C., Roos E.E. (1979): Seed storage of several horticultural species in liquid nitrogen (–196°C). HortScience, **14**: 628–630.

StatSoft (1994): Statistica for Windows (Volume I): General Conventions and Statistics I. StatSoft Inc., Tulsa.

Stewart-Wade S.M., Neumann S., Collins L.L., Boland G.J. (2002): The biology of Canadian weeds. 117. *Taraxacum officinale* G.H.Weber ex Wiggers. Canadian Journal of Plant Science, **82**: 825–853.

Zewdie M., Ellis R.H. (1991): Survival of tef and niger seeds following exposure to sub-zerotemperatures at various moisture contents. Seed Science and Technology, **19**: 309–317.

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