STRUCTURE, PHYSICAL CHARACTERISTICS, AND COMPOSITION OF THE PERICARP AND INTEGUMENT OF JOHNSON GRASS SEED IN RELATION TO ITS PHYSIOLOGY


INTRODUCTION

These investigations were undertaken in hope of explaining some features of the behavior of Johnson grass seeds during their initial dormancy, their period of after-ripening, and their germination. As has been shown elsewhere (15), Johnson grass seeds belong to that group whose well-matured embryos are never appreciably dormant, the dormancy of the intact fruit being imposed by its outer, nonliving structures. These include (1) the closely adhering, hard, brittle scales, (2) the fused pericarp and inner integument. Removal of the former hastens the germination and increases the germination capacity of the caryopses, whether these are freshly gathered, are fully after-ripened, or are in process of after-ripening, but does not appreciably affect the rate of after-ripening. Furthermore, removal of the fused pericarp and integument by corrosion with concentrated sulphuric acid or even its removal over one side of the embryo by means of a sharp needle induces the complete germination within three or four days even of freshly gathered grains which, without such treatment, would scarcely germinate at all in weeks or months.

It has also been shown (15, 16) that the germination of Johnson grass seeds is highly dependent upon the maintenance of alternating temperatures, that this sensitiveness to temperature conditions disappears upon the removal of the seed coverings, and that certain chemical substances exert a stimulating action upon their germination, particularly after the removal of the caryopses from the inclosing scales. These facts suggested that a study of the physical and chemical characteristics of the seeds, and especially of their pericarps and integuments, might help to explain their physiological behavior.

In marked contrast to the dormancy and germination physiology of Johnson grass seeds, Sudan grass seeds germinate very readily at ordinary temperatures, constant or alternating, without the lapse of any considerable period of after-ripening, and without any special preliminary treatment. Sudan grass seed has therefore been used for comparison with Johnson grass seed in the studies herein reported. The greater part of the work was done in the Hull Botanical Laboratory of the University of Chicago, and the remainder in the Seed-Testing Laboratories of the United States Department of Agriculture in Washington.

Both Johnson grass and Sudan grass have been described under several different names, and they have frequently been assigned to different species. Mr. A. S. Hitchcock considers Johnson grass as Holcus halepensis.
L. *Sorghum halepensis* Pois and Sudan grass as subspecies of the same, which he calls *Holcus halepensis sudanensis* (Piper) Hitchcock (*Andropogon halepensis sudanensis* Piper). The very close taxonomic relationship of the two forms increases the interest which attaches to the marked physiological differences between their seeds.

**STRUCTURE OF THE FRUITS AND CARYOPSES OF JOHNSON GRASS**

**I. EXTERNAL APPEARANCE**

Oakley (25), Vinall (30), and Youngblood and Conner (32) discussed the external appearance of the fruits of Johnson grass in comparison with those of Sudan grass. Hillman (17) later described in detail the fruits of these two kinds of grass, both with the scales on and with the scales removed.

Figure I, adapted from figure 4 in Hillman's paper, shows the external characters of the fruit of Johnson grass. The unhulled fruit or spikelet (A) is about 0.2 inch long and a little less than one-half as wide as long. It bears at its proximal end a smooth regular scar (a). The caryopsis (B) with its hyaline lemma is inclosed in two, straw-colored to black overlapping scales, the glumes. The outer scale, or lower glume, is convex and spear-shaped (A, I).

![Diagram of Johnson grass spikelets and caryopses](image)

The inner scale, or upper glume (c), is nearly flat, but slightly keeled. Its outer edge is inclosed by the overturned edge of the outer scale (d). The flattened inner side of the unhulled fruit usually bears two appendages, a rachis segment and the pedicel of a sterile spikelet (b, b). The distal ends of the glumes frequently are jaggedly broken (e) in many of the fruits of commercial lots. We have found that sufficient rubbing to cause a large amount of such breakage or even the very careful cutting away of the distal half of the glumes increases the germination of dormant lots even if none of the caryopses are removed from the scales.

The caryopsis (fig. 1, B) is about three-fourths as long as the unhulled fruit, oval or oval-elliptical in shape, dark reddish brown in color. The side of the caryopsis which was next to the outer scale is nearly flat and bears at its proximal end a small, roundish, very dark, and somewhat depressed area (f) where the pericarp extends across the hilum. At the extreme proximal end is the scar of the caryopsis (g). The side which was next to the inner scale is somewhat convex. At its proximal end is located the fairly large embryo (h), the position of which is clearly marked by a somewhat lighter color than that of the rest of the caryopses.
II. INTERNAL STRUCTURE OF THE CARYOPSIS

A large number of botanists have investigated the embryos of various grasses. Several of these, among whom are Bruns (8), Pammel (26), Guerin (14), Kennedy (19), and Sargant and Arber (27), have described the embryos and other organs of the caryopses of species of Andropogon, Sorghum, and other species of the tribe Andropogoneae.

Figure 2 shows a median sagittal section of a Johnson grass caryopsis. No attempt is made here to distinguish between integument and pericarp, as these structures are discussed and illustrated in later sections.

Besides the axial organs, the rather large embryo (A) includes the scutellum (a), the root sheath, or coleorhiza (i), and the epicotyl sheath, or coleoptile (k). The radicle (g) is directed toward the proximal end of the caryopsis. Its tip is covered by a well-developed root cap (h). The epicotyl (f), with the first two convolute leaves (x₁ and x₂) well formed and the rudiments of later leaves, extends toward the distal end of the caryopsis. Between the radicle and the epicotyl is a short, internode-like structure, the mesocotyl (e), which is variously interpreted as the fused hypocotyl and stalk of the cotyledon (27) or the elongated primary node (31).

The scutellum is expanded laterally into two wings which fold up around and almost wholly envelop the axial organs of the embryo. A portion of the upper edge of one of these wings is shown at (d). Wherever the scutellum is in contact with the starchy endosperm (B) the cells of its lower cell layer are elongated into the form of a columnar epithelium (b), which secretes diastase and possibly other enzymes for the conversion of the stored food of the endosperm. Along the entire length of that portion of the scutellum underlying the mesocotyl and epicotyl extends a central procambium strand (c), which enters the axial organs at the point of their insertion upon the scutellum and which sends off about a dozen small lateral branches and a short branch which extends under the radicle. These branches ramify throughout that face of the scutellum which is in contact with the starchy endosperm. Upon the germination of the caryopsis the elements of the central strand become differentiated, with the formation of spiral tracheae, and the cells of the columnar epithelium greatly elongate. These morphological changes can also be induced by wounding the endosperm region of the caryopsis or by a diseased condition of embryo or endosperm. The elongation of the epithelial cells, either generally over the whole face of the scutellum or locally, is always accompanied by corrosion of the starch grains in the underlying endosperm cells.

Just inside the coverings of the caryopsis (m) and extending entirely around the embryo and endosperm except at the region of the hilum and...
micropyle (p) is the aleurone layer (1), which comprises a single layer of cells.

Between the hilum, the starch-bearing endosperm, and the proximal end of the scutellum lies a light-colored mass of large, irregular, compressed, empty endosperm cells (r).

The coverings of the caryopsis consist of the fused product of the pericarp and inner integument—the latter of a single layer of cells which, at the distal end of the caryopsis, are much elongated and have very thick inner walls. The figure shows these elongated cells of the inner integument (s), a portion of one of the persistent styles (n), and the pedicel (o), with portions of the adhering lodicules. Usually, however, the caryopsis breaks from the pedicel at a point just distal to the insertion of the lodicules, so that these are not present on the hulled caryopsis.

The outer integument and nucellus have entirely disappeared in the mature caryopsis, with the possible exception of a portion of the former overlying the micropyle.

As already stated, complete or partial removal of the coverings over the embryo greatly increases the readiness with which Johnson grass caryopses germinate.

III. DETAIL OF THE PERICARP AND INNER INTEGUMENT

Figure 3 represents the pericarp and inner integument of a Johnson grass caryopsis as seen in cross section about midway from the proximal end to the distal end of the caryopsis. The drawings in this figure were made with camera-lucida and oil immersion lens from paraffin sections 15 microns in thickness, stained with iron alum haematoxylin. The material used had not been bleached or otherwise altered before embedding in the paraffin. Figure 3, A, was drawn from the flat (endosperm) side of the caryopsis opposite the end of the scutellum, and figure 3, B, from the rounded side over the end of the embryo (1) and adjacent endosperm cells (2).

The pericarp consists of several layers of cells of which only the outer epidermis (a) and the inner epidermis (b) are clearly distinguishable. The intervening layers consist of greatly compressed thin-walled cells, in which narrow cell lumena and intercellular spaces appear only at irregular intervals. One of these layers is the chlorophyll-bearing layer of the earlier stages of development of the caryopsis and in the mature fruit frequently contains starch grains. The outer epidermis is continuous and slightly undulating in surface contour and consists of rather large rectangular cells, with relatively thin walls and large lumena. The inner epidermis, as in other Gramineae, is fragmented longitudinally into long tubular cells, extending lengthwise of the caryopsis and connected with each other by their end walls, occasionally by their lateral walls, and by cells extending diagonally at infrequent intervals. In cross section these cells usually appear circular or broadly elliptical as in figure 3.

The inner integument (c) is a single-cell layer, continuous except at the hilum. The cells are much larger than any of the cells of the pericarp.
Their inner walls (d) and side walls (e) are very thick and dense, and dark brown in color; their outer walls are much thinner and less dense. There is a tendency for the outer walls to collapse into the cell lumen, which itself frequently becomes nearly or quite filled with a solidified, granular, slightly brown or yellowish mass of substance.

The outer walls and solidified cell contents are usually difficult to distinguish from each other. Together they are represented by the cross-lined areas (f).

Toward the distal end of the caryopsis the cells of the inner integument gradually increase in size and their inner walls increase in thickness, culminating in the great development shown in figure 2 (s). Over the embryo the inner integument is much thinner and somewhat lighter-colored than over the greater part of the endosperm—a feature which largely accounts for the lighter color of the embryo region of the caryopsis as contrasted with the endosperm region. The integument decreases in thickness also over the flat, or endosperm, side of the caryopsis proximally from the position indicated in figure 3, though on this side of the caryopsis its inner walls are always thick, dense, and very dark-colored. Proximally the inner integument ends in areas of special development at the micropyle and the hilum which will be described in detail in the following pages.

Figures 4 to 8, showing specialized areas of the pericarp and inner integument of Johnson grass caryopses, were all drawn with camera lucida from freezing microtome sections of fully imbibed seeds, the sections having first been decolorized on the slide with Javelle water, stained with methylene blue, and mounted in 75 per cent glycerin solution. The decolorizing process entirely removed the solid contents of the integument cells and somewhat increased the size of all cells. As the aleurone layer usually remained attached to the inner integument in the sections even when nearly all of the endosperm fell out, this layer is shown in the figures representing areas where it is present.

**PERICARP AND INNER INTEGUMENT AT THE DISTAL END OF THE CARYOPSIS**

Figure 4 represents a median sagittal section through the coverings of a Johnson grass caryopsis at its distal end. The inner integument (a) is much thicker than the combined thickness of the pericarp and the aleurone layer, and is much thicker toward the flat (endosperm) side of the caryopsis (A) than toward the embryo side (B), though this relatively great difference does not persist far from the distal end. The inner walls of the integument cells (1) are extremely thick and bear at intervals peculiar knoblike ingrowths (2) into the cell cavity. These ingrowths may be smooth but more frequently are studded with minute points. In the latter case, very infrequently one is found which before bleaching with Javelle water, but never after bleaching, exhibits double refraction in polarized light as if crystalline in structure. These ingrowths occur only near the distal end of the caryopsis. The long cells of the integument at the extreme distal end of the caryopsis sometimes extend far into the style and are broken off with the style by rough handling of the caryopsis. The relatively thin end walls of the integument cells at a little distance from the distal end are sometimes slightly folded (3) as if from the inward pressure of the pericarp, as it dries during maturation of the caryopsis.
The aleurone layer (b) is of relatively thick-walled cells, but the walls are not pigmented. Its thick walls, continuity, and persistent adherence to the integument are of interest in connection with the suggested protective roll of this layer in grass caryopses (7).

Of the pericarp only its outer epidermis (c) is clearly and definitely distinguishable in freezing microtome sections. The figure shows the base of one of the persistent styles (C).

**MICROPYLE AND SURROUNDING STRUCTURES**

Figure 5 represents a median sagittal section through the micropyle of a Johnson grass caryopsis. The position of the micropyle with reference to other structures can be seen by comparing this figure with figure 2. The aleurone layer (fig. 2, l; 5, a) on the embryo side of the caryopsis extends several cells beyond the proximal end of the scutellum (fig. 2, a; 5, b), but falls several integument cells short of reaching the micropyle.

The micropyle itself (fig. 5, c) is closed by the cells of the inner integument (d,d) which has so crowded in from all directions as to become turned back upon itself externally, the cells from opposite directions coming together but without the walls fusing. The inner cells of the double layer thus formed (e) are greatly elongated in a radial direction and have very heavy, densely pigmented inner walls. The cells of the outer layer (f) are considerably shorter and their outer (morphologically inner) walls are thick and heavily pigmented. On the side toward the hilum this reversed layer of inner integument cells extends within 2 or 3 cells of the edge of the hilum (g) and forms a conspicuous hump.
the opposite direction it extends only about half a dozen cells and its
surface forms a regular flat contour with that of the single layer of cells
of which the inner integument consists farther from the micropyle.
The pericarp over the micropyle as elsewhere consists of the very
distinct outer and inner epidermises and between these 3 or 4 cell thick-
nesses of thin-walled, elongated, irregularly arranged cells. The outer
epidermis (h) is of thick-walled cells with large lumena, rectangular in
section; the inner epidermis (i) of small, thick-walled, closely crowded,
heavily pigmented tubular cells, roundish or elliptical in sagittal section,
but changing between the micropyle and the hilum to compressed, rec-
tangular cells with much
thinner walls. At this
point the inner epidermis
of the pericarp is under-
laid by another layer of
small, thick-walled cells,
also heavily pigmented
(k), which extends unin-
terrupted over the mi-
cropyple from the hilum,
thinning out and gradu-
ally disappearing distally.
The origin of this cell layer
is not clear, but it may
be a persistent portion of
the outer integument in
which the micropylar
opening is entirely obliti-
erated. Over the area
where the reversed outer
layer of the inner integu-
ment ends next to the
hilum is a mass of small,
irregular, closely packed
pericarp cells (l) whose
very thick, densely pig-
mented walls are fused
with the walls of the integ-
ument cells upon which
they impinge. This group
of cells around the margin of the hilum is continuous with the cells of
the "closing tissue" of the hilum to be described in later pages. Thin-
walled pericarp cells (r) fill the hilum.

Where the caryopsis breaks irregularly from the pedical (m) the peri-
carp is supplied with a group of scalariform tracheids (n), most of which
end irregularly only a few cells from the break, while a few extend for a
short distance in rows in a tangential direction over the hilum. A con-
stant feature of this system of tracheids is its splitting into two branches,
one of which ends abruptly in a coiled knot, a few elements of which are
shown (o) in a direction toward the micropyle, while from the other
branch extend the rows of tracheids over the hilum (p). These latter
rarely extend over the circular hilum for more than one-third of its
diameter, and the underlying tissue of the pericarp is entirely nonvascular.

FIG. 5.—Mean sagittal section through the micropyle and neigh-
boring structures of a Johnson grass caryopsis: a, aleurone layer;
b, proximal end of scutellum; c, micropyle; d,d inner integu-
ment; e, its inner layer of cells at the micropyle; f, its outer layer
of cells at the micropyle; g, edge of the hilum; h, outer epidermis
of the pericarp; i, inner epidermis of the pericarp; k, layer of cells
locally underlying the inner epidermis of the pericarp, possibly a
persistent portion of the outer integument; l, group of closely
packed, thick-walled pericarp cells whose walls seem to be fused
with the walls of the inner integument cells; m, edge of irregular
scar of the caryopsis; n, scalariform tracheids of the pedicel; o,
coiled branch of tracheid system; p, tracheids in rows parallel to
the surface of the hilum; r, pericarp cells which fill the hilum; s,
large, empty, functionless endosperm cells which underly the
micropyle. X 180.
Under the micropyle is a group of large empty functionless endosperm cells (s) which continue also under the hilum and gradually give place to the starchy reserve cells of the endosperm.

**THE HILUM**

Since in a caryopsis the seed never becomes detached from its pericarp there is, of course, no true hilum, or seed scar. There is, however, in the caryopsis of the Andropogoneae, a large opening through the inner integument in the position corresponding to the hilum. Figure 6 represents this hilar orifice of a Johnson grass caryopsis in median sagittal section and figure 7 in median transverse section.

The cells of the inner integument are slightly turned outward at the margins of the hilar orifice (a, a). The group of small, compact, thick-walled, pigmented, pericarp cells mentioned in the preceding section are present outside of the margins of this orifice on all sides (b, b). They are particularly prominent in sagittal section on the side toward the micropyle (fig. 6, A) and are rather sparingly represented on the embryo side (fig. 6, B). Ninety degrees around the circumference of the hilar orifice from these points as shown in figure 7, they are very numerous, but not as thick-walled as at the longitudinal extremities of the hilar region. Stretching over this region from the points where these thick-walled pericarp cells fuse with the integument near the margins of the hilar orifice is a continuous stratum of several layers of pericarp cells with somewhat thickened walls (c), which, though forming a single tissue with the cells on both sides of them, differ from these in ways which are of great physiological significance. The radial contraction which characterizes all pericarp tissues in the dry, mature caryopsis, causing the very noticeable hilar depression, apparently reaches its maximum in this stratum, which also becomes intensely pigmented with a dark brown pigment and, with the thick-walled cells shown at b, b, forms a pro-
Protective cover to the large hilar opening through the inner integument. This cover we have designated as the "closing tissue" of the hilar orifice. As will be shown later, it is largely impervious to solutes and highly resistant to the action of 50 per cent chromic acid. In its protective function it supplements the inner integument with which it is structurally united through the fusion of the respective cell walls. Its intense pigment, showing through the overlying layers of pericarp cells, forms a circular black area which is always conspicuous in an external examination of the entire caryopsis. Around this closing tissue in all directions the degree of pigmentation abruptly decreases, though there is no sharp line between the very dark central region and the surrounding cells. In fact, when completely bleached with Javelle water, the cells of this dark central region and those above and below it are almost identical in appearance. The pericarp cells (e) within the margins of the hilar orifice are roughly cubical in shape and are arranged rather regularly in radial rows. In the absence of vascular bundle elements it is obvious that this mass of tissue—completely filling the hilar opening through the integument, and parenchymatous until pigmentation sets in during the maturation and drying of the caryopsis—acts as the only avenue for the conduction of nutrient materials from the vascular elements at the base of the pedicel and over the hilar region to the developing embryo and endosperm. Rows of long, thin-walled cells in the central layers of the pericarp tissue leading from the coiled branch of the vascular bundle of the pedicel and continuous with rows of cells within the hilar opening, doubtless also function as conducting elements. In the mature unbleached caryopsis, however, the dark central portion is strikingly differentiated from the surrounding structures. Moreover, the contrast here existing is accentuated by partial bleaching of microtome sections.

Fig. 7.—Median transverse section through the hilar orifice of a Johnson grass caryopsis: a, a, inner integument; b, b, compact group of thick-walled pericarp cells; c, "closing tissue" of the hilar orifice; d, outer epidermis of the pericarp; e, pericarp cells completely filling the hilar orifice in the integument. X 275.
with Javelle water. After the surrounding cells are almost completely
decolorized, the compact, densely pigmented central portion, the “closing
tissue,” still remains very dark, with the individual cell walls wholly
indistinguishable. Figure 8 represents in outline a median sagittal
section of a Johnson grass caryopsis which had received this partial
bleaching with Javelle water. On further treatment with Javelle water
the cell walls in this dark
closing tissue (f) also become
completely decolorized and
the compressed cells resume
their cubical shape as shown
in figures 6 and 7.

The relation of the clos-
ing tissue of the hilum to
the integument cells is fur-
ther shown in figures 9 and
10. The material from which
these figures were drawn was
from seeds which had been
treated with 50 per cent
chromic acid until the outer
layers of the pericarp, the coverings of the embryo, the proximal end
of the embryo itself, and the pericarp tissue within the hilar orifice
had been dissolved away. This left in the hilar region only the more
resistant integument and “closing tissue.” These were removed with
a sharp scalpel, bleached, washed and stained upon the slide, and
mounted in 75 per cent glycerin with thin strips of tissue paper under
the cover glass to protect the
now extremely delicate struct-
ures from crushing. The fig-
ures show only one cell layer
of the closing tissue, which is
really several cells thick.

Figure 9 is an external view
with the underlying margin of
the hilar orifice shown as a con-
tinuous heavy line (a). The
drawing was made with camera
lucida and represents ac-
curately the loose ends of the
cell walls (b) at the margin of
the tissue which had resisted
the corrosive action of the
chromic acid.

Figure 10 is a view from the
inner side of the hilum, looking out. The ends of the out-curving
integument cells (a) present a stereoscopic appearance, while the much
thinner-walled closing tissue (b) is shown in a lower focal plane as it
appeared through the hilar orifice.

COMPARISON OF JOHNSON GRASS AND SUDAN GRASS FRUITS AND CARYOPSES

Sudan grass seed differs from Johnson grass seed in certain minor
ways, some of which, however, are physiologically important. Both the
 unhulled fruits and the caryopses are slightly larger, flatter, and more
slender. The glumes are more fragile and more easily broken or removed, so that commercial lots of Sudan grass seed usually contain a considerably larger percentage of hulled caryopses than do Johnson grass seed. The caryopses are lighter colored, less glassy in appearance, and are more easily injured mechanically. The coat structures usually form conspicuous delicate folds or creases over the axial organs of the embryo, as the embryo shrinks during maturation and drying, while in Johnson grass caryopses the coverings are stretched rather tightly over the embryo even after the caryopsis is fully dried. In a Johnson grass caryopsis the micropylar prominence is usually the most proximal part of the inner integument, the embryo not extending farther forward than the micropyle. In a Sudan grass caryopsis, on the contrary, the end of the radicle and of the scutellum usually extend farther forward so that the inner integument is strongly arched forward from the micropyle to cover these organs. The Sudan grass embryo is therefore more exposed to mechanical injury than the Johnson grass embryo.

The micropylar structure is frequently less massive in Sudan grass caryopses than in Johnson grass caryopses. An open micropyle is rare in Johnson grass and somewhat more common in Sudan grass. One Sudan grass caryopsis was examined which had a nearly circular micropylar opening somewhat more than 100 microns in diameter through the integument.

Many Sudan grass caryopses are very light colored, and occasionally one is found which is almost white. In some such light colored caryopses the inner integument is poorly developed or almost lacking. In others it is as well developed as in the dark colored caryopses, but is less pigmented. Johnson grass produces no such light colored caryopses, and the inner integument is always well developed. Its thick inner wall, as well as the pericarp tissue, is more darkly pigmented than in Sudan grass caryopses.

Measurements of Coat Structures

Since removal of the coverings over the embryo of Johnson grass caryopses removes the restrictions to their germination at moderate and constant temperatures and makes them capable of germinating vigorously under the same conditions as Sudan grass caryopses, it was thought desirable to compare these coverings in the two kinds of seed. Measurements were therefore made of the minimum thickness of the coat.
structures over various parts of the caryopses in 25-micron median sagittal freezing microtome sections of a large number of caryopses. Table I summarizes these measurements for several portions of the caryopses, each entry being the average of the minimum thickness for five or six caryopses.

**Table I.—Minimum thickness in microns, of coat structures of Johnson grass and Sudan grass caryopses**

<table>
<thead>
<tr>
<th>Area measured</th>
<th>Small plump, dark Sudan grass caryopses</th>
<th>Small well-matured Johnson grass caryopses</th>
<th>Large, very light-colored Sudan grass caryopses</th>
<th>Large, very dark Sudan grass caryopses</th>
<th>Large, well-matured Johnson grass caryopses</th>
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<tbody>
<tr>
<td>Integument and pericarp:</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Front of coleorhiza</td>
<td>34</td>
<td>29</td>
<td>32</td>
<td>28</td>
<td>34</td>
</tr>
<tr>
<td>Over radicle</td>
<td>31</td>
<td>22</td>
<td>28</td>
<td>23</td>
<td>24</td>
</tr>
<tr>
<td>Over coleoptile</td>
<td>29</td>
<td>21</td>
<td>30</td>
<td>26</td>
<td>21</td>
</tr>
<tr>
<td>Over scutellum</td>
<td>36</td>
<td>23</td>
<td>31</td>
<td>26</td>
<td>25</td>
</tr>
<tr>
<td>At micropyle</td>
<td>72</td>
<td>60</td>
<td>79</td>
<td>52</td>
<td>66</td>
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<tr>
<td>At hilar orifice</td>
<td>80</td>
<td>71</td>
<td>65</td>
<td>64</td>
<td>83</td>
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<tr>
<td>Over middle of endosperm side</td>
<td>40</td>
<td>33</td>
<td>43</td>
<td>38</td>
<td>28</td>
</tr>
<tr>
<td>Integument at micropyle</td>
<td>35</td>
<td>42</td>
<td>52</td>
<td>48</td>
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<tr>
<td>Dark area over hilum</td>
<td>11</td>
<td>13</td>
<td>9</td>
<td>12</td>
<td>10</td>
</tr>
</tbody>
</table>

1 Each entry is the average for several caryopses.

In front of the point of the radicle and over the hilar orifice and micropyle the thickness of the pericarp and inner integument combined was greater in Johnson grass caryopses than in Sudan grass caryopses. These differences were related to the more forward position of the embryo in Sudan grass caryopses.

Over all other portions of embryo and endosperm where measurements were made the coverings of the Sudan grass caryopses were thicker than those of Johnson grass caryopses. They were thicker also in light-colored than in dark-colored Sudan grass caryopses, this being the result of a looser arrangement of the different layers, especially the starch-bearing layer, of the pericarp and a less contracted condition of the inner integument in the light-colored caryopses. Only in the very darkest Sudan grass caryopses were the coverings structures as compact as in Johnson grass caryopses.

The tendency for the pericarp tissues to fall loosely apart along the layers between the outer and inner epidermises was greater in sections of Sudan grass caryopses than in sections of Johnson grass caryopses.

**Resistance of Seed Coverings to Rupture from Heating in Water, and to Imbibition by the Embryo**

The differences in compactness of the coverings of Johnson grass and Sudan grass caryopses suggested that differences in mechanical resistance to rupture from the pressure of the expanding embryos might exist and might be significant in relation to the germination behavior of the caryopses. In an attempt to test the mechanical resistance of these
coverings, 100 each of the light-colored and dark-colored Sudan grass caryopses and of large and small Johnson grass caryopses were submerged in water in air-dry condition and slowly heated for three 20-minute periods with slow cooling between the periods of heating. The rate of heating was such that the water was just beginning to boil at the end of 20 minutes.

Scarcely any caryopses broke during the first period of heating, but all but a very few had broken by the end of the third period. The majority broke first over the endosperm. At the end of the third period of heating 42 small and 26 large Johnson grass caryopses and 9 dark and no light Sudan grass caryopses had broken over the embryo. The breaking of the coat structures of Johnson grass over the embryo and the failure of these to break in Sudan grass caryopses is evidently the result of their looser arrangement in the latter case and not of differences in resistance to pressure from within. The looser coverings of the Sudan grass embryo, and especially of those of the light-colored caryopses, allowed greater swelling of the embryos before these coverings were distended to their full capacity and at the same time gave better opportunity for the escape of gases. A natural corollary of this fact is that, under germination conditions, the Sudan grass embryo can imbibe a greater percentage of water without breaking the coverings than can Johnson grass embryos. If the force of imbibition of a Johnson grass embryo is insufficient to overcome the resistance of coat structures, the embryo will remain dormant on account of incomplete satisfaction of its water requirements. As a matter of fact actual tests with thick sections made with a freezing microtome and at once immersed in different solutions and examined with a microscope indicate that dormant Johnson grass embryos, even after long incubation under germination conditions, are about in equilibrium with 2-molar salt solution. The sections of the embryos contracted considerably in 4-molar salt solution and swelled very appreciably in water but underwent no appreciable change in 2-molar salt solution. Coat restrictions to water intake may, therefore, be important in imposing dormancy and resistance to germination here as with the seeds of many water plants (11, 12.)

COMPOSITION OF PERICARP AND INNER INTEGUMENT OF JOHNSON GRASS AND SUDAN GRASS CARYOPSES

Only a few microchemical tests were made by the authors. The results of these tests were verified and additional tests were made by Dr. Sophia Eckerson, of the University of Chicago and the Bureau of Plant Industry of the United States Department of Agriculture, for whose generous assistance we are greatly indebted. Table II summarizes Doctor Eckerson's results so far as they are significant in the present comparison.

The cell walls of the aleurone layer were of hemicellulose in both kinds of caryopsis. Although these walls are comparatively thick, it does not seem possible, in view of their composition and the much more resistant character of the integument and pericarp, to attribute to the aleurone layer any special protective function in uninjured caryopses.

The pericarp tissues contained the same elements in both kinds of caryopsis, except iron, which was present in the outer epidermis of Johnson grass and absent in that of Sudan grass, but pectic substances were much more abundant and the suberization was less in Sudan grass.
than in Johnson grass. The composition of the inner integument was quite different in the two kinds of caryopsis. In Sudan grass caryopses it consisted mainly of hemicellulose, the inner walls being somewhat, and the outer walls rather more impregnated with suberin. In Johnson grass caryopses, on the contrary, the outer walls contained cellulose and were somewhat suberized, while the inner walls as well as the closing tissue were suberin, strongly impregnated with fatty substance. The amount of fat, both in the walls and in the cell contents, was much greater in Johnson grass than in Sudan grass.

In addition to the data shown in Table II, all layers of the pericarp and the integument contained tannin in both kinds of caryopsis, but more in Johnson grass than in Sudan grass.

**TABLE II.—Composition of coat structures of Johnson grass and Sudan grass caryopses**

<table>
<thead>
<tr>
<th>Part examined</th>
<th>Composition.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Johnson grass caryopses.</td>
</tr>
<tr>
<td>Inner integument</td>
<td></td>
</tr>
<tr>
<td>Pericarp</td>
<td>Outer cell walls have very little suberin; some cellulose.</td>
</tr>
<tr>
<td>&quot;Closing tissue&quot; of the hilar orifice.</td>
<td>All cell layers contain pectic substances and are suberized; a little calcium; a little cellulose; iron in outer layer.</td>
</tr>
<tr>
<td></td>
<td>Suberin and considerable oil, which comes out after 10 to 15 minutes' warming in alcohol.</td>
</tr>
</tbody>
</table>

**COMPARATIVE RATES OF BLEACHING WITH JAVELLE WATER—CORRELATION WITH GERMINATION**

These tests were made with one lot of Sudan grass caryopses and two lots of Johnson grass caryopses, using only uninjured caryopses which had been removed from the scales by hand. The Johnson grass seed was nearly two years old and was therefore fully after-ripened. When fresh, No. 37001 was an unusually ready germinator, but the San Antonio lot was exceptionally resistant to germination. Almost 30 per cent of the naked caryopses of the former and less than 5 per cent of those of the latter germinated in 10 days at 25° C. At other temperatures, the comparison was similar. The caryopses were placed in small vials, and several times their volume of Javelle water was added. The Javelle water was renewed after 1, 2, 6, and 24 hours and at intervals of 24 hours thereafter until bleaching was complete. The first visible effect of the Javelle water was a pronounced darkening of the caryopses, probably due either to the action of the free alkali in the solution or to oxidations.
Caryopses of Sudan grass and Johnson grass No. 37001 became almost black in one-half hour, and those of the San Antonio lot in an hour. The initial blackening was followed by gradual bleaching, which was first apparent and proceeded most rapidly over the embryos and thence advanced around the endosperm at the proximal end, and at the same time toward the distal end over the entire circumference of the caryopsis. As the seeds whitened the bleaching solution darkened. More rapid loss of color in spots frequently gave a mottled appearance to the bleaching caryopses. The closing tissue of the hilar orifice and the inner walls of the very large integument cells at the distal end of the caryopses were the slowest to show the effect of the bleaching and the last to become completely bleached. Often the circular closing tissue of the hilum remained very dark 24 hours after all other tissue at the proximal end of the caryopsis was completely bleached.

In many Johnson grass caryopses the coverings over the edges of the scutellum and over the axial organs bleached more quickly than a little to either side of the axis, leaving two prominent dark lines the whole length of the embryo after the rest was white.

Table III shows in detail the progress of bleaching in the three lots of caryopses and the results of germination tests of the same lots of caryopses. The germination tests were made in 100 mm. Petri dishes with wet blotting paper as germination bed. Sudan grass was tested at room temperature and Johnson grass in an incubator at 26°C.

Examination of the data in Table III shows that Sudan grass caryopses bleached much more rapidly than Johnson grass caryopses and Johnson grass No. 37001 more rapidly than the San Antonio lot. Correlated with the difference in resistance of the two lots of Johnson grass caryopses to the action of Javelle water was a difference in the readiness with which they germinated. This latter difference, while only slight in the fully after-ripened condition at the time these tests were made, was, as previously indicated, very much greater when the seeds were fresh. Viability tests made after the third day by scratching the embryos along one side of the scutellum with a bent needle and returning them to the incubator for another day showed all the caryopses to be viable and capable of producing vigorous seedlings. The application of this method of determining the viability of Johnson grass embryos has been described elsewhere (15). A few of the most resistant Sudan grass caryopses bleached more slowly than a few of the least resistant Johnson grass caryopses. It is perhaps worthy of notice also in this connection that 1 per cent of the Sudan grass caryopses, though viable and potentially vigorous, did not germinate until after the coverings of the embryo had been opened—a process which induces the germination of the most resistant Johnson grass caryopses even before they have after-ripened.

Additional bleaching tests were made with the San Antonio lot of Johnson grass, using caryopses which had failed to germinate in three days at 26°C in comparison with others which had for three days been incubated as for germination except that germination was prevented by keeping them in an ice box. The caryopses remaining from the germination test bleached on the average more slowly than those which had been incubated in the ice box, so that none germinated. This seems to indicate that the caryopses of this lot which had germinated were in general those which would have bleached most rapidly in Javelle water.
TABLE III.—Bleaching with Javelle water and germination of Sudan grass and Johnson grass caryopses

<table>
<thead>
<tr>
<th></th>
<th>Sudan grass</th>
<th>Johnson grass No. 37001</th>
<th>Johnson grass, San Antonio, 1916</th>
</tr>
</thead>
<tbody>
<tr>
<td>First evidence of bleaching.</td>
<td>60 seconds, lighter over embryo.</td>
<td>15 minutes, lighter patches over embryo.</td>
<td>15 minutes less than in No. 37001.</td>
</tr>
<tr>
<td>First coloring of Javelle water.</td>
<td>60 seconds.</td>
<td>30 minutes.</td>
<td>60 minutes barely perceptible.</td>
</tr>
<tr>
<td>First &quot;axis&quot; completely bleached.</td>
<td>45 minutes.</td>
<td>45 minutes.</td>
<td>120 minutes.</td>
</tr>
<tr>
<td>First embryo completely bleached.</td>
<td>75 minutes.</td>
<td>120 minutes.</td>
<td>None in 6 hours; some nearly so.</td>
</tr>
<tr>
<td>First seed completely bleached except closing tissue.</td>
<td>3 hours, several in 6 hours.</td>
<td>None in 6 hours.</td>
<td>None in 6 hours.</td>
</tr>
<tr>
<td>After 24 hours:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Completely bleached.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nearly all bleached.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Little more than one-half bleached.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>About one-half bleached.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Less than one-half bleached.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Embryos bleached.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>All but distal end bleached.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>All but closing tissue bleached.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>All but distal end and closing tissue bleached.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Flat side bleached more than embryo.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>After 48 hours.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Completely bleached except small spots on a few caryopses.</td>
<td>17 per cent.</td>
<td>5 per cent.</td>
<td>1 per cent.</td>
</tr>
<tr>
<td>Nearly all bleached.</td>
<td></td>
<td>78 per cent.</td>
<td>6 per cent.</td>
</tr>
<tr>
<td>Little more than one-half bleached.</td>
<td>5 per cent.</td>
<td>68 per cent.</td>
<td>8 per cent.</td>
</tr>
<tr>
<td>About one-half bleached.</td>
<td>0 per cent.</td>
<td>19 per cent.</td>
<td>10 per cent.</td>
</tr>
<tr>
<td>Less than one-half bleached.</td>
<td>0 per cent.</td>
<td>2 per cent.</td>
<td>79 per cent.</td>
</tr>
<tr>
<td>Embryos bleached.</td>
<td>100 per cent.</td>
<td>98 per cent.</td>
<td>26 per cent.</td>
</tr>
<tr>
<td>All but distal end bleached.</td>
<td>0 per cent.</td>
<td>10 per cent.</td>
<td>8 per cent.</td>
</tr>
<tr>
<td>All but closing tissue bleached.</td>
<td>0 per cent.</td>
<td>6 per cent.</td>
<td>0 per cent.</td>
</tr>
<tr>
<td>All but distal end and closing tissue bleached.</td>
<td>0 per cent.</td>
<td>58 per cent.</td>
<td>0 per cent.</td>
</tr>
<tr>
<td>Flat side bleached more than embryo.</td>
<td>0 per cent.</td>
<td>0 per cent.</td>
<td>24 per cent.</td>
</tr>
<tr>
<td>After 72 hours.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Small spot on one caryopsis unbleached.</td>
<td></td>
<td>5 or 6 not completely bleached or nearly so.</td>
<td></td>
</tr>
<tr>
<td>After 96 hours.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Germination:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 day</td>
<td>98 per cent.</td>
<td>65 per cent.</td>
<td>44 per cent.</td>
</tr>
<tr>
<td>2 days</td>
<td>1 per cent.</td>
<td>3 per cent.</td>
<td>19 per cent.</td>
</tr>
<tr>
<td>3 days</td>
<td>1 per cent.</td>
<td>32 per cent.</td>
<td>36 per cent.</td>
</tr>
<tr>
<td>4 days</td>
<td>100.</td>
<td>100.</td>
<td>99.</td>
</tr>
<tr>
<td>Percentage viable.</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

100 seeds were used in each test, both for bleaching and germination.

Caryopses scratched at end of third day with a bent needle to induce germination.

RESISTANCE OF COAT STRUCTURES TO THE ACTION OF CHROMIC ACID: CORRELATION WITH GERMINATION

The resistance of the inner integument and the closing tissue in Johnson grass caryopses to the action of chromic acid has already been referred to and might be inferred from the fact that these consist wholly or largely of suberin.
The first treatments were with halved caryopses of three lots of fully after-ripened Johnson grass seed showing 100 per cent viability in comparison with halved caryopses of dark-colored and light-colored Sudan grass seeds. The caryopses, either in air-dry condition or after soaking in water, were cut in halves along a median sagittal plane with a sharp scalpel and were then immersed in 50 per cent chromic-acid solution, which was frequently changed. At the end of 24 hours' soaking in the chromic-acid solution some of the halved caryopses were washed in water and examined with the microscope. At this time considerable endosperm, white and normal in appearance, remained within the half-shells, but the majority of the embryos were entirely disintegrated. The coverings over the Sudan grass embryos were very considerably fragmented, and in most cases the remaining tissue was fragile and soft and tended to collapse into the empty embryo cavity. In the great majority of the Johnson grass half caryopses, on the contrary, the shells over the embryo cavity were intact or only slightly fragmented and remained stiff and brittle. Many of these were kept several days longer in 50 per cent chromic-acid solution without undergoing entire dissolution of the embryo coverings. Embryo coverings of caryopses of the Johnson grass lot which was most resistant to germination, especially before after-ripening, were more resistant to the action of the chromic-acid solution than embryo coverings of the other two lots.

Entire caryopses of these same lots of Johnson grass and Sudan grass and wheat grains from a lot showing 99 per cent germination in two days were next treated with 50 per cent chromic-acid solution in small vials each containing 100 caryopses. At 24-hour intervals the caryopses in the different vials were removed from the chromic-acid solution, thoroughly washed with sodium-bicarbonate solution followed by distilled water, and put to germinate. At the same intervals caryopses were withdrawn from another vial of chromic-acid solution for sectioning and microscopic examination.

All wheat embryos were uncovered and killed by the action of the chromic acid by the end of the first 24-hour period, though in the most resistant grains scutella and the larger part of the axial organs were still intact. Fragments of the outer coverings, sometimes including even the outer epidermis of the pericarp, remained.

With Sudan grass caryopses only the inner integument and the closing tissue of the most resistant individuals remained intact at the end of 24 hours' treatment. Occasional adhering remains of the outer coverings usually represented only the inner epidermis of the pericarp. The whole proximal end, both embryo and endosperm, tended to become slightly stained, showing the slight penetration of the acid. Only 1 per cent of the embryos of dark-colored caryopses were very weakly viable after 24 hours in the chromic-acid solution. Sixty-seven per cent of the embryos of dark-colored caryopses were wholly or partly disintegrated, while the other 33 per cent were not yet exposed by the disintegration of the inner integument. Light-colored caryopses were much less resistant than dark-colored caryopses, all embryos being more or less disintegrated and only a small distal portion of the endosperm remaining intact in several caryopses.
TABLE IV.—Resistance of the coverings of Johnson grass caryopses to the action of 50 per cent chromic acid

<table>
<thead>
<tr>
<th>Condition of caryopses:</th>
<th>Effect of varying length (days) of treatment with chromic acid.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0     1     2     3     5</td>
</tr>
<tr>
<td>Apparently not affected, or slightly darkened or mottled over embryo</td>
<td>45    12     7     2     5</td>
</tr>
<tr>
<td>Portions of embryo or its coverings (especially axial organs) more or less darkened or many after more than 1 or 2 days' treatment with part of embryo eaten away</td>
<td>50    83     93    98    98</td>
</tr>
<tr>
<td>Parts of endosperm eaten away through weak place in covering on flat side, usually opposite the embryo</td>
<td>5     5     5     5     5</td>
</tr>
</tbody>
</table>

Germination percentage at 26° C.:

<table>
<thead>
<tr>
<th>Condition of caryopses:</th>
<th>Effect of varying length (days) of treatment with chromic acid.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0     1     2     3     5</td>
</tr>
<tr>
<td>Portion of embryo or its coverings (especially axial organs) more or less darkened or many after more than 1 or 2 days treatment with part of embryo eaten away</td>
<td>65    40     12    3     0</td>
</tr>
<tr>
<td>Parts of endosperm eaten away through weak place in covering on flat side, usually opposite the embryo</td>
<td>3     4     2     3     0</td>
</tr>
<tr>
<td>Total germination after unscratched period</td>
<td>68    44     15    6     0</td>
</tr>
<tr>
<td>3 days</td>
<td>32    1     0     0     0</td>
</tr>
<tr>
<td>4 days</td>
<td>0     0     0     0     0</td>
</tr>
<tr>
<td>6 days</td>
<td>0     0     0     0     0</td>
</tr>
<tr>
<td>Total viable</td>
<td>100   45     15    6     0</td>
</tr>
</tbody>
</table>

Seed lot from San Antonio, 1917.

Germination percentage at 26°C.

<table>
<thead>
<tr>
<th>Condition of caryopses:</th>
<th>Effect of varying length (days) of treatment with chromic acid.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0     1     2     3     5</td>
</tr>
<tr>
<td>Portion of embryo or its coverings (especially axial organs) more or less darkened or many after more than 1 or 2 days treatment with part of embryo eaten away</td>
<td>34    73     78    94    94</td>
</tr>
<tr>
<td>Germination percentage at 26° C.</td>
<td>48    44     15    8     8</td>
</tr>
<tr>
<td>2 days</td>
<td>11    16     6     7     4</td>
</tr>
<tr>
<td>3 days</td>
<td>1     1      2     2     2</td>
</tr>
<tr>
<td>Total germination after unscratched period</td>
<td>60    61     23    15    4</td>
</tr>
<tr>
<td>3 days</td>
<td>40    2      1     0     0</td>
</tr>
<tr>
<td>4 days</td>
<td>0     0      0     0     0</td>
</tr>
<tr>
<td>6 days</td>
<td>0     0      0     0     0</td>
</tr>
<tr>
<td>Total viable</td>
<td>100   63     24    17    4</td>
</tr>
</tbody>
</table>
Table IV.—Resistance of the coverings of Johnson grass caryopses to the action of 50 per cent chromic acid—Continued

<table>
<thead>
<tr>
<th>Condition of caryopses:</th>
<th>Effect of varying length (days) of treatment with chromic acid.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
</tr>
<tr>
<td>Seed lot from San Antonio, 1916.</td>
<td></td>
</tr>
<tr>
<td>Apparently not affected, or slightly darkened or mottled over embryo...</td>
<td></td>
</tr>
<tr>
<td>Portions of embryo or its coverings (especially axial organs) more or less darkened or many after more than 1 or 2 days' treatment with part of embryo eaten away.</td>
<td></td>
</tr>
<tr>
<td>Parts of endosperm eaten away through weak place in covering on flat side, usually opposite the embryo.</td>
<td></td>
</tr>
<tr>
<td>Germination percentage at 26° C.:</td>
<td>44</td>
</tr>
<tr>
<td>1 day</td>
<td></td>
</tr>
<tr>
<td>2 days</td>
<td>19</td>
</tr>
<tr>
<td>3 days</td>
<td>0</td>
</tr>
<tr>
<td>Total germination after unscratched period 2</td>
<td>63</td>
</tr>
<tr>
<td>3 days</td>
<td>36</td>
</tr>
<tr>
<td>4 days</td>
<td>0</td>
</tr>
<tr>
<td>Total viable</td>
<td>99</td>
</tr>
</tbody>
</table>

1 The different lots are arranged in the order of increasing difficulty of germination, especially before after-ripening.
2 Embryos scratched at end of second or third day with a bent needle to induce germination.

Table IV shows the main features of the result with Johnson grass caryopses. These were remarkably resistant to the action of the chromic acid. The following points deserve special mention:

1. The resistance of the different lots to the action of the acid increased with increasing difficulty of germination, the readiest germinator (No. 37001) being most rapidly attacked by the acid and the poorest germinator (San Antonio, 1916) being most resistant.

2. The action of the acid for 24 hours or a longer period so reduced the resistance of the coat structures that practically all the embryos which were still viable germinated in 2 or 3 days without “scratching” whereas about 40 per cent of the controls failed to germinate until after scratching. In the most resistant lot (San Antonio, 1916) the percentage which germinated without scratching was actually increased by 24 hours' treatment. This recalls the action of concentrated sulphuric acid in removing the embryo coverings and thus inducing prompt and complete germination (15). In the case of the chromic acid the least resistant caryopses were killed within the first 24 hours, but a larger number of the most resistant caryopses were rendered easily germinable by the action of the acid.

3. The percentage of viable caryopses, and after the first 24 hours the percentage which germinated without scratching, decreased progressively with increased length of time in the chromic-acid solution.

4. In contrast to wheat and Sudan grass caryopses, which were killed within 24 hours, a small percentage of Johnson caryopses were still viable after 5 to 7 days.
A few Johnson grass caryopses had small, less resistant areas in the coat structures on the flat endosperm side opposite the embryo. The chromic acid, penetrating through these areas, ate small, deep holes into the endosperm. Such caryopses usually germinated if the integument over the embryo was still intact and the embryo itself uncolored by the acid. In fact even the starchy endosperm tissue, on account of its very hard, compact, glassy texture, seemed to be considerably more resistant to the corrosive action and to the penetration of the acid than was true of Sudan grass or wheat endosperms.

Nearly all caryopses which germinated after treatment with chromic acid belonged to the first class shown in Table IV “apparently not affected.” Of these nearly all produced strong vigorous seedlings when the length of the treatment did not exceed two days, with progressively weaker seedlings after longer treatments. All treated caryopses which germinated only after scratching produced very weak seedlings, while untreated caryopses which were brought to germination by scratching produced as vigorous seedlings as those which germinated without scratching.

Microscopic examination of sections of the most resistant caryopses showed the inner integument and the closing tissue of the hilar orifice intact even after seven days treatment. Frequently the outer walls of the integument cells after more than two days’ treatment were thoroughly disintegrated in places or so weakened and brittle that they broke away in sectioning, leaving only the thick inner walls covering the aleurone. All pericarp layers were present in places except after the very longest treatments, but these were greatly attacked at the hilar orifice, micropyle, and style, usually laying bare the closing tissue and sometimes also the micropyle within 24 hours. In most of the less resistant caryopses small portions of the integument were eaten away by the acid before coloring or disintegration of the underlying tissue began, but in a few of the less resistant and several of the more resistant caryopses there was evidences of local penetration of the acid sufficient to cause discoloration, through intact portions of the integument. The integument cells were on the average much more quickly destroyed over the embryo than elsewhere, so that after several days’ treatment the embryo coverings in many caryopses were largely gone and the embryos themselves entirely gone, leaving at the proximal end of the caryopsis only the integument cells on the flat side and the closing tissue of the hilar orifice. From such caryopses were obtained the integument portions from which figures 8 and 9 were drawn.

**RESISTANCE TO PENETRATION OF IODIN SOLUTION**

Brown, working with cereals (3) was the first to discover the existence of a nonliving, semi-permeable membrane surrounding any seed. He soon discovered (3, 4) that this membrane had selective qualities, allowing many solutes to pass through it readily, others only very slowly, and excluding still others. Schroeder (28) discovered a similar semi-permeable membrane in wheat grains, and showed that this membrane admitted both water and penetrant solutes mostly around the embryo and little if at all at the distal end of the grain. Collins (9) confirmed for barley Schroeder’s result with wheat. His results led him to believe that the entry of both water and solutes took place almost wholly through the micropyle, which, however, he was unable to locate exactly. All of these authors agree that iodin passes through the selective permeable
membrane in question more readily than most solutes, but Collins concludes that the barley grain does not appear to possess perfect impermeability to any solute.

To test the resistance of the coat structures of Johnson grass caryopses to the penetration of iodin, caryopses of the lot which had proved to be most resistant to the action of chromic acid and of Javelle water (San Antonio, 1916, Table III and IV) were immersed in a very dilute iodin potassium iodid (I₂KI) solution for three days and then tested for germination or sectioned for microscopic examination. Since both Johnson grass and Sudan grass caryopses usually contain starch in the embryos, the penetration of iodin solution in these can be observed to better advantage than in the cereals with their starchless embryos.

About 4 per cent germinated very weakly after scratching of the embryo with a needle. In these the needle wound healed, with darkening of the surrounding cells, much as in normal untreated caryopses. In all of these germinable caryopses the iodin had penetrated the coat structures and stained the underlying starch, either locally in small patches of the endosperm, or less frequently around the periphery of nearly the entire endosperm; but in none of them was any of the starch in the embryo stained. In some of them small areas of the embryo or of the endosperm portions underlying the scutellum stained pinkish brown, possibly from the penetration of iodin unaccompanied by the potassium ions which are necessary for the formation of the blue starch-iodin combination.

In the majority of the caryopses which failed to germinate, both of this lot and of No. 37001 treated at another time both in air-dry condition and after soaking in water, the iodin entered and stained the starch most promptly and abundantly along the margins of the scutellum, staining both endosperm and embryo starch. The stained areas gradually spread to cover the whole adjoining surfaces of the endosperm and the scutellum. From thence the stained areas advanced to the central organs of the embryo, and along the periphery of the endosperm from the under surface of the scutellum toward the distal end of the caryopsis, thus paralleling in general the course described by Collins for barley. The evidence seems to indicate that the iodin entered most readily through the hilar orifice, or the micropyle, or both, passing at once around the caryopsis and in a distal direction along the inner surfaces of the aleurone layer and of the scutellum. The passage of the iodin to the middle part of the proximal end of the scutellum and to the adjoining starchy endosperm cells seemed to be retarded by the intervening large, empty endosperm cells lying between the hilar orifice, micropyle, and proximal end of the scutellum, so that the distal end of the scutellum, though farther removed from the hilar region, became stained sooner than the proximal end. There were frequent undoubted cases also of local penetration of the iodin in some abundance through the uninjured integument. The iodin always passed only very slowly toward the center of the endosperm in a radial direction. In the periphery of the endosperm the extreme distal portion was almost invariably the last to stain. The penetration even around the embryo was not rapid enough to prevent the germination while in the iodin solution of about 16 per cent of one lot of caryopses which had previously been soaked in water in an ice-box. Of course germination proceeded only to the extent of breaking the coverings over the embryo, after which the embryos were promptly killed.

Sudan grass, wheat, and dent corn caryopses were soaked in weak I₂KI solution and examined for comparison with Johnson grass caryopses.
Only uninjured caryopses were used. In Sudan grass caryopses the iodin followed the same course as in Johnson grass caryopses but penetrated at the proximal end nearly twice as rapidly. In all of the wheat grains black bands appeared round at least a part of the base of the embryo within 2 hours and spread within 24 hours around and under the embryos and a half or more of the way to the distal end of the grains. There were also local areas of penetration elsewhere in a few grains. All embryos at the same time became stained light brick red. In the corn, black areas appeared within a very short time, first at the dent then generally at various points scattered over the grain. At the end of 24 hours a thin starch-stained zone just within the coat structures covered the entire grains except the thick hull at the extreme proximal end and sometimes a portion of the dent.

It is evident, therefore, that even the easily penetrating iodin solution found access to the embryos of Johnson grass somewhat more difficult than to those of Sudan grass and much more difficult than to those of wheat and corn.

**Penetration of Other Solutes; Possible Effect upon Germination**

In 2-molar and 4-molar solutions of sodium chloride, freshly harvested Johnson grass caryopses behaved at first as if they were protected by semipermeable membranes, imbibing quantities of water which decreased with increasing concentration of the solutions, losing water when transferred from the weaker solution with which they were in equilibrium to the stronger solution, and taking up water until they regained their previous weight when the reverse change was made. For some time they retained their viability when thinly covered with the salt solution, but by the end of several weeks they had taken up sufficient salt to kill all of the embryos.

Immersion of the freshly harvested caryopses for seven days in molar solutions of potassium sulphocyanid (KCNS), sodium sulphocyanid (NaCNS), and lithium nitrate (LiNO₃) killed all of the caryopses; nearly all were killed by immersion for seven days in molar solutions of potassium nitrate (KNO₃), sodium chlorate (NaClO₃), barium sulphocyanate (Ba(CNS)₂), and urea (CO(NH₂)₂); about one-half survived seven days' immersion in molar solutions of lithium sulphate (Li₂SO₄), barium nitrate (Ba(NO₃)₂), magnesium nitrate (Mg(NO₃)₂), and potassium tartrate (K₂C₄H₇O₆). Molar solutions of other salts for the same length of time caused less injury, and immersion in water for seven days caused no loss of viability.

Immersion of fully after-ripened caryopses for one or two days in 5 per cent acetic acid (CH₃COOH), 5 per cent hydrochloric acid (HCl), 5 per cent ammonium hydroxid (NH₄OH), 3 per cent alcoholic potassium hydroxid (KOH), 95 per cent alcohol, acetone, ether, chloroform, or xylol killed all of the caryopses; immersion for one day in 3 per cent sulphuric acid (H₂SO₄) killed 80 per cent of them; and immersion for two days in saturated calcium chlorid (CaCl₂) solution killed 20 per cent of them. The controls soaked two days in water, were uninjured. All of the solvents or solutes mentioned in this paragraph increased the subsequent rate of bleaching of the thoroughly washed caryopses with Javelle water and the rate of penetration of iodin solution; hydrates had the most effect, fat solvents next, followed by acids and by (CaCl₂), which had very little effect.
It can be seen from the preceding section that many solutes of various chemical nature pass through the membranes covering Johnson grass embryos. It has also been shown that certain chemical treatments greatly favor germination. These treatments include removal of the coverings over the embryo by means of concentrated sulphuric acid, treatment with chromic acid for a long enough time to weaken these coverings without killing the embryo, treatment with mercury salts, subjection to atmospheres with high concentration of carbon dioxide, and etherization. Furthermore, certain of the salts mentioned in the preceding section which were toxic in molar solutions slightly stimulated germination when used in tenth to hundred thousandth molar solutions and hydrogen peroxid in proper concentrations is also an efficient forcing agent.

The question arises: Do the beneficial effects of these chemical treatments result from stimulation of the embryo protoplasm or from the removal or lessening of coat restrictions? If the latter they may produce the effects observed either by increasing the permeability of the coat structures to solutes, thus admitting oxygen or releasing inhibitors which are held by them, or by decreasing the mechanical resistance of the coat structures to the expansion of the embryo.

Denny (13) has shown that tannins, lipoids, and pectic substances greatly decrease the permeability to water of seed coats which are impregnated with them, while suberized membranes were not significant in the seeds which he studied except as these membranes became impregnated with fatty substances, which he showed did decrease their permeability.

The pectic substances and hemicellulose in the coat structures of Sudan grass caryopses including the inner integument indicate that these membranes would probably take up water slowly, but in larger total amount than those of Johnson grass, thereby becoming more distended, with a greater weakening of their mechanical resistance. The greater abundance of tannin and of suberin and its associated fatty substances in the coat structures of Johnson grass, on the other hand, would tend to limit the total amount of water which they are able to absorb and their permeability to water and to substances in aqueous solution below the level obtaining for Sudan grass but should not effect the total amount of water which might in course of time pass through them if the embryo and endosperm were able to absorb it. These limitations would reach their maximum in the inner walls of the inner integument and in the closing tissue of the hilar orifice.

Johnson grass caryopses, either in the scales or with the scales removed, take up water so rapidly that limited permeability of their coverings to water can not be considered as a possible cause of their dormancy and germination physiology. In fact the freshly harvested, dormant, naked caryopses absorb water when immersed in it so that their total moisture content is about 50 per cent of their dry weight at the end of 24 hours, after which small amounts are absorbed. So far as water intake is concerned, therefore, the coat structures need be considered only as possibly limiting by their physical resistance, the total amount of water imbibed by the caryopses.

It is probable, however, that the substances which Denny (13) found limiting the permeability of membranes to water would also decrease
their permeability to substances in aqueous solution. We have shown that this is true so far as the effect of lipoid substances upon the penetration of I₂KI solution is concerned. If on account of restricted permeability metabolically developed inhibitors to germination were prevented from escaping from the embryo as suggested by Kidd (20) and by Mazé (23, 24), or if the concentration of oxygen within the embryo were thus maintained below minimum required for germination, dormancy would result.

It has been shown (16) that carbon dioxide in a wide range of concentration forces the germination of dormant Johnson grass caryopses, showing that Kidd's earlier hypothesis which indeed he and West (21) modified in a latter paper, does not apply in this case. We have found, furthermore, that increased partial pressures of oxygen in the atmosphere or even very high oxygen pressures are not effective in forcing the germination of Johnson grass. Limited oxygen supply, therefore, does not seem to play the rôle here that has been shown for dormant wild oats (1) Xanthium (10, 29) and other seeds; the forcing action of hydrogen peroxid on the germination of Johnson grass must apparently have some other explanation than increasing the oxygen supply of the embryo.

As for Mazé's hypothesis of acetic aldehyd as an inhibitor to germination Brown (4), Schroeder (28), Collins (9), and Brown and Tinker (5, 6) have shown that acetic aldehyd and similar compounds pass through the selective permeable membranes of wheat and barley rather readily. In our own work ether, acetone, chloroform, 95 per cent alcohol, and Xylol all entered Johnson grass caryopses in 24 hours at room temperature in sufficient quantity to kill the embryos. It is highly improbable, therefore, that acetic aldehyd would be kept in by the coats in sufficient concentration to hold the caryopses in a dormant condition for months or years under good conditions of moisture and aeration, as we have found to be the case with Johnson grass caryopses. The force of this argument is increased when we consider that if acetic aldehyd is present in the caryopses it is there as a product of respiratory activities, and that respiration is on a very low level in the dormant caryopses. If there were other possible water-soluble inhibitors present in the embryos, the same logic would apply to their removal.

If we turn now to the possible explanation of coat effects as related to the swelling of the embryo, we find the following situation: Sulphuric acid removes the coat structures; chromic acid weakens them; mercury salts probably tend to coagulate the coat colloids and may thus weaken the coat structures; lipid solvents dissolve a part at least of the lipid substances with which they are impregnated, thus increasing their permeability and probably also weakening their physical resistance; and salts other than those of mercury which increase germination (chlorates, sulphates, nitrates, sulphyocyanates) tend to increase the hydration of colloids and may thus weaken the coat structures. All these substances might be supposed to produce their beneficial effects upon germination, in part at least, by altering coat colloids. This can hardly be the case, however, with carbon dioxide, which probably passes through the pericarp and integument in solution and acts upon the embryo itself. Undoubtedly also all of the other substances which have been shown to stimulate germination pass through the covering membranes of the caryopses at least in very limited amounts. These membranes apparently
are not completely impermeable to any solute. These substances, even the highly toxic salts of mercury, may therefore reach the embryo in exceedingly small, subtoxic or only slightly toxic doses—much weaker than the solutions in which the caryopses are soaked—and stimulate it into growth. Moreover, treatment of freshly harvested Johnson grass caryopses with tenth molar to hundred thousandth molar solutions of hydrochloric, acetic, oxalic, citric, and tartaric acids and of sodium hydrate—all of which are colloid hydrators—did not increase their germination as we should expect if decreasing the coat resistance by increasing the hydration of coat colloids were the only factor involved.

Apparently with chromic acid the treatments which stimulate germination are such as just fall short of serious injury, exactly as Kidd and West (21) reported for dormant white mustard seed with a number of stimulating agents, which are not classifiable under any other head. Also some at least of the other substances which increase germination are toxic to the intact, dormant caryopses if employed in too great concentration. In the case of the wounding of the scutellum with a needle there is always definite, concrete evidence of a reaction of the living protoplasm in the prompt darkening (probably from suberization) of the cell walls along the wound surfaces. This reaction takes place only slowly if at all in dead embryos when these are scratched.

The argument set forth in the preceding paragraph might lead to the hypothesis that the favoring effect of various treatments upon germination is entirely the result of stimulation of the embryo protoplasm. This hypothesis, however, leaves unexplained one important earlier observation (15). If the distal ends of the dormant caryopses are cut off just back of the ends of the embryos and the embryo portions are put to germinate, the following reactions ensue. First, a slight distension of the starchy endosperm beyond the edges of the cut surfaces of the coat structures; second, after a day or two the cells of the epithelial layer of the scutellum begin to elongate in the region of the cut surface and the digestion of the starch begins in the endosperm cells underlying such areas of elongation exactly as in the early stages of normal germination of unmutilated caryopses; third, a few days later, normal germination.

The most probable explanation of this set of phenomena is the effect of an increased swelling capacity of the embryo due to the reduction of the mechanical pressure upon it, this time working from behind the embryo and effecting it more slowly than when the coverings were removed from the embryo itself. To be sure, the radicle and epicotyl break through their coverings when germination occurs exactly as in the germination of unmutilated caryopses, but as the result of growth forces which are greater than the imbibitional force of the partially imbibed embryo, and which could not be initiated so long as the swelling of the embryo was greatly restricted. And it has been shown in a previous section (see p. 205) that the embryos of dormant imbibed caryopses are not completely satisfied with water. Theoretically increased oxygen supply to the embryo or the removal of an inhibitor to germination may play a part also, but the theory of increased imbibition seems much more plausible.

Cereals and other grasses which were not after-ripened have been induced to germinate by this method of cutting the grain in two just back of the embryo. Others (2, 18) also have induced germination of cereals which were not after-ripened by wounding the endosperm, and have
attributed the result to effects upon oxygen or water absorption. To be sure, Kiessling (22) reported that coating the wounded endosperms with substances which he claims prevented increased imbibition as a result of the wounding did not prevent the stimulating effect upon germination and used this fact in support of his "stimulus" hypothesis. But it is difficult to accept this hypothesis, because the only avenue for the transmission of such a stimulus to the embryo through the medium of living cells appears to be along the aleurone layer, and even this path is interrupted along the adjoining faces of the scutellum and endosperm by a mass of compressed nonliving endospermic cell walls. Furthermore, in our experiments with Johnson grass caryopses the first evidences of vital and enzymic action were observed, not where the aleurone approaches the scutellum—that is, at the extreme distal end of the scutellum, but in the area nearest to the cut surface—that is, several epithelial cells from the extreme distal end.

There is one strong indication that the coat structures are more or less directly responsible for the dormancy and germination physiology of Johnson grass caryopses independently of any effect upon the embryo protoplasm of the operation of removing or weakening these structures, namely, the correlation which has been shown in previous sections between the resistance of these structures to certain reagents, on the one hand, and the germination of the fresh caryopses, on the other.

That differences in these coat structure occur as between different kinds of seed with different germination requirements might be wholly incidental and irrelevant. But when in addition similar differences occurring in different lots of the same kind of seed parallel in a logical manner differences in germination under identical external conditions, the parallelism can hardly be without significance. These considerations suggest that the inhibitory effect of the unusually tough and compact coverings of the embryo may be related primarily to a purely physical restriction of the imbibitional swelling of the embryo, especially its axial organs, a restriction which is enhanced by the location of these organs almost wholly enveloped by the massive wings of the scutellum and is increased also by the pressure of the tight-fitting scales within which the caryopses are inclosed. That such a physical restriction is paralleled by a decrease in permeability of the coat structures and an increase in their resistance to chemical attack is entirely natural, since the tensile strength, elasticity, and extensibility of the coat structures is determined by the degree of drawing together of the individual elements and the degree of their impregnation with substances which are insoluble in water and which confer relative impermeability. It should be stated, however, that actual proof of this hypothesis, which relates the tardy germination of Johnson grass seeds to restricted imbibition by their embryo, is, in fact, wanting. As already pointed out, certain facts indicate that other factors, perhaps involving direct stimulation of the embryo protoplasm, enter into the forcing of their germination by chemical reagents. A sharp distinction should be maintained between mechanical and chemical forcing of germination even when, as here, the same kind of seeds is used in both cases. The fundamental explanation may be quite different in the two cases.
SUMMARY

(1) Johnson grass seed is markedly dormant when first matured under ordinary conditions of storage, requires a number of months for complete after-ripening, and even when fully after-ripened will not germinate completely except with the use of alternating temperatures in a very warm temperature range. Seeds of its close taxonomic relative, Sudan grass, germinate freely with a wide range of temperature either constant or alternating, and without the intervention of any considerable period of after-ripening.

(2) Johnson grass caryopses are invested in the fruit with very hard, tight, usually darkly pigmented scales (glumes), the removal of which accelerates the germination of the caryopses and increases their germinating capacity.

(3) The coverings of the naked mature caryopses of both grasses consist of the fused pericarp and inner integument, the outer integument and nucellus having entirely disappeared, with the possible exception of a portion of the latter over the micropyle. The complete or partial removal of these coverings over Johnson grass embryos induces prompt and complete germination even of freshly matured caryopses, and under the temperature conditions for the germination of Sudan grass seed.

(4) The pericarp of both kinds of caryopsis consists of a continuous outer epidermis, a fragmented inner epidermis, and several intermediate layers of loosely arranged cells. One of these layers frequently contains starch. The outer epidermis does not have especially thick walls and is easily broken. The pericarp tissue breaks jaggedly at the pedicel when the caryopsis is removed from the scales, thus opening a passage for solutes into the pericarp tissue clear to the inner integument.

(5) The inner integument of both kinds of caryopses is highly developed and of large cells with very thick, darkly pigmented inner walls.

(6) The micropyle is usually completely closed by a massive, recurved development of the inner integument.

(7) The large circular hilar orifice contains no vascular elements, conduction from the vascular bundle of the pedicel and outer layers of the pericarp over the hilar region being by means of parenchymatous pericarp tissue which entirely fills the hilar orifice, and is fused with the inner integument at the hilar margins.

(8) A zone of this conducting pericarp tissue lying just outside the hilar orifice and including the elements which are fused with the inner integument becomes greatly contracted radially and darkly pigmented during the maturation of the caryopsis. This pigmented zone of the pericarp and the inner integument together constitute for the caryopsis an unbroken investment which is extremely resistant to the action of Javelle water and of chromic acid and has the quality of selective permeability, though it probably does not exclude any solute entirely. Penetrant solutes (iodin solution) enter much more readily at the proximal end of the caryopsis, probably through the hilar orifice, than elsewhere, but they also frequently enter locally in other places.

(9) The coverings of Sudan grass caryopses are more fragile and their embryos are less tightly inclosed and are so situated as to be more exposed to mechanical injury than is the case with Johnson grass caryopses.

(10) The coverings of the caryopses are thicker over the hilar and micropylar regions and in front of the point of the radicle in Johnson grass
caryopses than in Sudan grass caryopses, this difference being related to the more forward exposed position of the Sudan grass embryos. In other regions the coverings of Sudan grass caryopses are thicker than those of Johnson grass caryopses but are less compact and less darkly pigmented and offer less effective insulation to the embryo.

(11) The coverings of Johnson grass caryopses are much more resistant to the bleaching action of Javelle water and the corrosive action of chromic acid and are somewhat less readily penetrated by iodin than are those of Sudan grass caryopses. The lots of Johnson grass caryopses which are most resistant to the action of Javelle water and of chromic acid are most deeply dormant when fresh and most resistant to germination when after-ripened.

(12) The coverings of wheat caryopses are less resistant to the action of chromic acid than are those of Sudan grass caryopses.

(13) The coverings of wheat and corn are more readily permeable to iodin than are those of Sudan grass caryopses.

(14) Soaking Johnson grass caryopses in lipoid solvents kills the embryos and at the same time increases the rate of the subsequent penetration of iodin and the rate of subsequent bleaching in Javelle water.

(15) The inner integument and the various layers of the pericarp of Johnson grass all contain tannin compounds, and all are highly suberized, especially the inner wall of the inner integument, which consists of suberin impregnated with fats and to which is due the great resistance of the caryopses to the action of chromic acid. Probably tannin, suberin, and lipoids all increase the strength and diminish the extensibility of the coat membranes and decrease their permeability to solutes, and probably all are related to the inhibiting effects of the coverings of the caryopsis upon germination.

(16) While this hypothesis is not subject to exact proof it seems probable that the character of the coverings of Johnson grass caryopses limits the imbibitional swelling of the embryos and thus keeps their water content below the minimum required for the inception of germination at relatively low and constant temperatures. Removal of the distal ends, exposing the caryopses to increased imbibition, induces enzymic activity in the scutellum followed by germination, reversing the order which characterizes normal germination. It is possible that inhibitory substances are held within the coats and that these maintain the embryo in a dormant condition. The effective forcing agents may oxidize or precipitate these substances, or they may modify the permeability of the coat structures so they can diffuse. Breaking the coat structures would also lead to the exit of such materials.

(17) Chemical treatments which increase the germination of Johnson grass and the wounding of the embryo may involve both a reduced resistance of the embryo coverings to imbibition and a direct stimulus to the embryo protoplasm.
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