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GERMINATION OF CALLUNA VULGARIS (L.) HULL IN VITRO UNDER DIFFERENT PH-CONDITIONS

H. P. G. HELSPER and G. A. M. KLERKEN

Botanisch Laboratorium, afdeling Geobotanie, Toernooiveld, 6525 ED Nijmegen

SUMMARY

Seeds, collected from a stock of *Calluna vulgaris* (L.) Hull appeared to be polymorphic and four types could be distinguished: large-ribbed, small-ribbed, large-smooth, small-smooth. The seed types differ in germination response at pH = 5.6. Moreover, germination of *Calluna* seeds appears to be pH-dependent; pH = 3.2 is the critical value, at lower values germination decreases significantly.

Surface-sterilisation of the seeds also influences their germination percentage.

The distribution pattern of *Calluna* seedlings in the field is explained in connection with the pH.

1. INTRODUCTION

During many years of observation of sod and unsod heathlands on the Strabrechtse Heide (vegetation type *Genisto-Callunetum cladonietosum*) (DE SMIDT 1977) in the southern part of The Netherlands (cf. HELSPER et al. 1983) seedlings of *Calluna vulgaris* were rarely found. Only in sod areas treated regularly (once a year) with an overdose of marl (500 kg/ha = 50 g/m^2), seedlings were found (BENNEKER et al. 1979). Since the pH in the upper 5 cm (A_1-horizon) of the non-treated and marl-treated plots were 2.7 and 3.4, respectively (GROENENDAEL & ROOZEN 1978; KLERKEN 1983), the possibility that low pH retards germination was tested experimentally.

2. MATERIAL AND METHODS

Seeds of *Calluna vulgaris* were collected at the end of October 1982. Seed-bearing sprigs were collected and directly dried at 60 °C for several hours in the laboratory. The flowers, which then could be easily separated from the sprigs, were dried for some more days, till the seeds came free. The seeds were separated from the remaining flower parts by a fine meshed sieve (1 mm), dried for about 2 h at 30 °C and then covered with parafilm to keep them dry. Inspection of the seeds with a binocular (45 ×) revealed that dark-brown and light-brown seeds could be distinguished. The percentage of each seed type was determined in samples of 1000 seeds (N). Average seed weights were determined by weighing samples of 200 seeds of each seed type with an accuracy of 0.1 mg. Length and

Table 1. The germination percentages (%N), average weights, mean lengths, and mean width of four seed types of *Calluna vulgaris* (L.) Hull. Standard deviations are indicated.

Seed type	% N	Average weight (g)	Mean length (mm)	Mean width (mm)
ss	32	1.71×10^{-5}	0.35 ± 0.05	0.25 ± 0.05
sr	36	1.76×10^{-5}	0.35 ± 0.05	0.25 ± 0.05
ls	10	2.57×10^{-5}	0.55 ± 0.05	0.35 ± 0.05
lr	22	2.50×10^{-5}	0.55 ± 0.05	0.35 ± 0.05

width of 20 seeds of each type were determined under a binocular (45 ×) on graph paper.

Germination was carried out in Petri dishes on filter paper moistened with demineralised water acidified with H_2SO_4 in order to obtain the desired pH-range (BEKENDAM 1974, POËLL 1949). Compared with some other buffers (e.g. Mc Ilvain-buffer) these solutions had the advantage that growth of fungi did not occur. The pH-values tested were: 5.6, 5.0, 4.4, 3.8, 3.2, 2.9, 2.6 and 2.0. It was necessary to use acid-washed filter-paper to prevent pH changes during the experiment. A seed is considered germinated when the root is broken out

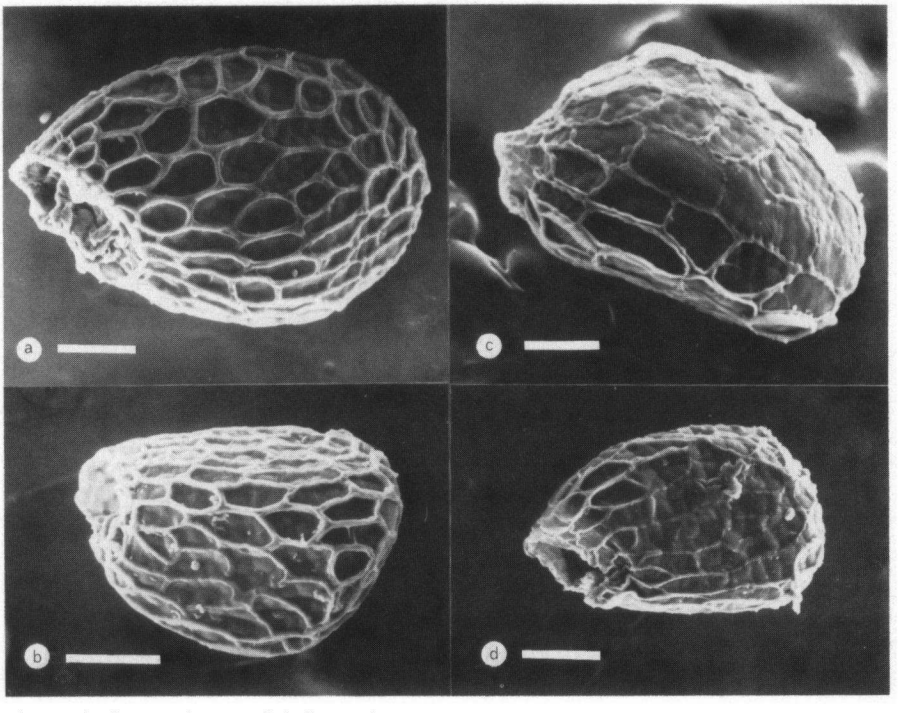

Fig. 1. The four seed types of *Calluna vulgaris*. a: large-ribbed (lr) seeds; b: small-ribbed (sr) seeds; c: large-smooth (ls) seeds; d: small-smooth (ss) seeds. Scale bars: 0.1 mm.

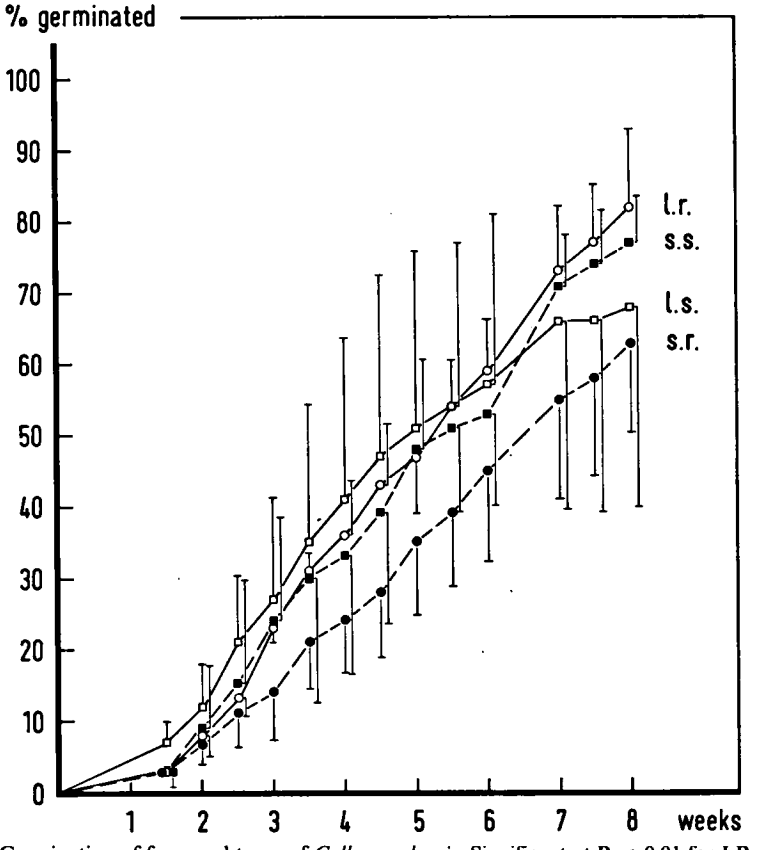

Fig. 2. Germination of four seed types of *Calluna vulgaris*. Significant at $P < 0.01$ for LR ↔ SR, $P < 0.05$ for LS ↔ SR, $P < 0.10$ for SS ↔ SR. Other combinations are not significant (one sided sign-test).

of the seed-coat, irrespective of the viability of the germ later on. Seeds were surface-sterilized in a solution of 140 ml demi-water plus 10 g $(Ca(OCl)_2$ during 30 minutes (KNUDSON 1929).

In every Petri dish 100 seeds were tested (see *fig. 3*). During germination the conditions were kept as optimal as possible by giving the seeds a daily photoperiod of 8 h light and 16 h darkness at 30 °C and 10 °C, respectively (GIMINGHAM 1972, BEKENDAM 1974), and by keeping the prevailing air humidity and the degree of moistness of the filter paper constant. Statistical analysis of the results was carried out by analysis of variance.

3. RESULTS AND DISCUSSION

We have distinguished dark-brown and light-brown seeds (see Material and Methods). The dark-brown seeds were strongly ribbed and varied in size; the

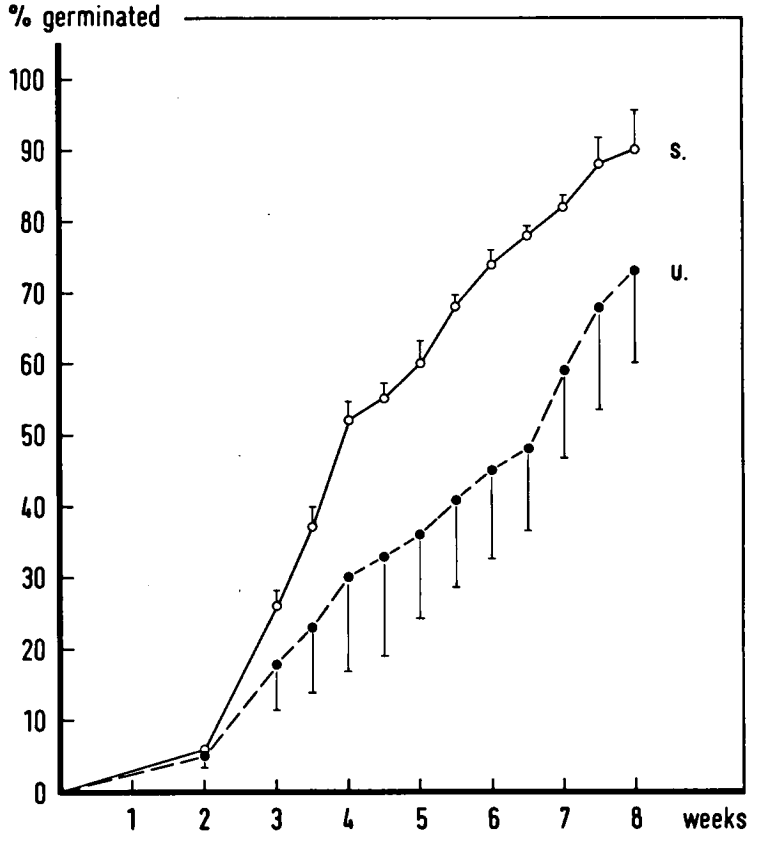

Fig. 3. Germination of sterilized (s) and unsterilized (u) seeds of *Calluna vulgaris* from a random mixture of the four types (*fig. 1.*). Significant at $P < 0.01$.

light-brown seeds were nearly smooth and varied also in size. So it turned out that four types of seeds could be distinguished, i.e. large-ribbed (lr) (*fig. 1a*), small-ribbed (sm) (*fig. 1b*), large-smooth (ls) (*fig. 1c*) and small-smooth (ss) (*fig. 1d*) (cf. BEIJERINCK 1940, BEWLEY & BLACK 1978, 1982). The results of *table 1* demonstrate that seeds of *Calluna vulgaris* are also polymorphic in size.

In addition, the four types of seeds also indicate a difference in germination capability at pH = 5.6 (*fig. 2*). After 8 weeks the percentage of germination of the large-ribbed (lr) seeds was 19% higher ($P < 0.01$) than that of the small-ribbed (sr) seeds. The germination percentages of both large and small smooth seeds are intermediate. However, the small-smooth (ss) seeds germinate better than the small-ribbed (sr) ($P < 0.1$).

Surface-sterilization of the *Calluna* seeds has a positive effect on the germination (*fig. 3*). After eight weeks 73% of the unsterilized seeds were germinated compared with 90% of the sterilized seeds ($P < 0.01$). Differences in germination

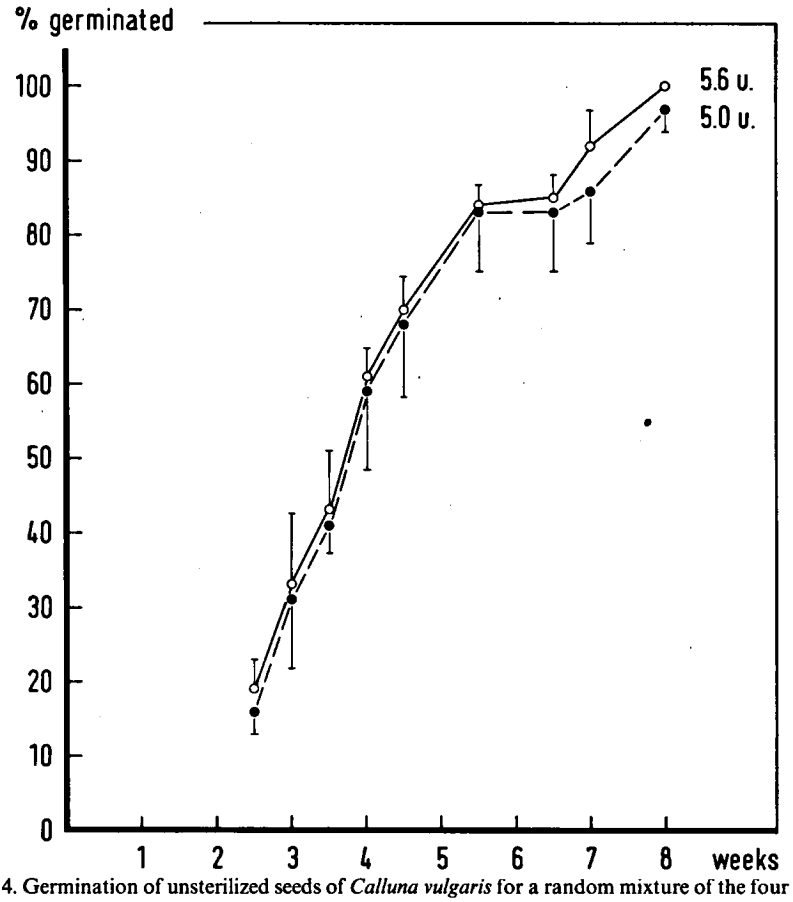

Fig. 4. Germination of unsterilized seeds of *Calluna vulgaris* for a random mixture of the four types at pH = 5.0 and 5.6.

between sterilized and unsterilized seeds has been reported earlier (BEWLEY & BLACK 1982).

The germination percentage of the seed mixture is affected by the acidity of the substrate (*figs. 4* and *5*). The critical point is at pH = 3.2. At lower pH-values, i.e. at pH 2.9, 2.6 and 2.0 germination strongly decreased and after five weeks all seedlings had died. At pH > 3.2 germination is more or less the same and after eight weeks nearly all seeds have germinated ($P < 0.01$ between pH = 3.2 and 2.9) and stayed alive. HARPER (1977) and BEWLEY & BLACK (1982) have found many examples of polymorphism in seeds of plants belonging to the Asteraceae, Poaceae, and others. Thus *Calluna vulgaris* appears to be another example of a plant with polymorphic seeds. BEIJERINCK (1940) has observed many forms of polymorphism within *Calluna vulgaris*, but only with regard to ramification, hairyness of the leaves, shape and colour of flowers, etc. Field observations, that seedlings of *Calluna vulgaris* are seldom found, except in sod areas treated

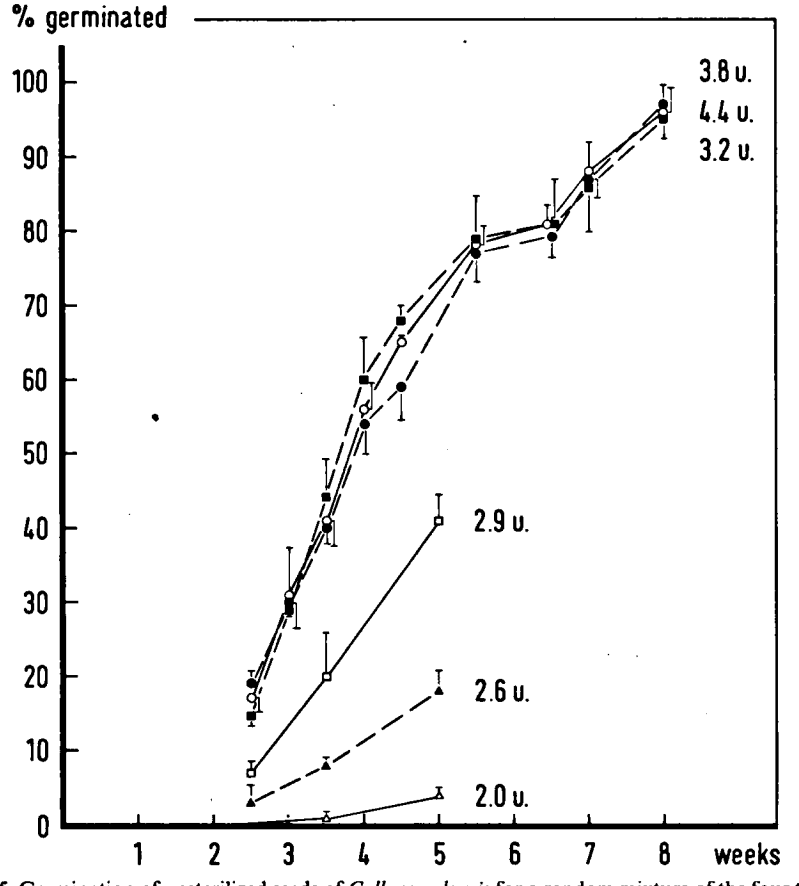

Fig. 5. Germination of unsterilized seeds of *Calluna vulgaris* for a random mixture of the four types at different pH's from 2.0–3.8. Significant at $P < 0.01$ for pH = 2.9–3.2. At pH = 2.9, 2.6, 2.0 all germinated seeds had died after five weeks.

with marl (BENNEKER et al. 1970), can now be explained by our findings that germination of heather seeds is strongly pH-dependent. It may be expected that treatment of sod-cut plots with marl will increase the germination of *Calluna* seeds.

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