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MECHANISM OF SEED DORMANCY IN *Ambrosia artemisiifolia*

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MECHANISM OF SEED DORMANCY IN *Ambrosia artemisiifolia*

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CHAPTER I

INTRODUCTION

Ambrosia artemisiifolia L., common ragweed, is a native North American annual, common throughout the temperate regions of the United States. The species is most abundant during the first year of secondary succession (Oosting, 1942; Thompson, 1943; Bard, 1952; Bazzaz, 1968) and is unable to invade grassy fields (Wagner and Beals, 1958; Gebben, 1965). Gebben (1965) experimentally correlated soil disturbance with high germination of common ragweed. Dickerson (1968) reported germination to decrease with increasing soil depth. Light enhanced the germination of ragweed seeds (Bazzaz, 1968) and the seeds which did not germinate in the dark entered a state of secondary dormancy (Bazzaz, 1970).

Seed dormancy is an adaptive characteristic which enables a plant to survive unfavorable growth conditions. Seeds of nonwinter hardy temperate plants, such as ragweed, may be dormant in the fall and require overwintering to break dormancy. In this case, dormancy prevents germination late in the growing season and insures seed survival until spring.

Recent review articles (Amen, 1968; Kelley, 1969; Black, 1970) attributed seed dormancy to a balance of endogenous germination inhibitors

and promoters. Elucidation of the inhibitor-promoter complex was largely a result of investigations involving exogenous and endogenous hormones.

Abscisic acid (ABA) inhibited the germination of ash seeds (Sondheimer and Galson, 1966), lettuce seeds (Khan, 1967), hazel seeds (Bradbeer, 1968) and apple embryos (Rudnicki, 1969). Various authors have identified ABA as the inhibitor in dormant seeds. The inhibitor concentration decreased during breaking of dormancy in peach (Lipe and Crane, 1966), ash (Sondheimer et al, 1968), walnut (Martin et al., 1969) and apple seeds (Rudnicki, 1969).

Gibberellin (GA) may be an important promoter in the termination of dormancy. Khan et al. (1957) and Khan (1960) reported that treatment with GA_3 resulted in the dark germination of light requiring lettuce seeds. Frankland (1961) demonstrated GA_3 stimulation of germination in dormant hazel seeds. Kallio and Piroinen (1959), Bradbeer (1968), Khan and Waters (1969), and Junttila (1970) also demonstrated a germination response to exogenous GA_3 in dormant seeds. Various authors have correlated breaking of dormancy with an increase in GA. Frankland and Wareing (1962, 1966) reported that stratification increased GA in hazel seeds. Ross and Bradbeer (1968) reported a hundred-fold increase in GA in hazel seeds in a favorable germination temperature after stratification. Chilling also increased GA content in birch (Eagles and Wareing, 1963) and ash seeds (Kentzer, 1966).

Exogenous indoleacetic acid (IAA) has been tested on the germination of many seeds, mostly without effects (Koller, 1962). Studies of endogenous auxin levels have also provided little evidence

to support their association with the germination process. Frankland and Wareing (1966) reported no difference in auxin content in dormant and nondormant beech and hazel seeds. However, Hemberg (1954) and Lane (1965), found that auxin activity increased in potato tubers and sunflower seeds respectively, during chilling.

In the present study the physiological basis for the ecologically important state of dormancy in ragweed seeds was investigated. Germination in light and darkness was correlated with stratification time, and the effect of exogenous GA_3 and IAA on germination of dormant seeds was determined. The content of endogenous inhibitor, auxin, and GA were also examined after various periods of stratification.

CHAPTER II

MATERIALS AND METHODS

Seeds of Ambrosia artemisiifolia were collected 7 miles east of Norman, Oklahoma, in October 1969 and 1970. They were cleaned and stored in dark bottles in an air-conditioned laboratory.

Dormant seeds were mixed with moist sand in petri dishes and chilled in a dark cold room at 5 C. The germination percentage was determined in continuous darkness and in alternating light and dark (16 hr light and 8 hr dark) at 27 C after 2, 4 and 8 weeks stratification.

Seed coats were removed from the dormant seeds by hand, and the seeds were scarified by clipping a piece from the base. Seeds were leached in continuous flowing water for 24 and 48 hr. The effect of GA_3 on germination was tested by continuously incubating the dormant seeds in 1.0, 0.5, 0.1, 0.05, 0.01 and 0.001 mg/ml solutions of GA_3. Some dormant seeds were allowed to continuously imbibe 10^{-3}, 10^{-4}, 10^{-5}, 10^{-6} and 10^{-7} M solutions of IAA. The germination percentage was determined by placing seeds on Whatman #1 filter paper disks in petri dishes in the dark or in alternating light and dark (16 hr light and 8 hr dark) at 27 C.

Assay for Indoleacetic Acid

Extraction and assay of IAA were accomplished by slight modifications of the procedures used by Eliasson (1969) and Alden and Eliasson (1970). One-gram aliquots of seed were ground with a mortar and pestle, and extracted with 50 ml ice-cold methanol for 1 hr at 5 C. The homogenate was filtered and the residue rinsed with three 10 ml aliquots of methanol at room temperature. Twenty-five ml of water were added to the methanol solution. The methanol was evaporated in vacuo at 35 C. The pH of the remaining aqueous residue was adjusted to 2.8 with 0.1 N HCL and subsequently partitioned 5 times with equal volumes of diethyl ether. The ether fraction was evaporated to dryness and the residue was made up to 10 ml with methanol and stored in a deep freeze.

Substances in the acid-ether fraction were further purified by descending paper chromatography. An aliquot of the sample extract was streaked along a 4-cm line on Whatman #1 paper previously washed with methanol-acetic acid-butanol-water (9.4:1:2.3:6.3, v/v) followed by distilled water. The chromatograms were developed to 40 cm with isopropanol-ammonia (sp. gr. 0.91) - water (100:14:6, v/v). After drying, the chromatograms were cut into 10 equal segments and assayed by the avena straight-growth test.

The variety Victory was used for the avena straight-growth test. Seeds were sterilized with 2.0% clorox for 5 min, rinsed with running tap water for 2 hr and planted embryo end down in vermiculite. They were grown for 72 hr at 25 C in the dark except for a 4-hr exposure to weak red light approximately 16 hr before use. Ten mm sections were

cut from coleoptiles 20-25 mm long, starting 2 mm below the tip. The sections were placed in a pH 5.0 glucose buffer solution containing 0.01 M K_2HPO_4, 0.005 M citric acid, and 1.6% glucose until a sufficient number of coleoptiles was obtained.

The segments of the chromatograms were cut into small pieces and placed in 2.5 ml of glucose buffer solution in 10 small beakers. Ten coleoptile sections were placed in each beaker which were sealed with Parafilm. The beakers were shaken on a reciprocal shaker at 25 C for 24 hr, after which the lengths of the coleoptile sections were measured with the aid of a photoenlarger. One beaker with 2.5 ml of glucose buffer, 10 coleoptile sections and a segment from an unloaded chromatogram corresponding to Rf 0.3 served as an indicator of coleoptile response with each test series. However, the control used as a base line in determining all test results was based on the mean coleoptile length obtained by assaying 2 unloaded chromatograms developed in the same solvent. The deviation of the mean control values was less than $\pm$ 0.14 mm for 99% of these control beakers.

Assay for Gibberellin-like Activity

The procedures used for GA extraction and assay were modified from Coomb et al. (1967a,b) and Jones and Varner (1967). One-gram seed lots were ground with a mortar and pestle and extracted with 50 ml of ethyl acetate for 8 hr, filtered, and reextracted with 50 ml ethyl acetate for 16 hr. The combined filtrates were evaporated to near dryness with a flash evaporator at 35 C and the residue brought to 5 ml with ethyl acetate. Aliquots of the sample extracts were spotted on 25 mm wide strips of Whatman #1 paper. The chromatograms were equilibrated over

the solvent (80% isopropanol) for 12 hr and developed (ascending) to 15 cm. After drying, the chromatograms were cut into ten 1.5 cm sections and assayed for gibberellin-like activity by the barley endosperm bioassay.

Barley seeds (huskless variety Himalaya) were cut transversely 4 mm from the distal end and the embryos were discarded. The endosperm pieces were sterilized in a 1% sodium hypoclorite solution for 30 min, followed by 4 washings in sterile water. Seeds were then soaked in sterile water for 24 hr at 5 C. The chromatogram segments were cut into small pieces, placed in 10 small sterile vials (24 mm wide) containing 1 ml sterile water and stoppered with cotton plugs. Two barley endosperm pieces were added to the vials and incubated for 24 hr at 30 C. After incubation the vials were analyzed for reducing sugar as described by Peleg (1960) using Somogyi's alkaline copper reagent (Somogyi, 1952). One vial containing only 1 ml sterile water and 2 endosperm pieces was used as a control with each test series. The deviation of the mean of these control values was less than $\pm$ 0.04% absorption at the 99% confidence level.

Assay for Phenolics

One-gram lots of seeds were ground with a mortar and pestle and boiled in isopropyl azeotrope (88% isopropanol: 12% water) for 5 min to stop enzymatic activity. They were then extracted by the method of Wilson et al. (1968). This method consisted of washing the seed material with boiling isopropyl-water (1:1, v/v), IBMW (isopropanol-benzene-methanol-water; 2:1:1:1, v/v) and boiling isopropyl azeotrope.

The residue was Soxhlet-extracted with isopropyl azeotrope for 24 hr followed by a second Soxhlet-extraction with isopropanol for 24 hr. The extracts were combined and evaporated to dryness in vacuo at 40 C. The residue was brought to a final volume of 10 ml with IBMW.

An aliquot of the extract was streaked along a 15-cm line on Whatman #1 paper previously washed with 5% methanol for 24 hr. The chromatograms were developed (descending) with KFW (methylisobutylketone-formic acid-water; 14:3:2, v/v) for 15 hr. After drying the 3 distinct bands visible under U.V. light were eluted with 5% methanol and absorption spectra were determined with a Beckman DB-G Spectrophotometer. Rf values were determined for the 3 spots in 6% acetic acid, IBW (isopropanol-butanol-water; 140:20:60, v/v), and BAW (butanol-acetic acid-water; 63:10:27, v/v). Maximum absorption values were used to determine the relative concentration of these phenols in dormant and stratified seeds. The concentration of chlorogenic acid was determined from the standard curve reported by Lehman (1970).

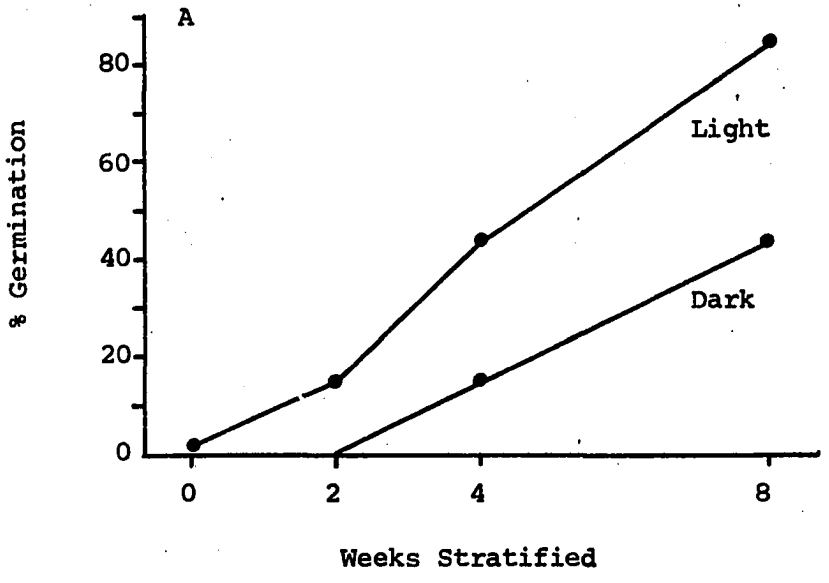
CHAPTER III

RESULTS

Freshly harvested Ambrosia artemisiifolia seeds failed to germinate in a favorable environment. Dormancy was broken by maintaining the seeds at a low temperature (5 C) under moist conditions. Seeds collected in 1969 required 8 weeks stratification for 50% germination in the light and 30% in continuous darkness. Eighty-four percent and 44% of the seeds collected in 1970 germinated in the light and continuous darkness respectively after 8 weeks stratification (Fig. 1). Germination did not occur during stratification in either case.

Removal of the testa from unchilled ragweed seeds (1969 harvest) broke dormancy in the light. However, seeds treated in the same manner did not germinate in continuous darkness (Fig. 2). Scarification significantly increased germination of dormant seeds in both light and dark although the maximum (less than 10%) was less than that of nondormant seeds (Fig. 3). Leaching had no effect on the germination of dormant seeds.

Gibberellic acid (GA_3) stimulated germination of dormant seeds in light but did not significantly affect germination in the dark (Fig. 4). Germination in the light was less than that in seeds stratified for 8 weeks. Scarification of dormant seeds followed by treatment with GA_3



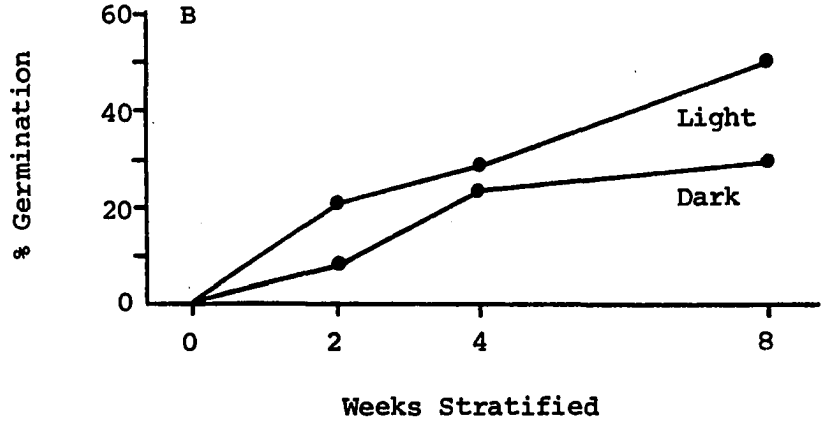


Figure 1. Effect of stratification on germination of ragweed seeds in light and dark: A, 1970 harvest, each point represents 150 seeds; B, 1969 harvest, each point represents 160 seeds. Germination recorded after 2 weeks.

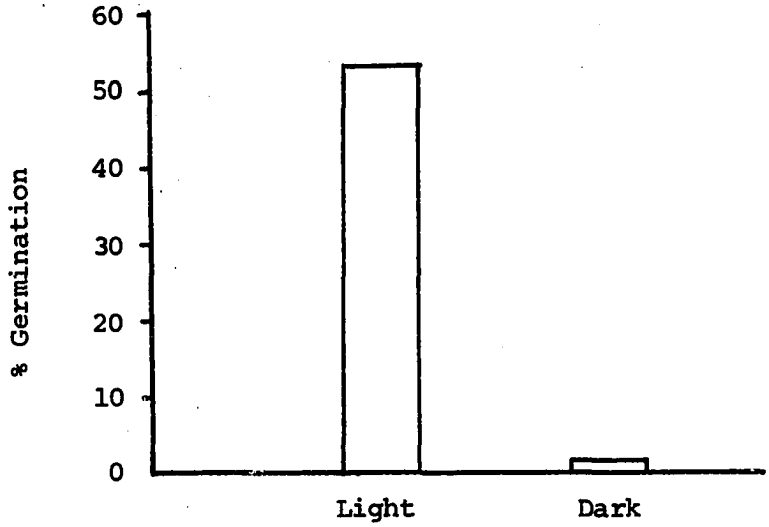


Figure 2. Effect of seed coat removal on germination of dormant ragweed seeds. Each bar represents 110 seeds.

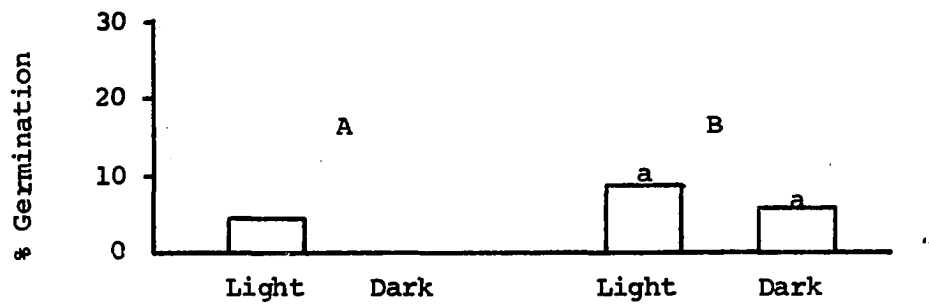


Figure 3. Germination of intact (A) and scarified (B) dormant ragweed seeds. Each bar represents mean of 3 replicates of 50 seeds each. a = significantly different from intact seeds at 5% level or better.

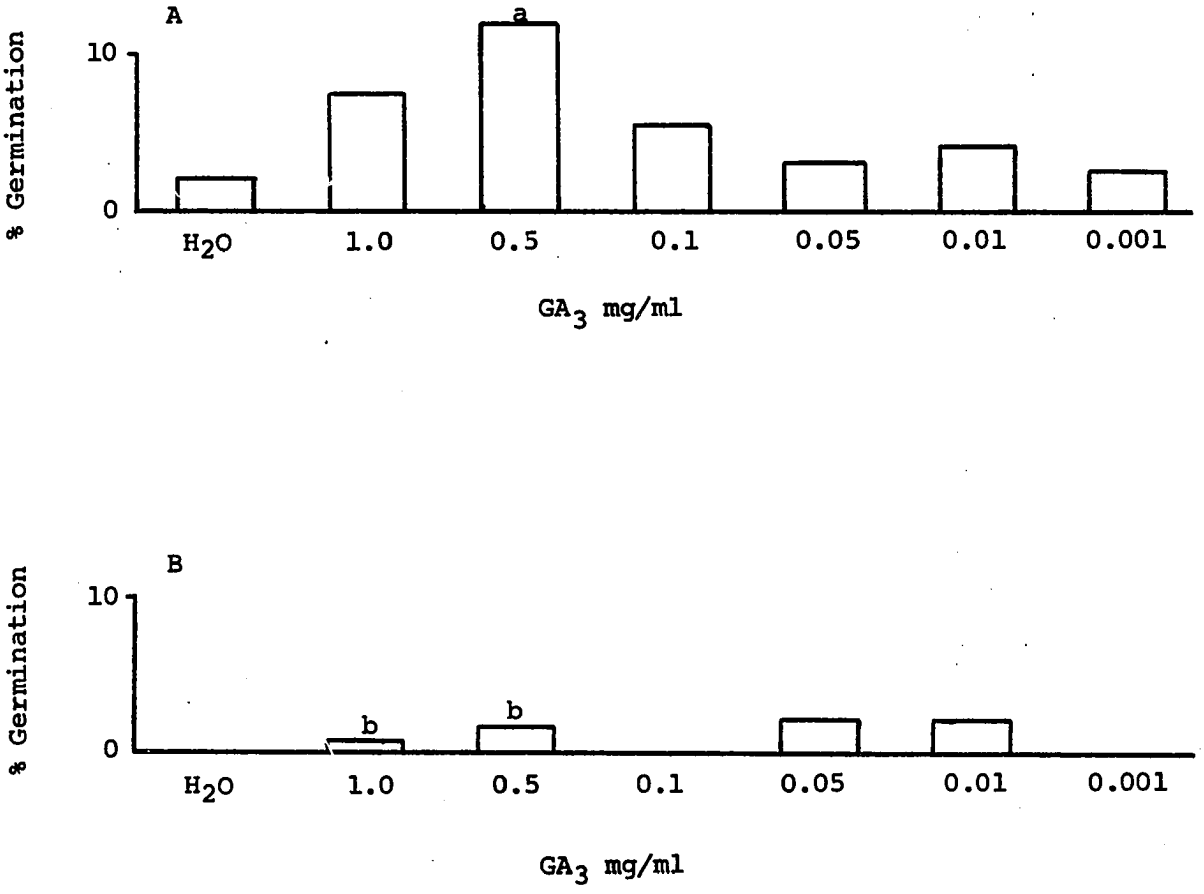


Figure 4. Effect of GA_3 on germination of intact dormant ragweed seeds: A, in light; B, in darkness. a = significantly different from H_2O control at 5% level or better; b = significantly different from germination in light at same GA concentration at 5% level or better. Each bar represents the mean of 3 replicates of 50 seeds each.

resulted in a maximum of 42% germination in the light and 16% in continuous darkness (Fig. 5). Indoleacetic acid did not break dormancy in intact or scarified seeds.

Avena coleoptile straight-growth assays (Fig. 6), (1970 harvest) revealed the presence of a growth promoter at Rf 0.3-0.4 and 2 zones of inhibition at Rf 0.1-0.3 and 0.5-1.0. After 2 and 4 weeks of stratification promoter activity significantly decreased at Rf 0.3-0.4 but increased at Rf 0-0.1; inhibitor activity completely disappeared at Rf 0.1-0.3 but was not affected at Rf 0.7-1.0. Stratification for 8 weeks resulted in the return of auxin activity at Rf 0.3-0.4 with a significant increase at Rf 0.2-0.3 and 0-0.1. Inhibitor activity was significantly less in seeds stratified for 8 weeks than in dormant seeds. The Ehrlich Reagent test indicated that promotion between Rf 0-0.1 was due to an indole compound. The Ehrlich test was negative, however, between Rf 0.2-0.3, the reported Rf for IAA on chromatograms developed with IAW (Villiers and Wareing, 1965).

Barley endosperm assays (Fig. 7) revealed GA activity in dormant seeds. The activity appeared at Rf 0.8-1.0 on chromatograms and a zone of inhibition at Rf 0.3-0.7. Qualitative and quantitative changes in GA and inhibitor content occurred after 2 and 4 weeks of stratification but no general trend was apparent. Eight weeks of stratification resulted in a slight increase in GA and a significant reduction in inhibitor content. The highest peak of GA activity was equivalent to approximately 5×10^{-4} µg GA_3/g seeds. Frankland and Wareing (1966) reported the concentration of GA to be about 10^{-4} µg/g dry weight in hazel seeds after chilling. Twenty-four hr in the light at 27 C were

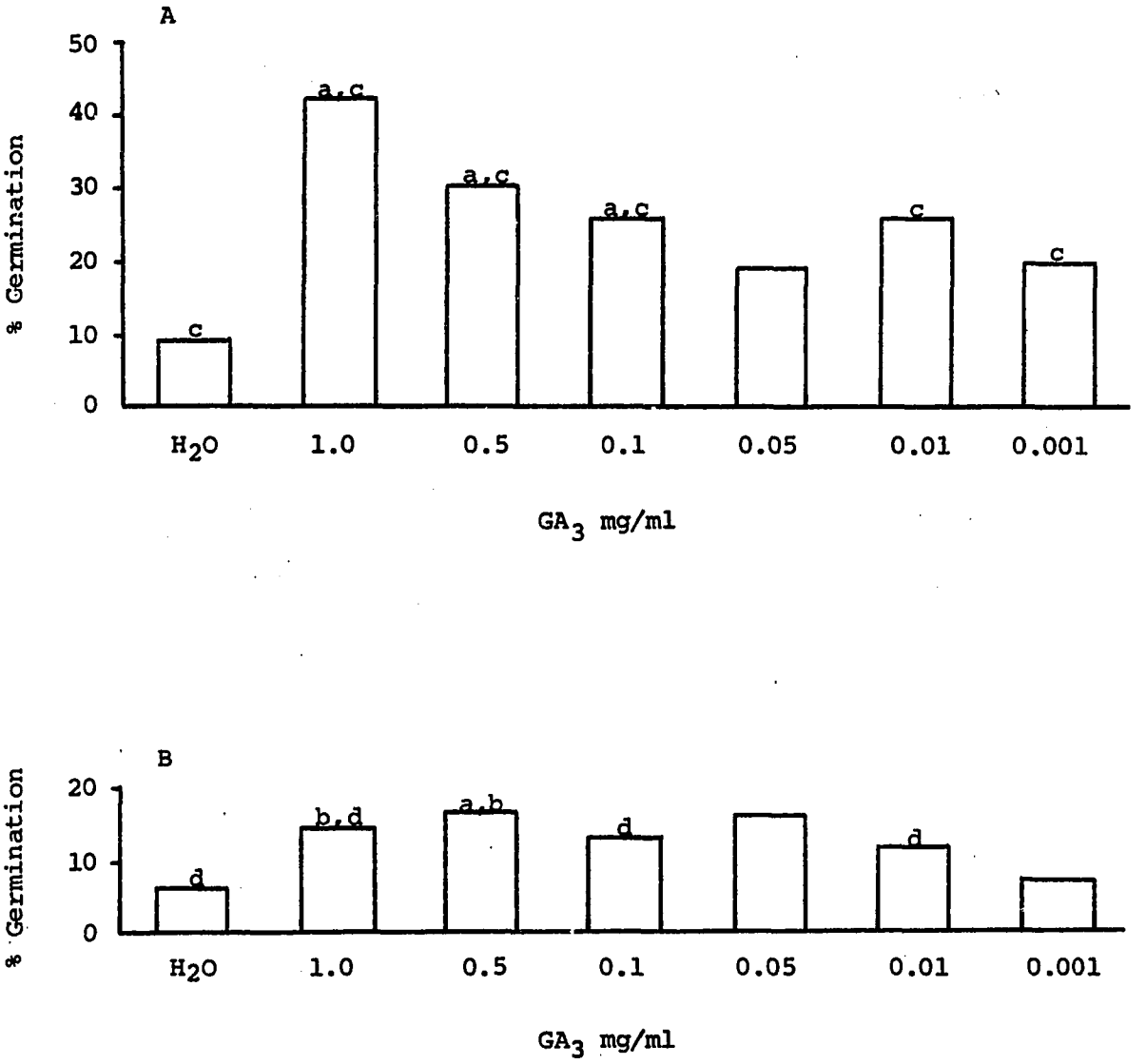


Figure 5. Effect of GA_3 on germination of scarified dormant ragweed seeds: A, in light; B, in darkness. Symbols a and b as in Fig. 4; c = significantly different from intact seeds (Fig. 4) in light at same GA concentration at 5% level or better; d = significantly different from intact seeds in dark at same GA concentration at 5% level or better. Each bar represents mean of 3 replicates of 50 seeds each.

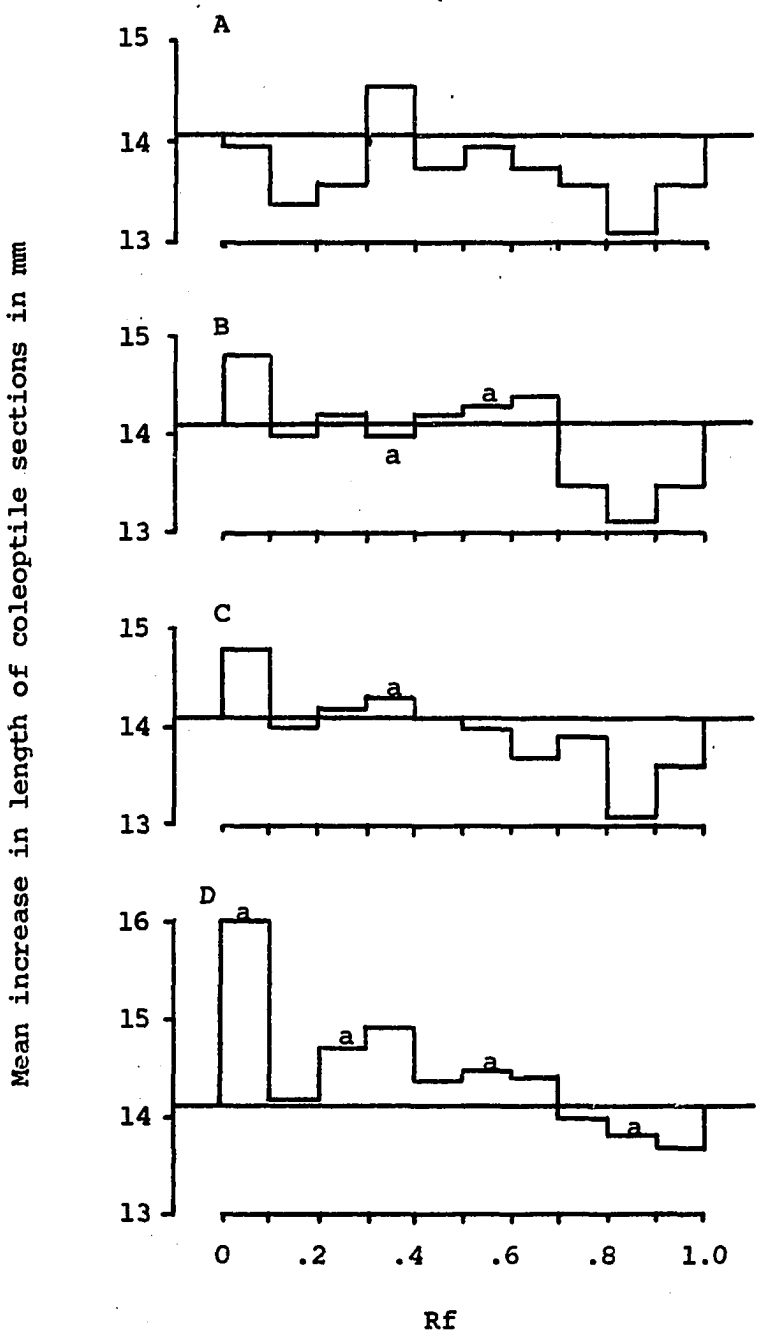


Figure 6. Auxins and inhibitors in dormant (A) and stratified (B, 2 wk; C, 4 wk; D, 8 wk) ragweed seeds determined by avena coleoptile test. Each bar represents mean of 40 coleoptiles. Horizontal line is the mean of controls. a = significant difference from dormant seeds at 5% level or better.

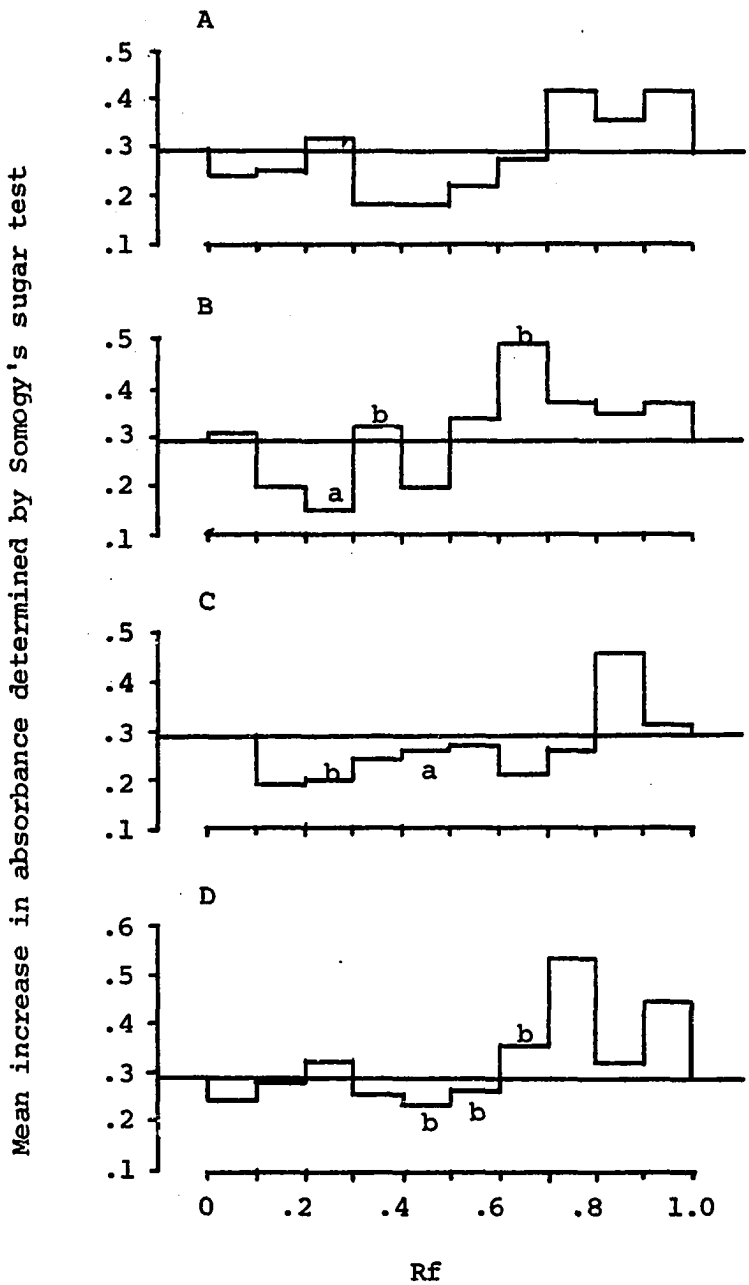


Figure 7. Gibberellin-like activity and inhibitors in dormant (A) and stratified (B, 2 wk; C, 4 wk; D, 8 wk) ragweed seeds as determined by the barley endosperm assay. Each bar represents mean of 4 tests. Horizontal line is the mean of controls. a = significantly greater or less than dormant at 5% level or better; b = significantly greater or less than dormant at 10% level or better.

required for 50% germination of ragweed seeds stratified for 8 weeks. Gibberellin content increased slightly in seeds which were placed under these conditions for 6 hr while GA content decreased in the dark (Fig. 8). In both cases inhibitor activity completely disappeared.

Three distinct fluorescent bands were observed on chromatograms of the seed extracts (Fig. 9). These bands were compared with scopolin, neochlorogenic acid, chlorogenic acid and Band 510 (4,0-caffeoylquinic acid). Band #1 appeared to be scopolin or neochlorogenic acid but rechromatography in other solvent systems, absorption spectrum, and fluorescence did not confirm this (Table I). The absorbance, fluorescence, and Rf's of band #2 indicated that the compound was chlorogenic acid. The migration in KFW and Rf values of band #3 in other solvent systems were not like any of the knowns. However, the absorption spectrum and fluorescence of band #3 were identical to chlorogenic and neochlorogenic acid.

The concentration of band #1 in seeds significantly decreased after 2 weeks of stratification and remained at a low concentration (Fig. 10). The concentration of chlorogenic acid was very high in dormant seeds and significantly increased during chilling, reaching a maximum of 4.43 mg/g seeds after 4 weeks. Band #3 increased significantly after 2 weeks of stratification and remained at a high level.

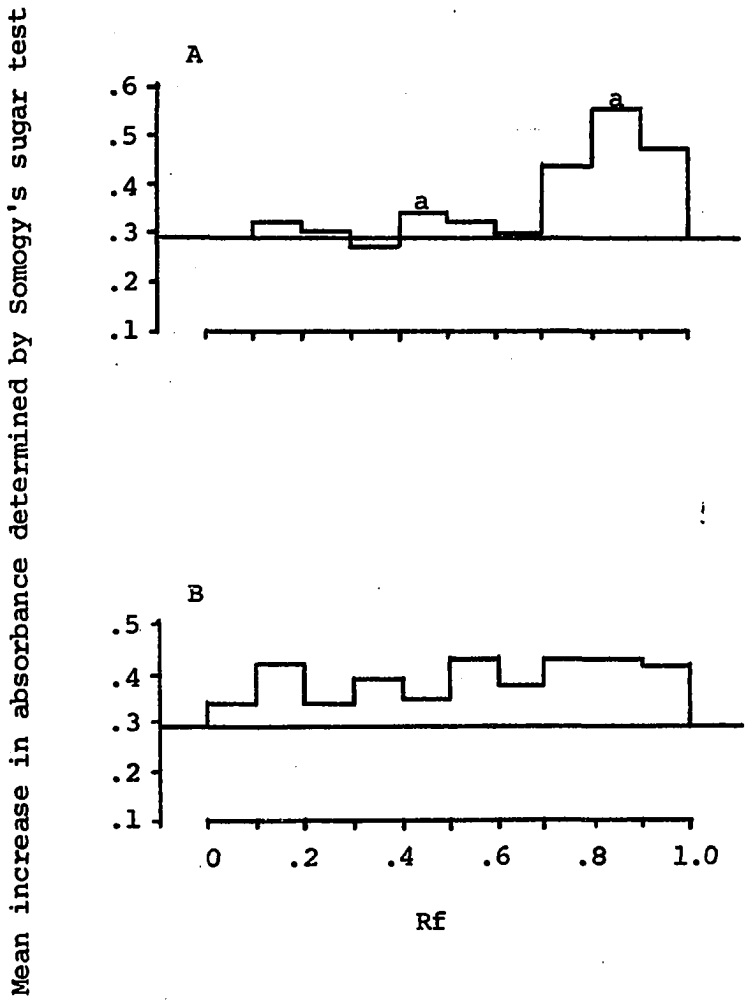


Figure 8. Gibberellin-like activity in stratified ragweed seeds after incubation in the light (A) and dark (B) at 27 C. Horizontal line is the mean of controls. a = significantly greater or less than activity in 8 wk stratified seeds (Fig. 7) at 5% level or better.

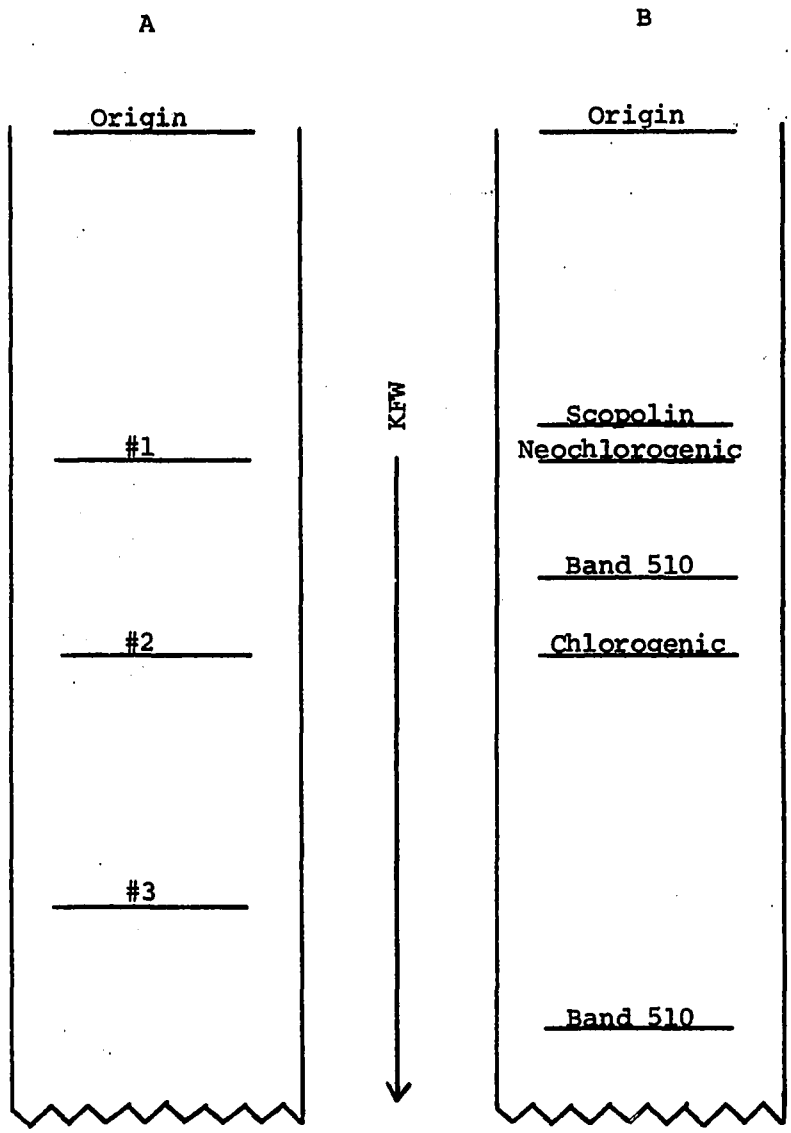


Figure 9. Migration of phenols from ragweed seeds (A) in KFW compared with knowns (B).

Table 1. Chromatography of phenols from ragweed seeds.

Compound	Rf's on Whatman #1[a]			Fluorescence under U.V.[b]		Maximum Absorption (nm)	
	BAW	6%	IBW	$-NH_3$	$+NH_3$	-NaOH	+NaOH
Chlorogenic acid	.62	.66 .81	.74	l. bl.	yel. gr.	324	380
Suspected Chlorogenic acid	.61	.64 .78	.74	l. bl.	yel. gr.	324	380
Neochlorogenic acid	.52	.70 .80	.69	l. bl.	yel. gr.	324	380
Unknown #1	.47	.30	.83	bl.	bl.	300	300
Unknown #3	.75 .45	.67	.44 .79 .86	l. bl.	yel. gr.	324	375

[a]Refer to text for solvent systems. Each Rf is an average of three runs.

[b]l, light; bl, blue; yel, yellow; gr, green.

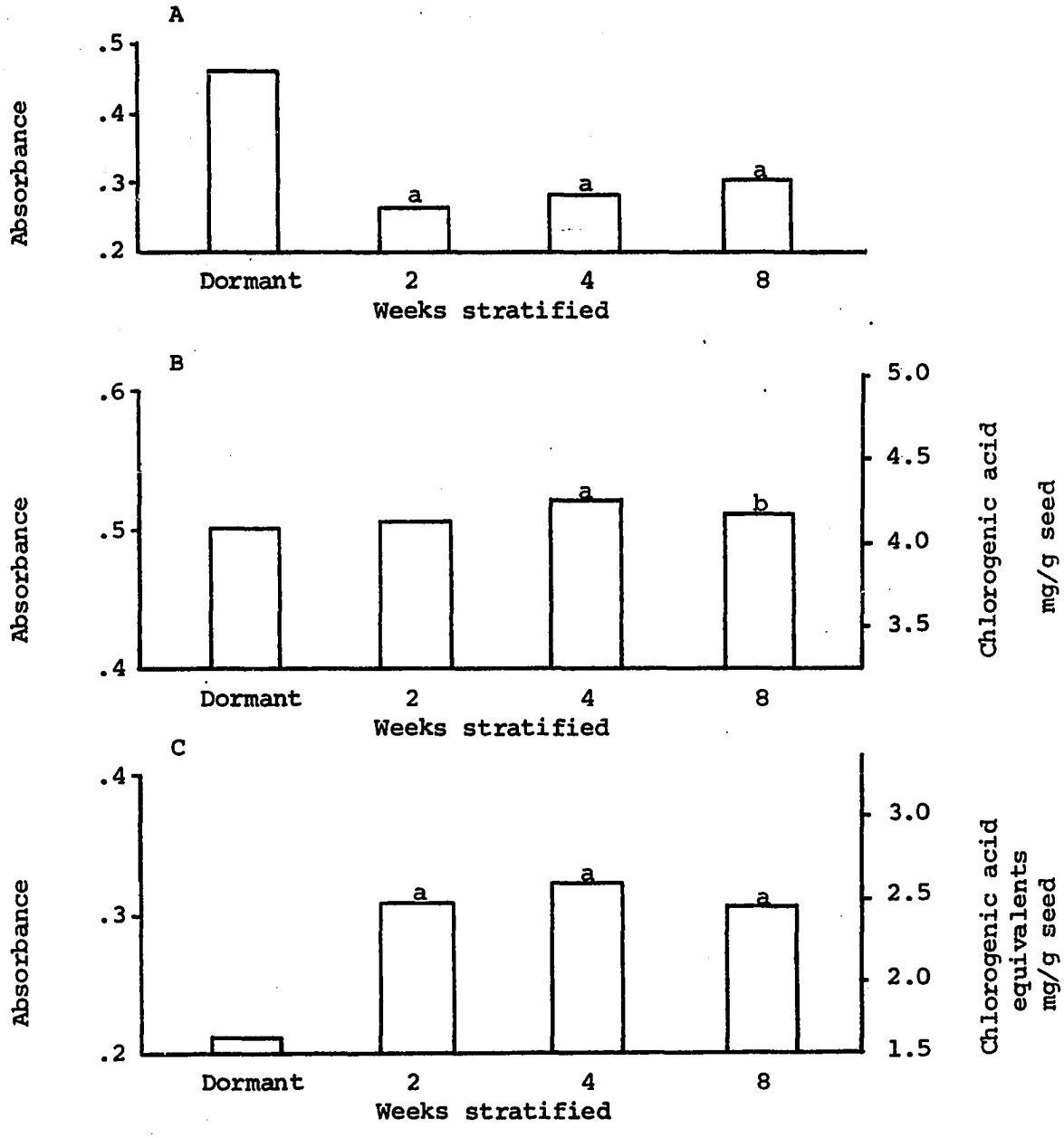


Figure 10. Effect of stratification on phenolic content of ragweed seeds: A, unknown #1; B, chlorogenic acid; C, unknown #3. a = significant difference from dormant at 5% level or better, b = significant difference from dormant at 10% level or better.

CHAPTER IV

DISCUSSION

Dormancy in seeds of common ragweed, was terminated by chilling in moist sand (stratification) for 8 weeks. The nondormant seeds readily germinated in alternating light and dark but not in continuous darkness. Bazzaz (1968) reported that stratified ragweed seeds germinated more readily in the light than in the dark. The nongerminated seeds in the dark entered a state of secondary dormancy which was broken by restratification. This mechanism may maintain a viable source of seeds from year to year (Bazzaz, 1970).

Many authors have noted the inability of ragweed to compete for space in abandoned fields for more than a few years or to invade fields with thick vegetation (Thompson, 1943; Wagner and Beals, 1958; Gebben, 1965). Dickerson (1968) suggested that this is due to an unfavorable germination temperature imposed by the dense vegetative cover. The results of the current study indicated that germination of ragweed seeds was inhibited by darkness. Therefore, shading by standing vegetation may also limit germination.

The embryos of freshly harvested ragweed seeds were nondormant in alternating light and dark but did not germinate in continuous darkness. Leaching did not affect germination and scarification

slightly stimulated germination of dormant seeds. This indicates that the seed coat does not maintain dormancy in ragweed seeds by the restriction of water uptake or gaseous exchange. This is the postulated mechanism of dormancy in many seeds (Barton, 1965). The evidence does indicate that dormancy may be due to a nonleachable inhibitor in the pericarp. Wareing (1965) reported nonleachable inhibitors present in the pericarp of both fleshy and dry fruits.

The stimulation of germination of dormant seeds by exogenous GA_3 is well documented (Khan and Waters, 1969; Junttila, 1970). The germination response of intact dormant ragweed seeds to exogenous GA_3 was low in the light and was not affected in the dark. Frankland (1961) reported scarification necessary to obtain a GA_3 response in hazel and beech indicating that the seed coats were impermeable to the GA_3 solutions. Scarification of dormant ragweed seeds increased the germination response to GA_3 in the light while germination was significantly less in the dark. This requirement for light cannot be explained by a difference in seed coat permeability. Thus stimulation by GA_3 was either inhibited in the dark or increased in the light.

Gibberellin content increased in ragweed seeds during stratification although the concentration remained relatively low after 8 weeks. These results were based on the activity of GA_3 which may not be an active component in the termination of dormancy in ragweed seeds. Brian et al. (1962) reported varying biological activities for GA. The increase in GA content in ragweed may increase the

activity of hydrolytic enzymes making stored carbohydrates available for the developing embryo (Simpson and Naylor, 1962; Bonner and Varner, 1965; Chrispells and Varner, 1967a).

A slight increase in GA content in ragweed seeds was observed during post-stratification. Ross and Bradbeer (1968) found GA concentration to increase about one hundred-fold in hazel seeds under favorable germination conditions 5 days after stratification. The time required for ragweed seed germination after stratification is approximately 24 hr which may be too short to observe an increase in GA. The decrease in GA content in the dark after stratification could be responsible for the observed low germination percentage under these conditions.

Exogenous IAA has been tested on the germination of many seeds mostly without any effects (Koller, 1962). This was also true in the case of dormant ragweed seeds. However, endogenous auxin activity increased in ragweed seeds during stratification. Hemberg (1954) found that auxin increased in potato tubers during chilling. In regard to seed dormancy, however, Wareing and Foda (1957) and Frankland and Wareing (1966) reported no difference in auxin content of dormant and nondormant seeds. The increased auxin activity in ragweed seeds may stimulate protein synthesis. Fan and Maclachlan (1967) demonstrated IAA stimulation of protein synthesis in general and cellulase synthesis in particular in pea epicotyls. Indoleacetic acid also reversed the effect of ABA on wheat coleoptile elongation (Andreae and Collet, 1968; Wurzburger and Leshem, 1969).

The quantity of auxin in the seed tissue may be regulated by phenols via the IAA-oxidase system. Monohydric phenols act as cofactors to the IAA-oxidase system and accelerate decarboxylation; dihydric phenols or polyphenols with at least one free hydroxyl group in the ortho-position on the aromatic ring inhibit decarboxylation of IAA (Hare, 1964). Chlorogenic acid, which was found in large quantities in both dormant and nondormant ragweed seeds, inhibits IAA-oxidase resulting in a high IAA concentration (Griffin, 1960; Henderson and Nitsch, 1962; Zenk and Muller, 1963; Pilet, 1964). Chlorogenic acid increased slightly in ragweed seeds during stratification. A phenol very similar to chlorogenic acid increased significantly during stratification and may also be an important inhibitor of IAA-oxidase. The identity of a phenol that decreased significantly during stratification is unknown. This is perhaps a monohydric phenol which serves as a cofactor for IAA-oxidase. Even at the low stratification temperature the changes in phenolic content occurred within 2 weeks.

Dormancy in many seeds has been attributed to high inhibitor content which decreases during breaking of dormancy. Inhibitor content was found to decrease in peach (Lipe and Crane, 1966), walnut (Martin et al., 1969) and apple seeds (Rudnicki, 1969) during stratification. Sondheimer, Tzou and Galson (1968) reported that ABA content decreased during stratification of ash seeds. Dormant ragweed seeds contained an inhibitor which decreased during stratification as shown by the avena coleoptile assay. The barley endosperm assay of ragweed seed extracts also revealed a decrease in inhibitor content during stratification. This inhibitor occurred at the Rf value reported for

abscisic acid (Kollman, 1970). Chrispells and Varner (1967b) reported ABA inhibition of gibberellin-stimulated α-amylase synthesis in the barley aleurone layer. The inhibitor was completely absent in ragweed seeds placed in a favorable environment after stratification. The observed decrease in inhibitor content during stratification and post-stratification could be an important aspect in the termination of dormancy in ragweed seeds. Amen (1968) suggested that inhibitors may be responsible for the lack of hydrolytic enzyme activity in dormant seeds.

CHAPTER V

SUMMARY

Dormancy in Ambrosia artemisiifolia seeds was broken by 8 weeks of stratification. Germination of nondormant seeds was greater in light than in continuous darkness. Embryos of freshly harvested seeds were nondormant. Leaching and scarification did not stimulate germination of the dormant seeds. Exogenous GA_3 slightly increased germination of intact dormant seeds and the effect was greatly increased by scarification. Germination was greater in the light in both tests. Exogenous IAA did not stimulate germination of dormant seeds.

Endogenous GA and auxin content increased during stratification and there was also a significant increase in GA during post-stratification at a favorable germination temperature. Inhibitors present in the dormant seeds decreased during stratification and post-stratification.

A high concentration of chlorogenic acid was present in dormant seeds and increased slightly during stratification. An unknown phenol very similar to chlorogenic acid in fluorescence and U.V. absorption significantly increased after 2 weeks of stratification. A significant decrease in the concentration of a second unidentified phenol occurred after two weeks of stratification.

It is proposed that dormancy in Ambrosia artemisiifolia may be controlled by an inhibitor-promoter complex. The dormant seed is characterized by high inhibitor and low promoter levels. In the nondormant seed the balance was shifted to favor the promoter. Evidence suggests that the inhibitor involved may be ABA and the promoters may be GA and auxin. The content of auxin may be partially controlled by the concentration of phenols.

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