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Peach Seed Dormancy in Relation to Endogenous Inhibitors and Applied Growth Substances¹

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Abstract. An inhibitor was present in both seed coat and embryo of a high and a low chilling cv. of unstratified peach seeds and its concn decreased as stratification proceeded. Embryonic tissue retained more of the inhibitor than the seed coat. As the concn of inhibitor decreased, seed germination increased. The inhibitor was tentatively identified as abscisic acid (ABA) by chromatography. A bound inhibitor was also present in the seed parts of both cvs., and its concn increased in the embryo as stratification proceeded. More ABA and bound inhibitor were present in the high-chilling cv. than in the low-chilling counterpart, indicating that they may be related as factors which cause a cv. to require long periods of chilling. Application of ABA reduced germination percentage on stratified seeds without seed coats. Application of gibberellic acid (GA) and N-benzyladenine (BA) combined had a synergistic effect in promoting germination of dormant seeds.

Eagles and Wareing (5) reported a growth inhibitor in birch leaves and named it "dormin." Later it was found to be the same compound as "abscisin II" (4), extracted from young cotton fruits (1). Recently, the name abscisic acid has been adopted for both dormin and abscisin II (2). A bound inhibitor, (+)-abscysil B-D glucopyranoside, has been described by Koshimizu et al. (8) in *Lupinus lutens* and Milborrow (13). This fact suggests that the glucoside may be the major rapid-storage product of ABA.

Abscisic acid is present in seeds which require chilling to germinate, and its concn, nature, or both are altered during a chilling treatment. Further, these alterations during dormancy have been correlated with the release of seeds from the dormant condition. Lipe and Crane (10) found ABA in peach seed coats, and reported its disappearance by the 6th week of stratification, after which the seed germinated. Martin et al. (11) observed a significant decrease of ABA in walnut kernels after 2 weeks of chilling, and after that, germination occurred. Lin and Boe (9) reported a decrease of ABA in plum seeds during a 90-day chilling period.

Growth inhibitors apparently have a profound effect on seed dormancy, but some research emphasizes a balance between growth-promoting and growth-inhibiting compounds. In *Corylus*

avellana seeds, GA concn increased slightly during chilling (6) and Mathur et al. (12) found an increase in GA₃ and GA₇ concn in peach seeds during stratification. Lin and Boe (9) noted an increase in GA-like substances in plum seeds during 90 days of stratification. A role for cytokinins and ethylene in dormancy was indicated by induced germination of certain species as a result of exogenous applications of BA and ethylene. Khan (7) suggested that the action of cytokinin was both antagonistic to the inhibitory effect of ABA and permissive to the action of GA₃.

We studied the changes of an acidic inhibitor and its bound form during stratification of peach seeds, and determined the effect of applied growth substances on germination of peach seeds.

Materials and Methods

We used 'Lovell' peach seeds, which require a long period of stratification, and 'Tetela' seeds, which require a very short period. The seeds, with endocarps removed, were soaked in water 24 hr before being stratified at 3°C in moist vermiculite. 'Lovell' samples were removed from stratification at 2-week intervals over a 3-month period while 'Tetela' seeds were removed at 5-day intervals during a 25-day period. A sample of seeds from each cv. was separated into seed coats and embryos for extraction and purification. Other samples were tested for germination.

Extraction and purification. Growth regulators were extracted with 80% methanol at 0°C for 72 hr, with the methanol being changed every 24 hr. The methanolic extracts

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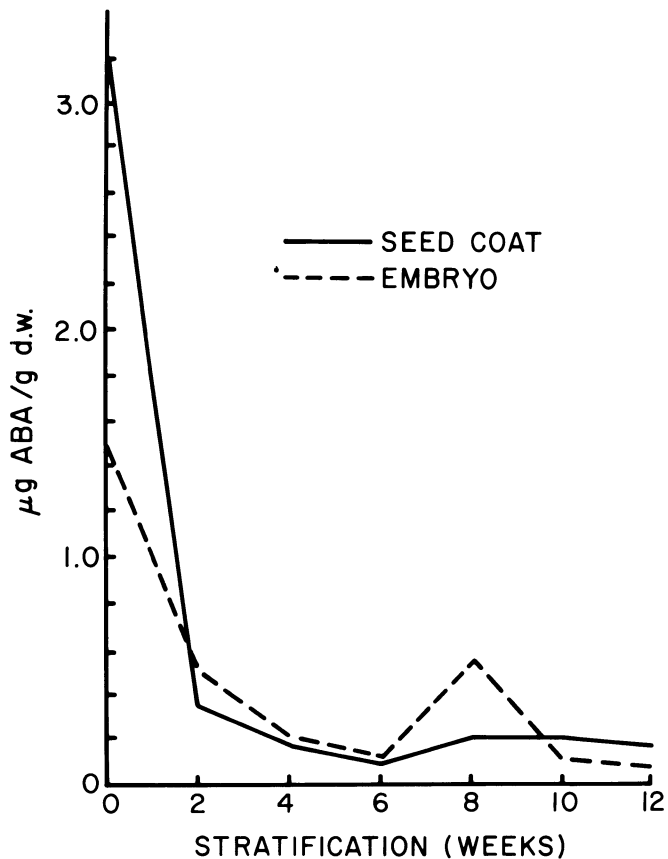


Fig. 1. Quantitative changes in concn of an acidic inhibitor in extracts of 'Lovell' peach seed coats and embryos at different stratification times. Calculations based on standards of ABA.

were combined, filtered, and evaporated under vacuum to the aqueous phase. The residue remaining on the filter was dried 3 days at 38°C and then weighed. After acidification to pH 2.8 with 1N H₂SO₄, the aqueous phase was partitioned 3 times with 10 ml ethyl acetate and 2 times with 5 ml anhydrous ethyl ether. The ethyl acetate and ethereal acidic phases were combined (Fraction I). They contained the acidic inhibitor. The remaining aqueous phase (Fraction II) was used to determine the presence of a bound inhibitor. The pH of fraction II was adjusted to 11.0 with 10% NaOH, and the fraction was heated 1 hr at 60°C. Then the pH was readjusted to 2.8 with 1N H₂SO₄, and the solution was partitioned with ethyl acetate and ether as previously mentioned. The acetate and ether partitions were then combined (Fraction III). Fractions I and III were evaporated under vacuum to dryness and then redissolved in a small volume of absolute methanol for further purification by instant thin layer chromatography (ITLC). The plates (type SAF) were developed twice in benzene:acetic acid:water (8:3:5 v/v/v), with the inhibitory zone appearing between R_f 0.05 and 0.3.

Bioassay. The presence of the inhibitor and its bound form in seed coats and embryos of both cvs. was detected by the wheat coleoptile assay (14). A standard curve of synthetic ABA was used to determine the relative inhibitor concn.

Seed germination and responses to exogenous growth regulators. 'Lovell' seeds were removed from stratification at 0, 2, 10, and 12 weeks, and ABA was applied at 0, 0.1, 0.2, 0.3, and 3.1 µg/ml. Seeds with these treatments on moist filter paper in petri dishes were held at 25°C for 15 days. Other 'Lovell' seeds were removed from stratification at 0, 2, 4, and 6 weeks and exposed to GA₃ at 0.02 and 2.0 ppm and BA at 1.0 and 100 ppm alone or combined and the seeds were germinated the same way.

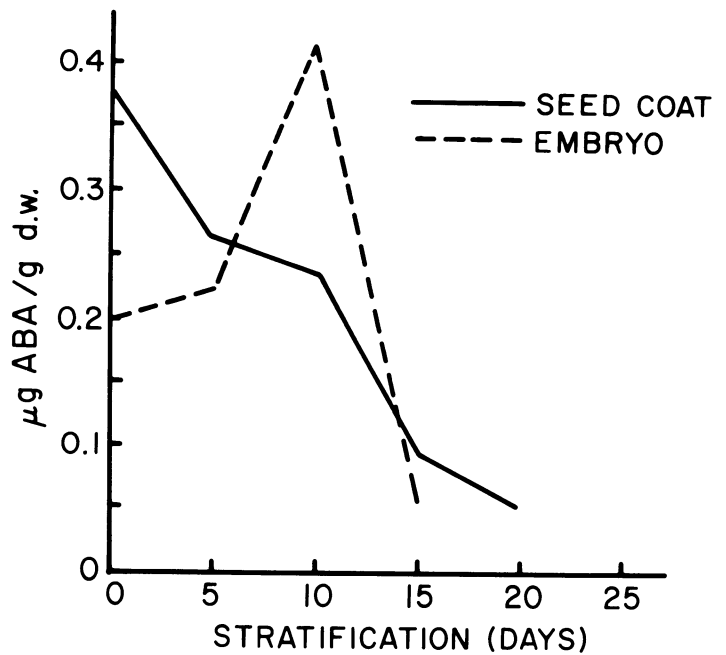


Fig. 2. Quantitative changes in concn of an acidic inhibitor in extracts of 'Tetela' peach seed coats and embryos at different stratification times. Calculations based on standards of ABA.

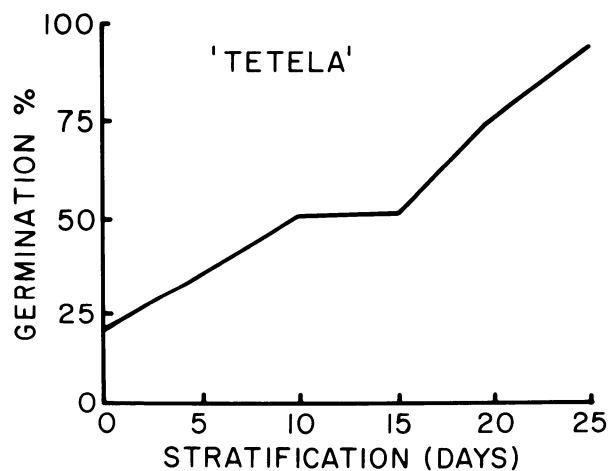
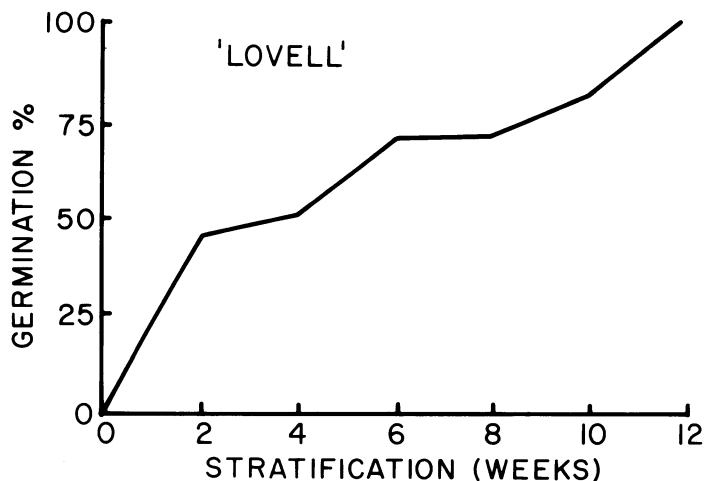


Fig. 3. Percent germination of 'Lovell' and 'Tetela' peach seeds after various times of stratification.

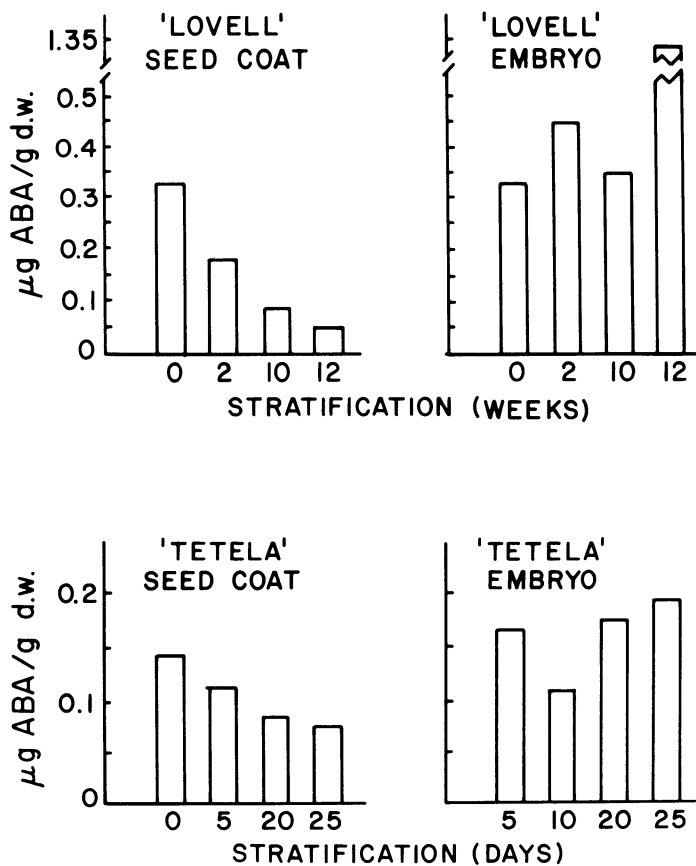


Fig. 4. Changes in concn of a bound inhibitor after hydrolysis of 'Lovell' and 'Tetela' peach seed coat and embryo extracts at different times of stratification. Calculations based on standards of ABA.

Characterization of the inhibitor. The inhibitor extract from nonstratified 'Lovell' seeds (Fraction I) was spotted on ITLC plates and developed in n-propanol:n-butanol:30% NH₄OH:water (6:2:1:2 v/v/v/v). Material in the inhibitory zone (R_f 0.7-0.9) was eluted with absolute methanol, and the eluate

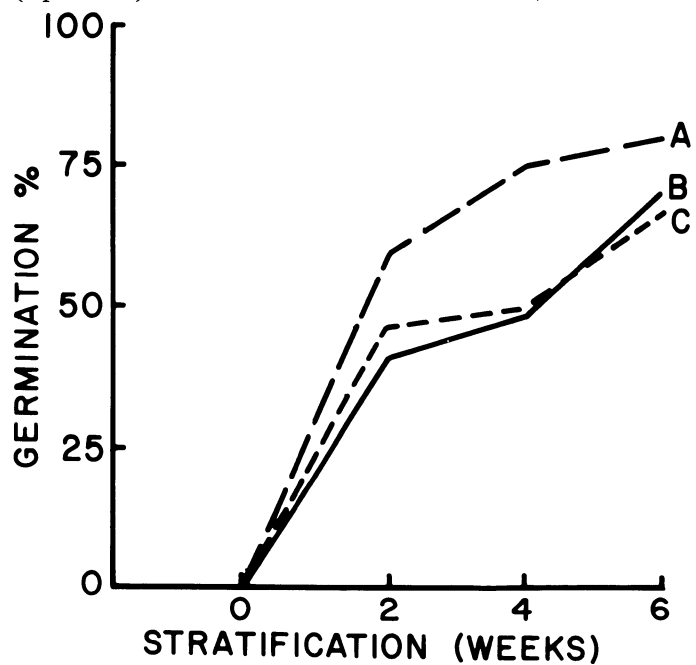


Fig. 6. Effects of GA and BA on germination of 'Lovell' peach seeds after different periods of stratification.
Treatments: A. GA₃ 2.0 ppm. + BA 100 ppm
B. GA₃ 0.02 ppm + BA 1.0 ppm
C. Control

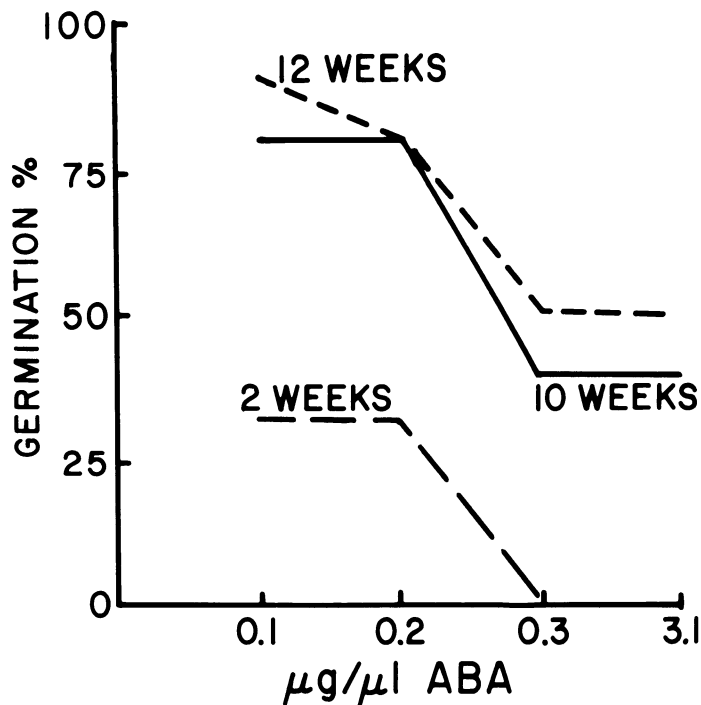


Fig. 5. Effect of abscisic acid and stratification for 2, 10, and 12 weeks on germination responses of 'Lovell' peach seeds without integuments.

was respotted on ITLC plates and developed with isopropanol:30% NH₄OH:water (8:1:1 v/v/v). Material in the inhibitory zone (R_f 0.65-0.85) was again eluted in absolute methanol and the eluate was respotted on thin layer chromatography silica gel plates and developed 3 times in benzene:acetic acid:water (8:3:5 v/v/v). A band of silica gel between R_f 0.25 and 0.45, which contained the inhibitor, was removed from the plate and the inhibitor eluted with ethyl acetate. The eluate was evaporated in a small tube to complete dryness, and then treated with diazomethane. The resulting methylated solution was dried, redissolved in 100 µl ethyl acetate, and chromatographed on a Varian 1520 dual aerograph equipped with a H flame ionization detector. In this system a 6-foot by 1/8-inch stainless steel column was packed with 3% SE-30 100/120 mesh, and the N carrier gas flow rate was adjusted to 40 ml/min. One µl of the treated extract was injected into the column, which was set isothermally at 160°C. Injector and detector temp were 205 and 210°C, respectively.

Results and Discussion

Seed coats and embryos of both cvs. contained an acidic inhibitor which decreased progressively to almost nonmeasurable concn as stratification proceeded (Fig. 1, 2). On a dry wt basis, the seed coat of 'Lovell' contained more than twice as much acidic inhibitor as did the embryo, 3.2 and 1.48 µg/g, respectively. The values for 'Tetela' were 0.4 in the seed coat and 0.2 µg/g in the embryo. The concn of inhibitor was closely related to seed germination. At high concn, germination percentage was low or zero (Fig. 3); but as stratification proceeded, the inhibitor decreased and the germination percentage increased. Later, as the concn in the seed coat declined, the inhibitor in the embryo seemed to become a controlling factor in germination. With the passage of time, perhaps some of the inhibitor in the seed coat was being leached by the moisture in the stratification medium, whereas other amounts were moving from the seed coat into the embryo. Further, tissues of the embryo may have a greater capacity to retain the inhibitor, and thus to affect its ultimate chemical form and distribution.

About half way through the stratification period, the

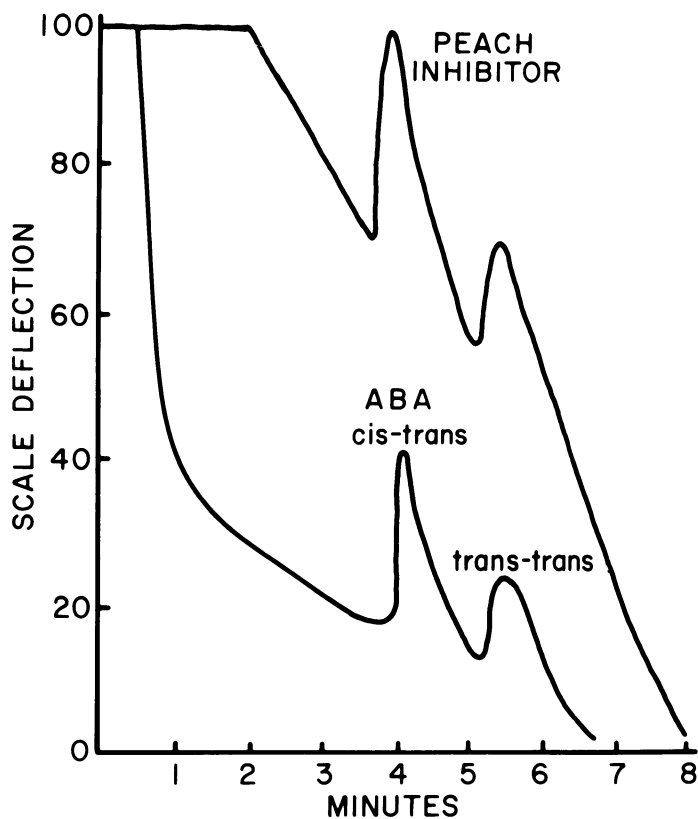


Fig. 7. Chromatogram obtained from injection of 1.0 μ l of methylated extract from peach seeds, equivalent to 1.5 g dry wt.

inhibitor concn increased in the embryos of both cvs., and then decreased (Fig. 1, 2). The increase coincided with a flat period in the germination curves (Fig. 3). This finding coupled with the reported decrease in GA₃ and GA₇ at the mid point in stratification of peach (12), support the inhibitor and promoter control proposal for inducing either dormancy or germination of seeds (3, 7).

A bound inhibitor was present in the extracts of seed coats and embryos of both cvs. (Fig. 4). In 'Lovell' embryos, the concn was 0.325 μ g/g dry wt at the start of stratification, the amount increasing progressively to 1.33 μ g/g by the end of stratification. In contrast, the concn in the seed coat decreased to 0.05 μ g/g. The same trends occurred in 'Tetela' seeds, but the concn were lower. The trends are not unequivocal but, generally, as the concn of the acidic inhibitor in the embryo decreased, that of the bound inhibitor increased. This supports the contention of Koshimizu et al. (8) and Milborrow (13) that the bound inhibitor may be a storage form of the acidic inhibitor. From our results, it appears that ABA accumulates in the embryo cells as the bound form and becomes inactive. At the same time there is an increase in growth promoter (12), and seeds are able to germinate.

High concn of ABA applied to 'Lovell' seeds without seed coats and stratified 2 weeks resulted in 0 germination whereas about 50% of those stratified 10 and 12 weeks germinated (Fig. 5). ABA did not completely retard germination by the 10th and 12th weeks, perhaps because of the increasing concn of promoter present (12). Cytokinins may also be involved at this time as it is known that BA is able to overcome the inhibitory effects of ABA (7).

Both GA₃ and BA promoted germination of stratified seed

up to 80% when applied together (Fig. 6) although no effect was observed with nonstratified seeds or when the compounds were applied singly. The results indicate that both growth regulators have a synergistic effect in breaking dormancy of peach seeds. It is known that under normal conditions of stratification, peach seeds produce GA₃ (12). Our applications of GA₃ did not induce seed germination without the presence of BA. Thus it is possible that germination in peach seeds may involve both GA and cytokinin.

At the beginning of stratification, 'Lovell' seed coats and embryos had higher acidic and bound inhibitor concn than did 'Tetela' seeds (Fig. 1, 2, 4). It is possible that the initial inhibitor level may be one of the factors which influence the amount of chilling required by a cv. If so, one could determine the amount of chilling required by measuring the concn of inhibitors and promoters before stratification. The fact that the methylated extract of the unknown inhibitor had a retention time similar to that of methylated standard ABA indicates that the peach seed inhibitor is similar, if not identical, to ABA (Fig. 7).

A hypothetical scheme to describe the internal control of peach seed dormancy could include the following: prior to stratification, ABA concn is sufficient to induce dormancy, and GA concn is low (12). As stratification proceeds, ABA decreases, and GA and cytokinins are synthesized or released from bound forms. Germination increases progressively until half the stratification requirement has been met. Abscisic acid increases simultaneously, but GA and cytokinins decrease, thus adversely affecting germination. Maximum germination occurs when stratification is completed, as GA and cytokinin concn are in their highest levels and ABA is present mostly in the inactive bound form.

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