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Effects of endogenous nitrate content of *Sisymbrium officinale* seeds on germination and dormancy

HARRO J. BOUWMEESTER*†‡, LENI DERKS*, JAKOB J. KEIZER* and CEES M. KARSSEN*§

**Department of Plant Physiology, Agricultural University, Arboretumlaan 4, 6703 BD Wageningen, The Netherlands; and †Department of Vegetation Science, Plant Ecology and Weed Science, Agricultural University, Bornsesteeg 69, 6708 PD Wageningen, The Netherlands*

SUMMARY

The nitrate content of seeds of the annual *Sisymbrium officinale* was raised by weekly nitrate fertilizations of the seeding mother plants. Nitrate content and germination of the seeds produced were positively correlated. Seed lots with different endogenous nitrate contents were buried at 10 cm in sandy loam. At regular intervals seeds were exhumed and germination was tested. Both the initial differences in endogenous nitrate content and the differences of germination rapidly diminished during burial because nitrate rapidly leached from the seeds. The sensitivity of exhumed seeds to applied nitrate strongly varied during this 1-year study. Sensitivity to nitrate was low in December and had increased in January–February probably because of low winter temperatures. A cold pretreatment in Petri dishes also strongly increased sensitivity to nitrate. Endogenous nitrate content of seeds collected in the field was much lower than reported in this study and therefore the differences in seed-nitrate content in the field probably are of limited ecological significance. A high endogenous nitrate content will stimulate germination only temporarily. In contrast, the changes in sensitivity in nitrate seem ecologically important because they will restrict germination of *S. officinale* to seasons suitable for survival of the seedlings.

Key-words: dormancy, endogenous nitrate, germination, nitrate sensitivity, *Sisymbrium officinale*.

INTRODUCTION

Nitrate is one of the most prominent chemical stimulants of germination. Promotive effects have been reported for a large number of weedy and ruderal annual species (e.g. Henson 1970; Vincent & Roberts 1979; Hilhorst *et al.* 1986; Bouwmeester & Karssen 1989, 1993a,b; Karssen & Hilhorst 1992). It might be expected that annual weed seeds will germinate better in nitrate-rich soils than in nitrate-poor field margins.

‡Present address: Centre for Agrobiological Research, Postbox 14, 6700 AA Wageningen, The Netherlands.
§Offprint requests to C.M. Karssen.

A high soil fertility may also stimulate germination of the next generation. Fawcett & Slife (1978) and Saini *et al.* (1985) reported a positive relationship between nitrate applications to plants of the annual *Chenopodium album* and the nitrate content of the seeds produced by these plants. Germination of these seeds in water increased with an increase in endogenous nitrate content.

Nitrate plays an essential role in the light-dependent germination of the annual *Sisymbrium officinale*. Hilhorst *et al.* (1986) showed that seeds of this species could only respond to light in the presence of nitrate. Hilhorst (1990a) hypothesized that nitrate may function as a 'cofactor' for the binding of phytochrome to a membrane-bound receptor. Sensitivity to nitrate declined during dormancy induction.

Exogenously applied nitrate stimulated germination of exhumed seeds of *S. officinale* throughout a period of 3 years (Bouwmeester & Karssen 1993b). The applied nitrate enabled the seeds to germinate during periods when this would not normally have been possible. In their studies with *C. album*, Saini *et al.* (1985) did not investigate whether an increased seed nitrate content also had a long-term effect on germination of buried seeds. In the present study we first investigated whether the endogenous nitrate content of *S. officinale* seeds could be raised and whether this stimulated germination. In addition, we studied the effect of a raised seed nitrate content on germination of seeds that had been buried for up to 1 year.

For this purpose, *S. officinale* plants were fertilized with a range of potassium nitrate concentrations to obtain seed lots with varying endogenous nitrate contents. Germination of these seed lots was tested. Seed lots that differed in endogenous nitrate content were buried in soil, and germination of exhumed seeds was tested during 1 year. Germination was tested in both water and nitrate to study whether endogenous nitrate affected sensitivity to exogenous nitrate.

MATERIALS AND METHODS

Plant cultivation

Seedlings of *Sisymbrium officinale* (L.) Scop. were collected in April 1987 in our laboratory garden in Wageningen from a uniform group of plants. Twenty-four plants were grown outdoors in pots (10 l) containing sandy loam and one plant per pot, and were watered as necessary. From the beginning of flowering until the harvest of seeds, plants were irrigated weekly with 250 ml of a KNO_3 solution of 0, 5, 10, 30, 59 or 119 mmol l^{-1}. Four plants were used per treatment. Seeds were harvested when mature and the seeds of the four plants of each treatment were combined. In 1988 the experiment was repeated, but seeds were harvested separately for each plant. Seeds were air-dried, cleaned and stored dry in closed plastic containers at 2°C.

Burial

From the 1987 experiment, three seed lots with different nitrate contents were divided into smaller lots of about 0·6 g (about 1500 seeds). These lots were packed in bags of fine mesh gauze, which were buried in sandy loam in a plastic net pot (diameter 10 cm), that provided good contact with the surrounding soil. To prevent loss of soil during handling the pots were lined with gauze. The soil surrounding the seeds prevented light from reaching the seeds during exhumation. The pots with the seeds were buried in the field in sandy loam at a depth of 10 cm.

Germination

Before burial on 4 December 1987 (week 0) and 8, 16, 29 and 53 weeks after burial, germination capacity of the seed lots was tested. Germination in week 0 was tested with triplicates of 50 seeds. Exhumed seed lots were divided into duplicate samples of varying number but close to 50. The exact number of seeds was determined afterwards by counting both germinated and non-germinated seeds.

Germination was assessed in cooled incubators (Gallenkamp, Crawley, UK, $T \pm 1$°C). Seeds were incubated in Petri dishes (diameter 50 mm) on one layer of filter paper (Schleicher and Schüll No. 595) moistened with the appropriate solution. Seeds were irradiated with a saturating 15-min dose of red light (620–700 nm). Red light was obtained by filtering light from six red fluorescent tubes (Philips TL 20W/15) through one layer of 3-mm plexiglas (red 501, Röhm and Haas, Darmstadt, FRG). The photon fluence rate at seed level was 11 μmol m^{-2} s^{-1}. Exhumed seeds were handled in dim green safelight, obtained by filtering light from one green fluorescent tube (Philips TL 40W/17) through two layers of yellow No. 46 and two layers of blue No. 62 Cinemoid filters (Strand Electric, London, UK).

Between 3 and 24 days after incubation, according to test temperature, when no additional germination occurred, both germinated and non-germinated seeds were recorded to determine final percentage germination. Protrusion of the radicle was the criterion for germination. Germination was tested in Milli-Q water and (on weeks 0, 8 and 53) 25 mmol l^{-1} KNO_3 at 2, 10, 15, 20, 25 and 30°C. Data were processed with the statistical package GENSTAT 5 (Procedure ANOVA; GENSTAT 5 Committee, Rothamsted Experimental Station, UK). The germinated fraction was transformed using an arcsine square root transformation to produce approximately normally distributed data with an equal variance. After ANOVA, least significant differences ($P<0.05$) were calculated and means (of transformed data) were compared. For ease of interpretation, data to be presented in tables and graphs were transformed back to percentages.

Nitrate measurements

Whenever seeds were exhumed for germination tests, subsamples of 50–100 mg (dry wt) were stored in 1·5 ml Eppendorf tubes at −20°C for later measurement of nitrate. In addition, in October 1988 seeds were exhumed for measurement of nitrate only (germination was not assessed). Seed moisture content was determined in a separate subsample of about 40 mg (dry wt) by weighing before and after drying for 1·5 h at 130°C. For measurement of nitrate in non-buried seeds, 100 mg of seeds were weighed in the Eppendorf tubes.

For nitrate measurement, seeds were homogenized in 1·5 ml of Milli-Q water with a tissue grinder (IKA-RW15, Janke und Kunkel KG, Staufen im Breisgau, Germany) for 1 min, after addition of some purified sea sand (Merck, Darmstadt, Germany). The homogenate was shaken for 15 min. After centrifugation for 15 min at 16 000 *g*, 750 μl of the supernatant was transferred to another Eppendorf tube containing 5 mg of activated charcoal CN-1 (Norit Clydesdale, Glasgow, UK). The Eppendorf tube was shaken for 15 min, centrifuged for 15 min at 16 000 *g* and the supernatant was passed through a MA 25 prefilter (Millipore, Etten-Leur, the Netherlands) (Hilhorst & Karssen 1989).

Nitrate of seeds exhumed in October and December 1988 was extracted similarly, but instead of a charcoal treatment, samples were purified by filtration over a small column

containing a few mg of Lichroprep RP-8 (particle size 25–40 μm, Merck, Darmstadt, FRG) on a MA 25 prefilter moistened twice with 500 μl of methanol. This purification step made the measurement more sensitive.

Samples of 20 μl were injected into a HPLC system (model 3500 B, Spectra Physics, Santa Clara, California, USA) equipped with a spectrophotometric detector (model 770, Spectra Physics) set at 210 nm and an integrator (model C-R1B, Shimadzu, Kyoto, Japan). The column was a Lichrosorb 10NH2 column (Chrompack, Middelburg, The Netherlands). The mobile phase consisted of 25 mM KH_2PO_4 pH 3·7 (Hilhorst & Karssen 1989). Nitrate levels were calculated on the basis of a linear relationship between concentration and peak height of nitrate standards. Nitrate was measured in duplicates and expressed on a dry weight basis.

Whenever seeds were exhumed, samples of the soil surrounding the seeds were dried on filter paper at room temperature and stored dry for later measurement of nitrate. Soil moisture content was determined in a separate subsample of about 40 g (dry wt) by weighing before and after drying for 8 h at 130°C. Air-dried samples were sieved over a 2-mm sieve and 3·0 g of soil was weighed out in a 40-ml polyethylene bottle. After addition of 30 ml of 0·01 mol l^{-1} $CaCl_2$ the bottles were shaken mechanically for 2 h. Subsequently, the bottles were centrifuged for 10 min at 2500 rpm (Houba *et al.* 1986). The supernatant was diluted five times and nitrate was determined after reduction to nitrite on a Cu–Cd column on an autoanalyser (Technicon Ltd, Swords Co., Dublin, Ireland) equipped with a sample controller (model 222, Gilson, France) at 550 nm. Soil nitrate content was expressed on a dry weight basis.

Total N measurement

Total N content was determined after wet digestion of dried samples by a modified Kjeldahl technique. The digestion mixture was made by dissolving 3·5 g selenium powder in 1 litre of concentrated H_2SO_4. Subsequently, 72 g of salicylic acid was dissolved in 1 litre of the sulphuric acid–selenium mixture. In a digestion tube, 2·5 ml of the digestion mixture was added to 300 mg of seeds dried for 16 h at 30°C. After 2·5 h the digestion tubes were placed in a heating block for another 2 h at 100°C. After cooling, three 1 ml aliquots of H_2O_2 (30%) were added at 10-s intervals, with careful but thorough mixing after each addition. Subsequently, the tubes were heated for 2 h at 330°C. The clear digest was made up to a 50 ml volume with demineralized water.

In the diluted digest total N was determined colorimetrically at 660 nm by a standard autoanalyser method (Technicon Ltd, Swords Co., Dublin, Ireland) equipped with a sample controller (model 222, Gilson France) (Anonymous 1978). Total N content was expressed on a dry weight basis.

Additional laboratory experiments

Germination of two seed lots from the 1987 experiment from plants that were fertilized with 250 ml of 5 and 119 mmol l^{-1} KNO_3 was also tested after pretreatment in Petri dishes in an incubator instead of outdoors in soil. Seeds of the two seed lots were pretreated for 41 days at 2°C in Milli-Q water of 0·1, 0·5, 1, 10 and 25 mmol l^{-1} KNO_3. After the pretreatment, seed nitrate content was determined in a duplicate sample after rinsing the seeds three times with Milli-Q water and germination was tested at 24°C with triplicates of 50 seeds. Differences in nitrate content of the two samples after pretreatment were tested using a distribution-free sign test (Hollander & Wolfe 1973).

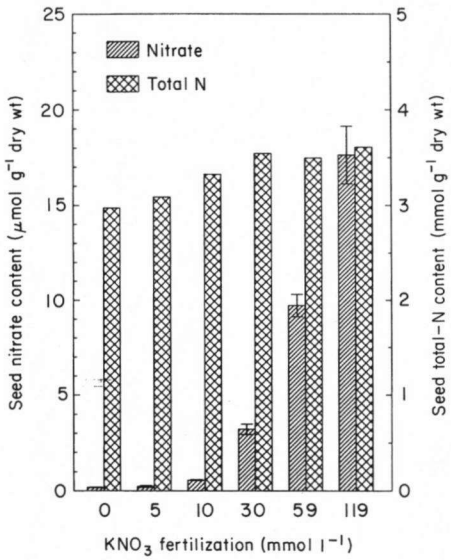

Fig. 1. The effect of KNO_3 fertilization of *Sisymbrium officinale* plants on nitrate and total N content of seeds produced. The plants were cultivated in pots in the open field and watered as necessary. Starting from the moment of flowering, once a week 250 ml KNO_3 solution was applied per plant. For each treatment, four plants were used. After harvest the seeds of these four plants were combined. Nitrate contents are means of duplicates ± standard error (vertical bars). Total N was measured in one sample only.

In the 1988 experiment, seed nitrate content was determined for each plant separately. After a pretreatment of 40 h at 15°C in Milli-Q water in darkness, triplicates of 50 seeds were irradiated with red light for 15 min and incubated at 24°C in water to test germination.

RESULTS

Application of KNO_3 increased the endogenous nitrate content of the seeds considerably (Fig. 1). The total N content increased by about 20%.

The red-light induced germination of the *S. officinale* seeds from the 1988 experiment showed a linear correlation (r=0·89) with the logarithm of the endogenous nitrate content (Fig. 2).

Burial

From the 1987 harvest, three seed lots were selected to be buried. They were from plants fertilized with 0, 30 and 119 mmol l^{-1} KNO_3, respectively, resulting in low, medium and high endogenous nitrate content: N_{low}, N_{med} and N_{high} (see Fig. 1 for data). Before burial, germination of the three seed lots differed significantly and correlated with endogenous nitrate levels (Fig. 3a; see Table 1 for statistical evaluation of means for all temperatures tested). Application of KNO_3 increased germination of the N_{low} and N_{med} seeds but not of N_{high} seeds (compare Fig. 3b with 3a; Table 1). Evidently, the endogenous nitrate of N_{high} was already saturating. Despite application of 25 mmol l^{-1} KNO_3, germination of the three seed lots still differed significantly (Table 1). The differences in germination of exhumed seeds were much smaller than for non-buried seeds (Fig. 3c, e).

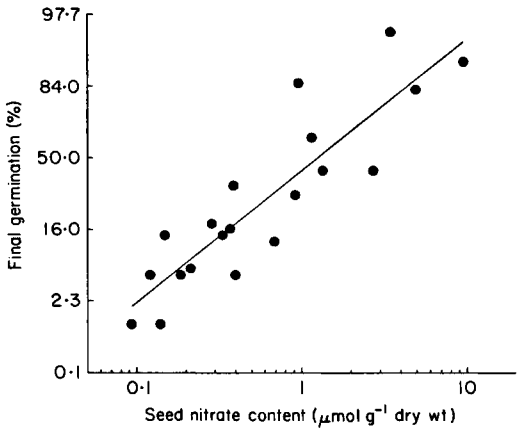

Fig. 2. Relationship between germination (probit scale) and endogenous nitrate content (log scale) of *Sisymbrium officinale* seeds. Triplicates of 50 seeds were pretreated for 40 h at 15°C in Milli-Q water, irradiated with 15 min red light and subsequently incubated at 24°C. Percentage germination was determined after 3 days. $y=4{\cdot}75^{*}\ x+1{\cdot}84$ (probits); $r=0{\cdot}89$.

Table 1. Changes in germination of three seed lots of *Sisymbrium officinale* with different initial nitrate contents: N_{low}, N_{med} and N_{high} obtained by weekly fertilization of flowering *S. officinale* plants with 250 ml of 0, 30 and 119 mmol l^{-1} KNO_3 respectively. Seeds were buried in December 1987 at a depth of 10 cm and exhumed after the indicated weeks of burial. Germination was tested in Milli-Q water (W) or 25 mmol l^{-1} KNO_3 (N) at 2, 10, 15, 20, 25 and 30°C. Before the test, seeds were irradiated with 15 min red light. Data are means for all temperatures. Means and significance of differences were calculated with ANOVA (see text for explanation). On each exhumation date, percentages germination followed by different letters are significantly different ($P<0{\cdot}05$)

Weeks of burial	Germination medium	Germination, % seed lot		
		N_{low}	N_{med}	N_{high}
0	W	3a	33b	70d
	N	27b	52c	69d
8	W	15a	38b	42b
	N	100c	100c	100c
16	W	89a	96b	95b
29	W	11ab	6a	16b
53	W	36a	42a	64b
	N	60b	67b	73b

Dormancy of *S. officinale* seeds was relieved during winter, re-induced in spring and again relieved in the next autumn (Fig. 4). The expression of the dormancy state depended on the temperature during the germination test. By early February, after 8 weeks of burial, germination at 2°C in water of all three seed lots had increased (Fig. 2a), whereas at 25°C, germination of M_{med} and N_{high} was lower than before burial (Fig. 4b). Like dormancy, sensitivity to nitrate fluctuated. By the end of January, seeds were extremely sensitive to nitrate, i.e. addition of nitrate strongly increased germination

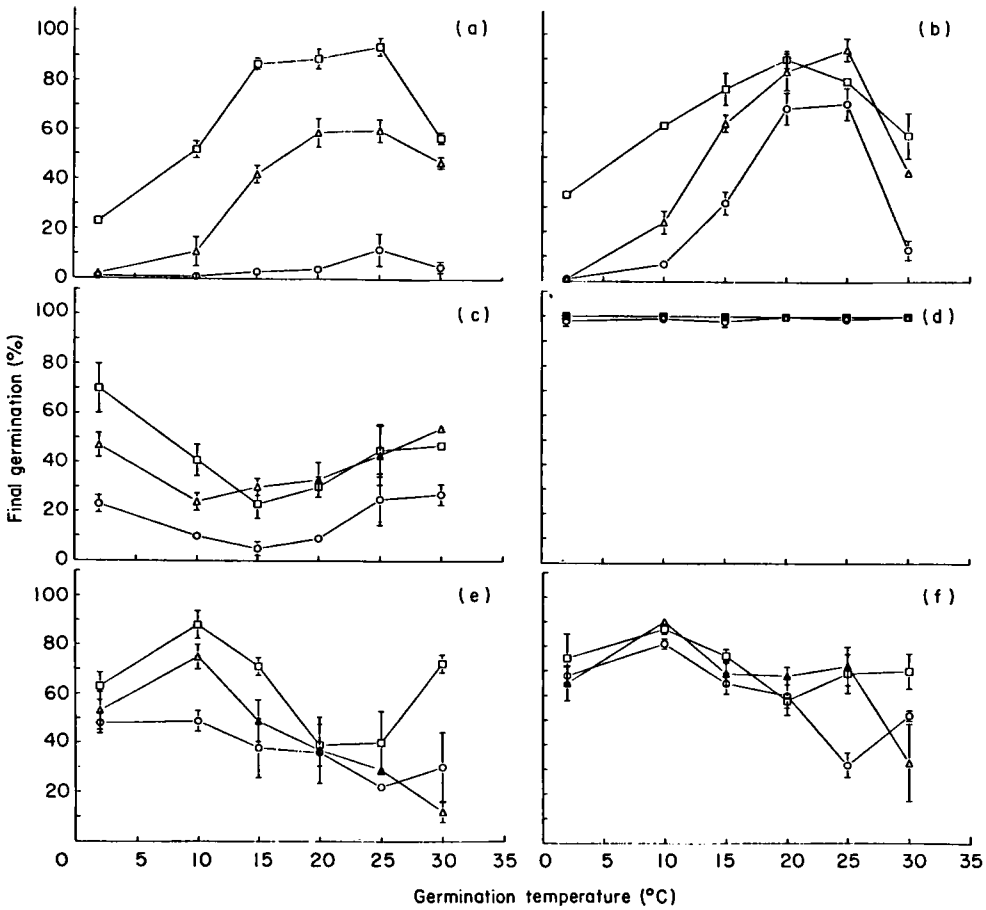

Fig. 3. Effect of a range of temperatures (indicated at the abscissa) and burial on germination of three seed lots of *Sisymbrium officinale* with low (○), medium (△) and high initial nitrate content (□) obtained by weekly fertilization of flowering *S. officinale* plants with 250 ml of 0, 30 and 119 mmol l^{-1} KNO_3, respectively. Seeds were buried in December 1987 at a depth of 10 cm and exhumed after 0 (a,b), 8 (c,d) and 53 weeks of burial (e,f). Germination was tested in Milli-Q water (a,c,e) and 25 mM KNO_3 (b,d,f). At the beginning of the test, seeds were irradiated with 15 min red light. Results are means of triplicates of 50 (a,b) or duplicates of approximately 50 seeds (c–f). Only standard errors (vertical bars) exceeding 2% are shown.

(Fig. 3c, d), whereas at the end of the experiment sensitivity was much lower (Fig. 3e, f). The optimal temperature for germination shifted from 20–25°C (non-buried seeds) to 2–10°C after a few months of burial.

Figure 5 shows the endogenous nitrate content of *S. officinale* seeds during burial. Endogenous nitrate was completely lost during the first weeks of burial. The nitrate content on 29 January was below the detection level. On later exhumation dates, endogenous nitrate could be detected again in the seeds. However, there were no consistent differences between the seed lots, nor seemed there to be a correlation between nitrate levels in seeds and soil.

The relationship between endogenous nitrate levels and germination was also studied under controlled conditions. Seeds of N_{low} and N_{high} lots—from plants fertilized with 5 and 119 mmol l^{-1} KNO_3, respectively (Fig. 1)—were incubated for 41 days at 2°C in

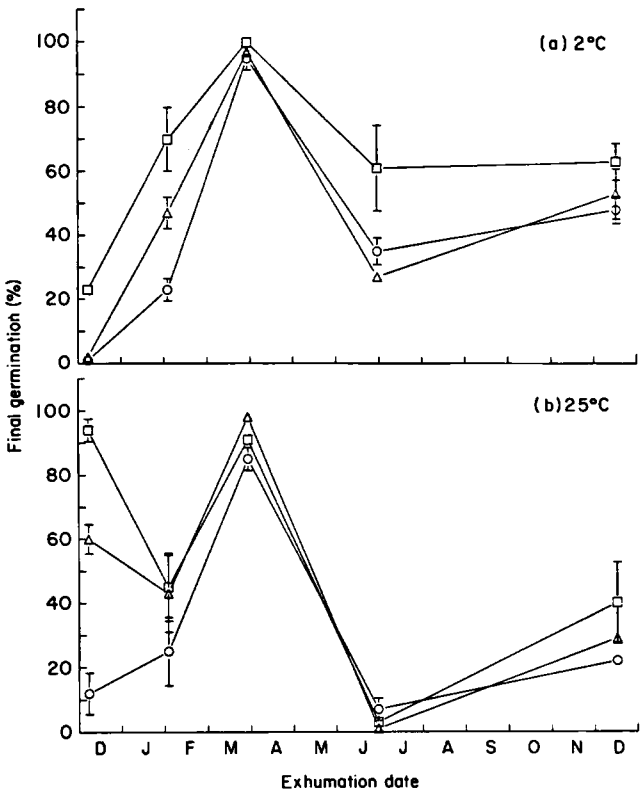

Fig. 4. Changes in the germination capacity of three seed lots of *Sisymbrium officinale* with low (○), medium (△) and high initial nitrate content (□) during burial in soil (data partly from Fig. 3). Seeds were buried in December 1987 at a depth of 10 cm and exhumed on the indicated dates. Germination was tested in Milli-Q water at 2 (a) and 25°C (b). At the beginning of the test, seeds were irradiated with 15 min red light. Results are means of triplicates of 50 (December 1987) or duplicates of approximately 50 seeds (on other dates). Only standard errors (vertical bars) exceeding 2% are shown.

a range of KNO_3 concentrations. After pretreatment in water or in KNO_3 concentrations not exceeding 1 mmol l^{-1}, the nitrate content of the seeds was below or close to the detection level (Fig. 6). Nevertheless, seeds germinated up to 100% at 0·5 and 1 mmol l^{-1} KNO_3. If seeds were pretreated in 10 or 25 mmol l^{-1} KNO_3, detectable amounts of nitrate were present in the seeds. Despite the high KNO_3 concentration in the imbibition medium N_{high} seeds still contained a slightly, but significantly higher nitrate level than the N_{low} seeds ($P<0·025$).

DISCUSSION

Endogenous nitrate in seeds

Fertilization of *S. officinale* plants with KNO_3 increased the nitrate content of seeds produced. There was a positive relationship, with a high correlation between endogenous nitrate levels and germination of non-buried seeds (Figs 2 and 3a) as was also reported by Fawcett & Slife (1978) and Saini *et al.* (1985) for *C. album*.

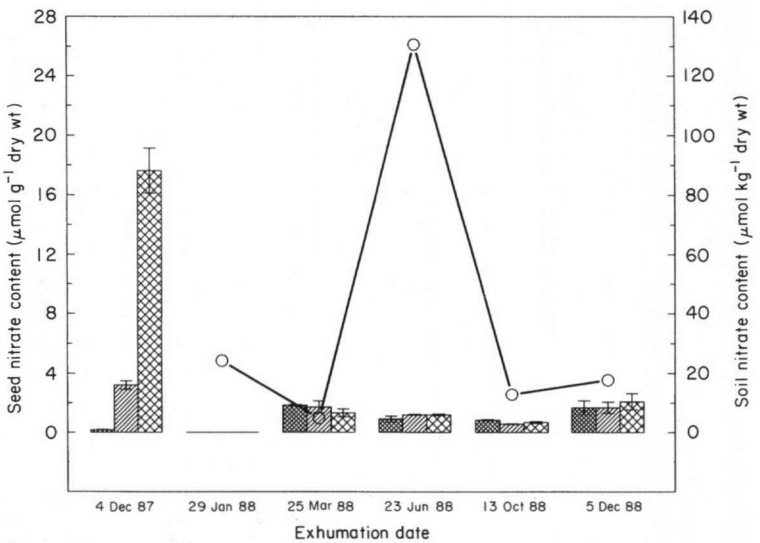

Fig. 5. Changes in endogenous nitrate content of seeds of *Sisymbrium officinale* with low (▩), medium (▨) and high (⊠) initial nitrate content (obtained as described for Fig. 3) during burial in soil. Also shown is the nitrate content of the soil (solid line). Soil nitrate was measured in one sample only. Seed nitrate contents are means of duplicates. Vertical bars indicate standard error.

Differences in nitrate content in seeds of annual weeds and ruderals may occur under field conditions. The experiments strongly suggest that they depend on differences in nitrate levels in soil during seed development. However, differences in nitrate accumulation in *S. officinale* seeds were much higher than those reported for *C. album* (Fawcett & Slife 1978; Saini *et al.* 1985; Bouwmeester 1990) and found in seed lots of *S. officinale* collected in the vicinity of Wageningen (H.W.M. Hilhorst, personal communication).

In the same field trial in which Fawcett & Slife (1978) obtained seeds of *C. album* with different endogenous nitrate contents, they found no difference in nitrate contents of seeds of *Abutilon theophrasti.* In parallel experiments with *Polygonum persicaria* we found no relationship between applied KNO_3 and seed nitrate level. Nitrate levels in seeds of *P. persicaria* collected both in trials and in the field were also about 10 times lower than in seeds of *C. album* grown under similar conditions (data not shown).

Differences between species in the accumulation of nitrate in seeds may be caused by differences in uptake or metabolism of nitrate. Van Beusichem *et al.* (1987) found rather large differences in the partition of nitrate reductase activity (NRA) over roots and shoot between pea (*Pisum sativum*), maize (*Zea mays*) and sunflower (*Helianthus annuus*). In pea, 45% of the NRA occurred in roots in contrast to 37% and 20% for maize and sunflower, respectively. Low NRA in roots may lead to higher nitrate contents in seeds, because more (un-reduced) nitrate is transported to the shoot.

Effects during burial

S. officinale seeds showed a clear dormancy pattern (Fig. 4) that was similar to previous results (Karssen 1980/81; Bouwmeester & Karssen 1989, 1993b). Between

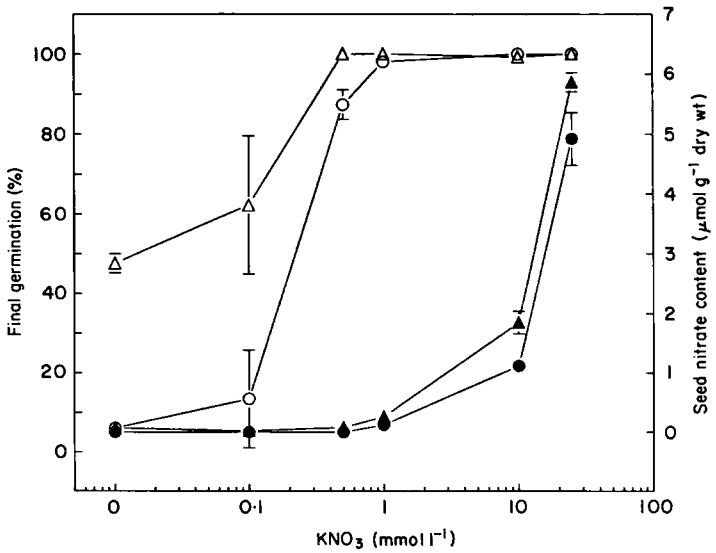

Fig. 6. Germination (open symbols) and nitrate content (closed symbols) of seeds with a low (circles) and high initial nitrate content (triangles), obtained by weekly fertilization of flowering *S. officinale* plants with 250 ml of 5 and 119 mmol l^{-1} KNO_3 respectively, after a pretreatment for 41 days at 2°C in Petri dishes in a range of KNO_3 concentrations. After 41 days, seeds from two Petri dishes were rinsed three times with Milli-Q water and surface-dried on a Büchner funnel. They were stored at −20°C for later nitrate measurement. Germination of seeds from three other dishes (50 seeds per dish) was tested at 24°C after 15 min red light, in the KNO_3 solution in which they were pre-incubated. Only standard errors (vertical bars) exceeding 2% (germination) or 0·1 μmol g^{-1} (nitrate content) are shown.

burial and the first exhumation date, germination of N_{high} and, less obviously, N_{med} seeds decreased when germination was tested at 25°C but not at 2°C (Fig. 4). At first sight this seems to indicate dormancy induction being visible at 25°C but not at 2°C. This observation was in contrast to previous burial experiments that showed that at the low winter temperatures during December and January, dormancy of *S. officinale* seeds was relieved (Karssen 1980/81; Bouwmeester & Karssen 1989, 1993b). These seemingly conflicting results are probably caused by nitrate leaching directly after burial, which was largest from N_{high} seeds (Fig. 5). Also, Hilhorst (1990b) hypothesized that part of the apparent dormancy induction at 15°C in water in Petri dishes was caused by nitrate leaching. The fact that germination of N_{high} in January at 2°C was higher than at the beginning of the experiment in spite of nitrate leaching (Fig. 4a) is probably attributable to the shift in optimum germination temperature for *S. officinale* to lower values, which occurs during the first winter after burial (Fig. 3; Bouwmeester & Karssen 1993b).

Hilhorst (1990b) showed that most of the endogenous nitrate leached from *S. officinale* seeds within the first 24 h of imbibition in water at 15°C. From *C. album* seeds, 97% of endogenous nitrate had leached within 7 days of imbibition in water at 2°C (Bouwmeester 1990). Hilhorst (1990a) hypothesized that nitrate in seeds is only loosely bound to structures on or close to the seed's covering tissues and therefore rapidly leaches upon imbibition. The presence of nitrate in the imbibition medium delays nitrate leaching (Fig. 6) and therefore may be slower in soil leaching. Nevertheless, the large differences in nitrate content and germination capacity that existed between seed lots at the beginning of the experiment also diminished during burial (Figs 3, 4 and 5; Table 1).

It is worth nothing that, apart from 29 January 1988, the nitrate content of buried seeds was always around 1–2 μmol g^{-1} (Fig. 5). This content is well above the minimum content required for germination, as shown in Fig. 2. Nevertheless, germination clearly fluctuated and even ceased in June (Fig. 4b) despite the fact that, by then, seed nitrate content was above 1 μmol g^{-1} (Fig. 5). Apparently, the nitrate content of the seeds is not the major factor regulating seasonal differences in germination. Derkx & Karssen (1993) showed that changes in dormancy in *S. officinale* seeds were caused by changes in light as well as nitrate sensitivity. The latter is confirmed by Fig. 3: the extent to which exogenous nitrate stimulated germination varied over the year. Nitrate sensitivity was low in unburied seeds (Fig. 3a, b), increased during winter (Fig. 3c, d) and decreased again during summer (Fig. 3e, f). A cold pretreatment in Petri dishes also increased nitrate sensitivity (Fig. 6). Moreover, the cold treatment increased nitrate sensitivity to such an extent that seeds germinated up to 50–100% at endogenous nitrate levels of less than 0·1 μmol g^{-1} (Fig. 6). Such a nitrate content would have been too low for germination of unburied seeds (Fig. 2).

Ecological importance of nitrate

The nitrate concentration in the soil solution fluctuates and can vary from virtually 0 to 50 mmol l^{-1} due to agricultural practices, plant growth and differences in moisture content and mineralization (Nye & Tinker 1977; Young & Aldag 1982). Because of variation in nitrate availability to the mother plant (spatially as well as in time) differences in nitrate content between seeds may occur. Immediately after seed shedding there may be enhanced germination but, upon imbibition, endogenous nitrate leaches and seed nitrate content may become a function of the surrounding soil nitrate (Goudey *et al.* 1988).

After 1 year's burial, N_{high} seeds still germinated at significantly higher percentages than N_{low} and N_{med} seeds (Table 1). Apparently, there is a long-lasting after-effect of the initial high nitrate content on germination of *S. officinale* seeds. However, as mentioned before, the endogenous nitrate level of the N_{high} seeds was extremely high as compared with samples collected in the field. Therefore, it is unlikely that increased nitrate levels in seeds under field conditions have a long-lasting effect on germination. The ecological advantage of this effect is that germination of seeds depends more on actual soil-nitrate levels than on levels present during the seed's development. The actual nitrate level may act as an indicator of suitable conditions for survival of seedings, e.g. gaps in the vegetation as suggested by Pons (1989).

Evidently, the strong changes in nitrate sensitivity of the seeds are of even greater ecological importance. Germination of seeds in periods of the year that are unfavourable for survival (summer for *S. officinale*) is prevented by strongly decreased nitrate sensitivity (=dormancy). Hence, germination is prevented during these periods even when there are high levels of soil nitrate, which would otherwise be enough to induce germination.

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