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# Seed Germination in *Chenopodium album* L.

## RELATIONSHIPS BETWEEN NITRATE AND THE EFFECTS OF PLANT HORMONES

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## ABSTRACT

**Effects of ethylene, gibberellins, and kinetin on the germination of two lots of *Chenopodium album* L. seeds, collected from the field in 1982 and 1983, were studied in relation to the availability of nitrate. The experiments were conducted in darkness and at temperatures ranging from 12 to 32°C. Ethylene induced over 75% germination in the 1983 seed but had little effect on the 1982 seed. Nitrate was only slightly promotive in either of the two seed lots. A combination of ethylene and nitrate, however, acted synergistically on 1982 seed, resulting in as much germination as that induced in 1983 seed by ethylene alone. In 1983 seed, a combination of ethylene and nitrate was only marginally more effective than ethylene. A similar relationship was observed in the effects of gibberellic acid$_{4+7}$ ($GA_{4+7}$) and nitrate on seeds from the two lots. The 1982 seed, which responded synergistically to combinations of nitrate with ethylene or $GA_{4+7}$ was found to contain an extremely low endogenous level of nitrate as compared to 1983 seed. Thus, high levels of either endogenous or applied nitrate appeared to enhance the germination response to ethylene or $GA_{4+7}$.**

**Kinetin had no effect on 1982 seed and only a small promotive effect on 1983 seed. There was no synergism between kinetin and nitrate in either of the seed lots.**

Seeds of many important weeds remain dormant for variable periods and may accumulate in soil to population densities as high as 10 to 100 million/ha (15, 18). Since these seeds exhibit sporadic release from dormancy, repeated measures are required to keep the weed population under control. If these seeds could be induced to germinate synchronously, weed control would become much more efficient. A variety of chemicals are known to break seed dormancy (2, 13), but ethylene and nitrate are particularly suitable for field application because of their low cost and lack of deleterious effects on the environment. We are investigating the effects of these chemicals on seed germination in *Chenopodium album* L. (Lamb's-quarters) with a view towards the development of strategies for a better control of this weed through enhanced and synchronized germination.

*Chenopodium album* is ranked among the most serious weeds of several major crops throughout the world (1). Freshly harvested seeds of this species are dormant. Although their germination has been shown to be promoted by ethylene (8, 11, 17) or nitrate (6, 14), the effectiveness of these chemicals has been found to be highly variable and to be modified by environment. These considerations have made it difficult to determine the feasibility of using ethylene or nitrate to enhance seed germination in the field.

The initial experiments reported here were done to assess whether a combination of ethylene and nitrate could induce uniformly high germination in this seed. These investigations led to the information on the influence of the endogenous and exogenous availability of nitrate on the response of seeds to hormonal treatments.

## MATERIALS AND METHODS

*Chenopodium album* L. seeds were collected in the fall of 1982 and 1983 from the University of Alberta Parkland Farm and kept in dry storage in airtight glass bottles at −24°C. These storage conditions did not affect the degree of dormancy over the period of experimentation.

Ethylene was administered either as ethephon (2-chloroethylphosphonic acid; Union Carbide) or in the gaseous form. Seeds were exposed to gaseous ethylene by placing open Petri plates inside flow-through plexiglass chambers, connected to a continuous flow of humidified air containing known amounts of ethylene. Potassium nitrate and $GA_{4+7}$ were dissolved in distilled $H_2O$. Kinetin was dissolved in 1 N HCl and diluted with distilled $H_2O$ to the HCl concentration of 0.001 N. In experiments with kinetin as a treatment, the concentration of HCl in solutions of other chemicals was also was made to 0.001 N and the pH of each solution was adjusted to 6.0 with 1% (w/v) NaOH.

Seeds were imbibed in 10-cm Petri plates, each containing one layer of Whatman No. 3 filter paper and 7 ml of the relevant solution or distilled $H_2O$. Each plate contained 100 to 150 seeds in all experiments, except that the interaction between $GA_{4+7}$ and nitrate was studied using 50 seeds per plate. Plates were sealed with parafilm to minimize moisture loss, and were incubated in the dark at temperatures ranging from 12 to 32 ± 0.1°C. The seeds were considered germinated when the protrusion of the radicle was visible with the naked eye. The extent of germination was recorded after 10 d of incubation. In the experiments where the rate of germination was studied, germination counts were taken from independent sets of plates on each day. This was done to eliminate any possible effect of seed movement during counting on germination.

Nitrate in the aqueous extracts of ground seeds was estimated by reduction to nitrite at 27°C in the methyl viologen system (10), using a commercial supply or purified *E. coli* nitrate reductase (Sigma Chemical Co.).

## RESULTS AND DISCUSSION

**Interactions between Exogenously Applied Plant Hormones and Nitrate.** *Ethylene and Nitrate.* Effects of ethephon (500 mg $l^{-1}$), $KNO_3$ (10 mM), or a combination of the two, applied to the two seed lots in the dark are shown in Figure 1. Less than 3% of the 1982 seeds germinated in controls at any of the temperatures

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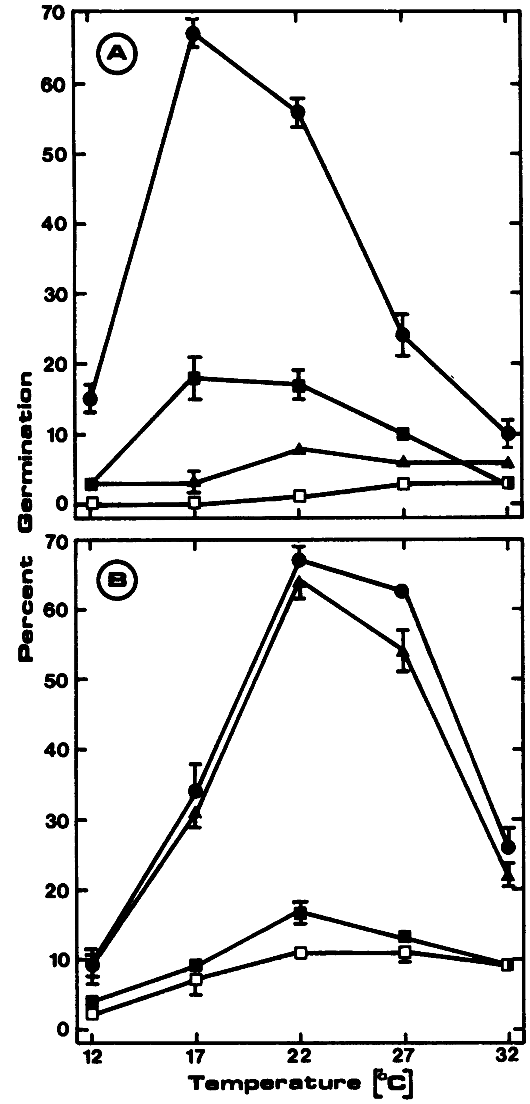

FIG. 1. Effect of ethephon (500 mg $l^{-1}$) and $KNO_3$ (10 mM) on the germination of *C. album* seeds incubated for 10 d at a range of temperatures in the dark. A, 1982 seed; B, 1983 seed. (□), Control; (▲), ethephon; (■), $KNO_3$; (●), ethephon + $KNO_3$. Vertical bars, SE.

between 12 and 32°C (Fig. 1A). Ethephon or $KNO_3$ slightly promoted germination when applied singly to these seeds. A combination of the two, however, acted synergistically resulting in 67% germination at 17°C.

Germination in the controls of 1983 seed was slightly higher than those of the 1982 seed (Fig. 1B). Potassium nitrate had no significant effect on 1983 seed at any temperature except 22°C where it promoted germination to 17% as compared to 11% germination in controls. The maximum germination, at respective optimum temperatures, following $KNO_3$ treatment was identical among the two seed lots. In contrast to 1982 seed, ethephon alone was as effective on 1983 seed as a combination of ethephon and $KNO_3$ was on either one of them (*cf.* Fig. 1, A and B). It was only at 27°C that the effect of a mixture of $KNO_3$ and ethephon on 1983 seed was statistically greater than that of ethephon alone (Fig. 1B).

The promotive effects of ethephon and nitrate were temperature dependent with optima at 17 and 22°C for 1982 and 1983 seed, respectively (Fig. 1, A and B). All the subsequent experiments were conducted at the optimum temperature for each seed lot.

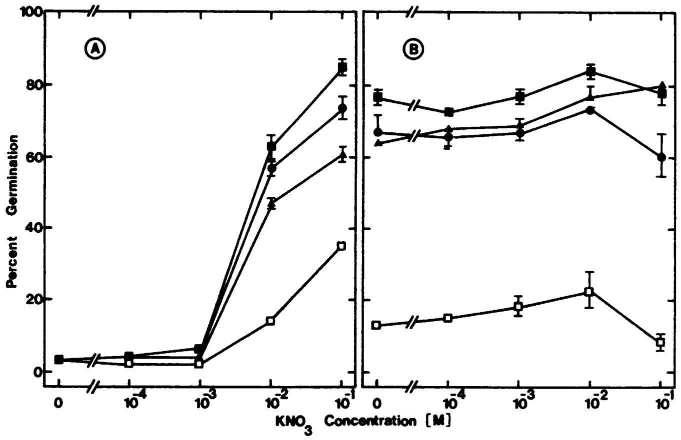

FIG. 2. Effects of a range of concentrations of ethephon and $KNO_3$ on the germination of *C. album* seeds incubated in the dark for 10 d. A, 1982 seed incubated at 17°C; B, 1983 seed incubated at 22°C. (□, ▲, ■, ●), Ethephon concentrations of 0, 10, 100, and 500 mg $l^{-1}$, respectively. Vertical bars, SE.

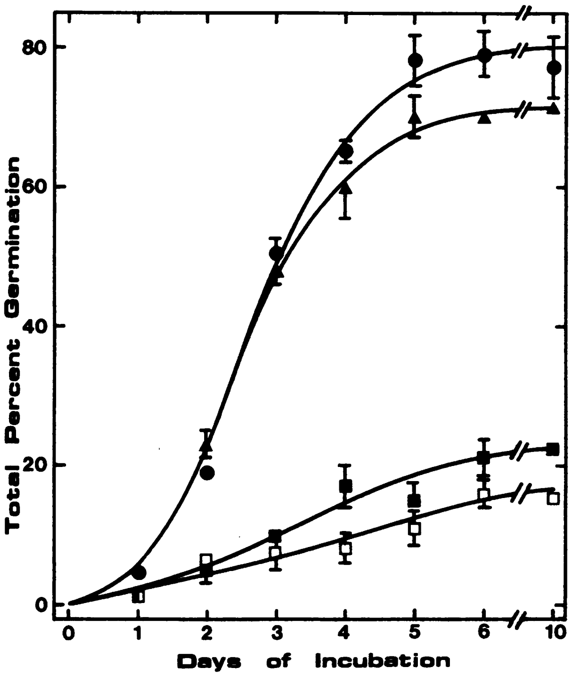

FIG. 3. Rates of germination of 1983 seed of *C. album* in controls (□) and following treatment with $KNO_3$ (■), ethephon (▲), and ethephon + $KNO_3$ (●). Seeds were incubated in the dark at 22°C. Concentrations of $KNO_3$ and ethephon were 10 mM and 100 mg $l^{-1}$, respectively. Vertical bars, SE.

Figure 2 shows the responses of the two lots of seeds to a range of concentrations of ethephon and $KNO_3$. When applied alone, the ethephon concentrations between 0 and 500 mg $l^{-1}$ had little influence on the germination of 1982 seed (Figs. 1A and 2A). Potassium nitrate concentrations up to 1 mM were without effect whereas 10 and 100 mM promoted germination (Fig. 2A). A synergism between ethephon and $KNO_3$ required a concentration of at least 10 mM nitrate in the medium. The most effective ethephon concentration in the presence of $KNO_3$ was 100 mg $l^{-1}$.

All concentrations of ethephon were highly effective in promoting the germination of 1983 seed (Fig. 2B). The most effective ethephon concentration was again 100 mg $l^{-1}$. The effect of $KNO_3$, alone or combined with ethephon, was relatively minor

Table I. *Effect of $GA_{4+7}$, Kinetin, and $KNO_3$ on the Germination of C. album Seeds*

Concentrations of $GA_{4+7}$, kinetin, and $KNO_3$ were 0.5, 0.05, and 10 mM, respectively. Seeds were incubated for 10 d in the dark. Incubation temperatures were 17 and 22°C for 1982 and 1983 seed, respectively. Each value denotes mean ± SE.

| Treatment | Germination | | Treatment | Germination | |
|---|---|---|---|---|---|
| | 1982 Seed | 1983 Seed | | 1982 Seed | 1983 Seed |
| | % | | | % | |
| Control | 1.7 ± 1.2 | 14.0 ± 4.2 | Control | 1.2 ± 0.2 | 7.9 ± 2.0 |
| $GA_{4+7}$ | 18.0 ± 3.1 | 43.3 ± 1.3 | Kinetin | 0.9 ± 0.3 | 25.9 ± 3.7 |
| $KNO_3$ | 16.7 ± 1.8 | 24.7 ± 4.7 | $KNO_3$ | 17.5 ± 5.0 | 17.0 ± 2.0 |
| $KNO_3$ + $GA_{4+7}$ | 52.0 ± 3.1 | 52.7 ± 4.1 | $KNO_3$ + kinetin | 15.4 ± 2.3 | 35.6 ± 2.8 |

and reached its maximum at a concentration of 10 mM.

Approximately 80% of the seeds could be induced to germinate by the application of 100 mg $l^{-1}$ ethephon to either one of the seed lots provided that the 1982 seed was also given 100 mM $KNO_3$.

Effects of gaseous ethylene at a concentration of 10 $\mu l$ $l^{-1}$, alone or in combination with 10 mM $KNO_3$, were studied on both seed lots. The results were in complete agreement with those obtained with ethephon, indicating that the interaction between nitrate and ethephon was attributable to ethylene. It has already been shown (12) that the effect of $KNO_3$ on germination can be ascribed to the nitrate ion since nitrate promoted germination regardless of the accompanying cation, whereas a series of cations including potassium had no promotive effect.

The rate of germination was studied in the 1983 seed lot. Germination commenced within 24 h of the beginning of incubation and the maximum germination in response to each treatment was obtained within 6 d (Fig. 3). The promotion of germination by ethylene and nitrate was, therefore, not merely a consequence of an increased speed of germination. In all experiments, final germination was recorded after 10 d of incubation for convenience of counting. Although the rate of germination of 1982 seed was not determined experimentally, maximum germination was always obtained in less than 10 d.

A study was made of the interactions of $GA_{4+7}$ or kinetin with nitrate to determine whether there was a general relationship between nitrate availability and the effects of growth regulators that have been shown to promote germination.

*Gibberellins and Nitrate.* $GA_{4+7}$, applied at a concentration of 0.5 mM (8), caused a moderate promotion of germination of 1982 seed, as did $KNO_3$ (Table I). When the two were applied together, their effect was synergistic, resulting in 52% germination. In 1983 seed, however, $GA_{4+7}$ alone induced 43% germination and there was only a minor additional effect of $KNO_3$.

*Kinetin and Nitrate.* Kinetin (0.05 mM) had no effect on 1982 seed and slightly promoted the germination of 1983 seed (Table I). There was no synergism between kinetin and $KNO_3$ in either one of the seed lots.

**Endogenous Nitrate Levels in the Seeds.** The nitrate levels in the two lots of seed were widely different; the nitrate content of the 1982 and 1983 seed being 9.9 ± 0.6 and 830.2 ± 42.4 $\mu g$ $g^{-1}$ dry weight, respectively. The seed that displayed a positive interaction between the plant hormones and exogenous nitrate, therefore, contained low amount of endogenous nitrate. There was no detectable nitrite in either of the seed lots. The reasons for the marked difference in nitrate content of the two seed lots are not known, but it has been shown (5) that the levels of nitrate in seeds could vary with the levels in the soil.

Synergistic effects of exogenous supplies of ethylene and nitrate on the germination of *Spergula arvensis* L. (11) and an accentuation of the ethylene-induced promotion of germination by preincubation of *Portulaca oleracea* L. seeds in nitrate (3) have been reported. Although endogenous nitrate contents of the seeds were not estimated, these reports suggest that the interactions among the effects of plant hormones and nitrate may be inherent in a variety of plants.

Nitrate promotes the germination of a wide range of species (7, 13) but its effect is best realized in combination with other factors such as temperature manipulations or light (6, 9, 14, 19). In view of the interaction of nitrate with growth regulators (Fig. 1; Table I), one wonders whether the environmental factors interact with nitrate by altering the availability of endogenous hormones. Plant hormones and environmental factors can often elicit identical responses in seeds, but a causal relationship between the two remains a matter of much controversy (2).

It is pertinent to consider the agronomic implications of these findings. Ethylene has been applied in the field to induce the germination of *Striga asiatica* (4), but sufficient physiological data for a widespread use of ethylene are lacking. On the other hand, increased nitrate fertilization of soil does not necessarily lead to any immediate enhancement of germination (5, 16), although it may reduce the dormancy of the next generation of seeds (5). Our results suggest that application of ethylene in combination with the manipulation of soil nitrate levels could be useful in the induction of uniformly high germination, thereby improving the efficiency of weed control.

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