THE ROLE OF ABSCISIC ACID IN PEACH (*Prunus persica* L.) SEED DORMANCY

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ABSTRACT

THE ROLE OF ABSCISIC ACID IN PEACH (Prunus persica L.) SEED DORMANCY

By

Prince Albert Bonamy

Abscisic acid (ABA) was identified in a methanol extract of dormant peach seed by combined gas liquid chromatography-mass spectrometry (GC-MS). ABA levels were measured during seed maturation using electron capture-gas liquid chromatography (EC-GC). Recovery of ^{14}C-ABA indicated that more than 50% of the ABA present in the extract should normally have been recovered by the procedure used for fractionation. ABA levels in the embryonic axes could not be related to the germination potential of embryos excised from maturing seeds. Drying and storage of seeds did not significantly affect total ABA content, although an increase in both free and bound ABA occurred in the embryonic axes. On imbibition, levels of both free and bound ABA dropped except for free ABA in the seed coat and bound ABA in the embryonic axes, neither of which changed significantly. Both free and bound ABA declined in all seed portions during stratification at both 5° and 20°C, yet only the 5°C treatment broke dormancy. Interruption of low temperature (5°C) stratification by 10 days at 27°C promoted final germination after 12 weeks, and increased ABA slightly, but not significantly. 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, as measured by GLC, was sufficient to account for only a small part of their biological activity. I therefore conclude that the level of ABA is not the major factor controlling the dormancy of peach seeds.

THE ROLE OF ABSCISIC ACID IN PEACH (*Prunus persica* L.) SEED DORMANCY

BY

Prince Albert Bonamy

A DISSERTATION

Submitted to
Michigan State University
in partial fulfillment of the requirements
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1976

DEDICATION

TO MY MOTHER,

LOUISE

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ABBREVIATIONS

The following abbreviations will be used in this dissertation.

ABA	abscisic acid
BA	N^6-benzyladenine
DPA	dihydrophaseic acid
EC-GC	electron capture gas-liquid chromatography
GA	denotes the series of gibberellins and use of a subscript denotes a specific gibberellin, as GA_1
GLC	gas-liquid chromatography
GC-MS	combined-gas liquid chromatography and mass spectrometry
IAA	indole-3-acetic acid
PPO	2, 5-diphenyl oxazole

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INTRODUCTION

INTRODUCTION

Viable seeds which fail to germinate on exposure to favorable environmental conditions of temperature, light, moisture and oxygen supply, are said to be in a state of dormancy (36). Such seed require special pretreatment to induce germination (43). Dormancy in many grains, e.g., Vicland oats (61) disappears during storage at room temperature, while dormancy of Rosaceous seeds may be broken by cold treatment (43).

Leopold (38) indicated that internal mechanisms regulating germination may be those involving (a) the enlargement and restriction of the embryo, and/or (b) growth substances in the seed. Agents that can terminate dormancy under certain conditions are (a) mechanical treatment such as scarification, (b) light, (c) temperature, and (d) certain chemicals.

Inhibitors have been implicated in the dormancy of seed of many temperate zone fruits. Abscisic acid (ABA) has been identified in peach (78), and measured in plum (39), and is thought to be one of the factors in the control of dormancy (35). The study of ABA and its role in dormancy may lead to a better understanding of this important phenomenon. ABA can be measured by GLC (62) which eliminates the need for extensive purification of extracts prior to bioassay, as well as interference from other inhibitors.

This study was designed to measure the level and distribution of ABA in peach seed during entrance into dormancy, and during the breaking of dormancy by chilling: and (b) to determine the effect of increasing the temperature prematurely during chilling, and thereby inducing secondary dormancy, on the levels of abscisic acid. Preliminary experiments were also conducted to determine the effects of exogenous growth regulators and extracts of non-stratified and stratified peach seed on germination of stratified seed.

LITERATURE REVIEW

LITERATURE REVIEW

Definitions

This review is concerned primarily with the literature relating to dormancy in Prunus seed. Dormancy is often separated into endogenous dormancy (also called rest, true dormancy, or constitutive dormancy) and exogenous dormancy (also called quiescence, external dormancy, or imposed dormancy).

Endogenous dormancy is a condition in the seed that delays or prevents germination under favorable environmental conditions and may be attributable to metabolic blocks, or the presence of inhibitors. (67). Exogenous dormancy involves external controls, for germination will occur if the seeds are placed under suitable environmental conditions (36).

Stratification is the practice of holding imbibed seeds in a moist environment, regardless of temperature (31), while low temperature (0° - 10°C) stratification is referred to as after-ripening (43). The chilling requirement prevents germination during unfavorable climatic conditions, and is viewed as a survival mechanism (74). Increasing the temperature prematurely may re-introduce dormancy, and then a second low temperature exposure may be required. Nikolaeva (47) and Janick et al. (31) both refer to this as secondary dormancy. On the other hand, the effects of several separated periods at low temperature may be cumulative (63).

Causes of Dormancy in Prunus Seeds

Crocker (13, 14) classified the various causes of dormancy as follows:

a. embryo immaturity

b. seed coat impermeability to water or gases

c. seed coat restriction to embryo growth

d. metabolic block within the embryo

e. a combination of the above

f. secondary dormancy

Recently, Kozlowski (36) re-classified these as follows:

a. morphologically mature but physiologically dormant embryos

b. rudimentary embryos

c. immature embryos

d. mechanically resistant seed coat

e. impermeable seed coat

Morphologically, the peach seed consists of an embryo surrounded by testa. Within the testa are thin layers of nucellar and endosperm tissue, but the cotyledons of the embryo make up the bulk of the seed, and contain the food reserves used by the developing seedling. The embryos of *Prunus* seeds are fully developed, but germination does not occur unless the seed coat (testa) is removed, or the seed after-ripened. If a mechanically resistant seed coat were responsible for dormancy, then low temperature stratification should reduce this resistance. However, Wong (78) found no difference in the resistance of seed coats of non-stratified *vs.* stratified peach seed. Thus, chilling does not appear to affect germination by weakening the seed coat in peach. On soaking in water, peach seeds become fully imbibed within 24 to 48 hours, indicating that the seed coat is not a barrier to the penetration of water.

Prunus embryos are morphologically mature but are physiologically dormant. Low temperature stratification of seed results in normal seedlings, while excised embryos germinated without low temperature exposure give rise to dwarfed seedlings (20).

Exogenous Control of Dormancy in Prunus Seeds

Seed Coat. As noted above, removal of the seed coat in *Prunus* results in embryo germination. For example, Chao and Walker (10) obtained 40 to 80% germination of non-chilled excised embryos of apricot and 50 to 70% germination of similarly treated peach embryos. However, the seedlings generally have short internodes and various abnormalities, and are not as vigorous as seedlings from stratified embryos (10, 20). Thus, in effect, the seed coat imposes dormancy upon the embryo, and can be thought of as an exogenous factor. Once the chilling requirement is completed, the seed coat is much less effective in restricting germination. The fact that seed coat resistance does not change with chilling (78) suggests that either inhibitors present in the seed coat prevent germination (see below), or that the non-chilled embryo is not sufficiently vigorous to break through the mechanical barrier provided by the testa.

Temperature. Pillay, *et al.* (49) working with cherry seeds, obtained consistently higher germination in seeds held at 7.2°C than in those held at 3.3°C. Chao and Walker (10) stratified apricot seed, with pericarp removed, for 0 to 4 weeks at 0°, 3.3°, 7.2°, 10°, or 22.2°C.

After 2 weeks highest germination occurred at 7.2°C, but after 4 weeks 95% germination was observed in seeds held at 3.3°C. Their data suggest an interaction between length of storage and temperature. However, since germination was recorded at the temperature of stratification, the results obtained may not reflect true germination potential. Suszka (68, 69, 70) held *P. avium* and *P. padua* seeds at 20°C for 2 to 4 weeks prior to after-ripening at 3°C, and observed that subsequent germination was greater than that of seeds held continuously at 3°C. Suszka (70) reasoned that in nature *Prunus* seeds first go through a period of warm moist stratification after the fruits ripen and drop from the tree. This is followed by a longer period of cold, moist stratification during which the seed undergo physiological changes that prepare them for germination. Joley (32) and Tehrani (71) confirmed Suszka's observations. Pollock (5) tested the effect of temperature upon the growth of embryos excised from non-stratified peach seeds. Embryos germinated at 19°C. showed no abnormalities, while those germinated at 23° or 27°C were dwarfed. He concluded that dwarfing symptoms were controlled by the temperature of germination.

When partially after-ripened peach embryos were transferred to 25°C in a moist environment, secondary dormancy was induced (33). However, if the embryos were dried at 5°C or 25°C for two days, and then stored at 26°C before re-imbibition, chilling was cumulative. Thus drying prevented the induction of secondary dormancy. These results differ from those of Flemion (19) and Coston (12) both of whom reported no inhibitory effect on germination of peach seed when chilling was interrupted by a period of time at 20°C to 25°C.

Light. Supplementary light accelerated the germination of excised embryos of peach, cherry, apricot, and plum seeds (56), but stratified seeds germinated in either light or darkness, suggesting that light plays no active role in Prunus seed dormancy under natural conditions.

Gases. Tissaoui (72) showed that exposure to N_2 broke dormancy in apple embryos even in the absence of chilling. Thus O_2 was not necessary for release from dormancy. No data are known from similar studies with Prunus seed.

Coston (12) observed that germination of peach seed was stimulated when either ethylene or ethephon was applied either before or after stratification at 0°C. However, abnormalities were observed following ethephon treatment.

Chemicals. Certain chemicals can partially substitute for chilling in Prunus seed. Tukey and Carlson (73) treated non-chilled seed of 23 cultivars of peach with thiourea, using concentrations of 0.005% to 0.5% and exposure periods of 2 to 16 hours. Thiourea at 0.25% resulted in 100% germination of 'Lovell' seeds. Dormancy of 6 additional cultivars was not broken, and all others showed less response than 'Lovell'. Seedlings from the treated seeds were dwarfed, and had shortened internodes and anomalous leaves, typical of seedlings from non-stratified excised embryos. Later, Carlson and Badizadegan (9) reported that thiourea was not as effective as BA or GA_3 in stimulating germination of seeds of several other peach cultivars after one month of stratification. Lipe (40) reported that both thiourea and GA_3 were able to break dormancy in peach seed. He noted that GA_3-treated seeds germinated slowly, and the resulting seedlings were not dwarfed, whilst thiourea-treated seeds germinated readily,but the seedlings showed typical symptoms of insufficient chilling.

Gray (23) was among the first to note that GA_3 could substitute for chilling in peach seed. Treatment of non-stratified seed with GA_3 (10 ppm) in agar medium resulted in 35% germination in 13 days, whereas the control seeds had not germinated after 53 days. However, seedling growth was not discussed. Chao and Walker (10) stimulated the germination of non-stratified peach and apricot seed with high concentrations (4,000 to 20,000 ppm) of GA_3, but only the apricot seedlings grew normally. However, application of 20 to 1,000 ppm GA_3 to excised embryos of peach and apricot resulted in normal seedling growth. Fogle (22) soaked _P. avium_ seed in GA_3 solution (100 ppm) prior to stratification. After 3 months at 5°C, 64% germination occurred in GA_3-soaked seed vs. 28% germination in water-soaked seed.

BA at 10 and 20 ppm stimulated germination in seeds of 3 varieties of peach after stratification for one month (9). On the other hand, non-stratified embryos or intact seeds of plum did not respond to 1,2,4 or 8 ppm BA (39).

The effects of GA and cytokinins may be additive or more than additive (synergistic). When GA_3 and BA were applied together to non-stratified peach seeds, no germination occurred (16); however, partially chilled seeds responded to treatment. No data were presented for the effects of the two chemicals alone. Lin and Boe (39) applied GA_3 and BA to non-stratified intact seeds and embryos of plum. Intact seed did not respond to treatment, but GA_3 (16 ppm) plus BA (8 ppm) resulted in 100% germination of the embryos compared to 25% for no treatment. However, GA alone at 32 ppm gave 62.5% germination, which was not significantly different from the effect of the mixture. The data are, however, suggestive of additive effects of GA and BA on germination. Effects on seedling growth were not discussed.

Naringenin, a growth inhibitor isolated by Hendershott and Walker (26) from peach flower buds, had no effect on germination of stratified peach seed or on seedling development (21).

Lipe (40) applied ABA (5.0 ppm) to excised embryos of peach and reduced germination from 83% (water control) to 5.6%. When Diaz and Martin (16) applied ABA to excised peach embryos and then stratified them for 2, 10, or 12 weeks, germination was also inhibited. However, the effect decreased with stratification, suggesting either that the inhibitory effect of ABA was being nullified by promoters produced at the low temperature, or that chilling increased the embryo's capacity to inactivate ABA. Similar results were reported by Rudnicki and Pieniazek (59) with apple (Pyrus malus L.) seeds.

Lipe (40) applied various combinations of IAA, GA, and ABA to dormant and non-dormant peach seed. ABA reduced the germination of non-dormant seed, as well as the response of dormant seed to GA.

Endogenous Control of Dormancy in Prunus Seeds

Three phases of seed dormancy are recognized by Amen (3), namely, induction, maintenance, and release. Relatively little information is available on induction in Prunus seeds. Weaver and Hough (76) noted that in the early-ripening peach cv. 'Raritan Rose', germination of excised embryos following 8 weeks at 4°C, was greater in samples taken 71 to 91 days after full bloom than those taken at 99 and 107 days. There was no apparent increase in inhibitor content (wheat coleoptile bioassay) or germination of excised embryos. Pillay (48) using the _Avena_ mesocotyl bioassay noted four growth promoters in methanol extracts of cherry seeds. He suggested that their disappearance at maturity might be related to the induction of dormancy. Bausher (5) used electron capture-GLC to follow ABA levels in seeds of 'Okinawa' peach as the embryo matured. The concentration at maturity was 30 ng/g fresh weight, which is probably far too low to have imposed dormancy, judging from the data of Lipe and Crane (41).

Studies have dealt with the maintenance and breaking of dormancy in _Prunus_ seeds.

Effect of the seed coat. As previously noted, the seed coat prevents the germination of non-chilled _Prunus_ embryos. The reasons for this are not well established. However, one can postulate that the effect is due either to chemicals in the seed coat which inhibit radicle growth, or to the mechanical resistance of the seed coat. If the latter, then Wong's (78) data suggest that the properties of the embryo, rather than those of the seed coat, change during after-ripening. The possible role of inhibitors is discussed below. Dennis (15) suggested the use of reciprocal crosses between cultivars or species which differed in chilling requirement as a test of the effect of the seed coat. If the seed coat controls dormancy, the chilling requirements of seeds from the two crosses should be similar to those of the female parent. Kester (34) made such crosses in almonds and almond/peach hybrids, and observed no maternal effect, suggesting that the seed coat has a purely mechanical effect.

Respiratory changes. Pollock and Olney (52) found that the rate of respiration increased with stratification in sour cherry (_P. cerasus_ L.) seed. They observed that dinitrophenol (DNP) stimulated respiration in dormant seed and that the effect declined with chilling. They suggested that blocks occurred in the electron transport system in dormant seeds, and that these were removed by chilling.

Later, LaCroix and Jaswal (37), compared O_2 uptake of dormant and non-dormant sour cherry seed in the presence and absence of dinitrophenol. They observed that DNP increased O_2 consumption 91% in dormant embryonic axes _vs._ only 18% in non-dormant axes, thus confirming the work of Pollock and Olney (52). The O_2 uptake of cotyledons was not affected by DNP, implying that the embryonic axis is the site of dormancy. They also noted that the C-6/C-1 ratio in the embryonic axis was constant during the 6 weeks of chilling, but decreased sharply at 7 weeks, suggesting a shift in respiratory pathway as dormancy was broken.

Auxins. Although IAA has been identified in extracts of developing sour cherry seed (28), Biggs (6) could find no marked changes in indole auxins in peach seeds as stratification time increased.

Gibberellins. Until recently, only GA_{32} had been identified in _Prunus_, occurring in developing seeds of apricot (11), and peach (79). However, G.C. Martin (personal communication) has now identified GA_{29} in immature plum (_P. domestica_ L.) seeds and fruits.

Although GA-like substances were detected by bioassay of methanol extracts of mature sweet cherry (_P. avium_ L.) seeds, Proctor and Dennis (55) were unable to correlate changes in concentration with changes in germination capacity during after-ripening.

Mathur _et al._ (42) reported that GA_3-like and GA_7-like compounds increased 1268% and 553% respectively during chilling of peach seed held at 0°C. Although there was a decrease at the eighth week of stratification, the concentrations increased at the 12th and 16th week. The concentration of GA_7-like substance was lower than that of GA_3-like substance throughout stratification. Germination increased from 0% for non-stratified seeds to 35% for seeds stratified for 4 weeks. No germination data were given for subsequent samples. However, the authors apparently used a spectrophotofluorometric method which is open to criticism. Lin and Boe (39), also using spectrophotofluorometry, found that dormant plum seed contained 0.125 μg/gm of GA-like substances, and that this increased to 0.17 μg/gm during the 90 day chilling period. They therefore suggested that increases in GA content are associated with the breaking of dormancy.

Cytokinins. Webb et al. (77) noted that butanol-soluble cytokinins in Acer saccharum seeds reached a maximum concentration after 20 days of stratification, then declined before dormancy was broken. Borkowska and Rudnicki (8) reported that both free and bound (released on acid hydrolysis) cytokinins increased during stratification of apple seed with a maximum at 5 weeks. No information is known concerning cytokinin levels in Prunus seed during stratification.

Inhibitors. Chao and Walker (10) inhibited the germination of embryos excised from non-chilled peach and apricot seeds with extracts prepared from similar seeds, and concluded that substances present in the seed coat were responsible for activity. However, Flemion and de Silva (21) could not correlate levels of inhibitors extracted from peach seeds with dormancy release.

Aitkins (2) identified the phenolic compounds mandelic acid and benzoic acid in peach seeds. These products of the hydrolysis of mandelonitrile were detected in non-stratified seed 72 hours after imbibition, but only trace amounts were noted during after-ripening. The concentrations (0.005 to 0.05 μg /g and 0.16 μg /g) of mandelic and benzoic acids, respectively, were not high enough to inhibit seed germination, and Aitkins (2) concluded that these compounds had no direct effect in peach seed dormancy.

Lipe and Crane (41) measured the levels of an ABA-like substance in peach seed extracts. Co-chromatography, UV absorption spectra, and bioassay with wheat coleoptile sections indicated that the inhibitor was ABA-like. They correlated the release of dormancy in peach seed with the disappearance of this substance. Although the inhibitor was present throughout the seed, its concentration was greater in the integuments than in the embryo. Application of the inhibitor extracted from dormant seeds to embryos excised from stratified seeds induced anomalies similar to those observed in seedlings from non-chilled embryos.

Diaz and Martin (16) investigated a similar compound in peach seed and showed that one of the components of the extract had the same retention time as ABA on GLC. They also reported a decline in the concentration of the inhibitor during chilling of two peach cultivars, 'Tetela' and 'Lovell', and this decline was correlated with an increase in the concentration of a similar inhibitor released by base hydrolysis. Milborrow (46) had observed that ABA could be released from its glucose ester in this manner. 'Tetela' seeds, which require only a few weeks of chilling, contained less inhibitor than 'Lovell' seeds, which must be chilled for 6 weeks, and more inhibitor was present in the embryo than in the seed coat.

Wong (78) unequivocally identified ABA in extracts of peach seeds by GC-MS. However, bioassay results indicated that levels in seed coat and cotyledon did not decrease significantly as stratification time at either 3°C or 20°C increased. Although a marked reduction occurred in the embryonic axis, the change was independent of temperature. Thus, although a decrease in ABA in the embryonic axis may be a prerequisite for germination, other factors, such as gibberellins, must be responsible for the effects of low temperature on germination.

Lin and Boe (39), using bioassay, reported a 32% decrease in an ABA-like substance in plum seeds after 30 days of chilling, with a subsequent 10% decline for the remainder of the 90 day chilling period.

Interaction of promoters and inhibitors. Dormancy control is often attributed to a balance of endogenous promoters and inhibitors (3) with dormancy maintenance being associated with the presence of high levels of growth inhibitors, and dormancy release with high levels of growth promoters. Amen (3) suggests that exposure to chilling temperature permits growth promoter production, which then triggers dormancy release. Flemion and de Silva (21) analysed extracts of peach seed during dormancy release. Their data indicate no correlation between the breaking of rest and the relative levels of promoters and inhibitors. However, Lin and Boe (39) noted that GA-like substances increased while growth inhibitory substances (ABA-like compounds) decreased in plum seeds exposed to chilling.

Khan (35) proposed that germination in some seeds is controlled by interaction between cytokinins, gibberellins, and inhibitors, with cytokinins playing a permissive role. Gibberellins are necessary for germination, but the presence of inhibitors, e.g., ABA, blocks their action. Cytokinins are capable of removing this block. However, Drury (17) states that this model could apply only to discrete data, and that germination of a population of seeds is a continuous function. Furthermore, available data on endogenous growth regulators in seeds which require chilling do not support the model. For example, the work of Webb, *et al*. (77) with *Acer* seed, suggests that *levels* of hormones do not determine the state of dormancy in the seed, but rather the *sequence of changes* in growth regulators. They showed that the levels of GA, ABA, and cytokinins were all low following chilling, but germination still occurred.

Although data are not available for peach, bioassay results with apple seeds indicated that GA-like substances reached a maximum after 4 weeks (64), cytokinins after 5 weeks (8), while ABA-like substances had declined to negligible levels after 3 weeks (58). Thus, if Khan's (35) hypothesis were correct, dormancy should have been broken after 4 weeks. However, 6 weeks were required for 50% germination, suggesting that the hypothesis does not hold in this case as well.

Summary

1. Removal of the peach seed coat allows germination, but the seedling grows slowly, and abnormalities occur if temperature of germination is greater than 20°C.

2. Holding seeds at 0 to 10°C for a specified period results in normal seedling growth even when the seed coat is intact.

3. The seed coat may either contain inhibitory substances or serve as a mechanical restriction to growth. Some workers (14, 16) have reported a decline in inhibitory substances in the seed coat, while others (21, 78) have found no such decline. The fact that no maternal (i.e., seed coat) effect is evident in seeds from reciprocal crosses of almonds and peach/almond hybrids which differ in chilling requirement (34) suggests that the seed coat effect is purely mechanical. Chilling does not appear to affect the mechanical resistance of the seed coat of peach (78). Thus germination potential is probably a function of embryo vigor, which is enhanced by chilling.

4. Chilling appears to prepare the embryo for growth. The changes observed in phosphate metabolism and respiration are indicative of growth potential, which perhaps is favored by growth substances produced during chilling. Inhibitors, including ABA, appear to decline, and promoters increase, leading to a favorable balance. However, endogenous regulators are usually present in lower concentrations than are necessary for stimulation or inhibition when applied externally (39) suggesting penetration problems or reduced sensitivity of the target organs. Several aspects of the role of ABA in _Prunus_ seed dormancy require further study. These include: (a) use of physico-chemical methods of measurement, rather than bioassay, (b) relation of ABA levels to the _induction_ of dormancy, as well as the _breaking_ of dormancy, (c) the effect of temperature in the

observed decline in ABA content during stratification, (d) the contribution of ABA to the observed inhibition by seed extracts, and (e) the role of esterification of ABA in the disappearance of the free acid during stratification.

SECTION ONE

THE ROLE OF ABSCISIC ACID IN PEACH (*Prunus persica* L.) SEED DORMANCY.

I. INDUCTION OF DORMANCY.

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THE ROLE OF ABSCISIC ACID IN PEACH (*Prunus persica* L.) SEED DORMANCY

I. INDUCTION OF DORMANCY

ABSTRACT

A peak observed on gas chromatography of a partially purified methanol extract of mature peach seeds was identified as ABA by combined gas chromatography-mass spectrometry (GC-MS). No germination occurred during seed maturation unless embryos were excised. Germination of excised embryos increased with maturity, and ABA content of embryonic axes and other seed parts did not appear to be related to germination potential. Drying and storage of seeds did not significantly affect total ABA content, although an increase in both free and bound ABA occurred in the embryonic axes. On imbibition, levels of both free and bound ABA decreased except for free ABA in the seed coat and bound ABA in the embryonic axis, neither of which changed significantly. In most cases examined, levels of free ABA paralleled those of bound ABA, suggesting that although the former may be converted to the latter, bound ABA is not a major source of free ABA.

Growth substances have been measured in developing seeds of peach (22, 21, 11), cherry (15, 9), and apricot (12), but only GA_{32} (24) and ABA (23) have been unequivocally identified in peach seeds. Using electron capture-gas liquid chromatography, Bausher (2) found that ABA increased in peach seeds as they matured, reaching a maximum concentration (30 mg/g fresh wt) when the embryo had reached full length. Germination potential was not determined but Bausher suggested that the level of ABA present at maturity was sufficient to impose dormancy.

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Using the Avena first internode bioassay, Pillay (15) observed that auxin-like substances decreased as cherry seeds matured, with no activity measurable at maturity. He suggested that this decrease in auxin-like substances might be the cause of rest. Hopping and Bukovac (10) identified IAA in sour cherry seeds, and noted a decrease in total auxin content in the seed as the fruit matured.

Bausher (2), found that cytokinin-like substances, as measured by the soybean callus assay, varied from 7.5 to 16.4 μg/g fresh wt. during embryo development of peach. His results suggest that high levels of cytokinin-like substances are associated with embryo growth and that a decline occurs when the embryo matures.

Dry storage may reduce the germination potential of seed. Haut (8) reported no significant effect of drying of cherry and peach seed on their germination during subsequent after-ripening. However, Fogle (6), found that dry storage prior to after-ripening reduced germination of cherry (P. avium L.) seeds to about 50% of those kept moist. Ross and Bradbeer (18) obtained similar results with hazel seeds. Balboa (1) reported a marked increase in ABA content in the embryonic axes of apple seeds sampled August 24 and subsequently stored for 4 months over $CaCl_2$. However, this was not true for mature seed sampled September 21.

The present work was undertaken to (a) identify ABA in mature peach seed, (b) relate ABA levels to entrance into dormancy, and (c) determine the effects of drying and storage of the seed on ABA content.

MATERIALS AND METHODS

Plant material and method of stratification. Mature peach seeds, cv. Halford were used for identification of ABA and for determining recovery of 2-^{14}C-ABA in fractionation. Pits from a California source were purchased from Hilltop Orchards, Hartford, MI, and were stored at 22 ± 1°C until used. Dry seeds were removed from the endocarp and soaked in distilled water for 48 hr prior to dissection. For comparison of ABA levels during maturation and storage, seeds, cv. Redhaven, were collected from mature trees at the Horticultural Research Center, East Lansing, MI. from July to September of both 1974 and 1975, and brought to the laboratory for immediate dissection.

Extraction of seeds and fractionation of extracts. All seeds were dissected into seed coat, cotyledons, and embryonic axis prior to extraction except where otherwise noted. The tissues were held at 0°C during dissection of 50-seed samples (*ca.* 15 min.), then covered with cold methanol and macerated in a Sorval Omni-mixer. Seed coats and cotyledons were ground in 250 ml of solvent, embryonic axes in 100 ml. The macerates were shaken overnight at 2° ± 1°C., filtered through cheesecloth and the filtrates were centrifuged. The supernatant was poured off and the tissued was re-suspended in fresh methanol and again centrifuged. The combined supernatant (*ca.* 500 ml for seed coat and cotyledons, 250 ml for embryonic axis) was evaporated *in vacuo* at 40° ± 1°C. The residue was redissolved in 25 ml of 0.1 M phosphate buffer pH 7.5 and partitioned (Fig. 1). For identification of ABA, an extract

Fig. 1. Procedure for fractionation of methanolic extracts of peach seed tissues to yield free and bound ABA fractions.

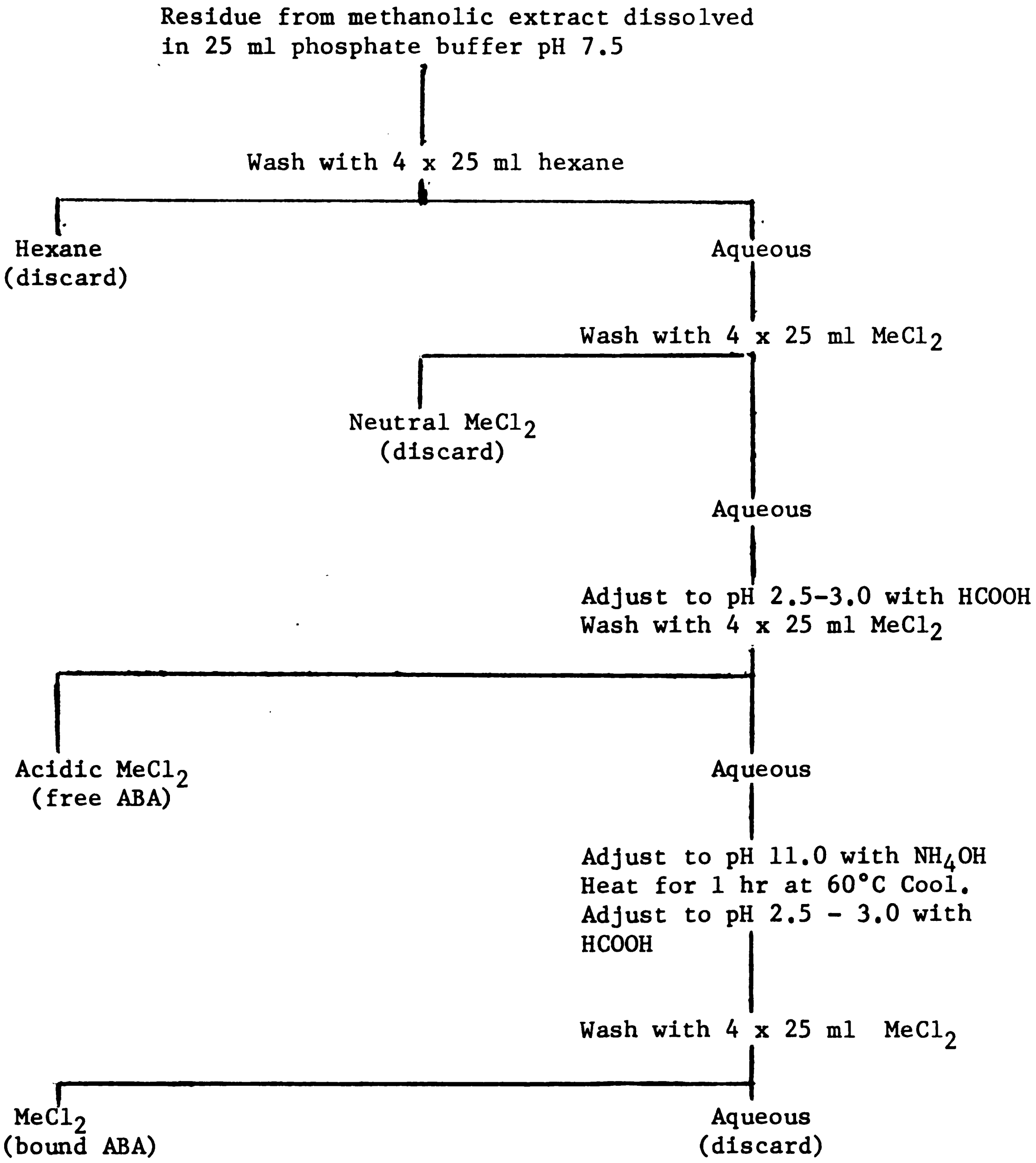
Residue from methanolic extract dissolved in 25 ml phosphate buffer pH 7.5
Wash with 4 x 25 ml hexane
Hexane (discard)
Aqueous
Wash with 4 x 25 ml $MeCl_2$
Neutral $MeCl_2$ (discard)
Aqueous
Adjust to pH 2.5-3.0 with HCOOH
Wash with 4 x 25 ml $MeCl_2$
Acidic $MeCl_2$ (free ABA)
Aqueous
Adjust to pH 11.0 with NH_4OH
Heat for 1 hr at 60°C Cool.
Adjust to pH 2.5 - 3.0 with HCOOH
Wash with 4 x 25 ml $MeCl_2$
$MeCl_2$ (bound ABA)
Aqueous (discard)

Fig. 1

of whole seeds (150 gm after imbibition) was prepared in a similar manner, except that larger quantities of solvent were used.

Methylation and GLC. Following evaporation of the dichloromethane, the residues from the free and bound fractions were methylated as described by Schlenk and Gellerman (19) and modified by Powell (16). Each residue was dissolved in 1 ml of ether/methanol (9:1, v/v). Carbitol (1.5 ml), KOH (1.0 ml, 60 g/100 ml H_2O) and an ethereal solution of diazald (1.5 ml at 115 mg/ml) were added in sequence to a 150 x 15 mm test tube, which was then connected by 'Teflon' tubing through 'Nalgene' stoppers to the tube containing the sample. The diazomethane generated on addition of diazald passed into the sample tube and methylated the organic acids present. The ether/methanol was then evaporated and the residue dissolved in ethyl acetate.

For quantitative analysis of ABA, one microliter of sample, representing 5 mg-eq of seed, 30 mg-eq of cotyledon, or 0.1 mg-eq of embryonic axis, was injected into a Packard 7300 gas liquid chromatograph. This was equipped with a ^{63}Ni foil electron capture detector and was operated either at 5 or 7.5 volts. The column (2 mm i.d. x 1.83 m) was packed with 3% SE 30 (methyl silicone) on 80/100 mesh Gaschrom Q. Column temperature was 210°C and inlet and detector temperatures were 26° and 270°C, respectively. The carrier gas was N_2 at a flow rate of 40 ml/min at 40 psi. Nitrogen scavenger gas was supplied to the detector at 70 ml/min.

Quantitation was based on peak height, using known quantities of synthetic cis, trans ABA (R. J. Reynolds Tobacco Co.) as a reference.

Retention time was also determined on a Hewlett-Packard 402B GLC using an electron capture detector and an XE 60 column (25% cyanoethyl, methyl-silicone) at temperature of 200°C. Inlet and detector temperatures were 260° and 270°.

Gas chromatography-mass spectrometry. For identification of ABA, the free fraction from 150 gm of whole seed was analysed, following methylation, on an LKB GC-MS interphased with a POP 8/I computer. The glass column (i.d. 2 mm by 1.84 m) contained 3% SP 2100 (methyl silicone) on Supelcoport (acid washed, silanized diatomite 100/120 mesh). Helium was used as carrier gas at a flow rate of 20 ml/min. Column and detector (flame ionization) temperatures were 200° and 290° C, respectively. The mass spectrometer was operated at 70 eV.

Recovery of 2-^{14}C-ABA. 2-^{14}C-ABA (Mallinkrodt Nuclear) was used to estimate how much ABA was lost during fractionation. In one experiment, methanol extracts were prepared following dissection of 100 non-stratified and 100 stratified (12 weeks at 5 ± 1°C) seeds.

In a second experiment, a methanol extract of 100 intact, non-stratified seeds was used. Following extraction 1.2×10^{-3} μCi of 2-^{14}C-ABA (specific activity 23 Ci/mole) was added to each sample, and the extracts were partitioned as described above. Ten ml of scintillation fluid (5 gm PPO and 100 gm naphthalene/liter in dioxane) was added to each fraction and the samples were counted for 10 min using a Beckman model LS 100 liquid scintillation counter. Counts were corrected for quenching and efficiency.

ABA levels during seed maturation vs. germination. Fruits were collected at weekly intervals from July 31 until fruit maturity in both 1974 and 1975, diameters of 10 fruits and weights of 10 seeds being recorded for each date. Two samples of 50 seeds each were analysed for ABA as previously described.

On each sampling date, 4 samples of 10 intact seeds and the same number of excised embryos were placed on 2 layers of moist filter paper in glass Petri dishes. Germination, defined as geotropic curvature of the radicle, was recorded after 10 days at 20 ± 1°C and 200 ft.-candles of fluorescent light.

Effects of drying and storage of seed on ABA content. 'Redhaven' seeds from the 1974 harvest were air-dried and stored in the endocarp for 12 months. One lot was dissected and extracted dry, the other imbibed before dissection. ABA was analysed as previously described.

All data on ABA content were analysed by analysis of variance, Duncan's (5) multiple range test being used for comparison of treatment means.

RESULTS

The peak assumed to be cis, trans-ABA in the extract had the same retention time as synthetic cis, trans-ABA on both supports (Table 1). None of the other peaks in the extract had retention times identical with trans, trans ABA or with either isomer of DPA.

GC-MS of the extract of non-stratified whole seeds showed the presence of ABA at the retention time of synthetic cis, trans-ABA. Major

Table 1. Retention times (min) of _cis_, _trans_ and _trans_, _trans_ isomers of Me-ABA and Me-DPA, and of ABA-like component in methylated extract from peach seed following gas chromatography on two column supports.

Column	Temp (°C)	Me-ABA (c,t)	Me-ABA (t,t)	Me-DPA (c,t)	Me-DPA (t,t)	Extract
SE 30	210	1.76	2.47	2.35	3.34	1.76
XE 60	200	1.49	--	2.16	3.00	1.49

fragments and intensities were in close agreement with the reference sample (Fig. 2), aside from fragments in the standard at m/e values greater than the molecular ion (M^+), which were probably due to impurities. Twenty-seven to 84% of the 2-^{14}-C-ABA added to extracts was recovered in the acidic dichloromethane fraction following fractionation (Table 2), recovery being less than 50% in only one of 9 samples. This represented from 70 to 92% of the total ABA recovered. Only 1 to 4% was recovered in the bound fraction.

Both fruit diameter and seed fresh weight increased steadily from mid-July until early September (Table 3). Intact seed failed to germinate, regardless of time of collection. Germination of excised embryos was low in July, increased sharply in early mid-August, and continued to increase until harvest (Table 3). Concentrations of both free and bound ABA in the embryonic axis were 10 to 100-fold that in other portions of the seed throughout maturation (Table 4), while levels in the cotyledons and seed coat were similar.

Expression of the data on a per seed basis (Table 5) showed that most of the ABA occurred in the cotyledons, which make up the greatest part of the seed (ca. 67% in mature seeds vs. 32% for the seed coat and less than 1% for the embryonic axis) on a fresh weight basis.

Levels of both free and bound ABA increased 3 to 4-fold in the seed coat during maturation in 1974; levels in the embryonic axis and cotyledons exhibited no particular trends (Table 4). In 1975 both free and bound ABA increased significantly during maturation in the embryonic axis but no trends were evident in other tissues. Data for the former are graphed in Fig. 3. No clear relationship between ABA content and

Fig. 2. Mass spectra of authentic *cis, trans*-ABA and of presumed ABA and of presumed ABA in extract of peach seeds. Intensities of major ions as percent of base peak.

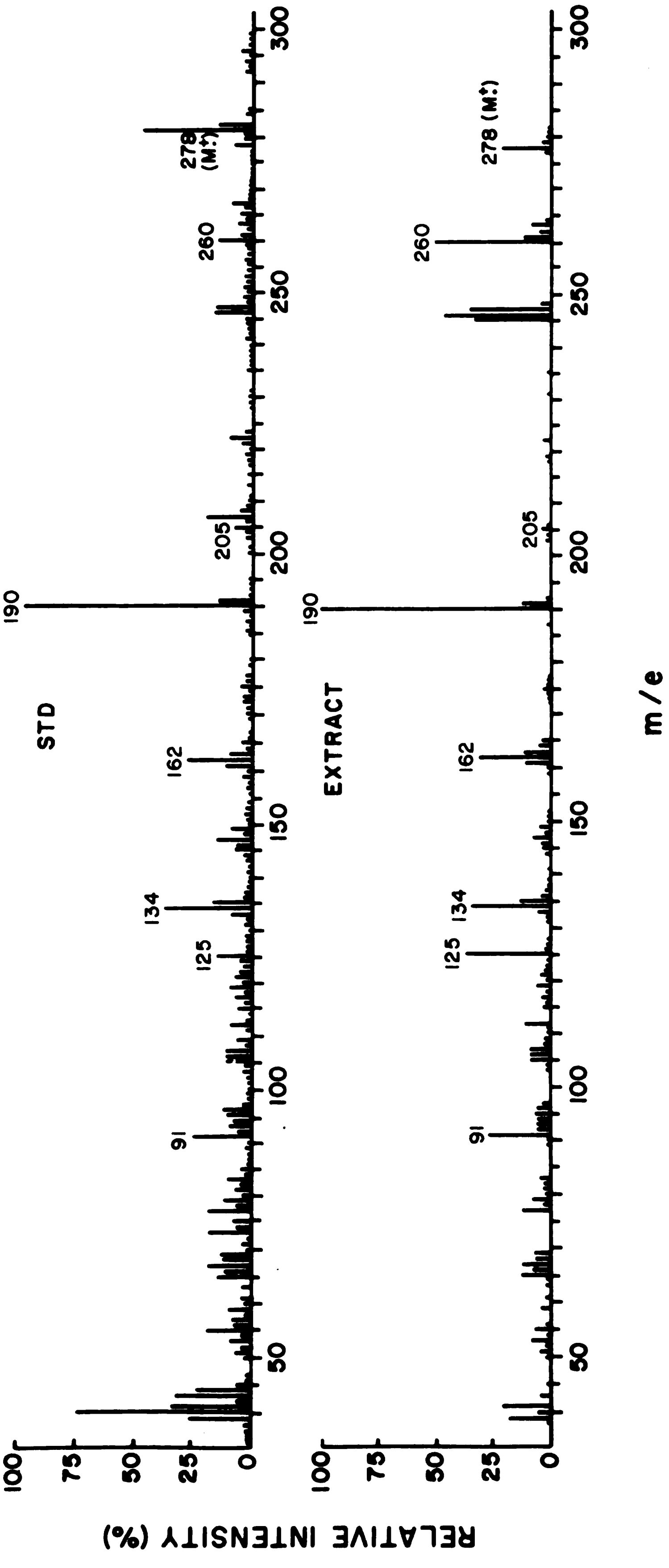
STD
EXTRACT
RELATIVE INTENSITY (%)
m/e
100
75
50
25
0
50
100
150
200
250
300
91
125
134
162
190
205
260
278 (M+)

Fig. 2

Table 2. Recovery of 2-^{14}C-ABA during fractionation of extracts of non-stratified and stratified peach seeds.

	Seed coat	Cotyledon	Embryonic axis	Intact seed	
	Imbibed only			Exp. 1	Exp. 2
Total recovery (%)	107	65	60	-	79
% of recovered counts					
Hexane	11	4	2	-	3
Neutral CH_2Cl_2	8	7	4	-	8
Acidic CH_2Cl_2	79 (84)[z]	84 (55)	92 (55)	-	89 (71)
Bound CH_2Cl_2	2	2	1	-	-
Aqueous residue	1	3	1	-	1
	Imbibed and stratified at 5°C for 8 wk.				
Total recovery (%)	72	60	31	87	80
% of recovered counts					
Hexane	8	4	1	2	2
Neutral CH_2Cl_2	4	5	4	7	10
Acidic CH_2Cl_2	85 (61)	84 (51)	88 (27)	88 (77)	87 (70)
Bound CH_2Cl_2	1	4	3	1	-
Aqueous residue	2	2	3	2	1

[z] Figures in parentheses indicate recovery as a % of total ABA added.

Table 3. Fruit diameter, seed weight, and germination of excised embryos during maturation of 'Redhaven' peach in 1974 and 1975. Means for 10 fruits, 10 seeds (wt.), or 4 x 10 seeds (germination).

	1974			1975		
Sampling date	Fruit diameter (mm)	Seed weight (mg)	% Germ.	Fruit diameter (mm)	Seed weight (mg)	% Germ.
July 16	35 a[z]	472 a	--	38 a	440 a	--
31	39 b	485 a	15.0 ab	39 a	443 a	20.0 a
Aug. 6	48 c	539 b	17.5 b	46 b	489 b	67.5 b
13	53 d	554 b	85.0 c	51 bc	527 bc	80.0 bc
22	58 de	559 b	87.5 cd	53 c	547 c	85.0 c
29	60 e	562 b	90.0 cd	57 c	568 c	90.0 c
Sept 7	65 e	573 b	97.5 d	--	--	--

[z] Within columns, means followed by the same letter are not significantly different from one another at the 5% level

Table 4. Effect of time of sampling on concentration of free (F) and bound (B) ABA (ng/g) in 'Redhaven' seed.

Sampling date	Seed coat		Cotyledon		Embryonic axis	
1974	F	B	F	B	F	B
July 31	8.8^{a}[z]	8.1^{ab}	4.8^{a}	3.9^{a}	126^{a}	265^{ab}
Aug. 6	7.7^{a}	6.0^{a}	9.9^{b}	3.2^{ab}	211^{ab}	323^{b}
13	9.0^{a}	7.3^{ab}	6.5^{a}	3.2^{a}	213^{ab}	207^{a}
22	18.9^{b}	15.9^{abc}	8.4^{ab}	7.8^{b}	267^{b}	327^{b}
29	13.9^{ab}	15.2^{abc}	8.4^{ab}	8.2^{b}	106^{a}	595^{c}
Sept 7	28.4^{c}	22.8^{bc}	9.6^{ab}	8.2^{b}	108^{a}	345^{b}
1975						
July 31	10.3^{a}	9.4^{a}	5.3^{a}	7.4^{a}	114^{a}	231^{a}
Aug 6	9.1^{a}	10.0^{a}	5.9^{a}	13.6^{b}	315^{c}	225^{a}
13	11.0^{a}	12.2^{ab}	12.1^{b}	6.1^{b}	351^{c}	378^{b}
20	6.9^{a}	10.7^{ab}	11.2^{b}	9.1^{ab}	212^{b}	364^{b}
Aug 27	11.0^{a}	17.7^{b}	3.9^{a}	6.6^{a}	298^{c}	373^{b}

[z] Within columns and years, means followed by the same letter are not significantly different from one another at the 5% level.

Table 5. Effect of time of sampling on total free (F) and bound (B) ABA (ng/seed) in developing 'Redhaven' peach seed.

Sampling date	Seed coat		Cotyledon		Embryonic axis	
1974	F	B	F	B	F	B
July 31	2.90^{a}[z]	2.54^{ab}	2.98^{a}	2.42^{a}	0.38^{a}	0.80^{a}
Aug 6	2.96^{a}	2.30^{a}	9.48^{c}	3.04^{a}	0.76^{b}	1.16^{a}
13	3.10^{a}	2.45^{ab}	6.38^{b}	3.15^{a}	0.80^{b}	0.78^{a}
22	5.45^{b}	4.64^{b}	7.98^{bc}	7.41^{b}	0.95^{b}	1.16^{b}
29	3.32^{a}	3.80^{ab}	7.31^{bc}	7.11^{b}	0.35^{a}	1.31^{b}
Sept 7	3.64^{a}	2.82^{ab}	7.32^{bc}	6.25^{b}	0.26^{a}	0.84^{a}
1975						
July 31	2.55^{b}	2.33^{ab}	2.69^{a}	3.79^{a}	0.34^{a}	0.66^{a}
Aug 6	1.61^{a}	1.75^{ab}	3.19^{a}	7.35^{b}	0.67^{b}	0.48^{a}
13	1.14^{a}	1.28^{ab}	4.82^{b}	2.42^{a}	0.55^{a}	0.60^{a}
20	0.80^{a}	1.24^{a}	6.54^{c}	5.32^{ab}	0.45^{a}	0.77^{a}
27	1.60^{a}	2.60^{b}	1.90^{a}	3.21^{a}	1.28^{c}	1.60^{b}

[z] Within columns and years, means followed by the same letter are not significantly different from one another at the 5% level.

Fig. 3. Concentrations of free and bound ABA in extracts of embryonic axes during maturation *vs.* germination of excised embryos.

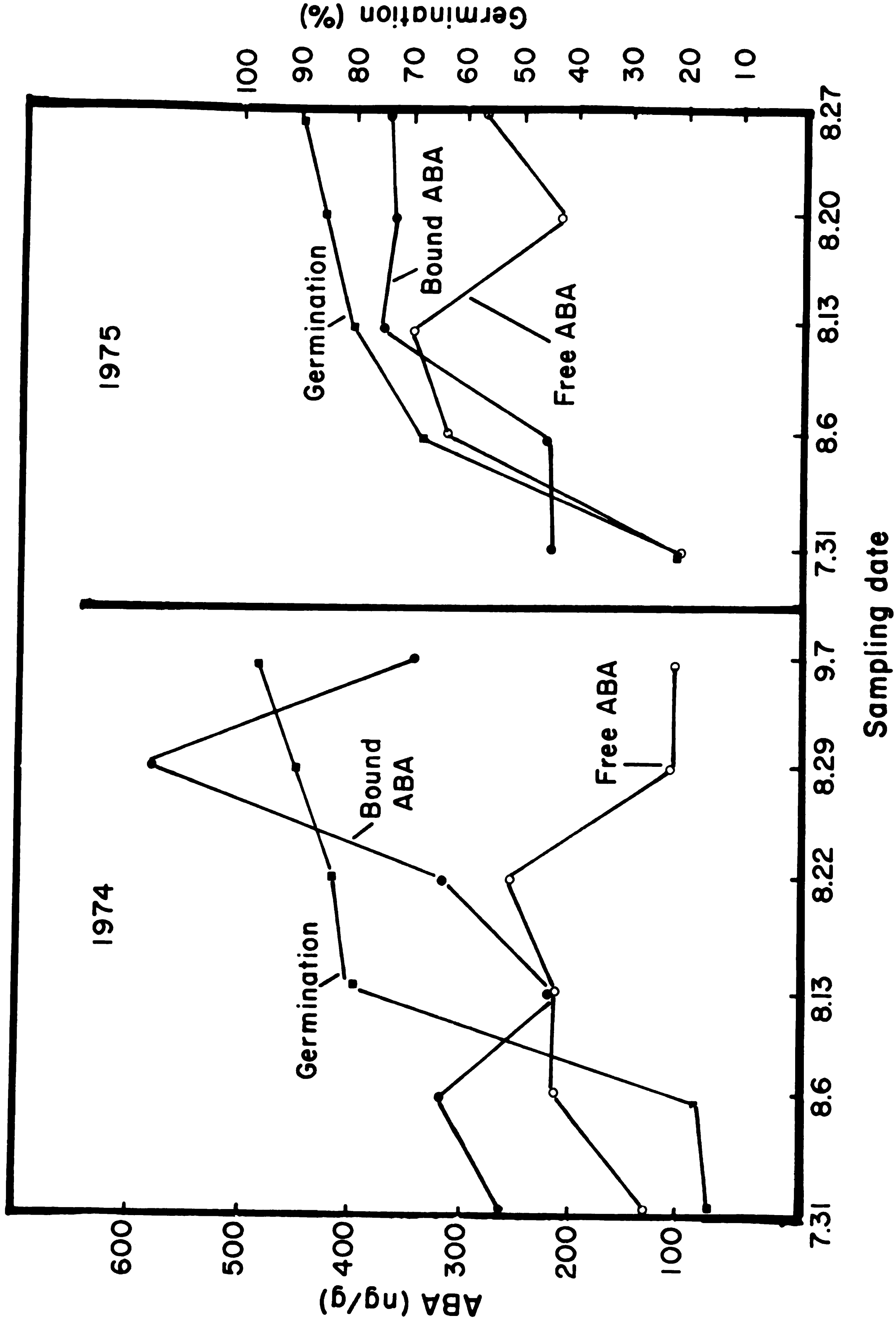
1974
1975
Germination
Bound ABA
Free ABA
Germination
Bound ABA
Free ABA
ABA (ng/g)
100
200
300
400
500
600
Germination (%)
10
20
30
40
50
60
70
80
90
100
7.31
8.6
8.13
8.22
8.29
9.7
7.31
8.6
8.13
8.20
8.27
Sampling date

Fig. 3

germination capacity is evident. The fact that ABA concentration increased in the seed coat in 1974 while content remained unchanged appears to reflect a decrease in seed coat weight during maturation. This relationship was not observed in 1975 however.

Total content of both free and bound ABA was not significantly affected by air-drying and storing in the laboratory for 1 year (Table 7), although ABA content of the embryonic axis alone increased 3 to 5-fold. Imbibition of the dried seeds had varying effects, depending on the tissue sampled and the form of ABA. Free ABA decreased 30% in the embryonic axis, 70% in the cotyledons, but only 7% in the seed coat, while bound ABA decreased 12, 81 and 72% in these tissues.

DISCUSSION

Wong's (23) identification of c,t-ABA in dormant peach seeds was confirmed in this work. The highest concentrations were present in the embryonic axes, confirming the observations of both Wong (23) for peach, and Balboa (1) for apple seeds. The maximum concn of ABA, calculated on a whole seed basis, ranged from 10 to 15 ng/g. These values are within the range of those determined by Bausher (2) for peach, cv Okinawa.

Balboa (1) showed that germination of excised apple embryos decreased as fruits matured; my data, on the other hand, show that germination of excised peach embryos increased with fruit maturation. The high rate of germination (90 to 98%) at harvest is in general agreement with other data for peach, e.g., those of Chao and Walker (3), who obtained

Table 6. Effect of drying and storage on ABA content. Total free and bound ABA content (ng/seed) of peach seed analysed fresh, after 1 year of dry storage, and after imbibition.

	Free ABA			Bound ABA		
	Fresh	Dry stored	Imbibed	Fresh	Dry stored	Imbibed
Seed coat	3.64^{a}[z]	3.47^{a}	3.24^{a}	2.82^{ab}	6.98^{b}	1.85^{a}
Cotyledon	7.32^{b}	6.80^{b}	1.99^{a}	6.25^{b}	6.70^{b}	1.26^{a}
Embryonic axis	0.26^{a}	1.86^{b}	1.19^{a}	0.84^{a}	2.54^{b}	2.23^{b}
Total	11.24^{b}	12.14^{b}	6.43^{a}	9.92^{b}	16.24^{b}	5.44^{a}

[z] Within tissues and forms of ABA, means followed by the same letter are not significantly different from one another at the 5% level.

57 to 72% germination of non-stratified excised embryos. Although ABA concn in the embryonic axis was high, it did not appear to affect germination capacity. Levels of free and bound ABA tended to parallel one another, in agreement with the data of both Mielke (13) for cherry buds, and Balboa (1) for apple seeds. These data do not support the suggestion of Diaz and Martin (4) that concentrations of free ABA and bound ABA tend to be inversely correlated. Although Seeley and Powell (2) have demonstrated the conversion of free ABA to a bound form, evidence for the reverse reaction is lacking. Harrison and Walton (7) demonstrated that ABA was converted to phaseic acid and thence to dihydrophaseic acid in bean (*Phaseolus vulgaris*) leaves. These compounds are metabolized in turn, and decline as levels of ABA drop, resulting in a parallel variation. Bound ABA may follow a similar pattern.

Drying and storage of peach seed increased the ABA content of the embryonic axis, but not of the remainder of the seed. On re-imbibition, free ABA decreased 30% in the embryonic axis, 70% in the cotyledons, and only 7% in the seed coat, while bound ABA decreased 12, 81 and 72% in these tissues. Had leaching been responsible for these losses, one would have expected a different relationship, with greatest losses in the seed coat, where exposure to the medium was maximum. Therefore, metabolic changes are implicated.

In summary, ABA level does not appear to be correlated with germination capacity of maturing peach seeds. Although the seed coat prevents germination, its maximum ABA content (September 7, 1974) was 28 ng/g, or approximately 0.05 ppm, assuming 50% recovery. This concentration would have had a negligible effect on germination, particularly in view of the fact that the concn in the embryonic axis was 4 fold higher at this time.

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SECTION TWO

THE ROLE OF ABSCISIC ACID IN PEACH (*Prunus persica* L.) SEED DORMANCY.

II. EFFECTS OF CHILLING

THE ROLE OF ABSCISIC ACID IN PEACH (Prunus persica L.) SEED DORMANCY

II. EFFECTS OF CHILLING

ABSTRACT

The relationship between levels of both free and bound abscisic acid (ABA) and dormancy in peach seeds was investigated. Concentrations were consistently higher in the embryonic axes of seeds than in the seed coats or cotyledons. Levels of both free and bound ABA declined in all seed portions during stratification at both 5° and 20° C, yet only the 5° treatment broke dormancy. Interruption of low temperature (5° C) stratification by 10 days at 27° C promoted germination after 12 wk, and increased ABA content slightly, but not significantly. 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. I conclude that the level of ABA is not the major factor controlling the dormancy of peach seeds.

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 (15).

Lipe and Crane (10) 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 (17) and Bonamy (3) have identified ABA in extracts of dormant peach seeds by combined gas chromatography-mass spectrometry, and Wong (17) 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 temperature, while that of embryonic axes declined markedly regardless of temperature. 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 (6) reported that an ABA-like inhibitor declined in peach seeds held at 3° C, 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 (11).

When embryos of partially chilled peach seeds were transferred to 25° C for 10 days, subsequent germination potential was reduced (8), and a longer exposure to cold temperature was required. Although this effect was not noted in peach seeds by either Flemion (7) or Coston (5), such "secondary dormancy" has been reported to occur in both apple (1) and cherry (14).

The purposes of my work were to determine: (a) the effects of duration and temperature of stratification on ABA levels in peach seeds, as determined by electron capture gas chromatography; (b) the effect of interruption of low temperature stratification on ABA content; and (c) the relationship between the ABA content of seed extracts and their effects on germination of non-dormant seed.

MATERIAL AND METHODS

Plant material and method of stratification. Peach pits, cv. Halford, 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° C. 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 ten 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 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 (3). 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 (3). Two replicate samples of 50 seeds each were used in all cases.

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

Effect of interruption of low temperature treatment on ABA content. Pits were held at 5° C for 42 days, then one group was transferred to 27° ± 1° C for 10 days, after which they were returned to 5°. The controls were held continuously at 5° C. Both germination capacity and ABA content of seeds were determined at intervals.

Effects of seed extracts on germination of stratified seeds. To determine the effects of stratification on germination-inhibiting activity of seed extracts, peach 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. The experiment was repeated, using both 2.5 and 5.0 seed equivalents per 5 ml. In this case, 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 biological activity.

RESULTS

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

Fig. 1. Free and bound ABA in peach seed as affected by time and temperature of stratification. ABA concentration (ng/gm) in embryonic axes *vs.* germination.

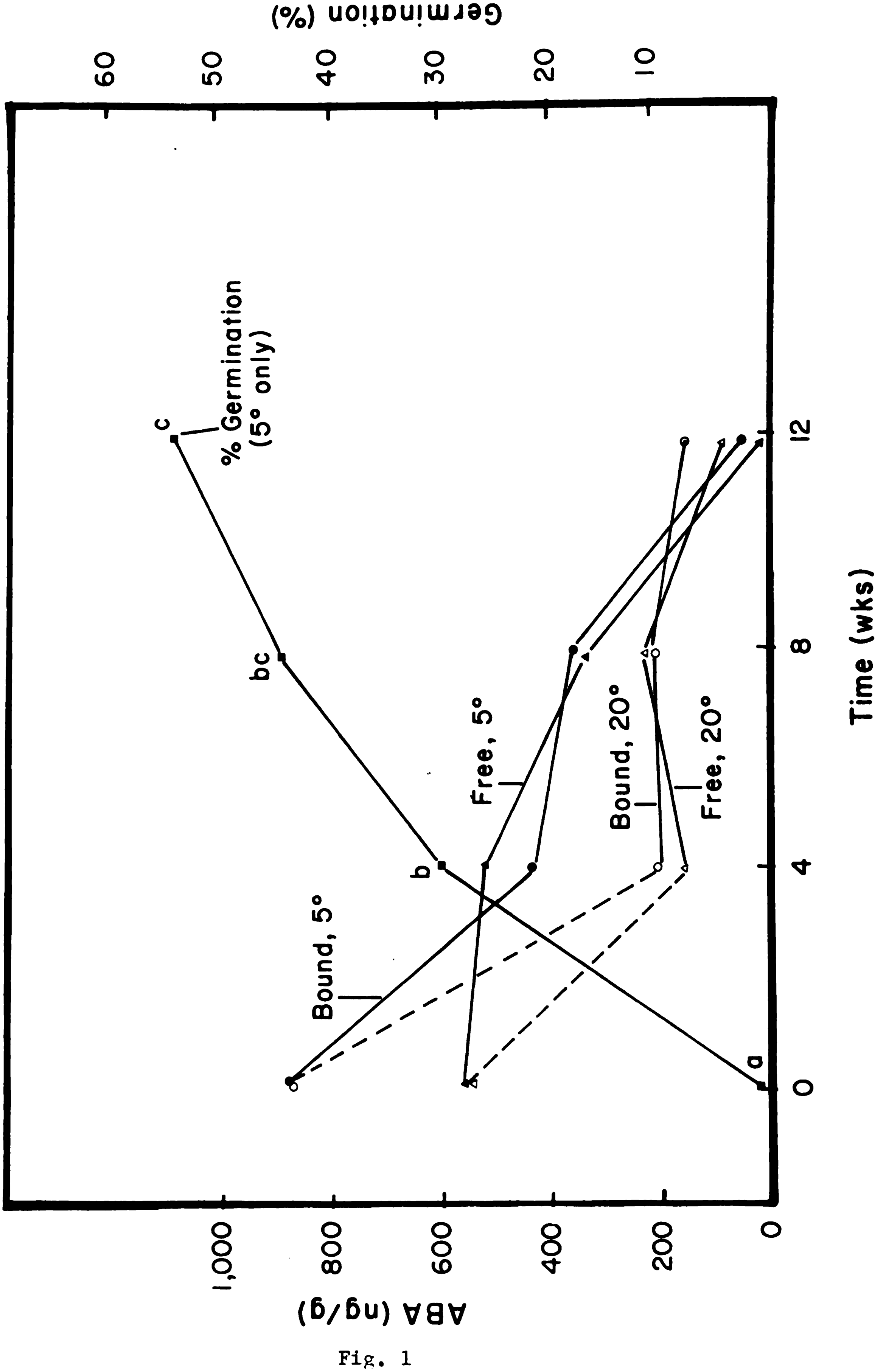

Germination (%)
60
50
40
30
20
10
% Germination (5° only)
c
bc
b
a
Bound, 5°
Free, 5°
Bound, 20°
Free, 20°
Time (wks)
0
4
8
12
ABA (ng/g)
1,000
800
600
400
200
0

Fig. 1

The concentrations 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 concentrations of both free and bound ABA in the embryonic axes declined during stratification at both temperatures (Fig. 1, Table 1). During the first 8 wk, the decline was more rapid at 20° than at 5° C., but the temperature effect was not significant after 12 wk. The approximate percentages of both free and bound ABA remaining in the embryonic axis after 12 wk of stratification were 5 (5°) and 15 (20°).

In the seed coat and cotyledons, ABA concentration also declined (Table 1), although the rate of loss was less rapid than in the embryonic axes. Percentages of free and bound ABA remaining after 12 wk were 5 and 15, respectively, in the cotyledons.

In non-stratified seeds, the ratios of free to bound ABA 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 concentration 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 weights of seed coat, cotyledons, and embryonic axes were 246.4, 510.0, and 3.0 mg,

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

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

[z] Within columns, means followed by the same letter are not significantly different from one another at the 5% level.

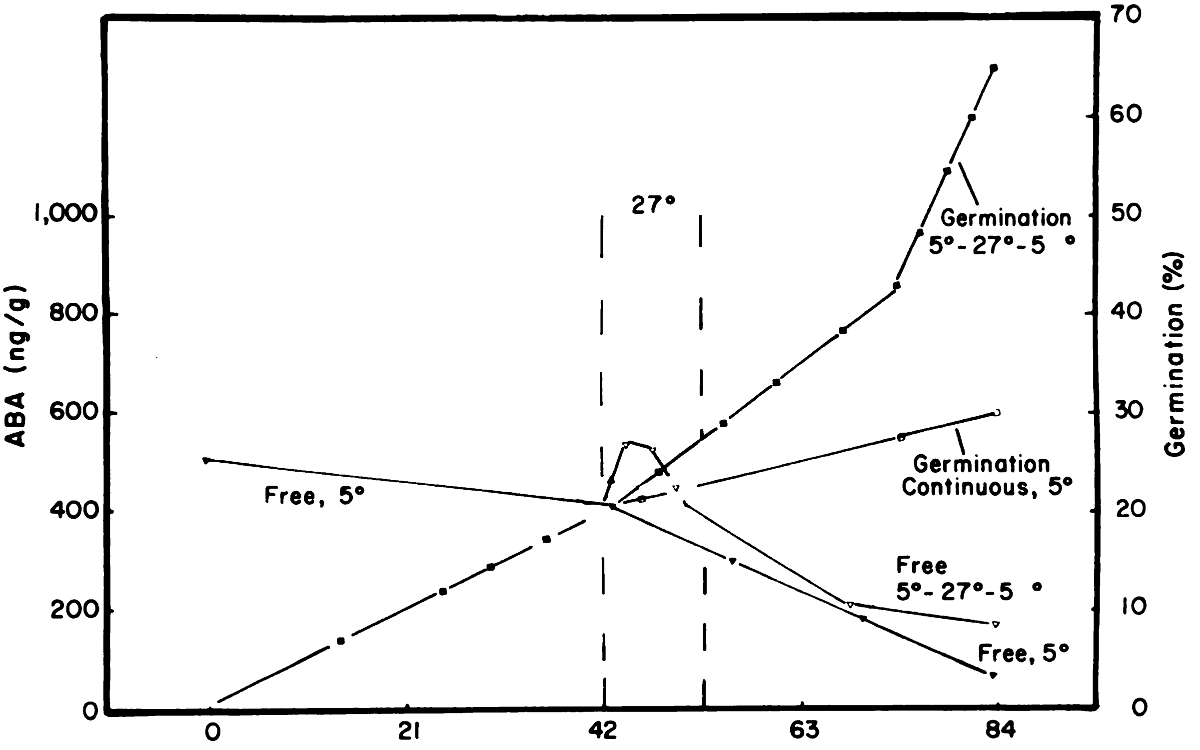
respectively, representing 32, 67, and 0.4% of the total weight of the seed. Values for total ABA in the whole seed indicate a loss of 90 to 95% of the free ABA and 75 to 90% of the bound ABA, during 12 wk of stratification.

Effect of interruption of low temperature on germination and ABA content. Germination of seeds held continuously at 5° C was low in comparison with previous experiments (see Fig. 2) and may not have reflected their true germination capacity. Transfer of seeds to 27° C for 10 days after 42 days at 5°C promoted final germination in comparison with the continuous 5°C treatment. This was unexpected, as this treatment has been reported to induce secondary dormancy in peach embryos (8) and other species (1).

An increase in concentrations of both free and bound ABA in the embryonic axis was noted following transfer to 27°C, although the increase (27.7%) was not statistically significant (Table 2). Slight increases (11.0%) were also noted in the bound ABA in the seed coat, and both free and bound ABA in the cotyledons. After 9 days at 27°C, levels had declined to or below those at the time of transfer (Table 2), with two exceptions, only one of which (bound ABA- cotyledons) was signficantly higher (5.3 ng/gm) than the initial level (3.9 ng /gm). After 80 to 84 days, ABA levels were higher in the seeds held at 27° C except for free ABA in the embryonic axis and bound ABA in the cotyledon.

Effect of extracts of stratified vs. non-stratified seeds on germination of stratified seed. In experiment 1 (Table 3) extracts of cotyledons and embryonic axes of non-stratified seed significantly

Fig. 2. Germination and free and bound ABA (ng/gm) in embryonic axes of peach seed as affected by removal from 5° to 27° ± 1°C.


ABA (ng/g)
Germination (%)
0
200
400
600
800
1,000
0
10
20
30
40
50
60
70
27°
Germination
5°-27°-5 °
Germination
Continuous, 5°
Free, 5°
Free
5°-27°-5 °
Free, 5°
0
21
42
63
84

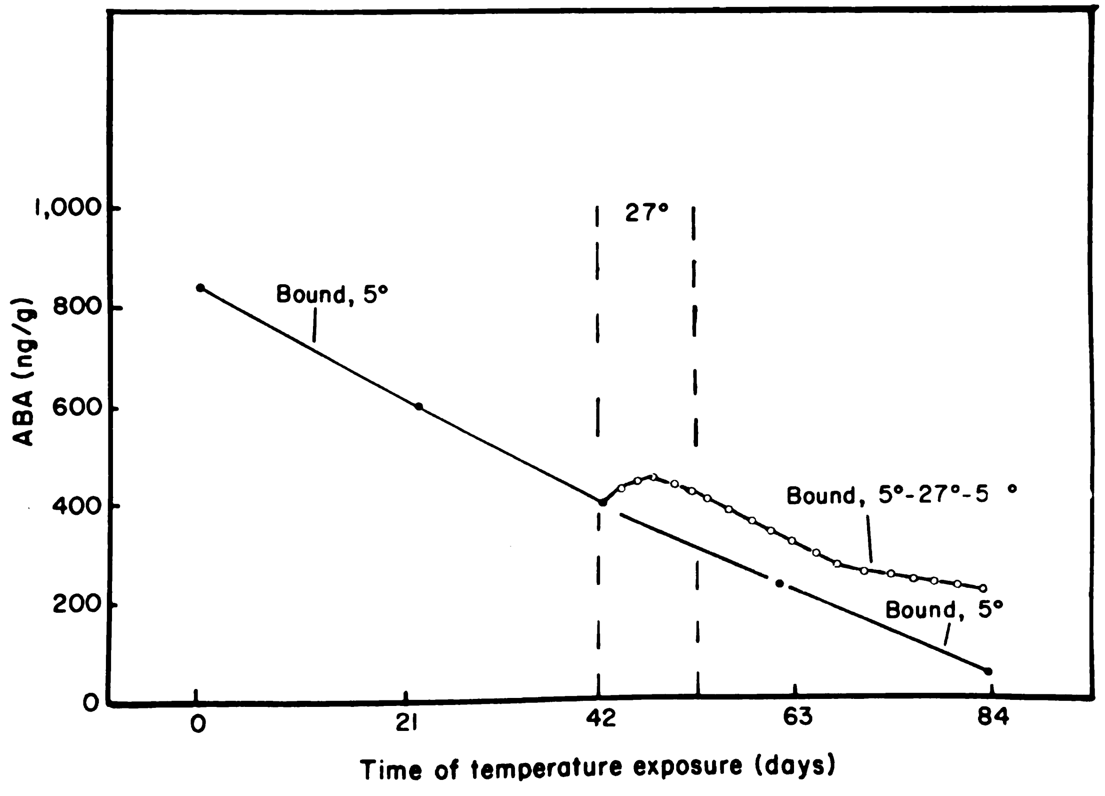

ABA (ng/g)
0
200
400
600
800
1,000
27°
Bound, 5°
Bound, 5°-27°-5 °
Bound, 5°
0
21
42
63
84
Time of temperature exposure (days)

Fig. 2

Table 2. Free (F) and bound (B) ABA (ng/gm) in peach seed as affected by removal from 5°C to 27°C.

Time (days)			Seed coat		Cotyledon		Embryonic axis	
at 5°	27°	5°	F	B	F	B	F	B
--	-	-	42.2^{d}[z]	16.9^{c}	6.1^{c}	5.2^{c}	504.2^{b}	839.2^{d}
42	--	--	17.7^{b}	9.2^{b}	4.4^{bc}	3.9^{b}	425.5^{b}	389.0^{c}
42	3	--	16.9^{b}	11.5^{b}	5.1^{c}	4.6^{bc}	543.4^{b}	436.9^{c}
42	6	--	9.7^{ab}	7.2^{b}	3.3^{b}	3.7^{b}	532.2^{b}	448.1^{c}
42	9	--	7.9^{ab}	6.3^{ab}	4.2^{bc}	5.3^{c}	424.1^{b}	449.4^{c}
42	10	14	28.3^{c}	7.0^{b}	4.3^{bc}	2.1^{a}	203.9^{a}	254.9^{bc}
42	10	28	14.7^{b}	7.7^{b}	3.7^{b}	1.9^{a}	173.9^{a}	216.9^{b}
84	--	--	3.7^{a}	2.2^{a}	1.5^{a}	1.5^{a}	56.9^{a}	42.7^{a}

[z] Means followed by the same letter are not significantly different from one another at the 5% level.

Table 3. Effects of extracts of non-stratified and stratified peach seed on the germination (%) of stratified seed.

	Extract of:	
	Non-stratified	Stratified
Seed coat	40^{abz}	50^{b}
Cotyledon	30^{a}	50^{b}
Embryonic axis	30^{a}	40^{ab}
Water control	55^{b}	

[z] Means followed by the same letter are not significantly different from one another at the 5% level.

inhibited the germination of stratified seed, while similar extracts of stratified seed had non-significant effects. 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.

In Experiment 2, extracts prepared from non-stratified seed inhibited germination in all cases (Fig. 3) but only the cotyledon extract had a significant effect. Extracts of stratified seed were less inhibitory than those of non-stratified seed with one exception (cotyledons, 2.5 seeds/5 ml.), and four of these extracts had no effect on germination.

ABA content of the extracts was determined by GLC and compared with their inhibitory activity relative to ABA (Table 4). Although there is a general parallelism between activity and ABA content, the quantities of ABA present are far too low to account for biological activity. 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 (6) that free ABA is converted to a bound form in the process.

Dormancy was broken only in seeds held at 5° ± 1° C, while the decline in ABA was noted at both 5° and 20° C (17). Wong, using bioassay, observed a similar temp - independent decrease in an ABA-like inhibitor in the embryonic axis of peach seeds during stratification. ABA levels

Fig. 3. Effect of peach seed extract and ABA on seed germination.

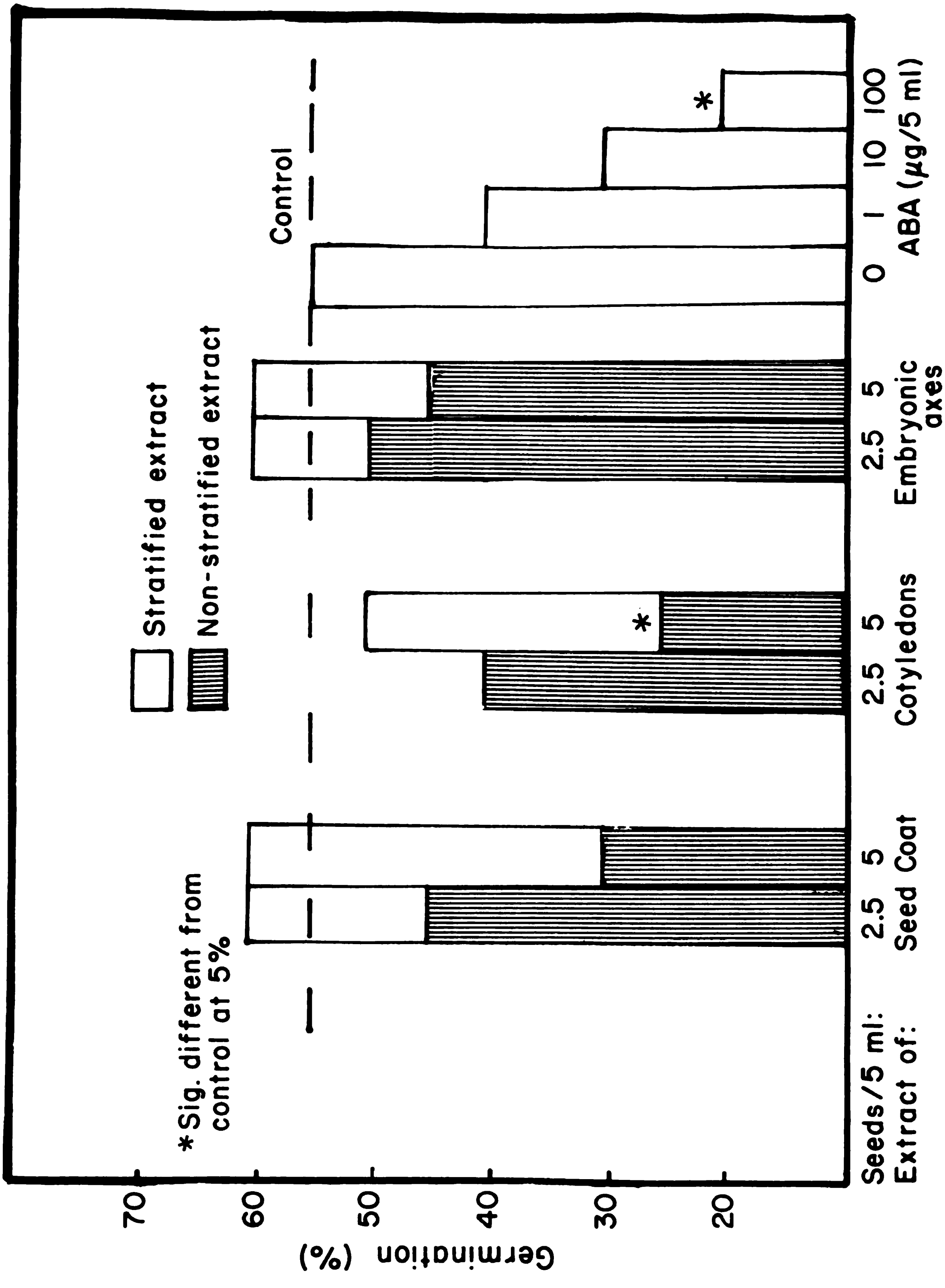

Germination (%)
70
60
50
40
30
20
Seeds/5 ml:
Extract of:
*Sig. different from control at 5%
Stratified extract
Non-stratified extract
Control
2.5
5
Seed Coat
2.5
5
Cotyledons
2.5
5
Embryonic axes
0
1
10
100
ABA (μg/5 ml)
*
*

Fig. 3

Table 4. Biological activity (ng ABA-eq per seed) of extracts of stratified and non-stratified peach seed as determined by assay with stratified peach seed *vs* ABA content (ng/seed) as determined by EC-GLC.

Extract	Non-stratified		Stratified		Stratified as % of non-stratified (GLC)
Seed coat	10,000	17.2	0*	0.9	6
Cotyledon	17,500	13.5	1000	3.5	20
Embryonic axis	460	6.5	100	0.4	2

*Germination of seed incubated with stratified extract was greater than germination of control.

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.

Interrupting the cold treatment with a 10-day period at 27° ± 1° C did not induce secondary domancy and, in fact, resulted in promotion of germination. Suszka (16) has noted a similar response when cherry and plum were stratified for short periods at 20° C prior to after-ripening at 3° C. ABA content of the seed rose slightly, but not significantly, as a result of this treatment, suggesting that had the ABA level risen sufficiently, dormancy might have been induced.

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 too low to account for all of the activity. These data suggest that other growth inhibitors are present which also decline with stratification.

These data, together with those for ABA content of maturing peach seed (3) parallel the observations of Balboa (2) concerning ABA levels in apple (Pyrus malus L.) seeds. In neither case is any clear relationship between ABA content and dormancy apparent. Growth promoters produced during chilling (9, 12, 4).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. (13) 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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SUMMARY AND CONCLUSIONS

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Abscisic acid was identified in methanolic extracts of dormant peach seeds by gas liquid chromatography-mass spectrometry. Germination of excised embryos increased as the seed matured, reaching 90 to 98% at harvest; at the same time, ABA concentration in the embryonic axes increased. Thus ABA levels could not be related with germination potential.

Drying and storage of seeds increased the ABA content in the embryonic axes, but on re-imbibition free ABA decreased 30% and bound ABA 81%; in the seed coat the decrease of free ABA was 7% vs 72% for bound ABA. Had leaching been responsible for the losses observed, a greater effect should have been shown by the seed coat.

Both free and bound ABA levels declined during stratification, yet only the cold stratification broke dormancy; therefore, ABA levels alone cannot be the controlling factor in maintaining dormancy in peach seed.

Interruption of the cold temperature by warm temperature resulted in promotion of final germination. ABA content increased slightly during the warm temperature exposure, but dormancy was not induced, suggesting that had ABA content risen sufficiently, re-stratification might have been necessary.

When stratified seeds were incubated with extracts of chilled vs non-chilled seeds, the latter were more active in inhibiting germination. However, the concn of ABA in the extracts, as analysed by GLC, was insufficient to account for the biological activity observed. These data suggest that other inhibitors are present which also decline at the low temperature.

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