

REVERSAL OF THE EFFECTS OF DETERIORATION
IN AGED SOYBEAN SEEDS
[GLYCINE MAX
(L.) MEER. CV. VICCJA]

BY

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To Martha

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Abstract of Dissertation Presented to the Graduate School
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REVERSAL OF THE EFFECTS OF DETERIORATION
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By

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Chairman: S.H. West, Ph.D.
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Deterioration of seed performance accumulates during storage. The seeds of most agronomic crops are significantly affected by this occurrence. Reversal or rejuvenation of aged seeds is therefore of primary interest.

The objective of this study was to test a contemporary hypothesis regarding rejuvenation of aged seeds. Experiments relying heavily on accelerated aging and priming were designed to test this hypothesis.

Information concerning both seed deterioration and its reversal resulted from the study. Seed tissue was shown by

tetrazolium staining to age most rapidly at symmetrical locations on the cotyledons. Aged tissue was in turn predisposed to imbibition injury which accounted for most of the loss of performance in aged seeds. When this injury was avoided by slow hydration, reversal of the sensitivity to imbibition injury was demonstrated. This reversal was a temperature dependent process. Slow hydration followed by dehydration improved the survival of seeds during accelerated aging demonstrating that loss of seed vigor was also reversible.

These results were consistent with the hypothesis that age related deterioration was reversed by pregermination metabolism. Alternative explanations may account for these results but the hypothesis tested could not be rejected based on the observations.

The significance of these findings applies to agronomy and to seed physiology. New information was learned regarding seed performance improvement through priming. This information may have application for increasing the performance of seeds after long term storage. The processes under study may relate to natural survival mechanisms in dry seeds.

CHAPTER I INTRODUCTION

One of the major problems with soybean seed production in Florida is deterioration during storage. The storage capabilities vary with the cultivar. Vicoja is among the most stable cultivars whereas Hardee seed loses vigor rapidly as determined by the accelerated aging seed vigor test.

This weakness in southern soybean seeds may have arisen inadvertently during the breeding process since storage characteristics have not been emphasized as a selection criterion. The place of origin in Southeast Asia indicates tropical compatibility in the genetic pool. Through the adaptation of soybeans to midwestern and northern U.S.A., seed lost resistance in tropical and subtropical environments to deterioration. The problem intensifies as cultivation of soybeans progresses into the tropics where there is a demand for high protein production on low nitrogen soils. Environmental effects of high humidity and temperature in the tropics preclude seed storage without expensive refrigeration.

Statement of Objectives

Seed deterioration was analyzed as several contributing causes including microflora, nonuniform tissue aging and imbibition injury.

Experimental evidence for hypothetical repair processes was also an objective. Additionally, investigations were directed toward providing new evidence to determine if repair is metabolic or spontaneous as contemporary research suggests.

In addition to providing a more thorough understanding of seed deterioration and possible repair processes, knowledge gained should be useful in efforts to improve seed performance including agronomic crops.

Literature Review

Original research publications are cited for specific information. In addition, there are books which provide a generalized resource for this study. Jensen has edited one of the most comprehensible texts available on cell biology (68). Lewin was reviewed for current knowledge of cytogenetics and cell structure (89).

On the subject of plant physiology, Leopold and Kriedemann (88), as well as Salisbury and Ross (148), are acknowledged. A current text on plant molecular biology by Smith and Grierson is now available (161). Plant biochemistry studies are well documented by Wareing (194),

Moore (97) and Nickell (106) for the status of growth regulator research. A review of plant biochemistry is provided by Tolbert (178).

Volumes specific for seed interest include the classic Yearbook on Seeds 1961, published by the USDA (163). Thompson's six volumes on seed technology were perused, but were, as the author states, an introductory edition (177). Kozlowski has three volumes with more emphasis on seed science but much of the information was dated (78, 79, 80). To learn the status of Soviet seed science, Ovcharov was reviewed (112). Perhaps the most elegant atlas on seeds was the USDA publication Seeds of Woody Plants (153). Copeland's excellent textbook (40) was used as a lead to several research publications of interest. For foundation knowledge in seed longevity research, Justice and Bass (69) must be mentioned. Khan's two volumes (74, 75) were most enlightening. For a pragmatic treatment of seeds, the book by Duffas and Slaughter (47) was of value.

On the subject of the plasma membrane, Bittar's three volumes (27) were read for a survey of membrane research. Tanford's book (169) The Hydrophobic Effect, completed the membrane literature review.

These references are not intended to represent a complete survey of the literature. An attempt has been made to include books and journal articles readily available to persons with an academic interest in seed science.

Hypothesis: Homeostasis as a Survival Mechanism

Seeds have at least two primary functions, propagation and survival. Survival can be further divided into two functions. The one more important to agronomic crops is survival by desiccation, a unique feature of some seeds (24). This type of seed is referred to as quiescent or orthodox (137). The second function is dormancy, which is found among both quiescent and recalcitrant seeds (110, 137, 183).

Dormancy provides the natural protection against seed deterioration while seed are still on the plant and after they are dispersed. Dormancy is generally perceived as a mechanism for delaying germination over time. There would be little survival value in dormancy, however, if it did not also reduce the rate of deterioration in seed so that germination also is distributed over time. (43, page 16)

The stress applied to seeds during storage is aging which in turn may be thought of as the spontaneous increase in disorder (entropy) described by the second law of thermodynamics (86). Background radiation, including heat, tends to randomize molecular organization (29, 113). Basic mechanisms for survival then must minimize entropy by continuous repair as is thought to be the case in dormancy (110, 188) or to reduce the entropy after imbibition as in the case of quiescent seed. A mechanism utilized by germ plasma storage centers is to reduce the entropy by low temperature storage of very dry seeds (14, 15, 16, 69, 141, 179, 181). Prevention of aging by desiccation and low

temperatures is of practical importance. The metabolic repair or homeostasis¹ concept is also of interest since, in nature, it may be the prevalent mechanism of dealing with the stress of aging. Detection of this repair activity has been reported by some investigators but denied by others.

In support of homeostasis. Early physiological support for dormancy as a survival strategy appeared in a 1953 report by Toole and Toole (180) who showed that the growth of lettuce was much more vigorous if the seeds were stored fully imbibed rather than dry. Villiers substantiated this finding in 1974 (189) with fully imbibed lettuce seeds. Villiers used thermal dormancy to prevent germination. Other experiments showed that intermediate imbibition would restore vigor (17) again supported by Villiers and Edgecumbe (190). This may be a common phenomenon according to Roberts and Ellis (141). The temporary wetting extended the vigor of the seeds after aging. Delouche and Nouyen (44) also suggested that dormancy may reduce the rate at which seeds lose vigor during storage. They based this on observations of moist, dormant rice sealed in glass jars for months without loss of vitality until its energy reserves were depleted.

¹ Homeostasis - a biological tendency to balance anabolism and catabolism (metabolic turnover and refurbishment)

Biochemical evidence for repair was provided by Gichner et al. (53) who chemically induced single-strand breaks into DNA. Radioactive thymidine was incorporated into the DNA in a manner which supported the repair hypothesis. Osborne and Sen (111, 155) have used similar technology to find a transient repair of accumulated DNA lesions after hydration of quiescent, aged seeds.

Microscopic support for the repair hypothesis is based on electron microscopy of membranes and light microscopy of metaphase chromosomes. In a series of publications (19, 20, 21, 22) repair of aged corn seeds was viewed as digestion of membranes by cytoplasmic organelles. Severe aging caused internal autolysis of the cell which began with collapse of the plasma membrane which causes it to shrink away from the cell wall (188). Microscopy was used by Clowes (38) who reported replacement of cells in the meristem of corn roots by replenisher cells following an X-ray exposure. Microscopy has also provided insight into repair of the genetic damage associated with aging.

Genetic evidence for injury and repair associated with aging was established in 1931 by Nilsson (107) and confirmed by Navashin (104) in 1933. Perhaps the first report of accelerated aging was by Cartledge et al. in 1936 (31). They used the aging treatment to produce chromosome aberrations. Others (189,190) have reported that if the seeds were stored fully imbibed, the chromosomes were

stable. Current work of this type includes that of Murata (100) and Roos (144), who like Cartledge (31), have shown that accelerated aging and natural aging produce chromosome aberrations. Most genetic damage is eliminated during plant development. Those mutations compatible with development lead to both chimeras and other phenotypic expressions.

Contradictory views concerning the hypothesis.

Roberts' (137) concluded, in a review of the control of viability during storage, that there is no connection between dormancy and longevity of seeds. In a reiteration of this position (139), he cites an argument based on the observation that most recalcitrant seeds are short lived. Perhaps the most emphatic objection to the concept of homeostasis is that pronounced by Bewley and Black in their 1982 text (26).

We might note, though, that at the present time no biochemical evidence has been presented for any turnover, synthetic, or (membrane) repair mechanisms in either imbibed-stored seeds or those subsequently germinated. (page 43)

Summarizing the review of literature concerning the question of repair mechanisms in seeds, the most pertinent works relative to this dissertation are the reports by Toole and Toole in 1953 (180), which were substantiated by Villiers in South Africa (189) and by Basu and Dhar (17) in India during the period 1974 to 1979. Roberts review (140) further supports the general finding that intermediate

wetting of some orthodox seeds can result in increased longevity. Still earlier reports suggest that soaking seeds in a limited amount of water improves their performance even after they are redried (76). In 1934, Chippindale (35) found that some grain seeds tolerated drought better if they were soaked in water before planting. McKee (96), in 1935, found that slightly sprouted, redried legumes and grasses grow more quickly. These studies support the results of this dissertation but since there are contradictory recent reports in the literature (41, 156, 157). Reasons for this discrepancy may include differences in cultivars and/or methodology.

Microflora and Seed Deterioration

Since bacteria require free water for growth, they are only evident during seed putrefaction. Even seed-borne pathogens have little effect on germination according to Christen (37). For a more comprehensive treatise on seed pathology, Neergaard should be consulted (105).

Fungi which affect seed deterioration are either genera specific for field conditions or storage fungi. Field fungi may affect deterioration before or during harvest. Any reduction in vigor before storage also increases the rate of deterioration in storage.

Storage fungi do not affect seeds if the seed moisture remains below critical levels (37). Relative humidity below

68 percent is sufficient to protect seeds against storage microflora.

Ultrastructural examination of Aspergillus glaucus, a common storage fungus, showed that the attack caused coalescence of the spherosomes (9). It was suggested that this event was cytotoxic.

Tao et al. 1974 (170) used chloramphenicol, puromycin and actinomycin-D to protect seed during accelerated aging from fungal and bacterial infection. Similarly, Royse et al. (146) used penicillin in concentrations ranging from 200 to 400 parts per million in dichloromethane, to effectively suppress the activity of Bacillus subtilis in soybean seeds during accelerated aging. This treatment increased the survival time of the seeds.

Regardless of Tao's previous report on the use of antibiotics during accelerated aging, its use was not mentioned in his 1981 study (200) with Woodstock. Also the use of fungicide treatment before accelerated aging is usually omitted.

Histology of Aged Seeds

Injury to seed tissue has been studied in relation to aging as well as the associated injury incurred during rapid hydration. Among the first symptoms of seed deterioration is inactivation of the mitochondrial dehydrogenase enzymes. Tetrazolium staining (55) forms the non-diffusible, formazan

pigment in viable cells. Non-viable cells remain colorless. Evans Blue is another vital stain used to identify patches of cells on the surface of seed cotyledons which were ruptured by soaking the seeds in water with the testa removed (48,50). This stain is passive in that healthy cells exclude the pigment. Cells with broken membranes are flooded with dye and are visible with light microscopy.

Seedlings often display necrotic patches, visible without magnification. These areas of dead tissue are consistent with Villiers' (188) description of groups of dying cells observed with the electron microscope.

Seeds from samples of low vigor, and with only partially impaired germination, often show these degenerative changes in isolated cells (or groups of cells) among apparently normal tissue, and it is assumed that, when extensive, these relate to the necrotic spots or areas seen in some low-vigor germinating seedlings. (188, page 45)

Notwithstanding this evidence for non-uniform susceptibility of seed tissue to the effects of aging, Sen (154) came to the opposite conclusion. By using radioactive precursors and autoradiography to detect repair after aging, her results indicated that all seed tissue was affected by aging in a uniform manner. Perhaps autoradiography failed to detect repair, whereas vital stains do not depend on this activity. It is not necessary to reconcile the differences between Villiers' histologic conclusions and those of Osborne. The tetrazolium reaction can be used to demonstrate that aging is uniform or non-uniform when seed tissue has symmetrical

areas which degenerate first under natural or accelerated aging.

Cytology of Plasma Membrane Injury

The cell's response to natural and accelerated aging has been studied on three levels. On the first level, chromosome aberrations are amenable to study under the light microscope in rapidly dividing metaphase cells of meristematic regions (101, 104, 107, 138, 139, 189). This abnormality often results in sectorial chimeras (143, 144) but most chromosome lesions are eliminated during plant development, especially during the pollen (haploid) stage. Few abnormalities are transmitted to the second generation (144).

Ultraviolet light microscopy has given visual, cytological information in dormancy imposed by phytochrome activation (131). Also the effects of plant hormones on calcium distribution have been monitored with these methods (152).

At the electron microscope level, aging during dormancy has provided another window for observing the repair process (185, 186, 187, 188). Organelles and membranes are evidently phagocytized during dormancy of Fraxinus excelsior, for example. This cytological view also supports Villiers' and Osborne's hypothesis of seed repair mechanisms and correlates with studies which have described the beneficial

effects moisture can have on preventing age related deterioration (17, 180, 189).

The most germane cytological study concerning the effect of aging on the plasma membrane contains electron micrographs (188) which depict plasmolysis after accelerated aging followed by collapse of the membrane and ultimate disolution of cell internal structure by autolysis. Collapse of the plasma membrane after aging could explain much of the electrolyte loss in imbibition studies.

Few or no cells show signs of possessing a functional plasma membrane, and the boundry layers of the cytoplasm have retracted from the walls. Keeping such seeds imbibed and periodically sampling for electron microscopy shows rapid degeneration of the cytoplasm in which the lipid bodies become confluent, and eventually total dissolution of the internal structure ensues. (188, page 45)

Just as the destruction of the plasma membrane follows an unexpected scenario, the construction of new membranes is equally unexpected. Chabot and Leopold (32), using freeze fracture transmission electron microscopy, indicated that new membrane material was seen "blebbing" from the cytoplasm to the plasma membrane. Buttrose (30) also used freeze-etch to monitor structural changes in seeds during hydration. Freeze-etch techniques complement earlier fixation which attempted to preserve the membranes in their dry state.

Microscopy has proven to be a powerful tool for studying the cytochemistry of aging in situ with vital stains. It has also offered direct observation of membrane destruction.

However, studies concerned with function of the membranes must use physiological techniques to monitor the permeability of the membranes as well as a biochemical approach to determine the activity of the membrane as it coordinates with the growth processes.

Plasma Membrane Physiology

As aging progresses, the first change observed in the seed is an increase in membrane permeability. The following events are in order of increased complexity, corresponding to a reduction in respiration which is also a membrane-dependent process (197,198). As the plant's ability to utilize energy reserves is reduced, there is a cascade of dependent events. Biosynthesis, such as protein and nucleic acid turnover, is correspondingly slowed, which in turn reduces growth and resistance of the plant to cope with stress of the process in emergence.

The plasma membrane has been identified as a likely weak point in the seed's resistance to aging (1,2,3). The association between the plasma membrane was confirmed by Parrish and Leopold (119) who demonstrated that aging decreased respiration and increased the electrolyte leakage of the membrane. The cause of the leakage was reported to be oxidation of the phospholipids (133), but this report was denied (132) by similar work conducted with soybeans. Peroxidation of the phospholipids has also been implicated

(164) in membrane deterioration. Analysis of tocopherol and the free radical content of seeds led Priestly et al. to conclude that aging does not affect the plasma membrane of soybeans in this manner (132, 133).

Proteins of the plasma membrane have been overlooked as part of the problem. According to Keenan et al. (72), 40 percent of the plant plasma membrane is protein on a dry weight basis. Only recently have purification techniques been improved (195) sufficiently to characterize the proteins by two-dimensional electrophoresis (160). Even more subtle alteration of proteins is suggested by tests such as the tetrazolium reaction which responds to dehydrogenase enzyme activity. The activity of proteins could be altered by aging via partial denaturation without altering their primary structure. The activity of proteins of the membranes may be an early event in the catastrophe cascade brought about by age. More severe aging leads to the ultimate catastrophe, death of the seed. Along with a reduction of growth, aging reduces the plant's turgor (120, 204, 205) which is dependent on membrane activity.

Physiological injury. A major part of injury to an aged seed occurs during imbibition (94, 95). Predisposition of aged seeds to soaking injury was reported by Woodstock and Tao in 1981 (200). At the same time Parrish and Leopold (119) reported that aging of seeds decreased respiration and increased electrolytes resulting from soaking. They

concluded that aging compromised the ability of the membrane to reform during hydration. Woodstock cited earlier reports in which seeds had been protected from imbibition injury by slowing the rate of water uptake. Pollock et al. (125,126,127,128) found that moist seeds, conditioned with water vapor, were less susceptible to chilling injury. Obendorf and Hobbs (108) also found that preimbibed seeds were protected from chilling injury. Another pertinent report was by Powell and Matthews 1978 (130), who used polyethylene glycol (PEG) to slowly hydrate pea embryos which resulted in less injury from this process. Woodstock and Tao (200) recognized that dry soybean embryos were also injured less if they were hydrated on germination paper moistened with 30 percent PEG rather than with water alone. The benefits to low vigor and accelerated aged (42) embryos were especially dramatic. Aged embryos recovered most but not all of their vigor following the PEG treatments. These authors thought that by slowing the water uptake, the plasma membrane had time to "repair" itself. This inference was not supported by data and must have related to Simon's hypothesis (158,159).

Cell injury can also result from membrane rupture during rapid imbibition (28,48,49,85,196), although rupture alone is probably an incomplete explanation (49). The structure of a large seed such as the soybean may incur structural damage as the external tissue swells more rapidly than the

center. It is unlikely that seeds in nature undergo the extremely rapid hydration found in this type of membrane study. The extreme situations may result in experimental artifacts. Nevertheless, insight can be derived from imbibition experiments concerning the permeability of the membrane as influenced by aging.

Spontaneous physiological repair. The "repair" hypothesis was based on an extensive literature review concerning membrane permeability (159). The explanation contended that the phospholipids of dry seeds assumed a quasi-laminar gel structure which required several hours to reorganize as the seed was hydrated. Such phase transition was investigated by Parrish and Leopold (118), but further studies by the same group were unable to support this concept (109).

Physiological priming and desiccation. These two seed treatment practices are reviewed together because they are often used in combination. There is no obligate relationship between them and each can be used separately as dictated by the circumstances.

The term priming was coined by Hedecker and Coolbear (63,64) who are credited with discovering its usefulness in invigoration of seeds. Priming is making a major contribution to agriculture and seed science, although the principle is not new. More than 60 years ago, in 1918, Kidd

and West (76) described the practice of slow seed hydration before planting as a treatment which improved productivity. Other historical reports include those of Chippindale (35) and McKee (96). Chippindale found that "soaking" increased the vigor of some Graminae. As an extreme example, McKee reported that, in addition to grasses, slightly sprouted, redried legumes grew more rapidly. A more recent report on the use of priming in agronomic practice describes the application of polyethylene glycol (PEG) to improve the rate and uniformity of cereal emergence (7). Priming has been a more common practice with vegetable seeds (201) and has been combined with the advent of fluid drilling technology.

Priming may not be limited to the use of PEG or other practices which may be difficult for both industry and farm managers to utilize. A new technique was reported by Perl and Feder (122), who found that water vapor alone could be used to prime pepper seeds. This report also helps to explain why Woodstock and Tao (200) found that accelerated aging for very short intervals of time actually improved soybean seed performance.

Since vigor is a controversial term, its 1979 AOSA definition follows:

Seed vigor comprises those seed properties which determine the potential for rapid uniform emergence and development of normal seedlings under a wide range of field conditions. (94, page 785)

The author (RLT) has used vigor as a relative term which allows growth-related measurements between treatments and controls to be compared. This concept does not conflict or contradict the defined concepts of rapid and uniform emergence; it does, however, consider these measurements on a relative basis.

While desiccation or redrying is a common practice, not all seeds are tolerant of this treatment. Adegbuyi et al. (4) found that treatment with PEG resulted in no beneficial effect on normal germination of herbage seed and redrying negated most effects. Most recently, a group of investigators (156,157) found that desiccation was injurious to soybean seeds after 36 hours of hydration.

Desiccation tolerance in higher plants is unique to seeds. Bewley reviewed this topic in 1979 (24). The plasma membrane of recalcitrant seeds cannot tolerate moisture levels below 40 percent (18). This is consistent with Bewley's belief that the plasma membrane may be a key factor in desiccation (24). Others (41) suggest that the reorganization of genetic material is a critical factor. Klein and Pollock (77) developed their views of desiccation through use of the electron microscope.

The point of no return has been studied as a means of determining what events in the seed prevent redrying. A likely associated event is the beginning of cell division (24). Cell division and cell expansion can be separated

experimentally (57), but no reports were found during this literature review which have used this technique to test Bewley's hypothesis (24).

The literature concerning priming and related fields of research is only beginning to emerge and should prove fertile. The simplicity of priming should promote its use as a common practice. Basu and Dhar (17) have been using moisture to invigorate seeds in India since 1974. They have found the practice to be applicable to a diverse variety of genera including grains and vegetables. For a subject so central to seeds with economic potential, priming is clearly a promising topic for research.

Growth Regulator Biochemistry

Biochemical processes of the plasma membrane are affected by aging just as is the permeability. Stimulation of this activity with growth regulators can increase the germination of aged seeds (61,123). One important growth regulator is fusicoccin which is reported by Marre' (92, 93) to stimulate proton extrusion. The nature of this activity is suggested by previous studies on plants with fusicoccin. The benefit of monovalent cations (123) is consistent with the mechanism of K^+/H^+ exchange during active transport.

The plant hormone ethylene is endogenously produced during germination (5,150,151). Like fusicoccin, ethylene is antagonistic to abscisic acid (ABA). Also like

fusicocin, ethylene has been reported to improve the germination of aged seeds. Both fusicocin and ethylene break dormancy, demonstrating a common mode in their activities. Fusicocin has been referred to as "super auxin" (97), and the association between ethylene production and auxins has been established (202).

Previous reports have examined the activity of ethylene on membrane permeability of the stomata (115,116). Stomata have been shown to open under the control of 2-chloroethyl phosphoric acid (ethephon), a water soluble source for ethylene (191). Since both fusicocin and ethylene open stomates, their activity seems to be similar to, or perhaps synergistic, with respect to proton transport.

An impairment in respiration and oxidative phosphorylation, both mitochondrial membrane-dependent processes, would in turn limit energy to support membrane dependent processes. Abscisic acid inhibits active transport by the plasma membrane of germinating seeds (39), but since abscisic acid does not inhibit respiration (23), it must antagonize fusicocin by affecting membrane permeability. While this may be a logical statement, it constitutes only an untested hypothesis.

Ethylene may have some activity in common with fusicocin. Unlike fusicocin, whose activity is limited to proton extrusion, ethylene is associated with other cellular events including sugar transport and metabolism

(176), but not starch hydrolysis (70). The synergism reported for ethephon (2-chloro-ethyl phosphoric acid) and kinetin in breaking dormancy in small cocklebur seeds (171) suggest possible synergism between ethephon and fusicoccin in low vigor, aged seeds as well as abscisic acid treated, dormant seeds. Tested alone, fusicoccin and ethephon antagonize abscisic acid induced dormancy in soybeans. Testing for synergism between fusicoccin and ethephon would provide new information regarding mechanisms of action they have in common. If synergism cannot be demonstrated, this information precludes practical applications of this combination. Antagonism between fusicoccin and ethephon would indicate that they are activating mutually exclusive or competitive events.

Table 1 relates references concerning the plasma membrane activity of seeds to the mode of growth regulator activity. The growth regulators include fusicoccin, ethylene and abscisic acid. The events starting with membrane-bound receptors and membrane-bound enzyme activity move upward in the table. As the proton pump is activated, growth is promoted by wall softening and increased turgor of the cell. If these events dominate, dormancy is broken and germination commences.

It is interesting to speculate that if ethylene is required by germinating seeds, then abscisic acid may induce dormancy by antagonizing the action of ethylene. According

TABLE 1

Literature on Membrane Activity of Growth Regulators

System, Activity and References		
Germination	Stimulation	5, 61, 123, 151
Dormancy		173
	Abscisic acid	23, 25, 91
	Ethylene	5, 67, 70, 71, 90, 171
Osmoregulation	Hormonal	165, 166
	PM permeability	62, 65, 114, 167
	Stomatal aperture	115, 116, 172, 176, 191
Membrane Activity	Adsorption of Ions	33, 117, 162
	Proton Pump	33, 34, 117, 135, 165, 166, 174
	Growth	135, 174, 182
	Fusicoccin	8, 12, 13, 39, 83, 84, 92, 93, 103, 123, 135, 147
	Receptors	6, 46, 66, 73, 121, 134, 168, 192

to this idea, inhibitors of ethylene synthesis such as amino-oxyacetic acid, AOA, should produce effects similiar to ABA.

It is recognized that the activity of growth regulators extends beyond regulation of the membrane activity. Table 2 summarizes these regulatory activities in seeds with supporting references. Two reports (114,167) suggest that the permeability of the membrane is primarily affected by growth regulators. This concept is based on artificial membranes. The permeability of phospholipid membranes can be altered with physiological concentrations of gibberellins (114) and kinetin (167). This would explain why, with the exception of ethylene, plant hormones are active in the 10^{-3} to 10^{-6} molar range.

TABLE 2

Literature on Regulatory Activity of Plant Hormones

System, Activity and References		
Energy Regulation	Sugar uptake	8, 60, 70, 129, 175, 176
Hormonal Regulation	Synergism	170, 171
	Antagonism	34, 36, 58, 124, 136, 172, 191
Biosynthesis	Hormone Synthesis	11, 52, 151, 193
	Induction	52, 54, 59, 150, 202
	<u>De Novo</u> Synthesis	10, 36, 114, 124, 167, 203
	Membrane Synthesis	51
Genetic	Second Messenger	61, 81, 84, 92, 93, 152
	Cell Division	83, 203

CHAPTER II MATERIALS AND METHODS

Microflora of Seed Deterioration

Seed Materials

Locally grown soybean seeds [Glycine max Merr. cv. Vicoja], were used in all experiments. The seeds were stored in an air-conditioned room (65 percent relative humidity), which resulted in an equilibrated seed moisture content of 11 percent on a fresh weight basis.

Control samples of seeds used as internal standards were important considerations in the experimental design. Factorial experiments included these controls.

Experimental Design-General

Most experiments were factorial with various levels of treatment, two for the chemicals and four for accelerated aging. The variables consisted of the chemicals, mode of application and application sequence, before or after accelerated aging. Controls were routinely used and served as a basis of comparison for data analysis.

Antimicrobial Chemicals

Streptomycin¹ was the only antibiotic evaluated. Several fungicides were tested singly and in combination. These fungicides were botran,² captan,³ maneb,⁴ and benomyl.⁵ Benomyl was used alone and mixed with captan and maneb. Other powdered mixtures which were used were captan/botran, captan/maneb, and streptomycin/captan/botran.

Several techniques were used for application. Chemicals were infiltrated into the seeds by imbibing them with saturated solutions of fungicides and streptomycin (400 p.p.m.). This application was essentially a combination of priming with chemical incorporation.

The application technique used as a routine was to tumble the seeds in captan/botran. Streptomycin and fungicides were applied to the seedcoats and were effective in preventing signs of fungal growth.

¹ Streptomycin structural name: 0 - 2 - dioxy - 2 - (methylamino) - alpha - L - glucopyranosyl - (1 to 2) - 0 - 5 - deoxy 3 C - formal - alpha - 2 - lyxofuranosyl - (1 to 4) - N, N' (aminoiminomethyl) - D - streptamine

² Botran structural name: 2,6 - dichloro -4- nitro-aniline

³ Captan structural name: 3a, 4, 7, 7a - tetrahydro -2- [(trichloromethyl) thio] -1H- isoindole - 1,3 (2H) - dione

⁴ Maneb structural name: [[1, 2 - ethanediybis [carbomodithioato]] (2-)] manganese

⁵ Benomyl structural name: [1 - [(butylamino) carbonyl] -1H- benzimidazol -2- yl] carbamic acid methyl ester

Additional protection from microflora was obtained by limiting the severity of accelerated aging. Selection of seeds was based on their growth relative to other sources.

Histology of Aged Seeds

Control seed samples consisted of high vigor, Vicoja seeds and were obtained from the Agronomy Department's seed storage facility. Other control seeds were subsamples of seeds which had been subjected to accelerated aging for 24 and 48 hours. Included also were other sources of Vicoja seeds from 1980 and 1982. Some experiments included other cultivars tested at various degrees of deterioration. Seeds harvested at maturity were also compared to postharvested seeds. Additionally, seeds primed after accelerated aging were tested.

Tetrazolium was used as a vital stain (55). It differentiates tissues according to dehydrogenase enzyme activity. Five dimensions of the tetrazolium experiments included natural aging, accelerated aging, priming, postharvest deterioration and cultivar differences.

Cytology of Plasma Membrane Injury

Injury and Fixation

Injury was accomplished by soaking seeds in water at 4 C. After two hours of excessively rapid hydration, the embryos were removed and fixed overnight in cacodylate buffered

glutaraldehyde (4 percent) at 4 C. The low temperature was used to slow temperature dependent processes within the cells.

The tissue was then rinsed three times with 0.1 M sodium cacodylate buffer, pH 7.2-7.5. Osmium tetroxide (1 percent) post-fixation also proceeded overnight in the cold.

Embedding was preceded by an alcohol dehydration series, including an overnight exposure to uranyl acetate. Acetone dehydration completed this process before the embryos were embedded in plastic using the Mollen-Hauer formulation. The maximum size of the tissue block was one cubic millimeter. The plastic resins were infiltrated in three stages, starting with 70 percent acetone and 30 percent plastic. This process allowed one hour for each stage. Finally, the samples were put into a 60 C vacuum oven and allowed to bubble until no longer foamy. The vacuum was released and the 60 C incubation continued overnight.

Microscopy

Embedded embryos were "blocked off" with razor blades then ultramicrotome sectioned with a diamond knife. Thick sections were cut through the central axis of an embryo and viewed without stain using a phase contrast microscope. This showed the orientation of tissue in the block. Thin sections were then cut and post-fixed with lead citrate. The Jewel electron microscope (JEM) was used for photography on film plates.

Physiology of the Plasma Membrane

Accelerated Aging

Just before the accelerated aging process, samples of seed were coated with captan and botran fungicides. Standard equipment and conditions of 100 percent relative humidity and 41 C were used for aging (42). However, nonstandard methods were used to expose the seed to this environment. Petri dishes (25 x 150 mm) were filled with a single layer of seed but remained uncovered in the aging chamber. An aluminum foil "tent" protected seeds from condensation. Treatment intervals were 20, 30, 40 and 50 hours. Moisture of 20 to 30 percent was obtained due to the aging process. This treatment was not severe enough to affect seed vitality but did affect the growth rate curve. After this treatment, the seeds were equilibrated to the original 11 percent moisture, by air-drying them for a week under storage conditions.

Controlling Water Uptake Rate

The rate of water uptake was controlled by varying the number of germination papers (Anchor, Inc.) wetted with a constant amount of water. Deionized water (20 ml) was placed in (25 x 150 mm) petri dishes; then in progression, one paper was placed in the first dish and five were placed in the last dish; 20 seeds were added to each dish. One paper served as the check, in that the water potential was

zero at the point of contact between the seed and paper. Each additional layer lowered that potential by reducing the availability of water. Imbibition was monitored by weighing the seeds at various time intervals. Transfer of seeds to petri dishes containing 20 milliliters of water and three papers allowed germination to begin in one day at 25 C. Four additional days were allowed for growth before measuring seedling weights or lengths. Each experiment was repeated and replicated at least twice for confirmation.

Automatic Seed Analyzer

In principle, the seed analyzer (ASA) records the electrical conductivity of a steep solution. Conductivity of the solution increases as the seeds are soaked. The electrolytes are of cytoplasmic origin consisting mainly of potassium salts of organic compounds.

The method used to determine electrolyte leakage as a function of hydration rate Figure 5, follows. Electrolytes absorbed by the germination papers during imbibition were measured after one hour of soaking the papers with a total of 60 milliliters of water. The automatic seed analyzer (ASA-610, Agro Sciences, Inc.) was used to measure the conductivity of 4 milliliter aliquots of the leached water. The conductivity of the solution was then expressed in microamperes. Differences in conductivity, due to treatments, were relative to controls. It was necessary to

soak the control paper, using the same methodology, to correct for background electrolytes.

Partial Priming

In some experiments seeds were partially primed by slow imbibition on four layers of paper for various time intervals then dried in open containers for at least 4 days. Imbibition was controlled at 25 C and 100 percent relative humidity unless otherwise indicated.

The term vigor is used to express the relative growth of treated to that of the control seedlings during the same time and under the same conditions. Growth was measured as fresh weight and expressed as the ratio of the root-hypocotyl to total seedling weight. Vitality refers to the percent of live seed based on germination or chlorophyll development. A new term, partial priming, is introduced to emphasize the short (24 hours) time required to fortify redried seed against soaking injury.

Biochemistry of Growth Regulators

Age versus ABA

Experimental design. The design was (5 X 2) factorial, replicated twice. Five levels of aging were tested against two levels of abscisic acid (ABA).

Preparation of the ABA solution. Cis-trans abscisic acid (molecular weight 264 and 95 percent purity) was the starting material. The free acid was not soluble in water but could be dissolved in an alkaline solution which converted the acid to a salt. In approximately 300 milliliters of water, contained in a 500-milliliter Erlenmeyer flask, 200 mg of sodium hydroxide was dissolved. Racemic ABA (66 mg) easily dissolved in the alkaline solution. Afterwards, sulfuric acid (0.01 M) and a glass electrode pH meter were used to titrate the solution to neutrality. The neutral solution was diluted to 500 milliliters. The pH of the final solution was 7.0.

Protocol. To each of the petri plates (25 X 150 mm) was added 2 milliliters of ABA (5×10^{-4} M), or 2 milliliters of a sham, followed by 10 milliliters of water. After mixing, the ABA concentration was 4×10^{-5} M. Two brown paper disks imbibed the liquid and 20 seeds were placed thereon. Four days at 25 C were allowed for germination and growth. Measurements were made on the total fresh weight of the seedlings in each plate and for the detached root-hypocotyls.

Data reduction used the percentage of root-hypocotyl of the total as a growth index. Since ABA reduced the rate of growth, it was necessary to normalize these growth percentages as a percentage of the unaged controls. This was done for the ABA treated and untreated series. The

interaction of ABA and accelerated aging could be determined by using the percentage of normalized growth with ABA to normalized growth without ABA, for each age category.

It was necessary to use triple ratios to correct for seed sampling error, ABA growth reduction and for reduction in growth due to aging. Using this method, the results then can be expressed in pure numbers (no units of measurement). The final percentages represent the effect ABA has on age. If the percentage decreases with age, then the isolated interaction is negative. The desirable outcome would be for the response to increase with age.

Age versus Fusicoccin and Ethylene

Preparation of the fusicoccin solutions. The publication of Cocucci and Cocucci (39) was used as a reference for this procedure. Fusicoccin (mol. wt. 670) was dissolved in ethanol (10^{-1} M). A stock solution 10^{-3} M was obtained by diluting the ethanolic solution with water.

Twenty-five milligrams of fusicoccin were dissolved in 0.5 milliliters of ethanol and then diluted to 38 milliliters with deionized water. A working solution of 10^{-5} M was prepared from this stock solution. Concentration at the final dilution was in the 10^{-6} M range.

Dose response. Concentration of fusicoccin was 1.5×10^{-6} M at final dilution. This value was based on 10^{-6} M, a

common concentration used by other investigators (39), and on the author's preliminary experiments. This concentration produced some dwarfing and also stimulated secondary root development. The concentration of ethephon (125 ppm) at final dilution (1:8,000) was selected by reducing the concentration until obvious stunting of the seedlings was stopped. The pH was not affected by this concentration of the acidic ethephon.

Protocol. Twenty seeds were used for each observation. The aged seeds were exposed to 24 hours of 100 percent relative humidity and 41 C, in an open petri dish protected from condensation by an aluminum "tent." Each petri dish also included two "brown" germination papers and a total volume of 12 milliliters. An additional five milliliters was added after seven days of incubation at 25 C.

Experimental design. The design was two-cubic, two concentrations for each of the three variables: age, fusicoccin and ethylene.

Statistics. Statistical analysis can be obtained from the design. Since the experiment was balanced, analysis of variance (ANOVA) may be applied to the data. Each of the eight treatments was run in triplicate and the experiment was repeated three times.

Data Analysis. Data were recorded after nine days of incubation. Three methods were used to interpret the data. The first was to use a graph. The second method was to tabulate the data as percent control for two groups, aged and unaged. The graph gives information in the third method (ANOVA) but statistical interpretation would not be as readily comprehended and would not be as appropriate for small differences due to treatments.

CHAPTER III RESULTS

Microflora and Seed Deterioration

In general, putrefaction was not common and streptomycin was limited to applications where bacterial activity was indicated by odor. Very high concentrations of streptomycin inhibited seedling growth. Another problem with coating seeds with streptomycin powder, also because this antibiotic is very hygroscopic, it increased the seed moisture when applied as a powder. The captan/botran mixture gave consistent protection from fungal growth in the petri dish germination test.

Histology of Aged Seeds

Tetrazolium stain defined areas of dead cotyledon tissue present on deteriorated seeds (Figure 1). These white areas of dead tissue were symmetrical between cotyledons. Areas most prone to aging were grouped loosely into several patterns. Similar patterns and symmetry were also observed on seedlings as necrotic spots. It was found that both accelerated aging and natural deterioration produce these results. There may be variations on this process. One exception was Vicoja 1980 which had a more uniform deterioration.

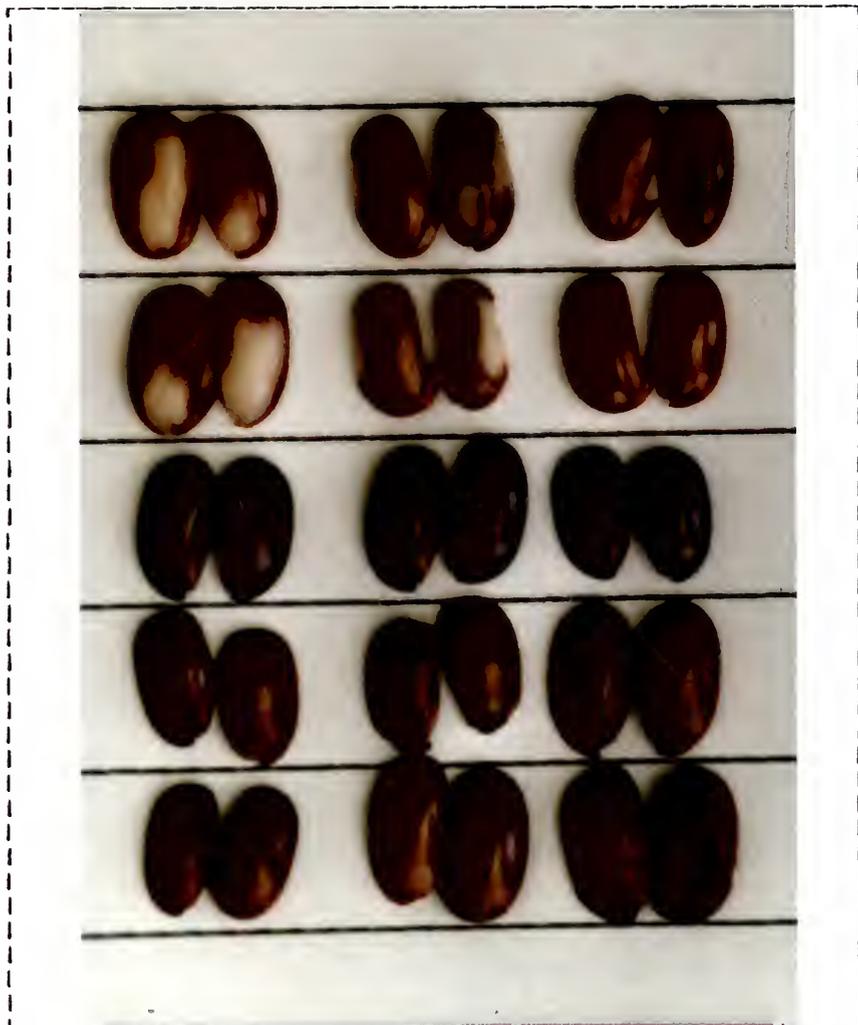


Figure 1: Differential Aging of Seed Tissue. Tetrazolium staining indicated that aging was not uniform in seed tissue. The pattern of aging was symmetrical. These symmetrical patterns occurred in several prevalent locations on the cotyledons. Both natural and accelerated aging produced this effect.

Tissue Excision Experiment

An additional facet of this histological examination of seed deterioration was to determine if the parts of the cotyledon most often affected by aging were important to the growth of the seedling. Parts of the cotyledons were excised with a scalpel and the wounds were coated with fungicide. These excisions removed approximately one-third of the moist seed mass. The most extreme treatment was to remove one of the cotyledons.

Compared to the controls, the rate of seedling growth after five days was not affected by removing part of the cotyledons. Removal of one cotyledon reduced growth. Location of the excision was not critical.

Automatic Seed Analyzer versus Tetrazolium

Results of this test were in agreement with the expectation that seeds which had the greatest extent of dead surface also had the highest ASA values. This observation was made by comparing two extreme categories of seeds, those with little evidence of dead tissue based on tetrazolium and those with extensive damage. The ASA values were consistent with the tetrazolium results, on an individual seed basis.

Cytology of Plasma Membrane Injury

Figure 2 shows the plasma membrane retracted from the cell wall. Ribosomes have escaped through the membrane but larger organelles remain inside the membrane.

Figure 3 confirms the particles as ribosomes because they form poly-ribosomes. The plasma membrane was also seen extending into the plasmodesmata in the cell walls.

Electrolytes escape from the symplastic to the apoplastic compartment. Membrane rupture is likely the basis of the seed analyzer (page 30).



Figure 2: Loss of Plasma Membrane Integrity. Excessively rapid water uptake burst the plasma membrane (M), allowing electrolytes to escape. Ribosomes (R) also escape but organelles remain as the membrane is plasmolysed.



Figure 3: Microscopic Identification. Particles were determined to be ribosomes (R) by identifying them with polyribosome (P) formation. The membrane was also determined to be plasma membrane (M) by its association with plasmodesmata (D) in the cell wall.

Physiology

Effects of Rapid Hydration

Water uptake was effectively slowed by additional layers of germination paper (Figure 4). Twenty milliliters of water completely saturated one layer of paper. Thus, one layer served as a control with the same matric potential as water. The uptake profiles were effectively modeled with a logarithmic linear transform corresponding to the following general equation:

$$Y = I + S \log X$$

The percent moisture on a fresh weight basis was the ordinate (Y). Hours of uptake were represented by the abscissa (X). The linear regression coefficient was at least 0.94. The terms (I) and (S) refer to the intercept and slope. Excluded from the model was the plateau portion of imbibition, 50 percent moisture and above.

Effect of age on uptake rate. The effect of aging on the rate of water uptake was determined from graphs similar to Figure 4 and was prepared from data on unaged seeds and seeds aged 30, 40 and 50 hours (Table 3). This information was essential for determining if aging predisposed seed to injury by weakening the plasma membrane by the velocity of water uptake. Since the uptake rates were logarithmic, one-

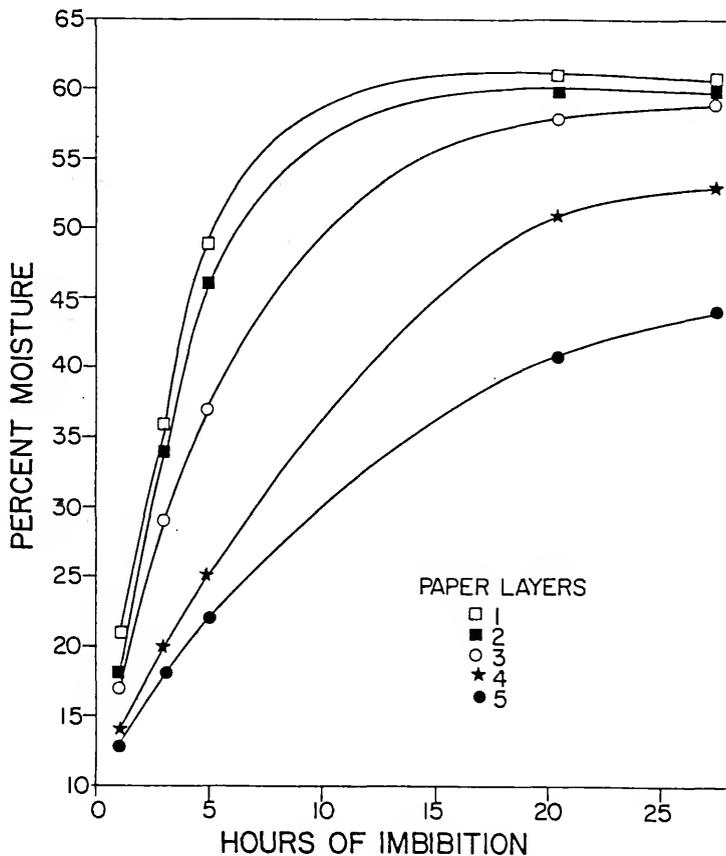


Figure 4: Controlled Rate of Water Uptake.

Soybean seed with an initial moisture of 11 percent were imbibed in petri dishes (25 x 150 mm) containing 20 milliliters of deionized water. Each plate also contained 20 seeds and one to five layers of germination paper. The graph symbol represents the number of paper disks per petri dish.

half maximum imbibition was used as a reference point to compare the rates of uptake versus aging treatments. Soybean seeds were found to reach 60 percent moisture before germinating; therefore, 30 percent moisture was equivalent to one-half maximum uptake. The data in Table 3 indicate that aging treatments did not influence the rate of imbibition. As the age of the seeds increased, there was no apparent reduction in the time required to reach one-half maximum imbibition for any of the five uptake rates tested.

TABLE 3
Effect of Aging on Rate of Water Uptake

Age	Layers of Paper				
	1	2	3	4	5
	<u>hours</u>				
00	1.18	2.0	2.6	6.0	7.0
30	2.0	2.3	3.1	6.5	9.0
40	1.9	2.2	3.6	6.6	10.0
50	2.0	2.6	4.9	7.5	9.7
<u>MEAN</u>	<u>1.9</u>	<u>2.3</u>	<u>3.6</u>	<u>6.7</u>	<u>8.9</u>
S.D.	0.1	0.3	1.0	0.6	3.0

Electrolyte Leakage. Electrolytes were measured from the papers used to imbibe seed. The data in Figure 5 indicate that aging increased the leakage of seed that experienced rapid water uptake. As the uptake rate was lowered with two and three papers, there was also a reduction in electrolytes lost by the seed. Four and five papers reduced the leakage of the most severely aged (50 h) to that of the unaged control.

Effects on growth. As uptake rate was controlled and electrolyte leakage was reduced, a concomitant improvement in vigor and vitality resulted. The extent to which growth could be increased depended on the extent of deterioration. The data of Table 4 correlate with the electrolyte data (Figure 5). Seed growth, after three levels of aging, was expressed as a percent of the growth of the unaged control. The term "vigor" was used for this variable. In general, vigor increased as the uptake rate was slowed with additional layers of paper. This dependence of leakage on imbibition rate increased as the age of the seeds increased.

Treatments that resulted in greater leakage also expressed the least growth. Growth of the seed then depended in part on the injury sustained at the time the seed was imbibed. Thus the elimination of imbibition injury was partitioned from the residual deterioration associated with aging.

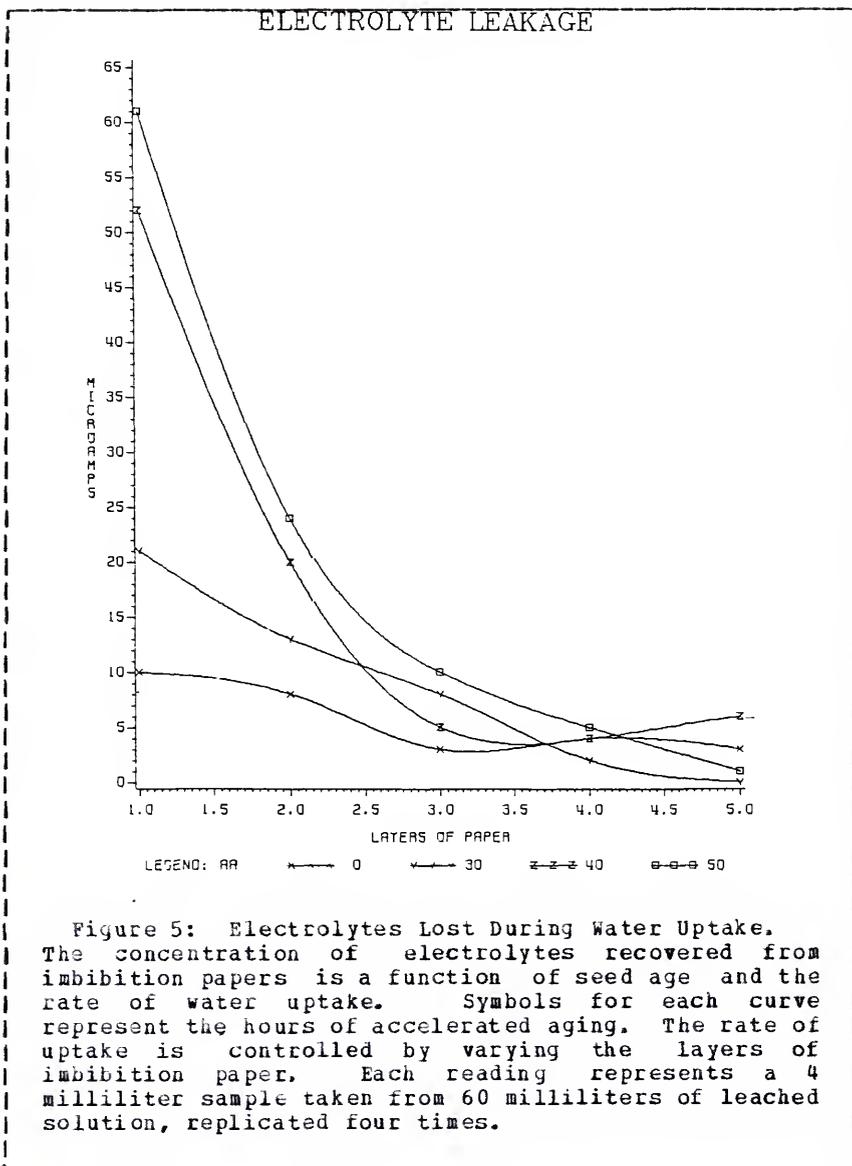


TABLE 4

Vigor as a Function of Age and Rate of Hydration

AA	Layers of Paper				
	1	2	3	4	5
<u>h</u>	<u>growth as percent of control</u>				
30	66	45	55	90	66
40	15	13	35	62	43
50	02	14	28	35	44

AA = accelerated aged (hours)

In addition to the growth of seedlings from aged seed being reduced by imbibition injury, the vitality or percentage of germination was also affected. Both aging and injury from soaking resulted in loss of vitality. The characteristic sigmoid curves of Figure 6 showed percentage of germination to be a function of age as well as the rate of hydration.

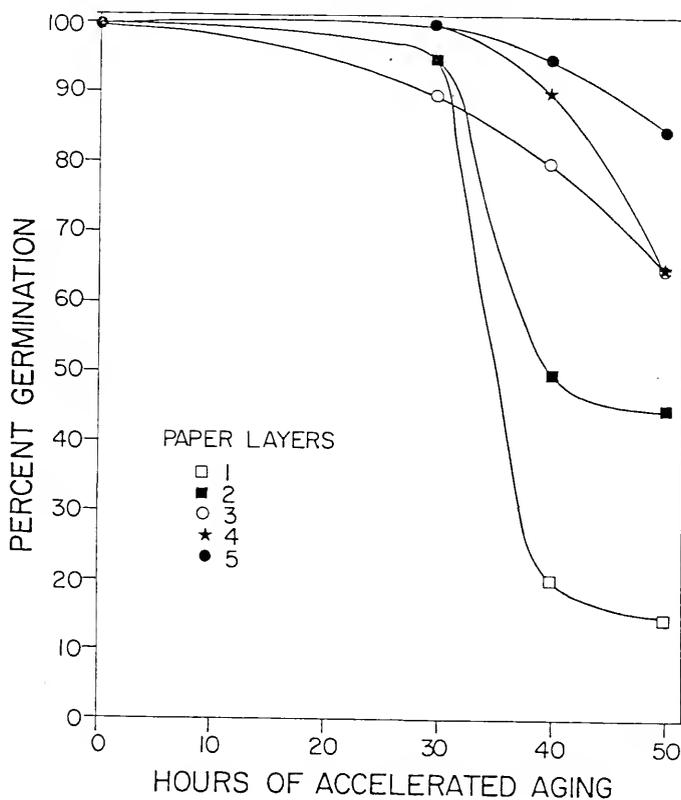


Figure 6: Effect of Hydration Rate on Aged Seeds. The vitality (percent germination) as a function of the hours of rapid aging and as a function of the control used to reduce the uptake rate. Each curve symbol represents the number of paper disks (one to five).

Effect of Priming on Electrolyte Leakage

Reversal of plasma membrane predisposition to imbibition injury was demonstrated (Figure 7). Seeds first primed, then redried, had one-third the conductivity of the controls. This reduction in electrolyte leakage during soaking was observed for the unaged control and each of the three levels of aging.

Possible explanations for the reduction of leakage in Figure 7 were tested to determine whether redrying was effective because it hardened the seed sufficiently to reduce the rate of water entry. Priming produced no detectable differences in the uptake rate (see Table 12). Reduction in leakage could not, therefore, be attributed to modification of the seedcoat or to other histological variables.

Additional evidence suggested that the plasma membrane was altered during partial priming. A primed versus aging factorial experiment similar to that represented in Figure 7 was conducted with beans split between the cotyledons. This removed the seedcoat as a moderator of water absorption. Results of the experiment with seed halves were similar to those with whole seeds (Figure 7). An exception to this was that the electrolyte loss was greater for half seeds than for whole, because of greater soaking injury.

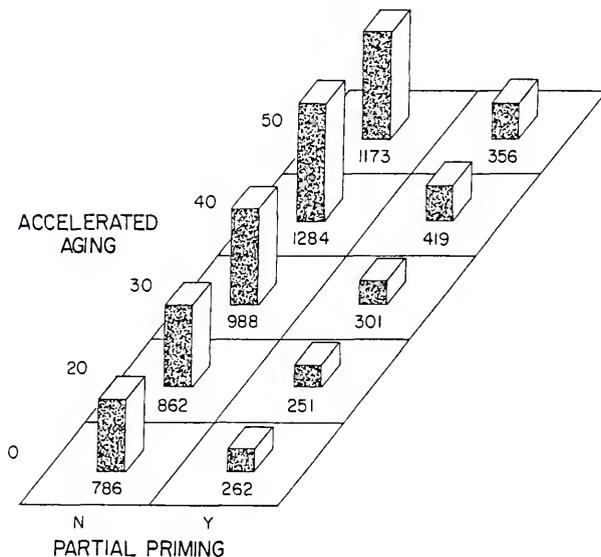


Figure 7: Effect of Priming on Electrolyte Leakage. A factorial experiment consisted of five levels of accelerated aging (0, 20, 30, 40 and 50 hours) with and without partial priming. All seeds were equilibrated to 11 percent moisture during storage. ASA data were collected after 9 hours of soaking and expressed as the sum of the microamperes, resulting from ASA data, for each of the 20 seeds per 10 treatments.

Effect of Aging on Primed Seeds

Table 5 shows that the vitality of seeds which had been primed was 140 percent that of the unprimed seeds, after 24 hours of accelerated aging. All seeds exposed to 48 hours of accelerated aging were much more severely injured but the vitality of primed seeds was increased to 320 percent that of the unprimed. All treatments were replicated twice. Each test plate contained approximately 85 seeds (10 grams). The vigor of seeds which survived all four treatments was noted two days after the vitality data were recorded. In general, the survivors of the primed seeds grew considerably taller than the surviving seeds of the unprimed controls.

TABLE 5

Effect of Accelerated Aging on Primed Seeds

No.	Treatment		Results	
	Primed	AA	% Live	% Increase
1	n	24	65	
2	y	24	92	140
3	n	48	24	
4	y	48	77	320

AA accelerated aging (hours)
% Increase in vigor over control

Priming-Temperature Dependence

Reversal of the predisposition to soaking injury was tested to determine whether temperature dependence could be established (Table 6). The aged (34 h) and unaged seeds were put into two groups exposed to imbibition at 4 C and at 25 C. The low temperature was expected to suppress metabolic activity. The low and high temperature treatments were again put into two drying conditions. Drying at 4 C minimized metabolism. Drying at 25 C allowed the moist seed to activate metabolic processes during this phase. The results are consistent with the hypothesis that repair was involved. Table 6 expresses the ASA data relative to those of the unprimed controls. Total suppression of repair, 4 C imbibition followed by 4 C drying, resulted in seeds that were statistically like the controls. The second and third pair of treatments gave similar results. The 4 C uptake and 25 C drying benefited both aged and unaged seed. The reverse, 25 C uptake and 4 C drying, was as effective. The most beneficial combination was 25 C uptake followed by 25 C drying. Accelerated aged and unaged (natural age) had the same ranks associated with the priming combinations but the aged seed had consistently higher values.

TABLE 6
Priming-Dependence on Temperature

Treatment		Results							
temp		aged				unaged			
wet	dry	rank	ASA	SD	%C	rank	ASA	SD	%C
4	4	1	85	8.6	111	1	68	12.9	113
4	25	2	57	0.9	74	2	50	3.7	84
25	4	3	50	8.6	65	3	38	9.2	64
25	25	4	47	4.7	61	4	28	2.9	47

ASA automatic seed analyzer (microamperes per seed per 4 ml); SD standard deviation (n=2); %C percent control

Effect of Soaking Temperature on Seed Leakage

Figure 8 contains the results from a test of Simon's phase transition hypothesis. Microampere values represent the averages of 20 seeds. The top curve (H) shows the increase in electrolyte leakage with age, when seeds were soaked at ambient temperatures. The second curve (Hp) was constructed from the ASA values of seeds primed after aging. Priming reduced the plasma membrane permeability which was consistent with other experiments.

The third and fourth curves repeat the profiles of the first and second but have much lower electrolyte values. The third curve (L) represents the aged treatments imbibed at 4 C in the refrigerator. Compared to the first curve

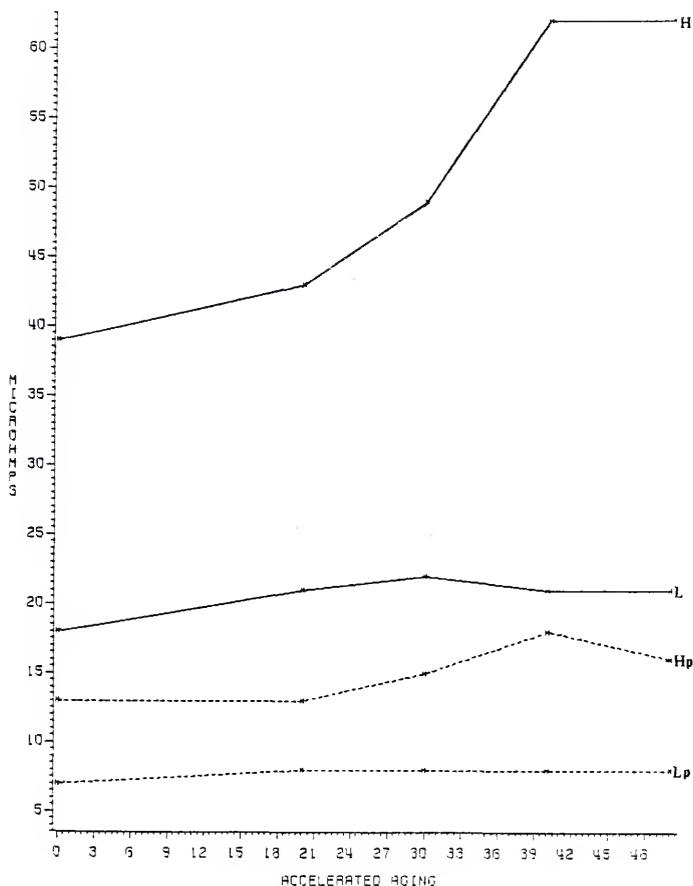


Figure 8: Effect of Low Temperature on Leakage. Soaking temperature (25 C) was represented by an (H). Seeds primed after aging then soaked at 25 C are represented by (Hp). Low temperature (4 C) soaking of five age categories, unprimed and primed after aging are denoted by (L) and (Lp) respectively.

(H), it was apparent the effect of the lower temperature was to decrease leakage of the seeds. The fourth curve represented seeds soaked at the lower temperature and primed (Lp) was also consistent in that the effect of the lower temperature was to reduce the leakage of primed seeds.

Additional measurements were taken at the conclusion of this experiment. The individual weights of the first 20 seeds from curve (H) were compared to the individual weights of the first 20 seeds from the (L) curve; the weight differences were highly significant using the Student's "t" statistical criteria. Seeds soaking for nine hours under refrigeration still had the wrinkled appearance indicating that imbibition was not complete. Seeds soaked at the higher temperature appeared smooth. Therefore, the lower temperature slowed the rate of water uptake.

Biochemistry of Growth Regulators

Age versus ABA

The objective of this experiment was to determine if ABA-induced dormancy has a beneficial effect on the performance of aged soybean seeds. The rationale was that ABA would delay germination sufficiently to allow the seeds' metabolism to be channeled into repairing the deleterious effects of aging. Aging treatments were sufficient to influence growth while gentle enough to minimize the effects on percent germination.

ABA dose response. In order to determine the concentration of ABA which would delay germination but would not require additional treatments to break dormancy, a dose response curve was generated. This curve, Figure 9, was modeled with a log-logit linear transformation. The dose of ABA (10^{-3} M) was expressed as the log of the ABA dose used in each test.

$$\text{logit} = \ln [P/(100-P)]$$

Linear regression analysis had a coefficient of the determination or (R^2) equal to 0.99. This analysis provided a new and effective mathematical model, judging from various alternatives described by Moore (97).

Isolation of ABA and age interaction. In Table 7 are treatments and primary data. Root-hypocotyl weights, in general, decrease with age when treated with ABA, but in this experiment the weight actually increased with age up to 30 hours, then started to decline. No effect of ABA was apparent on the unaged seeds. In both cases the root-hypocotyl was 22 to 23 percent of the total weight. After seven days of growth, the total weights (T) and root-hypocotyl weights (RH) were measured. The mean refers to the average of the two (RH/T) ratios.

In order to interpret Table 7 it was necessary to reduce the primary data as described in the methods protocol. Table 8 was constructed using the procedure previously described (page 32). Except for the unaged controls, ABA

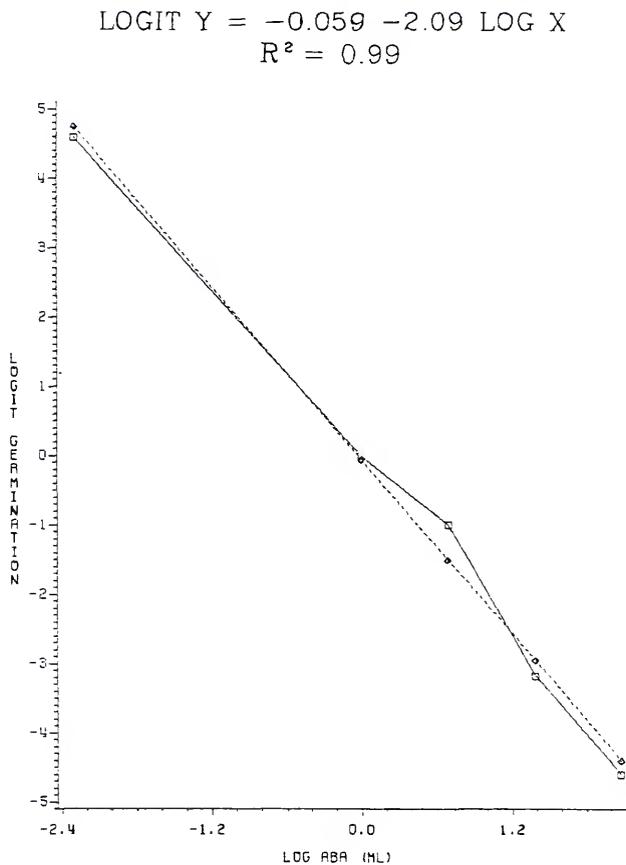


Figure 9: Dose Response to Abscisic Acid. Dose response to ABA was modeled with a log-logit linear regression. Each observation contained 50 seeds. Total volume of liquid was constant (15 ml), while the ABA dose varied from 0 to 8 ml. The conversion factor for milliliters added to the final molar concentration is (6.6×10^{-5}) . Data were recorded after five days of incubation at 27 C.

TABLE 7
Interaction of Age and Abscisic Acid

TREATMENT				RESULTS					
TRT#	AA	ABA	REP	T	RH	RH/T	MEAN	SD	CV
				g	g	%	%		
1	00	Y	1	6.23	1.37	22.0			
			2	6.20	1.44	23.2	22.6	0.8	3.7
2	20	Y	1	5.91	1.22	20.6			
			2	6.05	1.22	20.6	20.4	0.3	1.4
3	30	Y	1	5.67	1.17	20.6			
			2	5.97	1.16	19.4	20.0	0.9	4.3
4	40	Y	1	6.23	1.04	16.7			
			2	5.81	0.84	14.5	15.6	1.6	10.3
5	50	Y	1	5.72	0.86	15.0			
			2	.	.	.	15.0	.	.
6	00	N	1	6.65	1.50	22.6			
			2	6.66	1.49	22.4	22.5	0.1	0.6
7	20	N	1	7.11	2.09	29.4			
			2	6.86	2.20	32.1	30.8	5.5	17.8
8	30	N	1	7.19	2.10	29.2			
			2	7.40	2.53	34.2	31.7	3.5	11.0
9	40	N	1	6.76	1.64	24.3			
			2	7.09	2.19	30.9	27.6	4.7	17.0
10	50	N	1	5.37	1.04	19.4			
			2	.	.	.	19.4	.	.

T total weight; RH root hypocotyl weight; MEAN of two replications; CV coefficient of variation

results in less growth as expected since ABA reduces growth as well as inducing dormancy. If a variable called vigor is postulated and defined as growth relative to the unaged control, then this compensates for the reduction ABA has on growth relative to the unaged control. Vigor expressed as a percent then was corrected for the ABA effects on growth irrespective of age. To correct for age, aged ABA treatments were divided by the aged treatments without ABA and again expressed as a percentage. This new variable was called "vigor ratios" since it was the ratio of two vigor terms (with and without ABA). Vigor ratios then represent the isolation of the ABA age interaction. These data do not show a substantial interaction of ABA and accelerated aging. Perhaps a higher concentration of ABA would have given more definitive information. Statistical analysis by the generalized linear model may demonstrate significant interaction.

TABLE 8

Effect of ABA on the Vigor of Aged Seeds

TREATMENT		RESULTS		
ABA	AGE	GROWTH	GROWTH RATIO	VIGOR RATIO
Y	00	23	.	.
N	00	23	.	.
Y	20	21	91	
N	20	31	135	67
Y	30	20	87	
N	30	32	139	63
Y	40	16	70	
N	40	28	122	57
Y	50	15	65	
N	50	18	78	83

Age versus Fusicoccin and Ethylene

The rationale of this experiment was to determine if fusicoccin and ethylene, or its substitute, ethephon, interact synergistically. A synergistic response would indicate that each growth regulator has independent but complementary mechanisms of action. If, however, fusicoccin stimulates ethylene production, then it would be difficult to assign effects to a specific regulator. The objective was to find effective chemical means for low vigor seed stimulation.

After five days of growth, seeds were weighed before and after detaching the cotyledons and Table 9 contains the results.

TABLE 9
Interaction of Fusicoccin and Ethephon

OBS	TRT	REP	AA	FC	ET	ROOT
1	1	1	Y	N	N	0.39
2	1	2	Y	N	N	0.37
3	2	1	N	N	N	0.45
4	2	2	N	N	N	0.64
5	3	1	Y	Y	N	.
6	3	2	Y	Y	N	0.43
7	4	1	N	Y	N	0.50
8	4	2	N	Y	N	0.49
9	5	1	Y	N	Y	0.70
10	5	2	Y	N	Y	0.67
11	6	1	N	N	Y	1.09
12	6	2	N	N	Y	1.10
13	7	1	Y	Y	Y	0.55
14	7	2	Y	Y	Y	0.42
15	8	1	N	Y	Y	0.89
16	8	2	N	Y	Y	0.90

OBS observation; TRT treatment; REP replicate; ROOT root-hypocotyl weight (grams); AA accelerated aged

Table 10 summarizes the results relative to each of the two controls. In this experiment, ethephon stimulated root mass development. The roots appeared thick, short and root-

hairs were prominent. Seeds grown on two percent sucrose developed roots similar in appearance. Fusicoocin partially neutralized ethephon's effect indicating antagonism rather than the synergism expected. Fusicoocin did not reduce growth compared to the controls. At the end of five days of incubation, the total fresh weights and the root-hypocotyl weight were recorded for each petri plate. The percentage root-hypocotyl to total weight (%Tot) was used as the result. Each observation was relative to the aged or unaged control (%C), (Table 10).

TABLE 10
Growth Response to Fusicoocin and Ethephon

Treatment	Results			
	Aged		Unaged	
	%Tot	%C	%Tot	%C
Ethephon	13.0	159	19.0	202
Interaction	10.0	122	13.4	143
Control	8.2	100	9.4	100
Fusicoocin	8.3	101	9.1	97

%Tot percent of total weight (20 seeds); %C percent of control

Since the data in Table 9 contains a missing value, the generalized linear model was used for statistical analysis

(Table 11). No significant interaction was attributed to fusicoccin, with age and ethylene. All other interactions and main effects were significant or highly significant.

TABLE 11
Generalized Linear Regression Model

<u>SOURCE</u>	<u>DF</u>	<u>SUM OF SQ</u>	<u>MEAN SQUARE</u>	
MODEL	7	0.83559333	0.11937048	
ERROR	7	0.02730000	0.00390000	
CORRECTED TOTAL	14	0.86289333		
<u>R-SQUARE</u>	<u>C.V.</u>	<u>ROOT MSE</u>	<u>ROOT MEAN</u>	
0.968362	9.7680	0.06244998	0.63933333	
<u>SOURCE</u>	<u>DF</u>	<u>TYPE I SS</u>	<u>F VALUE</u>	<u>PR > F</u>
AA	1	0.23937190	61.38	0.0001
FC	1	0.03544835	9.09	0.0195
ET	1	0.45825016	117.50	0.0001
AA*FC	1	0.00000019	0.00	0.9946
AA*ET	1	0.06627273	16.99	0.0044
FC*ET	1	0.03402778	8.73	0.0213
AA*FC*ET	1	0.00222222	0.57	0.4750
MODEL F	30.61			0.0001

Variance alone did not give positive or negative information. Therefore, it was useful to use the graphic interpretation. As shown in Figure 10, no treatment produced less growth than the control. Ethephon was beneficial to both aged and unaged seedlings. Fusicoccin did not demonstrate a positive effect under these conditions. A combination of fusicoccin and ethephon reduced the benefit of ethephon alone.

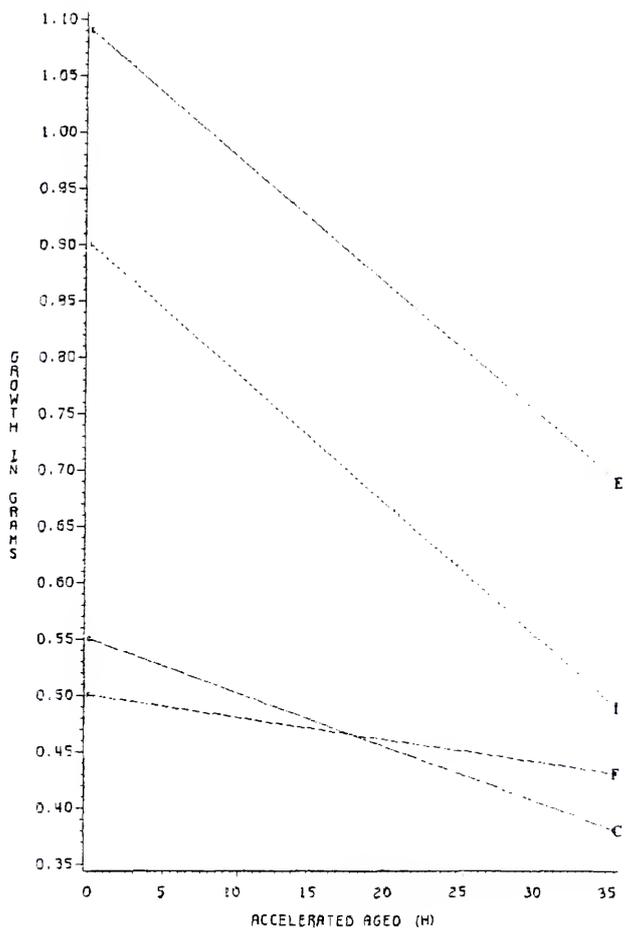


Figure 10: Age versus Fusicoccin and Ethylene. The root mass of each observation (20 seeds) was recorded after five days of growth at 25 C. Fusicoccin (F), ethylene (E), and their combined interaction (I) were applied to aged (34 h) and unaged seeds.

CHAPTER IV
SUMMARY AND CONCLUSIONS

Microflora of Seeds

Control of microflora prevented fungi from inhibiting the growth of seedlings. Use of at least two fungicides in combination, was a necessary protocol for these studies because aging weakened seeds and promoted fungal activity. Antibiotics have been used during accelerated aging, but use of fungicides for this purpose was not apparent during the literature survey.

Histology of Seed Deterioration

Symmetrical Necrosis

Black spots are frequently observed on emerging seedlings. The nature of these necrotic lesions was, in general, symmetrical. This injury may be related to differential aging of seed tissue as described below.

Tetrazolium Staining Pattern

Use of this stain on naturally aged and accelerated aged seeds showed symmetrical differential aging of the cotyledon tissue. This finding helps explain the nature of seed aging at the histologic level. The source of most seed leakage is probably from the unstained areas.

Cytology of Plasma Membrane Injury

Plasma Membrane Rupture

Electron-micrographs were obtained of cells with ruptured plasma membranes following rapid hydration. Some of the ribosomes were located between the membrane and the cell wall. Therefore, it is likely that cytoplasm would escape from these cells and constitute electrolyte leakage.

Physiology

Controlled Hydration Rate

A novel method was developed, allowing the hydration rate to be varied. Rapid water uptake injured seeds, especially when they were previously aged. By controlling the rate of hydration, injury during hydration could be varied and even prevented. Thus it was possible to partition imbibition injury and accelerated aging.

Partial Priming

A method was developed for priming seeds. It was also found that redrying could be applied to soybean seeds even after germination had begun.

The mechanism of priming was temperature dependent and probably metabolically dependent. Rejuvenation of seeds was indicated by at least three criteria: (1) Priming reduced electrolyte leakage (60 to 20 percent of controls). (2) Priming more than doubled the survival of seeds exposed to

accelerated aging. (3) Priming followed by redrying at 4 C did not affect leakage in aged or unaged seeds. Interpretation of this data was that metabolism suppressed by the low temperature was not sufficient for seed repair.

Biochemistry of Growth Regulators

Abscisic Acid Studies

Log-logit dose response of ABA. This mathematical model effectively described the inhibition by exogenous ABA on germination. Similar models were not found during the literature survey.

Antagonistic chemicals to ABA. Ethylene was the most effective treatment for reversing exogenous ABA dormancy. Other effective antagonist included thiourea and fusicoccin.

Effect on respiration by ABA. ABA was found to prevent an increase in respiration in imbibed seeds. Respiration increased during hydration until seed saturation, but ABA prevented an additional increase in respiration associated with germination.

Age interaction with ABA. The experiment was conducted with the hope that ABA could be used to improve the vigor of aged seeds by allowing the seed to channel metabolism into repair before starting the growth process. Unfortunately, no improvement in seed vigor could be detected as a result of

ABA treatment of aged seeds. Ethylene, on the other hand, did have a positive age interaction.

Fusicoccin Studies

Seedling morphology was affected by fusicoccin in the 10^{-6} M range and higher. With this evidence of biological activity, fusicoccin was shown to reduce the seedling growth attributable to ethylene stimulation. This activity was interpreted as antagonism between fusicoccin and ethylene. This activity was interpreted as antagonism between fusicoccin and ethylene. It seems reasonable that fusicoccin does not stimulate ethylene production, as is the case with some other growth regulators with auxin like activity.

Hypotheses Tested

Homeostasis

Several experiments in this study did support the homeostasis hypothesis (metabolically dependent repair, page 4). Conversely, no evidence could reject the hypothesis. Until this hypothesis fails to explain experimental observations, or until a more complete description is provided, this explanation must be accepted.

Simon's Hypothesis

This hypothesis (spontaneous repair, page 16), was found unsatisfactory when applied to observations encountered during this investigation.

Another report failed to support Simon. O'Neill and Leopold, 1982 (109) found no detectable phase transition in soybean phospholipids over the 0 C to 50 C temperature range.

CHAPTER V DISCUSSION

This study analyzed seed deterioration and repair using five approaches (microflora, histology, cytology, physiology and biochemistry). The analytical methods were progressively more detailed and on smaller scales.

Chronologically this work began with the use of growth regulators in an effort to refurbish the performance of aged seeds. Dormancy induced by abscisic acid was used to determine if repair took place during this period. The use of abscisic acid was complicated by additional steps to reverse the process. The very brief dormant period preceding germination was enough "dormancy" to improve performance. This led to a type of priming which was distinguished from the conventional technique (64) and was initially referred to as partial priming.

Beneficial effects of priming were evident with reduction in membrane permeability. Plasma membrane leakage was identified with cell injury and death. Prevention of membrane rupture improved seed vigor and vitality. Part of this improvement was explained by prevention of injury but repair processes were also evident.

Microflora and Seed Deterioration

Control of microbial activity with chemicals had a limited objective, which was to suppress fungal growth on seeds in the petri dish germination test. The results of these tests were used to establish a protocol which used captan/botran on all seeds unless otherwise noted.

This treatment effectively eliminated most signs of fungal growth. Avoidance of seed deterioration also reduced the fungal problem.

Questions remaining include the extent to which microflora affect the vigor of seeds before accelerated aging. Another question is the influence of microflora on the symmetrical, histological patterns visualized by tetrazolium staining.

It was assumed that the presence of most fungi was limited to dead tissue of the seedcoat. These saprophytic fungi may not penetrate the living aleurone layer. Another assumption was that absence of visual signs of fungi also indicated an arrest of fungal activity.

The design of the experiments used control treatments to minimize the unknown influence of microflora and reduce confounding due to their possible influence. Fungicides were used in earlier studies by Tao (170) and by Royse, Ellis and Sinclair (146). Few reports have considered the microflora component in relation to accelerated aging or its influence on the vigor of seed development.

Although microflora were of interest to the present investigation, it was not practical to pursue all of the avenues suggested. Emphasis was confined to the permeability of the plasma membrane in relation to aging.

Histology of Aged Seeds

This was integrated with other phases of the study and with information in the literature. For example, the contribution of microflora to the differential aging was unknown. Since most of the high vigor seeds developed symmetrical necrotic spots when accelerated aged for 24 hours, most of the effects are assumed to be physiologic. Microflora injury to the seed tissue may have been indicated only in selected seeds.

The correlation between tetrazolium analysis and seed analyzer results was consistent with the interpretation that dead, disrupted cells make major contributions to the ASA results rather than alternative concepts such as phase transitions of the phospholipids.

Certain inconsistencies of these tetrazolium results were also noted. Sen and Osborne (154,155) found that aging was uniform in rye embryos. Their materials and methods (monocots, isotopic precursors) were radically different from those used in the present study.

Another inconsistency within these experiments should also be mentioned. When seeds were aged then primed and

finally stained with tetrazolium, they developed areas of tissue without dehydrogenase activity. These tissues then may have lost enzyme activity without disruption of the cells. Osborne's (110) review credited the basis of tetrazolium staining to mitochondrial dehydrogenase enzymes. This raised another question: can cytoplasmic dehydrogenase enzymes also affect the tetrazolium stain?

The anatomical locations of the cotyledons most susceptible to aging were not critical to seedling development. The tissue excision experiments demonstrated that it was not the loss of necrotic tissue which affected growth of the seedling but only represented the more profound aspects of generalized deterioration.

Cytology of Plasma Membrane Injury

Knowledge obtained from cytological observations was integrated with the tetrazolium experiments as well as non-visual, physiological experiments dependent on the ASA.

An explanation consistent with the experiments of this study relies heavily on Villiers' (188) report. The description beginning on page 39 and the figures 2 and 3 are similar to that of Villiers'. His description of cell disruption was one accepted for this experiment. A basic difference between the present observation and Villiers' experiment was that he used soybean seeds rendered non-viable by accelerated aging. Rupture of the plasma membrane

by rapid water uptake probably occurred in both experimental situations. Villiers described collapse of the membrane followed by hydrolytic digestion of the cell contents. Severe cellular injury would explain electrolyte leakage during soaking without invoking Simon's phase transition hypothesis (159,158).

If the force of water ruptured the membrane, then the osmotic potential would tend to force electrolytes out of the cell. This process would shrink or collapse the membrane.

Additional questions concern the effect of predisposing the membrane to soaking injury and how this process can be reversed. These questions are discussed in the physiology sections.

Physiology of the Plasma Membrane

The results of the present study support the findings of Woodstock and Tao (200), in that accelerated aging (42) predisposed seed tissue to imbibition injury. The present study differed in its experimental approach and in extending previous work to gain insight into the reorganization of the plasma membrane. Evidence for this process was observed in both aged seeds and high vigor seeds.

Experimentally, hydration was slowed with matric potentials rather than an osmotic potential. This technique emphasized that the desired effect of slow hydration

resulted in the avoidance of physical injury to the seed. This technique also ruled out the possibility that polyethylene glycol was directly responsible for membrane restoration. In fact, polyethylene glycol probably slowed water uptake by increasing viscosity rather than by osmotic effects. Another consideration was that this chemical treatment is not entirely innocuous. Once imbibitional injury was avoided, the seed annealed the membrane. Annealing¹ was evident when primed and redried seed were no longer predisposed to soaking injury after accelerated aging. Furthermore, the repair process was temperature sensitive and probably metabolically dependent. It was of both practical and basic interest that the effects of aging was extrapolated from excised embryos (200) to whole seeds. The information gained has made it possible to increase seed vigor and resistance to the effects of aging.

Hypotheses considered in the course of these experiments included the following: First a hydrophilic/hydrophobic interaction of water with the plasma membrane phospholipids might bring about spontaneous reorganization. This explanation was not supported. The presence of water did not restore the membrane integrity at low temperatures. Although the temperatures may have prevented the phospholipid reorganization, it was expected that metabolism was the more sensitive to temperature.

¹ Anneal - to temper or toughen seeds against age induced predisposition to soaking injury.

Secondly, chilling was found to decrease electrolyte leakage, indicating that injury did not result from chilling. Therefore, the data in Table 6 are interpreted as evidence for the metabolic dependence of repair. Seeds that were imbibed and dried at low temperatures did not differ significantly from the controls for aged and unaged seed. The uptake of warm water followed by warm drying was the most beneficial treatment. This allowed the longest time and most favorable condition for metabolism. Protein activity may be implicated as part of the plasma membrane reorganization. On a dry weight basis, 40 percent of the plant plasma membrane is composed of proteins (72). Activity of these proteins may have affected membrane priming.

Thirdly, avoidance of injury during water uptake would explain the benefit of gradual hydration (125,130,200), without postulating a repair mechanism. Importantly, it was possible to separate plasma membrane repair from the confounding effects of imbibition injury.

It has been suggested that imbibition does not allow time for the membrane to reorganize (200). This rate-dependent process then results in leakage. Since leakage of redried seed was reduced by one-third, hysteresis was suggested. Once the membrane was organized by priming, it did not lose all of its organization and could organize more rapidly. Evidence against this hypothesis was that seeds had lower

ASA values at 4 C than at 25 C. If reorganization was the limiting factor, cold water should have decreased the rate of organization and subsequently increased leakage. Cold water did imbibe more slowly (102), but this treatment also decreased leakage. This evidence does not favor the hypothesis of Simon (158).

Test of Hypotheses

Metabolic repair. Interpretation of these results answered two questions. The first is "Does priming have an effect on aging?" The obvious answer was "yes." The second question was "How does priming affect aging?" The answer to this second question provides information about the priming process. Priming apparently does more than restore the plasma membrane. These results show that there was a total improvement in the vigor of the seeds which emphasizes the metabolic basis of priming and further supports the homeostasis concept of anabolic repair. Anabolism increased the vigor of the seeds. The accelerated aging treatment then became a vigor test and demonstrated the increase in vigor due to priming (82). That unaged seeds can benefit from priming was plausible since these seeds may have lost some of their maximum potential after a year of storage.

Other chemicals considered for use during priming include fusicoicin and ethylene. These chemicals may enhance seed vigor under stress conditions.

Spontaneous repair. Simon's concept of plasma membrane permeability has stimulated seed research for ten years (158). Experimental evidence supporting this concept includes that of Parrish and Leopold (118). Their results were based on protein leakage which occurred during soaking. Contradictory results were later found by O'Neill and Leopold (109). These investigators used differential scanning calorimetry to directly measure enthalpic changes in phospholipids during hydration. Interpretation of the current findings was also inconsistent with the phase transition concept.

According to that concept, the expected results would be an increase in electrolytes at the lower temperature. The rationale for these predicted results was that the lower temperature would slow the phase transition and hold the membrane in the permeable formation.

Simon's publication (158) calls attention to several deficiencies, which are apparent in retrospect. The application of the concept to seeds was extrapolated from research on brain phospholipids. Simon thought that in the dry state, the phospholipids formed the equivalent of micelles, although no microscopy supports this conjecture. His scholarly and stimulating report was based on a literature survey only, unsupported by experiments directed at determining its validity.

The role of water uptake in influencing the leakage of seed tissue was emphasized by the results of this experiment (Figure 8). Several experiments were subsequently performed to determine if priming reduced the leakage of seeds by reducing the rate of uptake. These experiments also failed to support this explanation for the priming phenomenon.

Results of physiological experiments are consistent with the homeostasis hypothesis. Conversely, Simon's hypothesis was not supported.

Biochemistry of Growth Regulators

ABA versus Accelerated Aging

Ultimately, use of this hormone was not necessary since other experiments in this study have shown that dormancy can be induced by redrying the seeds before they germinate or by reducing the availability of water. These other approaches have demonstrated the benefit "dormancy" has on seed performance.

Another consideration, evident in retrospect, is that both the control and ABA treatments would probably reverse some of the effects of aging in both treatments. Therefore, although ABA did not improve the "vigor" of aged seeds, this experiment does not contradict the homeostasis hypothesis.

Interaction of Fusicoccin and Ethephon

The antagonism between fusicoccin and ethylene may be limited to the concentrations used in these experiments. Perhaps the combined effect was excessive and became inhibitory. The potential for fusicoccin in stimulating rapid seedling emergence has been well documented by Khan (74). In defense of these results, the experiment improved on previous practices in which the same molar concentrations were used for each growth regulator (58). These practices failed to recognize the differences in dose responses. Perhaps dose selection should be based on the optimized value. Alternatively, the use of 50 percent maximum response would give a reference concentration which would prevent overdoses. Combining optimal concentrations could possibly have a negative effect by exceeding the limits of productive activity.

Suggestions for Future Research

This work tested an existing hypothesis and was unable to reject the possibility that dry seeds reverse the effects of deterioration after aging through metabolic turnover as a pregermination event. Additional future testing would attempt to inhibit protein synthesis chemically to determine if protein synthesis is required for the reversal of aging effects by priming.

APPENDIX
SUPPLEMENTARY EXPERIMENTS

Microflora and Seed Studies

Several interesting observations on microflora were obtained in some cases from formal experiments and on other occasions informally during work directed toward unrelated problems.

Fungal Survival

A grey mold was found that could grow on dead seeds at 41 C. This was the only seed fungus that was able to grow under the high temperature which is standard for the accelerated aging test. Seeds accumulated sufficient moisture (40 percent) to support fungal growth. The growth of fungi during aging was minimal since no signs were evident on seeds immediately after this treatment.

In another experiment, seeds with 11 percent moisture were sealed in a jar for 30 days at 41 C. The seeds did not survive but the fungus did. Seeds had more fungal growth after aging. This was probably because fungal growth was favored by nutrients which leaked from the seeds during imbibition.

Control of Fungal Growth

The use of fungicides was necessary for petri dish germination of aged seeds and for priming seeds. The reason for this requirement was that aging weakened the seeds and may also have made more nutrients available which stimulated fungal growth. Priming seeds at 15 C without fungicides resulted in severe fungal growth. Fungal activity in a petri dish reduced the growth of most seedlings not only those directly infected. Dead seeds would, at times, support fungal growth even though the seedcoat had been treated with fungicide.

Conversely, removal of nutrients leached from seeds and prevention of imbibition injury may explain why it was possible to germinate seeds without signs of fungi and without fungicides as is commonly done in growing bean sprouts. The seeds were rinsed with water several times a day, then drained. Care was taken to prevent evaporation. In this test germination could be controlled by limiting the seed moisture below 60 percent on a fresh weight basis.

Large numbers of seeds could be treated as in the above experiment for commercial production of bean sprouts in the food industry. The seed industry could also use this technology to prime seeds on a commercial scale without the use of PEG. Seed testing, with and without fungicides, may become a means for detecting harmful effects of microflora.

Histology of Seed Deterioration

Location of Seed Fungi

The location of most of the fungi was probably limited to the seedcoat. These fungi were probably saprophytes. Dusting the seeds with a dry powder prevented signs of fungal growth. Coating seeds with a fungicide slurry was effective and would be expected to penetrate the seedcoat. Fungal control was similar for the slurry and the dusting techniques.

Possible fungal growth within the live seed tissue was a topic of interest. It was possible by staining seed tissue, to locate fungus above the living aleurone layer. The organisms could be seen with a dissecting microscope. Only pathogens would be expected to be able to penetrate into living tissue.

Location of Hardness in Seedcoats

When a hard seedcoat was removed from the seed and imbibed with stain, the dye penetrated all but one cell layer, starting from the inside. Excluded from the stain was the exterior palisade layer. Only after some time was the stain able to penetrate these cells as they lost their impermeability.

Tetrazolium Staining Studies

The dead areas on deteriorated seeds may be related to microflora. The relationship of fungus to dead tissue is not understood at this time. For instance, does aging kill parts of the seed tissue and allow microflora to enter this dead tissue or are the dead areas predisposed to aging by microorganisms?

One experiment in which seedlings were grown on a dilute solution of tetrazolium showed that not all white areas were necessarily dead. The root and hypocotyl did not stain except for the root-tip and the junction between the root and hypocotyl. White, symmetrical and clearly defined patches developed on the cotyledons, indicating a lack of dehydrogenase activity in the unstained cells. Necrotic spots on seedlings were also symmetrical and may be the same areas identified with the tetrazolium stain. The extent of the dead tissue only indirectly indicates that injury had occurred in the other tissue as well. Surgical excision of parts of the cotyledons comparable to the necrotic areas had little effect on seedling growth. Removal of one cotyledon reduced growth, however. The presence of the insoluble formazan dye in the solution used to soak seeds indicated that cellular rupture was due to soaking injury.

Priming seeds after aging did not affect the subsequent tetrazolium stain. Penetration of the stain was observed by splitting the cotyledons and by making cross sections with a

scalpel. For the stain to penetrate the entire seed, it is suggested that the seeds be split between the cotyledons then imbibed directly in a tetrazolium solution.

Cytology of the Plasma Membrane

The cause of seed leakage was the question addressed by several preliminary experiments, suggested by current concepts, that aging causes deterioration of the membranes which results in loss of cytoplasmic electrolytes. Since about half of the membrane consists of protein, on a dry weight basis, perhaps there are alterations in membrane proteins which were associated with aging.

Proteins rather than phospholipids were selected for study because previous reports (132,133) indicated that phospholipids were not likely to be associated with the aging problem. Proteins were also selected for study because they are labile and therefore could be expected to be sensitive to environmentally imposed deterioration.

Experimentally, the object of this experiment was to characterize plasma membrane proteins in the embryos of two cultivars, Hardee and Vicoja, before and after accelerated aging. Protein characterization was ideally based on two-dimensional electrophoresis following ultracentrifuge purification. Aging was expected to produce an alteration in the protein composition. Also since cultivars differ in their aging rates, perhaps protein composition could account for this difference.

Membrane enrichment was accomplished by use of the discontinuous density gradient ultracentrifugation technique. Determination of purity was based on electron microscopy. Plasma membrane was distinguished from other membrane material by use of the Roland stain (142).

Membrane preparations were successfully extracted first from embryos and then from bean sprouts (Figure 11). Even though sufficient membrane quantity was obtained, the 70 percent purity was insufficient for further progress. Technology at the time limited preparations to "enriched" fractions. Since that time, a report has been published which makes it possible to obtain pure plasma membrane fractions (195).

Now that it is technically practical to obtain pure plasma membranes, the objectives of this early work may be attainable. Since experiments with both animal and plant tissues has identified "heat shock" proteins, it seems entirely likely that aged seeds would have a similar de novo synthesis response to injury (160). Additionally, evidence was obtained using other approaches (priming) which supported the concept of homeostasis or anabolic repair in pregerminated seeds.



Figure 11: Enriched Plasma Membrane. Seed embryos were used as a source of membrane. After the tissue was homogenized, differential centrifugation was used to sequentially remove tissue debris, nuclei and mitochondria. Plasma membrane was separated from other membranes by discontinuous, gradient ultracentrifugation. A purity of 70 percent was obtained with this method based on electron micrographs.

Physiology of Seed Deterioration

Priming Experiments

At least five points can be made which supplement an understanding of the priming effects based on physiology experiments. These experiments were conducted to answer the following questions: [1] What is the relationship between the maximum seed moisture used for priming and the leakage of redried seeds? [2] Does a divalent cation, such as calcium, benefit the priming process? This is a reasonable expectation since calcium had been shown by Gracen, in 1970, to stabilize the plasma membrane (56). [3] What is the "point of no return" for redrying seeds without loss of vitality? [4] Rather than repair of the plasma membrane, what other possible mechanisms may be responsible for the reduction in seed leakage after priming? For instance, is there sufficient moisture in the redried seed to slow the rate of hydration? [5] If seeds are redried to their original weight, do they return to their original moisture content?

Moisture versus priming effect. The relationship between maximum seed moisture used for priming and leakage was obtained for aged and unaged seeds. Figure 12 displays these results. The curves reflect the statistical analysis but more questions were raised by this experiment. The

first question is what determines the maximum priming effect once 60 percent moisture is attained? For instance, if 60 percent moisture produced the maximum priming effect then the line would be asymptotic to the X-axis. The second question or perhaps expectation is that the curve should be sigmoid because very low amounts of moisture would be proportionally less effective. Two separate experiments failed to obtain sufficiently consistent data to answer these questions with certainty. The limitation was finding a technique which gave the desired results. The first technique was to imbibe seeds to pre-selected moisture percentages, letting the time required vary. This was tedious and gave disappointing results. The second experiment was simplified in that time was controlled and the moisture was variable. There was some indication that moisture up to 30 percent increased seed leakage, but more than this amount lowered leakage. Additional factors contributing to the uncertainty of these findings were that only two points were affected, 20 percent and 30 percent moisture. Also the differences were not large.

Desiccation of bean sprouts. Experiments did show with certainty that when half of the seeds had germinated, it was possible to maintain vitality after redrying the seeds. The sprouted seeds survived, grew secondary roots, and demonstrated geotropic growth.

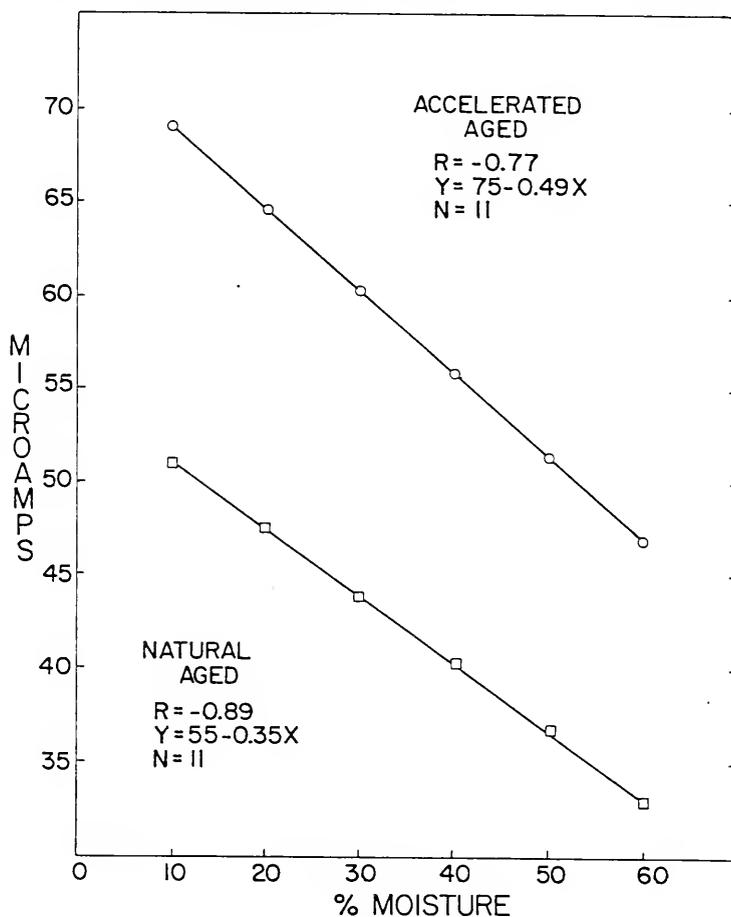


Figure 12: Seed Moisture Effect on Priming. Seed analyzer results (Y axis) are a function of the moisture used to prime the seeds. Each point represents average test results of 20 seeds, replicated twice.

Test of priming hypotheses. Priming reduced the leakage of seeds in experiments which have been repeated regularly. The explanation consistent with contemporary thought is that the plasma membrane is repaired, probably by metabolic processes. This explanation is satisfactory for all experiments which have been conducted to test this concept. It may not ultimately be true but it is a useful construct for conducting future experiments and does explain the results obtained thus far. Other experiments tested the alternative hypotheses that if priming followed by redrying reduced the rate of water uptake, then the reduction in electrolyte leakage may result not from repair of the plasma membrane but from reduced injury.

Table 12 contains the data from one of these experiments and is the basis for concluding that priming did not affect the rate of uptake (imbibition time) in this test. In order to determine if electrolyte leakage was reduced by reducing the rate of water uptake, two sources of primed seeds (Prime 1, Prime 2) were compared to three sources of unprimed seeds (Check 1, Check 2 and Check 3). The samples were imbibed on one layer of germination paper saturated with 20 milliliters of water. The weights of each sample were recorded four times during the eight hours of uptake. Priming did not affect the rate of uptake in this test.

Another hypothesis concerning the mechanism of priming was that residual moisture after priming protected the seeds

TABLE 12

Effect of Priming on the Rate of Imbibition

Uptake	Prime 1	Prime 2	Check 1	Check 2	Check 3
h	g	g	g	g	g
0	4.95	5.00	4.95	5.07	4.97
1	5.76	5.55	5.70	6.22	5.73
4	8.72	7.77	8.05	8.33	8.15
8	10.69	10.26	10.62	10.66	10.59

Uptake - imbibition interval (hours)

from soaking (and chilling) injury. The first experiment was designed to measure the leakage of seeds soaked in cold water as previously described. The second approach was to measure the residual moisture of the seeds after redrying. The thinking behind this experiment was that if even a small residual amount of moisture remained, then leakage of redried seeds may leak less than that of controls. Careful moisture measurements, made on oven drying control seeds and redried seeds at 106 C for 16 hours, showed no difference in moisture, even though replicates of these samples were very different on the ASA test.

The oven method was used in the previous experiment to compare the moisture content of two seed treatments. Another method used routinely was to start with 5.0 grams of seeds, prime, then redry to 5.0 grams. One concern with

this method was that respiration of the hydrated seeds used stored reserves. It was found that seeds redry to less than their original weight. About three percent of the seed dry weight was consumed during priming.

Another experiment compared seeds primed at 15 C and 25 C. Although both priming temperatures reduced ASA values to an equal extent, germination and growth was better after the 25 C priming treatment. This again suggests the importance of priming to the entire seed, not just to the plasma membrane. Pregermination events evidently not only restore the membranes but an increase in seed vigor becomes apparent when primed seeds are compared to unprimed controls.

Priming can increase germination rate. When primed and unprimed seeds were slowly hydrated for two days on five layers of germination paper, neither group had started to germinate until an additional 20 milliliters of water was added to the 20 milliliters already in each petri dish. Germination (broken seedcoat was the criterion) began the same day for the previously primed group of seeds. The following day primed seeds had an average of 21 percent germination whereas unprimed seeds had none. The second day almost all of the primed seeds had germinated whereas the average for the unprimed group was 16 percent. This experiment indicated that priming did increase the rate of germination even though the seeds had been redried. Priming and redrying were considered as separate components of the

partial priming technique. Page 31 contains descriptions of these procedures.

Similar experiments have indicated that germination and growth rates of seedlings increase if they are primed without redrying. This event may, however, depend on the moisture content of the seeds. For instance, seeds imbibed to 50 percent moisture may not grow more rapidly when the moisture is allowed to increase to the 60 percent required for germination. It may be possible to prevent cell expansion with moisture less than 60 percent. Cell division may have another limiting value. Metabolism would take place at still lower moisture. Therefore, it may be possible to use seed moisture as a means to separate each of these three processes.

Deterioration of Performance is Permanent

The reduction in growth rate of aged seeds was a permanent condition. When aged seeds were grown in a screen-house, most failed to germinate and those which did remained dwarfed. These stunted plants were grown along with unaged controls. Plants from aged seeds flowered with the control plants but they were smaller and had fewer and smaller root nodules. Part of the explanation for this stunting may be related to chromosome or genetic damage (145). However, if mechanical damage causes a similar growth reduction, then genetic damage does not offer a complete explanation (45).

Effects of Monovalent and Divalent Cations

Several tests were conducted to determine if salts, such as calcium chloride or potassium phosphate, affected the growth of seeds in otherwise standard germination tests. Concentrations were in the millimolar range for the salts and deionized water was the control. Each root length was measured after four days of growth. Root lengths were ranked and then plotted in ascending order. The growth curve was sigmoid for each seed treatment which reflected the expected normal distribution in growth rates among the seeds. Overlays of these plots provided a sensitive means of visualizing small differences among the treatments. Water alone was the least favorable medium for germination, but these differences were not large enough to justify routine use of these salts in germination tests.

A parenthetical comment is related to the distribution of growth among seedlings. Both germination and growth are described by sigmoid curves. Statisticians transform germination data to linearize the relationship between points of comparison. Growth data probably should be linearized before statistical analysis although this is not the practice. Before routine transformation, however, it should be shown that enough curvilinear relationship exists to justify the conversion.

Effect of calcium on priming. Further improvement in priming was sought through which fortified the water with 2

X 10^{-3} M calcium chloride solution as the medium. No difficulties were encountered due to the additional electrolyte but no benefit of this treatment could be detected. The calcium concentration tested was one based on the literature and since the effect was so negligible, no additional experiments were conducted.

Vacuum Drying to Prevent Seed Deterioration

Seed moisture on a fresh weight basis could be reduced from eleven percent to three percent by reducing the air pressure to one-third atmosphere for two days and the low moisture level retained by hermetically sealing. This approach may have economic advantages over continuous refrigeration as a means of maintaining seed performance. No adverse effect was detected from vacuum treatment. A very strong vacuum was also used to dry seeds without loss of vitality. Freeze drying seeds has been reported by Woodstock (199), but vacuum drying without freezing would have obvious advantages.

Stimulation of Growth By Slightly Aging Seeds

According to Woodstock and Tao (200), slightly aged seeds demonstrated improved performance. Also Perl in 1981 (122) reported a similar observation. The author conducted at least two experiments in an attempt to confirm these two reports.

In the first experiment, "dry" seeds were accelerated aged. Seeds, equilibrated to 11 percent moisture, were incubated at 41 C in a sealed jar. Samples of 20 seeds, aged 11 days in this manner (5 replicates), were compared to unaged seeds (4 replicates). After four days of growth at 25 C, no differences could be detected in total root-hypocotyl weights of the aged and unaged seeds by Student's "t" test:

$$t = [X1 - X2] / \text{SQRT} [(S1^2/N1) + (S2^2/N2)]$$

The symbols X1, X2 are the means, S1, S2 are the standard deviations. The number of replicates are N1 and N2.

In the second experiment, seeds were aged in the conventional manner on a wire screen over water at 41 C. Aging times in hours were 0, 6.5, 8.5, 22.0, and 28.5. The seeds were not redried before the germination test. Again growth was not improved by aging in any of the treatments.

There have been occasions in this study when slight aging did appear to stimulate the growth of seeds. This occurred in one instance when seeds were added to petri dishes containing two layers of germination paper and various amounts of water (1 through 16 milliliters). During 20 hours of aging at 41 C, growth was improved by the addition of some water. Up to two milliliters of water improved subsequent growth. Greater amounts were detrimental.

An explanation for these apparent contradictions can be advanced. In Perl's (122) work, the conditions were in

effect priming the seeds with water vapor which resulted in an improvement in vigor. Aging does not improve performance, but since priming does, and may occur as a result of moisture at the beginning of aging, the two effects may have become confounded.

Rates of Uptake and Leakage Compared

Three considerations were evident from specific experiments directed toward the following questions: (1) For a given sample of seeds, does the loss of electrolytes depend directly on the rate of water uptake? (2) What is the optimum soaking interval for the automatic seed analyzer? (3) When does the plasma membrane depend on respiration for active transport of ions across the plasma membrane?

Water Uptake Rate. The rate of uptake was described in the results chapter of this dissertation as a log-linear function between seed moisture and the log of time. Leopold recently described the rate of seed volumetric changes with a log-linear function (87).

$$-\ln [(m-a)/m] = kt$$

The maximum seed volume is (m), and (a) is the actual volume at any point in time. The proportionality constant, (k), indicates that seed volume increases at a constant rate which is proportional to the remaining possible expansion. Both Leopold's equation for seed volume and the equation

used in this study are applicable to water uptake data. Moreover, electrolyte loss conforms to similar profiles (99, 149). This suggests that deformation due to unequal volume changes throughout the seed may cause leakage from tissue injury. Figure 13 shows that the rate of water uptake is paralleled by the accumulation of electrolytes in the steep solution. Leakage and uptake rate was correlated during the first six hours.

One of the factors influencing the rate of water uptake is viscosity (184). The viscosity of PEG, not its osmotic potential, was likely responsible for Woodstock and Tao's findings (200). Probably, the viscosity of cold water reduces the leakage of soaking seeds (Figure 8).

Optimized Soaking Interval for the ASA. The manufacturer's recommended 24 hour soaking interval was not sensitive to differences in seed leakage between samples. This was apparent at the eight to twelve hour interval. For this reason, the shorter period was used for much of this work.

Effect of respiration on seed leakage. After reading Simon's review (158), it became apparent that lack of oxygen (anaerobiosis) could result in an increase in membrane permeability due to insufficient active transport. Consideration of this possibility along with another report by Mullett and Considine (99), who showed that electrolyte

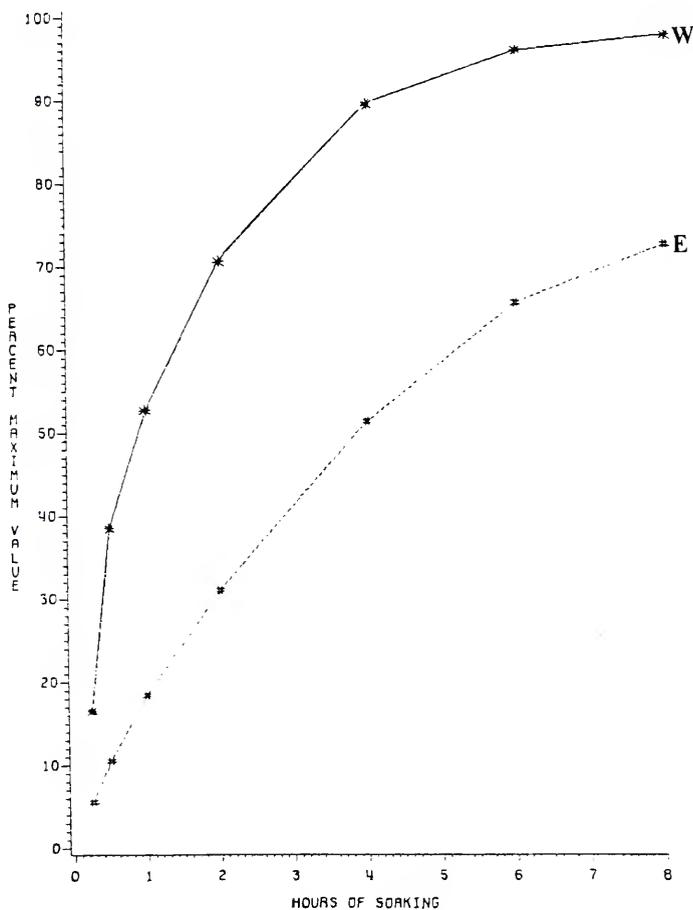


Figure 13: Rates of Hydration versus Leakage. Both the rate of hydration (W) and electrolyte leakage (E) conform to a log-linear relationship with time. This holds true when the seeds are imbibed on paper as well as when they are soaked in water.

loss increased with the depth of the seeds in water, it would explain these results. This would influence the ASA so seed leakage was partitioned into a dependence on rate of water uptake and air dependence.

An experiment (Figure 14) did give evidence for anaerobiosis in the ASA test, but this type of leakage does not contribute greatly to the ASA test results in less than 24 hours of soaking.

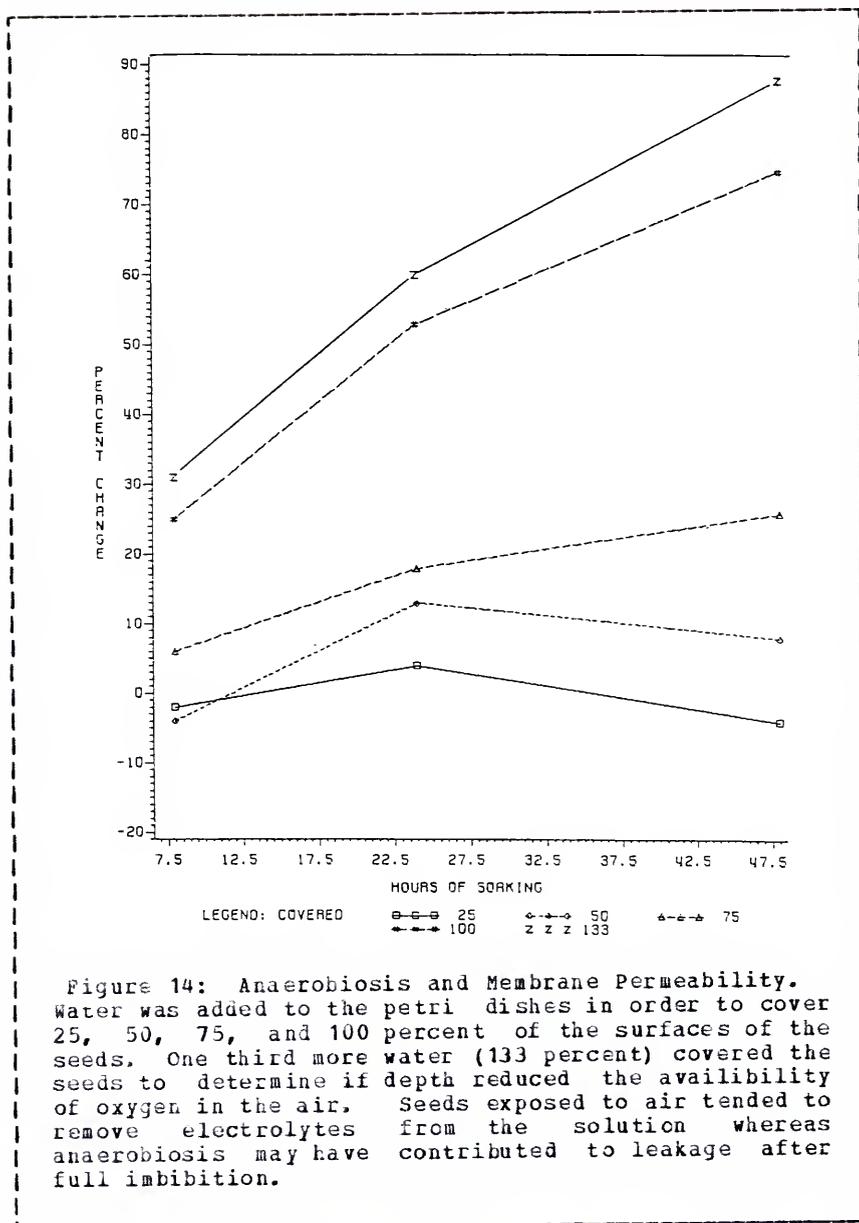


Figure 14: Anaerobiosis and Membrane Permeability. Water was added to the petri dishes in order to cover 25, 50, 75, and 100 percent of the surfaces of the seeds. One third more water (133 percent) covered the seeds to determine if depth reduced the availability of oxygen in the air. Seeds exposed to air tended to remove electrolytes from the solution whereas anaerobiosis may have contributed to leakage after full imbibition.

Biochemistry of Growth Regulators

Work which eventually evolved to partial priming, began with the use of abscisic acid to induce dormancy in seeds. The idea behind this work was that dormancy would alter the effects of aging on seeds by allowing homeostatic processes to repair age-related damage.

Another impetus to use growth regulators to study the effects of aging on seeds came from the work of Petruzzelli et al. (123). Fusicoccin as well as monovalent cations were shown to improve the germination of low vigor wheat. Dr. West was able to obtain a 25 milligram sample of fusicoccin from Dr. Marre¹ so we had an opportunity to incorporate the use of this chemical into studies concerned with stimulation of soybean seeds.

Considered in the subsections below are respiration studies, ABA dormancy, and the stimulation studies involving fusicoccin and ethylene. Respiration of dormant seeds was measured with an oxygen specific electrode (Yellow Springs Instrument Co., Inc.). This polarographic oxygen sensor responds to the oxygen dissolved in water. Respiration of seed samples were measured during a 10 minute interval as submerged seeds removed oxygen from the medium. Between measurements, seeds were not submerged so respiration could resume normally.

¹ Dr. E.E. Marre¹, Milan, Italy

Growth regulator experiments involved inducing dormancy and reversing dormancy with other growth regulators such as fusaric acid and ethylene. The effects of biosynthetic inhibitors, sucrose, and selected salts were also tested in an effort to enhance the mode of action for growth regulators and to gain insight into their mechanisms of action.

Respiration Experiments

Respiration was monitored during imbibition and early germination (YSI - oxygen electrode). The effects of accelerated aging on seeds was also measured with the same methods. Seeds which were dormant after abscisic acid treatment were tested to determine if their respiration was sufficient to support active metabolic repair processes.

Respiration versus imbibition. The increase in seed moisture corresponded to an increase in seed respiration until the seed reached 60 percent moisture. Oxygen uptake was 0.21 microliters per minute per seed, after six hours of imbibition. Twenty hours later, just prior to radicle emergence, respiration was doubled to 0.46 microliters per minute per seed.

These results confirmed other studies (26,98) and served to validate this methodology. In summary, respiration increased as a result of seed hydration until saturation. Afterwards, there was a plateau in water uptake and

respiration for almost 20 hours. As the radicle began to emerge, there was an increase in respiration, probably supplying energy for growth.

Respiration of Aged seeds. Respiration was reduced by aging. This was not a new observation (197), but the work provided experience and confidence in the methods.

Respiration of primed seeds. There was no improvement attributable to priming within the first 24 hours of hydration. This may signify that dry seeds which had been primed had a similar oxygen uptake versus time profile as unprimed seeds. It does seem likely that, at some later stage during this development, the respiration would exceed the unprimed controls if there is a concomitant increase in germination rate or growth rate due to priming.

Abscisic Acid Dormancy Induction and Reversal

A new model for the dose response was established with the log-logit linear transform (page 56). Once the optimum range was established for inducing dormancy, several methods were used which would reverse the effects of abscisic acid. Other growth regulators which were effective were fusicoccin, ethylene (as ethephon) and thiourea. Non-growth regulators explored included hydrogen peroxide, water elution, sucrose and selected nitrogen, phosphorous and potassium (NPK) salts. Other treatments tried to remove

abscisic acid by leaching seeds with water and by oxidation of ABA with hydrogen peroxide. Several of these treatments demonstrated a positive effect. No single treatment was as dramatic as the use of hormones, however.

Reversal of ABA dormancy was spontaneous when the dose was limited. Low doses of abscisic acid delayed germination and slowed growth. Restriction of dose rates was a practical way to avoid the necessity of dormancy reversal.

Another finding in the abscisic acid experiments which may be new, was its influence on seed respiration. Seeds which had been held dormant for a week had maintained respiration at the "plateau rate." Prevention of germination may have prevented increased respiration or vice versa.

Antagonistic Experiments

Fusicoccin antagonism. Factorial experiments used three concentrations of ABA versus three concentrations of fusicoccin. This design was used to obtain data for a statistical (SAS) procedure which optimizes the data on the basis of a three dimensional plot. The program supplies dummy variables, therefore, only nine treatments are required.

Fusicoccin in the 10^{-6} M concentration range did antagonize the effects of ABA in the 10^{-4} M range. This was confirmed by several related experiments.

Thiourea antagonism. Experiments with the same 3^2 design, demonstrated reversal of abscisic acid dormancy (1.3×10^{-2} M) by thiourea in the (3.5×10^{-2} M) range.

Ethylene antagonism. The design used to optimize the ethylene concentration (ethephon) was different from the previous optimization procedures. The maximum concentration of ethylene which reversed the effects of ABA without inducing dormancy was determined and used in subsequent experiments.

Serial dilutions of ethephon added to ABA dormant seeds promoted germination. Antagonism of 0.25×10^{-3} M abscisic acid was accomplished with (1.7×10^{-6} M) ethephon.

Mechanisms of ABA activity. Reversal of ABA dormancy by fusicoccin suggested that the mechanism of action for ABA was linked to the active transport by the plasma membrane. The basis of this suggestion was that fusicoccin is thought to have this single mechanism of action (93).

Fusicoccin Stimulation of Seeds

An overall objective of experiments with fusicoccin was to find seed treatments which would improve the performance of low vigor seeds. Also fusicoccin was combined with sucrose and/or salts with the purpose of finding a synergistic response. Other combinations included fusicoccin and growth regulators such as ethylene, as previously described in the results chapter (page 60).

Water potential. Germination paper was used to control the water availability to seeds germinating on a solution of fusicoccin. Three levels of water potential were combined with three concentrations of fusicoccin to determine if fusicoccin was able to promote germination under conditions of restricted water availability.

Fusicoccin in the 10^{-5} M range did stimulate germination of seeds growing in petri dishes when the water potential was reduced with germination paper. These results indicated that fusicoccin can increase the turgor of germinating seeds.

A similar experiment attempted to evaluate the effect of fusicoccin on turgor by stressing the seeds with 30 percent polyethylene glycol. This single concentration of osmoticum was too concentrated to permit germination even when the seeds were stimulated with fusicoccin. Several concentrations of osmoticum should be used to bracket the degree of stimulation due to the growth regulator.

Mode of action. Fusicoccin did alter seedling morphology. Concentrations in the 10^{-5} M range tended to produce short hypocotyls and stimulated secondary root formation. There was also a stimulation of chlorophyll development of seedlings in a manner characteristic of an auxin. But, unlike auxins, it is doubtful that fusicoccin acted through stimulation of ethylene biosynthesis.

Ethylene Biosynthesis and Stimulation

Experiments were conducted with amino-oxyacetic (AOA) acid and cobalt chloride to determine if fusicoocin stimulated endogenous ethylene production. The AOA (10^{-3} M) stunted seedling growth whereas the cobalt chloride (10^{-3} M) showed little effect. Exogeneous ethylene in the form of ethephon did not reverse the AOA retardation. This concentration of AOA was probably excessive.

It was not determined whether fusicoocin stimulated ethylene synthesis by use of AOA or cobalt ions. This was probably because the concentrations suggested by a review of the literature were inappropriate for this application. Amino-oxyacetic acid stunted growth so severely that no meaningful results were obtained. In contrast, cobalt in millimolar concentrations was ineffective in altering a growth response. Optimization of concentrations might have solved these problems.

Another approach, based on interaction of ethylene and fusicoocin, described in Chapter III on results, indicated that fusicoocin does not stimulate ethylene biosynthesis.

Mechanism of action. Both ethylene and fusicoocin break ABA dormancy. If fusicoocin stimulated endogenous ethylene synthesis, then this would indicate that ethylene and not fusicoocin antagonizes ABA.

Dormancy induction/reversal may be related to membrane permeability. The energy economy of the cell is dependent

on the semi-permeability of the membranes. Perturbation of this ability to produce chemical concentration gradients across membranes would reduce the rate of energy production to meet the demands for cell expansion and growth. Use of cell-free systems may avoid the inherent problem in isolating cause and effect relationships.

Mode of action. Both sucrose and ethylene stimulated the growth of root-hairs which suggest part of the ethylene response may be associated with mobilization of sugar within the seedling. This was observed in isolated embryos as well as whole seeds.

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BIOGRAPHICAL SKETCH

Three generations of Tildens have pioneered in Florida agriculture. Partly because of this precedent, it has been very satisfying for me to engage in agricultural research.

Education began for me in Winter Garden, Florida. High school graduation, however, was from Darlington School, Rome, Georgia, in 1958. This education continued at Stetson University in DeLand, Florida, where a B.S. degree in chemistry was earned.

An opportunity to use chemistry in a practical manner was provided during military service. This was at the Rocket Propulsion Laboratory, Edwards Flight Test Center, in California.

After returning to Florida in 1967, I was employed in pesticide research with the University of Florida to develop new, sensitive analytical methods. Several presentations and a publication resulted from this experience.

In 1970 my career continued as a research chemist in nuclear medicine at the Veterans Hospital, Gainesville. Developments in hormone analysis were communicated in a number of publications.

While employed by the VA, my association continued with the University. On a part-time basis, graduate credits were accrued and a Master of Education degree was awarded.

A desire to apply research findings to available products led to employment in private industry as a product development chemist (1977). Several research findings were manifest as commercial products for hormone analysis.

In 1980 my education resumed with the goal of applying my education and experience in chemistry to research problems in agriculture. The final goal was to earn the doctorate degree and to contribute to agricultural research. After earning a master's degree in horticultural science, I began work toward the doctorate in agronomy.

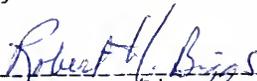
Fortunately, my wife Martha has understood the importance of this effort toward "self actualization." She has been very patient in this regard. This experience has also served as an example to our daughters, Beth and Becky, who take their education seriously.

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.



S.H. West, Chairman
Professor, Agronomy

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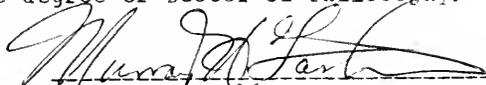
Robert H. Biggs
Professor, Horticultural Science

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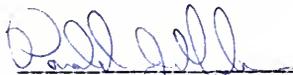
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Murray H. Gaskins
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I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.



Donald J. Huber
Assistant Professor, Horticultural
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This dissertation was submitted to the Graduate Faculty of the College of Agriculture and to the Graduate School, and was accepted as partial fulfillment of the requirements for the degree of Doctor of Philosophy.

April 1984

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