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Effects of Gibberellic Acid, Ethephon, and 1-Aminocyclopropane-1-Carboxylic Acid on Germination of *Amaranthus caudatus* Seeds Inhibited by Paclobutrazol

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Abstract. The specific inhibitor of gibberellin biosynthesis, (2RS,3RS)-1-(4-chlorophenyl)-4,4-dimethyl-2-(1,2,4-triazol-1-yl)pentan-3-ol (paclobutrazol), inhibited germination of *Amaranthus caudatus* L. seeds. Addition of gibberellic acid (GA_3), 2-chloroethylphosphonic acid (ethephon), or 1-aminocyclopropane-1-carboxylic acid (ACC) effectively antagonized inhibition. Ethephon was found to be the most efficient antagonist. The transfer of seeds after 1 day's incubation in paclobutrazol to solutions of GA_3 or ethephon reversed the inhibition, the effect increasing with increasing concentration of GA_3 or ethephon. Seeds incubated in paclobutrazol for 5 days decreased sensitivity to GA_3 and ethephon.

Gibberellins (GAs) are known to stimulate germination of dormant as well as nondormant seeds of several plant species (Bewley and Black 1982). The response of seeds to exogenous gibberellin may be regarded as an indicator of the role of GA in germination. One possible way to examine the role and function of gibberellins in germination is to observe the effect of inhibitors of GA biosynthesis on germination. Growth inhibitors such as (2-chloroethyl) trimethyl ammonium chloride (CCC) and 2′-isopropyl-4′-(trimethylammoniumchloride)-5′-methylphenyl-piperidine-1-carboxylate (AMO-1618) also inhibit the germination of seeds (Knypl 1967, Black 1969). These inhibitors block the formation of *ent*-kaurene (Graebe and Ropers 1978, Sembdner et al. 1980). More specific inhibitors such as α-cyclopropyl-α-(4-methoxyphenyl)-5-pyrimidinemethanol (ancymidol), 5-(4-chlorophenyl)-3,4,5,9,10-pentaaza-tetracyclo-5,4,$10^{2,6}$,$0^{8,11}$-dodeca-3,9-diene (tetcyclacis), and paclobutrazol, which inhibit formation of kaurenoic acid, are now known (Coolbaugh et al. 1978, Hildebrandt et al. 1982, Hedden and Graebe 1985). It was found that tetcyclacis inhibited germination of *Arabidopsis thaliana* (L.) Heynh. (Karssen et al. 1985) and A. *caudatus* L. seeds (Kępczyński 1986). The present research evaluates the interaction of paclobutrazol and gibberellic acid (GA_3), 2-chloroethylphosphonic acid (ethephon), and 1-aminocyclopropane-1-carboxylic acid (ACC) in the germination of *A. caudatus* L. seeds.

Materials and Methods

Commercially available nondormant seeds of *A. caudatus* L., harvested in 1982, were used for the experiments. Seeds were placed in 5-cm Petri dishes lined with filter paper moistened with 1.5 ml of distilled water or aqueous solutions of paclobutrazol alone or in combination with GA_3, ethephon, or ACC. In some experiments, seeds were incubated in solutions of paclobutrazol for 1 or 5 days, then transferred to the fresh medium: water or solutions of GA_3 or ethephon. The experiments were conducted in the dark at 24°C. All manipulations during dark incubation were performed under physiologically safe green light. Five replications with 50 seeds each were used, and each experimental variant was repeated. Seeds with radicles approximately 2 mm long were considered germinated. The number of germinated seeds was determined after time intervals specified for each experiment.

ACC was obtained from Sigma Chemical Company, ethephon from Union Carbide Agricultural Products Company, GA_3 from Polfa (Poland), and paclobutrazol (Cultar) from Imperial Chemical Industries.

Results

Paclobutrazol markedly affected the germination of *A. caudatus* seeds (Fig. 1). At 10^{-4} or 3×10^{-4} M, paclobutrazol almost completely inhibited germination after 3 days' incubation. To examine the interaction between gibberellic acid and paclobutrazol, seeds were incubated simultaneously in mixtures of the two compounds (Fig. 2A,B). At 10^{-4} M paclobutrazol, inhibition was completely reversed by 10^{-4} M GA_3, but at 3×10^{-4} M paclobutrazol, inhibition of germination was only slightly counteracted by 3×10^{-4} M GA_3. Inhibition caused by paclobutrazol could also be antagonized by the addition of ethephon (Fig. 2C,D). Ethephon at concentrations as low as 7×10^{-7} M almost completely counteracted the inhibition of germination by 10^{-4} M paclobutrazol. Compared to GA_3, ethephon was very efficient in reversing germination inhibition due to the presence of 3×10^{-4} M paclobutrazol. To determine if naturally produced ethylene is able to antagonize paclobutrazol-inhibited germination, ACC (a precursor of ethylene biosynthesis) was added. ACC effectively antagonized paclobutrazol-inhibited germination (Fig. 3). The inhibition due to 10^{-4} M paclobutrazol was completely antagonized by all concentrations of ACC evaluated, whereas inhibition by 3×10^{-4} M paclobutrazol was unaffected.

Preincubation of *A. caudatus* seeds in 10^{-4} or 3×10^{-4} M paclobutrazol

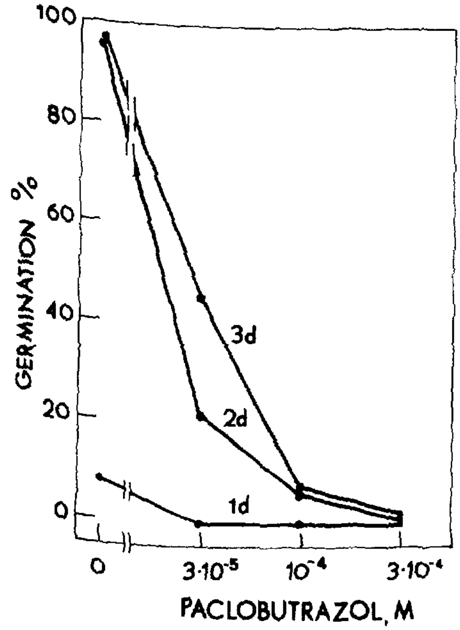

Fig. 1. The effect of paclobutrazol on germination of *A. caudatus* seeds in the dark at 24°C. 1d, 2d, 3d = days of germination. SD does not exceed ± 5%.

even for 1 day retarded seed germination (Fig. 4). Transfer of seeds that had been incubated for 1 day in paclobutrazol to solutions of GA_3 markedly reduced inhibition, the effect depending on the concentration of GA_3 (Fig. 4A,B). The transfer of seeds after 1 day's incubation in paclobutrazol to solutions of ethephon also antagonized inhibition, the effect increasing with increasing concentration of ethephon (Fig. 4C,D). However, the influence of ethephon on paclobutrazol inhibited germination was greater than that of GA_3 (Fig. 4). Prolonged incubation in paclobutrazol (5 days) made inhibition only partially reversible by GA_3 or ethephon (Fig. 5A,B). Again, ethephon was more effective than GA in this respect (Fig. 5).

Discussion

Our experiments with paclobutrazol suggest a role for endogenous gibberellins in the germination of *A. caudatus* seeds. Paclobutrazol, an inhibitor of gibberellin biosynthesis in seed tissues (Hedden and Graebe 1985), was found to be effective as an inhibitor of *A. caudatus* seed germination. The inhibition of seed germination due to the presence of paclobutrazol was markedly relieved by the addition of GA_3. Moreover, inhibition caused by the incubation of seeds for 1 day in paclobutrazol could also be overcome by GA_3. These facts, taken together with the inhibition of germination by tetcyclacis (an inhibitor of GA biosynthesis) and its reversal by GA_{4+7} (Kępczyński 1986), suggest that gibberellins have an important role in the germination of *A. caudatus* seeds.

Inhibition of seed germination caused by paclobutrazol was also antagonized by ethephon. It is worth noting that ethephon was more effective than GA

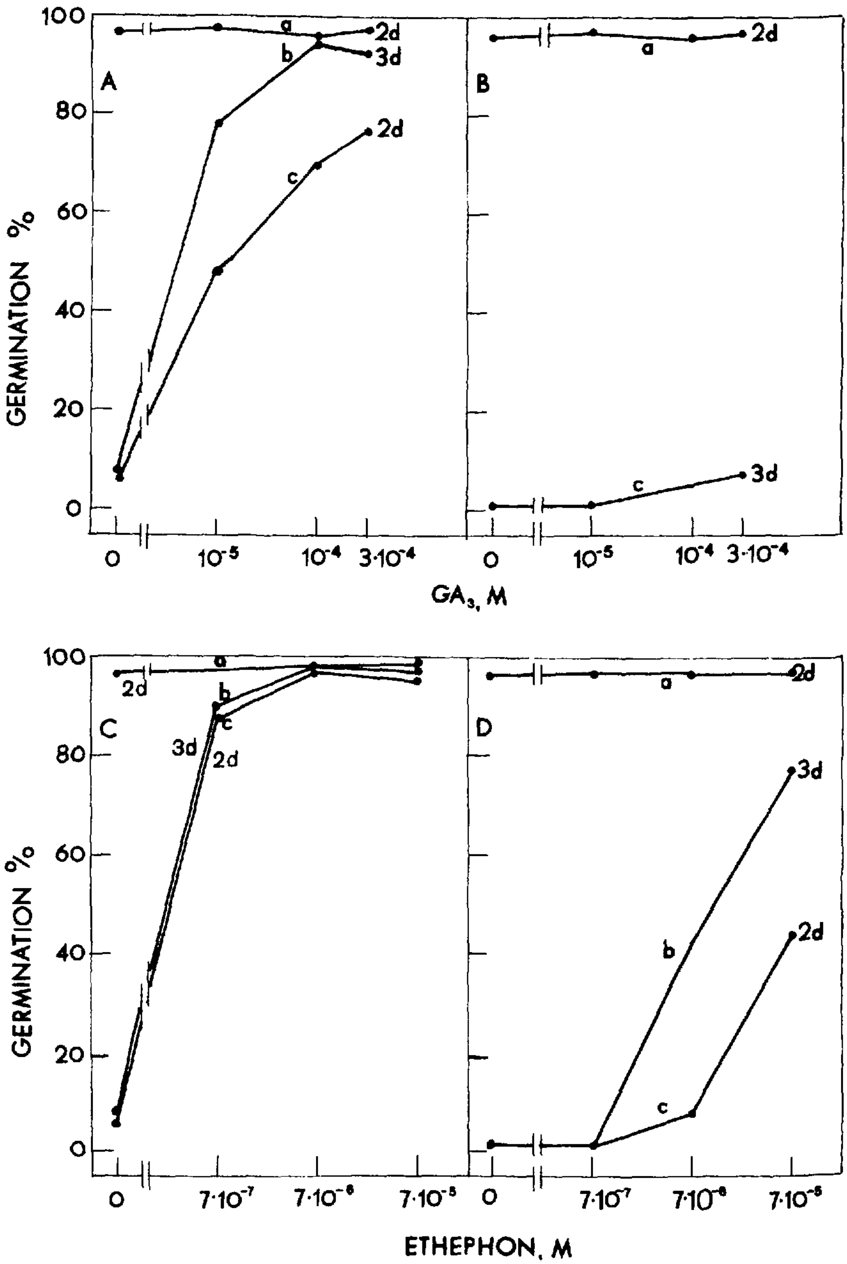

Fig. 2. The effect of GA_3 (A,B) or ethephon (C,D) on the inhibition of germination of *A. caudatus* seeds caused by paclobutrazol at 10^{-4} M (A,C) and 3×10^{-4} M (B,D). a—GA_3 or ethephon; b, c—paclobutrazol + GA_3 or ethephon. 2d, 3d = days of germination. SD does not exceed ± 11% (A), ± 6% (B), ± 14% (C), and ± 10% (D).

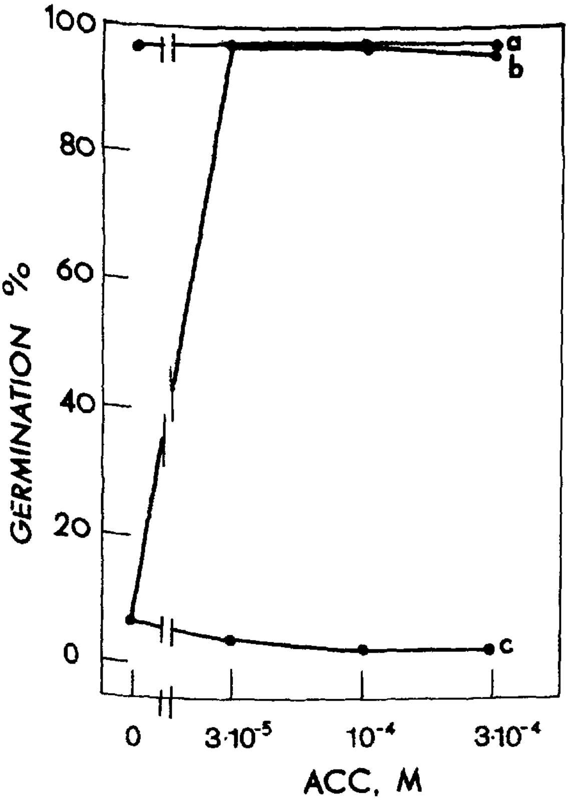

Fig. 3. The effect of ACC on the germination of *A. caudatus* seeds caused by paclobutrazol. a—ACC; b—ACC + 10^{-4} M paclobutrazol; c—ACC + 3 × 10^{-4} M paclobutrazol. Germination was recorded after 3 days. SD does not exceed ± 6%.

when applied simultaneously with paclobutrazol. Likewise, ethephon was found to be more efficient in reversing inhibition of *A. caudatus* (Kępczyński 1986) and *A. paniculatus* (Kępczyński and Kępczyńska 1988) seed germination due to the presence of tetcyclacis or paclobutrazol, respectively. Paclobutrazol-dependent inhibition can also be counteracted by ACC, a precursor of ethylene in *A. caudatus* seeds (Kępczyński and Karssen 1985). Reversal of paclobutrazol inhibition by ethephon or ACC appears to support the idea that gibberellins can be replaced by ethylene produced from ethephon or ACC (Kępczyński 1986).

Blocking the biosynthesis of endogenous gibberellins with prolonged preincubation (5 days) in paclobutrazol reduced the sensitivity of *A. caudatus* seed to exogenously applied GA$_3$ or ethephon. Possibly, long exposure to paclobutrazol impairs also sterol biosynthesis that cannot be overcome by GA$_3$ or ethylene. It was found that sterol biosynthesis in wheat is inhibited by the 2R,3R enantiomer of paclobutrazol (Lenton 1987).

The results reported here suggest that paclobutrazol inhibits *A. caudatus* seed germination as a result of its inhibitory effect on gibberellin biosynthesis. Data from the present paper and from Kępczyński (1986) support (1) the requirement of gibberellins for the germination of *A. caudatus* seeds and (2) the possibility of substituting endogenous gibberellins with exogenous GA or ethylene released from ethepon or synthesized from exogenously applied ACC.

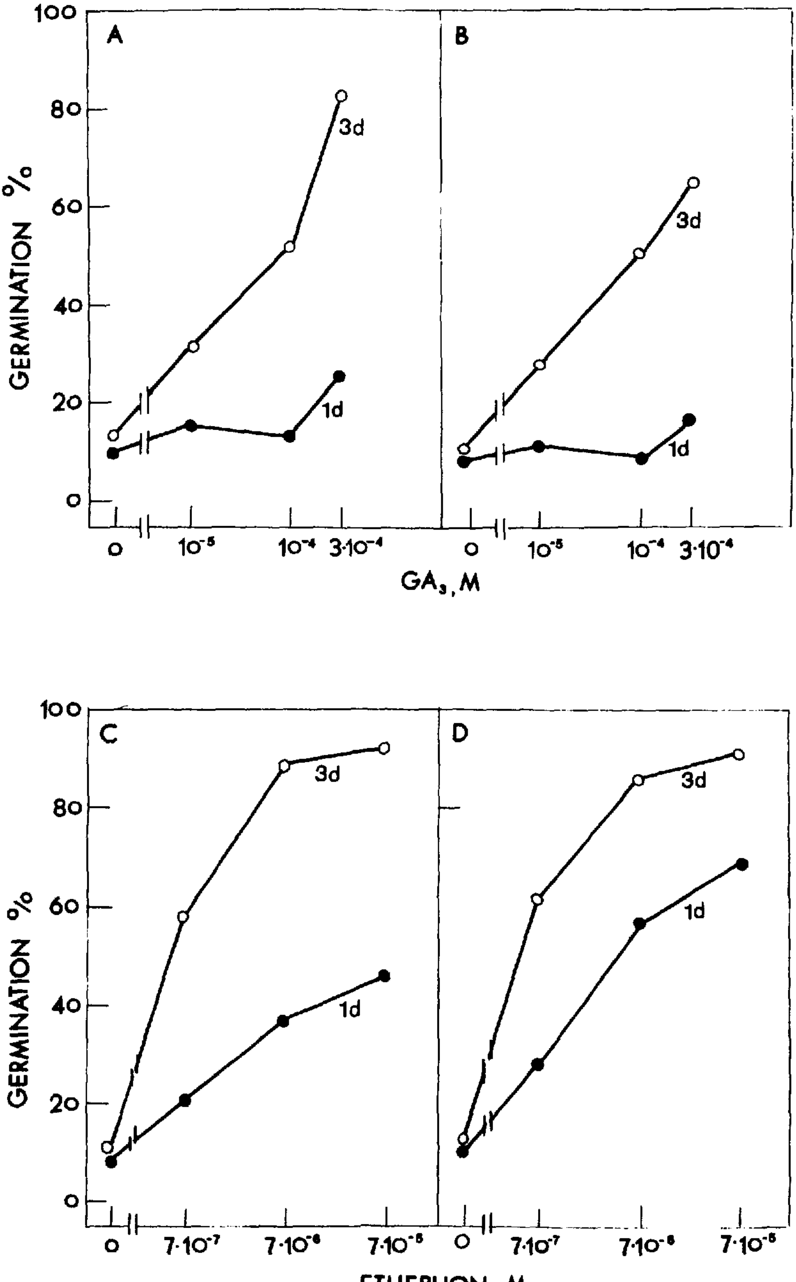

Fig. 4. The effect of GA_3 (A,B) or ethephon (C,D) on the germination of *A. caudatus* seeds preincubated 1 day in 10^{-4} M (A,C) or 3×10^{-4} M (B,D) paclobutrazol. 1d, 3d = days of germination after transfer. SD does not exceed ± 11%.

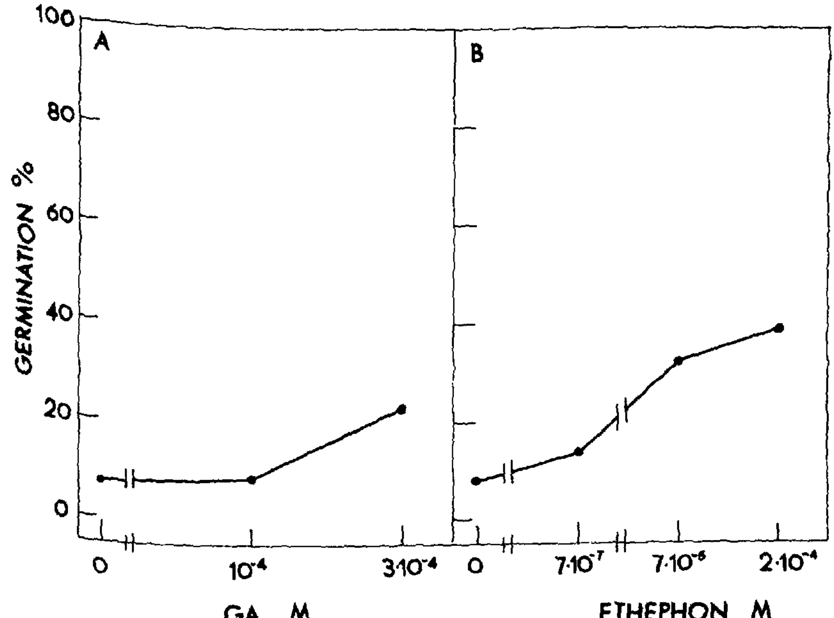

Fig. 5. The effect of GA$_3$ (A) or ethephon (B) on the germination of *A. caudatus* seeds preincubated 5 days in 3×10^{-4} M paclobutrazol. Germination was recorded 4 days after transfer. SD does not exceed ± 17%.

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