

Germination in declining and common herbaceous plant populations co-occurring in an acid peaty heathland

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SUMMARY

Antennaria dioica, *Arnica montana*, *Filago minima*, *Galium saxatile*, *Nardus stricta* and *Viola canina* are herbaceous plant species in The Netherlands which are declining and even locally extinct.

The germination biology of freshly collected seeds of these species was studied in relation to constant and alternating temperatures and was compared with that of some common co-occurring plant species. It is concluded that germination responses with respect to temperatures are unlikely to account for their declining situation.

Key-words: *Antennaria dioica*, *Arnica montana*, *Filago minima*, *Galium saxatile*, germination, *Nardus stricta*, *Viola canina*.

INTRODUCTION

The distribution of various herbaceous plant species, including *Arnica montana**, *Antennaria dioica*, *Galium saxatile*, *Nardus stricta* and *Viola canina*, character species of the alliance *Violion caninae*† have been declining in The Netherlands since 1950 (Mennema *et al.* 1985). This syntaxon classifies dry heathlands and unfertilized grasslands on moderately humid to dry, nutrient-poor acid, humic (podzolic) soils. Despite their preference for acid soils (Ellenberg 1979), the decline might be attributed to increased soil acidification (Van Dam *et al.* 1986). Nutrient-poor, poorly buffered (low contents of clay and organic matter), non-calcareous, sandy soils of moderate acidity (including cover sands) are the most susceptible to current levels of acid rain (Wiklander 1980; McFee 1983). In addition, it might be the result of the gradual replacement by grasses (e.g. *Deschampsia flexuosa*) which benefits the gradually increasing soil fertility by (1) progressive accumulation of organic matter in the topsoil resulting from changes in management practices during the last decades, and (2) input of air-born nutrients (NO_3^- , NH_4^+ , SO_4^{2-}) through acid rain. The topsoil of humic podzols is more beneficial to plant growth compared with other horizons (Ernst & Nelissen 1979). Increased soil fertility is likely to stimulate the competitive abilities of the grasses (Heil 1984).

It can be suggested that a decline in frequency or even local extinction of certain plant populations in acid peaty heathlands may be due to differences in the generative processes, in particular germination biology of the co-occurring herbaceous plant populations. It is

*Nomenclature for herbaceous species follows Heukels & van der Meijden (1983).

†Nomenclature for syntaxa follows Westhoff & Den Held (1969).

Table 1. Frequency of occurrence in The Netherlands (Hour-Square Frequency Class) in 1930 and in 1980 and map references of sites of seed collection of 22 herbaceous plant species which may co-occur in the *Violin caninae*. Hour-Square Frequency Classes follow Van der Meijden *et al.* (1983)

Species	Distribution according to Hour-Square Frequency Class		Map reference of sites of seed collection
	1930	1980	
<i>Violin caninae</i>			
<i>Luzula multiflora</i>	5	5	53°04'N,06°37'E
<i>Antennaria dioica</i>	6	4	53°30'N,06°10'E
<i>Arnica montana</i>	6	4	53°04'N,06°37'E
<i>Potentilla erecta</i>	6	7	53°04'N,06°37'E
<i>Galium saxatile</i>	7	6	53°04'N,06°39'E
<i>Viola canina</i>	7	7	53°04'N,06°39'E
<i>Danthonia decumbens</i>	8	7	53°06'N,06°01'E
<i>Nardus stricta</i>	8	7	53°06'N,06°01'E
<i>Festuca ovina</i>	8	8	53°04'N,06°37'E
<i>Junco (subuliflori)-Molinion</i>			
<i>Succisa pratensis</i>	8	6	53°08'N,06°38'E
<i>Quercion robori-petraeae</i>			
<i>Hieracium umbellatum</i>	8	7	53°04'N,06°37'E
<i>Hieracium laevigatum</i>	8	8	53°04'N,06°37'E
<i>Other species</i>			
<i>Filago minima</i>	7	6	53°04'N,06°39'E
<i>Juncus squarrosus</i>	7	6	53°04'N,06°37'E
<i>Agrostis canina</i>	7	7	53°06'N,06°01'E
<i>Deschampsia flexuosa</i>	7	7	53°04'N,06°37'E
<i>Campanula rotundifolia</i>	8	7	53°04'N,06°37'E
<i>Jasione montana</i>	8	7	53°04'N,06°37'E
<i>Anthoxanthum odoratum</i>	9	9	53°04'N,06°37'E
<i>Festuca rubra</i>	9	9	53°06'N,06°01'E
<i>Holcus lanatus</i>	9	9	53°06'N,06°01'E
<i>Rumex acetosella</i>	9	9	53°04'N,06°37'E

commonly observed that many grasses germinate in autumn immediately following seed shed, whereas herbs retard germination until spring (Grime 1979). It seems that most of the temperate grasses lack innate dormancy (no chilling-requirement) and are capable of germinating in light and darkness (non-photoblastic) over a wide temperature range. Because of their temperature-regulated germination response (easy germination at relatively high temperatures and poor germination at relatively low temperatures) many dicotyledons germinate in spring after fulfilment of a partial chilling requirement (Grime 1979; Pegtel 1983).

This paper compares the germination of a range of declining and common flowering plant populations, all of them may be found growing together.

MATERIALS AND METHODS

Seed collection

Seed (achene, caryopsis) collections of 22 flowering plant populations (Table 1) were made from many individuals, air-dried at room temperature for a maximum of 1 week and stored in paper bags at 4°C and 60–70% relative humidity.

Seeds of *Viola canina* and achenes of *Antennaria dioica* were sampled from potted individuals kept outside as a nursery stock at the experimental garden (Haren, The Netherlands). *Viola* came originally (1984) from a grassland site, and *Antennaria* from an inner dune site (1981).

Caryopses of *Agrostis canina*, *Danthonia decumbens*, *Festuca rubra*, *Holcus lanatus* and *Nardus stricta* were sampled in 1979 (De Boer 1981) from a nutrient-poor (P, N, K) fen meadow (litter fen), classified as Cirsio-Molinietum, on fen peat (prominent clayey earthened moss peat soil, Pegtel 1983).

The fruit sample of *Succisa pratensis* was taken in October 1980 from a dry heath vegetation (Pegtel 1986).

The only annual included in this study was *Filago minima*. This species is also supposedly declining by soil acidification (Van Dam *et al.* 1986). Achenes were drawn in 1984 from a population growing on non-calcareous, weakly acidic sand after its vegetation of the dominant *Calluna vulgaris* had been destroyed.

Seeds of *Anthoxanthum odoratum*, *Arnica montana*, *Campanula rotundifolia*, *Deschampsia flexuosa*, *Festuca ovina*, *Galium saxatile*, *Hieracium laevigatum*, *Hieracium umbellatum*, *Jasione montana*, *Juncus squarrosus*, *Luzula multiflora*, *Potentilla erecta* and *Rumex acetosella* were sampled in 1984 from a poorly developed Violion caninae on humic podzols in a weakly undulating cover sand overlying glacial till in the Pleistocene district in the north of The Netherlands (Pegtel 1987).

Germination experiments

Within 1 month after collection, batches of 50 seeds were spread on moist Whatman No. 1 filter paper in plastic Petri dishes. The dishes (three per treatment) were transferred to germination chambers.

The experiments lasted up to 150 days, depending on the species. Germinated seeds were counted and discarded. Seeds were considered to have germinated when the radicle protruded from the seed coat of dicotyledons or in the case of grasses protrude through the coleorhiza.

The breaking of dormancy. Depending on the results and on the amount of seeds available, seeds of some species were cold-stratified in a refrigerator at 5°C in the absence of light for various periods of time. Large batches of seeds were placed on either two moistened Whatman No. 1 filter papers or granulated hydrophylic rockwool (*Viola canina*) in 16 cm glass Petri dishes.

Seeds were removed from this pre-treatment condition and tested for ability to germinate at a range of constant and alternate temperatures or at a diurnal (12 h daily) temperature fluctuation of 20/10°C (*Viola canina*).

In a gibberellin pre-treatment, seeds of *Viola* were removed from dry storage, and batches of seeds were spread on granulated hydrophylic rockwool in 16 cm glass Petri dishes, moistened with either 4.0⁻¹ g l⁻¹ GA₃ or with 4.5 g l⁻¹ GA_{4/7} (both supplied as a commercial preparation 'Berelex') and transferred to a germination chamber at 25/15°C (12 h daily and light coinciding with the period of 25°C). Germination tests were conducted after the seeds had been rinsed with demineralized water, and were spread on moist granulated hydrophylic rockwool in plastic Petri dishes at similar temperature and light conditions. Seeds of *Rumex acetosella* were scarified.

Response to temperature. The influence of temperature upon mean final percentage of germination was studied at five constant and five diurnal (12 h daily) alternations over a

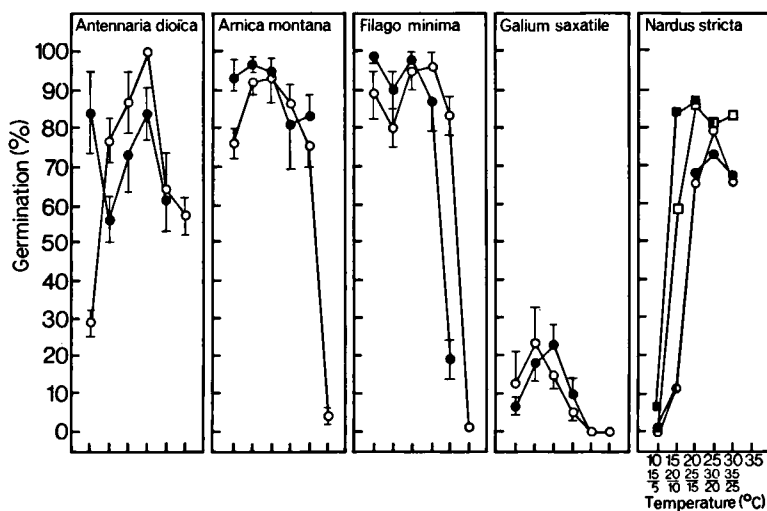


Fig. 1. Effect of various constant (○ □) and alternate (● ■) temperatures (12 h light daily) on the germination (%) of fresh (circles) and cold-stratified (at 5°C in darkness; squares) seeds of five herbaceous plant species: *Antennaria dioica*, *Arnica montana*, *Filago minima*, *Galium saxatile* and *Nardus stricta*. The vertical bars are standard deviations.

5°C-stepwise range of 10–30°C, using germination chambers. The temperature regimes applied are indicated along the horizontal axes of the graphs. The difference between day and night temperature was always 10°C. Light supply was always 12 h per day at an incident quantum flux density (PAR 400–700 nm) of at least $10 \mu \text{E m}^{-2} \text{s}^{-1}$ provided by cool-white 'Sylvania' (F 36 W/133 ST) fluorescent tubes and coincided with the period of high temperatures when temperatures alternated.

RESULTS

Germination in declining populations

The total germination percentages, at constant and alternate temperatures, of freshly collected seeds of some declining populations are presented in Fig. 1. *Antennaria*, *Arnica* and the annual *Filago* germinated substantially in the wide temperature range of 10–30°C. Substantial germination of seeds of *Nardus* only occurred in the constant temperature range of 20–30°C. At relatively low constant temperatures (10–15°C) germination was low. When caryopses of *Nardus* were cold-stratified, increased germination was only observed at 15 and 20/10°C, so widening the range of constant and alternate temperature regimes under which germination occurred. Compared to the former four species, *Galium* seeds germinated poorly (maximum 23%). Except for *Antennaria*, most populations germinated badly or not at all at 35°C. (*Nardus* was not tested at this temperature.) Germination of all five species was not increased with alternate temperatures.

Freshly collected seeds of *Viola canina* did not germinate at the temperature regimes applied. Moist-chilled dry-stored seeds were partly capable of germinating at the moderate temperature regime of 20/10°C (Fig. 2a). The length of cold stratification affected germination positively. Germination at 25/15°C was observed if dry-stored seeds were imbibed in the gibberellin GA₃ at 25/15°C (Fig. 2b). Prolonged periods of imbibition

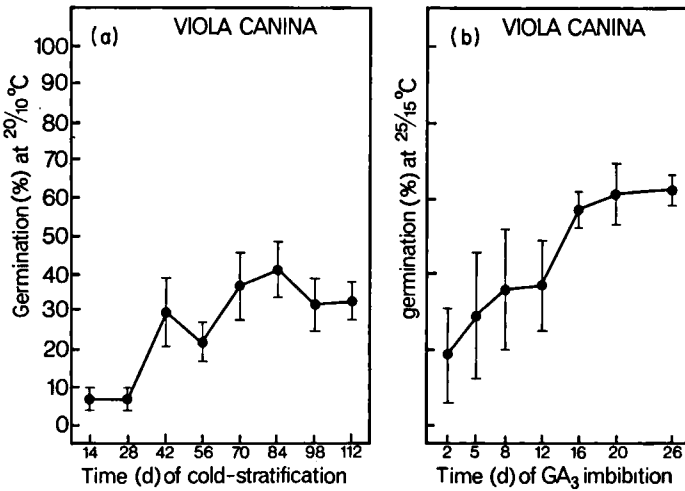


Fig. 2. Germination (%) of seeds of *Viola canina* at (a) 20/10°C (12 h light daily) after cold stratification (at 5°C in darkness), and at (b) 25/15°C (12 h light daily) after imbibition in 4.0 g l⁻¹ gibberellic acid (GA₃ at 25/15°C) and 12 h light daily) for various periods of time (days). The vertical bars are standard deviations.

stimulated germination. Such a gibberellin-promoted effect on the capability of the seeds to germinate at 25/15°C was not observed when the gibberellin GA_{4/7} was applied (results not presented in Fig. 2).

Germination of common monocotyledons

Figure 3 presents the temperature-affected total germination (expressed as a percentage) of freshly collected caryopses of a variety of monocotyledons. The species differed in their response to the various temperature regimes applied. Species such as *Deschampsia*, *Festuca ovina* and *Festuca rubra* germinated moderately, whilst the others attained more than 50% germination. The germination responses can roughly be grouped into three categories with respect to the shape of the curves.

1. A category of plant species which germinated optimally over a wide temperature range of 10–25 or 30°C (*Anthoxanthum*, *Deschampsia*, *Festuca*, *Holcus*).
2. A category of plant species which germinated poorly at 10–15°C, and optimally at a narrow temperature range (25–30°C: *Agrostis*; 20°C: *Juncus*).
3. A category of plant species which did not germinate at constant temperatures (*Danthonia*).

Germination at constant and alternate temperatures did not differ in *Anthoxanthum*, *Deschampsia*, *Festuca ovina* and *Luzula*. Alternate temperatures increased germination in *Agrostis*, *Danthonia*, *Festuca rubra*, *Holcus* and *Juncus*.

Danthonia caryopses when cold-stratified showed a shift in the germination response curve towards lower temperatures. Alternate temperatures increased germination slightly compared with the effect of constant temperatures.

Germination of common dicotyledons

Temperature-affected germination of freshly collected seeds of some common dicotyledons is presented in Fig. 4. Seeds of *Potentilla erecta* and scarified seeds of *Rumex acetosella* germinated poorly, presumably pointing to a high degree of dormancy. *Jasione montana*

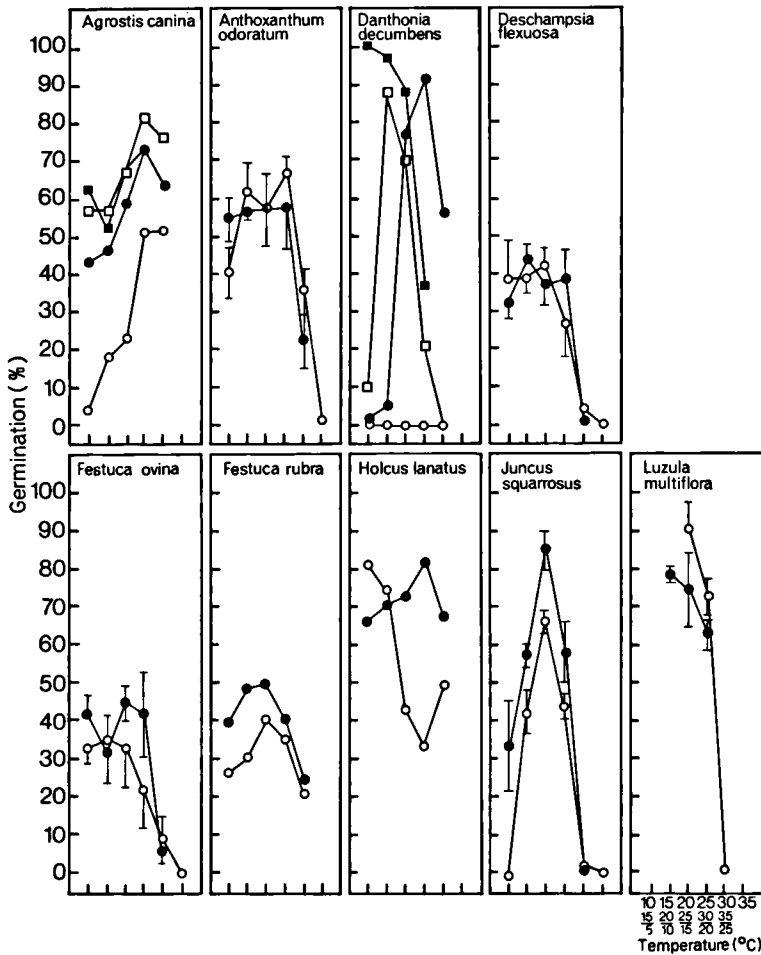


Fig. 3. Effect of various constant (○ □) and alternate (● ■) temperatures (12 h light daily) on the germination (%) of fresh (circles) and cold-stratified (at 5°C for 100 days; squares) seeds of common monocotyledonous plant species. The vertical bars are standard deviations.

germinated optimally over a wide temperature range of 10–25°C compared with the narrow temperature range of optimal germination in *Campanula rotundifolia*, *Hieracium umbellatum* and *Succisa pratensis*. *Hieracium laevigatum* hardly germinated at constant temperatures. Seeds of *C. rotundifolia*, *H. laevigatum*, *H. umbellatum* and *S. pratensis* germinated when temperatures alternated. This effect was not observed in *J. montana*.

As compared with freshly sampled fruits of *S. pratensis*, those which were cold-stratified germinated better and over a wider temperature range (10–30°C).

DISCUSSION

Most of the bulked fresh seed crops of the 22 species investigated show a high variation of germination with respect to temperature range and the level of final germination. At one extreme, seeds of *Anthoxanthum odoratum*, *Deschampsia flexuosa*, *Festuca rubra*, *Arnica montana*, *Filago minima*, *Antennaria dioica*, *Campanula rotundifolia* and *Jasione montana* germinate easily over a relatively wide range of temperatures, including relatively low

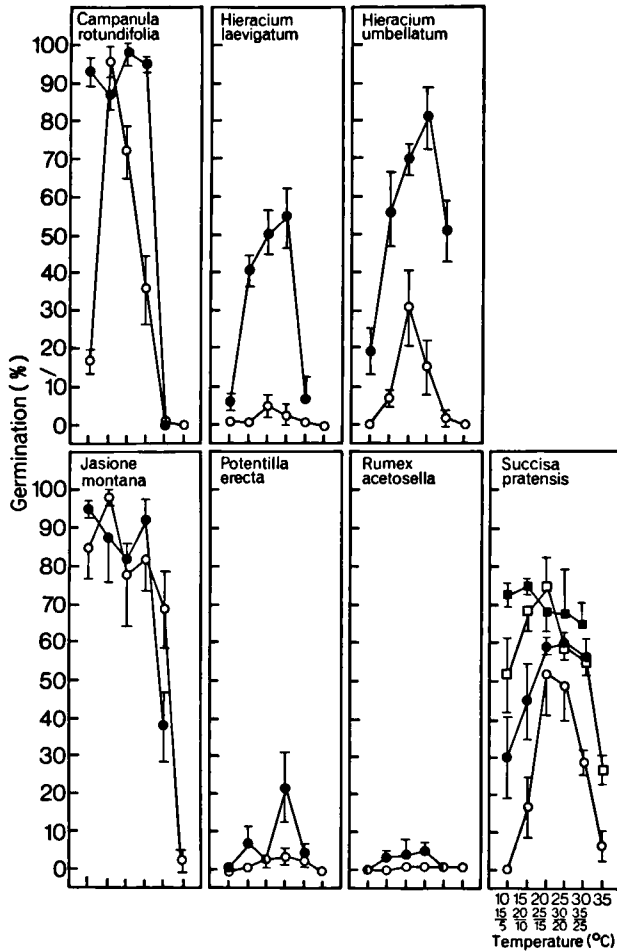


Fig. 4. Effect of various constant (○ □) and alternate (● ■) temperatures (12 h light daily) on the germination (%) of fresh (circles) and cold-stratified (at 5°C for 40 days; squares) seeds of common dicotyledonous plant species. The vertical bars are standard deviations.

temperatures. High temperatures are less beneficial, and alternate temperatures do not improve germination. Half of the seed crop of *Deschampsia flexuosa* and of *Festuca* species seems to be dormant. Another group of species, including the monocotyledon *Nardus stricta*, germinates easily only at relatively high temperatures up to an optimum and probably decreases in its germination response at still higher temperatures. Alternate temperatures may be beneficial. Species such as *Danthonia decumbens*, *Juncus squarrosus*, *Hieracium laevigatum*, *Hieracium umbellatum* and *Succisa pratensis* germinate within a restricted temperature range if they are not stratified at low temperature. For this group of species, alternate temperatures are, to some extent, beneficial to seed germination depending on the species, on the amplitude of temperature alternation, and may replace light requirement (Thompson *et al.* 1977), which is sometimes induced by burial (Wesson & Wareing 1969).

The function of this type of partial dormancy is comparable to true dormancy: it determines the timing of growth resumption whenever external conditions become

suitable. The timing is enforced by the effect of stratification (Vegis 1973). Such a germination pattern is typical of species adapted to a climate with a periodically recurring cold season, although not all the species follow this (such as *Holcus lanatus*).

At the other extreme, seed samples of *Viola canina*, (scarified) *Rumex acetosella*, *Potentilla erecta* and *Galium saxatile* appear to have a high proportion of dormant seeds. The dormancy of seeds of *Viola* is unlikely to originate from the seed coat (Silvertown 1984) because the chilling requirement can easily be replaced by pre-treatment with GA₃ (Fig. 2) at normal temperature regimes indicating that the seed coat is permeable to water and oxygen.

Concentrations of 4.0 g l⁻¹ GA₃ or 4.5 g l⁻¹ GA_{4/7} may be considered exceptional and potentially toxic. However, even higher concentrations of gibberellins have been used (Weaver 1972). Nevertheless, the GA₃ treatment proved to stimulate germination of *Viola canina*, and seedlings did not show any prolonged effect.

Depending on the populations (species), a seed sample seems to contain a variable proportion of both viable dormant and non-dormant seeds. To achieve maximum germination response, different (pre)-treatments are often required (Steinbauer *et al.* 1955; Thompson 1973). This phenomenon of germination polymorphism of bulked seed samples is commonly observed (Grime *et al.* 1981), and originates from (Thompson 1973): (i) genetic polymorphism between the seeds of different parent plants (genotypes), as demonstrated for *Stellaria media* (Van der Vegte 1978), and/or (ii) somatic polymorphism between the seeds of (a) individuals with the same genetical identity but grown under (slightly) different conditions, as in a patchy environment, and (b) individuals (within plant variability).

It is often observed that grasses germinate in autumn and herbs in spring (Grime 1979). However, such a separation in temperature-regulated germination response between monocotyledons and dicotyledons is not always appropriate, as indicated for autumn and spring germination of *Digitalis purpurea* (Van Baalen 1982) and for germination of dune annuals (Rozijn & Van Andel 1985). Monocotyledons such as *Agrostis canina* and *Nardus stricta* are likely to germinate in high numbers in spring. Half of the germination of caryopses of *Deschampsia flexuosa*, *Festuca ovina* and *Festuca rubra* will be postponed to next spring if after-ripening and/or requirement for chilling have been satisfied. This indicates that moisture supply is not always the overriding determinant of the time of germination of this group of species (Grime 1979). It may be predicted that the timing of germination of the declining dicotyledons (*Arnica montana*, *Antennaria dioica*, and *Filago minima*) as well as the germination of the common dicotyledons (*Campanula rotundifolia* and *Jasione montana*) will mainly be in autumn. These aspects certainly need further field studies and are therefore under investigation.

It may be concluded that germination responses with respect to the temperature of the seeds of the species *Antennaria dioica*, *Arnica montana*, *Filago minima*, *Galium saxatile*, *Nardus stricta* and *Viola canina* are not exceptional and are like those usually recorded for monocotyledons and dicotyledons. Therefore, temperature-regulated germination characteristics of these species are unlikely to account for their declining situation.

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