Seed dormancy in Pennsylvania smartweed and barnyardgrass

James Lowell Jordan
Iowa State University

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SEED DORMANCY IN PENNSYLVANIA SMARTWEED AND BARNYARDGRASS

Iowa State University

PH.D.

1981

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Seed dormancy in Pennsylvania smartweed
and barnyardgrass

by

James Lowell Jordan

A Dissertation Submitted to the
Graduate Faculty in Partial Fulfillment of the
Requirements for the Degree of

DOCTOR OF PHILOSOPHY

Department: Botany
Major: Plant Physiology

Approved:

Signature was redacted for privacy.

In Charge of Major Work

Signature was redacted for privacy.

For the Major Department

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For the Graduate College

Iowa State University
Ames, Iowa

1981

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GENERAL INTRODUCTION

Seed dormancy is the principal survival mechanism for annual weeds. Dormant seeds survive weed control practices, germinating seeds are vulnerable. Because dormant weed seeds may lose their dormancy and germinate after weed control operations are ineffective, dormant weed seeds limit the effectiveness of weed control practices.

Although termination of dormancy results in a germinable seed, dormancy termination does not inevitably result in germination. Dormancy can occur in different degrees, after a seed has been germinable, or from different factors (environmental or physiological). Because seed dormancy may not be the same for all seeds, the requirements for breaking seed dormancy may vary.

Seed dormancy may vary among seeds from the same plant or from different plants. One major variance in the weed habitat is competition stress from other plants (crops or weeds). Information about the effect of competition stress on weed seed dormancy would permit more accurate predictions of weed seed germination in a particular cropping season. This would be especially true for no-till agriculture since new weed seeds would not be buried and old weed seeds would not be brought to the surface.

Further research on seed dormancy is essential to a better understanding of the factors which promote germination. Then, research can be conducted on promoting germination of dormant seeds or enhancing dormancy to prevent germination.
LITERATURE REVIEW

The causes of seed dormancy and effective methods for breaking seed dormancy under laboratory conditions have been determined for many weed species. The difficulties of studying dormancy and relating it to observed germination patterns under field conditions have been compounded by the diversity of terminology proposed by various investigators. A useful classification system, now widely accepted, describes seed dormancy as being innate, enforced or induced (29). Innate dormancy describes the dormancy of seeds as they leave the parent plant and enter the soil habitat. Enforced dormancy is the condition in which nondormant seeds fail to germinate in conditions optimum for crop germination. Induced dormancy describes seeds which have lost innate dormancy; however, because they have passed through a long period of enforced dormancy, a dormant condition is acquired. Thus, the classification system of Harper (29) accommodates the occurrence of seeds of one species in all three phases in the same soil habitat and a sequence of events whereby seeds may lose dormancy, but fail to germinate. The classification system fits well the patterns of seed dormancy and germination observed in temperate zones of the northern hemisphere.

Enforced dormancy is maintained by environmental factors and germination may occur when the enforcing factor is removed. An example of germination following enforced dormancy is the germination of buried seeds when brought nearer the soil surface by plowing. Enforced dormancy has been attributed to high levels of CO₂, absence of light stimulus, lack
of temperature fluctuations and limited oxygen availability at the seed micorsite in the soil (20, 29).

A prolonged exposure to enforced dormancy promotes the onset of induced dormancy. High temperatures (65), restricted oxygen supply (33, 65), and high carbon dioxide levels (37) are important factors which promote induced dormancy. Although the inhibitory factor may be removed, induced dormancy will persist; persistence of dormancy, even in favorable germination conditions, distinguishes induced dormancy from enforced dormancy. Induced dormancy is similar to innate dormancy with regard to the factors which promote its termination (32). Innate dormancy has been called primary (20), natural (9), inherent (7), and endogenous dormancy (58). Innate dormancy is the initial dormancy present in a seed when the embryo ceases growth, although the seed may be still attached to the parent plant. Although most seeds have a period of innate dormancy, the intensity of this dormancy may be variable. Innate dormancy delays or prevents the onset of germination under conditions unfavorable for seedling development. Usually, innate dormancy results from the seed coat, the embryo, or from a combination of seed coat and embryo effects. The embryo may be dormant due to embryo immaturity or to physiological factors. Physiological factors which may cause innate dormancy include: (1) hormonal effects; (2) molecular changes; and (3) oxidative pathway changes.

Seed Coat Influences on Seed Dormancy

The seed coat is the interface between the seed and its environment. The seed coat may affect the seed regardless of favorable or unfavorable conditions. Two principal means by which seed coats affect seed dormancy
are by restricting water or oxygen passage into the seed. The most common effect of the seed coat is restriction of water entry into the seed. Thus, although a seed may be surrounded by water, its inability to imbibe water (hard seed coats) would prevent germination. Plant families which produce seeds with hard seed coats are: Asteraceae, Cannaceae, Chenopodiaceae, Convolvulaceae, Geraniaceae, Liliaceae, Malvaceae, and Solanaceae. Specific members of these families exhibiting hard seed coats include alfalfa, sweet clover, hollyhock, alfilaria, atriplex, asparagus, ornamental morning glory, canna and cherry tomato (5, 11, 17, 28, 30, 40, 43, 45).

The water restricting properties of some seed coats are a major factor in the extreme longevity of seeds of some plant species. Lupine seeds buried in a rodent's nest in northern Canada were still viable after 10,000 years (50). Sivori and Cigliano (61) reported successful germination of Cannaceae seeds that were 550 years old.

Although the hard seed coat condition may have a major effect on seed longevity and germination, knowledge about how water is excluded is still limited. Water exclusion appears to be a property of the outer cells of the seed coat (palisade or malpighian cells) (1, 18, 21). These are usually elongated cells which have caps that are embedded in a suberin matrix. External to the suberin matrix is a fine waxy cuticle. Ballard (4) does not consider cuticle a major barrier to water. The cuticle is stained with water based stains. Also, solvent removal of the cuticle does not improve water penetration into the seed (4).

The suberin layer is considered to be the main barrier to water
penetration (4). Because of the major role of suberized (and lignitized) palisade cells in water exclusion of the seed coat, attempts to break hard seed coat imposed dormancy involve modifying or disrupting the palisade cell layer of the seed coat. Treatments have included: acid or base treatments to dissolve the seed coat; scarification of the seed coat to cut through water excluding regions; raising the temperature to alter the seed coat; impaction (light blows to the seed) to cause separation of palisade cells; puncturing or piercing the seed coat to increase water entry; and removal of the seed coat to result in a naked seed.

Barriers to oxygen uptake

A second restriction of seed coats may be due to oxygen uptake into the seed. Reduced germination resulting from restricted oxygen uptake has been noted in Xanthium (19, 21, 59, 60, 64), Avena fatua (3, 31, 32), apple (66, 67), Phalaris (68), and Sinapis arvensis (22, 23, 24, 25). Edwards (25) suggested that the following processes may reduce seed coat permeability to oxygen: (1) high metabolic activity occurring in zones such as the aleurone layers; (2) phenol oxidation; and (3) musilagdevelopment. Hay (32) also noted reduced oxygen diffusion from the adherence of water and imbibed hulls around Avena fatua seeds; the reduction of oxygen supply to the embryo resulted in dormancy.

Physiological Influences on Seed Germination

Hormones

Five classes of plant hormones are known. They are auxins, gibberellins, cytokinins, abscissic acid, and ethylene. Gibberellins and
abscissic acid have a predominant role in seed dormancy and germination.

Gibberellins can induce germination in seeds which require darkness (Phacelia tanscetifolia Benth.), light (Grand Rapids lettuce), dry storage (Avena fatua L.), or stratification (hazel nut). Amen (2) and Galston and Davies (27) suggested that GA induces germination via amylase production; they based their hypothesis largely on prior research (27, 48, 69).

Previously, Paleg (47, 48) and Yomo (69, 70) had shown that barley endosperm produces amylases in response to GA application. However, Chen and Varner (16) reported that new synthesis of amylases is not a prerequisite for germination of wild oat (Avena fatua L.) or barley (Hordeum). Chen and Varner (16) reported that amylases were not responsible for the gibberelllic acid stimulation of lettuce seed germination because lettuce seeds store lipids, not starch. Chen and Varner (14, 15) concluded that gibberellins function by reducing or removing specific metabolic block(s). Bieleski et al. (8) reported that GA induces fatty acid β-oxidation and glyoxylate enzyme formation in wheat aleurone, indicating a possible role for GA in germination that does not involve amylase production.

Whereas, gibberellins promote germination, abscissic acid (ABA) generally inhibits germination. At lower concentrations, ABA also inhibits root growth (14). The inhibitory effects of ABA can be reversed by GA$_3$ and by kinetin (39, 62). Khan (36) suggested that the role of cytokinins in germination is to permit release from ABA induced dormancy.

When ABA levels are high enough in the seed to cause dormancy, prechilling or stratification reduces the level of ABA and, thus, releases
the seed from dormancy (39). In apple seeds, for example, reduction of ABA levels progresses with stratification. Rudnicki (57) reported that the longer apple seeds were stratified, higher concentrations of synthetic ABA were needed to inhibit germination. Lipe and Crane (41) reported similar observations made on ABA levels of peach seeds that had been prechilled and then germinated.

**Molecular changes**

Molecular changes related to seed dormancy involve enzyme activity and activation. Enzyme activity and activation are related directly to the water content of the seed.

Seed maturation is followed by rapid dehydration, during which the water content of the seed becomes suboptimal for germination. During the decrease in water content of the seed, respiration and protein synthesis also decline (38, 42). The decrease in respiration is directly associated with a decrease in enzyme activity. For example, when the relative water content of pea seed cotyledons decreases below 55 percent, sharp reductions of succinate and malate dehydrogenase activities occur (38). The decrease in enzyme activity may result from a decrease in enzyme synthesis and a normal enzyme turnover; a change which may result from changes in RNA metabolism and ribosome function (35, 56). The decrease in enzyme synthesis may also be associated with changes in the endoplasmic reticulum (ER) and with the loss of membrane bound polysomes (49).

Enzymes may also be converted to an inactive form during rapid dehydration. For example, an inactive form of glucosidase can be
extracted from peas. Mild proteolysis can activate the enzyme (44).

When imbibition occurs, an increase in respiration occurs. The rise in respiration may result from the synthesis or the activation of hydrolytic and/or respiratory enzymes. The enzymes of glycolysis (26), the pentose phosphate pathway (10, 12, 26, 42), the tricarboxylic acid cycle (26), and the glyoxylate cycle (10, 12, 42) increase during imbibition. Enzymes associated with phosphate and nitrogen metabolism, and with protein, carbohydrate, and lipid utilization also increase with imbibition (26).

**Pentose phosphate pathway**

The most research on metabolic pathways related to seed dormancy has been conducted on the role of the pentose phosphate pathway in seed dormancy and germination. Roberts (51, 52, 53, 54, 55, 56) has proposed the pentose phosphate pathway as the pivotal pathway in germination. He has attributed all dormancy breaking techniques as to having a primary influence on the pentose phosphate pathway (56). He believes that stimulation of the pentose phosphate brings upon the onset of germination.
SECTION ONE.

THE EFFECT OF MAIZE COMPETITION

ON WEED GROWTH

AND SEED PRODUCTION
ABSTRACT

Plants of four weed species, yellow foxtail (*Setaria lutescens* (Weigel) Hubb.), green foxtail (*Setaria viridis* (L.) Beav.), Pennsylvania smartweed (*Polygonum pensylvanicum* L.) and velvetleaf (*Abutilon theophrasti* Medic.), were grown free from competition and in competition with maize plants. Vegetative and reproductive parameters were measured at two locations in central Iowa during 1979 and 1980. Height of Pennsylvania smartweed and velvetleaf plants increased for plants in competition with maize plants. Plant weight, number of branches, stem diameter, number of inflorescences, number of flowers or fruit per inflorescence, inflorescence length, and average number of seeds produced per plant decreased for Pennsylvania smartweed and velvetleaf plants grown with maize competition. Plant growth and seed production for green and yellow foxtail also decreased for plants grown with maize competition. Reduction in weed seed production was over 90% for each of the four weed species when grown with maize competition. Also, minimum, average, and maximum ratios of weed seed production for maize versus no maize competition were calculated. The minimum difference in seed production occurred for green foxtail plants. One green foxtail plant growing without maize competition could produce as many seeds as five green foxtail plants growing with maize competition. The maximum difference in seed production occurred for Pennsylvania smartweed plants. One Pennsylvania smartweed plant growing without maize competition could produce as many seeds as 402 Pennsylvania smartweed plants growing with maize competition.
INTRODUCTION

Many weed species compete effectively with crop plants for nutrients, water, and light. As the density of dry matter production of weeds increases, a proportional decrease in crop yield is observed (4, 5, 6, 8, 10, 13). Weed control practices become necessary to reduce weed density and minimize the decrease in crop yield. Increasing the density of crop plants (7) and rotating the crop sequence (1, 9, 12) may diminish competitive effects of weeds. Both crop density and crop rotation utilize maximizing the ability of crops to compete with weeds. Pavylchenko and Harrington (11) reported competition from crops may affect the growth habits of weeds.

Normally in the crop habitat, weed and crop plants grow in close proximity to each other. A neighboring plant is thus an external force that may limit plant growth and reproductive capacity (2). Harper and Gajic (3) have demonstrated the effects of competition stresses on the reproductive capacities of plant populations.

Although weed control measures are designed to increase the competitive advantage of crop plants over weed plants, less research has been directed to the effects of crops on weeds. The seed production potentials of low order weed infestations are important components of economic injury level and associated IPM considerations. If weed control measures are effective, weed plants should be less hardy and have a reduced reproductive capacity. For example, weeds that commonly compete with maize should have different growth habits and seed production when growing with maize and without maize competition. The objectives of this
research are to investigate the effects of maize competition on the growth habit and seed production of Pennsylvania smartweed (Polygonum pensylvanicum L.), velvetleaf (Abutilon theophrasti Medic.), green foxtail (Setaria viridis (L.) Beauv.), and yellow foxtail (Setaria lutescens (Weigel) Hubb.).
MATERIALS AND METHODS

Two maize fields near Ames, Iowa were selected for the maize-weed competition research. The first field was at Curtiss Farm which has a soil type of Webster silty clay loam (O.M. 5%; pH 6.5). The second field was at Hind's farm which has a soil type of Colo silt loam (O.M. 4%, pH 6.5). Both fields were fertilized each year with 200 kg urea N, 60 kg P and 90 kg K per hectare. Neither field was treated with herbicides in either 1979 or 1980. Both fields were planted with maize (Pioneer 3780) in 75 centimeter (30 in) row spacings in May 1979 and May 1980.

Both fields had uniform distributions and high densities of Pennsylvania smartweed, velvetleaf, green foxtail, and yellow foxtail infestations. Field research plots were three 30 m by 6.75 m plots for each maize-competition and noncompetition tests for each weed species. The plots were arranged randomly in each field and replicated 3 times.

When the maize plants reached the five leaf stage, the weeds were thinned by hand. Also, maize plants were thinned to nine maize plants per meter of the crop row. In the noncompetition plots, the maize plants were removed. The spatial distribution of the 14 Pennsylvania smartweed or velvetleaf plants per plot is shown in Fig. 1. For green and yellow foxtail, 10 weed plants were retained per meter of the crop row. The plot diagram for the green and yellow foxtail plots is shown in Fig. 2. Weed spacing for noncompetition and competition tests was the same.

Vegetative parameters were measured during mid August when the maize
was two meters tall. Seven velvetleaf or Pennsylvania smartweed plants were removed from each velvetleaf or Pennsylvania smartweed plot. The weed plants removed were spaced alternately with the weed plants remaining. The following parameters were measured: fresh weight per plant in kg (with a spring scale balance); number of branches per plant; stem diameter at the soil line (with a Verner calibrator); and number of inflorescences per plant.

From the 21 velvetleaf or Pennsylvania smartweed plants from each competition regime at each locality, 100 inflorescences were randomly removed. The number of flowers (or seeds) per inflorescence were determined. The inflorescence length (for Pennsylvania smartweed) or diameter (for velvetleaf) were then measured.

For green and yellow foxtail, the following measurements were taken from seven plants that had been removed from the central portion of each green or yellow foxtail plot: height (with a meter stick); fresh weight (with a spring scale balance); number of tillers per plant; and number of inflorescences per plant. Fifty inflorescences were randomly harvested from the central portion of each plot. Flowers or seeds per inflorescence and inflorescence length were then measured. Seed production per plant was calculated mathematically from the number of inflorescences per plant and the number of flowers (or seeds) per inflorescence. Measurement data from both fields and years were pooled according to the weed species and to the maize competition level.

The ratios of possible seed production for weeds growing with maize competition and for weeds growing without maize competition were obtained
mathematically. The ratio of the average differences was obtained by dividing the average number of seed produced from plants with maize competition by the number of seed produced from plants without maize competition. The minimum difference was calculated by using the values possible of seed production if the plants without maize competition produced one standard deviation less seeds than average; simultaneously, the with maize competition plants would produce one standard deviation more seeds than average. For the maximum difference, the without maize competition plants produced one standard deviation more seeds than average; whereas, the with maize competition plants produced one standard deviation less seeds than average.

Stress index calculations for corn, furnished by the Agronomy Department,\(^1\) showed no significant differences in water stress periods between 1979 and 1980, which might have affected corn-weed competition. Since the patterns of competition were so similar, data for the two years were combined.

---

\(^1\)Dr. Robert H. Shaw, Department of Agronomy, Iowa State University. Personal communication.
Fig. 1. Distribution of the 14 velvetleaf or smartweed plants in a 30 m long plot with 3/4 m spacing between crop rows. For without maize competition plots, weed spacing the same; however, the maize plants were removed from the two rows between the weed rows, from the two rows on either side of the weed rows, and from the weed rows themselves.
Fig. 2. Distribution of green or yellow foxtail plants in a 30 m long plot with 3/4 m spacing between crop rows. Weeds were only allowed to grow in rows 3 to 6; maize (without weeds) was allowed to grow in rows 1, 2, 7, and 8 in maize competition plots. However, all maize plants were removed from rows 1-8 in without maize competition plots; otherwise, weed spacing remained the same as in maize competition plots. Growth habit measurements and seeds were taken from the middle portions of rows 4 and 5 to eliminate the border effect.
RESULTS AND DISCUSSION

Weed plants growing in a maize field had different growth habits and reproductive capacities. Only two parameters increased with maize competition, the heights of Pennsylvania smartweed (Table 1) and velvetleaf (Table 2). The difference in height response of Pennsylvania smartweed and velvetleaf versus the response of green foxtail (Table 3) or yellow foxtail (Table 4) may be attributed to the difference in root systems. Pennsylvania smartweed and velvetleaf have tap roots, whereas green and yellow foxtail have fibrous root systems. Pavylchenko and Harrington (11) noted a high degree of competition between the fibrous root system of grass weeds competing with the fibrous root systems of grass crop plants. Under such a situation, the monocot weed could be under greater stress than a dicot seed for water and nutrients. With less water and nutrient stress, the dicot weeds would be better able to respond to light limitations than noncot weeds.

Generally, maize competition with weeds resulted in the reduction of the growth parameters measured (Tables 1-4). However, the reduction of the vegetative parameters was variable according to species. The length or pod diameter (for velvetleaf) had the least amount of change with maize competition. The greatest effect in maize competition tended to be in plant fresh weight (92 to 99% reduction) and the number of inflorescences per plant (82 to 97% reduction).

The number of inflorescences per Pennsylvania smartweed plant (97% reduction) (Table 1) and velvetleaf plant (90% reduction) (Table 2)
was a larger factor in the reduction of seed production than the number of flowers or fruits per inflorescence (45% and 7% reduction respectively). However, for green and yellow foxtail, the reduction of number of seeds per spike (79% and 59%) was as important in reducing potential seed production as the reduction of the number of spikes per plant (83% and 82% respectively) (Tables 3 and 4).

Potential seed production per plant was greatly reduced for plants of the four weed species when grown with maize competition (Tables 1-4). One Pennsylvania smartweed plant, growing by itself, can produce the equivalent number of seed and, possibly seedlings, as 400 Pennsylvania smartweed plants growing in a maize field (Table 5). Because of the greater seed production of a weed plant growing without maize competition than with maize competition (Table 5), future weed populations are greatly enhanced by weeds which are allowed to grow at the edges of fields or in noncultivated areas. To minimize future weed populations, it is necessary to control weeds growing without crop competition in addition to controlling weeds growing with crop competition.
Table 1. Effect of maize competition on growth and seed production of Pennsylvania smartweed (*Polygonum pensylvanicum* L.)

<table>
<thead>
<tr>
<th>Parameter measured</th>
<th>Without maize competition</th>
<th>With maize competition</th>
<th>Change (reduction –; increase +)</th>
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<tr>
<td>Height (cm)</td>
<td>71 ± 9</td>
<td>170 ± 8</td>
<td>+147</td>
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<tr>
<td>Plant weight (kg) (wet)</td>
<td>4.11 ± 1.12</td>
<td>0.07 ± 0.01</td>
<td>-99</td>
</tr>
<tr>
<td>Branches (No)</td>
<td>274 ± 27</td>
<td>5 ± 4</td>
<td>-98</td>
</tr>
<tr>
<td>Stem dia (cm) (soil line)</td>
<td>2.2 ± 0.5</td>
<td>1.0 ± 0.1</td>
<td>-61</td>
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<tr>
<td>Inflorescences (no)</td>
<td>217 ± 24</td>
<td>6 ± 4</td>
<td>-97</td>
</tr>
<tr>
<td>Flrs. or frt. (per infl)</td>
<td>62 ± 6</td>
<td>34 ± 5</td>
<td>-45</td>
</tr>
<tr>
<td>Infl. length (cm)</td>
<td>4.2 ± 1.4</td>
<td>3.3 ± 1.3</td>
<td>-21</td>
</tr>
<tr>
<td>Seeds (No/ave. pl.)</td>
<td>13,400 ± 2,600</td>
<td>200 ± 160</td>
<td>-98</td>
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Table 2. Effect of maize competition on growth and seed production of velvetleaf (*Abutilon theophrasiti* Medic.)

<table>
<thead>
<tr>
<th>Parameter measured</th>
<th>Without maize competition</th>
<th>With maize competition</th>
<th>Change (reduction -; increase +) %</th>
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<tr>
<td>Height (cm)</td>
<td>154 ± 9</td>
<td>176 ± 9</td>
<td>+14</td>
</tr>
<tr>
<td>Plant weight (kg) (wet)</td>
<td>2.23 ± 1.07</td>
<td>0.08 ± 0.01</td>
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</tr>
<tr>
<td>Branches (No)</td>
<td>18 ± 5</td>
<td>1 ± 0</td>
<td>-94</td>
</tr>
<tr>
<td>Stem dia (cm) (soil line)</td>
<td>3.4 ± 1.2</td>
<td>1.0 ± 0.3</td>
<td>-71</td>
</tr>
<tr>
<td>Infl. or pods (No)</td>
<td>352 ± 28</td>
<td>35 ± 10</td>
<td>-90</td>
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<td>Seeds (No) (per pod)</td>
<td>15 ± 1</td>
<td>14 ± 1</td>
<td>-7</td>
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<tr>
<td>Pod dia (cm)</td>
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<td>2.2 ± 1.3</td>
<td>0</td>
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<tr>
<td>Seeds (no/ave. pl.)</td>
<td>5,300 ± 800</td>
<td>500 ± 200</td>
<td>-90</td>
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Table 3. Effect of maize competition on growth and seed production of green foxtail (*Setaria viridis* (L.) Beauv.)

<table>
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<tr>
<th>Parameter measured</th>
<th>Without maize competition</th>
<th>With maize competition</th>
<th>Change (reduction −; increase +)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Height</td>
<td>137 ± 6</td>
<td>63 ± 9</td>
<td>−54</td>
</tr>
<tr>
<td>Weight (kg) (wet)</td>
<td>1.33 ± 0.75</td>
<td>0.10 ± 0.01</td>
<td>−92</td>
</tr>
<tr>
<td>Tillers (No)</td>
<td>21 ± 5</td>
<td>3 ± 2</td>
<td>−86</td>
</tr>
<tr>
<td>Spikes (no)</td>
<td>36 ± 4</td>
<td>6 ± 4</td>
<td>−83</td>
</tr>
<tr>
<td>Spike length (cm)</td>
<td>9.0 ± 3.1</td>
<td>4.0 ± 2.3</td>
<td>−56</td>
</tr>
<tr>
<td>Seeds per spike (No)</td>
<td>659 ± 29</td>
<td>137 ± 19</td>
<td>−79</td>
</tr>
<tr>
<td>Seeds (No/ave. pl.)</td>
<td>23,700 ± 3,600</td>
<td>800 ± 650</td>
<td>−97</td>
</tr>
</tbody>
</table>
Table 4. Effect of maize competition on growth and seed production of yellow foxtail (*Setaria lutescens* (Weigel) Hubb.)

<table>
<thead>
<tr>
<th>Parameter measured</th>
<th>Without maize competition</th>
<th>With maize competition</th>
<th>Change: (reduction -; increase +)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Height (cm)</td>
<td>135 ± 6</td>
<td>50 ± 10</td>
<td>-63</td>
</tr>
<tr>
<td>Plant weight (kg) (wet)</td>
<td>1.54 ± 0.38</td>
<td>0.10 ± 0.01</td>
<td>-94</td>
</tr>
<tr>
<td>Tillers (No)</td>
<td>16 ± 4</td>
<td>7 ± 5</td>
<td>-56</td>
</tr>
<tr>
<td>Spikes (No)</td>
<td>17 ± 4</td>
<td>3 ± 2</td>
<td>-82</td>
</tr>
<tr>
<td>Spike length (cm)</td>
<td>7.9 ± 1.7</td>
<td>4.0 ± 1.4</td>
<td>-49</td>
</tr>
<tr>
<td>Seeds per spike (No)</td>
<td>238 ± 15</td>
<td>98 ± 6</td>
<td>-59</td>
</tr>
<tr>
<td>Seeds (No/ave. pl.)</td>
<td>4,000 ± 1,300</td>
<td>300 ± 200</td>
<td>-93</td>
</tr>
</tbody>
</table>
Table 5. Ratios of potential seed production of weed plants growing with maize competition versus potential seed production of weed plants growing without maize competition

<table>
<thead>
<tr>
<th>Weed</th>
<th>Minimum</th>
<th>Average</th>
<th>Maximum</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pennsylvania smartweed</td>
<td>30:1</td>
<td>68:1</td>
<td>402:1</td>
</tr>
<tr>
<td>Velvetleaf</td>
<td>8:1</td>
<td>11:1</td>
<td>20:1</td>
</tr>
<tr>
<td>Yellow foxtail</td>
<td>14:1</td>
<td>30:1</td>
<td>182:1</td>
</tr>
<tr>
<td>Green foxtail</td>
<td>5:1</td>
<td>13:1</td>
<td>53:1</td>
</tr>
</tbody>
</table>
REFERENCES CITED


SECTION TWO.

PARENTAL STRESS AND PRECHILLING EFFECTS ON

PENNSYLVANIA SMARTWEED (POLYGONUM PENSYLVANICUM L.)

SEED DORMANCY AND GERMINATION
ABSTRACT

Pennsylvania smartweed (Polygonum pensylvanicum L.) seeds were harvested from plants growing free from competition and in competition with maize plants. All seeds were dormant when harvested. After 15 weeks of prechilling at 2°C in 33% moist soil, 4% of the seeds from plants with maize competition germinated and 35% of the seeds from plants without maize competition germinated; after 30 weeks of prechilling, the germinations were 94% and 92% respectively. When viewed with a scanning electron microscope, seed coats of seeds from plants with maize competition appeared well channeled; but, seed coats of seeds from plants without maize competition were compact. Seed dormancy of seeds from plants growing without maize competition may result from an impermeable seed coat. When the embryos were investigated using a transmission electron microscope, more lipid bodies were present in the embryo epidermis in seeds from plants with maize competition (than in seeds from plants without maize competition). Also, intercellular junctions occurred in embryos of nonprechilled seeds from plants with maize competition; similar junctions did not occur in embryos of prechilled seeds or seeds from plants without maize competition. Apparently, dormancy of seeds from plants growing with maize competition may tend to result from embryo immaturity.
INTRODUCTION

The termination of weed seed dormancy and the onset of germination is a critical point in conducting effective control measures. Whereas, a dormant weed seed is essentially immune from weed control measures, germinating weed seeds are extremely vulnerable to both cultivation and herbicides.

A seed may be dormant because it is formed dormant (innate dormancy), becomes dormant (induced dormancy), or is under conditions unfavorable to germination (enforced dormancy)(2). Of these three types of dormancy, innate dormancy is most directly affected by growth conditions under which it matured on the parent. Innate dormancy begins while the seed is still attached to the parent plant (10).

Different parental photothermal environments can affect the germinability of redroot pigweed (*Amaranthus retroflexus* L.) seeds (5). Seed germinability is also affected by the time of the day in which the seed is harvested (8) or by differences in seeds shed that have the same apparent maturity (1). Much of the physiological heterogeneity of seeds has been attributed to the parental environment (5, 6, 13). Since seeds are affected by the parent plant, research needs to be conducted on the affect of parental stress (due to competition with other plants) on the dormancy and germinability of the offspring.

Previously, Jordan (3) reported that Pennsylvania smartweed (*Polygonum pensylvanicum* L.) growth habit was affected by competition with maize. For example, plant weight, number of branches, and number of seeds that could be produced per weed plant were reduced when the weed plant
was subjected to maize competition. Because seed production was reduced by maize competition, the effect of parental stress on the seed dormancy of the offspring was investigated.
MATERIALS AND METHODS

Seed collection and Preparation

The term "seed" in this investigation refers to the entire dispersal unit. However, the correct botanical term for the dispersal unit is an achene. An achene is a dry, one seeded indehiscent fruit. The achene wall will be referred to as being a seed coat. The germinating structure is the seed, not the fruit. Because this is a dormancy and germination investigation, the research centers around the unit in its entirety as it relates to dormancy and/or germination.

To collect seeds, aluminum screens (18 x 16 mesh) were placed below the Pennsylvania smartweed plants used in Section One of this thesis. Screens were placed on the ground below the Pennsylvania smarcweed plants on Sept. 1. Seeds were removed from the screens on Sept. 29. Because seed production of Pennsylvania smartweed plants with maize competition was low (Section One), additional seed collection plots were utilized at each location. After the seeds were harvested, they were pooled according to collection site and maize competition regime. The seeds were stored in muslin bags at 24 C and in dry air. Prior to use, the calyx was removed from the seeds by vigorous agitation of the muslin bags. Debris was removed with a seed blower.

Seed Measurements

Weight, thickness, and volume of Pennsylvania smartweed seeds were measured. Four replications of 5,000 seeds were weighed from each collection site. Three hundred seeds from each competition regime from
Each collection site were removed randomly from the other seeds. A micrometer was used to measure the thickness of the seeds (across the thinnest portion of the seed).

Volume was determined by placing 4 replications of 5,000 seeds into graduated cylinders and then adding water. The difference in apparent volume and volume of water added was calculated to determine the volume of seeds present. Seeds were dried immediately in a 35°C forced air oven for 2 hours to minimize imbibition.

Soil Preparation

Colo silt loam (O.M. 4.0; pH 6.5) was collected from the upper 2 cm of soil at Hinds farm on Oct. 1, 1979. The soil was packed into muslin bags which were flattened to 5 cm thickness. The soil cakes were dried in a forced air oven at 105°C for three days. The oven dried soil was then ground to flour-like fineness with a meat grinder. To determine approximate water holding capacity, 50 g of soil was placed in a funnel (with a Whatman No. 1 filter paper in it), water was added and the soil was allowed to drain by gravity for 30 minutes. The initial versus the final weight were used to determine water holding capacity. Ten replications were conducted on the water-soil tests. The soil maintained a 33% moisture level; a level used in subsequent experiments.

Prechilling and Germination Conditions

Fifty grams of oven dried soil was placed in a 9 cm plastic petri dish. On top of the soil, two 10 cm by 10 cm squares of cheesecloth were placed. One hundred seeds were placed on the cheesecloth and then covered
by two more pieces of cheesecloth. Fifty more grams of soil were then placed over the seeds and cheesecloth, and the petri dish cover was then put in place. When moist soil was desired, 33 ml of deionized water was added to the soil. Seeds were prechilled while in soil (in petri dishes) as described previously. Seeds were prechilled in oven dry soil or 33% moist soil at 2 C or 14 C (Table 2). All seeds were prechilled in the dark.

Each week for six weeks, ten replications of seeds from each competition regime at each collection site and from each prechilling regime were removed for germination tests. To insure adequate water supply to the seeds, 33 ml of water was added to the petri dishes that contained oven dry soil. Seeds were allowed to germinate for one week at 29 C or 35 C in the dark. Each germination temperature had half (5) of the ten replications previously removed for each competition regime at each collection site and from each prechilling regime. Because of the limitation of the number of seeds available for use, seeds were allowed to prechill at 2 C in the dark for 15 or 30 weeks prior to germination testing. Germination of seeds prechilled for 15 or 30 weeks was conducted at 35 C only. No seeds were tested from 7 to 14 weeks of prechilling or from 16 to 29 weeks of prechilling.

Seeds that were nicked prior to germination testing were not prechilled and not allowed to imbibe water. A no. 11 surgical scalpel was used to chip away approximately 5 mm² from the embryo end of the seed. There were four replications from each competition regime at each collection site. Data for each collection regime were pooled for each experiment.
Scanning Electron Microscopy

Scanning electron microscopy techniques were used to investigate the seed coat. Seeds were processed for a cross-sectional view of the seed coats by cryofracturing the seed. To obtain a cross-sectional view of the seed coats, fresh seeds were wrapped in parafilm, immersed in liquid nitrogen, and fractured with a razor blade. Cryofractured seeds were then mounted in silver paste that had been applied to the upper surface of standard JEOL SEM stubs. To insure direct viewing of the cryofractured seed coat, the cryofractured surface was parallel to the surface of the stub. All specimens were coated with a 308 Å thick layer of gold:palladium (60:40) in a sputter coater to improve resolution of the seed surface. A JEOL JSM-35 scanning electron microscope was used to view the seeds. Photographs were taken with Polaroid 665 film.

Transmission Electron Microscopy

Embryo preparation for transmission electron microscope observations began by excising (by hand) the embryos of unimbibed seeds and seeds that had prechilled for 30 weeks (at 2°C in 33% moist soil). The excised embryos were put immediately into vials containing 2% glutaraldehyde buffered with 0.1 M sodium cacodylate buffer. The material was postfixed with 0.1 M sodium cacodylate buffered 2% osmium tetroxide at 4°C for 12 hours. The material was rinsed 5 times with sodium cacodylate buffer (10 times per rinse) and prestained with 2% uranyl acetate (in water) for 12 hours at 4°C. Stained material was dehydrated thru a gradated acetone series and, subsequently, processed thru a gradated propylene oxide.
series. The embryos were then embedded in PolyBed^R 812 resin. Ultrathin sections (60 to 70 nm) were cut on a Serval MT Ultramicrotome with a DuPont diamond knife. Ultrathin sections were mounted on formvar and carbon coated 100-mesh grids. Sections were stained with Reynold's lead citrate (9) at 24 C for 10 minutes. Transmission electron microscope examinations were made using a Hitachi 8 electron microscope at 50 kV. Only the epidermis of the embryo cotyledons was investigated.
RESULTS AND DISCUSSION

Although competition with maize affected the growth habit and seed productivity of Pennsylvania smartweed (3, 4), the gross morphology of the seeds produced appeared identical. The weight, thickness, and volume of the seeds were identical (Table 1). Also, the same prechilling and soil moisture regimes resulted in a breaking of dormancy of both groups of seeds (Table 2). Initially, all freshly harvested seeds were dormant, regardless of competition regime of the parent plants (Fig. 1). After three weeks of prechilling at 2°C, seeds from the with maize competition plants began to germinate. After four weeks, seeds from the without maize competition plants were still dormant; seeds from the with maize competition plants reached 2% germination. After five weeks (Fig. 1) up through fifteen weeks (Table 3) of prechilling at 2°C, seeds from the with maize competition plants did not increase in apparent germinability. Germination was 5 ± 1% at five weeks, but only 4 ± 2% after 15 weeks of prechilling. During the period from five to fifteen weeks, however, the seeds from the without maize competition plants increased in germinability from 13 ± 1% (at 35°C) to 35 ± 8%. Differences between the two groups of seeds were also noted when the seed coats were nicked. If a portion of the seed coat was nicked away, more seeds from the without maize competition plants germinated than seeds from the with maize competition plants (6 ± 4% and 42 ± 9% respectively)(Table 3). Differences between seeds — collected from plants growing with maize competition or without maize competition — were also evident when the seeds were investigated using scanning electron microscopy. Seeds that had been harvested from plants without maize
competition had dense, poorly channeled seed coats (Fig. 2); whereas, seeds that had been harvested from plants with maize competition had well channeled seed coats (Fig. 3). Apparently, the seed coat may be more responsible for imposing dormancy in seeds from plants growing without maize competition than with maize competition. The difference in dormancies may be due to differences in seed coat permeability to water and oxygen.

To determine if the embryo also had an effect on Pennsylvania smartweed dormancy, transmission electron microscopy was used. Lipid bodies were apparently present in greater density in the epidermis (of the embryo cotyledons) of seeds removed from plants with maize competition (Figs. 5 and 7) than without maize competition (Figs. 4 and 6). Apparently, although fewer seeds were produced by plants with maize competition (3), more lipid bodies could be formed per seed. The epidermis of seeds from maize competition (but not prechilled) had intercellular junctions present (Figs. 5 and 8). The seed was probably not only storing more lipids, it was maturing slower. During the 30 weeks of prechilling, the junctions between cells disappeared (Fig. 7); at the same time, germinability increased from 6 ± 4% to 94 ± 5%. Junctions between cells did not appear in seeds of plants which were without maize competition.

Pennsylvania smartweed seed dormancy is apparently more than a seed coat imposed dormancy, as proposed by LaCroix (7). Instead; while the thick-walled palisade cells were forming in the seed coat, the embryo could have also been maturing. The seeds from plants without maize competition could have had more mature embryos; whereas, the seeds from
plants with maize competition could have had less mature embryos. While dormancy of seeds from plants without maize competition may tend to be seed coat induced, dormancy of seeds from plants with maize competition may tend to be caused by an immature embryo.

Prior to germination, some morphological changes which occur in seeds from both competition regimes are similar. During prechilling for 30 weeks in moist soil, protein bodies were digested (Figs. 6 and 7). Similar prechilling effects have been noted in yellow foxtail prior to release from dormancy (8, 11). After an embryo is no longer immature, developmental processes could be similar to an embryo that was initially mature when the seed was shed.

Actual developmental changes of seeds in controlled prechilling of a laboratory may not be directly paralleled by seeds stratified in the field. In the field, the tillage layer of the soil frequently becomes frozen. Below freezing conditions were shown to be nonconducive to terminating dormancy (Table 2). However, the freezing and thawing of soil on seeds may result in stresses on the seed coats which may alter seed coat permeability to water or oxygen.

Regardless of whether the seeds are prechilled in the laboratory or stratified in the field, termination of innate dormancy depends largely upon the nature and degree of innate dormancy. For Pennsylvania smartweed seeds, seeds may be produced by parental plants growing under varying levels of competition stress which would result in seeds ranging from highly dormant to slightly dormant. It is possible that the differences in the innate dormancies of seeds (due to parental stress) may
be partially responsible for the small percentage of Pennsylvania smartweed seeds which germinate each year. Stoller and Wax (12) reported that only a fraction of the viable Pennsylvania smartweed seeds germinate each year; the seeds that germinate do so in a short period of time each spring. It is not known if the marginally germinable seeds (which did not germinate in the germination flush) acquire an induced dormancy or need more stratification to overcome the remaining vestiges of innate dormancy. More experimentation is needed to investigate the following: first, the formation of innate dormancy of seeds from stressed versus nonstressed plants; second, the changes which occur which cause dormant seeds to become germinable; and, third, the requirements for breaking the dormancy of prechilled (or stratified) seeds that have been in a favorable environment for Pennsylvania smartweed germination.
Table 1. Fresh weight, thickness, and volume of Pennsylvania smartweed seeds (achenes), taken from plants grown with and without maize competition.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Germination</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>+Maize</td>
</tr>
<tr>
<td>Weight (wet) (g)(^a)</td>
<td>34.0 ± 1.3</td>
</tr>
<tr>
<td>Thickness (mm)</td>
<td>0.989 ± 0.022</td>
</tr>
<tr>
<td>Volume (ml)(^a)</td>
<td>29.5 ± 1.4</td>
</tr>
</tbody>
</table>

\(^a\) per 5,000 seeds (achenes).
Table 2. Germination of smartweed seeds from plants grown with and without maize competition and placed in soil with different moisture and temperature regimes for 6 weeks

<table>
<thead>
<tr>
<th>Prechilling temperature</th>
<th>Soil moisture</th>
<th>Germination observed</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>+Maize</td>
</tr>
<tr>
<td>2C</td>
<td>0</td>
<td>No</td>
</tr>
<tr>
<td>2C</td>
<td>33</td>
<td>Yes</td>
</tr>
<tr>
<td>-14C</td>
<td>0</td>
<td>No</td>
</tr>
<tr>
<td>-14C</td>
<td>33</td>
<td>No</td>
</tr>
</tbody>
</table>
Fig. 1. Germination of Pensylvannia smartweed seeds prechilled in 33% moist soil at 2 C for up to 6 weeks. Seeds had been harvested from plants growing with or without maize competition.
COMPETITION WITH MAIZE --- WITHOUT MAIZE

I LSD 0.05

% MAXIMUM PESW GERMINATION AT 29°C

% MAXIMUM PESW GERMINATION AT 35°C

WEEKS PRECHILLED AT 2°C
Table 3. Germination (at 35C) of Pennsylvania smartweed seeds that had been prechilled 15 or 30 weeks at 2 C, in the dark, in soil with 33% moisture, or that had achene walls which had been nicked. Seeds were harvested from plants growing with or without maize competition

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Germination +Maize</th>
<th>-Maize</th>
</tr>
</thead>
<tbody>
<tr>
<td>15 weeks prechilled</td>
<td>4 ± 2</td>
<td>35 ± 8</td>
</tr>
<tr>
<td>30 weeks prechilled</td>
<td>94 ± 5</td>
<td>92 ± 4</td>
</tr>
<tr>
<td>Achene wall nicked</td>
<td>6 ± 4</td>
<td>42 ± 9</td>
</tr>
</tbody>
</table>
Figs. 2 and 3. Scanning electron micrographs of cryofractured Pennsylvania smartweed seed coats.

2. Seed coat of a seed taken from a plant growing without maize competition. X2666.

3. Seed coat of a seed taken from a plant growing with maize competition. X327.
Figs. 4 to 8. Transmission electron micrographs of the epidermis of Pennsylvania smartweed embryo cotyledons. L = lipid bodies. PB = protein bodies.

4. Epidermal cell section of a seed taken from a plant growing without maize competition. Seed has not imbibed water and has not been prechilled. X4,400.

5. Epidermal cell section of a seed taken from a plant growing with maize competition. Seed has not imbibed water and has not been prechilled. Note the junctions which occur between the cells (arrows). X4,400.

6. Epidermal cell section of a seed taken from a plant growing without maize competition. Seed has been prechilled for 30 weeks at 2C in 33% moist soil. Note the change in the protein bodies (vs. Fig. 4). X4,400.

7. Epidermal cell section of a seed taken from a plant growing with maize competition. Seed has been prechilled for 30 weeks at 2C in 33% moist soil. Note the change in the protein bodies and the lack of junctions between cells. X4,400.

8. Closer view of a junction between two epidermal cells of unimbibed, nonprechilled seeds taken from a plant growing with maize competition.
REFERENCES CITED


SECTION THREE.

BARNYARDGRASS (*ECHINOCLOA CRUS-GALI* (L.) BEAUV.)

SEED DORMANCY AND GERMINATION: THE EFFECT OF

ULTRAFREEZING IN LIQUID NITROGEN TO $-196\,^\circ$ C
ABSTRACT

Barnyardgrass (Echinochloa crus-gali (L.) Beauv.) seeds were ultra-
frozen in liquid nitrogen (-196°C) for 5 minutes and thawed for 1 hour in
24°C air. Germination of seeds not frozen was 60%; germination of seeds
ultrafrozen 10 times was 99%. To determine if the seed coat was fractured
by ultrafreezing, the seeds were examined with a scanning electron micro-
scope. No cracks were apparent in the surface of intact seeds ultrafrozen
10 times. No cracks or structural changes were noted between cryo-
fractured seed coats that had been ultrafrozen once or 11 times. When
the seed coats were viewed with a transmission electron microscope, no
differences were noted between seeds ultrafrozen 10 times or not ultra-
frozen. Water imbibition (percent weight increase) was the same for seeds
not ultrafrozen and seeds ultrafrozen 10 times. Therefore, it is unlikely
that ultrafreezing caused an increase in seed coat permeability to water.
When the secretory epithelium of unimbibed seeds ultrafrozen 0, 4, or 10
times was viewed using a transmission electron microscope, changes in the
structure of lipid bodies were noted. Following water imbibition, lipid
bodies reformed to a state similar to that present before ultrafreezing.
Although the lipid bodies reformed, protein bodies were digested much
faster in secretory epithelium of seeds ultrafrozen 10 times than in seeds
not ultrafrozen. Seeds were also germinated in 10^{-4}M concentrations of
intermediates from glycolysis, the citric acid cycle, the pentose
phosphate pathway, the glyoxylic acid cycle, and other metabolic path-
ways. Utilization of substrates was most enhanced for chemicals common
to the later steps in glycolysis and the citric acid cycle.
INTRODUCTION

Seed dormancy may result from unfavorable environmental conditions (enforced dormancy) or from physiological factors (innate or induced dormancy). Enforced dormancy can result from an insufficient water or oxygen supply, or from temperatures that do not enhance germination (3). Innate dormancy can be attributed to the embryo, the seed coat, or a combination of the embryo and seed coat effects. After-ripening, including prechilling or stratification and hormone treatments with gibberellins have been used to overcome embryo dormancy in grass seeds (4, 13). Also, several techniques are commonly used to overcome seed coat imposed dormancy. Techniques used include scarification (chemical and mechanical), mechanical removal of the seed coat, and nicking or piercing the seed coat.

Ultrafreezing with liquid nitrogen has also been used to overcome seed coat imposed dormancy (1, 2). Ultrafreezing of seeds has been reported to overcome seed coat induced dormancy by causing small cracks to form in the seed coat. The cracks would then allow greater water imbibition into the seed (1, 2).

Because ultrafreezing may reduce seed dormancy, ultrafreezing with liquid nitrogen was used to break barnyardgrass seed dormancy. By investigating the effect of ultrafreezing on the seed coat and embryo, information about barnyardgrass seed dormancy and germination could be obtained.
MATERIALS AND METHODS

Seed Collection and Preparation

The term "seed" in this research refers to the entire disposal unit, including the lemma, palea, and caryopsis, with or without the glumes attached. The term "seed coat" refers to the lemma and palea. Barnyardgrass seeds with glumes attached were collected from plants in naturally growing populations in two maize fields near Ames, Iowa. To insure that mature seed were collected, muslin bags, 45 cm x 30 cm, were placed over the developing racemes of four to six barnyardgrass plants per bag; bags were placed over the developing racemes on Aug. 1, 1979 and the open ends of the bags were tied shut. On Sept. 19, 1979, the bags and seed were removed from the tops of the barnyardgrass plants. The seeds from each site were pooled. The seeds were stored in muslin bags at 24°C and in dry air. Prior to use, the glumes were removed from the seeds by rubbing the seeds between hands. Debris was separated from the seeds by using a seed blower.

Ultrafreezing and Seed Germination

Ultrafreezing of barnyardgrass seeds was achieved by pouring liquid nitrogen (-196°C) over barnyardgrass seeds that were in a beaker. Liquid nitrogen was poured continuously until the seeds were covered by three cm of liquid nitrogen. By adding liquid nitrogen when the liquid nitrogen level reached the upper layer of the seeds, the seeds were kept frozen at -196°C for four minutes. The liquid nitrogen surrounding the seeds was allowed to evaporate; subsequently, the seeds were allowed to thaw for one
in 24 C air. After the ice on the outside of the beaker had melted, the seeds were thoroughly mixed by stirring. Samples of seeds were removed for testing. To repeatedly ultrafreeze the seeds, the seeds left in the beaker were alternately ultrafrozen with liquid nitrogen and thawed for the same periods of time previously mentioned. Alternate ultrafreezing and thawing was conducted on three groups of seeds taken from each collection site. Seeds that floated on top of the liquid nitrogen were discarded. Each time the seeds were ultrafrozen, seeds that had floated on the liquid nitrogen were not filled and were not viable. After the seeds had been ultrafrozen up to ten times, three replications of 100 seeds from each ultrafreezing were germinated.

To germinate the seeds, they were placed between two pieces of Whatman No. 1 filter paper (nine cm dia.) in a dispo ten cm dia. plastic petri dish. Five ml of deionized water was added to moisten the filter paper. Two ml of deionized water was added to each petri dish two, four, and six days later to maintain adequate moisture in each petri dish. Germination was recorded daily for one week; the number germinated for each test was averaged for each day.

Water Imbibition and Weight Loss

Barnyardgrass seeds were allowed to imbibe water for 12 hours at 35 C in the dark. Percent imbibition was based on the difference between initial weight and weight after water imbibition. To determine weight loss, seeds were placed in a 105 C forced air over for 24 hours. Percent weight loss was based on the difference between initial weight and weight after drying in a forced air oven. Three replications of 100 seeds
ultrafrozen ten times were used from both collection sites and the three ultrafreezing series conducted previously. Nine replications of 100 seeds not ultrafrozen were used from both collection sites for each test.

Scanning Electron, Transmission Electron and Light Microscopy

Barnyardgrass seeds for scanning electron microscopy (SEM) observations were processed for direct observation of the intact seed coat surface and for a cross sectional view of the seed coat (by cryofracturing the seed). Only the palea portion of the seed coat was investigated in this research. For observation of the seed coat surface, barnyardgrass seeds, not ultrafrozen and ultrafrozen ten times, were mounted directly in silver paste that had been applied to the upper surface of standard brass JEOL SEM stubs. Cross sectional views of the seed coat were obtained. Seeds were wrapped in parafilm, immersed into liquid nitrogen, and fractured with a razor blade; a process called cryofracturing. The seeds were fractured across the midpoint of the seed perpendicular to the longest axis. Cryofractured seeds were mounted in the same manner as non cryofractured seeds in silver paste on JEOL SEM brass stubs. To insure direct viewing of the cryofractured seed coat, the cryofractured surface was parallel to the surface of the stub. All seeds were coated with a $308 \, \text{Å}$ thick layer of gold:palladium (60:40) in a sputter coater to improve resolution of the seed surface. A JEOL JSM-35 scanning electron microscope was used to view the seeds. Photographs were taken with Polaroid 665 film.

Seed coat and embryo preparation for transmission electron microscope (TEM) observations began by excising (by hand) the seed coats and embryos
from unimbibed seeds ultrafrozen and thawed 0, 4, or 10 times. Also, embryos from seeds (not ultrafrozen and ultrafrozen ten times) that had imbibed water for 1, 4, or 16 hours (under germination conditions used previously) were also studied. The excised seed coats and embryos were separated and put immediately into vials containing 2% glutaraldehyde buffered with 0.1 M sodium cacodylate (pH 7.2). The seed coats and embryos were fixed in glutaraldehyde for 24 hours at 4 C. After fixing, they were rinsed 5 times (10 minutes per rinse) with 0.1 M sodium cacodylate buffer. The material was postfixed with 0.1 M sodium cacodylate buffered 2% osmium tetroxide at 4 C for 12 hours. The material was rinsed 5 times with sodium cacodylate buffer (10 minutes per rinse) and prestained with 2% uranyl acetate (in water) for 12 hours at 4 C. Stained material was dehydrated thru a gradated acetone series and, subsequently, processed thru a gradated propylene oxide series. The seed coats and embryos were then embedded in Polybed\textsuperscript{R} 812 resin. Sections were cut on a Serval MT Ultramicrotome with a DuPont diamond knife. Ultrathin sections (60 to 70 nm) were mounted on formvar and carbon coated 100-mesh grids. Sections were stained with Reynolds lead citrate (10) at 24 C for 10 minutes. Transmission electron microscope examinations were made using a Hitachi 8 electron microscope at 50 kV. Only the secretory epithelium was investigated in the embryo sections.

For light microscopy, thick sections (1.5 μ) were sectioned from the same blocks of resin embedded seed coats that had been ultrathin sectioned for transmission electron microscopy. Thick sections were also cut on a Servall MT Ultramicrotome with a DuPont diamond knife and placed
directly on glass slides. Thick sections were stained with 2.0% borax and toluidine blue (in water, w/v) for 15 minutes. Light micrographs were taken using a Leitz Ortholux.

Substrate Tests on Germination

After 0 or 4 ultrafreeze and thaw cycles, 3 replications of 3 tests of 100 seeds were germinated in the same manner as the seed germination tests previously described. The paper was moistened with 5 ml of 10^{-4}M substrate solutions from the pentose phosphate pathway (Table 2), the glycolysis pathway (Table 3), citric acid cycle (Table 4), and the glyoxylate cycle (Table 5). Also, other substrates from various pathways were used (Table 6); 0.1% (v/v) Tween 20 in water was also used in one test (Table 6). Because a sustained level of germination (for seeds in water) had been reached in 2 days, seeds were germinated in the dark at 35 C for 2 days; total germination was recorded. The change in germination for each substrate was calculated from subtracting the number of seeds germinating with water from the number of seeds germinating in the presence of a substrate.

To help distinguish between substrate and ultrafreezing effects, T-tests were calculated for the changes in germination (from water standards) for each substrate added. The T-test values were calculated using the following formual: $T$-test value $= (m_0 - m_4)/(\sigma_0^2/n_0^{-1} + \sigma_4^2/n_4^{-1})^{1/2}$.

The terms used in the T-test equation are as follows: $m_0$ is the difference in the germination means of not ultrafrozen seeds in substrate solution versus not ultrafrozen seeds in water; $m_4$ is the difference
in the germination means of ultrafrozen seeds in substrate solution versus ultrafrozen seeds in water; \( \sigma_0^2 \) is the standard deviation of not ultrafrozen seed germination in substrate solution versus germination of not ultrafrozen seeds in water; \( n_0 \) is the total number of replications for not ultrafrozen seeds in water or substrate (\( n_0 = 18 \)); \( \sigma_4^2 \) is the standard deviation of ultrafrozen seed germination in substrate solution versus germination of ultrafrozen seeds in water; and \( n_4 \) is the total number of replications for ultrafrozen in water or substrate (\( n_4 = 18 \)).

T-test values are positive if a greater increase in germination occurred for not ultrafrozen seeds than for ultrafrozen seeds. T-test values are negative if a greater increase in germination occurred for ultrafrozen seeds than for not ultrafrozen seeds.
RESULTS AND DISCUSSION

Germinability of barnyardgrass seeds (*Echinochloa crus-galli* (L.) Beauv.) increased after the seeds had been repeatedly ultrafrozen with liquid nitrogen (-196 °C) (Fig. 1). The change in germinability increased most rapidly at 1 ultrafreeze (60% to 71%) and from 7 to 9 ultrafreezes (77% to 98%). There were plateaus of germinability from 1 to 6 ultrafreezes and above 9 ultrafreezes (Fig. 1). Thus, there might have been more than one major physiological response leading to increased barnyardgrass seed germinability.

Previous research on the increase of germinability had been conducted using seeds that were relatively impermeable to water (1, 2). Although germinability of barnyardgrass seeds increased with ultrafreezing, the amount of water imbibed by ultrafrozen seeds did not increase (Table 1). Also, the amount of apparent dry matter in the barnyardgrass seeds not ultrafrozen and ultrafrozen 10 times was the same (Table 1). Because Barton (1) and Busse (2) used quick thaw methods after ultrafreezing the seeds, cracking of the seed coat could be likely. The barnyardgrass seeds in this investigation were thawed slowly to minimize stresses which occur during thawing.

When the seeds were viewed using a scanning electron microscope, seeds that had not been ultrafrozen appeared identical to seeds that had been ultrafrozen 10 times (Figs. 2 and 3). Closer examinations of the seed surfaces also did not reveal any morphological changes or any cracks appearing with ultrafreezing (Figs. 4 and 5). When the seeds were cryofractured and the seed coats viewed with a scanning electron
microscope, no cracks or morphological changes were apparent in the seed coats which would have resulted from increased numbers of ultrafreezes (Figs. 6 and 7).

No cracks or morphological changes appeared in the seed coats of seeds that had been ultrafrozen 10 times (versus seeds not ultrafrozen) (Figs. 8 and 9). Closer examinations with a transmission electron microscope also showed no changes in seed coat structure with ultrafreezing. The cuticle of seeds ultrafrozen 10 times appeared identical to the cuticle of seeds not ultrafrozen (Figs. 10 and 11). Finally, secondary wall areas in the seed coat were identical between seeds ultrafrozen 10 times and seeds not ultrafrozen (Figs. 12 and 13). Thus, because of the identical response of not ultrafrozen seeds and ultrafrozen seeds to water imbibition and loss (Table 1), and because of the lack of morphological changes in seed coats (Figs. 2 to 13), ultrafreezing probably does not affect the seed coats by causing minute cracks in the seed coats. Rather, ultrafreezing probably has an effect on the barnyardgrass embryo.

When the secretory epithelium (of the embryo) was observed with a transmission electron microscope, ultrastructural differences were apparent which resulted from ultrafreezing (Figs. 14 to 19). In seeds that had not imbibed water or been ultrafrozen, the lipid bodies of the secretory epithelium were dispersed throughout the cell (Fig. 14). The lipid bodies themselves had a definite shape and appeared homogenous in texture (Fig. 15). When unimbibed seeds had been ultrafrozen 4 times, fewer lipid bodies appeared in the inner portions of the cells (Fig. 16).
The lipid bodies that were present are different from the lipid bodies in seeds never ultrafrozen. Invaginations occurred at the borders of the lipid bodies. The lipid bodies began to appear grainy and to have less definite borders (Fig. 17). By 10 ultrafreezes, lipid bodies were virtually absent from the cells, except as rows just below the plasmalemma (Fig. 18). The lipid bodies that remain had no invaginations and no definite borders (Fig. 19). Although the ultrastructure changed, seed germinability had increased from 60% with no ultrafreezes to 98% with 10 ultrafreezes.

Lipid bodies did not remain dissociated after water imbibition commenced. Within an hour after imbibition started, lipid bodies reformed in the seeds which had been ultrafrozen 10 times (Fig. 21). After an hour of water imbibition, the secretory epithelium of seeds not ultrafrozen appeared identical to the secretory epithelium of seeds ultrafrozen 10 times (Figs. 20 and 21). Enzymatic activity, however, did change due to ultrafreezing 10 times. Protein bodies in the secretory epithelium of seeds ultrafrozen ten times had already started to become digested after 4 hours of water imbibition (Fig. 23). A similar digestion pattern of protein bodies was not noted in seeds never ultrafrozen (Fig. 24). After 10 hours, the differences in protein body digestion were more noticeable. Protein bodies in seeds ultrafrozen 10 times were being digested (Fig. 24). Protein bodies in seeds never ultrafrozen were not being digested (Fig. 25).
Substrate Tests on Germination

Several physiological changes occur when seed dormancy terminates and germination is initiated. Hormones, storage proteins, lipids, carbohydrates, and numerous metabolic pathways become involved as a seed becomes a seedling. Because of the complexity of the living seed, researchers have not agreed upon which physiological process leads to dormancy termination and germination initiation.

The pentose phosphate pathway has been most frequently proposed to be the crucial pathway in seed dormancy. Activation of the pentose phosphate pathway has been suggested to be the initial step in germination (11, 12). Evidence for the role to the pentose phosphate pathway includes the stimulation of germination of dormant seeds by methylene blue, pentose phosphate intermediates, and various other compounds that directly affect the pentose phosphate pathway (11, 12). However, when three pentose phosphate intermediates and methylene blue were supplied exogenously to seeds that had been never ultrafrozen or ultrafrozen four times, positive T-test values were obtained (when percent germination increases were compared) (Table 2). This indicates that the pentose phosphate pathway was not affected by ultrafreezing.

The latter portion of glycolysis (Table 3) and the citric acid cycle (Table 4) were both affected by ultrafreezing. Thus, not ultrafrozen seeds did not utilize 3-phosphoglycerate, 2-phosphoglycerate, and pyruvic acid, while ultrafrozen seeds did utilize these compounds. The latter portion of the glycolysis pathway after dihydroxy acetone phosphate and glyceraldehyde-3-phosphate may be inactive in dormant seeds. Prior to the
formation of glyceraldehyde-3-phosphate, the compounds produced in
glycolysis can be directed to the pentose phosphate pathway which
apparently remains active during dormancy (Table 3). The latter portion
of the citric acid cycle may also be inactive or have reduced activity
in dormant seeds; succinic acid, fumaric acid, and malic acid stimulated
ultrafrozen seeds more than seeds not ultrafrozen (Fig. 3).

Although similar to the citric acid cycle, the glyoxylate cycle is
probably not responsible for dormancy in barnyardgrass seeds. Glyoxylic
acid is extremely inhibitory to germination of seeds that have been ultra-
frozen 4 times; not ultrafrozen seeds were less susceptible to glyoxylic
acid, though germination was still reduced (Table 5). Thus, initiation of
the glyoxylic acid cycle may not occur at the onset of germination.

The pentose phosphate pathway, glycolysis, and the citric acid cycle
contain reactions which liberate sufficient energy to produce ATP from ADP
and NADH from NAD+. The formation of energy rich compounds, especially
ATP, has been shown to increase substantially after the onset of water
imbibition (8). When ATP and NADH were added exogenously to the seeds,
both ultrafrozen and never ultrafrozen seed germination increased (Table 6).
Because positive T-test values were obtained, ATP and NADH utilization may
be independent of ultrafreezing. Whereas ATP promoted germination, ADP
caused a reduction in germination (Table 6). The increased level of germi-
nation for ultrafrozen seeds was negated. Thus, ADP may be involved in
maintaining dormancy.

Nitrogen-containing compounds, glycine and KNO₃, also affected germi-
nation (Table 6). Amino acids may stimulate germination by increasing the
cytokinin content (16). The utilization of glycine in promoting germination does not appear to be affected by ultrafreezing (Table 6). Therefore, glycine may not be involved in the initial reactions of germination. 

KNO₃ also stimulated the germination of ultrafrozen seeds, but reduced the germination of not ultrafrozen seeds. Although KNO₃ is a major dormancy-breaking agent for seeds of many species (17, 14), the actual mechanism by which KNO₃ affects dormancy is still not well-understood.

The effect of ultrafreezing on metabolic systems may be related directly to the cellular ultrastructure of the seed. When Tween 20, a surfactant, was added to never ultrafrozen seeds, germination approached 100%. However, Tween 20 reduced the germination of seeds that had been ultrafrozen (Table 6), possibly by modifying the reorganization of lipid bodies during water imbibition. Surfactants have been shown to alter cellular ultrastructure (15), change the activities of enzymes (5,6) and affect metabolic pathways (7, 9).

Generalizations regarding the metabolic pathways in seed germination must take into account the changes resulting from ultrafreezing treatments. Also, exogenously supplied compounds may be metabolized to different compounds, may be involved in different pathways, or may not be taken up by the seed due to permeability barriers. These considerations preclude extensive speculation from the results obtained under the conditions of these experiments.
Fig. 1. Total barnyardgrass germination (%) vs. number of ultra-freezes (-196°C with liquid nitrogen) after 1 week at 35°C.
Table 1. Fresh weight, moisture content, and percent imbibition of barnyardgrass seeds that had been ultrafrozen 0 or 10 times with liquid nitrogen

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Fresh weight (g)</th>
<th>Weight Loss 24 h/105°C</th>
<th>Imbibition 12 h/35°C</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 freezes</td>
<td>0.231 ± 0.006</td>
<td>6.6 ± 1.6</td>
<td>18.6 ± 4.4</td>
</tr>
<tr>
<td>10 freezes</td>
<td>0.228 0.007</td>
<td>5.6 ± 2.0</td>
<td>18.7 ± 2.4</td>
</tr>
</tbody>
</table>
Figs. 2 to 5. Scanning electron micrographs of seeds that had been ultrafrozen 0 or 10 times with liquid nitrogen (-196°C).


3. Ultrafrozen 10 times. Note the similarity between this seed and the one shown in Fig. 2. X25.

4. Not ultrafrozen. X2,000.

5. Ultrafrozen 10 times. No difference existed between the surface of this seed and the seed not ultrafrozen. X2,000.

Figs. 6 and 7. Scanning electron micrographs of cryofractured seeds that had been ultrafrozen 1 or 11 times with liquid nitrogen (-196°C).

6. Seed coat of a seed ultrafrozen once. X400.

7. Seed coat of a seed ultrafrozen 11 times. Note the similarity of the seed coat to the seed coat shown in Fig. 6. Also, note the lack of cracks. X400.
Figs. 8 and 9. Light micrographs of barnyardgrass seed coats of seeds that had been ultrafrozen 0 or 10 times with liquid nitrogen (-196 C).


9. Ultrafrozen 10 times. Note similarity to Fig. 8. Also, note the lack of distortions or cracks resulting from ultra-freezing. X700.

Figs. 10 to 13. Transmission electron micrographs of seed coats of seeds that had been ultrafrozen 0 or 10 times with liquid nitrogen (-196C).


Figs. 14 to 19. Transmission electron micrographs of the secretory epithelial cells (a tissue of the embryo) taken from seeds that have been ultrafrozen 0, 4, or 10 times. Seeds had not imbibed water or had been prechilled.

14. Secretory epithelium of a seed not ultrafrozen. Lipid bodies were dispersed throughout the cells. X5,720.


16. Secretory epithelium of a seed ultrafrozen 4 times. Lipid bodies were more concentrated toward the perimeters of the cells. X5,720.

17. Closer view of the lipid bodies in the secretory epithelium of a seed ultrafrozen 4 times. Invaginations (arrows) were present at the perimeters of the lipid bodies. X108,680.

18. Secretory epithelium of a seed ultrafrozen 10 times. Lipid bodies were mainly concentrated at the perimeters of the cells. X4,250.

19. Closer view of the lipid bodies in the secretory epithelium of a seed ultrafrozen 10 times. Lipid bodies did not have a definite border and had a grainy appearance. X80,750.
Figs. 20 to 25. Protein body (PB) digestion in the secretory epithelium of seeds that have never been ultrafrozen or ultrafrozen 10 times. X12,000.

20. Not ultrafrozen. Seed had imbibed water for 1 hour.
21. Ultrafrozen 10 times. Seed had imbibed water for 1 hour.
22. Not ultrafrozen. Seed had imbibed water for 4 hours.
23. Ultrafrozen 10 times. Seed had imbibed water for 4 hours.
24. Not ultrafrozen. Seed had imbibed water for 16 hours.
25. Ultrafrozen 10 times. Seed had imbibed water for 16 hours.

Note the greater protein body degradation of secretory cells of seeds ultrafrozen 10 times than of seeds not ultrafrozen.
Table 2. Changes in the germination (from water standards) of barnyard-grass seeds ultrafrozen 0 to 4 times with liquid nitrogen and germinated in the presence of $10^{-4}$M pentose phosphate intermediates. Methylene blue is also included because it is a stimulator of the pentose phosphate pathway.

<table>
<thead>
<tr>
<th>Intermediate</th>
<th>Change in germination</th>
<th>T-test values</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0 freezes</td>
<td>4 freezes</td>
</tr>
<tr>
<td>6 Phospho gluconate (trisodium salt)</td>
<td>32 ± 5</td>
<td>18 ± 3</td>
</tr>
<tr>
<td>Glyceraldehyde 3 phosphoric acid</td>
<td>32 ± 5</td>
<td>19 ± 3</td>
</tr>
<tr>
<td>Ribose 5-phosphate (disodium salt)</td>
<td>36 ± 4</td>
<td>20 ± 3</td>
</tr>
<tr>
<td>Methylene blue</td>
<td>26 ± 4</td>
<td>12 ± 5</td>
</tr>
</tbody>
</table>
Table 3. Changes in the germination (from water standards) of barnyard-grass seeds ultrafrozen 0 or 4 times with liquid nitrogen and germinated in the presence of 10^{-4}M glycolysis intermediates

<table>
<thead>
<tr>
<th>Intermediate</th>
<th>Change in germination</th>
<th>T-test values</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0 freezes</td>
<td>4 freezes</td>
</tr>
<tr>
<td>Fructose</td>
<td>19 ± 3</td>
<td>22 ± 3</td>
</tr>
<tr>
<td>Fructose 6-phosphate</td>
<td>35 ± 3</td>
<td>20 ± 4</td>
</tr>
<tr>
<td>(Potassium salt)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fructose 1,6-diphosphate</td>
<td>36 ± 3</td>
<td>15 ± 3</td>
</tr>
<tr>
<td>(trisodium salt)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dihydroxy acetone phosphate (DHAP)</td>
<td>27 ± 3</td>
<td>16 ± 5</td>
</tr>
<tr>
<td>(lithium salt)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glyceraldehyde-3-phosphoric acid</td>
<td>32 ± 5</td>
<td>19 ± 3</td>
</tr>
<tr>
<td>3-Phosphoglycerate (calcium salt)</td>
<td>-47 ± 4</td>
<td>15 ± 4</td>
</tr>
<tr>
<td>2-Phosphoglycerate (sodium salt)</td>
<td>-20 ± 5</td>
<td>18 ± 5</td>
</tr>
<tr>
<td>Pyruvic acid</td>
<td>-23 ± 4</td>
<td>-5 ± 4</td>
</tr>
</tbody>
</table>
Table 4. Changes in the germination (from water standards) of barnyard-grass seeds ultrafrozen 0 to 4 times with liquid nitrogen and germinated in the presence of $10^{-4}$M citric acid cycle intermediates

<table>
<thead>
<tr>
<th>Intermediate</th>
<th>Change in germination</th>
<th>T-test values</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0 freezes</td>
<td>4 freezes</td>
</tr>
<tr>
<td>Oxaloacetic acid (OAA)</td>
<td>24 ± 4</td>
<td>19 ± 2</td>
</tr>
<tr>
<td>Acetyl CoA (Lithium salt)</td>
<td>31 ± 4</td>
<td>18 ± 3</td>
</tr>
<tr>
<td>Citric acid</td>
<td>37 ± 5</td>
<td>22 ± 2</td>
</tr>
<tr>
<td>Isocitric acid</td>
<td>34 ± 4</td>
<td>21 ± 3</td>
</tr>
<tr>
<td>Coenzyme A (Lithium salt)</td>
<td>29 ± 7</td>
<td>21 ± 4</td>
</tr>
<tr>
<td>Succinyl CoA (Sodium salt)</td>
<td>30 ± 4</td>
<td>20 ± 4</td>
</tr>
<tr>
<td>Succinic acid</td>
<td>4 ± 5</td>
<td>16 ± 5</td>
</tr>
<tr>
<td>Fumaric acid</td>
<td>10 ± 6</td>
<td>14 ± 5</td>
</tr>
<tr>
<td>Malic acid</td>
<td>1 ± 6</td>
<td>8 ± 5</td>
</tr>
</tbody>
</table>
Table 5. Changes in the germination (from water standards) of barnyard-grass seeds ultrafrozen 0 to 4 times with liquid nitrogen and germinated in the presence of $10^{-4}$M glyoxylate cycle intermediates

<table>
<thead>
<tr>
<th>Substrate</th>
<th>Change in germination</th>
<th>T-test values</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0 freezes</td>
<td>4 freezes</td>
</tr>
<tr>
<td>Oxaloacetic acid (OAA)</td>
<td>24 ± 4</td>
<td>19 ± 2</td>
</tr>
<tr>
<td>Acetyl CoA (Lithium salt)</td>
<td>31 ± 4</td>
<td>18 ± 3</td>
</tr>
<tr>
<td>Citric acid</td>
<td>37 ± 5</td>
<td>22 ± 2</td>
</tr>
<tr>
<td>Isocitric acid</td>
<td>34 ± 4</td>
<td>21 ± 3</td>
</tr>
<tr>
<td>Glyoxylic acid</td>
<td>-22 ± 7</td>
<td>-64 ± 5</td>
</tr>
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</table>
Table 6. Changes in the germination (from water standards) of barnyard-grass seeds ultrafrozen 0 to 4 times with liquid nitrogen and germinated in the presence of various substrates (10^{-4}M). Tween 20 was used at 10^{-1\%} (v/v) concentration in water

<table>
<thead>
<tr>
<th>Substrate</th>
<th>Change in germination</th>
<th>T-test values</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0 freezes</td>
<td>4 freezes</td>
</tr>
<tr>
<td>Tween 20</td>
<td>35 ± 3</td>
<td>-9 ± 3</td>
</tr>
<tr>
<td>ADP (sodium salt)</td>
<td>-17 ± 7</td>
<td>-31 ± 8</td>
</tr>
<tr>
<td>Glycine</td>
<td>32 ± 3</td>
<td>23 ± 4</td>
</tr>
<tr>
<td>ATP (disodium salt)</td>
<td>23 ± 6</td>
<td>18 ± 4</td>
</tr>
<tr>
<td>KNO3</td>
<td>-8 ± 7</td>
<td>12 ± 4</td>
</tr>
<tr>
<td>NADH</td>
<td>27 ± 3</td>
<td>16 ± 5</td>
</tr>
</tbody>
</table>
REFERENCES CITED


GENERAL DISCUSSION

The results of this investigation dealt with different aspects of weed seed dormancy. Dormancy was related to environmental growth conditions of the parent plants. Dormancy termination was accompanied by changes in embryo cell structures. The termination of dormancy might not be dependent on the pentose phosphate pathway for all weed seeds.

Seed dormancy is the major survival mechanism of annual weeds. Termination of dormancy defines the time when weeds pose a potential problem in crop production. However, since seed germination does not always follow termination of dormancy, weed control methods must be predicated on germination of weed seeds. Crops and weeds germinate together in the shared soil habitat and are subjected to control methods which exploit differences in seedling growth. Control methods based on minimizing seed germination have wide application in conservation tillage systems where the triggering effect of tillage on weed seed germination is lost to control methods. Laboratory studies of weed seed dormancy hold high potential for an improved understanding of the precise germination requirements of weed seeds in the microsite environments of an undisturbed spring seedbed.

Weed-crop Competition and Weed Seed Dormancy

In the field, weeds grow under different competition and microclimate regimes. How competition or the microclimate affects the parent weed plant and the weed seed produced has been the object of recent research. Although this dissertation is about weed seed dormancy, competition itself has more importance than having an effect on weed seed
dormancy. Without being able to compete with the crop, the non-crop plant would not be a weed. In fact, the inability of some weeds to compete with specific crops has been a basis for crop rotation. Two measures of effective crop competition with the weeds are as follows: first, a change in the weed growth habit of the weed so that the weed plants are less hardy; and second, a reduction in the number of weed seeds produced. Maize competition with four species of weeds resulted in smaller weed plants which produced fewer seeds. The importance of weed plants without crop competition is accentuated by the larger number of seeds produced per plant. The seeds produced have the potential of leading to future weed infestations. From the integrated pest management viewpoint of economic thresholds, the seed production of a weed growing with maize competition is not as economically significant as one growing without any competition. If economic thresholds for weed seed production are determined, the stress conditions of the parent plants should be considered.

Along with changing the seed production of four species of weeds, maize competition also resulted in different kinds of Pennsylvania smartweed seed dormancies. When Pennsylvania smartweed plants were grown with maize competition, dormancy of seeds tended to be due primarily to immature embryos. However, seeds from plants grown without maize competition were dormant because of impermeable seed coats. Because of the different natures of seed dormancy from plants with different competition stresses, information about the conditions under which the seed developed to predict germination responses in the field is
important. Simply removing and germinating Pennsylvania smartweed seeds from the soil does not adequately predict the germination response during the spring germination flush. Furthermore, differences in weed seed dormancy may be localized in the field, depending on previous weed-crop competition levels. Finally, sampling seeds from specific areas of a field does not take into consideration disturbances in the entire soil habitat.

Ultrafreezing and Barnyardgrass Seed Dormancy

Barnyardgrass seeds had an embryo-induced dormancy. Contrary to the hypothesis by Busse (11) and Barton (6) that ultrafreezing releases seed dormancy by affecting the seed coat, this study showed that ultrafreezing released seed dormancy by affecting the barnyardgrass seed embryo. Second, contrary to the hypothesis of Roberts (55) that all seed dormancy release is related to the activity of the pentose phosphate pathway, during germination, ultrafreezing apparently affected barnyardgrass seed dormancy by affecting glycolysis and the citric acid cycle. Before any generalizations can be made about seed dormancy or germination as affected by ultrafreezing, further tests would have to be conducted on other ecotypes of barnyardgrass seeds and on seeds of other species.

When ultrafreezing experiments are conducted with other barnyardgrass ecotypes, differences in the germination response to ultrafreezing may be encountered. Barnyardgrass seed dormancy is peculiar to the ecotype investigated (34). For example, in the Philippines, some barnyardgrass seeds germinate immediately after being shed; all viable seeds germinate after 3 months of being shed from the parent plant.
However, in the United States, barnyardgrass seeds commonly have a 4 to 48 month dormancy (34). Second, barnyardgrass seeds that have never been buried do not need a photoinduction period to germinate. However, barnyardgrass seeds that are buried in soil acquire a dependency on light for germination (63). In Iowa, where the seeds were collected, barnyardgrass seeds normally do not germinate in the same year as they were shed. Thus, during the burial or overwintering period, dormancy characteristics may change. Laboratory studies on seed dormancy have limited direct correlation to weed seed dormancy in the field. Thus, the results of this investigation may reflect a different pattern of dormancy termination than that encountered with seeds in the soil habitat.
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