AN ANALYSIS OF THE GIBBERELLIC AND ABSCISIC ACID CONTENT OF WHITE ASH SEEDS

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Abstract. Using high performance liquid chromatography, the content of gibberellic acid (GA) and abscisic acid (ABA) was examined in white ash seeds *(Fraxinus americana)* ranging in age from 1 to 10 years. The youngest seeds exhibited the highest level of GA, after which the GA content decreased rapidly. The greatest concentration of ABA was found in 4-yr-old seed. Germination tests showed that germination rate could be enhanced in older seeds with lower concentrations of exogenous GA. Furthermore, the germination of seeds receiving no exogenous GA showed a high positive correlation to fluctuations in the endogenous GA:ABA ratio. Thus, the initiation of germination in white ash seeds appears to be affected by the relative concentrations of these two hormones.

Seed dormancy provides an obvious advantage to the survival of plants as it tends to restrict germination to an environment suitable for seedling establishment and survival. Responses to environmental stimuli, such as light or stratification, cause an increase in promoter hormone levels or a decrease in inhibitor hormone levels (Varner, 1965). Thus, a favorable environment can initiate germination by appropriately influencing the endogenous hormone balance of seeds.

Dormancy is an aspect of growth cessation characterized by partial decline in metabolic activity (Moore, 1979). The inception and termination of dormancy are regulated by an equilibrium between growth promoter and growth inhibitor activity (Noggle & Fritz, 1976), most prominent of which are gibberellic acid (GA) and abscisic acid (ABA). During seed development, the growth promoter (GA) may be in excess; but as the equilibrium shifts to the inhibitor (ABA), the inception of dormancy begins or is extended. Once the seed enters a dormant stage, germination probably occurs when the hormone balance shifts back in favor of GA.

The purpose of the present study was to examine the levels of GA and ABA in seeds of white ash *(Fraxinus americana)* ranging in age from 1-10

years. After determining the endogenous levels of GA and ABA in various-aged seeds, treatments with exogenous GA, in amounts great enough to shift the hormone equilibrium to GA, were applied to dormant seeds in an effort to induce germination. Only total GA was measured in these experiments. No attempt was made to separate and characterize individual gibberellins.

Materials and Methods

Extraction of GA and ABA. Seeds of white ash ranging in age from 1-10 years were obtained from the Forestry Sciences Laboratory at Starkville, MS. One-hundred seeds from each age class were homogenized in 80% methanol (v/v) and agitated over a 24-hr period. The homogenate was then centrifuged at 1500 G for 10 min, filtered through Whatman No. 1 filter paper, flash evaporated and stored at 5°C.

Separation and purification of GA and ABA. The evaporated samples described above were reconstituted with 5% methanol in 0.2 M acetic acid, centrifuged at 1200 G for 10 min and filtered twice — once through a 5µ PTFE filter and then through a 0.2µ Fluoropore filter. Aliquots of each sample (1cc) were injected into a high performance liquid chromatograph and eluted in a linear methanol gradient (0-100% in 30 min) in 0.2 M acetic acid (Soundheimer et al, 1968; Barendise & Van de Werken, 1980). Samples of the eluate were collected at 2-min intervals and bioassayed with pea seedlings *(Pisum sativum)* to determine the presence of ABA and GA (Moore 1967). Endogenous quantities of GA and ABA were determined in each sample by comparing chromatographic peaks from different-aged seeds to comparable peaks for known quantities of the same two hormones.

Germination of white ash seed. Seeds ranging in

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age from 1-10 years were de-pericarped, weighed and placed in distilled water. After 24 hrs, the seed were re-weighed to determine the quantity of water absorbed. This quantity of water was used to prepare solutions of GA (see Table 3) that were of sufficient concentration to overcome the inhibitory levels of endogenous ABA determined previously in similar-aged seeds.

Two-hundred seeds each from lots of 1-10 year-old white ash seed were de-pericarped and placed in either GA solution (prepared as above to overcome the inhibitory effect of endogenous ABA) or in distilled water. After 24 hrs, the seed were transferred to petri dishes and allowed to germinate under conditions described previously (Bonner, 1970). After 35 days, the formation of curvature at the basal end of each seed was noted and recorded as being indicative of germination. Germination rates for seeds varying in age from 1-10 yrs were compared to determine if older seeds germinated at a faster rate than younger seeds of the same species.

Results and Discussion

The presence of GA and ABA was determined using the bioassay technique outlined earlier. Growth of hormone-treated and untreated (control) pea seedlings 10 days after treatment is shown in Table 1. Inhibited growth suggests the presence of ABA. The data show that both root and shoot weight were greater for GA-treated plants and less for ABA-treated seedlings. These data confirm the presence of both hormones in the eluate from homogenized white ash seeds.

The quantities of GA and ABA found in seed of different ages are presented in Fig. 1. The youngest seed had the highest GA content, after which endogenous levels of this hormone decreased steadily over the sampling period. Thus, for the first year or so, GA levels are high thereby promoting germination. By the time these seeds are 3 yrs old, however, GA levels are declining quite rapidly, thereby creating conditions unfavorable for germination. With ABA, 1 yr-old-seed exhibited the lowest concentration of this hormone (Fig. 1). Endogenous levels of ABA increased significantly from 1 to 4 years, after which the quantity decreased steadily throughout the sampling period. This steady decline is probably the result of natural chemical degradation of ABA over time. These data suggest that endogenous ABA levels in white ash seed are initially low, allowing conditions favorable for germination. This is followed by an increase in the concentration of ABA, a condition which tends to inhibit germination in this species. Thus, the initial hormonal equilibrium (high GA, low ABA) seems to favor germination, while a subsequent shift in the concentration of these hormones (lower GA, higher ABA) seems to favor dormancy.

As suggested earlier, it is not the concentration of GA or ABA per se that seems to exert the greatest influence on the initiation of germination in white ash seeds, but rather the equilibrium that exists between these two hormones. Table 2 shows the relationship between GA and ABA for different-aged white ash seeds. During the first year, the concentration of GA is appreciably greater than that for ABA. Extrapolating the data in Table 2 for 2-yr-old seeds, we see that the hormones are in approximate equilibrium at age 2. By 3 years of age, and for the remainder of the

Table 1. Comparison of root and shoot weights (± standard deviation) for pea seedlings 10 days after treatment with eluate corresponding to observed peak levels of GA and ABA extracted from seeds of *Fraxinus americana* L.

Tissue	Treatment	Fresh weight (g)	Dry weight (g)
Roots	ABA	0.0837 ± 0.016	0.0152 ± 0.004
	GA	0.2126 ± 0.009	0.0328 ± 0.012
	Control	0.1672 ± 0.016	0.0229 ± 0.010
Shoots	ABA	0.6230 ± 0.015	0.0752 ± 0.001
	GA	0.9640 ± 0.016	0.1277 ± 0.001
	Control	0.8129 ± 0.007	0.0985 ± 0.002

Table 2. The effect of age on the relationship of endogenous GA and ABA in seeds of *Fraxinus americana* L.

Age of seed (yrs)	Hormone in excess	Difference between ABA and GA (ug)	GA:ABA ratio
1	GA	0.41	1.44
3	ABA	0.28	0.78
4	ABA	0.67	0.59
5	ABA	0.32	0.75
6	ABA	0.30	0.75
7	ABA	0.23	0.80
10	ABA	0.20	0.82

sampling period, ABA is always present in amounts exceeding those for GA. The quantity of ABA thus remains in excess until dormancy is broken by the production of endogenous GA.

Examination of Fig. 1 reveals a difference in the slope of chemical degradation of GA and ABA. ABA shows a decidedly faster rate of degradation from years 4-10 than does GA. The mechanism responsible for the increased rate of ABA breakdown is not yet clearly understood, but this phenomenon could function as a natural safety valve to ensure the initiation of germination. As both hormones undergo chemical degradation,

Table 3. Germination of GA-treated and untreated seeds of Fraxinus americana L. ranging in age from 1-10 years.

Age of seed	Calculated amount of	Percent germination[z]	
(yrs)	GA absorbed[y] (ug)	GA-treated	Untreated
1	——	——	72
3	0.28	79	4
4	0.67	75	1
5	0.32	94	4
6[x]	0.30	——	——
7	0.23	82	5
10	0.20	80	7

[z] Based on the average germination of 100 seed for each age class.

[y] Based on quantity of water abosrbed over a 24-hr period and calculated to be at a concentration greater than endogenous ABA levels found in similar aged seed.
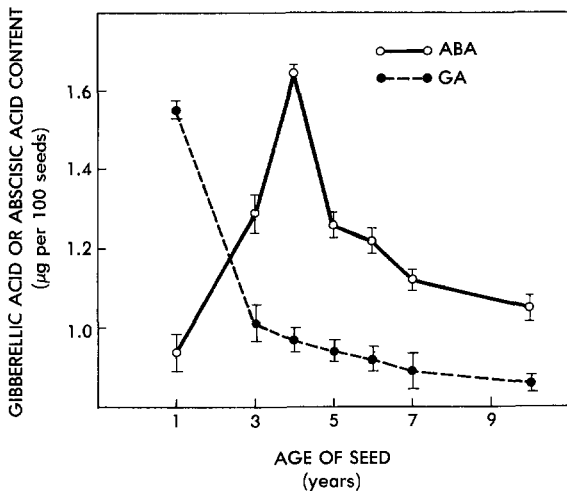
[x] No results; seed contaminated with Penicillium.

Fig. 1. Comparison of GA and ABA content in 1-10 year-old Fraxinus americana L. seed. Each point represents the mean of 3 determinations ± the standard error (vertical bars).

the differences in the concentration between them decreases. Thus, the seed becomes more susceptible to environmentally-stimualted endogenous GA production. The decreasing difference between GA and ABA might also ensure the germination of the seed before essential proteins contained in the embryo are deactivated.

The results of germination tests (described earlier) conducted to investigate the hypothesis suggested above, that the promotion of germination in older white ash seeds requires less endogenous GA than promotion of germination in younger seeds, are reported in Table 3. All of the GA-treated seeds had a germination rate comparable to the 1-yr-old seeds which required no exogenous GA to germinate. These data indicate that older seeds can be made to germinate with lower concentrations of exogenous GA than younger seeds. Furthermore, the germination rate of seeds which received no endogenous GA treatment shows a positive relationship to fluctuations in the GA:ABA ratio. From these data we may conclude that for white ash seeds the initiation of germination is, in most cases, regulated by the relative concentrations of GA and ABA.

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