

The effects of paclobutrazol, abscisic acid, and gibberellin on germination and early growth in silver, red, and hybrid maple

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Abstract: Silver maple (*Acer saccharinum* L.) seeds were used to examine the mechanisms regulating recalcitrant seed germination aiming towards the development of a biochemical method to temporarily inhibit germination without refrigeration. Treatment of fresh silver maple seeds with the cytochrome P₄₅₀ inhibitor paclobutrazol delayed germination and reduced root and shoot extension. The inhibitory effect of paclobutrazol plus abscisic acid (ABA) on the shoots of silver maple could not be overcome by a brief treatment with gibberellic acid A₃ (GA₃). Red maple seeds (*Acer rubrum* L.) were almost completely inhibited by the treatment with paclobutrazol alone, and this inhibition could not be overcome by a brief treatment with GA₃. In the red × silver hybrid, root growth was little affected by paclobutrazol plus ABA; however, shoot growth was markedly inhibited by paclobutrazol, and this inhibition was sharply increased by the combination with ABA but partially alleviated by GA₃. Treating fresh silver maple seeds with paclobutrazol for 12 h followed by storage under ideal germination conditions, in the presence of 1 mM ABA, completely prevented germination for 1 week. Root extension was completely restored by the continuous provision of GA₃ along with the ABA.

Résumé : Des semences d'érable argenté (*Acer saccharinum* L.) ont été utilisées pour étudier les mécanismes régissant la germination chez les semences récalcitrantes, afin de développer une méthode biochimique pour inhiber temporairement la germination sans utiliser le refroidissement. Le traitement de semences fraîches d'érable argenté à l'aide du paclobutrazol, un inhibiteur du cytochrome P₄₅₀, a retardé la germination et réduit le développement des racines et des pousses. L'effet inhibiteur du paclobutrazol combiné à l'acide abscissique (ABA) sur les pousses de l'érable argenté n'a pu être levé par un court traitement à l'acide gibbérellique A₃ (GA₃). La germination de semences d'érable rouge (*Acer rubrum* L.) a été presque complètement inhibée par le paclobutrazol employé seul; cette inhibition n'a pu être levée par un court traitement avec GA₃. Pour les hybrides issus du croisement entre l'érable rouge et l'érable argenté, la croissance racinaire a été peu affectée par le paclobutrazol combiné à l'ABA. La croissance des pousses a toutefois été grandement inhibée par le paclobutrazol et cette inhibition s'est accentuée lorsque le paclobutrazol était combiné à l'ABA; l'inhibition était partiellement réduite par l'application de GA₃. Des semences fraîches d'érable argenté traitées au paclobutrazol pendant 12 h et entreposées par la suite sous des conditions idéales de germination, en présence de 1 mM d'ABA, n'ont pas germé pour 1 semaine. Le développement des racines fut complètement rétabli par un apport continu de GA₃ combiné à l'ABA.

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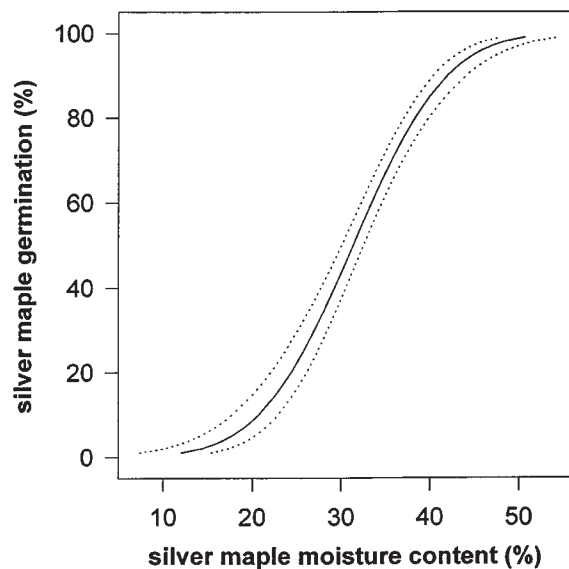
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Introduction

Based on their response to low temperatures and desiccation, seeds can be classified into two groups, orthodox and recalcitrant (Roberts 1973). Orthodox seeds, which are shed from the tree with a low water content (5–10%), tolerate low temperatures and desiccation and can maintain viability when stored for long periods (i.e., 30 years or more) under conventional storage conditions. Recalcitrant seeds, which are shed from the tree with a high water content (i.e., 35–65%) are intolerant to low temperatures and desiccation, and it can be difficult to maintain viability during collection, transportation, and processing of the seed. Recalcitrant seeds can be stored for short duration (i.e., 14 days to 3 years; King and Roberts 1979). The genus *Acer* includes trees that produce seeds that exhibit a spectrum of characteristics from recalcitrant to orthodox. Silver maple (*Acer saccharinum* L.) produces seeds that are intolerant to drying and long-term storage (Tylkowski 1984; Brown 1995). Red maple (*Acer*

Fig. 1. The effect of air-drying on seed viability in silver maple seeds. The broken lines are 95% confidence limits.

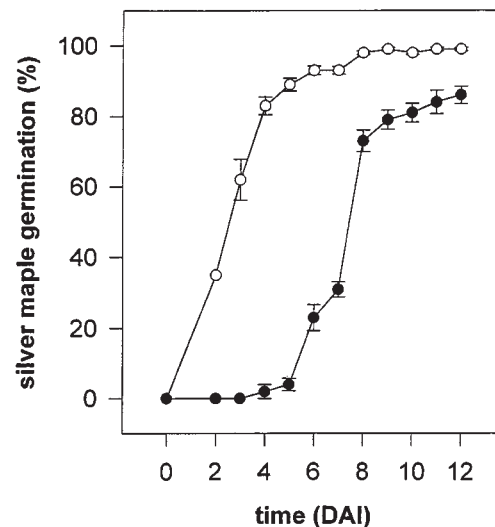


rubrum L.) produces seeds that are orthodox in behaviour, that may display seed-coat induced dormancy (Webb and Dumbroff 1969), and that can be stored for long duration (USDA Forest Service 1948). *Acer saccharinum* × *A. rubrum* hybrid trees have been produced and form viable seeds that possess characteristics intermediate to the parents (Brown 1995). Many tropical and temperate trees that produce recalcitrant seed are important in reforestation and conservation programs. Problems associated with the loss of viability during collection and processing and the short storage life of these seeds can have serious impacts on these programs.

Abscisic acid (ABA) regulates many processes associated with embryo development, germination, and dormancy, while gibberellic acid (GA) is also involved in the regulation of germination and dormancy (Hetherington and Quatrano 1991). In general, endogenous ABA levels and embryo sensitivity to exogenous ABA decline during seed maturation and drying or after abscission and imbibition (Bewley and Black 1978; Finch-Savage et al. 1992). The germination of some recalcitrant seeds is similar to that of ABA anabolic mutants, which block ABA synthesis in the embryo axis (Rock and Zeevart 1991) and of ABA-insensitive mutants displaying a lower sensitivity to the inhibiting effects of ABA (McCarty et al. 1991). Seeds with these mutations, i.e., alternative forms of seed regulatory genes and naturally recalcitrant seeds can either germinate precociously (before the completion of seed development) or germinate viviparously (on the maternal plant) (Koornneff et al. 1982; Robertson 1955; Brown 1995), indicating that ABA metabolism may be involved in the regulation of recalcitrant seeds germination. In addition, other mutants obviate the requirement for gibberellin during germination resulting in a triazole-insensitive phenotype (Jacobsen and Olszewski 1990).

The capacity of triazoles to selectively inhibit gibberellin anabolism and ABA catabolism make them useful diagnostic tools for establishing the role of these hormones in physiological processes (Grossmann 1990). The triazole family of

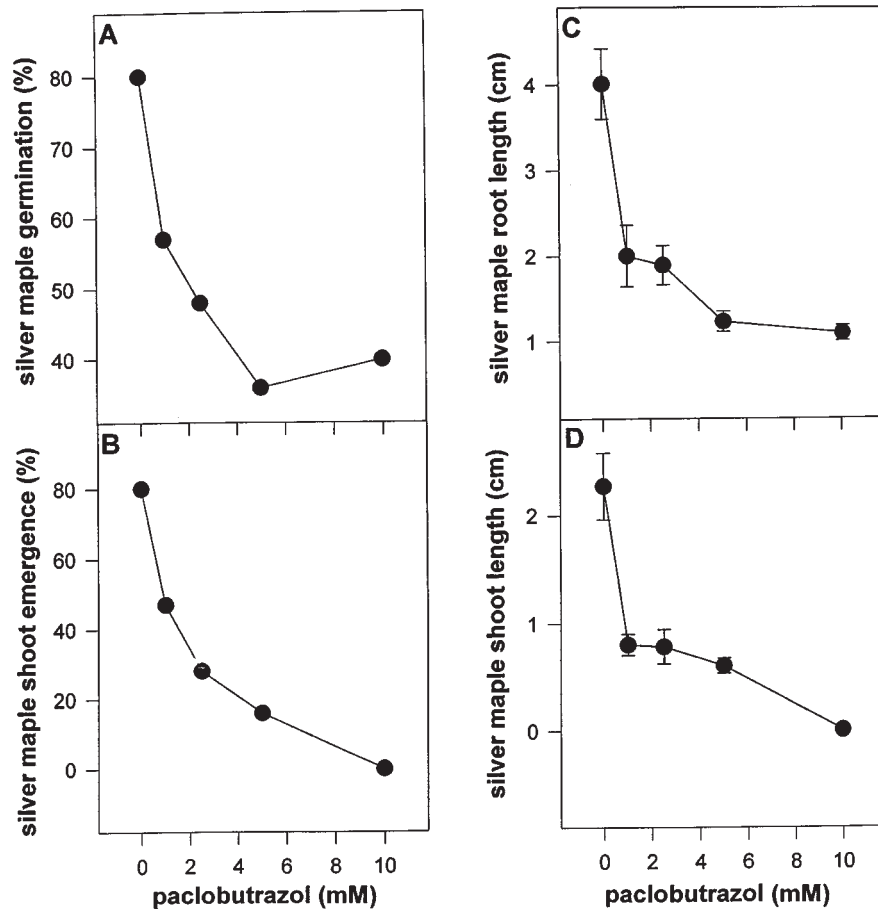
Fig. 2. The time course of the inhibition of silver maple seed germination by paclobutrazol. The effect of treatment with 1 mM paclobutrazol for 12 h on the germination of fresh silver maple seed and fresh seeds was measured over time. Fresh-picked seeds (50% MC) were treated with paclobutrazol or deionized water for 12 h immediately after collection, rinsed briefly in water, and left to dry for 12 h prior to germination tests as described in Materials and methods. ○, untreated. ●, 1 mM paclobutrazol. Values are the means ± SE from three independent experiments each with $n = 30$.



compounds all contains three conserved nitrogen atoms within a five-member ring that presumably binds to and inhibits cytochrome P_{450} enzymes. Cytochrome P_{450} monooxygenases are a family of heme-containing metalloenzymes that may donate hydroxyl groups to carbon from molecular oxygen (Sponsel 1995). Triazole-type monooxygenase inhibitors markedly reduce the cytochrome P_{450} dependent 8'-hydroxylation of ABA (Gillard and Walton 1976; Zeevart et al. 1990) that is required for ABA turnover resulting in little production of the primary catabolite, phaseic acid (Zeevart et al. 1990). Hence, the application of a triazole can lead to the accumulation of ABA within the treated tissue (Asare-Boamah et al. 1986; Rademacher 1992). Triazole-type inhibitors also inhibit the biosynthesis of gibberellin at the oxidation of *ent*-kaurene to *ent*-karenoic acid step resulting in lower endogenous gibberellin levels (Coolbaugh and Hamilton 1976). The capacity for preventing sterol ring synthesis also makes triazoles powerful antifungal agents.

In this study, the triazole-type cytochrome P_{450} inhibitor paclobutrazol ((2RS,3RS)-1-(4-chlorophenyl)-4,4-dimethyl-2-(1,2,4-triazolyl)-pentan-3-ol) was used to examine the role of gibberellin synthesis and ABA turnover in the germination of silver, red, and hybrid maple seeds and to determine whether a paclobutrazol seed treatment can be of benefit in maintaining silver maple seed viability. A progressive series of experiments to establish the conditions for preparing fresh silver maple seeds and to determine the appropriate timing and concentrations of paclobutrazol and ABA applications to prevent germination was conducted from 1991 to 1997.

Fig. 3. The dose response of silver maple seeds to paclobutrazol. The effect of a range of paclobutrazol concentrations on (A) percent germination, (B) percent shoot emergence, (C) root extension, and (D) shoot extension. Partially dried silver maple seeds were treated with the indicated concentration of paclobutrazol for 12 h prior to rinsing and germination tests. Values are the means \pm SE ($n = 25$).



Materials and methods

Silver maple seeds were collected from various sources in Ontario in different crop years between 1991 and 1997. Red maple and silver \times red maple hybrid seeds were from selected trees that produced seed which in previous years displayed intermediate and strong dormancy (J. Marshall and B. Wang, unpublished).

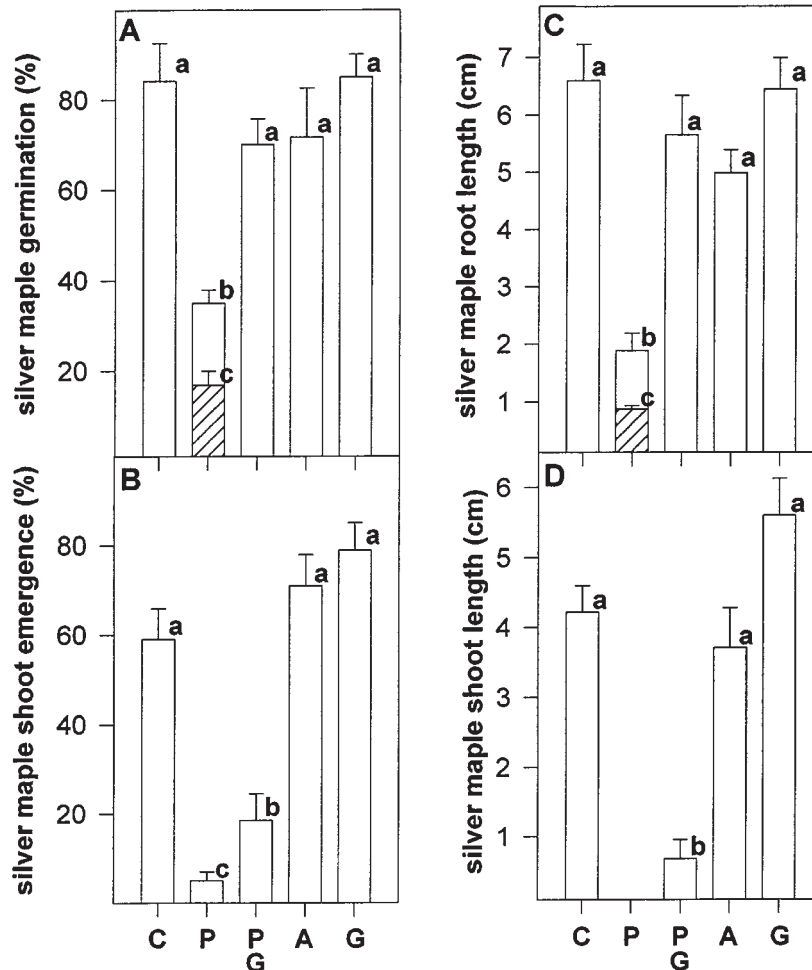
For germination, the seeds were placed in the bottom of a Petawawa germination box (Wang and Ackerman 1983), covered with the treatment solution, and a plastic grid placed over the seeds to keep them below the surface of the solution. The seeds were held on a laboratory bench at room temperature for 12 or 24 h as indicated. The highly dormant red maple seeds were germinated with and without stratification on moist Kimpac cellulose at 4°C for 30 days. The seeds were treated with water, or aqueous solutions of paclobutrazol, ABA, GA₃ and (or) their combination at concentrations indicated in the figure captions, prior to rinsing with water followed by germination tests. For paclobutrazol treatments followed by the continuous presence of ABA, the seeds were soaked in water or 1 mM paclobutrazol for 24 h, rinsed in water, and then germinated on Kimpac soaked in 1 mM ABA, 1 mM GA₃, or the combination of paclobutrazol and ABA or paclobutrazol and GA₃. One additional set of untreated controls were immediately germinated on Kimpac, i.e., not soaked in water for 24 h prior to germination (no difference was observed between the two types of controls). Germination and extension values were obtained after 7 days of germination.

Seed moisture content was determined by measuring fresh mass (FM) followed by drying in a forced draft oven at 105°C for 16 h and cooling at ambient temperature and humidity before the determination of dry mass (DM). Percent moisture content (%MC) was then calculated as $\%MC = (FM - DM)/FM$. Air drying of silver seeds was performed on a laboratory bench under ambient temperature and humidity. Silver and hybrid seeds were dried to 40% MC, and red maple seeds were dried to 10% MC.

For germination tests, the seeds were incubated on cellulose pads that had been moistened with 100 mL of distilled water and suspended on a plastic grid above an additional 110 mL of distilled water in the reservoir of the germination box. The boxes were placed in a controlled environment chamber (Conviron, Winnipeg, Man.) with cycles of 8 h light : 16 h dark under fluorescent light with an intensity of 12 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ at 20°C and 85% relative humidity for at least 15 days. Germination was defined as the emergence of 0.2 cm of root or shoot from the samara. Seeds were dissected with a razor and root and shoot length measured to the nearest millimetre. Percentage germination values were corrected by the amount of unfilled or nonviable seed as determined by visual inspection for a bright green aleurone layer and the absence of discoloured tissue.

Paclobutrazol was obtained as a 250 g/L suspension from ZENECA Agro (Stoney Creek, Ont.). Synthetic ABA and GA₃ were obtained from Sigma (St. Louis, Mo.). Regression analysis, ANOVA, multiple comparisons, and χ^2 tests were performed with SAS software (SAS Institute Inc., Cary, N.C.).

Fig. 4. The effect of paclobutrazol, ABA, GA₃, and their combination on (A) percent germination, (B) percent shoot emergence, (C) root extension, and (D) shoot extension of fresh silver maple seeds. Hatched bars show seeds left in the presence of 1 mM paclobutrazol and not rinsed with water prior to germination tests. Treatments are as follows: C, control; P, 1 mM paclobutrazol; A, 50 μM ABA; G, 50 μM GA₃. Values are the means ± SE ($n = 3$ in Figs. 4A and 4B; $n = 30$ in Figs. 4C and 4D). Bars with different letters are significantly different by ANOVA followed by multiple t tests.



Results

The effects of air drying on silver maple seed viability

Fresh silver maple seeds were found to maintain full viability when dried to 40% MC. Polynomial regression of the relationship between percent germination and air-dried moisture content yielded the equation: germination (%) = $100.41 + 23.64(\%MC) - 2.093(\%MC^2) + 0.0855(\%MC^3) - 0.0015(\%MC^4) + 9.52 \times 10^{-6}(\%MC^5) + 1.16 \times 10^{-9}(\%MC^6)$ that fitted the data with a r^2 value of 0.99 and produced trendless residuals. The predicted survival function and 95% confidence intervals are plotted in Fig. 1. Silver maple seeds survived drying to 40% MC; however, survival rapidly declined below this value, and viability was severely reduced below 30% MC.

The effect of paclobutrazol on silver maple seed germination

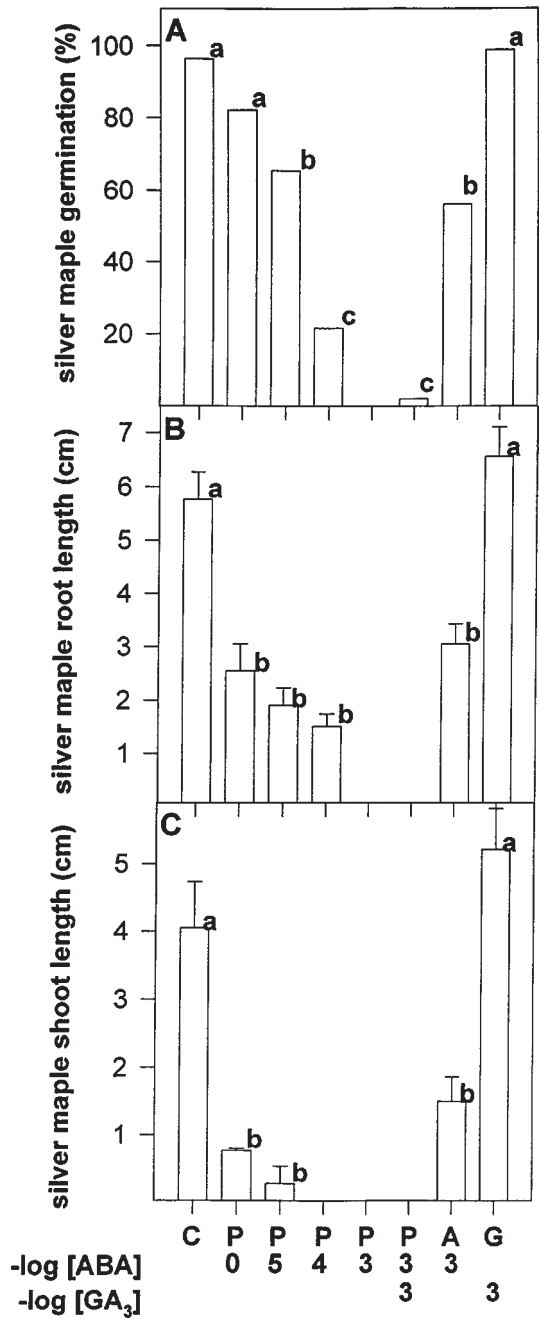
Treatment of seeds with 1 mM paclobutrazol delayed germination by 50%, five days after imbibition (DAI), but 80% of the treated seed germinated by 12 DAI (Fig. 2). The max-

imum difference between control and treated seeds was observed at 5 DAI. Nevertheless, 7 DAI was selected for the measurement of all subsequent experiments to be able to detect enhanced or decreased germination upon addition of GA and (or) ABA. One millimolar paclobutrazol treatment of seeds that were partially dried and stored at 4°C prior to testing, resulted in a significant, but not complete, inhibition of root and shoot emergence and extension (Figs. 3A–3D) and was thus selected for subsequent experiments. Higher levels of paclobutrazol did not reduce root emergence below 40% in partially dried seed (Fig. 3A), although shoot emergence and root and shoot extension were progressively inhibited or abolished at higher concentrations (Figs. 3B–3D).

The interaction of paclobutrazol and GA₃ in silver maple seeds

In fresh silver maple seeds treated immediately after harvesting with paclobutrazol, root emergence and extension were sharply inhibited, and this effect was completely reversed by the provision of GA₃ (Figs. 4A and 4B). In contrast, the

Fig. 5. The interactive effects of paclobutrazol treatment combined with increasing levels of ABA on (A) germination, (B) root length, and (C) shoot length of fresh silver maple seeds. Treatments are as follows: C, control; P, 1 mM paclobutrazol; A, ABA; G, GA₃. Concentrations of ABA and GA₃ are expressed as $-\log(\text{molar concentration})$. Values are the means ($n = 90$; Fig. 5A) and means \pm SE ($n = 30$, Figs. 5B and 5C). Bars with different letters are significantly different by ANOVA followed by multiple t tests.



emergence and extension of shoots from fresh seeds was nearly abolished by paclobutrazol treatment, and this effect was only partially reversed by GA₃ (Figs. 4C and 4D). A 12-h treatment with 10⁻⁵ M ABA had no effect on root or shoot emergence and development. The effect of

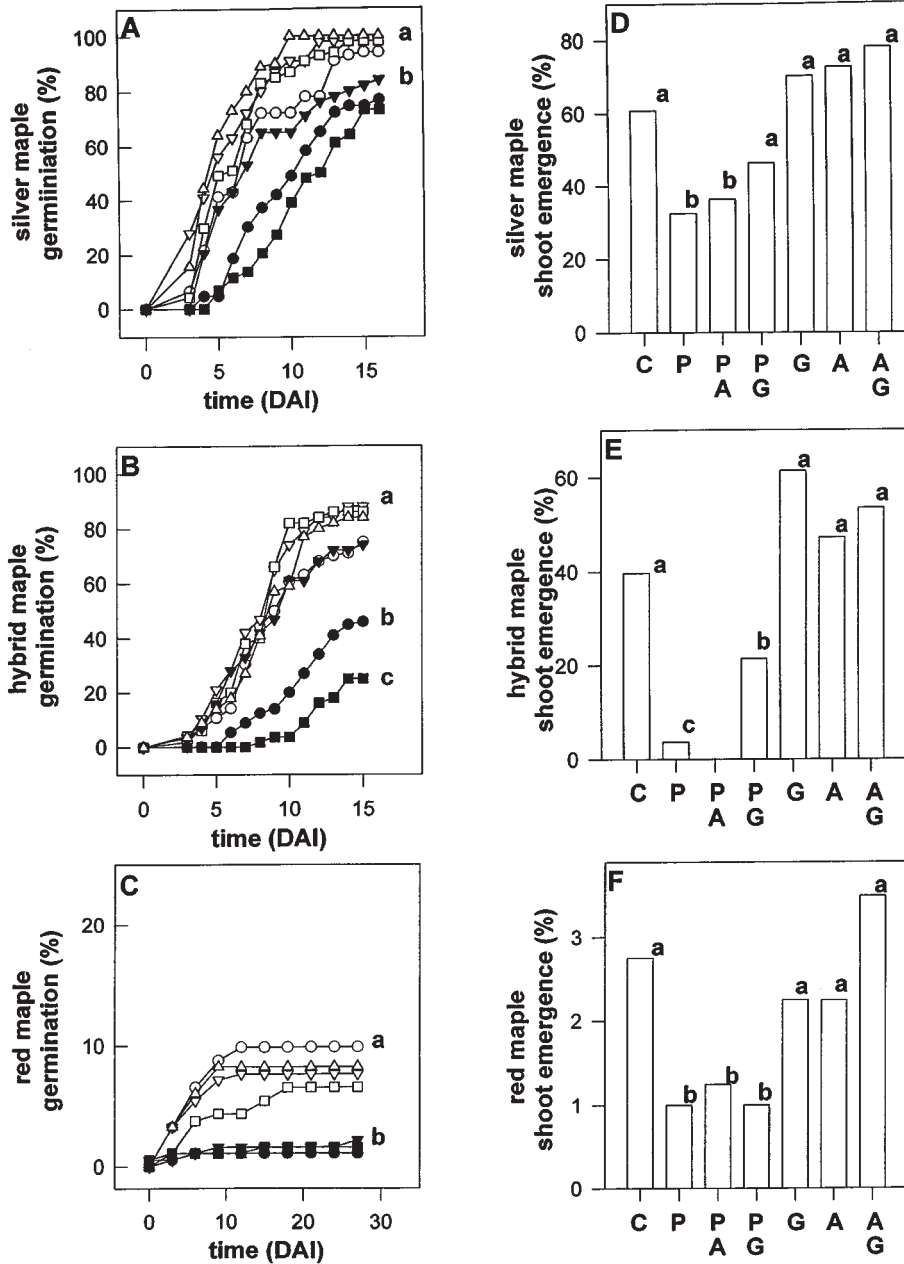
paclobutrazol was more pronounced when the inhibitor was not washed from the seeds (Fig. 4, cross-hatched bars).

The interaction of paclobutrazol and ABA in silver maple seeds. In 10⁻³ M ABA-treated fresh silver maple seeds, germination was inhibited and root or shoot extension was similar to the paclobutrazol treated seeds (Figs. 5A–5C). However, the combination of 10⁻³ M ABA with paclobutrazol completely abolished root elongation, and this inhibition could not be overcome by 10⁻³ M GA₃. Shoot extension was significantly reduced by the combination of paclobutrazol plus 10⁻⁵ M ABA and abolished by the combination with 10⁻⁴ M ABA. The combination of paclobutrazol and 10⁻⁵ M ABA inhibited seed germination. No germination and thus no root extension were observed at ABA concentrations of 10⁻³ M (Fig. 5). The inhibition of germination, root extension, and shoot extension caused by the combination of paclobutrazol and ABA could not be overcome by GA₃. After 12 days of incubation, most seeds treated with paclobutrazol alone, and about one half of the seeds treated with 1 mM ABA alone germinated (Fig. 5).

The interactions of paclobutrazol, ABA, and GA₃ on silver \times red maple hybrid cross germination and development. As noted above, partially dried silver maple (40% MC) still germinated well, but their sensitivity to paclobutrazol or hormone treatments was greatly reduced. Germination of partially dried silver maple was delayed by about 3 days but attained levels comparable with controls by 15 DAI (Fig. 6A). In the partially dried silver maple seeds, the inhibition of root emergence and extension by paclobutrazol was no longer recoverable by GA₃. In stored seeds, silver maple shoot emergence and extension was also inhibited by paclobutrazol (Figs. 6A and 6D), and the inhibition was more severe in shoots than in roots. The paclobutrazol-induced inhibition of shoot and root emergence in silver maple was not increased by 10⁻⁵ M ABA nor abolished by 10⁻⁵ M GA₃ (Figs. 6A and 6D). In contrast to the high levels of germination in silver maple seeds, germination of the silver \times red maple hybrid cross was intermediate (Fig 6B), while only 10% of highly dormant red maple seeds germinated (Fig. 6C). This low level of germination in red maple was observed with and without stratification. Red maple shoot emergence was nearly abolished by paclobutrazol and, in contrast to silver maple, showed little improvement by the addition of GA₃ (Fig. 6C). Nevertheless, given the potent inhibition of red maple shoot emergence by paclobutrazol it is not possible to comment on the interaction of the cytochrome P₄₅₀ inhibitor paclobutrazol with the exogenously added hormones (Fig. 6F). In marked contrast, silver \times red hybrid seed root emergence was inhibited by paclobutrazol. This inhibition could be markedly enhanced by low concentrations of ABA but was overcome by GA₃ (Fig. 6D). Hybrid shoot emergence was inhibited by paclobutrazol and abolished by the combination of paclobutrazol plus ABA, and the inhibition by paclobutrazol was overcome by GA₃ (Fig. 6E).

Shoot extension was enhanced by the availability of GA₃ in partially dried silver maple and red maple seeds (Figs. 7D and 7F) but not in hybrid maple (Fig. 7E). Hybrid root and shoot emergence were sharply reduced or abolished in the presence of paclobutrazol plus ABA, but this effect was partially overcome by the provision of GA₃. Moreover, hybrid

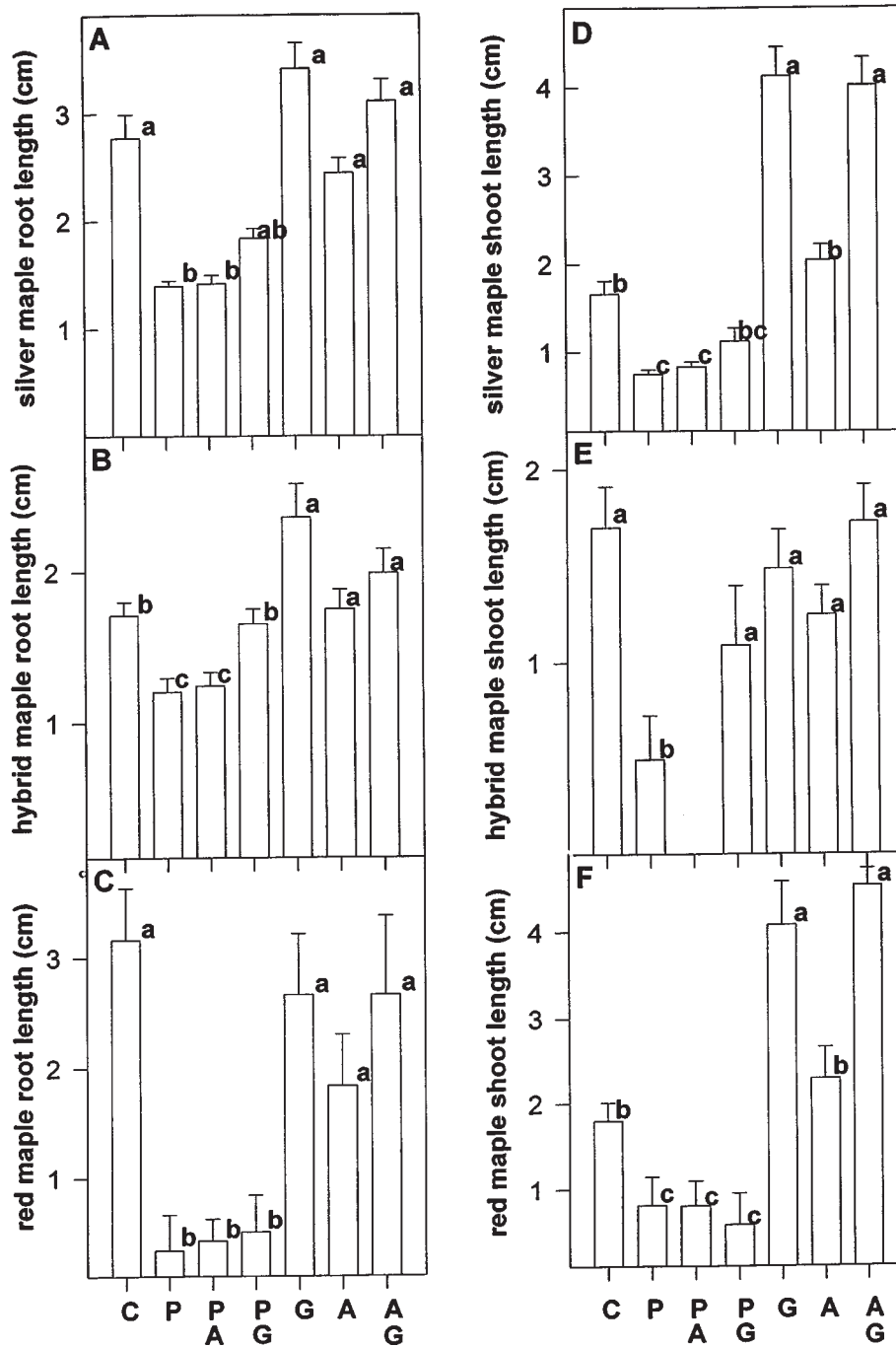
Fig. 6. The interactive effects of ABA and GA on germination and shoot emergence in silver, red and silver × red maple hybrid cross. The effect of 12-h treatments with paclobutrazol, ABA, GA₃, or their combinations on the germination of (A) silver maple, (B) hybrid maple, and (C) red maple. Treatments are as follows: ○, untreated; ●, 1 mM paclobutrazol; □, 50 μM ABA; ▽, 50 μM GA₃; ▽, paclobutrazol and GA₃; ■, paclobutrazol and ABA; △, ABA and GA₃. The effect of 12-h treatments with paclobutrazol, ABA, GA₃, or their combinations on percent shoot emergence of (D) silver maple, (E) hybrid maple, and (F) red maple. Treatments are as follows: C, untreated; P, 1 mM paclobutrazol; A, 50 μM ABA; G, 50 μM GA₃. Shoots were sampled at the last day of the corresponding root treatment. Values are means (*n* = 40 for silver maple and hybrid maple, and *n* = 120 for red maple). Bars with different letters are significantly different by individual χ^2 tests.



shoot extension, but not root extension, was also inhibited by paclobutrazol plus ABA and the paclobutrazol-inhibited extension was partially restored by GA₃ (Fig. 7E). In marked contrast to hybrid root emergence, the inhibition of hybrid root extension in response to paclobutrazol was distinctly muted in comparison with both parent species (Fig. 7B). Moreover, root extension in the hybrid was significantly stimulated by GA₃ alone in contrast to both parent species.

In an attempt to store recalcitrant seeds at ambient temperature, fresh silver maple seeds were treated for 12 h with paclobutrazol followed by a continuous exposure to ABA, or ABA and GA (Fig. 8). The constant exposure of paclobutrazol-treated seeds to ABA resulted in very low rates of germination. A further treatment with GA₃ resulted in the full recovery of root extension but not shoot extension. The inhibitory effects of the paclobutrazol treatment

Fig. 7. The effects of paclobutrazol, ABA, GA₃, and their combinations on root and shoot extension: (A) silver maple root; (B), hybrid maple root; (C), red maple root; (D) silver maple shoot; (E) hybrid maple shoot; and (F) red maple shoot. Treatments are as follows: C, control; P, 1 mM paclobutrazol; A, 50 μM ABA; G, 50 μM GA₃. Values are means ± SE (*n* = 40 for silver maple and hybrid maple, and *n* = 120 for red maple). Bars with different letters are significantly different by ANOVA followed by multiple *t* tests.



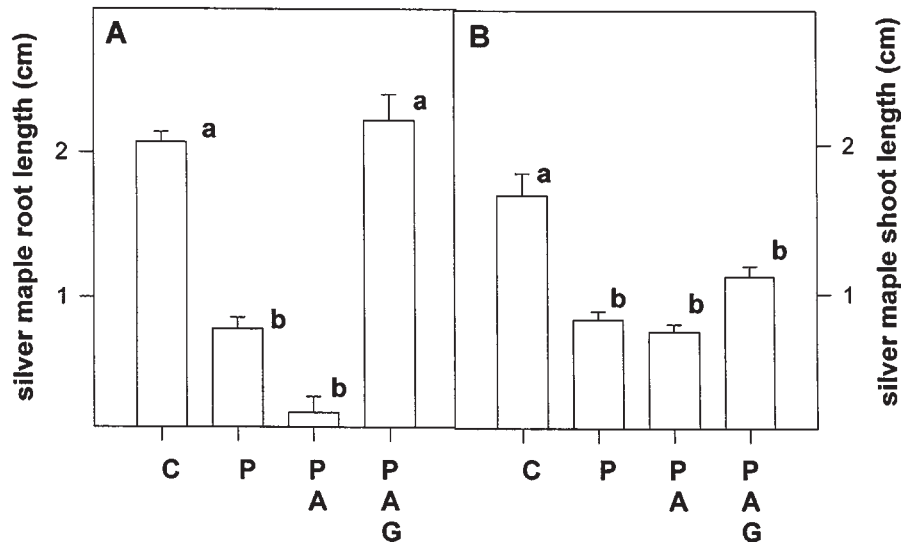
did not diminish with time, and after 40 days of growth in soil, seedlings from treated seeds (that were washed before sowing) remained severely stunted (not shown).

Discussion

In a series of experiments executed in a brief period in each spring over the course of 8 years, we examined the role of ABA and GA in the germination of the recalcitrant seeds

of silver maple using the cytochrome P₄₅₀ inhibitor paclobutrazol. It has been postulated that cytochrome P₄₅₀ inhibitors can be used to examine the roles of GA synthesis and ABA turnover in physiological processes, such as seed germination (Grossmann 1990). Thus, our results provide some insights on the possible role of cytochrome P₄₅₀ mono-oxygenases and their function in GA and ABA metabolism in the germination of recalcitrant, hybrid, and orthodox maple seed.

Fig. 8. The effect of paclobutrazol followed by continuous ABA and (or) GA₃ on silver maple shoot length (A) and root length (B). The seeds were treated for 12 h with paclobutrazol and germinated in the presence of ABA and (or) GA₃. Treatments are as follows: C, control; P, paclobutrazol 1 mM; A, 1 mM ABA; G, 1 mM GA₃. Values are the means \pm SE ($n = 30$). Bars with different letters are significantly different by ANOVA followed by multiple *t*-test comparisons.



The interaction of the cytochrome P₄₅₀ inhibitor and phytohormones is dependent on dose, duration, and timing of the treatment. We have provided evidence that both GA synthesis and ABA turnover seem to play a role in the regulation of recalcitrant silver maple seed behaviour. The sensitivity of the fresh seeds to ABA in the presence of paclobutrazol would indicate that ABA catabolism proceeds readily in the silver maple seeds. However, the apparent importance of ABA turnover in the seeds changed with time after separation from the tree, such that fresh seeds were more affected by ABA than the partially dried seeds. The greater sensitivity of the shoots versus the roots to nonmetabolized ABA would suggest that the role of cytochrome P₄₅₀ dependent processes may vary between tissues.

ABA turnover and silver maple seed germination

In our initial experiments, the treatment of fresh silver maple seeds with 10⁻⁵ M ABA had little effect on germination or subsequent root and shoot elongation (Fig. 4). However, treatment with paclobutrazol reduced germination as well as root and shoot elongation, processes that could not be fully recovered by treatment with GA₃. Hence, we suggest that the effects of paclobutrazol did not result from the inhibition of GA₃ synthesis alone (Coolbaugh and Hamilton 1976). Furthermore, treatment of fresh silver maple seeds with high concentrations of ABA (10⁻³ M) alone had some inhibitory effect on seedling development. The combination of exogenous ABA with the triazole, revealed a sensitivity to the presence of ABA, especially in the shoots (Fig. 5). We speculate that the sensitivity to ABA may indicate that a cytochrome P₄₅₀ dependent activity, which catabolizes ABA, could play a permissive role in the control of silver maple germination and subsequent shoot development. Nevertheless, this assumption was not tested by the direct measurement of ABA levels. In fresh seeds, shoot development was far more sensitive to the presence of nonmetabolized ABA,

suggesting the role of ABA metabolism in the regulation of the shoot growth (Fig. 5).

Comparison of recalcitrant and orthodox seed

Germination of partially dried silver maple seeds was less inhibited by paclobutrazol or ABA than fresh seeds (Figs. 6 and 7). The loss of sensitivity to paclobutrazol in partially dried silver maple seeds would indicate that the sensitivity of the seeds to ABA declined with time, that the endogenous ABA was catabolized (Finch-Savage et al. 1992), or that GA₃ was synthesized rapidly after the seed is separated from the tree. When the sensitivity of silver maple seeds to paclobutrazol and ABA was compared with that of hybrid and red maple seeds under identical conditions, some interesting patterns emerged. Elongation was partially inhibited by paclobutrazol in silver maple roots, strongly inhibited in hybrid maple shoots but not roots, and nearly abolished in red maple roots. Moreover, in hybrid maple the interaction of ABA and paclobutrazol resulted in a strong inhibition of shoot elongation. Taken together these results would suggest that the inhibition of cytochrome P₄₅₀ mediated catabolism of ABA in the dormant red maple seed, as reflected in the shoots of the silver \times red hybrid, played a role in the orthodox seed dormancy and not in the recalcitrant silver maple seeds.

Interaction of ABA and GA

The notion that seed germination may be regulated, in part, by the balance between the stimulatory effect of GA₃ and the inhibitory effect of ABA has been previously suggested (Bewley and Black 1978). The rapid synthesis of GA₃ and the concomitant turnover of ABA could account for the fresh silver maple seed phenotype. Our results may indicate that the rapid germination of silver maple seeds, like seeds from other recalcitrant tree species (Finch-Savage et al. 1992), might require the turnover of ABA and the

synthesis of GA to initiate germination. Hence, seed germination could be regulated by the expression of members of the cytochrome P₄₅₀ family of enzymes that are required in both of these processes. In this regard, seeds that carry a mutant form of a factor that regulates gene expression has been shown to result in insensitivity to ABA and viviparous germination (McCarty et al. 1991). In addition, mutant seeds that carry a defective regulatory factor no longer require the presence of GA₃ to grow and thus show a triazole-insensitive phenotype similar to that observed in silver × red hybrid roots (Jacobsen and Olszewski 1990).

Practical applications

Commercially available cytochrome P₄₅₀ inhibitors and gibberellins may have some beneficial use in the collection and transport of recalcitrant seeds to nurseries, especially in the tropics where refrigeration is not available. The inhibition of germination by a brief treatment of the fresh seeds with paclobutrazol at room temperature followed by storage in the presence of ABA, could provide a biochemical means of storing moist seeds at ambient temperatures. Paclobutrazol was found to cause severe stunting of the seedlings, indicating that this growth regulator may not be useful for improving storage of recalcitrant seeds. Nevertheless, the capacity of GA₃ to partially overcome the inhibition of germination and completely overcome the inhibition of root extension suggest the possibility to optimize a method that provides the best balance of inhibition followed by recovery and subsequent growth. Triazole treatments could have some short-term effects on growth; nevertheless, pretreatment of seeds has been reported to increase the stress hardiness of seedlings (Shen and Zeng 1993), which may be of some importance in the reforestation of tropical forests.

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