SEED DORMANCY IN SETARIA LUTESCENS

by

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INTRODUCTION

Seed dormancy is the major adaptation of annual weeds which permits them to survive and flourish in spite of the many agronomic practices designed to favor the establishment of the crop. As a result of this particular adaptation, propagules of weed species may remain dormant or in the non-growing state for extended periods. During this time, varying percentages of seed may encounter that combination of environmental conditions which results in the termination of dormancy. Thus a year to year crop of weeds is produced and new supplies of dormant seeds are added to those already present in the soil. An understanding of the specific conditions responsible for the maintenance, and for the termination of dormancy in weed seeds is basic to the development of superior control methods.

The present study was an attempt to explain the observed changes in the dormancy of Setaria lutescens (Weigel) F. T. Hubb, in response to the natural environment and to investigate the mechanisms underlying the maintenance and termination of dormancy in this species. Setaria lutescens was chosen because it met the important requirements for a suitable experimental material. Adequate supplies of both dormant and non-dormant seed were available, methods were known for breaking the dormancy, and the species is a serious weed pest of the region.

LITERATURE REVIEW

Seed dormancy is an important factor in the continuance of many plant species, including weeds. In temperate zones it is a survival mechanism which prevents the fall germination of newly matured seed of species which are not winter hardy. The problems of seed dormancy have been studied extensively during the past half century, and recently the continuing search for new herbicides has emphasized renewed research interest in the field. Dormancy may be attributed to a number of biochemical, physiological and physical factors. The sequence of scientific endeavor in the field will be outlined by considerations of the more important contributing factors.

Observed Dormancy Patterns

In many species of the temperate zones, freshly matured seeds are dormant and require varying periods of after-ripening before germination may occur. Several exceptions to this general condition and variations in the requirements for after-ripening have been noted. Environmental conditions during maturation, and regional differences have provided more valid explanations of the observed differences in behavior of seeds than have genetic differences. Typical of these have been the wide variations in dormancy reported for _Avena fatua_.

Bibbey (10) found that in Canada the germination of

freshly collected seeds of _Avena_ _fatua_ differed by 90 percent
among two lots of seed collected in the same field in two
successive years. Hay and Cumming (28) reported that, if
dormancy was developed in their strains of Canadian _Avena_
fatua, it persisted only a few weeks under laboratory storage.
Thurston (51, p. 76) however, states that "in spite of all the
factors that may affect dormancy, freshly collected samples
of _A. fatua_ in England are usually 95 to 100 percent dormant
and dormancy is retained for at least 9 to 12 months under
laboratory storage."

The importance of environment is evident from the work
of Thurston (51, p. 77) who reported that four Canadian sorts
of _Avena_ _fatua_ grown at Rothamsted in 1958 had 100 percent
dormant seeds at harvest, germination was occasional during
the winter of 1958, but a flush of spring germination occurred
in mid-March of 1959. Therefore, he concluded, the difference
between Canadian and British wild oats may depend on the grow-
ing or storage conditions rather than on genetical differences.
Chepil (13) found that all of the 58 species he studied showed
periodicity of germination, some germinating in the spring,
some in the fall or winter. The importance of photoperiod was
demonstrated by Lona (35), who found that the germination of
Chenopodium _amaranticolor_ was higher if the plants matured on
short days rather than long days. His explanation was that
thinner coats developed under short days than long days.

Similar results were observed by Thurston (52) in _Avena_ _ludoviciana_ in England. He found that the seeds developing on the plant become viable before they become dormant, and dormancy developed during the latter stages of ripening but persisted only a short time in the seeds which matured first. He states that seed dormancy probably depends on weather conditions during maturation. Color of seeds and dormancy has been associated in _Halogeton glomeratus_; Williams (57) found that brown and black seeds are produced in a ratio of one to three. Black seeds germinated readily but brown did not germinate under laboratory conditions. Brown seeds are produced first as a periodic response to the shortening of days in mid-summer, while black seeds are formed later on during short days. Brown seeds are dormant and may germinate years after formation, resuming the continuity of the plant.

Dormancy as Determined by Seed Structure

Impermeability of seed coats to water uptake and gas exchange has been considered a major contributing factor to this type of dormancy. Many investigators indicate that the outside layer of cells of the coats prevents the entrance of water. White (56) found that in small leguminous seeds the cuticular layer over the palisade cells determined the impermeability, while in the larger leguminous seeds the outer portion of the palisade cells was involved also. The physical

condition and chemical composition of this outer layer was
related to water uptake in the work done by Raleigh (43). He
found that as seeds of Gymnocladus canadensis hardened during
maturation, pectic substances made the cells impermeable to
water. Hamly (24) indicated that the suberin caps over the
cells of the palisade layer caused water impermeability in
sweet clover seeds. In some species this may be overcome by
treating the seeds with chemicals. Shaw (46) obtained germi-
nation of Nelumbo lutea seed by treating them with acetone and
then placing them in water. She assumed that fatty substances
were dissolved out of the stomatal cavities which extended
deeper than the palisade layer, allowing water to enter. When
ether was used as a solvent the embryo was killed. Similar
results were obtained by McKeever (36) who found that soaking
the seeds of Robinia psuedo-acacia for 10 to 120 minutes with
wax solvents, acetone, ether, etc. speeded the germination of
treated seeds by 10 to 15 days.

Impermeability of membranes to gases is an important
factor in the maintenance of dormancy in many species. Brown
(12) studied permeability of the seed coat membrane of
Cucurbita pepo. He found that the outer membrane was much
less permeable to oxygen than the inner membranes. His re-
sults indicated that the living tissue of the inner membrane
limited oxygen exchange, since the outer membrane was discon-
tinuous at the micropylar region. Killing this tissue in-

creased diffusion rate of gases. Atwood (2) found that dormant A. fatua seed would germinate when placed in an atmosphere of high oxygen tension. Breaking the seed coat produced the same results. Toole et al. (54) suggested however, that manipulation of the seed during seed coat rupture may produce associated effects not related to actual change in seed coat. Christie (14) in studies of dormancy in A. fatua found a slower rate of germination when the enclosing lemma and palea were removed. The importance of nutrient mobility was emphasized by Crocker (15, p. 104). He quoted unpublished research by Flemion with embryos from seed of Pinus nigra. Flemion found that the embryos stored under moist conditions at 5°C. for one month grew faster than those kept at room temperature. Crocker states: "In coniferous seeds much of the stored food is in the endosperm. The difference in the growth of the embryo from the low temperature stratification and that from the non-stratified seed may be explained by the movement of nutrients and the accessible food from the endosperm during the month of stratification."

Metabolic Aspects of Seed Dormancy and Germination

Failure of seed germination may be due to metabolic blocks to embryo growth, originating in the embryo itself or in tissues external to the embryo. Control of germination may be exerted through the influence of naturally occurring

chemical inhibitors or may result from the absence of certain required biochemical intermediates. Other seeds have specific light requirements which in all probability are linked with chemical reactions.

Evenari (21) in a recent review of the literature on germination inhibitors has emphasized their role in the maintenance of seed dormancy. Some of the inhibitors reported to occur in seeds include, ammonia, hydrogen cyanide, ethylene, essential oils, alkaloids, unsaturated lactones, and unsaturated acids. Black (11) reported two inhibitor regions on chromatograms of aqueous extracts of hull and caryopsis of _Avena fatua_ L. Evenari (20) reported a study by Köller where inhibitors assumed a vital role in the survival of species growing in arid habitats. Seeds of _Atriplex dimorphostegia_ contain a water soluble inhibitor in the bracts. The amount and solubility of the inhibitor determined the amount and duration of the rainfall required before germination could proceed and thus insured adequate soil moisture for seedling growth.

A number of species produce seeds which have either a light requirement for germination or which are inhibited by exposure to light. The phenomenon appears to be related to a number of physiological responses to light. The subject was reviewed by Toole _et al_. (54) and Toole (53).

Termination of dormancy may be accomplished in several

ways. Some species respond to low temperature stratification
for several months in moist medium at temperatures of 0° to
10°C. This procedure imitates nature's methods of after-
ripening in temperate zones. Crocker (15, p. 90) in his book
presents a table that includes 90 species that respond to low
temperature stratification. However, other species require
dry storage. Crocker and Barton (16, p. 119) published a list
of 50 species in which dry storage terminated dormancy.

In spite of extensive research in the field of seed
physiology, relatively little of it has dealt with the bio-
chemical changes accompanying the breaking of dormancy and the
initiation of germination. Eckerson in 1913 (19) followed the
chemical changes which occurred during after-ripening of
several species of Crataegus seeds, using chemical, micro-
scopic, and physiological methods. She found proteins, fats
and small amounts of soluble sugars, stored mostly in the
cotyledon. The chemical changes were an initial increase in
acidity associated with an increase in water-holding capacity,
and an increased catalase and peroxidase activity. Pack (40)
working with seeds of several juniper species described these
changes: increase in water imbibition and pH, especially in
the embryo; increase in the degree of dispersion of stored
fat; decrease in stored fat and proteins with increase of
sugars and starches; translocation of fatty substance from
endosperm to embryo; increase of soluble protein and hydroly-

sis; increase in respiration; increase in respiratory quotient; doubling of catalase activity; and rise of vigor of seeds as shown by their resistance to fungal infection. All of these were accompanied by growth of the embryo.

More recently Barton in 1961 (5) and 1962 (4) has reported biochemical studies, done at the Boyce Thompson Institute, with dormant epicotyls of *Paeonia suffruticosa* Haw. The biochemical studies indicated the presence of the following organic acids; pyrrolidone carboxylic, succinic, malic and citric. The first two were present in small amounts, but did not seem to be related to dormancy. Malic accumulated in dormant endosperm and dormant embryo. Citric acid increased upon water absorption, was low in the embryo, and increased with germination. The ratios of citric to malic in the endosperm before and after germination were 1:1 and 3:1 respectively. Sugars detected were fructose, glucose, sucrose, raffinose and one unidentified sugar. Fructose increased in the embryo and endosperm of non-dormant seeds. As germination proceeded glucose remained the same and raffinose and sucrose decreased in the endosperm. Barton (8) investigated nitrogen metabolism in the same species. She found that the free amino acids, alanine and glutamine, were present in larger quantities in non-dormant than in dormant epicotyls, and that asparagin was most abundant in dormant epicotyls.

LaCroix (32) working with *Polygonum pensylvanicum* seed,

found, in electron photomicrographs, numerous electron-dense particles in the nucleoli of cells from embryos of non-dormant embryos. These particles were not present in dormant embryos. He concluded that this difference may indicate basic changes in metabolic pattern which are coincident with the transition from the dormant to non-dormant state.

Germination of seeds may be associated also with other factors. Bernard (9) working with orchids proposed that germination of these seeds was dependent upon the infection by certain strains of fungus, but his case for the need of fungus was weakened by the failure of germination in inoculated seeds. Knudson (31), working with orchid seeds of Cattleya and Laelia found that a fungus provided soluble organic substances, particularly sugars. When a suitable nutrient medium was supplied, germination was obtained in the absence of the fungus.

Respiration and mold growth have been associated as shown by the work done by Milner and Geddes (37). They measured the oxygen and carbon dioxide production of soybeans containing about 18.5 percent moisture. Their results indicated that respiration curves were similar in form to those of microbiological population growth curves. They killed the seeds by heating them at 50°C., a temperature not lethal for mold growth. Similar results were obtained with Triticum aestivum by Oxley and Jones (39). They found that carbon dioxide was

produced from microorganisms growing in the pericarp, and removal of embryo showed the same respiratory rates as intact seeds.

A further interesting potential role of respiration in seeds was advanced by Livingston (34). He concluded that the water produced in respiration may be an important source of water during the early stages of germination. Babcock (3) concluded that metabolic water sufficient for all the vital processes of the plant is provided by the slow oxidation taking place as a result of direct respiration. In seeds that are not germinating this could be essential for maintaining viability.

The utilization of fats stored in the endosperm, in the early stages of germination has been reported by several investigators. Barton (7) found that the R. Q. values for germinating seeds of *Amaranthus* *retroflexus*, were approximately 0.7, shortly after being moistened despite the fact that chemical analyses showed 47.3 percent carbohydrates and only 7.8 percent lipids in the caryposes. Fats were concentrated mainly in the embryo. Stiles and Leach (49) working with *Fagopyrum* *esculentum*, found that the small reserves of fats were consumed at a very early stage of germination even though the principal food reserves were starch. Crombie and Comber (17) studied the metabolism of fats in germinating seeds of *Citrullus* *vulgaris* and found a 75 percent reduction in fat

content after 24 hours exposure of seeds to germination condi-
tions.

The metabolism of sugars and organic acids during germi-
nation has been traced in the following studies. Taüfel et
al. (50) working with peas and soybeans found traces of
glucose and fructose and measurable quantities of raffinose
and starch. During soybean germination oligosaccharides
decreased, sucrose increased, and maltose was detectable.
Later on maltose disappeared completely while sucrose in-
creased, in peas and beans. Lisitsyn (33) working with
Fraxinus excelsior could find no appreciable changes in
carbohydrate during the early stage of germination. Solda-
tenkov and Mazurova (48) studied organic acid fractions during
the ripening and germination of Triticum aestivum seeds. They
found acetic, malic, citric and formic acids in both seedlings
and mature seeds.

MATERIALS AND METHODS

Techniques of laboratory seed germination, isolated embryo culture and chemical analysis were employed during this study of seed dormancy in <u>Setaria</u> <u>lutescens</u>. Mature seed was harvested from wild populations of <u>S</u>. <u>lutescens</u>, and was stored at approximately 5°C, until used in experiments. Seed lots were designated by the year of harvest. The term "seed" will refer henceforth to the spikelet which includes glumes, lemma, palea and caryopsis. The structures exterior to and enclosing the caryopsis are termed collectively the "hull".

Germination Tests

Two filter papers were placed in the bottoms of standard size petri plates, 9 cm in diameter. The papers were moistened with 5 ml of deionized water, 25 seeds or caryopses were spread uniformly on the surface of the paper and germinated under alternating temperatures of 20° and 30°C (15 hours at 20° and 9 hours at 30°). Germination percentages were recorded at two and six days after placing in the germinators. Germination is defined, for purposes of this study, as that stage of growth when the radicle was five millimeters or more in length. Unless otherwise indicated, the lemma and palea were removed prior to germination tests, to eliminate the effects of the hull on water uptake and seed germination.

Embryo Culture Techniques

Lemma and palea were removed using tweezers and razor blade. Prior to embryo excision, the caryopsis was soaked in water at approximately 5°C for three hours to facilitate removal of the embryo; in some experiments the soaking period was omitted to avoid possible diffusion of inhibitors from the endosperm. Scalpels and forceps were dipped in 50% ethyl alcohol and flame sterilized prior to use. Embryo isolations were done under a dissecting microscope at 50x magnification. All manipulations were carried out in a transfer chamber to minimize contamination. The chamber was described by LaCroix (32). All glassware used in embryo culture procedures was soaked in sulfuric acid-sodium dichromate, glass cleaning solution, then rinsed in tap and distilled water. Deionized water, used in preparation of culture media, was obtained by passing distilled water through a Barnstead demineralizer. Reagent grade chemicals were used in preparation of media. Embryos were cultured on a semi-solid culture medium, based on that described by Rappaport (44) with some modifications. Since embryos from non-dormant seed grew normally on this medium, no additional modifications of the media were utilized. The following stock solutions were prepared:

Stock Solution A

$Ca(NO_3) \cdot H_2O$	23.6 g
KNO_3	8.5 g
KCl	6.5 g
H_2O to 500 ml	

Stock Solution B

$NaH_2PO_4 \cdot H_2O$	1.35 g
$MgSO_4$	3.02 g
$MnSO_4 \cdot H_2O$	0.76 g
H_2O to 250 ml	

Stock Solution C

A chelated form of iron was prepared using the method of Jacobson (30). The ethylene diamine tetra-acetic acid was obtained from Eastman Chemical Company. One milliliter of the solution provided five parts per million of iron in one liter of nutrient medium.

Basic medium was prepared according to the following schedule:

1. 20 g sucrose was added to 1 liter of water, and the solution was brought to a boil.

2. With constant stirring, 8 g of agar (Difco, Special Noble) was added slowly, and the mixture boiled until the agar was completely in solution.

3. The solution was allowed to cool; then 5 ml of solution A, 2.5 ml of solution B, and 1 ml of solution C

were added.

4. The medium was poured into embryo culture flasks and
    autoclaved for 20 minutes at 15 lb pressure.

Biochemical Analyses

Quantitative analyses of sugars and long chain fatty
acids, qualitative analyses of sugars and organic acids, and
spectrophotometric analyses of lipid fractions were made for
non-dormant, dormant and dead caryopses of S. lutescens.
Analyses were made both before and after exposure of seed to
germination conditions for 48 hours. This time interval was
sufficient for complete germination of non-dormant caryopses.
A Warburg apparatus was used for respiration experiments.
Methods are summarized below and additional details of pro-
cedure are included in the presentation of results.

Sugar determinations

Experimental samples of caryopses were ground to pass a
40-mesh screen and extracted in a semimicro Sexhlet apparatus.
Reducing sugar determinations were made on cleared, deleaded
extract, using the ceric sulfate method described by Hassid
(26, 27). Sucrose was hydrolized at room temperature with
yeast invertase. Standard glucose curves were prepared each
week, and used in the determinations of reducing sugars and
sucrose. Results were expressed in milligrams of sugar per

100 caryopses.

Fatty acid determinations

Benzene fractions extracted from ground caryopses were evaporated under vacuum, and transferred with 2 ml of hexane to a thin neck reaction flask. The methylation procedure was based on that described by Selvey (45) with some modification. For long chain fatty acid analysis ten microliters were fed to a Perkin-Elmer vapor fractometer Model 154.

Qualitative analyses

The caryopses were ground and then refluxed for 30 minutes with 80 percent ethanol. The extract was centrifuged, the lipid fraction removed, and the remaining extract passed through appropriate ion exchange columns to separate it into three fractions containing the amino acids, organic acids and sugars, respectively. The fractions were evaporated to dryness under vacuum at 39°C and stored at -5°C for later use. The procedures followed in the separations were based on those described by Racusen (42).

Qualitative comparisons of experimental caryopses samples were made on the bases of appropriate methods of one dimensional paper chromatography. Chromatographic techniques were used to characterize partially the carbohydrates and organic acid fractions, but no attempt was made to include the amino

acid fractions. Spectrophotometric analyses of lipid frac-
tions were carried out on a Beckman DU spectrophotometer.

RESULTS FROM STUDIES OF SEED DORMANCY
IN SETARIA LUTESCENS

The present study has included an investigation of artificial and natural methods of terminating dormancy, a study of the roles of endosperm, embryo and hull (lemma and palea) in the maintenance of dormancy and an attempt to characterize dormancy on the basis of differences in the chemical composition of the caryopses from dormant and non-dormant seed.

Seed produced by Setaria lutescens is generally dormant when matured, and this dormancy may be maintained for extended periods, when the seed is stored dry, at cool temperatures. After-ripening under natural conditions in the fall and winter, and low temperature stratification in the laboratory were known to terminate dormancy effectively. Preliminary studies of the seed samples available initially or collected during this investigation revealed striking differences in the dormancy of various seed lots. Adequate supplies of both dormant and non-dormant seed were available. These included 1959 dead, 1960 non-dormant and 1961 and 1962 either dormant or non-dormant, depending on date of harvest. The terms dormant and non-dormant refer to the condition of the caryopses with hulls removed.

Anatomical Considerations

Careful examination of S. lutescens seed indicated that the separate roles of hulls, embryo and endosperm in the maintenance of dormancy should be determined initially.

Lemma and palea

The mature caryopsis of Setaria lutescens seed is enclosed by the lemma and palea, referred to commonly as the hull. Preliminary experiments with the non-dormant, 1960 seed lot indicated that the hull impaired water absorption and delayed germination. Data presented in Table 1 illustrate this delaying effect of the hull on germination of non-dormant caryopses. The effects of hulls in preventing germination of non-dormant caryopses suggested a possible role of inhibitors in the hull. This was investigated by leaching experiments, using cold water in the closed recirculating system described by LaCroix (32).

Leaching in cold water increased germination of intact seeds or of seeds with hulls ruptured. After-ripening for eight weeks on the soil surface in the field roughly approximated water leaching. Removal of lemma and palea resulted in rapid and complete germination of caryopses. When non-dormant seeds were placed in the field, the inhibitory effect of the hull was eliminated partially. When intact, dormant seeds of

Table 1. Germination percentages of non-dormant seeds (1960 lot) of S. lutescens following water leaching in the laboratory or after ripening in the field

| | Leaching time in hours | | | | After-ripening 8 weeks in field |
	0	24	48	96	
Entire seed	29	62	55	74	59
Entire seed with hull clipped	58	73	66	89	90
Caryopses alone	98	100	98	100	98

S. lutescens were leached in cold water, germination of caryopses was not increased appreciably. These data are summarized in Table 2.

The hull of mature S. lutescens seed may be either black or green in color. No differences in dormancy condition were

Table 2. Germination of dormant caryopsis of S. lutescens after leaching with water

| Leaching time in hours | Percentage germination after: | |
	six days	ten days
0	5	5
24	8	8
48	8	20
96	6	20

found to be associated with hull color in any of the seed lots used in this study.

When the water leachate from 100 hulls was used as the moisture source for 100 non-dormant caryopses, no reduction in germination was observed. A suggestion of inhibition of seedling growth was noted, but was not substantiated in all four replications.

Data presented later, in water uptake studies, showed no difference in the rate and amount of water absorbed by dormant and non-dormant entire seeds. These results demonstrated an inhibitory effect of the hulls on germination of non-dormant caryopses. This effect may be due to germination inhibitors in the hull, to mechanical constriction of the caryopsis or to a combination of both. The effects of the hull in maintaining seed dormancy were independent of caryopsis dormancy, even though the same after-ripening conditions eliminated hull effects partially and terminated caryopses dormancy. Since removal of hulls did not promote germination of dormant caryopses, and since major research effort was concerned with the condition of embryo and endosperm, the decision was made to conduct all remaining germination tests with the lemma and palea removed. This procedure effectively eliminated the effects of hulls in the evaluation of caryopses dormancy.

Embryo

Embryo culture techniques, described previously, were followed in experiments to determine the dormancy condition of embryos of S. lutescens seed. The embryos were removed without soaking the seeds previously in water. This prevented possible diffusion of inhibitors of growth factors from endosperm to embryo. Embryos were excised with the scutellum attached, since previous experience indicated that the scutellum attachment facilitated embryo growth. Embryos were grown at 20° - 30°C. Several experiments were conducted; the results from a typical one are summarized in Table 3. Germination of embryos from dormant seed exceeded 85 percent, while germination of dormant caryopses was only five percent. On the basis of these data, embryo dormancy was excluded as a major factor in the maintenance of seed dormancy.

The possible diffusion of inhibitors from endosperm to

Table 3. Growth of isolated embryos of Setaria lutescens on a basic nutrient medium

Seed lot	Condition of seed	Number of excised embryos	Germination after 12 days	Percentage germination of embryos	Percentage germination caryopses
1960	non-dormant	45	39	86	100
1962	dormant	45	38	85	5

embryo when the seed was soaked in water prior to embryo isolation was investigated. Seeds were soaked in water for three hours at 5°C before removing the embryo. The results confirmed the absence of embryo dormancy. However, growth was faster when caryopses were soaked before embryo isolation, suggesting a possible diffusion of growth factors from endosperm to embryo. The role of the endosperm in seed dormancy was investigated further by transplanting embryos from dormant and non-dormant seeds into dormant and non-dormant endosperms. Major difficulties in techniques included fungal contamination from endosperm tissue and a lack of close contact between endosperms and transplanted embryos. No consistent inhibitory effect was observed in the growth of embryos transplanted into dormant endosperms. However, growth of embryos was slow compared with isolated embryos even when transplanted into non-dormant endosperms.

Seed Dormancy Patterns Observed in S. Lutescens

The range of caryopsis dormancy encountered in various seed lots is illustrated in Table 4. The 1960 seed lot was non-dormant while those of 1961 and 1962 were essentially dormant. Figures 1-5 illustrate the caryopses and embryos of dormant and non-dormant seeds during exposure to germination conditions. The 1960 and 1961 seed had been stored at 5°C. Germination tests conducted in 1962 showed that there was no change

Table 4.   Germination of caryopses of <u>Setaria lutescens</u>

Seed lot	Percentage germination after:	
	two days	six days
1959	0	0
1960	96	96
1961	6	30
1962	0	5

in the dormancy condition of either 1960 or 1961 seed follow-
ing storage either at 5°C or at room temperature in the
laboratory.   Dates of harvest for the 1959 and 1960 seed lots
could not be established with certainty; 1961 and 1962 seed
lots were harvested during the last week of September in each
year.   The observed dormancy conditions of the respective lots
suggested that date of maturity of the seed might be related
to dormancy.

Accordingly, <u>S</u>. <u>lutescens</u> seed was harvested by hand from
scattered wild populations during the period July-October in
1962.   Results of germination tests on fresh seed are sum-
marized in Table 5.   Seed was dormant on all collection dates
except July 15.   These results suggested that in general a
high percentage of seed which matured after August 15 would be
dormant; in many seasons most seed of <u>S</u>. <u>lutescens</u> matures
after August 15.   Furthermore, under natural conditions mature
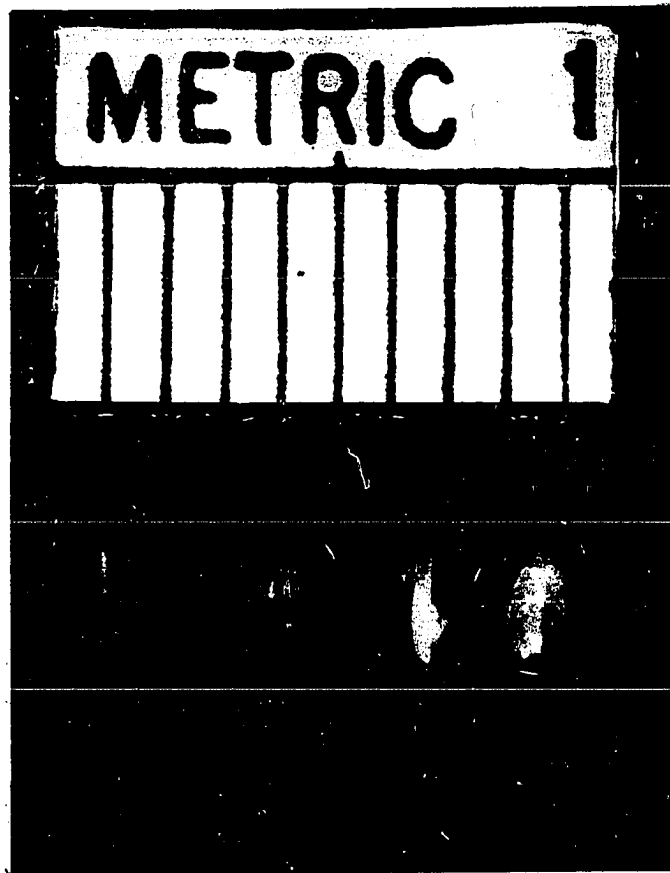
Figure 1. Seed and caryopsis of <u>Setaria</u> <u>lutescens</u>

from left to right: mature seed, caryopsis
(lemma and palea removed), isolated embryo,
endosperm

Figure 2. Dormant caryopses of <u>Setaria lutescens</u>
after 24 hours under germination
conditions

Figure 3. Non-dormant caryopses of <u>Setaria lutescens</u>
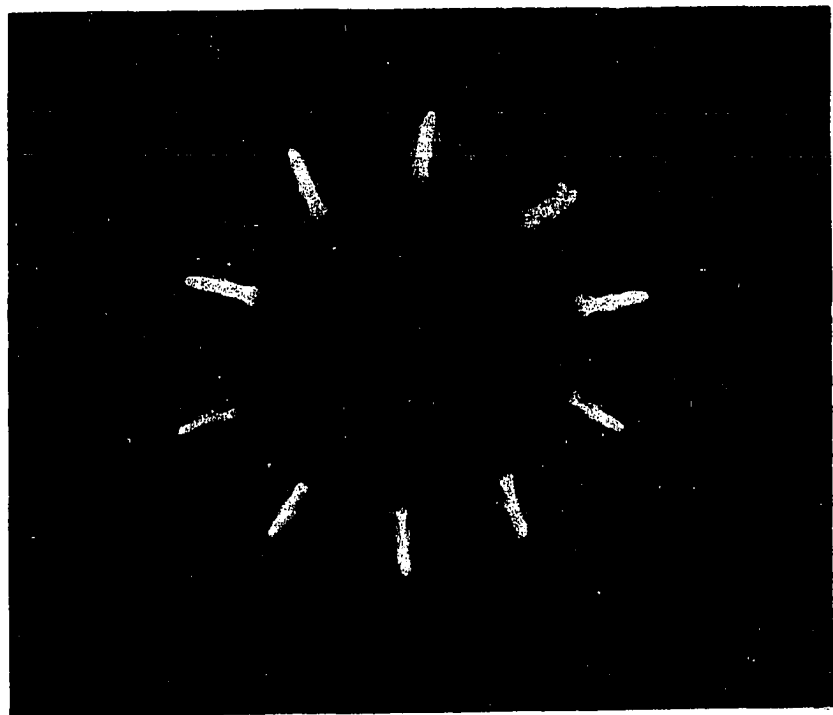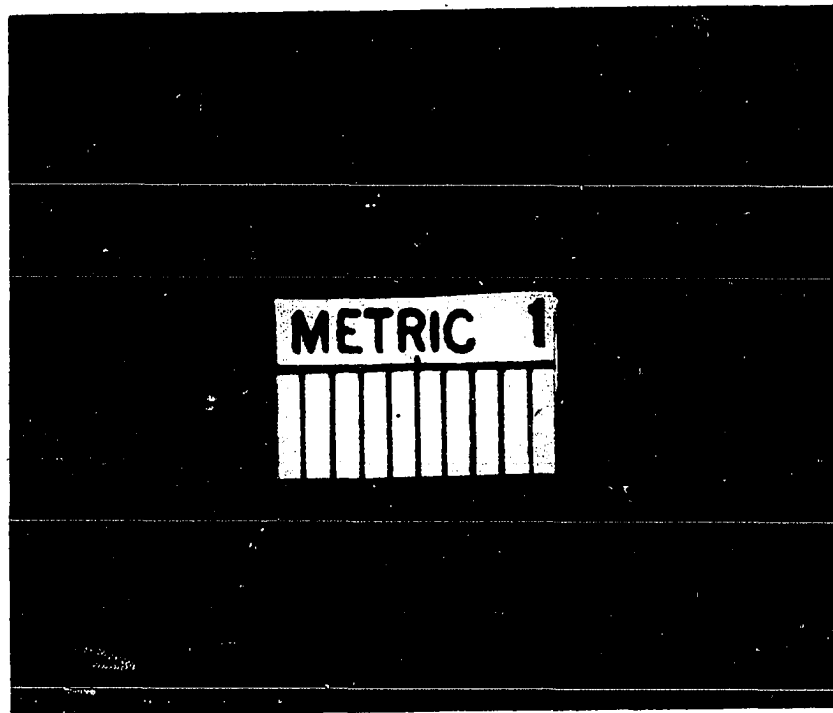after 24 hours under germination conditions

Figure 4. Dormant caryopses of _Setaria lutescens_ after
ten days under germination conditions. Note
the lack of elongation

Figure 5. Seedlings of _Setaria lutescens_ after two
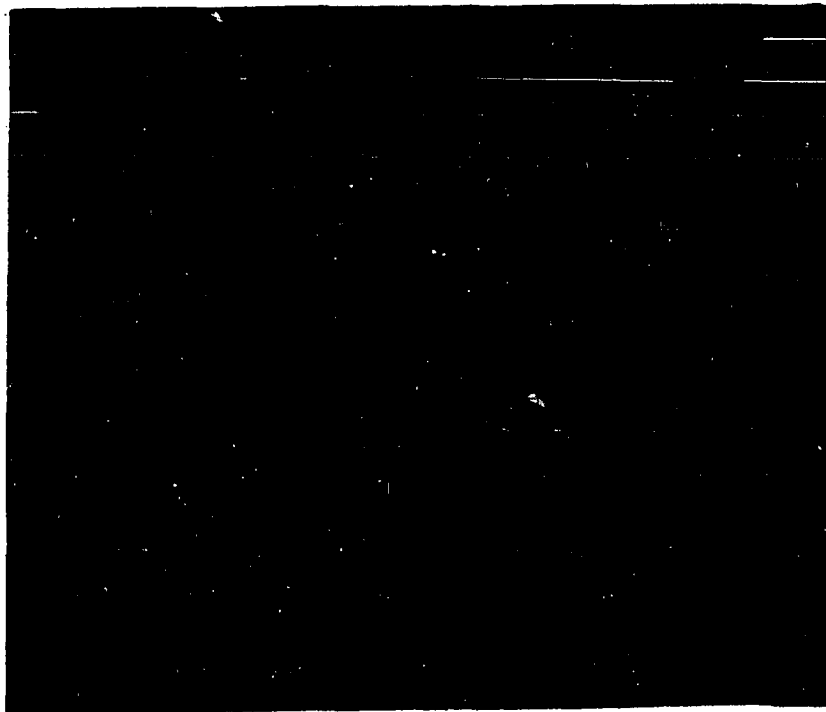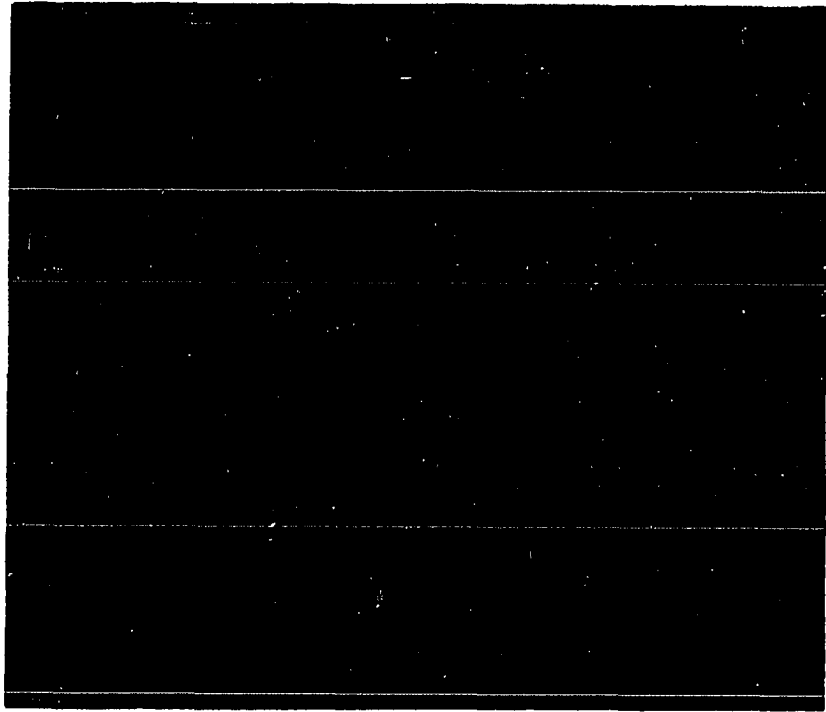days under germination conditions

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Table 5. Germination percentage of caryopses of Setaria
lutescens collected at various intervals during
the growing season of 1962

Collection date	Percentage germination
July 15	66
August 15	16
September 26	10
October 5	5
October 25	5

seeds fall to the ground and are exposed to rainfall and
fluctuating temperatures. The effect of these conditions on
seed dormancy was tested by collecting seed from the soil
surface during November and December. The results of germina-
tion tests of these samples, contained in Table 6, revealed a
high percentage of non-dormant caryopses present in both years.
These results were used in designing experiments to break
artificially the dormancy of freshly harvested seed and seed
stored at low temperatures.

Since seed collected in July were non-dormant and seed
collected in September were dormant, a possible photoperiod
effect was investigated. S. lutescens seedlings were exposed
to 14, 16, and 18 hours of light photoperiod under greenhouse
conditions during the winter of 1961-62. Three pots contain-
ing three plants each were placed in each photoperiod treat-
ment and replicated three times.

Results of this experiment indicated that photoperiod was

Table 6. Germination percentage of <u>Setaria</u> <u>lutescens</u> caryop-
         ses collected from soil surface at several dates
         in 1961 and 1962

Collection date	Percentage germination after six days
November 8, 1961	88
November 10, 1962	70
November 20, 1962	80
December 20, 1962	96

not important in the development of dormancy, since freshly

harvested seeds were highly dormant with all photoperiod

treatments. Differences were obtained in dates of flowering

and panicle production (Table 7). With a 14-hour photoperiod

Table 7. Panicle production from twenty seven <u>S</u>. <u>lutescens</u>
         plants, exposed to photoperiods in the greenhouse

Days after planting	Day length in hours 14	Day length in hours 16	Day length in hours 18
60	4	0	0
70	8	0	0
80	12	0	0
90	20	0	1
100	29	2	4
110	38	6	11
120	47	12	18

the first panicles were observed two months after planting. Photoperiods of sixteen and eighteen hours resulted in the development of panicles three months after planting. Seed set was relatively low with 16 and 18 hours photoperiods.

Experimental Methods of After-Ripening

These experiments were designed to investigate after-ripening effects of relative humidity and to verify the observed effects of fall moisture and temperature conditions on the termination of seed dormancy.

Relative humidity

Solutions of glycerol and water of relative humidities of 32, 50, 87 and 100 percent, prepared according to Washburn (55, p. 291), were used to maintain the different humidities at 25°C. A closed glass system similar to that described by Osborne (38) was used. Three wire baskets containing 500 seeds were placed in a glass tube, 3 cm in diameter, and were exposed to a constant flow of air which was passed first through the appropriate mixture of glycerol and water. A small aquarium pump was used to maintain air flow in each closed system.

Initially, and after 1, 2, 4, 12, and 24 weeks seeds were removed from the glass tubes and moisture and germination percentages were obtained. The results of the germination
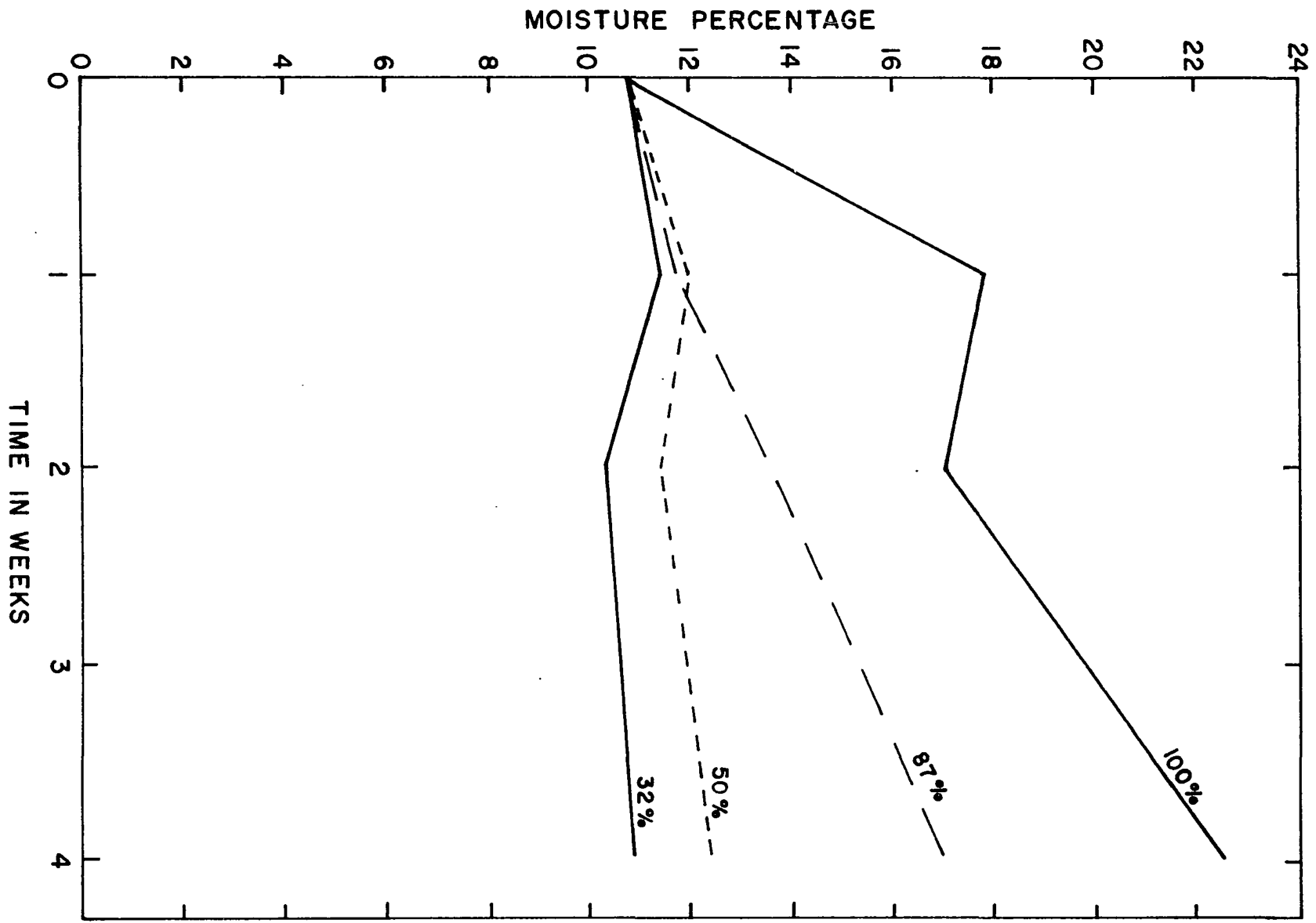
tests are presented in Table 8. Higher relative humidities of 87 and 100 percent increased the germination in the first two weeks, but exposure of seeds at 100 percent relative humidity for 24 weeks decreased germination to zero percent; possibly the seeds were killed. At 32 percent relative humidity germination was not increased. The results suggest that high relative humidity at 25°C is not a good method for after-ripening seeds of Setaria lutescens.

Moisture percentages of intact seed, on dry weight basis, were obtained from triplicate samples of 100 seeds each. Results are illustrated in Figure 6. The initial moisture percentage was 10.8 percent. No major changes were observed in seeds stored at 32 percent relative humidity. There was only a slight change at 50 percent relative humidity. When seeds were subjected to 100 percent relative humidity, the moisture percentage went up from 10.8 to 17.9 percent in one

Table 8. Effects of relative humidity on the germination of Setaria lutescens caryopses. Values in percentages

| Relative humidity | Exposure time in weeks | | | | | |
	0	1	2	4	12	24
32	30	41	18	28	20	30
50	30	45	53	48	53	51
87	30	66	73	48	53	61
100	30	68	75	50	43	0

Figure 6. Moisture uptake over a four-week period for
        entire freshly-matured seeds of S. lutescens,
        stored at 32, 50, 87 and 100 relative
        humidities

week, and after four weeks increased to 22.5 percent. Water uptake was rapid in the first week, fell off during the second week, and then increased steadily with storage at 100 percent relative humidity.

Low temperature stratification

Low temperature stratification is one of the most effective methods of terminating seed dormancy, and is essentially imitative of fall weather conditions in temperate zones. Dormant seeds harvested in 1962 were mixed with soil in wire baskets and exposed to storage temperatures of 0, 5, 10, 20, and 25°C. Additional variables were dry and moist sterilized and non-sterilized soil. After twelve weeks storage, seeds were removed and germination percentages of caryopses were obtained. The results are summarized in Table 9. The results show that germination was stimulated with storage at 0° to 10°C

Table 9. Germination percentages of caryopses of S. lutescens, 1962 seed lot after storage for 12 weeks in moist or dry soil at five temperatures

| Soil conditions | Storage temperatures °C | | | | |
	0	5	10	20	25
Sterilized dry	0	0	8	8	8
Sterilized wet	80	68	84	60	56
Non-sterilized dry	4	0	8	4	4
Non-sterilized wet	30	44	80	28	16
None dry	0	0	0	8	8

under moist conditions, but low germination was observed at
the same temperatures under dry conditions; indicating clearly
that moisture was necessary to terminate dormancy in this
species. Seed, exposed to air without the addition of soil
remained dormant. Higher temperatures improved germination
under moist conditions, but the optimum temperature was 10°C.
Dry seed exposed without soil indicated the effects of soil
in maintaining a moist environment. Storage in moist soil at
10°C, terminated dormancy effectively and roughly duplicated
the results obtained under fall field conditions in other
tests. This experiment demonstrated the importance of mois-
ture in breaking dormancy and indicated that 10°C was optimum
for this seed lot. Germination was somewhat lower when seed
were stored in moist non-sterile soil. None of the seeds
germinated under storage conditions, even at 20 and 25°C,
demonstrating the inhibitory or delaying effect of the hulls
on caryopsis germination.

In a follow-up experiment, dormant seeds of 1962 seed lot
were stored at various depths in the soil, during the period
September to December, 1963. The seeds were mixed with moist
soil and contained in small wire basket. Seeds were removed
at 2-week intervals, the hulls removed and germination tests
run on the caryopses. These data, summarized in Figure 7,
showed that maximum germination was obtained after eight weeks
storage, and indicated that depth of burial was not critical.

Figure 7.   Germination of S. lutescens caryopses, 1962
            seed lot, following storage at various soil
            depths in the field during October and
            November, 1962

Dormancy was terminated effectively by the cool, moist storage conditions encountered.

Additional methods, evaluated in this study, proved ineffective. These included exposure of entire dormant seeds to boiling water, 80% ethanol, benzene, Skelly A and chloroform-methanol mixtures. Some of these treatments decreased seed viability as tested by isolated embryo cultures, but did not terminate caryopsis dormancy. The use of activated charcoal as a germination medium for dormant seeds did not stimulate germination.

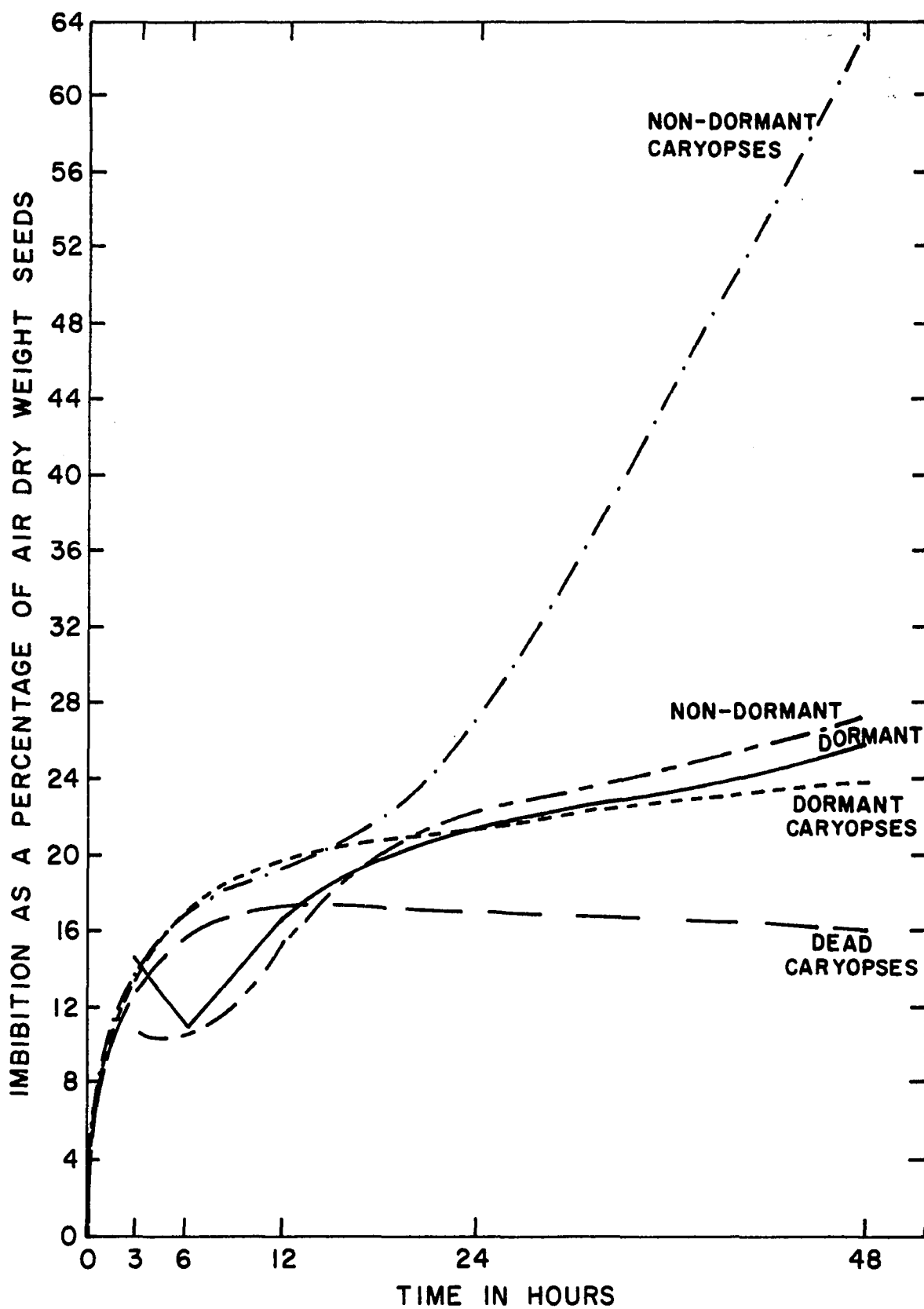# BIOCHEMICAL CHARACTERIZATION OF DORMANCY
IN SETARIA LUTESCENS

The observed patterns of seed dormancy encountered in S.
lutescens, the availability of both dormant and non-dormant
caryopses, and the absence of embryo dormancy, led to study
of the endosperm in an attempt to characterize dormancy on a
biochemical basis. These experiments included the determina-
tion of water uptake and respiration rates, as well as esti-
mates of sugar fractions, organic acid components, and lipid
fractions. Differences in chemical composition of dormant and
non-dormant seeds were negligible, except for an unidentified
compound present in the endosperm of dormant seeds. The
occurrence of this compound could not be associated directly
with the dormant condition, except as it was present in
significantly larger quantities in dormant seeds.

Seed lots used, were 1960 non-dormant, 1959 non-viable
and 1962 dormant. Determinations were made at the beginning
and the end of a 48-hour germination period. The 1959 seed
lot was not viable, either by seed germination or excised
embryo tests. It was included in these experiments in an
attempt to explain the non-viable condition and to determine
whether the seed was dead or merely in a different condition
of dormancy.

Water Uptake Studies

Absorption of water precedes germination, but germination may not always follow water uptake. Water uptake at 25°C by bare caryopses and by entire seeds was determined for four experimental seed lots. Three samples of 25 seeds from each lot were weighed and placed in petri dishes containing filter paper and water. Seed samples were removed and weighed after 3, 6, 12, 24 and 48 hours exposure to water. The imbibed seeds were surface dried before weighing. The results are summarized in Figure 8. Water absorption in dormant and dead seeds reached a maximum after 12 hours, and there was little change during the remainder of the 48-hour period. Non-dormant caryopses showed a rapid uptake of water associated with germination and radicle emergence. Water uptake curves for dormant and non-dormant entire seeds were almost identical and except for an unexplained drop in moisture level during the 3-6 hour exposure period were not appreciably different from that observed with caryopses of dormant seed. The similarity of the curves during the 24-48 hour period indicates strongly that impermeability of the hull to water is not an important factor in the maintenance of dormancy. The considerable lag in water uptake by non-dormant entire seeds as compared with naked caryopses was reflected in much slower rates of germination. This lag in water uptake and delay in germination may be due to inhibitors present in the hulls, although results

Figure 8. Water absorption curves for various lots of
          entire seed and caryopses of S. lutescens

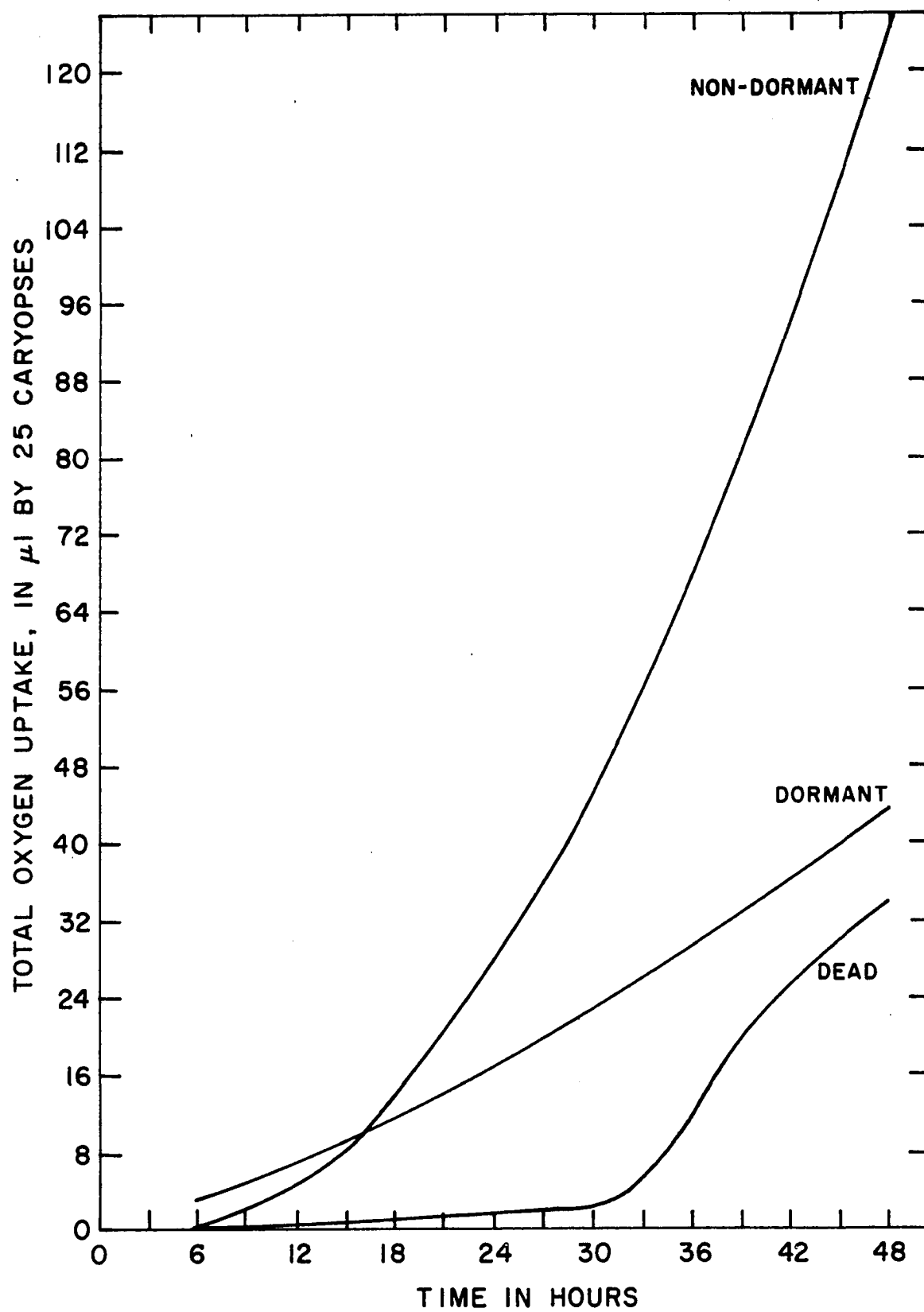from leaching experiments supported this explanation only
partially.

Respiration Studies

Rates of oxygen uptake were determined for dormant, non-
dormant and dead seeds. Hulls were removed and the caryopses
surface sterilized in the same calcium hypochlorite solution
used in embryo culture experiments. Duplicate samples of 25
caryopses were placed in Warburg vessels containing 0.5 ml of
sterile water. Readings were taken at one-hour intervals for
a period of 48 hours, at a temperature of 27°C, and under
light. The results are illustrated in Figure 9. Non-dormant
caryopses show a rapid oxygen uptake while dormant seeds
showed a steady rate of oxygen uptake over the 48 hour period.
The indicated oxygen uptake for dead seeds probably reflected
respiration of microorganisms not destroyed by surface steri-
lization of caryopses.

Carbohydrate Constituents of Seeds

Quantitative determinations of reducing sugars (glucose)
and the non-reducing sugars (sucrose) hydrolyzed by invertase,
were obtained for dormant, non-dormant, and dead caryopses of
S. lutescens. Triplicate samples of 100 caryopses each,
ground to pass a 40 mesh screen, were placed in a semi-micro
Soxhlet apparatus and refluxed for eight hours with 20 ml of

Figure 9. Oxygen uptake curves for various lots of
          caryopses of S. lutescens

80 percent ethanol. The extract was evaporated almost to dryness, 15 ml of water added, and the sample was cleared with 2 drops of saturated neutral lead acetate. The extract was filtered and taken to a volume of 25 ml in a volumetric flask containing 1 ml of dipotassium phosphate (K_2HPO_4, 125 g/l) to precipitate any excess of lead. Flasks were placed in the refrigerator for five hours until the lead phosphate precipitate had settled.

Reducing sugar determinations were made with duplicate samples of 5 ml of the cleared, deleaded extract using the ceric sulfate method of Hassid (26, 27). Five milliliters of alkaline potassium ferricyanide was added to 5 ml portions of ethanol extract in an 8-inch test tube. The solution was heated in a water bath at 100°C for 15 minutes. Tubes were cooled for 4 minutes in tap water and 5 ml of 5 N. H_2SO_4 was added to the samples, and they were titrated with ceric sulfate. Reducing sugars were estimated from weekly prepared standard glucose curves. Results were expressed as milligrams of glucose per 100 seeds.

Sucrose determinations were made with duplicate samples of 5 ml of cleared, deleaded extract. Sucrose was hydrolyzed, at room temperature for 18 hours, with 1 drop of yeast invertase. Titration was done with ceric sulfate and sucrose content determined by subtraction. Standard sucrose curves were prepared weekly. Results were expressed as milligrams of

sucrose per 100 seeds. The results summarized in Table 10 revealed no difference in sucrose or glucose content between dormant and non-dormant caryopses, and indicated that non-reducing sugars were slightly more abundant than reducing sugars. Dead caryopses had little or no sucrose, but content of reducing sugars was about double that present in either dormant or non-dormant caryopses. These data suggested that the sucrose fraction had been hydrolyzed in dead 1959 seed lot.

Experiments to duplicate the 1959 seed lot, by killing seed were undertaken. Non-dormant 1960 seeds, were soaked in water for 18 to 20 hours to raise the moisture percentages approximately from 10 to 18 percent. Soaked and dried seeds were stored for 5 days in incubators at 35°, 45°, and 50°C. Samples were taken after 5 days and germination percentages recorded. The results in Table 11 show that germination of moist seeds was reduced markedly after storage at 45° and 50°C.

Table 10. Reducing (glucose) and non-reducing sugars (sucrose) in dormant, non-dormant and dead caryopses of S. lutescens

Year of harvest	Condition of caryopses	Sugar content in mg/100 caryopses		
		Reducing	Non-reducing	Total
1959	dead	4.15	0.00	4.15
1960	non-dormant	2.10	2.50	4.60
1961	dormant	1.98	2.80	4.78

Table 11. Effect of temperature on germination of soaked and dry seeds of S. lutescens. Non-dormant seeds were used

Temperature	Condition of caryopses	Percentage germination
35°C	dry	98
35°C	soaked	98
45°C	dry	88
45°C	soaked	52
50°C	dry	80
50°C	soaked	0

However, these temperatures were not detrimental to germination of dry seeds. Germination was checked 10 days later and it was found that moist seeds at 45°C had 0 percent germination and dry seeds 92 percent. Sugar determinations on these same seed lots revealed the same pattern as observed with 1959 seeds.

Sucrose content was decreased markedly and glucose was doubled in killed seeds. These results suggest that moist seeds were killed at 45° to 50°C, and the sucrose hydrolized.

In a further experiment, the changes in glucose and sucrose content were determined after the first 48 hours of exposure to germination conditions. Dormant, non-dormant, and dead caryopses were placed in a petri dish containing 2 filter papers and 5 ml of water. Seedlings and non-germinated caryopses were removed at the 48th hour, dried at room temperature,

and then ground, extracted and analyzed for glucose and sucrose. The results in Table 12 showed a marked decrease in the sugar content of dead caryopses, after 48 hours exposure to germination. This resulted probably from extensive organism growth on dead seeds. Glucose and sucrose content of dormant caryopses was decreased, indicating an increased rate of metabolic activity, despite low germination percentages. Non-dormant seeds showed an increase in glucose and slight increase in sucrose; this increase in sugars could be due to hydrolysis of starch.

Fatty Acids

Long chain fatty acid constituents were determined for dormant (1961), non-dormant (1960) and dead (1959) seeds of S. lutescens. Analyses were made on the dry caryopses and of caryopses or seedlings after 48 hours exposure to germination

Table 12. Carbohydrate changes during the first 48 hours of germination of dormant, non-dormant and dead caryopses of S. lutescens

Year of harvest	Condition of caryopses	Sugar content in mg/100 seeds		
		Glucose	Sucrose	Total
1959	dead	.70	.01	.71
1960	non-dormant	3.15	2.01	5.16
1962	dormant	1.40	2.50	3.90

conditions. One hundred caryopses or seedlings were ground and extracted three times in 10 ml of benzene, the extract was evaporated and methylated following the procedure described by Selvey (45). Fatty acid extracts were transferred to reaction flasks with 2 ml of hexane which was then evaporated to dryness with rotary evaporator at 50°C. Two ml of methanol containing two percent sulfuric acid were added to the dry residue and the flask stopper was fastened securely with a rubber band and placed in an oven at 55°C for two hours. The flask was removed, 1 ml of distilled water was then added, shaken, and 0.2 ml of hexane were added, shaken, and enough water was added to bring the hexane-lipid portion up into the neck of the reaction flask.

Ten microliters of the hexane layer were injected to a Perkin-Elmer vapor fractometer Model 154. The chromatogram was developed and the area under the curve for each fatty acid in each chromatogram was computed. These were added to obtain the total area under the curves and the data for each fatty acid were expressed as a percentage of the total area. Data are contained in Table 13. Total content of long chain fatty acids was not significantly different for dormant, non-dormant and dead caryopses. Three most abundant fatty acids were linoleic, oleic and palmitic; stearic and linolenic acids were present in small amounts. No differences in the percentages of each acid in dormant, non-dormant and dead seeds were

Table 13. Long chain fatty acid content on dormant, non-dormant and dead caryopses of S. lutescens. The results are expressed as percentage from the total content

Year of harvest	Condition of caryopses	Fatty acid content in percentage of the total area under the curve					Total area under the curve
		Palmitic	Stearic	Oleic	Linoleic	Linolenic	
1959	dead	6.0	0.98	15.20	76.50	0.97	836
1960	non-dormant	5.45	0.61	15.30	77.00	1.25	978
1961	dormant	5.50	0.62	14.95	78.4	0.62	964

Table 14. Long chain fatty acid content, of dormant, non-dormant, and dead caryopses of S. lutescens, after 48 hours exposure to germination. Results are expressed in percentages of the total areas under the curve

Year of harvest	Condition of caryopses	Fatty acid content in percentage of the total area under the curve					Total area under the curve
		Palmitic	Stearic	Oleic	Linoleic	Linolenic	
1959	dead	6.30	1.20	14.00	77.30	1.60	1123
1960	non-dormant	6.20	1.35	15.35	75.60	1.50	955
1961	dormant	5.80	1.10	15.20	76.50	1.40	884

observed. Linoleic and oleic acids had not been oxidized in the dead seeds.

Changes which occurred in the fatty acid content during the first 48 hours of exposure of seeds to germination are summarized in Table 14. Fatty acid consumption during the first 48 hours is not significantly different in any of the seed lots investigated.

Further Qualitative Chemical Characterizations

Additional attempts to characterize dormant and non-dormant caryopses on the basis of meaningful biochemical differences were limited to qualitative determinations, using the techniques of paper chromatography and elementary spectrophotometric analysis. Caryopses before germination and seedlings or ungerminated caryopses after 48 hours exposure to germination conditions were utilized for comparisons of non-dormant, dormant and dead seed lots.

Paper chromatography

Ground samples of experimental caryopses were refluxed for 30 minutes with 25 ml of 80 percent ethanol, centrifuged, washed three times with 10 ml of benzene to remove fats, and the supernatant evaporated almost to dryness. The volumes were then taken to five ml and passed through a set of two ion exchange columns, each 20 cm long and 8 mm in diameter,

arranged with one column dripping into the second. One hundred ml of deionized water were added and free amino acids were isolated on the top column, organic acids were isolated in the bottom column and sugars passed through in the percolate from the columns. Amberlite IR-120 C. P. was used in the top column and Amberlite IR-45 was used in the bottom one, according to the methods of Racusen (42) and Aronoff (1). Amino acids were eluted with 100 ml of 3N, NH4OH and organic acids were eluted with 100 ml of 3 percent HCl. The three fractions (carbohydrates, amino acids and organic acids) were evaporated to dryness under vacuum and washed and evaporated three more times with 5 ml of deionized water. The fractions were transferred to 5 ml vials, evaporated once more and stored at 5°C.

Qualitative assays of the sugar and organic acid fractions were carried out with paper chromatography methods. Preliminary tests with the amino acid fraction indicated serious problems in technique, and no further effort was expended.

Sugars

The sugar fraction was dissolved in water and spotted on Whatman No. 1 filter paper. The technique was one-dimensional, double run, using a solvent composed of five parts n-butanol, one part acetic acid and 2 parts water, described by Hough

(29). Color was developed by the methods described in Experiments in Biochemistry (22). Sugars were identified by comparison with known sugars.

Sugars identified in all three seed lots, both before and after the 48 hour germination period were fructose, glucose, sucrose, raffinose, and two unidentified oligosaccharides with low Rf values. Sucrose was present in non-dormant and dormant caryopses, but not in dead caryopses. Glucose was most abundant in all seed lots at both times of sampling. These results indicate that dormancy was not associated with a distinctive pattern of sugar components in the carbohydrate fraction.

Organic acids

Organic acids were identified by one dimensional paper chromatography. The solvent was formulated with ethyl formate, formic acid and water, in the proportions 12:5:3 respectively, with 0.015 percent w/v of bromophenol blue and 0.05 percent v/v of sodium formate added, according to the method described by Hartley (25). Three major acids detected were citric, malic and isocitric; malic acid was the most abundant. These three acids were present in all three seed lots, non-dormant, dormant and dead, and no major changes in the pattern were observed after 48 hours exposure to germination.

The results from experiments with the organic acid and

sugar fractions, indicated that on the basis of these routine preliminary qualitative tests, there were no important differences by which the dormant caryopsis condition could be characterized positively. However when the lipid fraction from dormant and non-dormant caryopses was exposed to ultra violet light, white fluorescence was observed. This prompted spectrophotometric analyses of the lipid fractions.

Spectrophotometric Analysis of Lipid Fractions

Preliminary tests of lipid fractions were done on a Cary recording spectrophotometer, Model 14. When the spectrum of each solution was measured in 1.00 cm path-length cuvettes in the range of 250 mμ to 700 mμ, two independent absorption peaks were noted, one at 280 mμ and one at 315 mμ. When the ratio of the absorbancy at 280 mμ to that at 315 mμ was calculated, it was significantly greater for the lipid fraction from dormant caryopses than for that from non-dormant caryopses. Further experiments demonstrated that it was not necessary to remove the lipid fraction from the eighty percent ethanol extract prior to spectrophotometric analysis. The following procedure was used in further experiments with dormant and non-dormant caryopses.

1. 100 caryopses from dormant and non-dormant seeds were ground to pass a 40 mesh screen, and placed in a 40 ml centrifuge tube.

2. Ten ml of 80 percent ethanol were added and stirred
   for one minute with an air-driven stirrer. The
   homogenate was centrifuged at 3000 rpm for ten minutes.

3. The supernatant was retained, 10 ml of the 80 percent
   ethanol was added to the residue and the sample was
   centrifuged again. The extractions were carried
   three times.

4. The supernatants from the three successive extrac-
   tions were combined and brought to a 100 ml volume
   with 80 percent ethanol.

5. Readings were taken in the range 275-320 mμ on either
   a Cary recording or a Beckman DU spectrophotometer.

6. The ratios of readings at 280 and 315 mμ were com-
   puted and the values were analyzed statistically.
   Treatment means were compared on the basis of a
   multiple range test described by Snedecor (47).

Additional procedures included: use of seed of uniform
moisture percentage, use of a single batch of 80 percent
ethanol for a particular experiment and the adjustment of the
volume of final ethanol extract to a basis of 1 ml per cary-
opsis extracted. Using these techniques, a series of experi-
ments was conducted to compare various seed lots of S.
lutescens.

A comparison of non-dormant, dead and two lots of dormant
seed based on spectrophotometric analysis is summarized in

Table 15. Absorbancy readings were consistently higher at 280 mμ than at 315 mμ for all samples tested.

At the 280 mμ peak, dormant seeds showed highest absorbancy values, and non-dormant the lowest values. When the ratios (280/315) of the values were computed, they were relatively constant among replicates within each experimental seed lot. Higher ratios were associated with dormant seeds and lower ratios with non-dormant and dead seeds. Variance analysis of the ratios reported in Table 16, show highly significant differences between ratios for dormant and non-dormant seed. There was no difference between the two lots of dormant seed.

In a second experiment caryopses from two lots of non-dormant seed and one lot of dormant seed were extracted and analyzed by the spectrophotometric method. The results are summarized on Table 17. The calculated ratios were higher than in the previous experiment, but a significant difference between ratios for dormant and non-dormant seeds was observed again.

When a supply of non-dormant 1962 seed became available after exposure under fall field conditions, a similar comparison was made between dormant and non-dormant caryopses from initially identical seed lots. The results, compiled on Table 18, demonstrated once more the distinct differences among the calculated ratios for dormant and non-dormant caryopses. The

Table 15. Spectrophotometric absorption at 280 mμ and 315 mμ for ethanol soluble fractions from dormant and non-dormant seeds of S. lutescens

| Year of harvest | Condition of seed | Rep 1 | | Rep 2 | | Rep 3 | | Rep 4 | | Calculated ratios A280 mμ/A315 mμ |
		280 mμ	315 mμ	280 mμ	315 mμ	280 mμ	315 mμ	280 mμ	315 mμ	Mean*
1959	Dead	530	340	540	350	447	300	540	352	1.53[a]
1960	Non-dormant	455	278	470	291	375	245	417	265	1.58[a]
1961	Dormant	498	275	590	305	483	247	499	244	1.93[b]
1962	Highly dormant	650	335	704	352	545	291	580	306	1.93[b]

*Ratios indicated by the same letter were not significantly different at P = 0.05.

Table 16. Analysis of variance of values for the ratio
(280 mμ/315 mμ) of readings of spectrophotometric
analysis of the 80 percent ethanol fraction of
dormant, non-dormant and dead seeds of **S. lutescens**

Source of variation	Degrees of freedom	Sum of squares	Mean square	F
Replications	3	0.009	0.003	
Treatments	3	0.561	0.187	46.75**
Error	9	0.036	0.004	
Total	15	0.606		

**Significant at P = 0.01.

ratio value for non-dormant 1962 seed was higher than for 1960

or 1961 non-dormant seed lots, and further checking revealed

that the 1962 non-dormant seed lot had a higher moisture per-

centage than other lots when caryopses were extracted.

The experiment was repeated, using caryopses which had

been dried at 100°C for 24 hours prior to ethanol extraction.

The data, summarized in Table 18, confirm previous results and

show the importance of uniform moisture percentages in experi-

mental seed lots when analyzed by this method. There were no

differences in ratio values among non-dormant lots and all

were significantly lower than those obtained for dormant seed.

These data suggest then, the presence of an unidentified

substance which absorbs light at 280 mμ. The substance was

not isolated, but its presence was indicated by spectrophoto-

Table 17. Spectrophotometric absorption at 280 mμ and 315 mμ, for ethanol soluble fractions from dormant and non-dormant caryopses of S. lutescens

Year of harvest	Condition of seed	Rep 1		Rep 2		Rep 3		Rep 4		Calculated ratios 280 mμ/315 mμ Mean*
		280 mμ	315 mμ	280 mμ	315 mμ	280 mμ	315 mμ	280 mμ	315 mμ	
1960	non-dormant	327	130	260	100	180	67	280	97	2.66[a]
1961	non-dormant	314	131	309	130	265	104	276	117	2.42[a]
1962	dormant	409	129	437	120	414	102	380	86	3.82[b]

*Ratios indicated by the same letter were not different significantly at P = 0.05.

Table 18. Calculated values for ratios of readings at 280 mμ and 315 mμ, for three caryopses lots of S. lutescens, with and without drying at 100°C prior to ethanol extraction. Values are averages of four replicates

| Year of harvest | Condition of seed | Calculated values for absorbancy ratios A280 mμ/A315 mμ Treatment before extraction* | |
		None	100°C for 24 hours
1960	non-dormant	1.90[a]	1.98[a]
1961	non-dormant	1.95[ab]	1.81[a]
1962	non-dormant	2.39[b]	1.90[a]
1961	dormant	2.86[c]	2.35[b]
1962	dormant	3.31[c]	2.84[b]

*Ratios indicated by the same letter were not different significantly at P = 0.05.

metric analysis. The significantly greater amounts of the material indicated in dormant seed does not prove that it is a factor in the maintenance of dormancy. The substance was located in the endosperm, was not detected in the embryo and did not diffuse to the embryo during the 48 hour exposure of caryopses to germination conditions. It was soluble in 80 percent ethanol, in benzene, in Skelly A and in a lipid solvent composed of a mixture of two parts chloroform and one part methanol, described by Folch (23). It was insoluble in water and in acetone. Where caryopses were sectioned by

freezing microtome and the sections examined under a fluorescence microscope, the fluorescence was observed to be in the cell walls of the endosperm tissue.

DISCUSSION

At least two mechanisms are operative in the maintenance of seed dormancy in <u>Setaria</u> <u>lutescens</u>. One is conditioned by the caryopsis, the other by the hull. Most samples of freshly matured seeds had a high percentage of dormant caryopses which did not germinate when the hulls were removed. Caryopsis dormancy was terminated readily by the combination of low temperature and high moisture, but seeds after-ripened in this way did not germinate appreciably until the hulls were removed. Dormancy imposed by the hulls appeared to result from a combination of effects, including impermeability to water, possible mechanical constriction of the caryopsis, and probably inhibitors in the hull. Water leaching promoted the germination of intact seeds with non-dormant caryopses, but the leachate did not inhibit germination of non-dormant caryopses. Isolated embryos from either dormant or non-dormant caryopses grew normally on nutrient medium. This suggests that embryo dormancy is not a factor in seed dormancy with <u>S</u>. <u>lutescens</u>, but does not exclude the possibility that excision itself in some way stimulates the embryo to growth.

Evidence from this study suggests that <u>S</u>. <u>lutescens</u> has a seed dormancy pattern comparable to that of many temperate zone, annual weeds. Freshly matured seeds are dormant, even though the isolated embryos will grow. Both hull and caryopsis

dormancy are evident at this stage. After-ripening at low temperatures and high moisture terminates the caryopsis dormancy under field conditions in the fall, in eight weeks or less. Depth of seed placement in the soil in the field did not modify the effectiveness of the after-ripening process under cool, moist conditions. This is in agreement with the observations of Dawson and Bruns (18) from their depth of seeding studies with S. lutescens and other annual grass weeds. The effects of hulls and environment, however prevent germination until the following spring. This pattern of dormancy is comparable to that observed by LaCroix (32) in Polygonum pensylvanicum, except that dry storage of S. lutescens did not result in the development of embryo dormancy as reported by LaCroix. The intense caryopsis dormancy encountered in S. lutescens, persisted for at least 18 months under cool, dry storage, with no evidence of embryo dormancy. Pollock and Toole (41) have discussed a similar type of dormancy in Ambrosia artemesiifolia. Seeds of this species mature in late summer and remain dormant until the germination block is removed by the low temperature storage conditions of the winter.

Among the seed lots available during this study, the 1960 lot showed a complete lack of caryopsis dormancy. The exact conditions under which this 1960 lot had matured and was harvested could not be determined. However, occurrence of this seed lot with non-dormant caryopses suggests that under some

conditions, non-dormant seed may be produced, although it was not accomplished experimentally in this study. The lack of dormancy in freshly harvested seed has been noted by other investigators, including Bibbey (10), and Thurston (51) who harvested non-dormant <u>Avena fatua</u> in some seasons. The ready availability of adequate supplies of <u>S</u>. <u>lutescens</u> seed with either dormant or non-dormant caryopses and the lack of embryo dormancy in either, prompted further study of the chemical composition of the endosperm.

Attempts to characterize dormancy or the lack of it, on the basis of biochemical differences, is an approach which has received only limited attention from investigators of seed dormancy. Barton (5), writing in 1961, states that, "in spite of the great amount of work done in the field of seed physiology comparatively little has dealt with the biochemical changes accompanying germination process." Numerous investigators, including Eckerson (19) 1913, and Pack (40) 1921, have suggested possible differences in the metabolic mechanisms of dormant and non-dormant seeds, but none has offered conclusive proof that the observed changes which occur during the transition of a dormant seed to a germinating one, were anything more than the symptoms and end products of processes which were not measured in the studies reported.

The present work with <u>S</u>. <u>lutescens</u> has not revealed any striking differences in the chemical constituents of dormant

and non-dormant caryopses, except to suggest the occurrence
of an unidentified and not yet isolated substance in the lipid
fraction of caryopses, and to indicate its presence in signif-
icantly higher quantities in dormant seeds than in non-dormant.
Other compounds characterized by chemical analyses were pres-
ent in both dormant and non-dormant caryopses and there was no
indication of a significant difference, either in quantity or
quality which might serve to describe the dormant condition
in biochemical terms. The carbohydrate fraction contained
mainly starch, glucose, fructose, sucrose and raffinose. Dur-
ing the first 48 hours of germination there was an increase in
free sugars in non-dormant seeds, resulting probably from
starch digestion. Barton (5) 1961 found the same sugars in
Paeonia suffruticosa. While S. lutescens is a starchy seed,
quantitative analyses indicated a small but uniform consump-
tion of the major fatty acids, oleic, linoleic, and palmitic,
during the first 48 hours of germination. Similar results
were observed by Barton (7) and Stiles (49). They found that
the small reserves of fats in the germinating seed were con-
sumed during early stages of seedling development even though
starch was the principal food reserve of the seed.

While the results of this study of S. lutescens indicated
no significant differences in the carbohydrate, organic acid,
and fatty acid fractions of dormant and non-dormant caryopses,
the possibility of such differences remains. The techniques

employed may not detect the important differences or compounds which may be synthesized differentially, but utilized immediately.

The absence of sucrose in the non-viable 1959 seed lot appears unimportant to the biochemical characterization of dormancy. When dormant and non-dormant seed were moistened and exposed to 50°C temperature the seed was rendered non-viable and sucrose was absent. The oxygen uptake curves and the carbohydrate depletion rates for these seeds suggested microbial activity. Similar results were noted by Milner and Geddes (37). These results with 1959 dead seed and the experimentally killed non-dormant seed suggest that freshly harvested mature seeds may be killed by careless handling and storage. Such conditions would occur rarely, if ever under natural conditions.

The unidentified substance detected by spectrophotometric analyses of the lipid fractions from caryopses, was present in significantly greater amounts in dormant seeds than in non-dormant. The substance was not detected in the embryo. It was not isolated and hence was not characterized beyond its presence in the lipid fraction of the endosperm tissue. Its role, if any, in the maintenance of caryopsis dormancy is not clear from the present work. Speculation on this point suggests two possible mechanisms, involving either inhibition of germination or a modifying effect on cytoplasmic permeability

of cells.

The case for possible growth inhibitors in the endosperm is weakened considerably by the evidence that there was no diffusion of inhibitors to the embryo when dormant caryopses were soaked for a number of hours prior to excision of embryos. Furthermore, the embryos of dormant caryopses showed some cell division and limited growth, when exposed to moisture and temperature conditions conducive to germination of non-dormant caryopses. When such embryos were isolated, they grew normally on the nutrient medium. This suggests more likely then a possible mechanism whereby the substance may modify the permeability of cells in the region of the scutellum and thus interfere with the movement or even the synthesis of plant foods stored in the endosperm. Water uptake curves suggested that there was little difference in the water uptake of dormant and non-dormant seeds, provided the hulls were intact.

This study has clarified the general patterns of seed dormancy in S. lutescens, has developed the major techniques and procedures for the harvest, storage and after-ripening of seed, and has delineated the more promising areas for future research. Biochemical studies have demonstrated the probable futility of trying to characterize dormancy on the basis of the usual sugar, fatty acid and organic acid components of the metabolic systems usually encountered. The changes observed in these components are probably incidental to, or the result

of, other mechanisms which control the termination of seed dormancy. The presence of the unidentified substance detected by spectrophotometric analysis may also be merely incidental to the dormant condition.

Under natural conditions in the field, a majority of S. lutescens seed are ready to germinate in the spring following the year of maturation. The fate of seeds which do not germinate in the spring, the factors which limit complete germination of a given seasonal crop and the dormancy characteristics of those seeds which do not germinate appear important areas for future research. The mechanisms which maintained caryopsis dormancy in dry storage under laboratory conditions may not necessarily be characteristic of dormancy in the field, but a further clarification of the dormancy characteristics of this seed seems a reasonable initial approach.

The wide variation in dormancy among seed lots encountered in this study underlies the importance of using seed which exhibits a dormancy pattern characteristic of the species, and suggests the dangers which may attend the use of seed harvested and stored under conditions which fail to simulate the natural environment. A further difficulty encountered was the tendency for germination conditions to contribute to the termination of dormancy whenever extended germination periods were used.

SUMMARY

1.   Seed dormancy in <u>Setaria</u> <u>lutescens</u> is maintained by the separate and combined effects of both hulls and endosperm. Embryo dormancy was not a factor in the seed dormancy of this species.

2.   The patterns of seed dormancy observed and the effectiveness of natural and simulated conditions of cool, moist storage or exposure in terminating dormancy suggest this probable sequence under field conditions. Matured seed falls to the ground in a dormant condition. Exposure to cool, moist, autumn climate of the temperate zone promotes the termination of caryopsis dormancy. The inhibitory effects of the hull and the adverse environmental conditions prevent germination until spring. In this study, 90 percent or more of seed left outside over winter on the ground, germinated in the spring. This suggests that failure of seed to germinate in the spring following the year of maturation is not due primarily to seed dormancy.

3.   The occasional collection of mature seed lots with non-dormant caryopses indicated that the development of caryopsis dormancy was conditioned by environmental factors during maturation. Attempts to reproduce such conditions experimentally were not successful.

4.   The complete lack of embryo dormancy in the presence of

both caryopsis- and hull-conditioned dormancy suggests the presence of inhibitors in these regions of the seed. Leaching experiments did not confirm the presence of such inhibitors. The fact that isolated embryos grew on nutrient medium does not prove conclusively the absence of embryo dormancy, since the physical effects of caryopsis manipulation may have provided a stimulatory effect. The experimental results do, however, support the view that embryo dormancy was not an important characteristic of observed seed dormancy.

5. Attempts to characterize dormancy on the basis of known organic acid, fatty acid and carbohydrate constituents were not successful. Quantitative or qualitative analyses revealed no significant differences in these fractions between dormant and non-dormant caryopses.

6. An unidentified substance, detected by spectrophotometric analyses of the lipid fractions from caryopses, was present in significantly greater amounts in dormant seeds than in non-dormant. The substance was not detected in the embryo. Its role, if any, in the maintenance of caryopsis dormancy is not clear from the present work. Speculation on this point suggested two possible mechanisms. The possible effects of the substance in limiting cell permeability in the region of the scutellum was considered a more probable explanation than one based on growth inhibitor effects.

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