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COMPARATIVE GERMINATION ECOLOGY OF THREE SHORT-LIVED MONOCARPIC BORAGINACEAE*

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SUMMARY

Germination of seeds and seed bank dynamics of *Cynoglossum officinale, Echium vulgare* and *Anchusa arvensis* were studied under natural conditions. The effects of constant and alternating temperature, soil moisture and light were studied in controlled environments. Additionally, for *Cynoglossum officinale* moist-chilling requirements and the effect of depth of sowing on germination were investigated. The various ways in which *Cynoglossum officinale, Echium vulgare* and *Anchusa arvensis* live with mortality-risks related to germination and survival of seeds, are discussed.

In the field, *Cynoglossum officinale* germinates from February till May, *Echium vulgare* from March till November and *Anchusa arvensis* from March till July.

These three species have a small persistent seed bank. *Cynoglossum officinale* seeds can persist on the soil surface and on the infructescences, the latter seed bank may last for two years. At 2 and 15 cm depth buried seeds germinated within one year. After three years at 2 cm depth 85% of the *Echium vulgare* seeds and *c.* 40% of the *Anchusa arvensis* seeds had germinated. At 15 cm depth these percentages were *c.* 25% and *c.* 5%, respectively.

The maximum number of *Cynoglossum officinale* seeds germinated at 0–10 °C 12D/12L and 12% soil moisture. The light response proved to be different for seeds from different populations. A six weeks moist-chilling period can break dormancy. The highest germination percentage was found for a depth of sowing of 1 cm. *Echium vulgare* germinated quickly under a wide variety of temperature and soil-moisture conditions. The highest germination percentage was found at 20–30 °C 12D/12L and 6–12% soil moisture. The seeds germinated in darkness just as well as in light. The maximum number of *Anchusa arvensis* seeds germinated at 15–25 °C 12D/12L and 6% soil moisture. The light response proved to be different for seeds from different populations.

A classification of the species could be made, though not strictly, in a species with such characteristics that the mortality risk is reduced in space and time (*Cynoglossum officinale*) and species with characteristics through which the risk is reduced in space and spread in time (*Echium vulgare* and *Anchusa arvensis*).

1. INTRODUCTION

A very risky period in a plant's life is the period of germination and establishment. In comparison with perennials short-lived plants relatively often envisage this risky period. However, plants have various characteristics through which they can live with the mortality-risks related to germination and establishment. A phenomenon shown by most 'biennials' is the possibility to delay flowering and seed production by becoming a short-lived monocarpic perennial. In this

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way, and by genetic variability, the risk is spread over different individuals within one population. Within one individual the risks may be spread by spreading of flowering and seed production in time, either in one year (indeterminate monocarpic species) or over different years (polycarpic species). Besides this, plants show a continuum of strategies connected with germination and survival of seeds ranging from:

a) spreading the risk over the seeds by making many small seeds, and
b) reducing the risk per seed by making few large seeds.

In category (a) the risk can be spread in space or in time, either realized by long-distance dispersal, or by a long-lived seed bank and/or spreading of germination in time, respectively. Species with such characteristics that the risks are spread in space will generally occur in short-lived habitats that are unpredictable in space, whereas species with characteristics through which the risks are spread in time will be found in short-lived habitats that are unpredictable in time.

In category (b) the risk can be reduced in space and time too. In space the risk is reduced by short-distance dispersal resulting in germination close to the parent plant. This will be met with in long-lived habitats, predictable in space. But also a long-distance dispersal can be risk-reducing, viz. when combined with particular behaviour of the dispersing vector which guarantees a safe site for germination of the dispersed seeds. The risk is reduced in time when the species has a well-timed germination which occurs only in a period with minimal mortality risk.

On the other hand, a very-short-lived seed bank may be a mechanism to reduce the risks of predation or loss of viability. Those species with such characteristics that the risk is reduced in time will occur in habitats predictable in time. The predictability of the habitat is an important factor determining which life-cycle strategy is favoured.

Especially biennial plants are well-adapted to colonization of open, disturbed habitats which are intermittently available (HARPER 1977; HART 1977; WERNER 1977; GRIME 1979; VAN DER MEIJDEN & VAN DER WAALS-KOOI 1979; GROSS 1980).

The present study concerns the strategies connected with germination and survival of seeds of three short-lived species *Cynoglossum officinale* L., *Echium vulgare* L. and *Anchusa arvensis* (L.) Bieb., all Boraginaceae.

To investigate whether mortality risks are spread or reduced in time, natural germination and survival of seeds in the soil were studied in time. Information about the latter was obtained by sampling the natural seed bank as well as an artificial seed bank protected from predation. Because light, temperature, temperature-fluctuations and soil-moisture are known to play an important role in seed germination and thus in the process of building up a seed bank, the effects of those factors on germination were studied. Also germination under controlled conditions gave information about the species having a well-timed germination or a germination which is spread in time.

Under controlled conditions *Cynoglossum officinale* seeds are known to germinate slowly without pretreatment (FREIJSEN 1975; FREIJSEN et al. 1980; LHOTS-

KÁ 1982). On the contrary natural germination mainly occurs in a short period in March. Because the winter period may be causing this quick, well-timed germination, the effect of moist-chilling was studied.

An attempt is made, for this group of short-lived plants, to discuss the interrelationship of germination characteristics, longevity of seeds in the soil, size of seeds, seed production, dispersal, lifespan, survival time of plant populations and habitat. How the species are equipped to meet the risks related to germination and survival of seeds in the seed bank is discussed.

This paper forms part of an investigation into the population ecology of biennial plants in relation to the unpredictability in time and space of the environment.

2. MATERIALS AND METHODS

2.1. The investigated species

Cynoglossum officinale (referred to as *Cynoglossum*) and *Echium vulgare* (referred to as *Echium*) are short-lived perennials. Under "favourable" conditions they may have a biennial life cycle. *Anchusa arvensis* (referred to as *Anchusa*) is usually an annual, completing its life cycle within 12 months. It survives winter as seed.

In the Dutch dunes *Cynoglossum* has a relatively wide ecological amplitude. Individual plants sometimes produce some thousands but mostly some hundreds (50–800; VAN LEEUWEN & VAN BREEMEN 1980) of seeds (nutlets), which weigh *c.* 20 mg and are *c.* 6 mm large. Populations of *Cynoglossum* are long-lived, compared with *Echium* and *Anchusa*. All thirteen populations marked in 1977 were still present in 1981.

Echium, in the dunes, has a narrow ecological amplitude compared with *Cynoglossum*. It produces thousands of seeds (nutlets) which have a mean weight of about 2.7 mg and are *c.* 2.5 mm large. Populations of *Echium* are relatively long-lived. From fourteen populations only one had disappeared in 4 years (*c.* 7%).

In the study area, *Anchusa*, like *Echium*, has a narrow ecological amplitude compared with *Cynoglossum*. It produces upto about 250 seeds (nutlets) per plant. The seeds weigh 3–6 mg and are *c.* 4 mm large. Populations of *Anchusa* are relatively short-lived. Out of seventeen populations nine disappeared in 4 years (*c.* 55%).

The dispersal distance for most of the seeds of the investigated species, is less than 1 m, though occasionally dispersal over greater distances is possible.

2.2. Germination in time in the dune area

Information on natural germination was collected in 1978 from various populations in Meijendel, a dune area north of The Hague, the Netherlands, superintended by the Dune Waterworks of The Hague. For a detailed description of the area see BOERBOOM (1960).

The germination of *Cynoglossum* was studied in populations of an exposed habitat (C.e.), a sheltered habitat in and around a sea-buckthorn scrub (C.s.) and an intermediately sheltered habitat (C.i.).

Natural germination of *Echium* was studied in a roadside population (E.r.) and a population in and around a *Salix repens* scrub (E.s.).

Information from the germination of *Anchusa* was collected from a roadside population (A.r.) and a population in the shade of a *Populus* grove (A.g.).

A more detailed description of the site of the populations in the form of relevés will be published elsewhere.

Depending on the spatial heterogeneity of the vegetation and of the population size eleven to thirty permanent plots of 50 × 50 cm were chosen randomly (in situations with a mosaic vegetation) or regularly (in gradients) within each population.

2.3. Natural seedbank

2.3.1. Seedbank in and on the ground

The natural seed bank was investigated by taking soil samples separated in three layers: 0–1 cm, 1–5 cm and 5–15 cm depth. In the laboratory these subsamples were dried at room temperature and the seeds were retrieved by sieving. In this way 80–95% of the seeds in the samples could be collected (tested by putting a known number of seeds in dune sand, collected from a depth at which no "natural seeds" occurred).

The following categories were distinguished:

(a) dormant seeds.
  (i) seeds in enforced dormancy, germinating in the laboratory under favourable conditions.
  (ii) seeds in innate and induced dormancy, not germinating under favourable conditions, but viable (T.T.C. test (Moore 1973)).
(b) dead seeds, (T.T.C. test).
(c) germinated seeds (opened along preformed fracture lines).
(d) predated seeds, seeds with traces of predation and the contents eaten.
(e) fragments, only when recognizable specifically.

Samples from the seven populations were taken twice, in early spring before germination and in autumn when germination had practically stopped. In the latter period seed rain had not yet started or was prevented by removing infructescenses in the direct neighbourhood. In every population between nine and twenty samples were taken, depending on size of the population and heterogeneity of the habitat.

2.3.2. Above-ground seed bank of Cynoglossum

To investigate to what extent the infructescences of *Cynoglossum* contribute to a seed bank, the time lapse between seed ripening and primary dispersal was determined. Because wind was expected to be a main factor causing primary dispersal, an exposed and a sheltered habitat were compared. As exposed habitat one plot of 4 × 8 m was chosen, as sheltered habitat a sea-buckthorn scrub (population C.s.) was studied. Randomly thirty permanent plots of 2.5 × 2.5 m were chosen.

Within this population three types of plots could be distinguished:
s = well-sheltered plots within the sea-buckthorn scrub,
e = plots at the edge of the scrub, with sheltering from one side,
o = open, exposed plots, just outside the scrub.
In the plots all infructescences were marked with plastified iron rings. Seeds on these infructescences were counted over a period of two years.

2.4. Germination under controlled conditions

Seeds were collected in the dunes of Meijendel. Unless otherwise stated a mixture from different populations was used, which had been stored in envelopes at room temperature. Germination experiments were performed in cabinets at alternating temperature regimes (12 h darkness, low temperature and 12 h light, high temperature). Germination took place in closed 9 cm diameter plastic Petri dishes on Whatman no. 1 filter paper, wetted with demineralized water. Once in every two days germination was checked, demineralized water added and germinated seeds were counted and removed. Criterion for germination was the emergence of the radicle. The experiments were stopped after a two-week period of less than 1% germination of the remaining seeds.

At the end of the experiment the viability of the remaining seeds was tested with the T.T.C. test. Differences were tested with the χ^2-test of independence at the 5% level or Fisher's exact probability test, with a two-sided significance level of 0.05.

2.4.1. Moist-chilling requirements of Cynoglossum seeds

Seeds were moist-chilled by putting them on wetted filter paper in 15-cm-diameter Petri dishes at a temperature of 5 °C. After chilling periods of 0,2, 6 and 12 weeks four replicates of fity seeds each were placed at a 0–10, 5–15 and 10–20 °C regime.

2.4.2. The effect of constant and alternating temperature

Cynoglossum seeds were pretreated by moist-chilling for 5 weeks. Eight replicates of twenty-five seeds were then transferred to each of the temperature regimes 5 and 10 °C constant, and 0–10, 5–15 and 10–20 °C alternating (12D/12L).

For *Echium* and *Anchusa* six replicates of twenty-five seeds each were incubated at three constant temperatures 10, 20 and 25 °C, and at six alternating temperatures ranging from 0–10 °C to 25–35 °C.

2.4.3. Temperature-moisture interaction

Humus-poor sand from Meijendel was air-dried at room temperature and sieved (mesh width 2 mm). The sand was held at 24 (saturation), 12, 6 and 3% moisture (by weight). Maximum deviation from these values due to desiccation during the experiment was 24 ± 2.8, 12 ± 2.6, 6 ± 2.0, and 3 ± 2.1, respectively. The seeds were pushed into the sand surface to guarantee sufficient contact.

For *Cynoglossum* two replicates of 100 seeds each, were put in 19 cm diameter Petri dishes at alternating temperatures 0–10, 5–15 and 10–20 °C.

Echium seeds from four populations were tested in separate Petri dishes. Nine dishes with twenty-five seeds each were incubated per treatment at alternating temperatures 15–25, 20–30 and 25–35 °C.

Anchusa seeds from two populations were tested separately in eight Petri dishes with twenty-five seeds each at 10–20, 15–25 and 20–30 °C.

2.4.4. The effect of light

Petri dishes were either incubated at a 12hD/12hL regime (light-treatment) or a 24 h-darkness regime (darkness-treatment). The dishes containing seeds for the darkness experiment were filled, closed and subsequently wrapped in aluminium foil to obtain complete darkness. The dishes were put at the optimal alternating temperature for germination and were not opened during the experiment. When closing the dishes, vaseline was used to prevent water loss.

Cynoglossum seeds were collected in the three populations described previously, viz. C.e, C.s. and C.i., and were pretreated (moist-chilling) at 5 °C, for 6 weeks. From each population six replicates of twenty-five seeds each were used per treatment.

For the experiment with *Echium* seeds, collected in population E.s., four replicates of twenty-five seeds were used per treatment.

Anchusa seeds were collected in A.r. and A.g. Eight replicates of twenty-five seeds were used per population per treatment. The percentage viability of fresh seeds was also determined with the aid of a T.T.C. test.

The germination percentages were determined when germination had stopped in the light treatment.

2.4.5. The effect of depth of sowing on the fate of Cynoglossum seeds

Seeds were placed in pots with permanently moist sand at five sowing depths (0, 1, 10, 25 and 50 mm). Two replicates of fifty seeds each were incubated at 18–20 °C and a 8D/16L regime. To accelerate germination the seeds were slightly damaged by cutting a small piece of the fruit-coat. Emergence was noted when the cotyledons penetrated the sand-surface. Subsequently the sand was sieved to investigate whether germination had occurred without emergence.

2.5. Seed bank experiments in the dune area

2.5.1. Artificial seed bank in the soil (long-term)

Seeds of the three species were buried in their natural habitat at 2 and 15-cm depth in four replicates of 200. Predators were excluded by plastic netting (mesh width *c.* 1 mm). The replicates were collected again 9, 17, 26 and 33 months after burial. Then germination was studied at 0–10 °C for *Cynoglossum*, 20–30 °C for *Echium* and 15–25 °C for *Anchusa*. After 2 weeks without further germination, viability of the remaining seeds was tested (T.T.C. test).

Five different categories of seeds were distinguished:

(a) not recovered,

(b) germinated in the field (opened along the preformed fracture line),
(c) enforced dormant (germinated in the laboratory at favourable conditions),
(d) induced (plus innate) dormant (not germinating in the laboratory but still viable according to a T.T.C. test),
(e) dead (T.T.C. test).

2.5.2 Articifical seed bank of Cynoglossum above, on and in the soil (short-term)

To study the behaviour of *Cynoglossum* seeds on the infructescences during winter and early spring an artificial infructescence was created by means of a bamboo stick with a bag of plastic netting (mesh width *c.* 2.5 mm) containing fresh seeds at the natural height. This artificial infructescence was placed in population C.s. in December 1980.

The germination of 200 of these seeds was tested after 2 and 4 months.

Moreover, in December 1980 6 × 200 seeds from 1980 and 6 × 200 seeds from 1979 (still attached to the infructescences in 1980) were put into bags made of plastic netting (mesh width *c.* 1 mm) together with some dune sand. Three bags with seeds of each of the two year classes were buried at 2 and 15-cm depth at the same site as in the long-term experiment). On the surface 3 × 200 seeds per year-class were sown and covered with the same plastic netting as used for the bags. Germination of 200 seeds was tested after 2, 4 and 7 months.

At the start of the experiments the remaining seeds were stored at *c.* 5°C. Immediately after collecting, at the start of the experiment and after 2 and 4 months, germination of 8 × 25 seeds of each year-class was tested. After the germination at favourable conditions the same categories of remaining seeds were distinguished as in the long-term experiment.

3. RESULTS

3.1. Germination in time in the dune area

In the three populations of *Cynoglossum* almost all seedlings appeared in March and April, the main germination period being March (*fig. 1*). *Echium*, on the contrary, was germinating from the beginning of March up to November. The roadside population E.r. started later than the population in *Salix repens* scrub E.s. The germination-peak-period was July and April, respectively. In spite of habitat differences, germination in the two *Anchusa* populations was synchronized from March till July.

These observations suggest *Cynoglossum* to have the lowest temperature optimum closely followed by *Anchusa* and then by *Echium*, the latter obviously having the broadest temperature and moisture amplitude for germination as germination occurred under the most different weather conditions found from March till November.

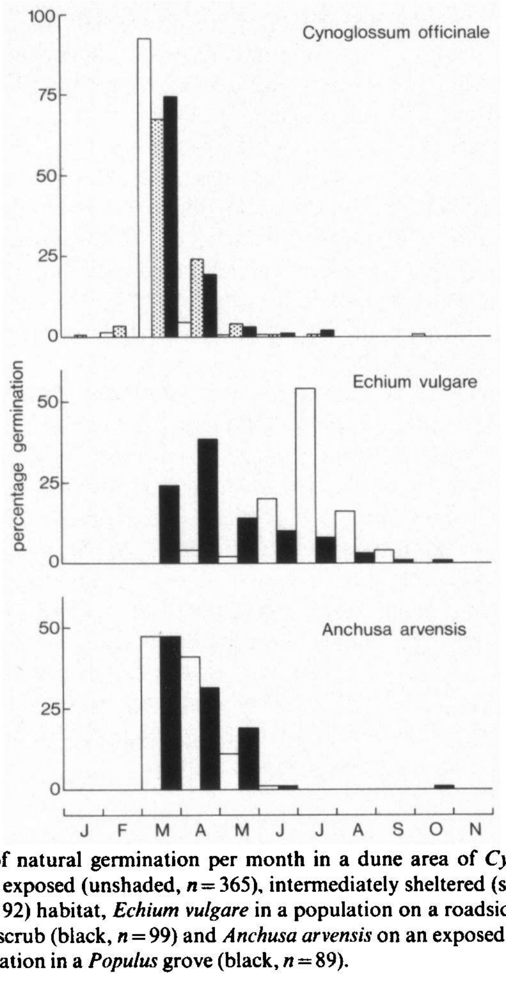

Fig. 1. Percentages of natural germination per month in a dune area of *Cynoglossum officinale* in a population in an exposed (unshaded, $n = 365$), intermediately sheltered (stippled, $n = 279$) and sheltered (black, $n = 192$) habitat, *Echium vulgare* in a population on a roadside (unshaded, $n = 50$) and in a *Salix repens* scrub (black, $n = 99$) and *Anchusa arvensis* on an exposed roadside (unshaded, $n = 93$) and in a population in a *Populus* grove (black, $n = 89$).

Table 1. Density of viable seeds and seed fragments in the seed bank before and after (between parentheses) germination in the field (i.e. before the new seed rain) found in the top 15 cm of the soil in different dune populations. *Cynoglossum officinale* in exposed (C.e.), sheltered (C.s.) and intermediately sheltered (C.i.) habitat, *Echium vulgare* at a roadside (E.r.) and in a *Salix repens* scrub (E.s.) and *Anchusa arvensis* in a *Populus* grove (A.g.) and an exposed roadside habitat (A.r.).

	Cynoglossum officinale			*Echium vulgare*		*Anchusa arvensis*	
	C.e.	C.s.	C.i.	E.r.	E.s.	A.g.	A.r.
No. of viable seeds/m^2	47 (53)	124 (12)	44 (35)	0 (13)	25 (59)	83 (47)	169 (106)
No. of fragments/m^2	79 (141)	12 (6)	124 (0)	11 (0)	63 (118)	77 (49)	442 (584)
No. of samples	19 (10)	15 (15)	20 (10)	16 (20)	14 (9)	15 (15)	11 (10)

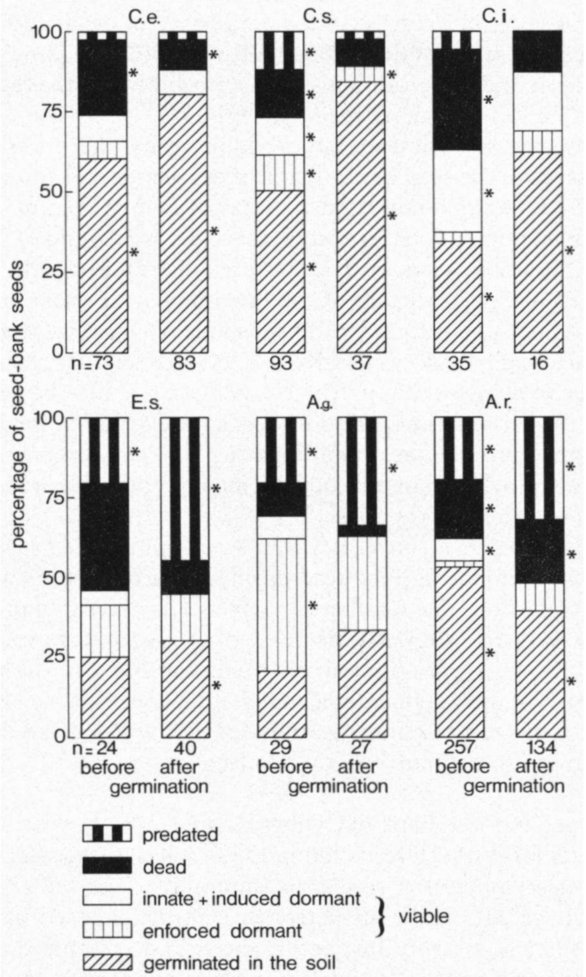

Fig. 2. Percentages predated (partly eaten, more than 75% left), dead, innate and induced dormant, enforced dormant and germinated seeds in the seed bank of *Cynoglossum officinale* in a population in an exposed (C.e.), sheltered (C.s.) and intermediately sheltered (C.i.) habitat, *Echium vulgare* in a *Salix repens* scrub (E.s.) and *Anchusa arvensis* in a population in a *Populus* grove (A.g.) and in an exposed roadside habitat (A.r.) before and after the field germination period.
n is the number of seeds found in the soil samples (12 cm diameter, 15 cm depth). See for the number of samples *table 1*.

* means clustered according to the dispersion coefficient $D = \frac{S^2(n-1)}{\bar{x}}$, $\alpha = 0.05$

3.2. Natural seedbank

3.2.1. Seedbank in and on the ground

The most pronounced reduction, after germination, in the number of viable seeds as well as in their relative share in the seed bank was observed in *Cynoglossum* population C.s. (*table 1*; *fig. 2*). It is unlikely that mortality caused this reduction because of the decrease in the percentage of dead seeds. The relative amount of predated seeds and seed fragments was about the same after germination, so predation or deterioration was not contributing to the relative decrease of viable seeds, unless seeds were predated entirely.

According to the relative decrease of viable seeds, there was a substantial turn-over of seeds in the seed bank. Viable seeds were never found deeper than 5 cm; most of them were found in the top layer of 1 cm of the soil.

In both *Echium* populations an absolute increase was found of the viable seed bank after germination (*table 1*). The clustered distribution pattern of the seed bank (indicated in *fig. 2*; because of the low number of seeds found, E.r. was left out of consideration) is thought to be responsible for this *"generatio spontanea."* The percentage viable seeds of E.s. decreased slightly after germination (*fig. 2*), not due to dying off but partly to predation and may be partly to germination. After the germination period no seeds were found in innate or induced dormancy. Apparently weather conditions had broken dormancy. Viable seeds were found down to 15-cm depth, but the majority of these were in the upper 5 cm.

The viable seed bank of *Anchusa* was in A.r. about twice as large as in A.g. Like in *Echium* no innate and induced dormant *Anchusa* seeds were found on the second sample date. The number of viable seeds decreased in both populations (*table 1*). A.g. also showed a decrease of the percentage viable seeds, not due to dying off, but to germination and predation (*fig. 2*). A.r. showed an increase of the percentages fragmentated and predated seeds, which may be caused by the decrease of the percentage germinated seeds. Viable seeds were found down to 15-cm depth, but mostly in the top 1-cm.

3.2.2. Above-ground seed bank of Cynoglossum

In both habitats most of the seeds fall in the first 4 months after ripening (*fig. 3*). In the first December after seed formation only 6% of the original number of seeds is still present on the infructescences in the exposed habitat, against *c.* 38% in the sheltering scrub. Just before the start of germination in the field, these figures had dropped to 2% and 25% in the exposed and the sheltered habitat, respectively. Within the scrub, the plots with the three distinquished categories of sheltering showed a similar difference. In the open spots the seeds fall sooner than at the edge of the scrub, and the latter again fall sooner than those at the most sheltered spot. Within the scrub the infructescence-seed bank lasts more than two years.

3.3. Germination under controlled conditions

3.3.1. Moist-chilling requirements of Cynoglossum seeds

The highest germination percentage was found after a pretreatment of 6–12 weeks moist-chilling at 0–10 °C (average 5 °C) (*fig. 4*). Seeds germinate faster at the higher temperature regimes and when the moist-chilling pretreatment period is longer (*table 2*).

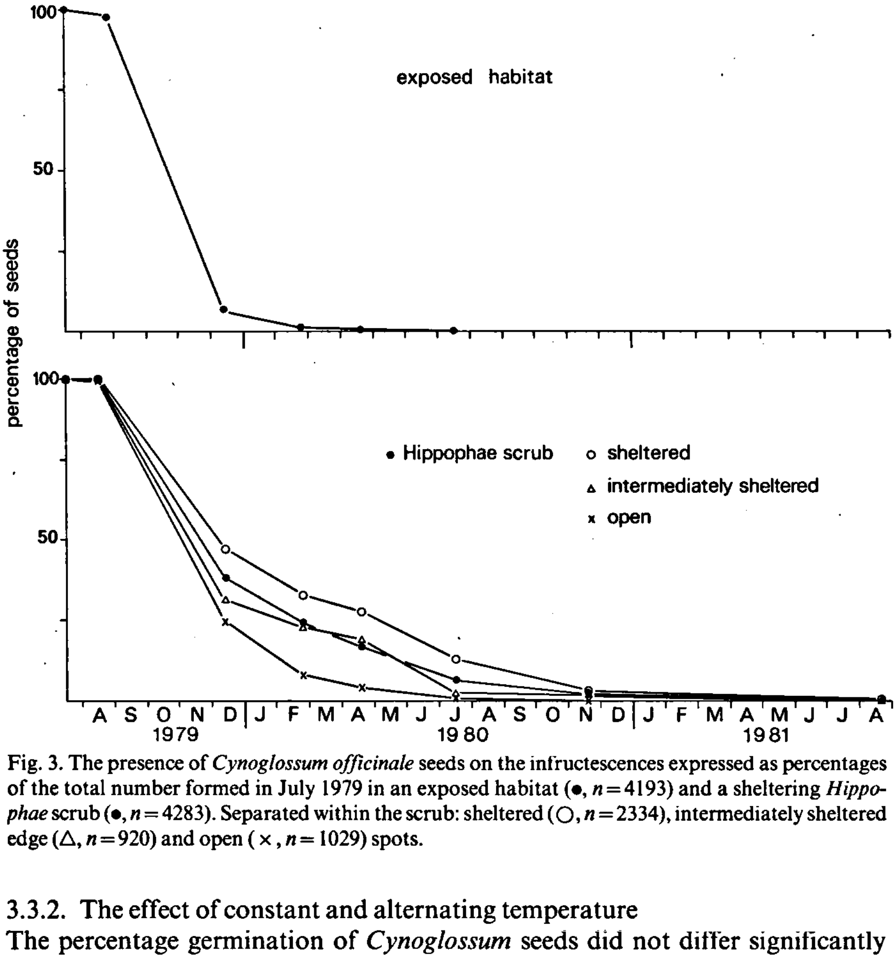

Fig. 3. The presence of *Cynoglossum officinale* seeds on the infructescences expressed as percentages of the total number formed in July 1979 in an exposed habitat (●, $n = 4193$) and a sheltering *Hippophae* scrub (●, $n = 4283$). Separated within the scrub: sheltered (○, $n = 2334$), intermediately sheltered edge (△, $n = 920$) and open (×, $n = 1029$) spots.

3.3.2. The effect of constant and alternating temperature

The percentage germination of *Cynoglossum* seeds did not differ significantly between the various alternating temperature regimes (*table 3*). At 5 °C constant temperature, however, germination was significantly better than at the alternating regimes $P < 0.01$), whereas at 10 °C constant temperature a significantly lower germination ($P < 0.01$) was found.

The highest germination in *Echium* was found at 25–35 °C alternating and 25 °C constant temperatures (*table 3*). Germination at alternating temperatures always was significantly higher than germination at the corresponding mid-point constant temperature ($P < 0.01$). During the experiment fewer seeds died at 25–35 °C than at the other temperature regimes. When relating the germination to the total number of seeds minus the dead seeds at the end of the experiment, highest germination percentage was found at 20–30 °C, which, however, was not significantly different from the germination percentage at 25–35 °C.

The highest germination percentage for *Anchusa* was found at 15–25 °C which was not significantly different from 10–20 and 20–30 °C (*table 3*). Again germina-

Table 2. Mean germination time (days) for *Cynoglossum officinale* seeds 100 days after sowing at different temperature regimes (12D/12L) and moist-chilling periods ($n = 200$).

Moist-chilling period (weeks)	Temperature regime (°C)		
	0–10	5–15	10-20
0	81.3	40.5	16.0
2	52.6	23.2	8.8
6	12.4	5.2	3.3
12	19.7	4.6	3.9

Table 3. Percentages germinated seeds (mean number per Petri dish ± S.D.) of *Cynoglossum officinale* (8 × 25), *Echium vulgare* (6 × 25) and *Anchusa arvensis* (6 × 25) at various constant and alternating temperature regimes (12D/12L).

Alternating regimes (°C)	*Cynoglossum*	*Echium*	*Anchusa*
0–10	42.0(10.5±3.2)a	4.0(1.0±0.9)a	4.0(1.0±3.7)a
5–15	45.0(11.3±3.3)a	12.0(3.0±1.4)b	19.3(4.8±1.9)b
10–20	40.5(10.1±2.9)a	43.3(10.8±2.5)c	24.0(6.0±2.5)bc
15–25	—*	62.0(15.5±3.0)d	32.7(8.2±2.7)c
20-30	—*	79.3(19.8±1.6)e	32.0(8.0±1.1)cd
25–35	—*	94.0(23.5±1.1)f	18.7(4.7±1.2)b
Constant regimes (°C)			
5	89.5(22.4±2.0)b	—*	—*
10	18.0(4.5±2.6)c	1.3(0.3±0.5)a	2.0(0.5±0.8)a
20	—*	28.7(7.2±2.5)g	13.3(3.3±1.5)b
25	—*	62.7(15.7±1.9)d	20.0(5.0±1.3)bd

Different characters (within columns) indicate significant differences at the 5% level.
—* not determined.

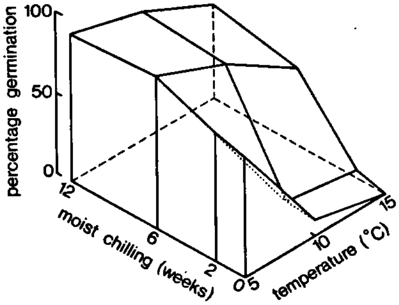

Fig. 4. Germination percentage of *Cynoglossum officinale* in relation to alternating temperature and moist-chilling period ($n = 200$). The average of each of the three temperature ranges of 10°C is indicated.

tion appeared to be better at alternating temperatures than at the corresponding mid-point constant temperature though the difference was not significant for 20–30 °C alternating and 25 °C constant temperature.

3.3.3. Temperature-moisture interaction

The maximum germination percentage was reached for *Cynoglossum* at 0–10 °C and 12% soil moisture, for *Echium* at 20–30 °C and 12% soil moisture and for *Anchusa* at 10–20 °C and 6% soil moisture (*table 4*). (Because the *Echium* and *Anchusa* seeds from the different populations did not behave essentially different, the results were pooled.) Maximum germination of *Cynoglossum* seeds tends to occur in somewhat higher soil-moisture conditions than *Echium* and *Anchusa* seeds.

Of all used combinations of temperature and soil moisture, germination rate of *Cynoglossum* is highest at 5–15 °C and 6–12% (excluding experiments with less than 10 seedlings). The germination rate of *Echium* and *Anchusa* is about

Table 4. Percentages germination and mean germination time (M.G.T.) of seeds of *Cynoglossum officinale* ($n = 200$), *Echium vulgare* ($n = 225$) and *Anchusa arvensis* ($n = 200$) for different soil moisture and temperature combinations.

Cynoglossum officinale temperature (°C):	0–10		5–15		10–20	
moisture (%)	germination (%)	M.G.T. (days)	germination (%)	M.G.T. (days)	germination (%)	M.G.T. (days)
24	62.5 ab A	57.8	21.0 a B	75.9	0.0 a C	–
12	73.0 a A	57.5	43.5 b B	39.1	7.0 a C	81.7*
6	58.0 b A	56.2	36.0 b B	41.7	0.5 a C	49.0*
3	25.5 c A	82.5	14.0 a B	46.4	1.0 a C	14.0*

Echium vulgare temperature (°C):	15–25		20–30		25–30	
moisture (%)	germination (%)	M.G.T. (days)	germination (%)	M.G.T. (days)	germination (%)	M.G.T. (days)
24	83.1 a A	3.1	82.7 a A	3.8	71.6 a B	2.7
12	91.1 b A	2.9	95.1 b A	3.2	83.6 b B	2.9
6	92.4 b A	3.2	94.2 b A	3.1	73.8 a B	3.3
3	88.0 ab A	4.5	85.3 a A	4.8	52.0 c B	4.9

Anchusa arvensis temperature (°C):	10–20		15–25		20–30	
moisture (%)	germination (%)	M.G.T. (days)	germination (%)	M.G.T. (days)	germination (%)	M.G.T. (days)
24	60.5 ac A	4.3	47.0 a B	3.4	63.0 a A	4.5
12	71.5 ab A	4.8	69.0 b A	3.7	76.0 b A	4.5
6	80.5 b A	5.2	73.0 b A	4.8	75.5 b A	4.9
3	74.0 b A	4.8	68.5 b A	4.9	63.5 a A	5.4

Different characters (within columns) and different capitals (within rows) indicate significant differences at the 5% level.

Table 5. Percentages germinated and dead seeds (mean number per Petri dish ± S.D.) from populations of *Cynoglossum officinale* (6 × 25), *Echium vulgare* (4 × 25) and *Anchusa arvensis* (8 × 25) at the optimal alternating temperatures, 0–10 °C, 25–35 °C and 20–30 °C, respectively, in light and darkness.

Population	Light (12 h darkness/12 h light)		Darkness (24 h darkness)	
	germinated	dead	germinated	dead
C.e.	26.7(6.7±3.7)a	20.0(5.0±3.7)A	81.3(20.3±1.9)b	8.0(2.0±1.7)B
C.s.	56.0(14.0±5.9)a	12.0(3.0±2.1)A	73.3(18.3±3.9)b	4.7(1.2±0.8)A
C.i.	60.0(15.0±7.2)a	9.3(2.3±1.4)A	62.0(15.5±6.3)a	3.3(0.8±1.0)A
E.s.	92.0(23.0±4.9)a	6.0(1.5±1.3)A	84.0(21.0±2.6)a	10.0(2.5±1.3)A
A.r.	20.5(5.1±3.8)a	59.0(14.8±3.0)A	20.5(5.1±1.8)a	20.0(5.0±1.9)B
A.g.	28.5(7.1±2.6)a	59.0(14.8±3.3)A	40.5 (10.1±3.4)b	47.5(11.9±2.7)A

Different characters (germinated) or capitals (dead), indicate significant differences at the 5% level, within rows for *Cynoglossum* (C.e., C.s. and C.i.) and *Echium* (E.s.) or within rows as well as within columns for *Anchusa* (A.r. and A.g.).

the same for alle used circumstances. *Cynoglossum* seeds germinate much slower than *Echium* and *Anchusa* seeds.

3.3.4. The effect of light

Seeds from populations C.e. and C.s. germinate significantly better in darkness than in light ($P < 0.01$) (*table* 5). There is no significantly different germination in light or darkness for seeds from population C.i. During the experiment significantly more seeds from C.e. had died in light than in darkness ($P < 0.01$). The same but not significant effect was found for seeds from C.s. and C.i.

For E.s. the percentage of germinated as well as the percentage of dead seeds are not significantly different in light or darkness.

When fresh, the *Anchusa* seeds from population A.r. and A.g. proved to have the same viability percentage so it is possible to compare the amounts of germinated and dead seeds at the end of the experiment between the populations. Contrary to seeds from open habitat (A.r.), seeds from the grove habitat (A.g.) germinated better in darkness than in light. During the experiment significantly more seeds from the open habitat (A.r.) died in the light treatment than in darkness ($P < 0.01$).

The two *Anchusa* populations did not show significant differences in germination and mortality in the light treatment. In darkness the seeds from the grove habitat germinated significantly better than the seeds from the open habitat ($P < 0.01$). During the experiment in darkness significantly more seeds from the grove habitat died than from the open habitat (the grove being not the optimal habitat for *Anchusa*) ($P < 0.01$).

3.3.5. The effect of depth of sowing on the fate of Cynoglossum seed

Maximal germination and survival in sand occurred at a depth of 10 mm (*fig.* 5). Probably the lower germination percentage at 0 mm was caused by the desiccation of the soil surface. At 50-mm depth there was some germination but none of the seedlings emerged.

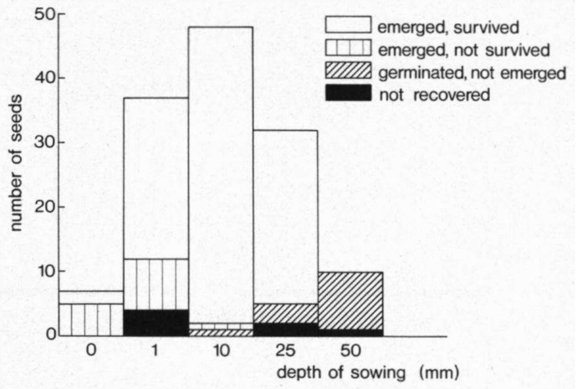

Fig. 5. Fate of *Cynoglossum officinale* seeds 53 days after sowing, in relation to the depth of sowing ($n = 100$ for each depth); individuals that emerged and survived, seedlings that emerged and died, seedlings not emerged and seeds not recovered.

3.4. Seed bank experiments in the dune area

3.4.1. Artificial seed bank in the soil (long-term)

Seeds of the three investigated species behaved very differently in the soil (*fig. 6*). *Cynoglossum* had no persistent seed bank. All seeds had germinated in a period of 9 months after burial at 2-cm as well as 15-cm depth. *Echium* and *Anchusa* on the contrary showed long-lived seed banks. For both species there was a decrease in dormant seeds at 2-cm depth, due to germination. At 15-cm depth there was hardly any loss in dormant seeds after an initial germination flush. The latter was higher for *Echium* than for *Anchusa*. Both species showed a transition from innate (induced) dormancy to enforced dormancy.

3.4.2. Artificial seed bank of Cynoglossum above, on and in the soil (short-term)

At the start of the experiment practically all "old" seeds (formed the year before collecting them from the infructescences were in induced dormancy, agains only 25% of the "fresh" seeds (formed in the year of collection, *fig. 7*).

Nearly all buried seeds ("old" and "fresh") germinated within 4 months. Most surface seeds did not germinate, but more fresh than old seeds did. So the surface seed bank will, for a relatively great part, consist of old seeds. Temporarily (about April, 4 months after the start), most of the remaining surface seeds are in induced dormancy.

Dormancy can be induced in "fresh" seeds by storing them at *c.* 5 °C. "Old" seeds were already dormant when still on the infructescence. They remained dormant during storage. So their position on the infructescence induces dormancy, which will be partly caused by low winter temperatures.

"Fresh" seeds on the soil surface germinate well in spring. Seeds shed after a period of 'hanging' on the infructescence will stay dormant over a longer time. One favourable spring may not be sufficient to break dormancy.

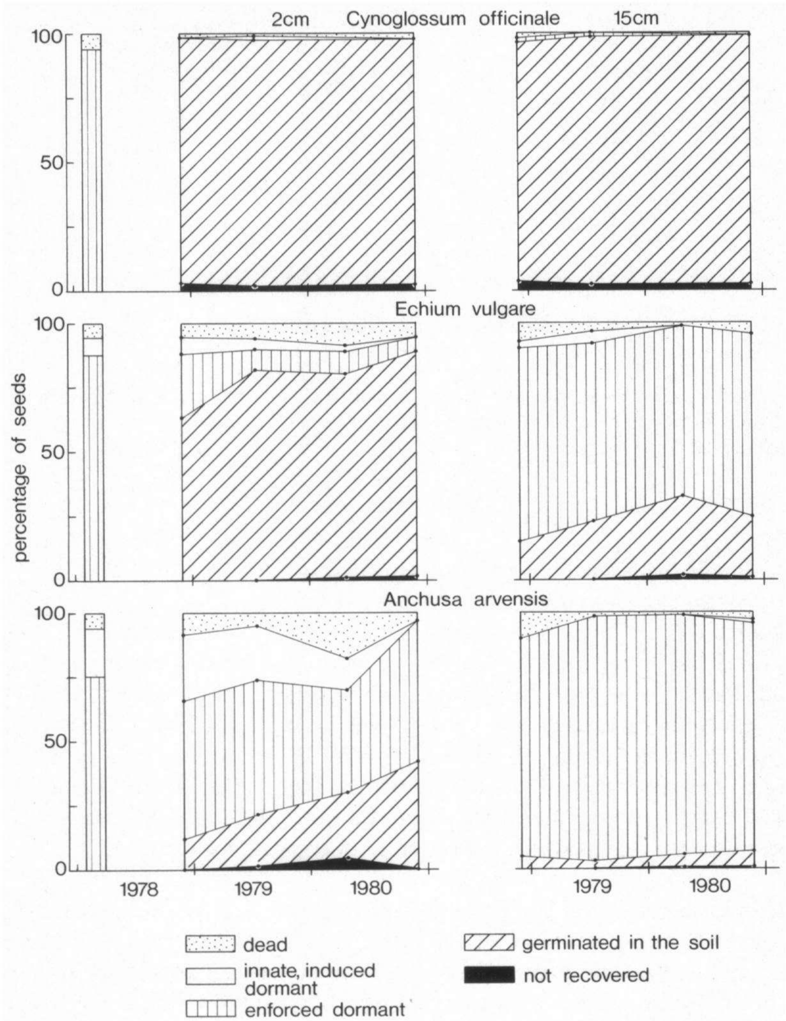

Fig. 6. Fate of seeds buried in dune sand (protected against predators) at 2-cm and 15-cm depth of *Cynoglossum officinale* ($n = 200$), *Echium vulgare* ($n = 200$) and *Anchusa arvensis* ($n = 200$). The left column indicates the germination capacity and dormancy state of the seeds at the start of the experiment.

4. DISCUSSION

4.1. Germination

4.1.1. Cynoglossum officinale

The most important seed-dormancy-breaking agents are temperature, light and nitrate (Roberts 1972). Dormancy of *Cynoglossum* seeds can be broken by nitrate (Freijsen et al. 1980) and by moist-chilling (Lhotská 1982; this paper).

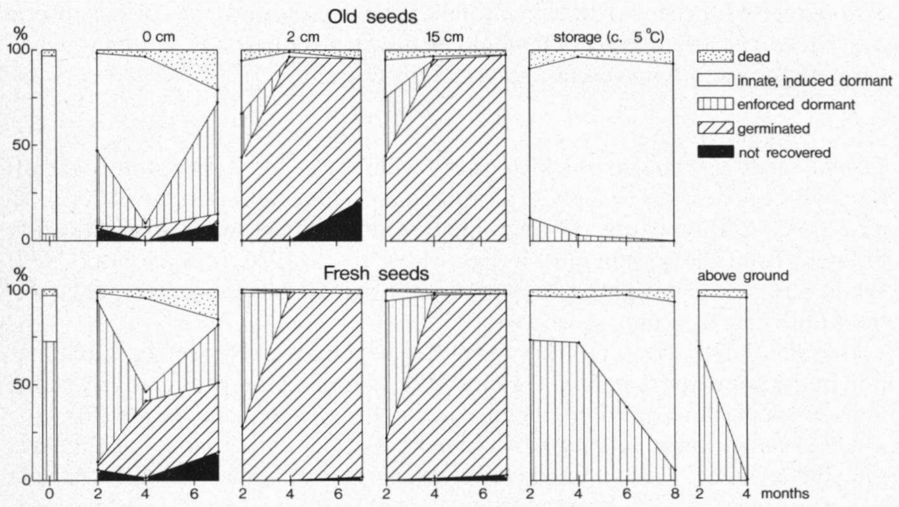

Fig. 7. Fate of *Cynoglossum officinale* seeds ($n = 200$) buried in sand (protected against predators) at different depths, above ground and under cold storage (old seeds, formed the year before collection, but still present on the infructescences; fresh seeds, formed in the year of collection). The left column indicates the germination capacity and dormancy state of seeds at the start of the experiment. Sample months were February, April and July.

The time of germination in early spring is brought about by the low, fluctuating temperatures at which, in laboratory conditions too, maximal germination is found (FREIJSEN et al. 1980; this paper), but fluctuations in temperature are not necessary for germination. A species germinating at a constant temperature is, as far as the germination temperature is concerned, not depending on disturbed sites.

Seeds of *Cynoglossum* from the exposed habitat population and from the population in a scrub germinated better in total darkness than in a 12D/12L regime. Seeds from the intermediately sheltered habitat (population C.i.) germinate in darkness as well as in the dark/light regime. So darkness stimulates or has no effect on the germination of *Cynoglossum*. Light may be one of the factors which can cause the building up of a surface-laying seed bank.

Most *Cynoglossum* seeds germinated at a depth of 1 cm, a depth at which the soil moisture is relatively high and fluctuates less (compared with the soil surface).

With regard to temperature, soil moisture and light *Cynoglossum* can germinate in spring in a wide variety of habitats (after a pretreatment of 6-weeks moist-chilling no nitrate is necessary for germination). The moist-chilling period always occurs in winter and soil-moisture and temperature will be appropriate for germination.

The germination characteristics, innate dormancy broken by several weeks of cold stratification and rapid germination after the end of the winter, fit in a cold-avoiding syndrome for germination (see ANGEVINE & CHABOT 1979). It also agrees with the fact that in winter the above-ground part of the rosette dies back. An early frost period would be disastrous for young seedlings, when germination in autumn was not prevented by dormancy.

4.1.2. Echium vulgare

Echium seeds germinated quickly under a wide variety of temperature and soil-moisture conditions. The highest germination percentage was found at 20–30 °C and 6–12% soil moisture. The germination in darkness was not significantly different from the germination in light. MITCHELL (1926, in SALISBURY 1952) found germination in light only. Possibly the origin of the seeds or storage conditions did affect their light sensitivity.

Germination in the dunes was spread from March till November. Germination in the population on humus-rich soil (E.s.) and hence with relatively stable moisture conditions was more regular than in the roadside population (E.r.)

The germination of *Echium*, which can occur under a wide variety of temperature and soil-moisture conditions in the dunes, may be spread in time by drying out of the soil surface or by intermittent availability of germinable seeds.

4.1.3. Anchusa arvensis

Because *Anchusa* proved to have an intermediate temperature response for highest germination percentage and a broad soil-moisture amplitude, it is expected to germinate in time between *Cynoglossum* and *Echium*, which in fact was found. Additionally autumn germination may occur. In that case *Anchusa* surviving the winter as a rosette and flowering the next year, can live longer than 12 months.

The difference in light response of seeds from an open (roadside population, A.r.) and a shadowed habitat (grove population, A.g.) may have adaptive value. More seeds from the grove situation germinated in darkness. At the end of the experiment in darkness more seeds from the shaded habitat had died than from the open habitat. Furthermore more seeds from the open habitat died in light than in dark germination conditions. So a seed bank can be build up in the open habitat, where the seeds are blown over by sand, better than in the grove habitat. Whether this difference in the light sensitivity of the *Anchusa* seeds is induced by the environmental conditions or caused by a genetic difference needs to be investigated.

4.2. Seed bank

The three investigated species have a rather small persistent seed bank. According to the seed bank typology of THOMPSON & GRIME (1979) they all belong to Type IV, which confers the potential for regeneration in circumstances where disturbance is temporarily and/or spatially unpredictable, but not all properties assigned to species with a Type IV seed bank are found in the investigated species.

4.2.1. Cynoglossum officinale

According to its large seeds, its germination in darkness and at low temperatures, *Cynoglossum* has type II properties of species with a transient seed bank, which should be found in herbs colonizing vegetation gaps created by seasonally predictable damage and mortality in the vegetation. The buried seed population forms such a transient seed bank with seeds germinating within one year after being buried.

According to SALISBURY (1952) the large food reserves of seeds such as those of the hound's tongue are mainly utilised in root production so that at an early stage of development and long before the wetter period of the year has passed their roots have penetrated to a depth in the sand where the moisture content is not only higher but subject to far less frequent or pronounced fluctuation. The induced dormancy in April as has been found in surface seeds has adaptive value in reducing the risk of desiccation of the young seedling. In July this induced dormancy is lost again but in this period no germination will occur under natural conditions due to the prevailing temperatures.

The seeds on and above the soil surface behave differently from those in the soil and form a persistent seed bank. One *Cynoglossum* plant may contribute to different seed-bank types, but also populations may differ in behaviour. Exposed populations have no or just a very small persistent seed bank, because seeds, mostly shed before winter, will be buried by windborne sand and germinate soon. In this way the seed bank in an exposed habitat will be more of Type II, whereas in a "scrub population" the seed bank will be more like Type IV (*sensu* THOMPSON & GRIME 1979). This reasoning is built up in the light of the results of the experiments with an artificial seed bank.

Cynoglossum certainly has a persistent seed bank in scrubs and sheltered habitats in the form of old-seed-bearing infructescences and of the dormant seeds on the ground. A population in open habitat may become extinct when after a catastrophe all seedlings have died. New invasion will be possible by seeds formed by plants in scrubs. This type of seed bank seems to have adaptive value in a mosaic habitat such as found in a scrub with open spots.

In the exposed habitat (population C.e.) seeds were still present in the seed bank after the germination period. Probably the habitat where moss covered 20% of the area was still suitable for the presence of a surface-laying seed bank.

The statement of SALISBURY (1952), that germination of *Cynoglossum* is intermittent and can be spread over a period of 8 years, must be seen in the light of a surface-laying dormant seed bank in a habitat without windborne sand.

At first sight the lack of a dormancy mechanism in buried *Cynoglossum* seeds is not consistent with the general behaviour of short-lived monocarps. However, *Cynoglossum* seeds under natural circumstances will hardly ever be found at this depth. The hooks on the fruit coat, which are assumed to be useful as dispersal aids, will also be useful to keep the seeds on the soil surface, where they can stay dormant for over one year. Furthermore they possibly serve as anchors to keep the seeds within the microhabitat of the parent plant. This is useful for a species in a predictable environment.

Cynoglossum occurs typically near rabbit burrows and disturbed sites (VAN OOSTSTROOM & REICHGELT 1961; WESTHOFF & DEN HELD 1969; GRUBB 1976). This may be the result of seed dispersal effected by rabbits, but could also be the outcome of the relatively more abundant germination at those spots where the seeds are easily buried.

4.2.2. Echium vulgare

Echium is able to germinate in the seed bank at a depth of 2 and 15 cm. Probably the seedlings can bridge a 2-cm layer of sand (within one year more than 60% germinated at that depth). Seedlings from seeds germinated at 15-cm depth do not emerge and die.

According to ROBERTS (1972) most mortality of seeds appears to be due to the breaking of dormancy mechanisms and the subsequent germination while buried in the soil. COOK (1980) and VAN LOENHOUD & DUYTS (1981) also mention losses from the seed bank by subterranean germination for other species. *Echium* takes an intermediate position in seed-bank longevity between *Anchusa* and *Cynoglossum*. It was known already that *Echium* seeds remain viable in the soil over a number of years (SALISBURY 1961).

Indications for the existence of a seed bank are various. After the removal of landmines from Meijendel following World War II, large areas of the dunes coloured blue with flowering *Echium*. The same occurred after the digging activities by the Dune Waterworks (BOERBOOM 1960). The new plants establishing after those large scale diggings obviously stem from seed-bank seeds (may be after the first establishment and seed setting of fewer plants).

In the natural seed bank we found very few seeds, which, however, may be due to the small sample size and to a clustering of seeds in the soil. But even a small persistent seed bank is important in such situations.

4.2.3. Anchusa arvensis

When buried at 15-cm depth hardly any germination or deterioration is found in *Anchusa* seeds. Innate or induced dormancy disappeared completely after 9 months stay at 15 cm and about 3 years at 2-cm depth. The same phenomenon of loss of dormancy after burial has been found for *Stellaria media* by ROBERTS & LOCKETT (1975).

The longevity of the seeds in soil is likely to be overestimated because predation was excluded in our experiments. In an experiment in which seeds were sown in a natural habitat of the three species and predation was not excluded up to 90% of the seeds disappeared within 2 months after sowing (VAN BREEMEN & VAN LEEUWEN 1983). In this experiment sometimes fragments of seeds were left behind by predators, but predation of whole seeds and secondary horizontal dispersal may also have occurred (samples were taken down to 10 cm, so seeds which had moved downwards were also counted). Like GRUBB (1977) stated, differences in survival under natural conditions may be more closely related to palatability, digestability and the incidence of predators than to inherent differences in longevity.

4.3. Strategy

The following picture of the strategy of the three short-lived monocarpic Boraginaceae *Cynoglossum, Echium* and *Anchusa* cannot be complete because not all life-stages of the plant are observed.

In this study numerous differences in germination and seed-bank characteristics were found. *Cynoglossum* produces relatively few, large seeds, most of which fall close to the parent plant. The seed bank is relatively short-lived and the germination is well-timed. All these characteristics point to a strategy of reducing the mortality risks connected with germination and survival of seeds in space as well as in time. *Cynoglossum* is, as far as the investigated characteristics are concerned, expected to live in a habitat predictable in space and time. In fact *Cynoglossum* populations are long-lived and the characteristic habitat of the species (*Hippophae*-scrub) is more stable than the habitats of both other species.

Echium produces thousands of small seeds, the seed bank is relatively long-lived and germination is spread all over the summer season. All characteristics are suitable for spreading the risk in time. the dispersal distance is low, most seeds falling in the microhabitat of the parent plant, thus reducing the risks in space. *Echium* is therefore expected to live in a habitat unpredictable in time, but predictable in space. The characteristic habitat of *Echium* (road verges) is predictable in space, but exactly when a disturbance (necessary to bring seeds out of the seed bank to the soil surface) will occur is unpredictable..

The seed bank of *Anchusa* is long-lived and yearly germination is spread over a period of about 4 months. Through these characteristics risk is spread in time. Therefore *Anchusa* is expected in a habitat unpredictable in time. *Anchusa* populations are relatively short-lived, which drops a hint to the unpredictability. However, *Anchusa* invests a relatively big portion of its total seed weight in one seed (compared with *Echium* it produces less and somewhat heavier seeds) and most of the seeds fall close to the parent plant. Altogether *Anchusa* also shows characteristics for reducing risks in space.

The classification of the three investigated species in a species with such characteristics that the mortality risk is reduced in space and time (*Cynoglossum*) and species with characteristics through which the risk is reduced in space and spread in time (*Echium* and *Anchusa*) should not be considered a strict one. Seeds from different populations of the same species have demonstrated the existence of different germination and seed-bank characteristics: a*Cynoglossum* population was found in which the risks were relatively more spread and an *Anchusa* population in which the risks were relatively more reduced in time.

The ranking of species in a continuum from r-selected species with small seeds, dispersing into unstable, early successional habitats, to K-selected species with large seeds in mature stable climax vegetation (HARPER et al. 1970) leads to similar combinations of characteristics as found in this study. COOK (1980) places species in a continuum from species adapted to dispersal in time with a.o. small seeds, short-lived plants, long-lived seed bank and unstable populations of plants to species adapted to dispersal in space with a.o. large seeds and fruits, long-lived plants, short-lived seed bank and stable populations of plants. Delayed reproduction (dormancy and diapause) has been discussed by LEVINS (1969) as an adaptive response to environmental uncertainty, which is somewhat similar to the spreading of germination in time.

The trend found in this study is very similar to the ideas of LEVINS (1969), HARPER et al. (1970) and COOK (1980), but it originated from a somewhat different approach, viz. a study of the ways in which plants are equipped to meet the mortality risks related to germination and survival of seeds; the reduction of risk per seed and the spreading of risk over different seeds in space and time.

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