

mination, particularly in view of the fact that the concn in the embryonic axis was 4-fold higher at this time.

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Abscisic Acid Levels in Seeds of Peach. II. Effects of Stratification Temperature¹

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Abstract. The roles of free and bound abscisic acid (ABA) in the breaking of dormancy in seeds of peach (*Prunus persica* (L.) Batsch) were investigated. Concentrations were consistently 10- to 100-fold higher in the embryonic axes of seeds than in the seed coats or cotyledons. Free and bound ABA declined 75 to 95% during stratification at both 5° and 20°C, yet only the 5° treatment broke dormancy. Extracts of chilled seeds were less inhibitory to germination of non-dormant seeds than were extracts of non-chilled seeds, but ABA content of the extracts was sufficient to account for only a small part of their biological activity.

Many temperate tree seeds require cold stratification (after-ripening) before germination and normal seedling growth can occur. This treatment is thought to reduce levels of growth inhibitors, such as ABA. Lipe and Crane (6) noted a decline in an ABA-like inhibitor during after-ripening of peach seeds, and demonstrated that both this inhibitor and synthetic ABA could inhibit the germination of non-dormant peach embryos. Wong (10) and Bonamy and Dennis (2) have identified ABA in extracts of dormant peach seeds, and Wong (10) measured the levels of an ABA-like inhibitor during stratification at 3° and 20°C. The inhibitor content of seed coats and cotyledons was not significantly affected by stratification at either temp, while that of embryonic axes declined markedly regardless of temp. Since dormancy was broken only at 3°, Wong concluded that the ABA-like inhibitor was not the only controlling factor in dormancy. Diaz and Martin (4) reported that an ABA-like inhibitor declined in peach seeds held at 3°, and that this decline was correlated with an increase in an inhibitor which could be

released by base hydrolysis. They suggested that the latter might be ABA released from its glucose-ester (7).

The purposes of our work were to determine the effects of duration and temperature of stratification on ABA levels in peach seeds, as determined by electron capture gas chromatography, and the relationship between the ABA content of seed extracts and their effects on germination of non-dormant seed.

Materials and Methods

Plant material and method of stratification. 'Halford' peach pits, obtained from a California source, were purchased from Hilltop Nurseries, Hartford, MI, and were stored at 22 ± 1°C until used. Prior to extraction of seed tissues or stratification, pits were soaked in tap water for 48 hr, then placed in plastic bags. The bags were drained of excess water, closed, and held at either 5 ± 1° or 20 ± 1°. The bags were opened at approximately 2 wk intervals for aeration.

Evaluation of germination capacity. At each sampling date, endocarps were removed and 4 samples of 10 seeds each were held at 20 ± 1°C in Petri dishes lined with moist filter paper. Germination, indexed by protrusion of the radicle through the seed coat, was recorded during a 10-day period.

Extraction of seed tissue. Seeds stratified for varying lengths

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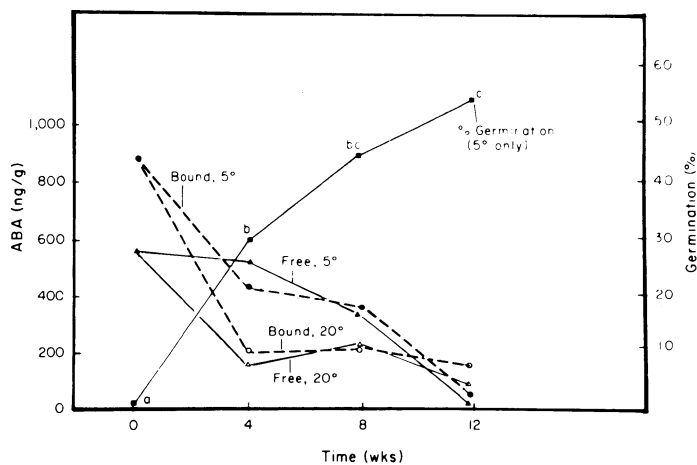


Fig. 1. Free and bound ABA (ng/g) in embryonic axes of peach seeds as affected by time and temp of stratification, and germination at 20°C of seeds held at 5°C for 0 to 12 weeks. Seeds held continuously at 20°C failed to germinate. Superscripts (germination data) indicate mean separation by Duncan's multiple range test, 5% level. Significant differences for ABA concn are indicated in Table 1.

of time were dissected into seed coats, cotyledons, and embryonic axes, the tissues were extracted with cold methanol, and the extracts were fractionated as previously described (2). Residues from the acidic dichloromethane fraction (free ABA) and a similar fraction after base hydrolysis of the water residue (bound ABA) were methylated with diazomethane, and aliquots were gas chromatographed using an electron capture detector (2). Two replicate samples of 50 seeds each were used in all cases.

Effect of duration and temp of stratification on ABA content. Seeds were removed from pits stratified at both 5 ± 1°C and 20 ± 1°C after 0, 4, 8 and 12 wk, extracted, and the quantity of ABA in the extracts was determined.

Effects of seed extracts on germination of stratified seeds. To determine the effects of stratification on germination-inhibiting activity of seed extracts, pits were stratified for 7 wk at 5°C. The seeds were then dissected, extracted with methanol, and the extracts processed as described above. Non-chilled, imbibed seeds were handled in a similar manner. The residues from the free acidic dichloromethane fractions were dissolved in water. Five ml aliquots equivalent to 5 seeds were placed on filter

papers in Petri dishes, and 10 stratified (12 wk at 5°C) seeds added to each dish, with 2 replicate dishes per treatment. The experiment was repeated, using both 2.5 and 5.0 seed equiv. per 5 ml. In the latter experiment, ABA controls were included (0, 1, 10, and 100 µg/5 ml), and ABA content of the extracts was determined by gas chromatography for comparison with their activity in inhibiting germination.

Results

Effects of duration and temp of stratification. Germination of seeds held at 5°C increased steadily, reaching 55% after 12 weeks (Fig. 1), while seeds stratified at 20°C did not germinate.

The concn of both free and bound ABA were 10 to 50 times as high in the embryonic axis as in the seed coat or cotyledons. The concn of both free and bound ABA in the embryonic axes declined during stratification at both temp (Fig. 1, Table 1). During the first 8 weeks, the decline was more rapid at 20°C than at 5°C, but the temp effect was not significant at 12 weeks.

In the seed coat and cotyledons, ABA concn also declined (Table 1), although the rate of loss was less rapid than in the embryonic axes. Bound ABA concn in the cotyledons was significantly greater at 20°C than at 5°C after 12 weeks, but other effects of temp were non-significant.

In non-stratified seeds, the ratios of free to bound ABA concn in the seed coat, cotyledons, and embryonic axis were 2.3, 0.8, and 0.6, respectively. These ratios varied somewhat during stratification, but no meaningful relationship with dormancy was apparent.

When the data were expressed on a per seed basis (Table 1), 7 to 26% of the free ABA occurred in the embryonic axis, 19 to 54% in the seed coat, and 29 to 72% in the cotyledons. Comparable values for bound ABA were: embryonic axis = 9 to 24%; seed coat = 12 to 31%, and cotyledons = 36 to 75%. Although the seed coat and cotyledons contained the largest amounts of ABA, the high concn in the embryonic axis caused this portion of the seed to contain a surprisingly large proportion of the ABA, considering its small size. Typical fresh wt of seed coat, cotyledons, and embryonic axes were 246.4, 510.0, and 3.0 mg, respectively, representing 32, 67, and 0.4% of total seed wt. Values for total ABA in the whole seed indicate a loss of 90 to 95% of the free ABA and 75 to 90% of the bound ABA during 12 weeks of stratification.

Effect of extracts of stratified vs. non-stratified seeds on germination of stratified seed. Extracts of cotyledons and embryonic axes of non-stratified seed significantly inhibited the

Table 1. Free (F) and bound (B) ABA in peach seeds as affected by duration and temp of stratification.^Z

Time (wk)	Temp (°C)	Seed coat		Cotyledons		Embryonic axis		Whole seed	
		F	B	F	B	F	B	F	B
<i>ABA concn (ng/g)</i>									
0	—	40.5c	17.6c	4.5b	5.8c	577.9e	907.8d	—	—
4	5	30.5bc	7.1ab	5.3b	4.2bc	528.9e	439.1c	—	—
	20	12.6ab	5.8ab	5.0b	1.9ab	160.4b	213.7b	—	—
8	5	21.5b	13.6c	2.2a	2.2ab	355.3d	373.2c	—	—
	20	16.2b	7.7b	1.8a	1.6a	245.9c	221.3b	—	—
12	5	1.9a	2.4a	1.3a	1.6a	27.9a	49.3a	—	—
	20	2.4a	3.3ab	0.6a	3.2b	93.6ab	155.9ab	—	—
<i>Total ABA (ng/seed)</i>									
0	—	12.0c	5.19d	5.89b	7.59b	2.54d	3.99d	20.44c	16.79c
4	5	6.1b	1.41ab	6.42b	5.08b	2.51d	2.09c	15.01bc	8.59b
	20	3.5ab	1.59b	5.42b	2.04a	0.70b	0.94b	9.66b	4.58a
8	5	4.5b	2.82c	2.68ab	2.68ab	1.88c	1.97c	9.05b	7.48b
	20	3.2ab	1.52ab	1.74a	1.55a	0.94b	0.85b	5.89ab	3.93a
12	5	0.4a	0.52a	1.57a	1.93a	0.14a	0.25a	2.17a	2.71a
	20	0.4a	0.58a	0.57a	3.47ab	0.35ab	0.59ab	1.35a	4.65a

^ZMean separation within columns and methods of expressing results (concn vs. total) by Duncan's multiple range test, 5% level.

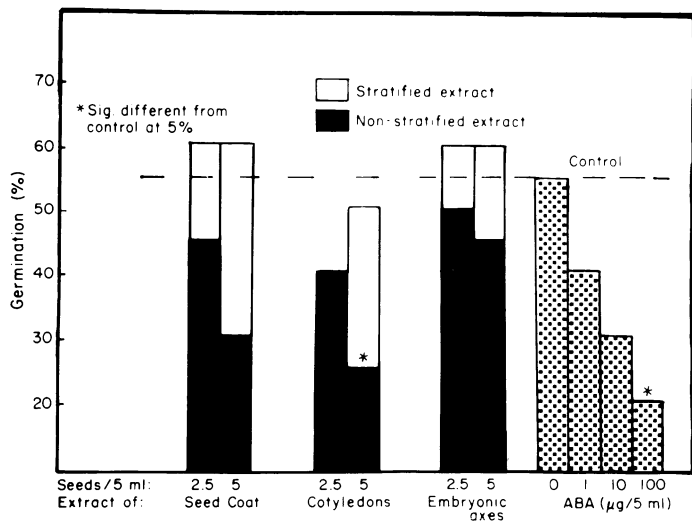


Fig. 2. Effect of peach seed extracts and ABA on germination of peach seeds stratified for 12 weeks at 7°C. Extracts were prepared from seeds held at 5°C for 0 (non-stratified) or 7 (stratified) weeks.

Table 2. Effects of extracts of non-stratified and stratified peach seed on the germination of stratified seed.^Z

Seed part	Germination (%)	
	Non-stratified extract	Stratified extract
Seed coat	40ab	50b
Cotyledon	30a	50b
Embryonic axis	30a	40ab
Water control	55b	

^ZMean separation by Duncan's multiple range test, 5% level.

germination of stratified seed, while similar extracts of stratified seed had non-significant effects (Table 2). Extracts of seed coats from both treatments had non-significant effects. The activity of the extract prepared from embryonic axes was particularly high, considering the small amount of tissue represented.

Extracts prepared from non-stratified seed inhibited germination in all cases in a second experiment (Fig. 2), but only the cotyledon extract had a significant effect using 2 replications (2 × 10 seeds). Extracts of stratified seed were either inactive or less inhibitory than those of non-stratified seed with one exception (cotyledons, 2.5 seeds/5 ml).

A standard curve was constructed by plotting log ABA concn vs. % inhibition of peach seed germination (data in Fig. 2), and ABA content of the extracts was estimated, using means for both levels of each extract. The actual ABA content of the same extracts was determined by GLC. Although biological activity and ABA content were generally parallel, the quantities of ABA present were far too low to account for activity (Table 3). However, the effect of stratification in reducing ABA content was again confirmed.

Discussion

Both free and bound ABA declined during stratification, hence no evidence was obtained to support the suggestion (4) that free ABA is converted to bound form in the process.

Dormancy was broken only in seeds held at 5°C ± 1°C, while the decline in ABA was noted at both 5°C and 20°C. Wong (10),

Table 3. Biological activity (ng ABA-eq per seed) of extracts of stratified and non-stratified peach seed as determined by assay with stratified peach seed and ABA content (ng/seed) as determined by EC-GLC.

Extract	Non-stratified		Stratified		Stratified as % of non-stratified (GLC)
	Assay (ng ABA-eq per seed)	GLC (ng/seed)	Assay (ng ABA-eq per seed)	GLC (ng/seed)	
Seed coat	1100	17.2	0 ^Z	0.9	5
Cotyledon	3200	13.5	220	3.5	26
Embryonic axis	110	6.5	0 ^Z	0.4	6

^ZGermination of seed incubated with stratified extract was greater than germination of control.

using bioassay, observed a similar temp-independent decrease in an ABA-like inhibitor in the embryonic axis of peach seeds during stratification. ABA levels alone therefore cannot be the controlling factor in the dormancy of peach seeds. This does not rule out the possibility that chilling permits the synthesis of a promoter, thus increasing the promoter/ABA ratio.

The inhibitory effects of seed extracts upon germination of stratified seeds declined with stratification. Although ABA content paralleled activity in most instances, the quantities of ABA present were far too low to account for all of the activity. These data suggest that other growth inhibitors, possibly phenolic compounds, are present which also decline with stratification.

Our data, together with those for ABA content of maturing peach seed (2), parallel the observations of Balboa-Zavala (1) concerning ABA levels in apple seeds. In neither case is any clear relationship between ABA content and dormancy apparent. Growth promoters produced during chilling (3, 5, 8) may be responsible for the breaking of dormancy. On the other hand, rates of synthesis or degradation may be more important in controlling growth. However, Sondheimer et al. (9) reported no difference between chilled vs. non-chilled ash (*Fraxinus americana* L.) embryos in ability to metabolize either ABA or zeatin. Obviously, much work remains to be done before a clear understanding is obtained of the effects of chilling in breaking dormancy.

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