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Reproductive behaviour of *Lotus alpinus* (DC) Schleicher

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Variation within *Lotus corniculatus* L.s.l. from Switzerland. II. Reproductive behaviour of *Lotus alpinus* (DC) Schleicher

by

Krystyna URBANSKA-WORYTKIEWICZ, Othmar SCHWANK and Alessandro FOSSATI

Contents

1. Introduction
2. Materials and methods
3. Results
 - 3.1. Breeding behaviour
 - 3.2. Fertility relationships between diploids and tetraploids
 - 3.3. Germinating behaviour
 - 3.4. Seedling establishment in Alpine climatic conditions
 - 3.5. Notes on the vegetative growth and the population structure
4. Discussion
 - Summary - Zusammenfassung
 - References

1. Introduction

This paper is the second in a series dealing with the variation occurring within the group of *Lotus corniculatus* L. from Switzerland. In a previous publication, a preliminary report on chromosome numbers and the cyanogenesis has been presented; it was found that *Lotus alpinus* (DC) Schleicher occurring above the timberline is differentiated into two chromosomal races viz. diploid ($2n=12$) and tetraploid ($2n=24$), the distribution of most-

ly homoploid populations being strongly influenced by edaphic factors as well as the altitude above sea level (URBANSKA-WORYTKIEWICZ and WILDI 1975). Later investigations confirmed this pattern of microdifferentiation (URBANSKA-WORYTKIEWICZ and SCHWANK, in preparation).

Lotus alpinus is polymorphic as far as the cyanogenesis is concerned (URBANSKA-WORYTKIEWICZ and WILDI 1975). The results obtained so far suggest that the respective frequency of cyanogenic and non-cyanogenic individuals within the population might be related, on the one hand, to the type of substrate and on the other hand to the level of polyploidy. Given the fact that the population structure in plants is primarily influenced by the reproduction system(s), a study on the reproductive behaviour of *Lotus alpinus* was undertaken. The present paper deals with the breeding system, fertility relationship occurring between the two chromosomal races as well as their germinating behaviour, notes on the vegetative growth and the population structure being also presented.

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2. Material and methods

Plants used in the breeding experiments as well as the seed material studied for germination originated from natural populations (Table I). The diploids were found only in acidic crystalline substrate whereas the tetraploids occurred in acidic silicate, carbonate and serpentine soils. The material was correspondingly coded, the set Si 12 being used for the diploids, whereas the tetraploids were respectively given the symbols Si 24, Ca 24 and Se 24. This code is referred to the following chapters and shall be applied as well in our forthcoming publications.

The plants chosen for the study on the breeding behaviour were indi-

Table 1. Origin of the material

Coll. No.	Sample code	Station	Altitude a.s.l.
I. Breeding experiments:			
35	Si 12	BE; Lauberhorn	2470 m
51	Si 12	OW; Titlis-Laubergrat	2400 m
84	Si 12	VS; Höchtailligrat	2940 m
142	Si 12	GR; Piz Nair	2700 m
147	Si 12	GR; Piz Lagalb	2450 m
153	Si 12	GR; Fuorcla Sulej	2750 m
II. Germination studies:			
27	Se 24	GR; Totalp	2250 m
128	Si 24	GR; Parpaner Rothorn	2200 m
181	Ca 24	GR; Aroser Weisshorn	2600 m
193	Ca 24	GR; Cassons	2350 m
314	Ca 24	GR; Grünhorn	2450 m
330	Ca 24	GR; Schiahorn	2400 m

ividually potted in a garden soil mixed with the sand (about 3:1). They all were cytologically tested prior to the experimental pollinatins. The cyanogenesis was controlled in the standard picric acid test. The breeding experiments were carried out in 1977-1978.

The seed material was collected in the wild on August 26/27, 1976. Pods were first dry-stored whole at room temperature until December 15, 1976; subsequently, the seeds were taken out from the pods and, save for the stratified series, kept at room temperature until May 2, 1977. The first stratification series comprised 240 seeds corresponding to the four categories i.e. Si 12, Si 24, Ca 24 and Se 24; each of the samples consisted of 60 seeds and was stratified at + 2°C from December 15, 1976 to February 2, 1977. The second series consisted exclusively of Si 22, 225 seeds being stratified from

February 23, 1977 to May 2, 1977.

The main part of observations on the germinating behaviour of *Lotus alpinus* was carried out in two experimental series (Table 2 and 3).

Table 2. Experiment I (2.2.1977-1.5.1977): seeds sown in various substrates, each variant represented by a single Petri dish. Stratified and non-stratified series respectively marked with + and -.

Medium	Number of seeds							
	Sil2+	Sil2-	Si24+	Si24-	Ca24+	Ca24-	Se24+	Se24-
Acidic silicate	30	30	30	30	-	30	-	30
Carbonate	-	30	-	30	30	30	-	30
Serpentine	-	30	-	30	-	30	30	30
Moist blotting paper	30	30	30	30	30	30	30	30

Table 3. Experiment II (2.5.1977-8.6.1977): seeds sown in moist blotting paper. Number of Petri dishes is given in brackets.

Sample	Stratification	Number of seeds
Si 12	+	225 (9)
Si 12	-	150 (6)
Si 24	-	180 (6)
Ca 24	-	300 (6)
Se 24	-	360 (6)

The tetraploid seeds originating from carbonate soils that were later tried for scarification, were stored at room temperature from August 26/27, 1976 to May 2, 1977, then transferred to a refrigerator and kept at approx. + 4°C until January 9, 1979.

Both the breeding experiments as well as the studies on the germinating behaviour of *Lotus alpinus* were carried out in conditioned chambers. The following program was set:

day temperature + 17°C
 night temperature + 10°C
 air humidity 70 %
 light: 16 h.-photoperiod, max. intensity 12500 Lux.

Paralelly to the laboratory experiments, a small field trial was started to chek on the germination and the seedling establishment of *Lotus*

alpinus in Alpine conditions. The pots placed in the experimental plot of the Geobotanical Institute at the Strelapass (2300 m a.s.l.) were respectively filled with acidic silicate, carbonate and serpentine soils. The soils used in the experiments originated from neighbouring regions.

The pods were sown on August 28, 1976 and left over winter (Table 4); the first survey was performed on August 3, 1977 i.e. about eleven weeks after the snowmelt.

Table 4. Field trial at the Strelapass: arrangement of the samples

Soil taken from	pH	Number of the snow seeds			
		Si 12	Si 24	Ca 24	Se 24
Acidic silicate	4.5.	240	160	155	240
Carbonate	7.3	240	160	155	240
Serpentine	7.1	240	160	155	240

The experimental plot at the Strelapass is rather wind-exposed with the snowmelt setting in early in season. Had the vegetation cover not been removed there, the *Caricetum firmae* would have occurred as a representative plant association. The conditions of the garden are rather extreme for *Lotus alpinus* that unusually keeps off strongly wind-exposed stations.

3. Results

3.1. Breeding behaviour

The studies on the breeding behaviour of diploids and tetraploids were carried out in five series of experimental pollinations:

- A. Not emasculated flowers were bagged and left without any interference;
- B. Not emasculated flowers were pollinated with pollen from other flowers of the same individual;
- C. Emasculated flowers were pollinated with pollen from other flowers of the same individual;
- D. Emasculated flowers were pollinated with pollen from another donor

belonging to the same population;

E. Emasculated flowers were pollinated with pollen from a donor representing a different population with the same level of polyploidy.

The pods were collected in about two months; the evaluations of the breeding behaviour was based upon the pod setting rates as well as the seed output observed in the studied material.

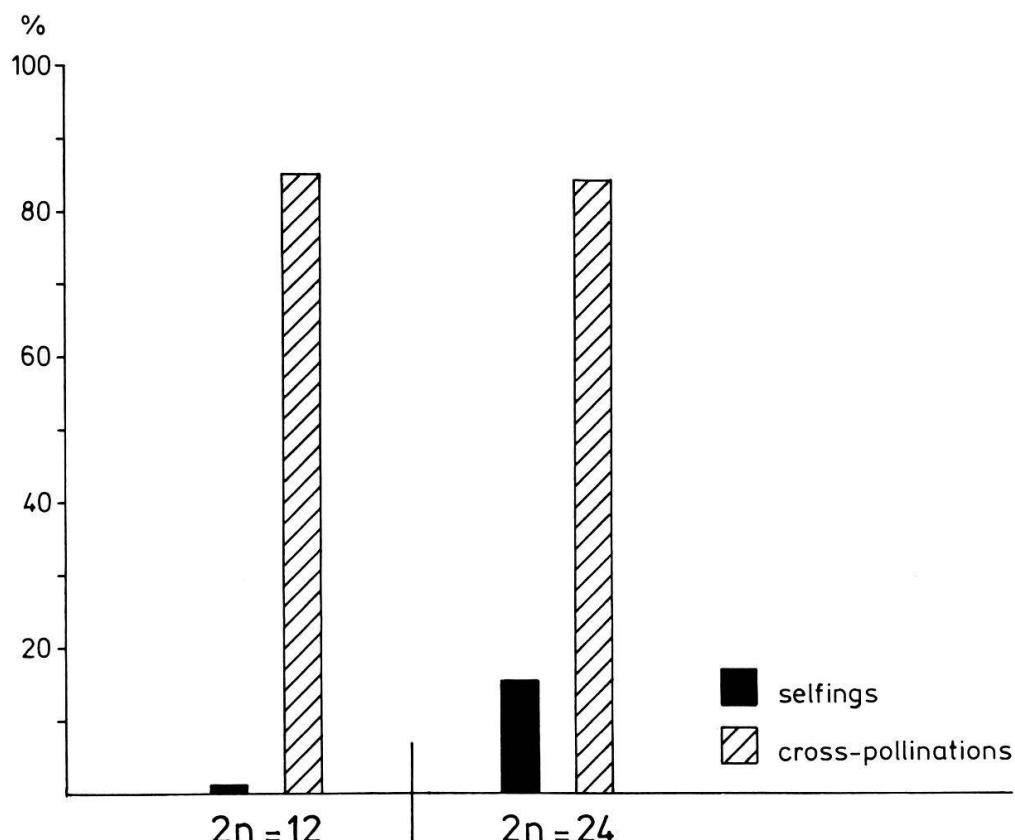


Fig. 1. *L. alpinus*: pod setting in selfings and intrapopulational crosses.

3.1.1. Diploid individuals

The studied diploids proved to be nearly completely self-incompatible (Fig. 1, Table 5); selfings resulted only in four pods containing each a single seed. The cross-pollinations, on the contrary, yielded a reasonably good seed output; however, a rather pronounced individual variation was observed in the number of seeds per pod. The pod setting rates in cross-pollinations were comparably high on the intra- and the interpopulational level,

the respective values being 85.2 and 80.8 per cent. No particular differences were observed in the breeding behaviour of cyanogenic and acyanogenic individuals; the four pods obtained in selfings were all produced by the HCN-negative plants but the material was far too limited to decide whether they were fortuitously developed or might reflect any established pattern of behaviour.

It should be noted that the first-developed flowers responded better to experimental pollinations and apparently were most resistant to the mildew than those appearing later; it would be advisable to study this aspect of behaviour in natural populations of *L. alpinus*.

Table 5. Diploid *L. alpinus* (2n=12); pod setting and seed output in selfings and cross-pollinations

Collection No.	HCN	Pollinations		- pod setting - seed output	
		A	B	C	D
35/3	+	5-0-0	6-0-0	6-0-0	6-5-19
35/5	-	6-0-0	6-0-0	6-0-0	6-6-14
35/8	-	5-0-0	5-0-0	5-0-0	5-3-12
35/9	+	5-0-0	5-0-0	5-0-0	5-5-21
51/2	+	6-0-0	5-0-0	5-0-0	5-4-18
51/3	+	6-0-0	5-0-0	5-0-0	7-5-23
51/5	-	5-0-0	5-1-1	5-0-0	5-5-17
51/7	+	5-0-0	5-0-0	5-0-0	5-5-18
51/8	+	5-0-0	5-0-0	5-0-0	5-4-19
84/1	+	5-0-0	5-0-0	5-0-0	7-7-11
84/2	+	5-0-0	5-0-0	5-0-0	5-5-18
84/3	-	5-0-0	5-1-1	5-0-0	5-5-21
84/5	+	5-0-0	5-0-0	5-0-0	5-4-14
84/9	+	5-0-0	5-0-0	5-0-0	5-5-22
142/1	-	5-0-0	5-1-1	6-0-0	6-5-25
142/8	-	5-0-0	5-0-0	5-0-0	5-3- 9
142/10	-	8-0-0	5-0-0	5-0-0	5-4-16
142/15	-	5-0-0	5-0-0	5-0-0	5-5-19
147/2	+	5-0-0	5-0-0	5-0-0	5-3-11
147/3	+	5-0-0	5-0-0	5-0-0	5-4- 7
147/4	+	5-0-0	5-0-0	5-0-0	5-5-18
153/1	-	6-0-0	7-1-1	7-0-0	8-6-17
153/6	-	5-0-0	5-0-0	3-0-0	7-4-22
153/7	-	8-0-0	6-0-0	6-0-0	9-5-18
153/8	-	7-0-0	5-1-1	5-0-0	5-4-11
					5-4-21

Note. A, B, C, D refer to various pollination series (see p. 66); the first cipher in each triplet corresponds to pollinations, the second - to the number of pods, the third - to the number of seeds.

3.1.2. *Tetraploid individuals*

The tetraploid plants studied in the course of the present study were predominantly allogamous; some individuals, however, proved to be partly self-compatible, the seeds developing both in open as well as forced selfings. Interestingly enough, all the seeds obtained from selfings were produced by acyanogenic individuals (Fig. 1, Table 6).

Table 6. Tetraploid *L. alpinus* (2n=24); pod setting and seed output in selfings and cross-pollinations

Collection No.	HCN	Pollinations		pod setting		seed output	
		A	B	C	D	E	
27/1	-	5-0- 0	5-0- 0	5-0- 0	5-5-30	5-3-19	
27/3	-	5-0- 0	5-2-12	5-1- 6	5-5-36	5-4-16	
27/4	-	5-0- 0	5-0- 0	5-0- 0	5-4-24	5-3- 6	
27/5	-	5-1- 4	5-4-11	5-3- 8	5-3-17	5-4-19	
27/6	-	5-0- 0	5-0- 0	5-0- 0	5-5-34	5-5-28	
128/1	-	5-0- 0	5-0- 0	5-0- 0	5-4-26	5-2- 7	
128/2	-	6-0- 0	5-0- 0	5-0- 0	5-5-31	5-5-21	
128/4	-	6-4-14	5-4- 9	5-3-15	5-5-22	5-4-18	
181/1	-	5-0- 0	5-0- 0	5-0- 0	5-4-32	5-1- 4	
181/2	-	5-0- 0	5-0- 0	5-0- 0	5-5-38	5-4-26	
181/3	+	5-0- 0	5-0- 0	5-0- 0	5-1- 5	5-5-24	
181/4	-	5-0- 0	5-0- 0	5-0- 0	5-3-39	5-4-16	
181/5	-	5-3- 8	5-4-14	5-4- 9	5-4-21	5-4-19	
193/2	+	5-0- 0	5-0- 0	5-0- 0	5-5- 4	5-5-32	
193/4	+	5-0- 0	5-0- 0	5-0- 0	5-5-19	5-4-27	
193/5	+	5-0- 0	5-0- 0	5-0- 0	5-3-18	5-4-11	
193/6	-	5-2- 7	5-4-12	5-1- 0	5-4-23	5-4-17	
314/5	-	5-0- 0	5-0- 0	5-0- 0	5-3-16	5-3-17	
314/8	-	5-0- 0	5-0- 0	5-0- 0	5-5-34	5-3- 8	
314/9	-	5-3- 9	5-2- 6	5-2- 5	5-5-41	5-4-14	
314/10	-	6-0- 0	5-0- 0	5-0- 0	5-5-24	5-4-15	
330/2	-	5-0- 0	5-0- 0	5-0- 0	5-5-17	5-5-14	
330/3	-	5-0- 0	5-0- 0	5-0- 0	5-4-28	5-1- 3	
330/4	-	5-2- 9	5-3-13	5-3-18	5-4-12	5-4-19	
330/5	-	5-0- 0	5-0- 0	5-0- 0	5-5-29	5-5-21	

The pod setting rates reached 15.1 % in selfings, whereas those observed in intra- and interpopulational crosses represented 84.8 and 75.2 per cent, respectively. It is interesting to note that the number of seeds obtained in intrapopulational crosses was much higher than that developing in crosses between various populations, a comparable trend being also found in

the diploids (Table 7). It can be supposed that the observed differences were partly influenced by an individual variation in number of seeds per pod and, on the other hand, probably related to varying degrees of compatibility occurring between different populations.

Table 7. *L. alpinus*; number of seeds obtained in selfings and cross-pollinations.

Chromosome number	Total	Selfings	Cross-pollinations	
			Intrapopulational	Interpopulational
2n=12	724	4 0.5 %	440 60.8 %	280 38.7 %
2n=24	1230	189 15.4 %	620 50.4 %	421 34.2 %

Thus, *Lotus alpinus* can be characterized as a predominantly outbreeding taxon with the self-incompatibility mechanisms operating mainly at pre-mating stages. Self-compatible individuals tend to occur more frequently in tetraploids than in diploids and it cannot be excluded that a partial self-compatibility is in some way related to non-cyanogenic individuals. However, further studies are indispensable to get a more precise information in the latter subject.

3.2. Fertility relationships between diploids and tetraploids

The fertility relationships occurring between the two chromosomal races of *L. alpinus* were studied in reciprocal crosses (Table 8); the observations on the pod setting and the seed production were followed by a germination test carried out during eleven months.

The obtained results suggest complex incompatibility barriers preventing a gene exchange between diploids and tetraploids. In most cases the pollinated flowers did not develop at all; sometimes, the pods developed yet were empty; numerous seeds were mishaped and/or empty; last but not least, the seeds have not germinated becoming completely rotten by the end of the germination test.

The unsuccessful experimental crosses between diploids and tetra-

Table 8. *L. alpinus*; experimental crosses between diploids and tetraploids. Female parents listed first; number of sound-looking seeds given in brackets.

Cross type	Number of pol- linated flowers	Number of pods	Number of seeds	Germination
2n=12 x 2n=24	200	17 7.5 %	41 (18)	nil
2n=24 x 2n=12	200	3 1.5 %	9 (9)	nil

ploids corroborate the authors' previous observations in the wild (URBANSKA-WORYTKIEWICZ and WILDI 1975, SCHWANK 1977), no triploid individuals having been found within natural populations. It seems therefore that the incompatibility plays a main role on the control of a gene exchange between diploid and tetraploid populations of *Lotus alpinus*; the very narrow contact zone of the two chromosomal races may accordingly be considered as a subsidiary factor.

3.3. Germinating behaviour

3.3.1. Global germination rates

For a general evaluation of the germination behaviour of *Lotus alpinus*, data from both laboratory experiments (see p. 72) were pooled. As far as the diploids are concerned, only the seeds sown in a moist blotting paper were taken into consideration, the material sown in various substrates being insufficient for a comparison. On the other hand, all the tetraploid series were evaluated together, for no relevant differences occurred between samples sown in various substrates. The authors are aware of the fact that this somewhat arbitrary grouping might to some extent influence a detailed interpretation of the results; for this reason, only preliminary remarks are presented. Our investigations do, however, reveal some patterns in the germinating behaviour of *Lotus alpinus* that not only permit to distinguish between diploids and tetraploids but also suggest a racial differentiation occurring on the tetraploid level.

On the whole, the germination rates in *L. alpinus* were low, not exceeding 30 %. The germination in diploids began later and proceeded much

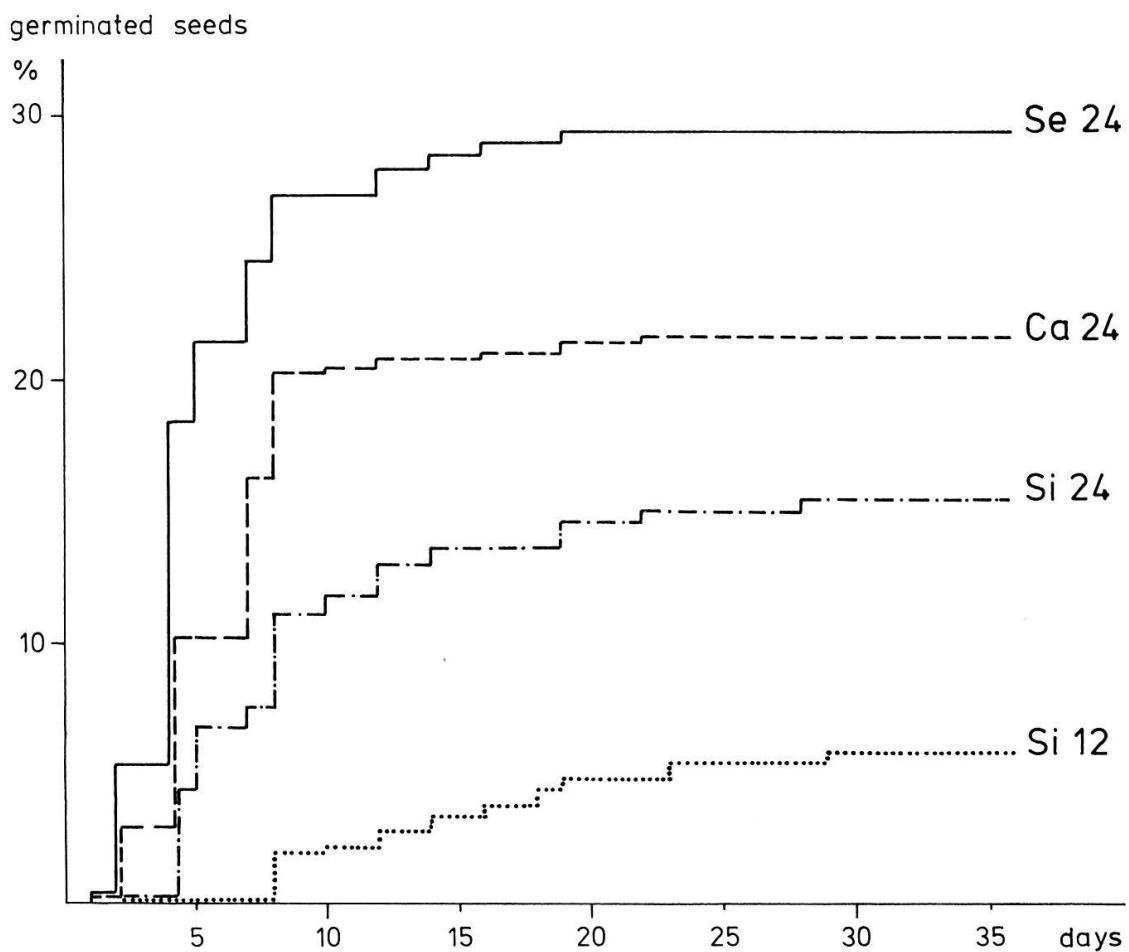


Fig. 2. Global germination rates observed in *Lotus alpinus*.

slower than in tetraploids. The global germination rates found in the 12-chromosomal material were twice to four times lower than those found in tetraploids (Table 9, Fig. 2); the differences proved to be significant in the Chi square test:

$$\text{Chi}^2 = 21.00; \text{Chi}_+^2 (\alpha = 0.001, \text{Df } 1) = 10.8$$

It is very interesting to note that the differences occurring between tetraploids originating from various substrates in the wild were also statistically significant, the following pattern being found:

$$\begin{aligned} \text{Si 24 : Ca 24 : Chi}^2 &= 3.98; \text{Chi}_+^2 (\alpha = 0.05, \text{Df } 1) = 3.841 \\ \text{Ca 24 : Se 24 : Chi}^2 &= 4.69; \text{Chi}_+^2 (\alpha = 0.05, \text{Df } 1) = 3.841 \end{aligned}$$

Table 9. Global germination rates in *Lotus alpinus* calculated for the 35th day of the experiment.

Sample	Number of seeds	Number of germinated seeds	Germination rate %
Si 12	555	33	5.9
Si 24	360	55	15.3
Ca 24	480	105	21.8
Se 24	540	160	29.6

3.3.2. Stratification experiments

The stratification at + 2°C had a diversified effect upon the germinating behaviour of diploids and tetraploids. In the diploid samples, the influence of the stratification was rather pronounced (Fig. 3): non-stratified seeds began to germinate soon after sowing but only a minute percentage of germinated seeds was observed, the reached maximum of 2 % remaining unchanged from the 14th day on; by contrast, the stratified seeds began to germinate a few days later than the control series, but the germination proceeded regularly reaching by the 35th day a maximum of 10 %. The differences occurring between stratified and non-stratified material were statistically significant in the Chi square test:

$$\text{Chi}^2 = 19.6; \text{Chi}^2_+ (\alpha = 0.001 \text{ DF } 1) = 10.8$$

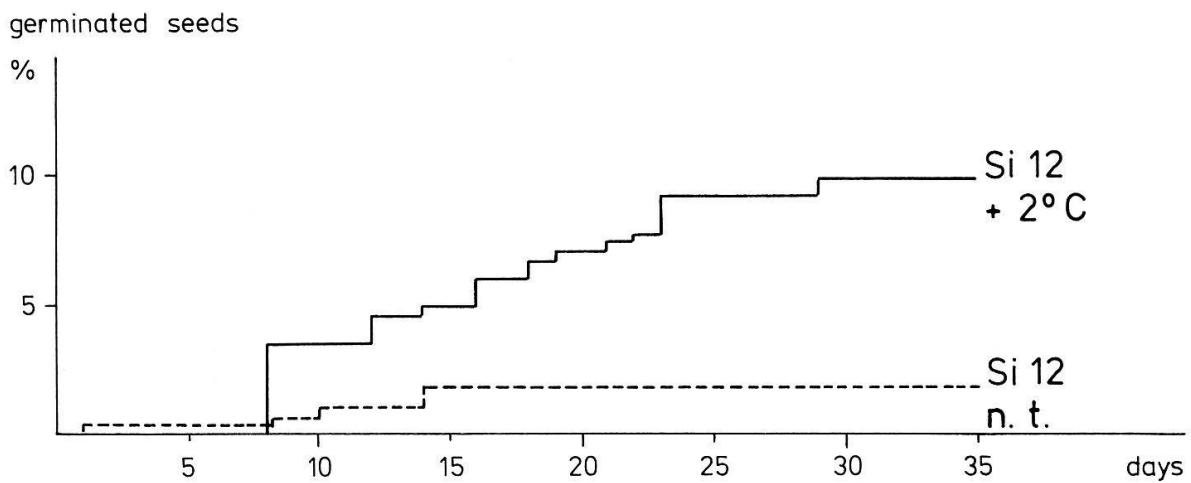
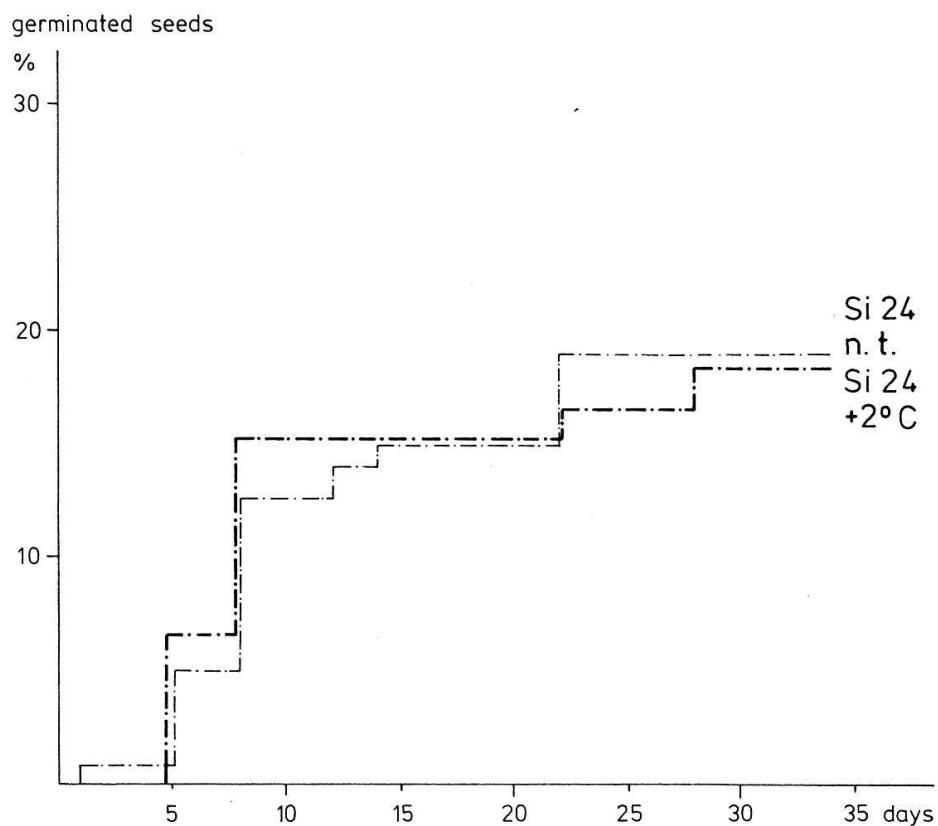
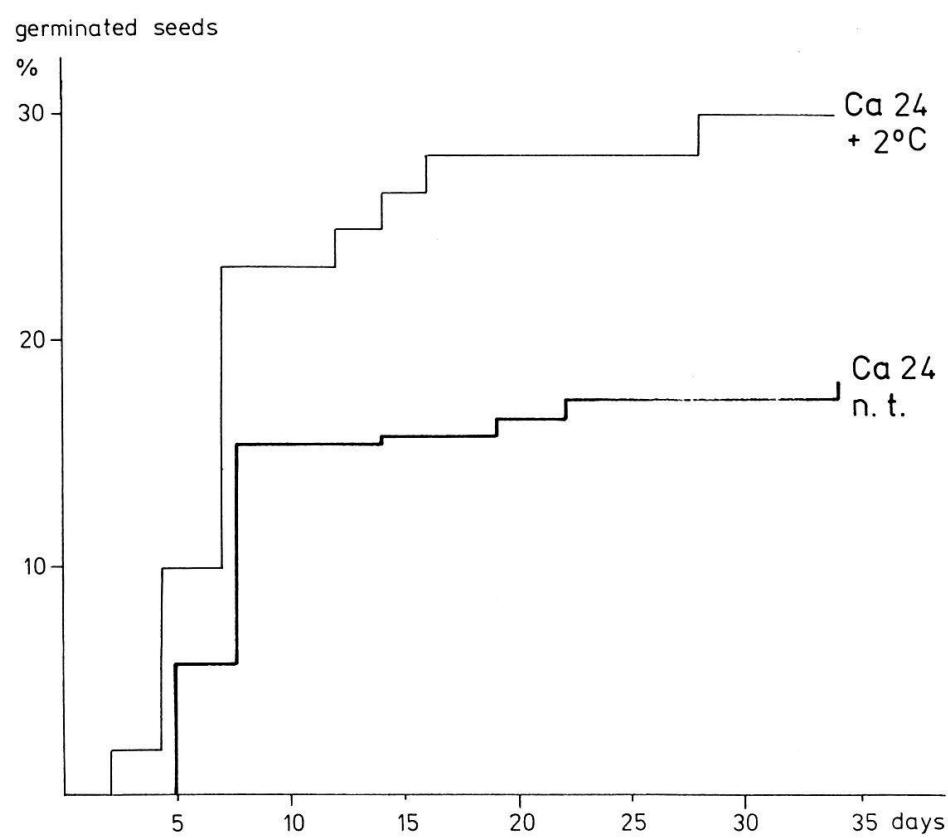
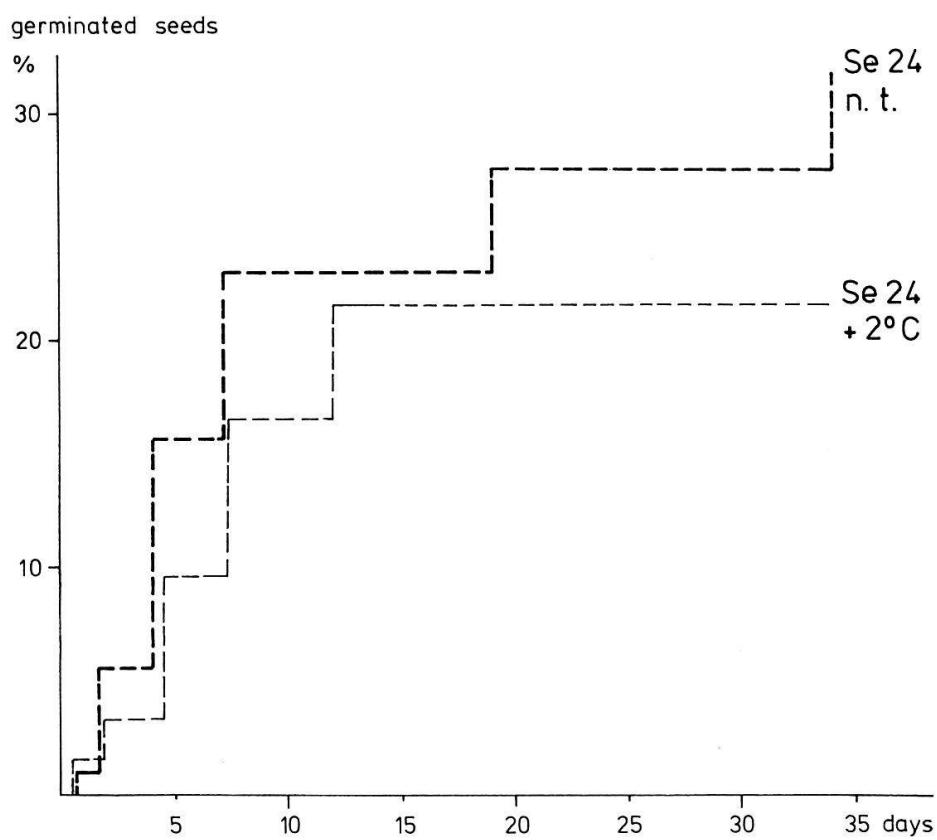


Fig. 3. Influence of the stratification upon the germination of diploid *Lotus alpinus* ($2n=12$) from acidic silicate soil. (n.t. = no treatment).

As far as the tetraploids are concerned, the response to the stratification was different in each of the three samples originating from various substrates. Differences between stratified and non-stratified samples from acidic silicate soil were virtually none, the germination rate being approximately 20 % in either series (Fig. 4). In the material from serpentine soil, non-treated seed germinated even better than stratified ones, the germination rates being 32 vs 22 per cent, respectively (Fig. 5). On the other hand, the samples from carbonate soils responded rather favourably to the stratification, reaching above 30 % of germinated seeds whereas the maximal rate ob-



Figs 4-6. Tetraploid *Lotus alpinus* ($2n=24$): germination in stratified and non stratified series from three different substrates.
 4. Acidic silicate. 5. Serpentine (see opposite page).
 6. Carbonate (see opposite page). (n.t. = no treatment).



served in the non-treated series was less than 20 % (Fig. 6). The tetraploid material was not evaluated statistically, for the amount of seeds studied in particular was too small.

3.3.3. Scarification experiments

Preliminary studies in influence of scarification upon the germinating were carried out so far only in tetraploid samples from a carbonate soil; the very promising results deserve nevertheless a special mention.

The scarification was performed with a razor blade, two alternative methods viz. excision of the hilum or dorsal incision in the seed coat being applied (Fig. 7).

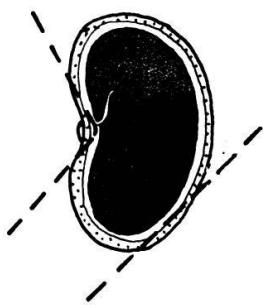


Fig. 7. Alternative scarification methods used in *Lotus alpinus*: a. Excision of the hilum. b. Dorsal incision in the seed coat.

As in numerous other *Leguminosae*, hard seeds of *Lotus alpinus* responded well to scarification. The excision of the hilum resulted in a more than 20 % improved germination that proceeded rather regularly. The optimal treatment, however, proved to be a dorsal incision in the seed coat; the material scarified in this way germinated in 100 % within only seven days after sowing (Fig. 8). The differential response to scarification suggests that the factors inhibiting the germination are precisely localized within the seed coat. Further studies on the influence of scarification upon the germination of *Lotus alpinus* are continued.

The scarification experiments were carried out in a two-and-a-half-year-old sample Ca 24 which offered a good opportunity to observe the effect

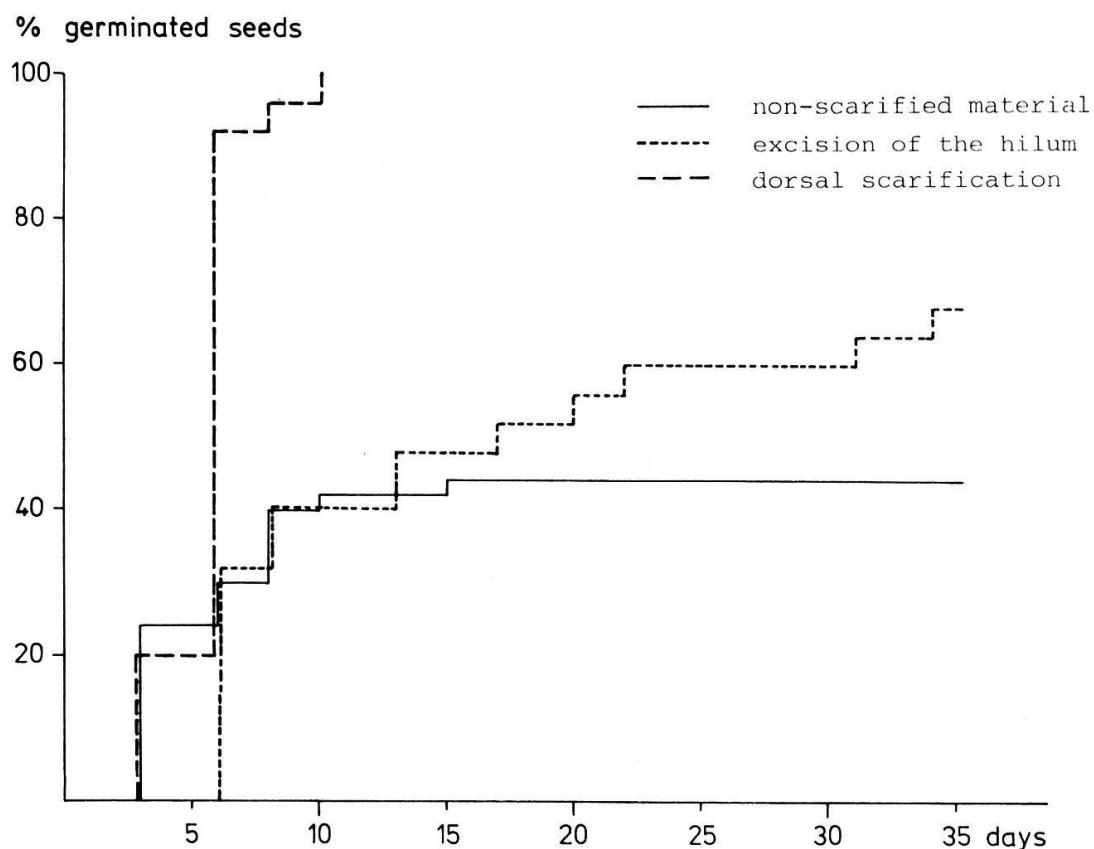


Fig. 8. *Lotus alpinus* (2n=24): influence of scarification upon the germinating behaviour.

of ageing upon the germinating behaviour. It was found that the long-stored material of *Lotus alpinus* germinated much better than the freshly harvested one (40 vs. \approx 17 percent) and also showed improved germination rates as compared to the stratified series that originated from the same station but were younger (Fig. 8, see also Fig. 6). Only the material from a carbonate soil has been studied so far, but a comparative study on seed longevity in plants from other substrates should be most desirable.

3.4. Seedling establishment in Alpine climatic conditions

Out of the numerous seeds sown at the Strelapass, only a limited number of seedlings was found in the first year after sowing (Table 10, Fig. 9).

The slow establishment observed at the Strelapass corroborates the results obtained in the study on the germinating behaviour of *Lotus alpinus*

Table 10. The 1977 survey at the Strelapass: number of established seedlings.

Sample code	Number of seedlings *		
	Acidic silicate	Carbonate	Serpentine
Si 12	1 (240)	1 (240)	0 (240)
Si 24	2 (160)	2 (160)	0 (160)
Ca 24	1 (155)	2 (155)	1 (155)
Se 24	2 (240)	11 (240)	2 (240)

* number of sown seeds is given in brackets

and further suggests that the seed dormancy may offer different aspects in either of the two chromosomal races of this taxon. As far as the behaviour of particular tetraploid samples is concerned, two features should be noted: 1/ the pattern was very similar in the material from acidic silicate and that from carbonate; 2/ the performance of the material from serpentine was positively superior to that of two other samples (Table 10, Fig. 9).

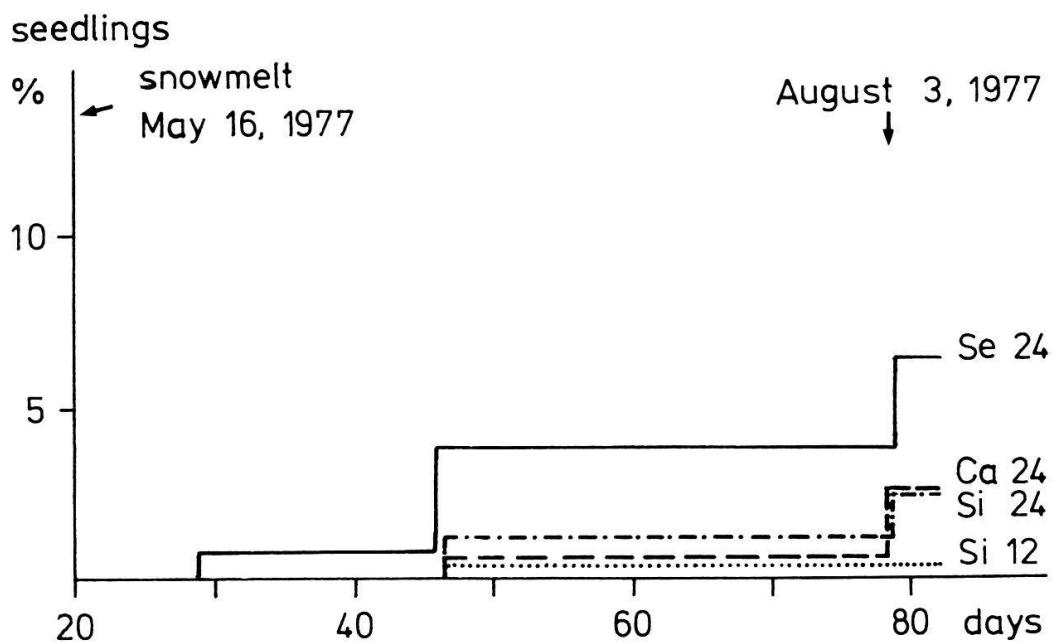


Fig. 9. Seedling establishment in the Alpine garden at Strelapass.

3.5. Notes on the vegetative growth and population structure

The vegetative growth in *Lotus alpinus* usually results in the formation of a firmly rooted rosette. The main root is long, thick and lignified in older plants; numerous secondary roots, in contrast, are for the most part thin and not deep-reaching into the soil.

The clonal development proceeds by means of shallow rhizomes producing secondary shoots in some distance from the main rosette; the whole clone usually remains interconnected. The clone formation in *L. alpinus* seems to be strongly density-dependent: plants occurring in open sites most frequently form large compact rosettes with none or only a few secondary shoots, whereas individuals inhabiting grassy slopes tend to develop clones of a complicated structure. Numerous slender shoots intermingle with other species within a given surface which renders difficult the identification of particular clones. Culture experiments carried out in uniform conditions suggest that some differences in growing habit are genetically fixed and indicate a racial differentiation influenced by the type of substrate occurring on the tetraploid level (URBANSKA-WORYTKIEWICZ and SCHWANK, in press; SCHWANK 1977).

The size of population in *Lotus alpinus* greatly varies both in diploids as well as in tetraploids. Particular populations seem to be rather stationary in number, but they may represent any variant between an extremely small population (2-5 individuals) and a large continuous one (180 and more plants). As far as the age-specific population structure is concerned, all the studied surfaces invariably represented the same pattern: reproducing adults and/or sterile rosettes were main components, seedlings being remarkably rare or absent; it should be noted, however, that the present remarks are based on field observations carried out mostly in the middle of the vegetation season and have to be completed by the surveys performed soon after the snowmelt.

4. Discussion

The reproductive strategy of *Lotus alpinus* comprises several variable components. Let us consider some genetical and ecological factors that may influence this variation.

In some plant species, mixed selfing and outcrossing may be balanced by the reproductive behaviour of different genotypes. In *Vicia faba*, for instance, the products of selfings tend to be outbreeders and vice versa (DRAYNER 1956, 1959, HOLDEN and BOND 1960). *Borago officinalis* represents a similar case, the homozygotes being obligate outbreeders whereas the heterozygotes are self-compatible (CROWE 1971). On the other hand, the breeding system in numerous Angiosperms proved to be controlled by environmental factors that may change the level of outcrossing or even suppress totally the sexual reproduction. This influence is well-exemplified by *Lithospermum carolinense* where outcrossing rates vary from less than 60 % to 100 % according to the density-dependent production of cleistogamic flowers (LEVIN 1972); in *Cardamine amara*, the successfull outbreeding is determined by the population structure and in particular by the density of self-incompatible clones (URBANSKA-WORYTKIEWICZ, in press). The variation in the breeding behaviour may as well be seasonal e.g. In *Myosurus sessilis* where the self-pollination occurs during the first part of the year and outcrossing later in season (STONE 1959).

As far as *Lotus alpinus* is concerned, the varying level of outcrossing seems to be related in the first place to particular genotypes. The results obtained in the course of the present study strongly suggest an individual variation in number of seeds per pod. BUBAR (1958) has shown that there was a considerable variability in the rate of the ovule development in self-sterile species of *Lotus*, whereas in autogamous species the ovules matured simultaneously; it is conceivable that a comparable phenomenon might directly influence the seed output in *L. alpinus*, partly self-compatible individuals having more receptive ovules at a given time than self-incompatible ones.

The differential fecundity as a fitness measure is of a particular interest in *L. alpinus*, especially as far as the tetraploids are concerned, for the seed output invariably was the best in acyanogenic individuals. A comparable trend was previously found in wild populations of *L. corniculatus* by JONES (1962). Similarly, FOULDS and GRIME (1972) observed in *Trifolium repens* cultivated under stress conditions that both a general performance as well as the sexual reproductive vigour in acyanogenic plants was superior to that found in cyanogenic ones; the British authors supposed that the observed differences might result either from a pleiotropic effect on genes responsible for the cyanogenesis or their linkage to the genes which influence the sexual reproduction. Whatever the mechanism operating in *L. alpinus* may be, the differential fecundity in this taxon positively deserves detailed investigation on the population level.

The germinating behaviour represents a further important feature in the reproductive strategy of *L. alpinus*. The rather complex pattern of variation observed in this respect seems to be influenced both by genetical as well as ecological factors. The seed dormancy apparently has a general importance for *L. alpinus* occurring above the timberline; however, the statistically significant differences observed not only between diploids and tetraploids but also between tetraploids originating from different substrates suggest an evolutionary divergence. The seed dormancy occurring in *L. alpinus* is apparently due to inhibitors localized in the seed coat; it might reflect an opportunistic adaptation to specific niche conditions where a repeated freeze-thaw or an abrasive scarification with soil particles might trigger off the germination. It is very interesting to note that a precise scarification dramatically improved the germination in tetraploid *L. alpinus* originating from carbonate soils, for this factor might decide which seeds germinate in the wild and which do not. As elegantly demonstrated by HARPER and BENTON (1966), even in a controlled homogenous environment the number of seeds recruited into a germination population is determined not only by individual properties of each seed, but also by its precise position, contact with substrate and so on. Further studies on the germinating behaviour of *L. alpinus* are very promising.

The seedling establishment in *L. alpinus* is obviously influenced by extreme conditions occurring within the alpine zone. It is possible that

temporary soil draughts as well as the needle ice activity, generally considered as important factors operating above the timberline (BLISS 1971) might play a rôle in the rejuvenation of some populations in *L. alpinus*. On the other hand, the limited number of seedlings observed both in the field trial as well as in the wild might partly result from the selection eliminating unfit genotypes at the very first life stage.

An apparently undiminished viability of two-and-a-half-year-old seeds of *L. alpinus* indicates that the seed bank in alpine populations is based upon contributions made over several years. As recently pointed out by LEVIN (1978), the seed pool allows a covariance between fitness and absolute seed production; a differential contribution to the seed bank may accordingly have the same consequences as the directional selection. At this point, we are brought back to the intriguing problem of the cyanogenesis in *Lotus alpinus*.

The acyanogenic, self-compatible tetraploids yielded the highest seed output in experimental pollinations and it may be supposed that they largely contribute to the seed bank in the wild, numerous seeds being carriers of acyanogenic genotypes. However, the cyanogenesis within the *Lotus corniculatus* group is controlled by dominant genes and the tetrasomic inheritance (DAWSON 1941); a certain balance in proportion of acynogenic/cyanogenic genotypes might thus be expected in the seed pool. Curiously enough, the pattern of distribution of adult cyanogenic/acyanogenic individuals within the populations of *Lotus alpinus* is not uniform, but apparently influenced by the type of substrate as well as the altitude above sea level (URBANSKA-WORYTKIEWICZ and WILDI 1975; URBANSKA-WORYTKIEWICZ and SCHWANK, in press; URBANSKA-WORYTKIEWICZ and SCHWANK, in preparation). A rather distinct relation between cyanogenesis and altitude was previously found in *Trifolium repens* by DADAY (1954). It should be noted, however, that the few samples studied by the British author mostly originated from the colline-subalpine zone and the substrate remained unspecified. Still more important, the material of DADAY was obtained from wild seeds in an experimental greenhouse; the observed variation was therefore potential rather than actual, the frequency of adult plants in natural populations being undoubtedly influenced by the stabilizing selection. A detailed population study on *Trifolium repens* from various substrates and higher altitudes is most advisable.

In conclusion, an interesting coincidence occurring between the origin and the behaviour of the studied seeds in non-treated material should be mentioned. The sample Se 24 originating from a uniformly acyanogenic population manifested the best germination, the seedling establishment in the field trial being also the best (Table 10, Fig. 9). On the other hand, the samples Si 24 and Ca 24 both originating from mixed populations consisting of cyanogenic and acyanogenic plants; germinating rates as well as the seedling establishment were approximately the same in both samples, their performance being inferior to that of the material from serpentine soils. It is obviously too early to decide whether the observed tendencies are fortuitous or the reproductive strategy in *Lotus alpinus* might be expressed by different tactics in cyanogenic individuals. The present study does, however, indicate that the reproductive behaviour represents a very important aspect in the problem of substrate-influenced microdifferentiation occurring in the alpine populations of this taxon. Further investigations are in progress.

Summary

Lotus alpinus ($2n=12, 24$) proved to be predominantly allogamous, the varying level of outcrossing being probably influenced by an individual variation in seed number per pod as well as the degree of inter-compatibility between particular populations. All the partly self-fertile individuals were acyanogenic. It is supposed that the differential fecundity in *L. alpinus* is related to particular genotypes. Strong incompatibility barriers block the gene exchange between the two chromosomal races.

The germinating behaviour and the seedling establishment in *L. alpinus* are apparently influenced both by the degree of polyploidy as well as the type of substrate. The highest germination rates occurred in tetraploids from serpentine (Se 24); the lowest rates were found in diploids (Si 12), whereas the samples Si 24 and Ca 24 represented intermediary values. The same trend appeared in the limited seedling establishment at the Strelapass (2300 m a.s.l.). Responses to stratification were also differentiated. The mechanical scarification improved and accelerated the germination, in particular when precisely applied (dorsal incision). Two-and-a-half-year-old seeds showed an undiminished viability in germination trials.

Wild populations of *L. alpinus* mostly consisted of adults, seedlings being exceedingly rare or absent in the middle of the season. The clonal growth proceeding by means of shallow rhizomes apparently was density-dependent.

In conclusion, possible relations between cyanogenesis and reproductive behaviour of *L. alpinus* are briefly considered.

Zusammenfassung

Lotus alpinus ($2n=12, 24$) erwies sich als vorwiegend allogam, wobei der Prozentsatz des durch Fremdbestäubung hervorgerufenen Samenansatzes wahrscheinlich durch die individuelle Variation in der Samenzahl pro Hülse sowie durch den Grad der Interkompatibilität zwischen den verschiedenen Populationen beeinflusst wird. Alle der teilweise selbstfertilen Individuen waren acyanogen. Es wird vermutet, dass die unterschiedliche Fruchtbarkeit bei *Lotus alpinus* mit verschiedenen Genotypen zusammenhängt. Der Genaustausch zwischen den zwei Chromosomenrassen wird durch starke Unverträglichkeitsbarrieren verhindert.

Das Keimverhalten und das Aufkommen der Keimlinge bei *L. alpinus* wird offenbar durch den Grad der Polyploidie und durch die Gesteinsunterlage bestimmt. Die höchsten Keimraten wurden bei den Tetraploiden vom Serpentin (se 24) beobachtet, die niedrigsten bei den Diploiden (Si 12); Proben von Tetraploiden auf Silikat (Si 24) und Karbonat (Ca 24) verhielten sich intermediär. Aehnliche Ergebnisse wurden für die beschränkte Entwicklung der Keimlinge auf dem Strelapass (2300 m ü.M.) erhalten. Stratifikationsexperimente zeigten ebenfalls unterschiedliche Resultate. Die Skarifikation verbesserte und beschleunigte die Keimung, besonders wenn der Eingriff am Rücken des Samens erfolgte. 2 1/2-jährige Samen zeigten in Keimtesten eine unveränderte Lebensfähigkeit.

Natürliche Populationen von *L. alpinus* setzen sich hauptsächlich aus ausgewachsenen Individuen zusammen; Keimlinge waren in der Mitte der Vegetationsperiode ausserordentlich selten oder fehlten. Die vegetative Ausbreitung durch oberflächliche Rhizome scheint von der Dichte der Vegetation abzuhängen.

Zu Abschluss werden kurz mögliche Beziehungen zwischen der Cyanogenese und dem Fortpflanzungsverhalten von *L. alpinus* diskutiert.

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