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Response of *Amaranthus retroflexus* L. seeds to gibberellic acid, ethylene and abscisic acid depending on duration of stratification and burial

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Abstract Freshly harvested, dormant seeds of *Amaranthus retroflexus* were unable to germinate at 25 and 35 °C. To release their dormancy at the above temperatures, the seeds were stratified at a constant temperature (4 °C) under laboratory conditions or at fluctuating temperatures in soil or by outdoor burial in soil. Fully dormant, or seeds stratified or buried (2006/2007 and 2007/2008) for various periods were treated with exogenous gibberellic acid (GA_3), ethephon and abscisic acid (ABA). Likewise, the effects of these regulators, applied during stratification, on seed germination were determined. The results indicate that *A. retroflexus* seed dormancy can be released either by stratification or by autumn–winter burial. The effect of GA_3 and ethylene, liberated from ethephon, applied after various periods of stratification or during stratification, depends on dormancy level. GA_3 did not affect or only slightly stimulated the germination of non-stratified, fully dormant seeds at 25 and 35 °C respectively. Ethylene increased germination at both temperatures. Seed response to GA_3 and ethylene at 25 °C was increased when dormancy was partially removed by stratification at constant or fluctuating temperatures or autumn–winter burial. The response to GA_3 and ethylene increased with increasing time of stratification. The presence of GA_3 and ethephon during stratification may stimulate germination at 35 °C. Thus, both GA_3 and ethylene can partially substitute the requirement for stratification or autumn–winter burial. Both hormones may also stimulate germination of secondary dormant seeds, exhumed in September. The response to ABA decreased in parallel with an increasing time of stratification and burial up to May 2007 or March 2008. Endogenous GA_n, ethylene and ABA may be involved in the control of dormancy state and germination of *A. retroflexus*. It is possible that releasing dormancy by stratification or partial burial is associated with changes in ABA/GA and ethylene balance and/or sensitivity to these hormones.

Keywords *Amaranthus retroflexus* · Burial · Germination · Plant hormones · Primary dormancy · Stratification

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Introduction

Seeds of numerous species are not able to germinate after harvest at conditions favorable for the germination process. These seeds are considered as primarily dormant. Such dormancy is induced during seed development and maturation on plants. Seed dormancy is common in wild plants; it may ensure the ability of species to survive natural catastrophes, and decrease competition between individuals of the same species (Finkelstein et al. 2008). Furthermore, it allows seeds of numerous weeds to persist in soil for many years. Under natural conditions, dormant seeds are exposed to fluctuating environmental factors e.g. light, temperature, moisture and the presence of gases which lead to cyclical changes in the dormancy state (Finkelstein et al. 2008). Seed dormancy can be released during burial, cold stratification and dry after-ripening, or by chemicals (Bewley and Black 1994). Dormancy release may involve changes in concentrations of abscisic acid (ABA), gibberellins (GA_s) and ethylene and/or a change in sensitivity to these hormones (Hilhorst 2007; Kępczyński and Kępczyńska 1997; Matilla 2000). Application of exogenous GA_3 and ethylene can partially or completely remove dormancy in seeds of many plant species.

Amaranthus retroflexus is an important weed, which infests soil in many parts of the world (Holm et al. 1997), including Poland. As has been shown, buried seeds of *A. retroflexus* can remain viable for at least 6–10 years (Costea et al. 2004). Dormancy levels of these seeds have been found to vary due to, among other factors, temperature during maturation, fertilization of soil and competition between cultivated plants (Costea et al. 2004). Dormant *A. retroflexus* seeds could germinate fully in darkness at 35–40 °C (Kępczyński et al. 1996) or only partially at 35 °C (Schonbeck and Egley 1981; Kępczyński et al. 2003b). Earlier studies have shown a role for plant hormones in regulating the dormancy state in *A. retroflexus* seeds. Ethylene, ethephon, an ethylene releasing compound, and 1-aminocyclopropane-1-carboxylic acid (ACC), a precursor of ethylene biosynthesis induced germination of primarily dormant *A. retroflexus* seeds (Schonbeck and Egley 1981; Kępczyński et al. 1996, 2003b). Likewise, gibberellic acid could remove dormancy in these seeds (Kępczyński et al. 2003b). It was also shown that dormancy release by GA_3 involved ethylene biosynthesis and action (Kępczyński et al. 2003b). However, there is no data comparing the effects of stratification and burial on releasing dormancy of *A. retroflexus* seeds. Similarly, no research has been conducted on the response of these seeds, originating from the same harvest, stratified or buried for various periods, to gibberellin, ethylene and abscisic acids.

The aim of the present study was to determine the effects of GA_3, ethephon or ABA (1) applied after various periods of stratification, during stratification at constant temperature (4 °C) or after stratification at fluctuating temperature and (2) applied after various periods of burial in soil at seasonal fluctuating temperature on *A. retroflexus* seed germination at 25 and 35 °C in darkness.

Materials and methods

Plant material

Amaranthus retroflexus L. seeds were collected from wild populations in September 2006, near Lubniewice in Poland. The inflorescences were stored in open air then shaken gently to remove seeds. 10 days after collection, clean seeds were stored at −20 °C until required.

Stratification

Experiment 1

Seeds (0.9 g) were placed in 12 cm diameter Petri dishes lined with three layers of filter paper moistened with 12 ml distilled water and sealed with parafilm. Petri dishes were placed in darkness at 4 °C for 20 weeks. Every 2 weeks the seeds were removed and the effects of GA_3 (10^{-4}, 3×10^{-4}, 10^{-3} M), ethephon (3×10^{-6}, 10^{-5}, 10^{-4} M) and ABA (10^{-6}, 10^{-5}, 10^{-4} M) on their germination at 25 and 35 °C in darkness determined.

Experiment 2

Seeds were incubated at 4 °C on 3 layers of filter paper moistened with distilled water or solutions of GA_3 (10^{-3} M), ethephon (10^{-4} M) and ABA (10^{-4} M) in 12 cm Petri dishes sealed with parafilm. After 4, 8 and 12 weeks of stratification, seed germination in water, at 35 °C, in darkness was determined.

Experiment 3

Seeds (0.9 g) were placed in 12 cm diameter Petri dishes lined with three layers of filter paper moistened with 12 ml distilled water, sealed with parafilm and covered with aluminum foil. Then, on 12 November 2007, the Petri dishes were placed outdoors at of 10 cm depth below the soil surface. After 4 weeks, the seeds were removed in darkness under green light and the effects of GA_3 (10^{-4}, 10^{-3} M), ethephon (10^{-5}, 10^{-4} M) and ABA (10^{-6}, 10^{-5}, 10^{-4}M) on germination at 25 and 35 °C in darkness determined. In 2007 the average temperature at 5 cm below the surface of soil was 4.0 (November) and 3.3 °C (December). The lowest temperature in November was −2.7 and in December −7.6 °C. Readings were taken by Szczecin Department of Institute of Meteorology and Water Management (IMGW-PIB).

Burial

On 6th November 2006 and 4th November 2007, nylon bags which contained 12 g of seeds, harvested in 2006, were placed in boxes made of metallic net. They were covered with 10 cm of soil taken from the field where the population of *A. retroflexus* plants used for seed collection had grown. After various periods of burial, seeds were removed in darkness and incubated in water, GA_3 (10^{-3} M), ethephon (10^{-4} M) and ABA (10^{-4} M) solutions in darkness at 25 or 35 °C.

Germination

Seeds (3 replications of 50) were incubated in 6 cm diameter glass Petri dishes on one layer of filter paper moistened with 1.5 ml distilled water or appropriate solutions of GA_3, ethephon or ABA. Seeds were incubated in darkness for up to 7 days. Germinated seeds were counted every day. Seeds were regarded as germinated when

the radicle protruded through the seed coat and was longer than 2 mm. All manipulations were performed under green light.

Results were presented as a percentage of seed germination after seven days of incubation. Furthermore, the rate of germination was presented according to Timson (1965) by calculating the value of Σ_7 ($\Sigma_7 = 700$ means that 100 % of seeds germinated on the first day).

Statistical analysis

The average ± standard deviation (SD) of three independent determinations from 50 seeds each are presented. Data have been analyzed for significance using one-way or two-way ANOVA (Statistica for Windows ver. 9.0, StatSoft Inc., Tulsa, Oklahoma, USA). Duncan's multiple range test was used for determination of significant differences between germination values ($P \leq 0.05$).

Results

Effect of GA_3, ethephon and ABA on seed germination after various periods of stratification

Germination at 25 °C

Freshly harvested *A. retroflexus* seeds did not germinate at 25 °C (Fig. 1a). Stratification increased seed germination percentage at 25 °C as time progressed. 16 and 20 weeks of stratification resulted in ca. 40 % germination. GA_3, a well-known dormancy releasing factor, did not affect germination of dormant, non-stratified seeds. GA_3, at its lowest concentration, i.e. 10^{-4} M, did not affect germination of seeds stratified up to 16 weeks and only slightly increased the percentage of germination after 20 weeks. However, a higher concentration of this stimulator, 3×10^{-4} M, improved germination beginning from week 4 until the end of the 20 week stratification period. After 16–20 weeks of stratification ca. 70 % of seeds were able to germinate due to the effect of GA_3 applied at the above concentration. An application of GA_3 at the highest concentration, 10^{-3} M, caused ca. 90 % of seeds to germinate after just 4 weeks of stratification; after this period only ca. 10 % of seeds could germinate in water.

Non-stratified seeds responded to ethephon at 10^{-4} M, the highest concentration used; ca 25 % of seeds germinated (Fig. 1a). This regulator, in all applied concentrations, markedly increased seed germination after various periods of stratification, the effect being dependent on its concentration and duration of stratification. The response to ethephon was more evident after 4 weeks of stratification. The stimulatory effect of ethephon at 3×10^{-6} and 10^{-5} M increased with the length of stratification and reached a maximum after 20 weeks; resulting in 80–90 % germination. The highest germination improvement was observed after ethephon application at 10^{-4} M; ca. 80 % of seeds germinated just after 8 weeks when in the control only about 10 % of seeds germinated. ABA, known to be responsible for seed dormancy maintenance, at 3×10^{-6} and 10^{-5} M slightly inhibited germination of partially stratified seeds; the effect decreased with increasing time of stratification (Fig. 1a). At 3×10^{-5} M, the highest concentration, ABA inhibited germination of stratified seeds for longer than 4 weeks. After 16–20 weeks of stratification only ca 10 % of seeds were able to germinate in the presence of ABA at the above concentration.

A beneficial effect of a prolonged period of stratification on seed germination was also evident when germination rate was considered (Fig. 2a). Stratification for the period between 4 and 20 weeks increase the rate of seed germination over 3 times. Only at its highest concentration did GA_3 increase the rate of germination of seeds stratified for various periods. Ethephon also increased the rate of germination of seeds stratified for various periods (Fig. 2a). After 4 weeks of stratification this compound was effective only at the highest concentration, but with an increasing duration of stratification a stimulatory effect was evident at lower concentrations of ethephon. At 10^{-5} and 3×10^{-5} M, ABA lowered the rate of germination but the effect was significant only in seeds stratified for 20 weeks (Fig. 2a).

Germination at 35 °C

Dormant seeds did not able to germinate at 35 °C (Fig. 1b). Stratification for only 4 weeks caused ca. 65 % of seeds to germinate. Extension of the stratification period did not markedly increase germination; germination reached 75 %. At 10^{-3} M, GA_3 caused ca. 25 % germination of non-stratified seeds. At concentrations below 10^{-3} M, this regulator did not affect germination of seeds stratified for various periods. However, GA_3 at 10^{-3} M allowed for 80–90 % seed germination independently of a period of stratification. Ethephon at 10^{-4} M increased germination of non-stratified seeds up to almost 40 % (Fig. 1b). This compound, applied even at the lowest concentration, i.e. 3×10^{-6} M, caused that almost all seeds were able to germinate after just 4 weeks of stratification; 90 % seeds germinated. At 3×10^{-6} M, ABA partially inhibited the germination of stratified seeds (Fig. 1b). ABA's inhibitory effect clearly occurred at 10^{-5} and 3×10^{-5} M concentrations, as only 10–15 % of seeds stratified for 4 weeks germinated in comparison to 65 % in the control. The extent of inhibition decreased with prolonged stratification.

The rate of germination at 35 °C (Fig. 3) was higher than at 25 °C (Fig. 2) after every period of stratification.

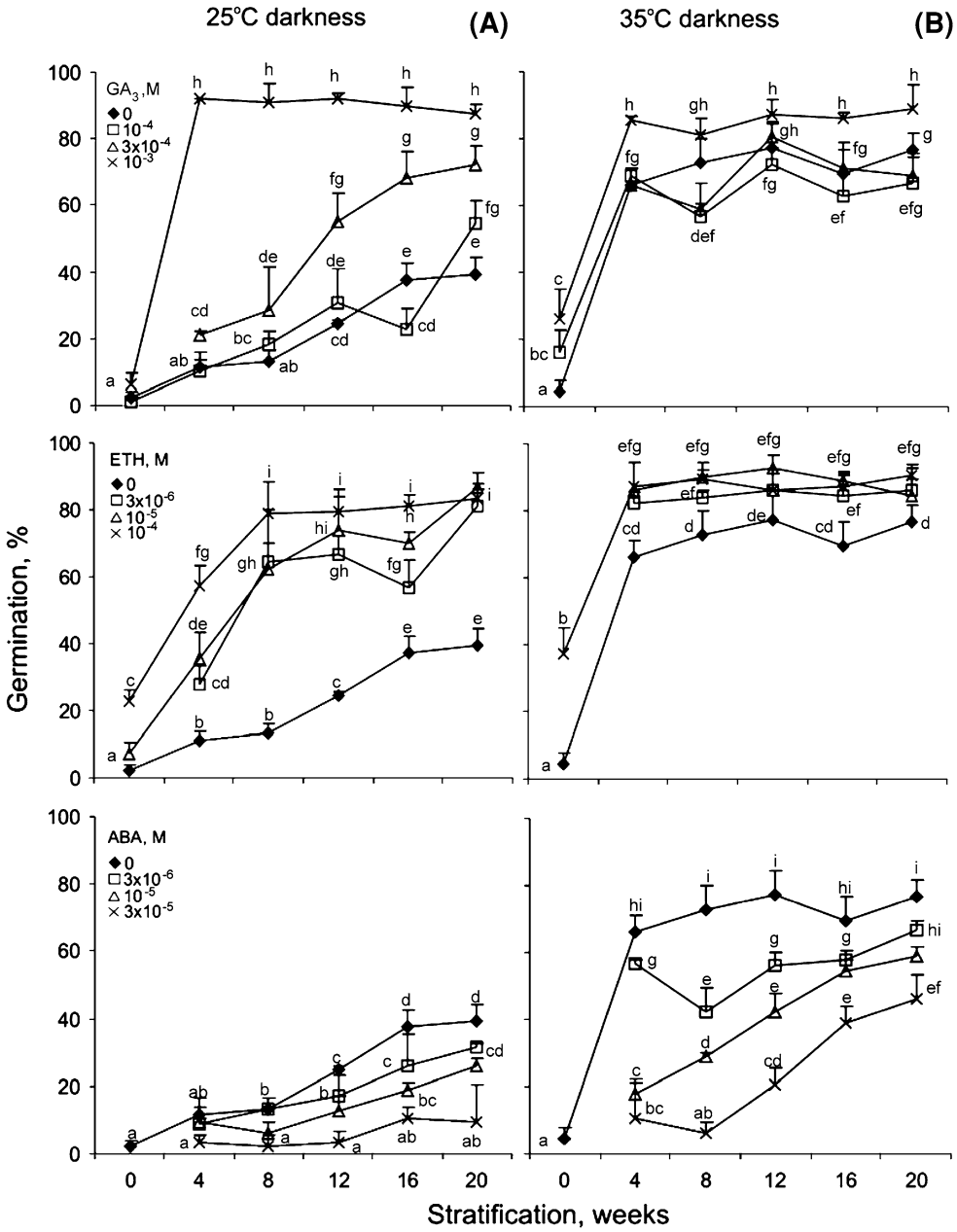
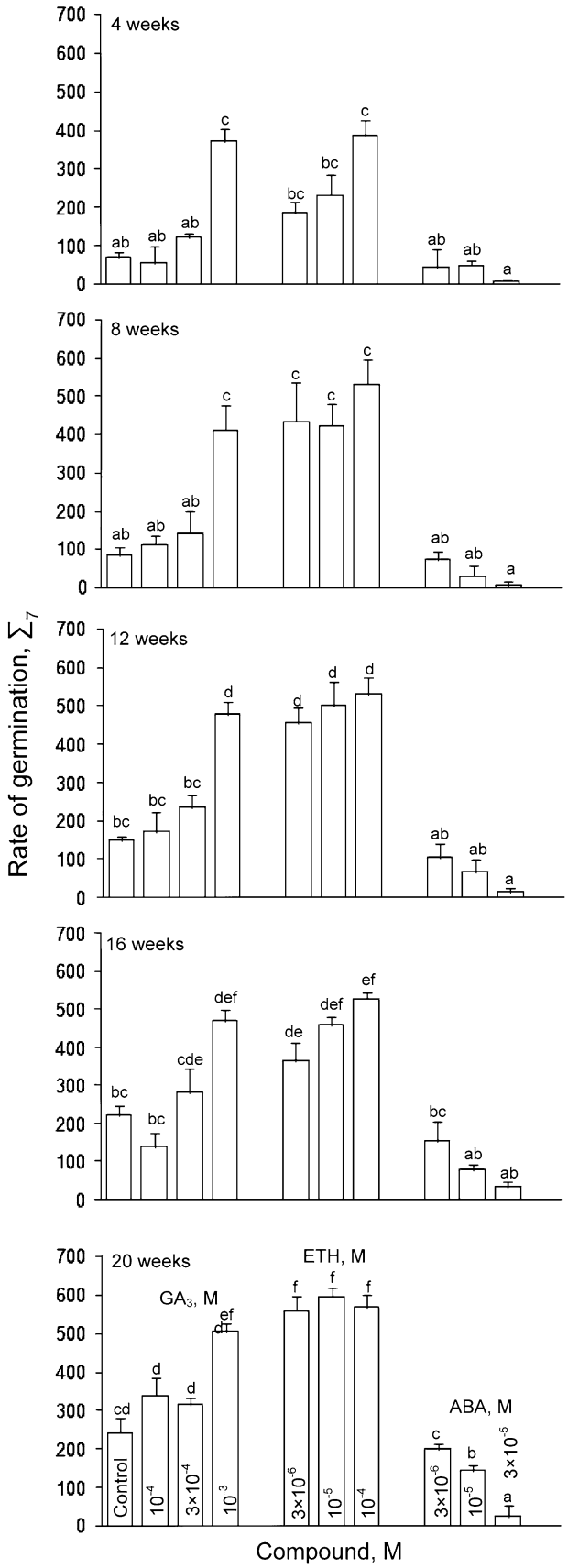


Fig. 1 Effect of GA_3, ethephon and ABA on percentage germination of *A. retroflexus* seeds at 25 (**a**) and 35 °C (**b**) after various periods of stratification at 4 °C. Germination was determined after 7 days of incubation. The *vertical bars* indicate ± SD. Two-way ANOVA with the Duncan post hoc test was used to determine significant differences. Mean values with *different letters* (*a–i*) are significantly different ($P \leq 0.05$)

Both GA_3 and ethephon did not or only slightly increased the rate of germination (Fig. 3). ABA markedly decreased the rate of germination of seeds stratified for 4–20 weeks when applied at 10^{-5}–3×10^{-5} M (Fig. 3).

Effects of GA_3, ethephon and ABA presence during stratification on subsequent seed germination

GA_3, ethephon and ABA were applied during stratification conducted for 4, 8 and 12 weeks, and then germination at 35 °C determined. Seeds stratified for 4 and 8 weeks germinated to 50 and 60 % respectively (Fig. 4a). After 12 weeks of stratification, 75 % of seeds germinated. GA_3 and ethephon allowed almost all seeds to germinate after stratification for 8 weeks. During the 4 week stratification period, the application of ABA resulted in markedly inhibited germination; around 20 % seeds germinated in comparison to 50 % in the control. The inhibitory effect of ABA disappeared after a prolongation of the stratification time. GA_3 and ethephon increased, whereas ABA decreased, the rate of germination after 4 weeks of stratification (Fig. 4b). After a prolonged stratification in the presence of ABA the germination rate was similar to that in control.



◀**Fig. 2** The effect of GA_3, ethephon and ABA on the rate of *A. retroflexus* seed germination at 25 °C after various periods of stratification at 4° C. The *vertical bars* indicate ± SD. Two-way ANOVA with the Duncan post hoc test was used to determine significant differences. *Columns* with *different letters* (*a–f*) are significantly different ($P \leq 0.05$)

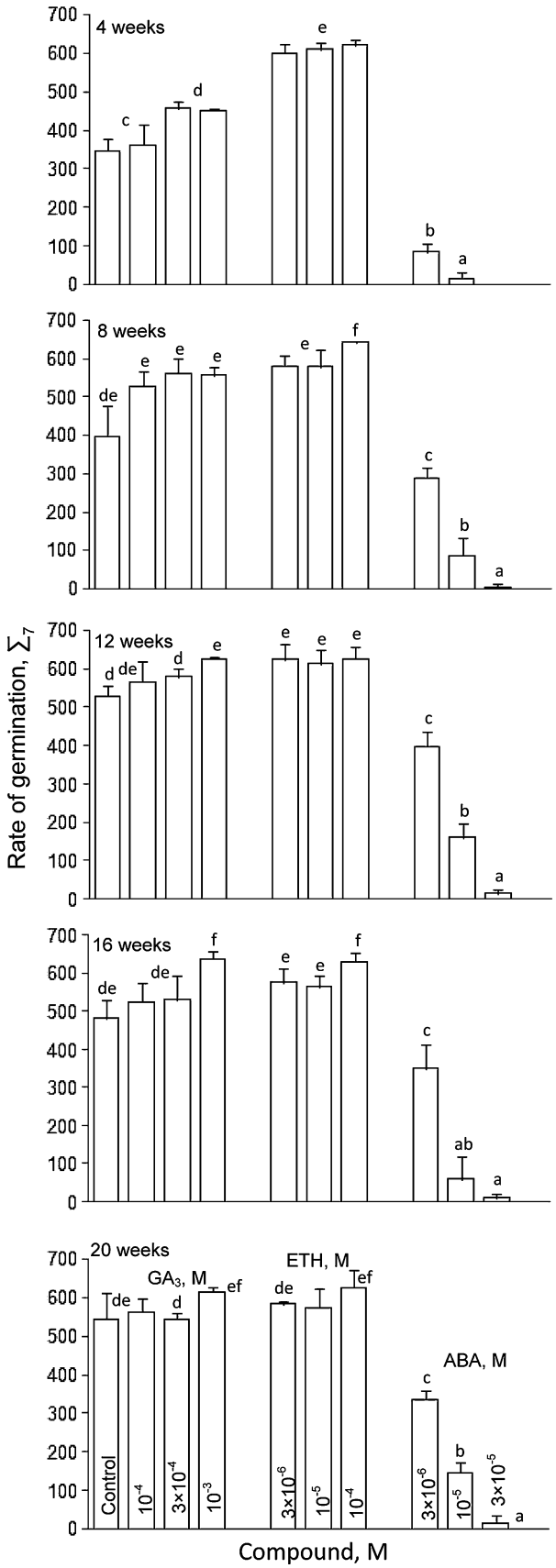
Effect of GA_3, ethephon and ABA on germination, at 25 and 35 °C, of seeds stratified under natural conditions in soil

Both GA_3 and ethephon, at the highest concentrations, increased the percentage of germination at 25 °C of seeds stratified in Petri dishes placed in soil for 4 weeks, from November to December (average temperature 5 cm below the surface of soil ranging 4.0–3.3 °C and minimal temperature ranging from −2.7 to −7.6 °C; Fig. 5a). GA_3 stimulated germination more effectively than ethephon, whereas ABA did not affect germination. At 35 °C, GA_3 and ethephon enabled the germination of almost all stratified seeds (Fig. 5c). ABA completely inhibited germination when applied at the highest concentration. GA_3 and ethephon strongly increased the rate of germination at 25 and 35 °C (Fig. 5b, d). Strong inhibition of seed germination rate resulting from the application of 10^{-4} M ABA occurred only at 35 °C (Fig. 5d).

Effect of GA_3, ethephon and ABA on seed germination after various periods of outdoor burial in soil

Germination at 25 °C

Seeds were buried for various periods and their germination in the presence of the various regulators at 25 °C was determined. One and two months of burial (with average temperatures ranging from 4.4 to 5.6 and minimal temperatures ranging from −1.2 to 2.3 °C; Fig. 6a) during the experiment started in November 2006, resulting in ca. 60 % seed germination at 25 °C (Fig. 6c). Prolonged burial until March and May 2007 increased germination to 90 and 75 %, respectively. Further extension of the stratification period decreased the percentage of seed germination. The lowest level of germination, 25 %, was noted in the case of seeds recovered in September 2007. The germination of recovered seeds increased again in December 2007 and reached 75 %. Both GA_3 and ethephon increased the germination of seeds exhumed from December 2006 to February 2007; 80–95 % seeds germinated. ABA lowered germination of the seeds buried from November 2006 till April 2007, from about 60 to 90 % in control to about 10–35 %. The effect of this hormone decreased with stratification time extended until May. ABA also markedly



◀**Fig. 3** The effect of GA_3, ethephon and ABA on the rate of *A. retroflexus* seed germination at 35 °C after various periods of stratification at 4° C. The *vertical bars* indicate ± SD. Two-way ANOVA with the Duncan post hoc test was used to determine significant differences. *Columns* with *different letters* (*a–f*) are significantly different ($P \leq 0.05$)

inhibited germination of seeds exhumed in December 2007. In the presence of ABA only ca. 20 % seeds germinated in comparison to 75 % of seeds in the control group. In the experiment started in November 2007, the highest level of germination at 25 °C was observed when seeds were buried during the period of December–March (average temperatures ranging 1.8–3.4 and minimal temperature ranging from −3.3 to 0.1 °C; Fig. 6b); the percentage of seeds that germinated reached 55–60 % (Fig. 6d). Then, similar to the previous experiment, their germination ability lowered during summer months and then increased again when seeds were removed from soil in December 2008. GA_3 enhanced germination of seeds removed from soil in January and February; 90 % of the seeds germinated. Ethephon increased germination of seeds buried up until March; 75–90 % of the seeds germinated. After an extended period of burial, however, seeds did not show such a sensitivity to these hormones. ABA inhibited germination of seeds exhumed in December 2007, January 2008 and February 2008 (Fig. 6d).

GA_3 and ethephon increased not only percentage germination at 25 °C, but also its rate (Fig. 8a).In contrast to ethephon, GA_3 did not affect the rate of germination of seeds removed from soil in December. Ethephon was more effective than GA_3 in increasing the rate of germination of seeds buried until January. Seeds recovered in February responded similarly to ethephon and GA_3 (Fig. 8a). ABA strongly inhibited the rate of germination during the whole burial period (Fig. 8a).

Germination at 35 °C

Germination at 35 °C of seeds buried from 2006 November until May 2007 ranged between 80 and 95 % and then markedly decreased to 20 and 30 % for seeds exhumed in June and September, respectively (Fig. 7a). Seeds removed from soil in December 2007 recovered the ability of germination and 80 % of seeds germinated. The burial of seeds from November 2007 until February 2008 resulted in germination of 80–95 % of seeds (Fig. 7b). Again, the -seeds showed lower germination after burial until September and an increase in germination percentage was observed in December. GA_3 and ethephon increased germination only in the case of seeds exhumed in September 2007 (Fig. 7a). In both experiments ABA inhibited germination of buried seeds at all periods (Fig. 7a, b). The

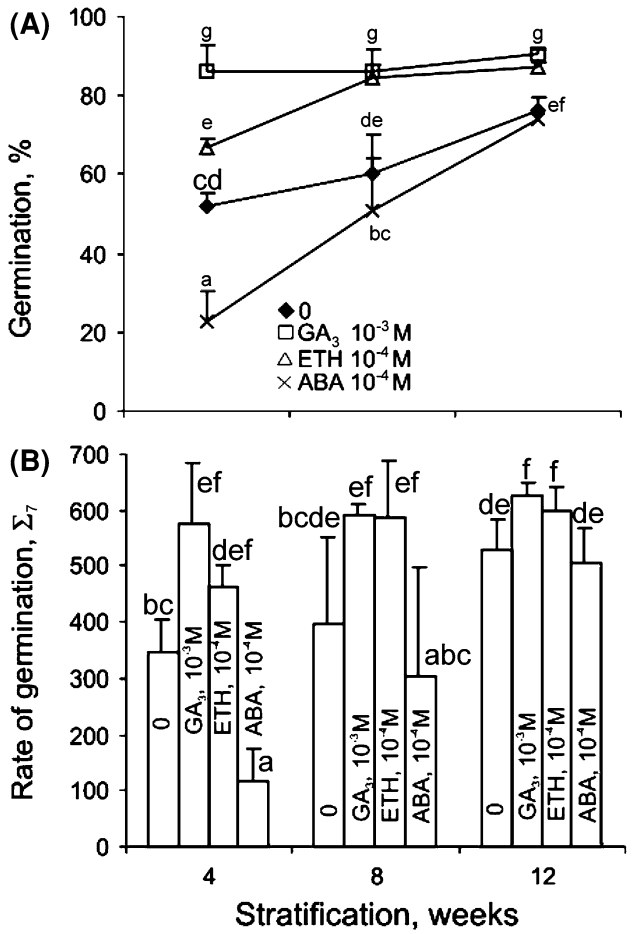


Fig. 4 The effect of stratification of *A. retroflexus* seeds at 4° C in the presence of GA_3, ethephon and ABA on percentage (**a**) and rate (**b**) of germination at 35 °C. The *vertical bars* indicate ± SD. Two-way ANOVA with the Duncan post hoc test was used to determine significant differences. Mean values with *different letters* (*a–g*) are significantly different ($P \leq 0.05$)

rate of germination also showed seasonal changes, being similar from December to March, lowered until September and increased again in December (Fig. 8b). GA_3 and ethephon did not affect the rate of germination excluding seeds removed in September. ABA strongly inhibited the germination rate of seeds buried for various periods.

Discussion

Burial

It has been demonstrated previously that primarily dormant seeds of *A. retroflexus* cannot germinate in darkness at 25 °C and can either germinate fully or partially at 35 °C (Kępczyński et al. 1996; Kępczyński et al. 2003b). In the present study, the seeds of *A. retroflexus* were not able to germinate at 35 °C in darkness, indicating a high level of primary dormancy (Fig. 1). These seeds were considered fully dormant. Primarily dormant seeds of *A. retroflexus*, similar to previous studies (Egley 1989), showed cyclic changes in dormancy as indicated by varying germinability following their recovery from soil after varying periods of burial. Primary dormancy of *A. retroflexus* seeds buried in 2006/2007 (Fig. 6c) and 2007/2008 (Fig. 6d) seasons was completely or partially removed, probably mainly due to low temperatures during late autumn and winter (Fig. 6a, b). A primary role played by seasonal soil temperature changes in temporal changes of seed dormancy of summer annual *A. retroflexus* had been postulated previously (Omami et al.1999). Decreased germination during summer months is probably associated with an induction of secondary dormancy in these seeds caused by increasing temperatures as has also been suggested by Egley (1989). A possible induction of secondary dormancy by high temperature has been shown in an experiment with *A. caudatus* seeds (Kępczyński and Bihun 2002). In addition, *A. quitensis* and *A. palmeri* seeds have shown a seasonal dormancy pattern (Faccini and Vitta 2005; Jha et al. 2010), indicating that the behavior of seeds kept in soil is characteristic for the genus *Amaranthus*. Similarly, dormancy of other annual species has been released due to a low temperature during burial and reinduced by a - high temperature (Karssen 1995). Burial at low temperatures, between November 2006 and May 2007 or between November 2007 and March 2008, enabled partial or total germination not only at 35 °C (Fig. 7), but also at a lower temperature, i.e. 25 °C (Fig. 6). Thus, releasing dormancy in these seeds was expressed in their different ability to germinate at the above temperatures. Low temperatures, during November and December, also removed secondary dormancy, since seeds were again able to germinate at 25 and 35 °C. The occurrence of such temporal changes in dormancy of buried *A. retroflexus* seeds has been interpreted as an adaptation to enhance survival when seeds are in the soil, especially when they are deeply buried (Omami et al. 1999). However, it has been shown that there is no close relationship between dormancy and persistence (Thompson et al. 2003).

Ethylene, liberated from ethephon, only partially released dormancy in fully dormant seeds (Fig. 1). Burial for the first months, from November 2006 to February 2007 and from November 2007 to March 2008, increased the - seed response to ethylene at 25 °C, but not at 35 °C. The seeds which passed into secondary dormancy due to burial during summer months, showed lower response to ethylene at 25 °C than primary dormant seeds. A similarly low response of secondary dormant *A. retroflexus* seeds to ethylene had been reported by Egley (1989). GA_3, in contrast to ethylene, very slightly affected fully dormant seeds only at 35 °C (Fig. 1). Seeds buried for various

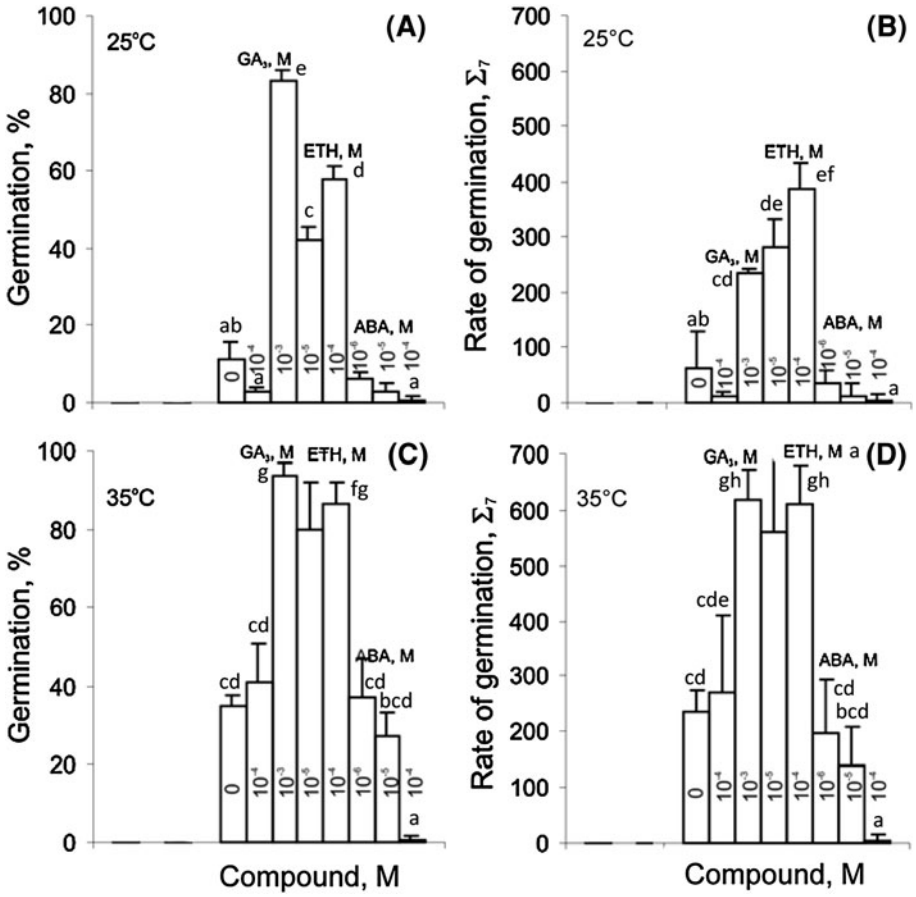


Fig. 5 The effect of GA_3, ethephon and ABA on percentage (**a, c**) and rate (**b, d**) of germination at 25 (**a, b**) and 35 °C (**b, d**) of *A. retroflexus* seeds after 4 weeks of stratification in soil. The *vertical bars* indicate ± SD. Two-way ANOVA with the Duncan post hoc test was used to determine significant differences. *Columns* with *different letters* (*a–h*) are significantly different ($P \leq 0.05$)

periods, showed similar responses to GA_3 and to ethylene (Figs. 6, 7). However, ethylene was more effective than GA_3 10 times lower concentration of ethephon than GA_3 was required. The response to ABA decreased until May 2007 (Fig. 6c) and March 2008 (Fig. 6d), and increased again when secondary dormancy was partially removed due to low temperature next autumn. Increased response of *A. retroflexus* seeds to exogenous ethylene and gibberellin might be associated with an increasing synthesis and/or increased tissue sensitivity to these hormones during the first months of burial. Conversely, releasing dormancy due to burial associated with a decreasing response of seeds to ABA may involve a lowering content and/or decreased sensitivity to this hormone. The above hypothesis can be supported by results of experiments with seeds of *Arabidopsis* ecotype Cape Verdi Isle (Cvi) (Footitt et al. 2011). Dormancy cycling in seeds of this plant was related to changing synthesis of ABA and GA_3. Dormancy increased during winter as soil temperature declined and the expression of ABA synthesis and gibberellic acid catabolism genes increased. Then dormancy declined in spring and summer, while endogenous ABA decreased and GA synthesis genes increased. It was also suggested that ABA signaling and sensitivity are more likely regulators of dormancy in these seeds than the absolute level of ABA.

Stratification

Stratification, an effective way of alleviating seed dormancy in summer annuals (Bewley and Black 1994), released *A. retroflexus* seed dormancy gradually over time (Fig. 1). Seeds stratified at 4 °C for 4 and 20 weeks were only partially able to germinate at 25 °C but germinated almost completely at 35 °C. Thus, stratification at a constant temperature (Figs. 1, 2, 3, 4) was less effective in releasing dormancy than autumn–winter burial of seeds (Figs. 6, 7, 8). Since stratification at naturally occurring fluctuating soil temperatures (Fig. 5) had a similar effect to stratification at a constant temperature in an incubator, other factors might probably be responsible for the difference between the effects of burial and stratification. In the soil, seeds are exposed not only to fluctuating temperatures, but also to several other factors such as compounds solutions, gases and soil microorganisms, which may affect the dormancy state of seeds. Microorganisms are considered the primary source of biologically active substances in soil (Rodríguez-Gacio et al. 2009). Plant growth promoting rhizobacteria, that colonize roots, have been applied to a wide range of agricultural species for the purpose of the growth enhancement, including increased emergence (Kloepper et al. 1980, 1991). Naturally occurring soil

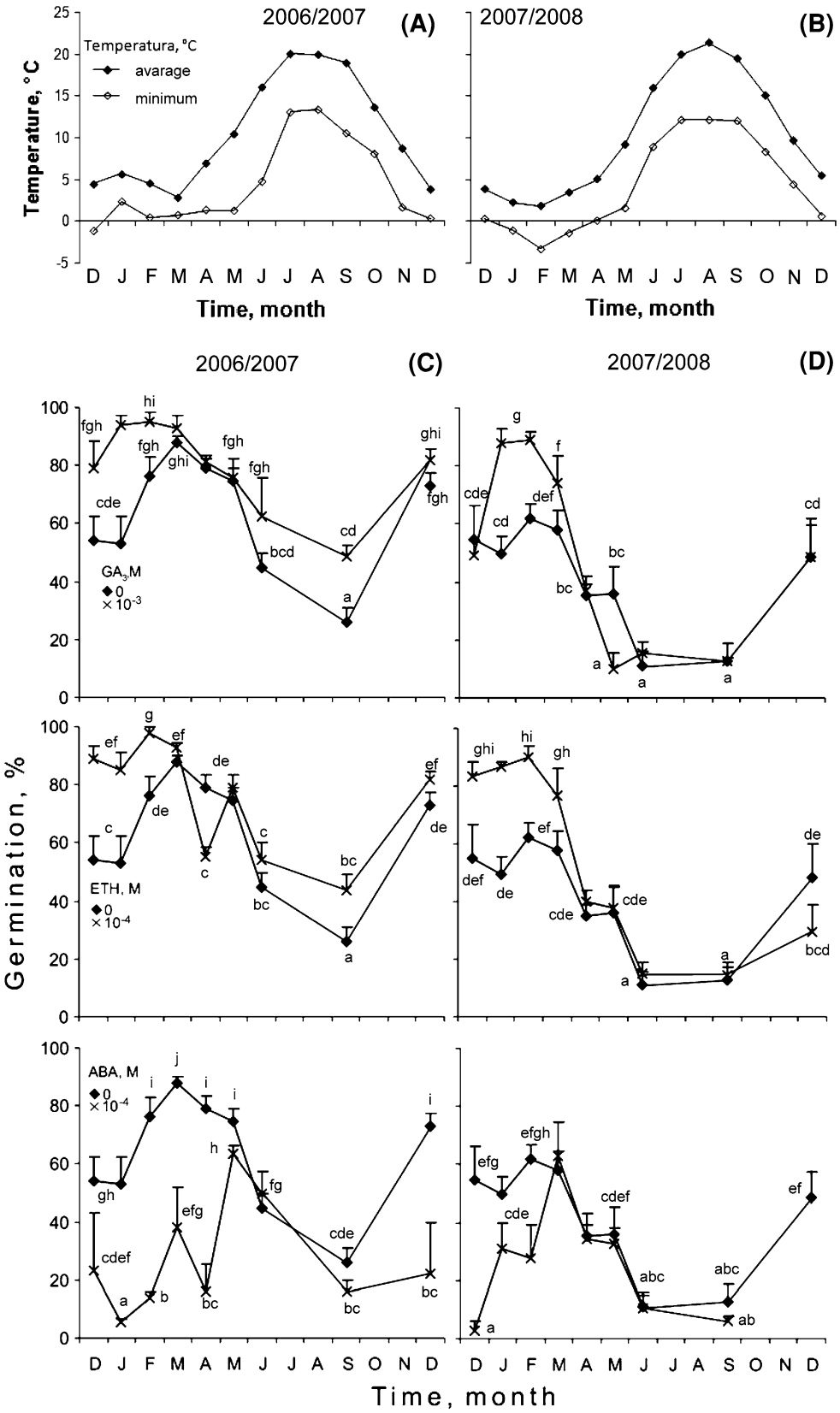


Fig. 6 Seasonal changes in temperature (**a, b**) and response to GA_3, ethephon and ABA at 25 °C of *A. retroflexus* seeds buried in 2006–2007 (**c**) and 2007–2008 (**d**). Average monthly and minimum temperatures at depth of 5 cm based on readings taken by Szczecin Department of Meteorology and Water Management. The *vertical bars* indicate ± SD. Two-way ANOVA with the Duncan post hoc test was used to determine significant differences. Mean values with *different letters* (*a–j*) are significantly different ($P \leq 0.05$)

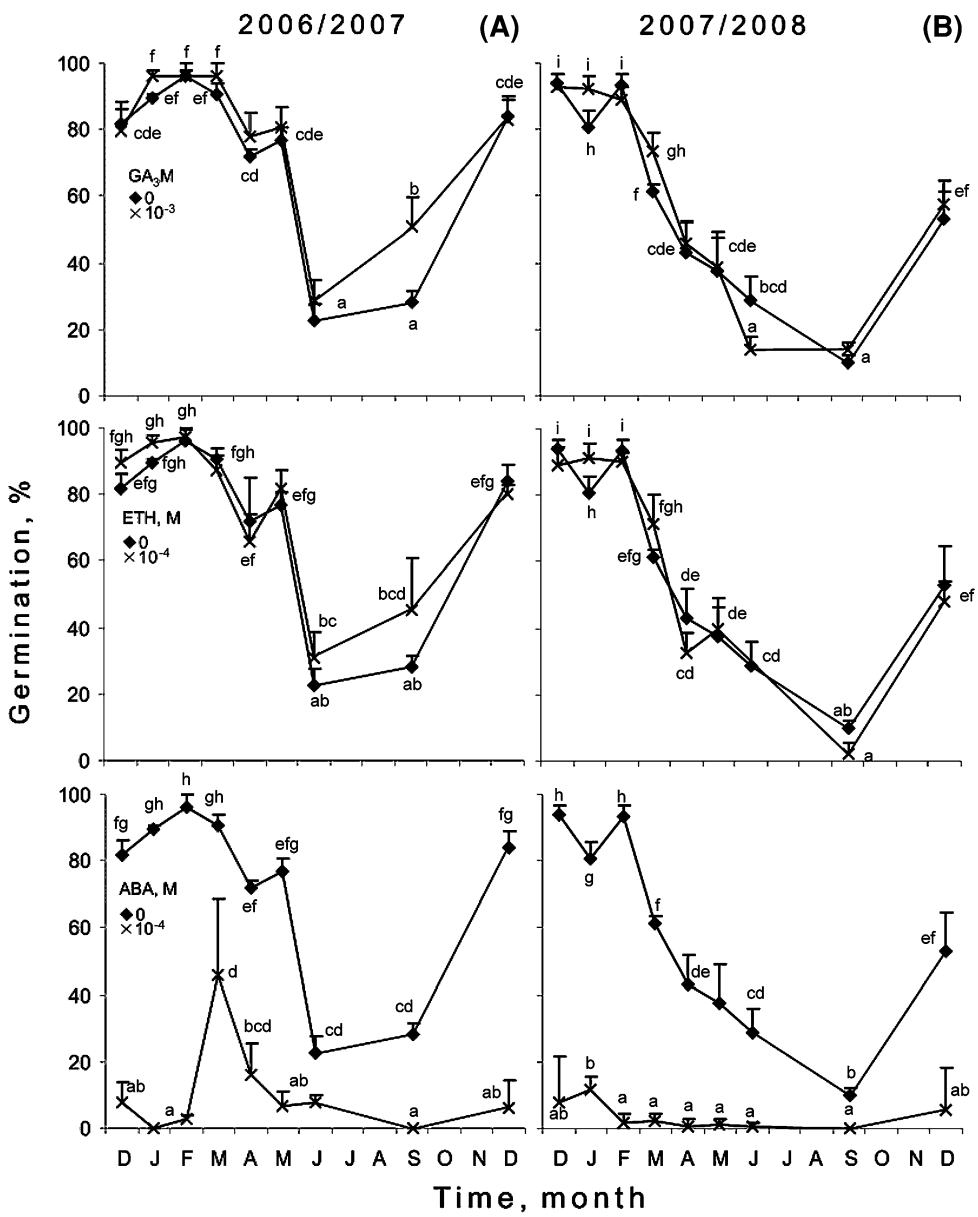


Fig. 7 The effect of GA_3, ethephon and ABA on percentage germination of *A. retroflexus* seeds at 35 °C after various periods of burial in 2006/2007 (**a**) and 2007/2008 (**b**). The *vertical bars* indicate ± SD. Two-way ANOVA with the Duncan post hoc test was used to determine significant differences. Mean values with *different letters* (*a–i*) are significantly different ($P \leq 0.05$)

microorganisms such as *Azospirillum*, *Azotobacter* and *Pseudomonas* may increase *Zea mays* seed emergence and plant growth (Gholami et al. 2009). However, there is no evidence that the soil seed banks may be affected by hormones (Rodríguez-Gacio et al. 2009).

The stimulatory effect of GA_3 and ethylene on germination of partially dormant seeds was much more evident at germination temperature 25 °C (Figs. 1a, 2) than at 35 °C (Figs. 1b, 3). Similar to the case of burial, ethylene was more active in stimulating germination of partially dormant seeds than GA_3. Both hormones, applied after or during stratification, are able partially to replace the requirement for cold stratification of partly dormant seeds (Figs. 1, 4). The response of stratified seeds to GA_3 at 25 °C, gradually increased as the time of stratification of *A. retroflexus* seeds was progressing (Fig. 1). In experiments with other species it was also shown that the sensitivity to GA_3 increases with the progress of dormancy release (Cadman et al. 2006; Kucera et al. 2005; Finch-Savage and Leubner-Metzger 2006). Results received in experiments with *Arabidopsis thaliana* showed that stratification may remove dormancy of GA-deficient mutants by increasing sensitivity to gibberellins (Karssen et al. 1989). Releasing dormancy can also be associated with an increasing gibberellin biosynthesis during stratification as has been shown in an experiment with *Arabidopsis* seeds (Yamauchi et al. 2004). It has also been found that the *GA 20ox1* gene involved in GAs biosynthesis is induced by stratification and GA_3, which releases dormancy in *Fagus silvatica* (Calvo et al. 2004). Similar, to the case of GA_3, the response to ethylene at 25 °C was also gradually

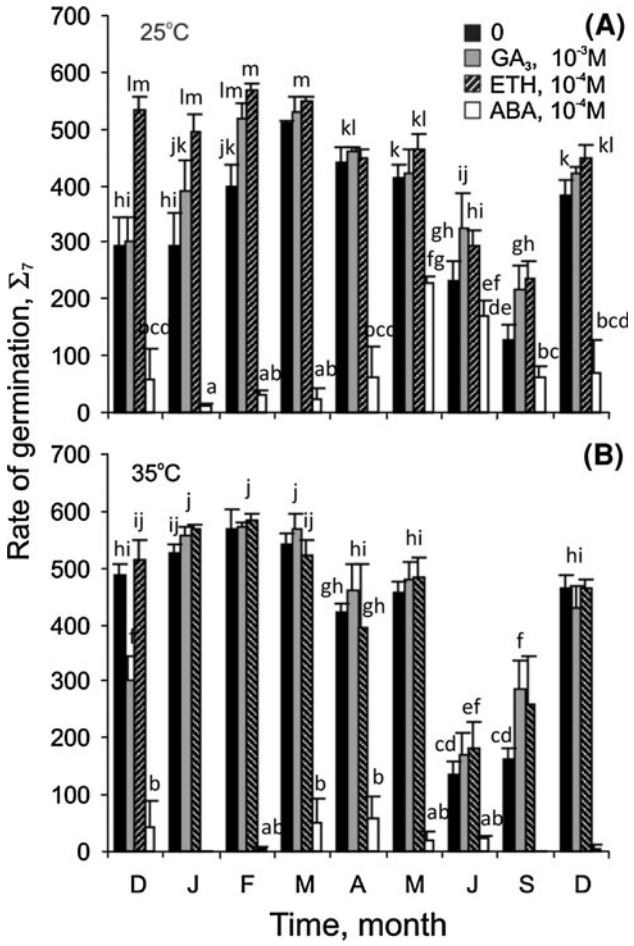


Fig. 8 The effect of GA_3, ethephon and ABA on the rate of germination at 25 °C (**a**) and 35 °C (**b**) of *A. retroflexus* seeds buried for various periods in 2006/2007. The *vertical bars* indicate ± SD. Two-way ANOVA with the Duncan post hoc test was used to determine significant differences. *Columns* with *different letters* (*a–m*) are significantly different ($P \leq 0.05$)

increased as the time of stratification of *A. retroflexus* seeds increased (Fig. 1). A transition from dormancy to germination caused by stratification of *A. retroflexus* seeds might be connected with an increasing ethylene biosynthesis. Excised apple embryos have been observed to produce progressively greater amounts of ethylene with increasing periods of stratification (Kępczyński et al. 1977). Previously it was found that dormant *A. retroflexus* seeds contained less, immediate precursor of ethylene biosynthesis, ACC, than non-dormant seeds (Kępczyński et al. 2003b). A cross-talk between ethylene and gibberellins in control of dormancy releasing was found. It was suggested that ethylene biosynthesis and action were involved in releasing *A. retroflexus* seed dormancy by GA_3 (Kępczyński et al. 2003b). Ethephon, effective in releasing *Fagus silvatica* seeds dormancy stimulated the expression of *GA 20ox1* gene (Calvo et al. 2004). The ACC oxidase gene expression, ACC content and ethylene production were increased due to treatment of these seeds with GA_3 (Calvo et al. 2004). In contrast to GA_3 and ethylene, the response of *A. retroflexus* seeds to ABA, applied after or during stratification, was decreasing as the time of stratification was progressing(Figs. 1, 4). Transition from seed dormancy state of *F. sylvatica* (Le Page-Degivry et al. 1997), *A. thaliana* (Ali-Rachedi et al. 2004), and *Chamaecyparis nootkatensis* (Schmitz et al. 2000) to germination can be associated, with decreasing ABA biosynthesis due to stratification. The loss of dormancy of *A. thaliana* seeds is related to the increased sensitivity to GA, decreased sensitivity to ABA and ABA/GA balance (Cadman et al 2006). Moreover, a crosstalk between ABA and GA with other signals including ethylene must be taken into account (Rodríguez-Gacio et al. 2009). Releasing dormancy in *A. retroflexus* seeds by stratification and ethylene might involve interaction between endogenous ABA and ethylene. Exogenous ABA has been found to decrease the effect of ethylene on germination of non-dormant *Amaranthus caudatus* seeds (Kępczyński et al. 2003a).

Stratification partially or almost completely released dormancy in primary dormant *A. retroflexus* seeds. Furthermore, dormancy in these seeds can be removed during burial in late autumn and winter, probably mainly due to a low temperature. Autumn–winter burial during the above seasons was found to be more effective than stratification in removing dormancy. Seeds buried during summer, due to higher temperatures, passed into secondary dormancy which was released probably by low temperatures in October and November. Both the burial until February 2007 or March 2008 and stratification increased response to GA_3 and ethylene. Response to ABA was decreasing as the stratification time was progressing and as the times of burials were progressing until May 2007 or March 2008 in the two conducted experiments. Endogenous GA_n, ethylene and ABA might probably be involved in the control of dormancy state and germination of *A. retroflexus*. Releasing dormancy by stratification or partial burial could be associated with a changing in ABA/GA and ehylene balance and/or sensitivity to these hormones.

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