THE HARD SEED MECHANISM IN *CONVOLVULUS ARVENSIS L.* AND THE INFLUENCE OF ENVIRONMENTAL VARIABLES UPON GERMINATION

by

ROBERT HAROLD CALLIHAN

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APPROVED:

Redacted for privacy

Assistant Professor of Farm Crops

In Charge of Major

Redacted for privacy

Head of Department of Farm Crops

Redacted for privacy

Chairman of School Graduate Committee

Redacted for privacy

Dean of Graduate School

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INTRODUCTION

Field bindweed (*Convolvulus arvensis L.*), or wild morning-glory is considered a primary noxious weed in most of the United States, due to its persistence on agricultural lands. It has been a serious weed in many countries, creating problems familiar to agronomists throughout most of the world. Under favorable conditions it is vigorous and robust, while in unfavorable environments it is weak and spindly; but even then it is an extremely persistent perennial herbaceous plant. For these reasons, considerable research effort has been focused on methods for controlling or eradicating the weed.

Many methods for control have been devised; several have been to a large degree successful, especially since the advent of modern herbicides. However, even after thorough eradication of the growing plant, reinfestation by seedlings occurs. Records show that the period elapsing between plant eradication and seedling reinfestation can be at least 27 years (43). Although modern methods of bindweed eradication by chemical application are greatly advanced, reinvasion by seedlings is counteractive. Reinvasion is usually attributed to dormant seed in the soil. Seed dormancy is common in the plant world, and

field bindweed is only one plant among many with this characteristic.

Seed dormancy has challenged the initiative of researchers in plant physiology and agronomy for many years. The mechanism of dormancy in some species has been elucidated in the past few years. Some examples of causes of dormancy are impermeable seed coats as in legume seeds, the presence of growth inhibitors as in Xanthium seeds, low concentrations of phosphate acceptors as in cherry seeds, the requirement of activation by light as in lettuce seeds, and the absence of essential substrates for cellular components as in tree peony seeds. Seed dormancy begins during maturation. The degree of dormancy is often related to the environmental conditions under which the seed is formed. The type of dormancy varies with species. Thus special germination requirements are needed for different dormant species.

Besides the dormancy problem, seeds of many species are rather specific with regard to environmental conditions for germination. Among the environmental factors which are known to affect germination are temperature, moisture, atmospheric gasses, and chemicals.

Impermeable seed coats are characteristic of many species of the Leguminosae. However, seeds of many other families are known to be impermeable. Most of these

families are composed of primarily weedy species as represented in the Solanaceae, Geroniaceae, Malvaceae, Chenopodiaceae, Portulacaceae, and Convolvulaceae. The mechanism of dormancy in bindweed seed is considered to be seed coat impermeability, or a "hard seed" dormancy.

Until more is understood of the characteristics of germination in bindweed seed, success in eliminating dormant seed as a reinvasion factor will probably arise empirically, if at all. This research attempts to determine the mechanism of hard seed and germination characteristics of bindweed seed to provide a broader base of fundamental information useful in development of control methods.

REVIEW OF LITERATURE

Moisture

The fact that apparently sound seeds of some plants fail to imbibe water and germinate has directed agronomists and seed physiologists along diverse paths of investigation. Impermeability as a dormancy-causing condition was recorded as early as 1876 by the German botanist, Friedrich Nöbe, in his work entitled Handbuch der Samenkunde (30), wherein he attributed impermeability to a waxy coating. However, he apparently had not entered into active investigation of the topic.

Perhaps the first actual investigation on the extent of seed coat impermeability was conducted by the Italian scientist, Guiseppe Gola, who published in 1905 that the condition was to be found in about 300 species of plants including species in Leguminosae, Cistaceae, and Malvaceae (17, p. 57-62). This was the first of a number of investigations carried on by scientists in various institutions in the early 1900's.

Rostrup, in 1896 (34, p. 53) treated seeds with acid to terminate the dormant condition, thus initiating the first work on actual treatment of seeds. Since that time, various methods for breaking seed-coat dormancy have been

investigated, including the use of extreme pressure (13), electrical currents (16), freezing (5, 29), impaction (19), hot water (6), infrared heat (32), and presoaking of alcohol (36). Various early methods of scarification were discussed by Rose (33, p. 425-427). Since then various scarification methods came into use both for experimental purposes and for commercial and farm use (14, 40, 32).

Experiments of early workers indicated the area of impermeability of most seeds was the palisade layer, or the cuticle covering palisade layer (19, p. 346). White (51, p. 372) and Raleigh (31, p. 273-275), concluded that a layer of cuticle over the palisade cells of the seed coat was responsible for impermeability in many small seeded legumes. Raleigh found that pectic substances in the seed coat of Gymnocladus caused impermeability with maturity.

Lute (27, p. 30) stated that impermeable alfalfa seed became permeable when the tips of the Malpighian, or palisade cells of the seed coat were planed through.

It has been postulated that, in many impermeable seeds, when natural water absorption finally occurs, it occurs through cracks in the seed coat caused by weathering

or by the action of soil microflora. This has been shown to be the case in some legume seeds (2, p. 355-360; 44, p. 618-619).

Simpson and Adams (37, p. 22-23) found that the primary site of water absorption in cotton seeds is the chalaza. They concluded that some uptake occurred through the micropyle, but none through the seed coat. Christiansen and Moore (7, p. 582) found that impermeable cotton seed contained a chalazal plug which was impermeable due to a high concentration of lignin and/or pentosans. This chalazal plug was rendered permeable by hot water, thus permitting the seed to absorb water.

It has been suggested that weathering and soil microorganism action render hard seed coats permeable under natural conditions (6, p. 475; 37, p. 22).

Rudolfs (35, p. 293) found, in studies with various seeds, that there was a marked difference among genera and species in their water absorption rates. Legumes (alfalfa, soybeans, white lupine, and Canada field peas) showed higher absorption rates than wheat, corn, watermelon, and buckwheat. Absorption rates were progressively retarded by increasing the osmotic pressure.

Shull (39, p. 31) concluded that water absorption phenomena in seed were complex processes which depended upon a number of factors, both external and internal. He

determined the imbibitional power of seeds for Xanthium by subjecting them to osmotic forces as high as 1000 atmospheres created by salt solutions of varying concentrations. He discovered that these seeds attracted water from a medium which exerted an osmotic pressure of 1000 atmospheres. Using this study as a calibration of his Xanthium seeds, he was able to determine with fair accuracy the force with which moisture is held in a soil by allowing seeds to germinate in the soil under varying moisture contents.

Shull concluded that the osmotic force exerted by the salt solutions was of the same nature as that exerted by the soil solution, and that the osmotic force exerted may be referred to as soil moisture tension. Since Shull's work, this idea has found wide acceptance among plant physiologists and soil scientists (3, p. 210; 28, p. 98-99). On the basis of the work done by Shull and others, Baver (3, p. 210) considered moisture absorption by seeds as one of the most important techniques with which the capillary potential of a soil can be determined.

Hunter and Erickson (21, p. 108-109), found that for germination of sugar beets, corn, rice, and soybean seeds each species had to attain a certain moisture content. Each required a soil moisture tension not to exceed a maximum which varied considerably among the species used.

Some had the ability to germinate at a much higher tension than others, i.e. 3.5 atmospheres for sugar beets, 6.6 atmospheres for soybeans, 7.9 atmospheres for rice, and 12.5 atmospheres for corn, at a temperature of 25° C.

Helmerick et al. (20, p. 560-562) studied wheat germination and early growth under controlled limited moisture environments, and found that moisture could be controlled through the use of various osmotic concentrations of mannitol, which, according to Collander and Barlund (9, p. 60) is an inert nonelectrolyte which probably enters plant cells slowly if at all. Thimann (42, p. 600) concluded that mannitol was the best chemical found to physically limit water uptake in a plant without affecting the metabolic activity of the plant cells.

Uhvits (46, p. 284), studying the germination of alfalfa seed under osmotic stress, found that NaCl exhibited a toxic effect on alfalfa seed germination when used to obtain osmotic tensions. This observation complements the conclusions of Ayers. Mannitol, however, gave the desired osmotic tensions without the toxic effect. There has been general acceptance of mannitol as a compound useful in producing osmotic tensions, since the work of Uhvits.

Stiles (41, p. 221-222) working with corn and cotton seed, concluded that apparently seeds exist with hydric,

mesic, or xeric germination adaptations, or tendencies toward these types of responses. There were marked differences among species and varieties within species with regard to seed coat hygroscopicity, rate of water uptake by endosperm and cotyledons, and final amount of water absorbed at germination. Stiles also found that the seeds used displayed differences in hygroscopicity and imbibitional absorption capacity throughout the uptake and germination stages.

Temperature

Research has been conducted on germination response of many cultivated plant seeds and weedy plant seeds to temperature variation (11, p. 87-90; 49). Therman optima vary widely among species, but usually correspond roughly to habitat growing season temperatures. Some seeds have been found to be rather specific in temperature requirements whereas other types have displayed little sensitivity to temperature variations.

Many seeds seem to germinate best when subjected to diurnally alternating temperatures, with a relatively long cooler period of about 16 hours alternating with a short warmer period of about 8 hours. Certain seeds germinate more rapidly at constant temperatures or may even be slowed up by alternating temperatures. Others

require periods of low-temperature stratification before they will germinate. Various combinations of these requirements may be necessary to germinate certain seeds.

Brown and Porter (6, p. 487) at Iowa germinated bindweed seed at temperatures ranging from .5° C. to 40° C., and found most rapid germination at 30 and 20-30° C., indicating that the seeds used were not responsive to temperature fluctuations.

Light

In the large majority of photosensitive seeds, light favors germination, but is not absolutely necessary. Kinzel (11, p. 121) in 1913, made limited tests with 964 species and found that light enhanced germination to some degree in 70 percent of them, inhibited germination to some extent in 27 percent, and had no effect on only 3 percent of the species. It appears from his study that although some types of photoresponses are common within a family, taxonomic or morphological similarities seem to have little bearing on photoreaction in seeds, for dissimilarities are very common within a taxon.

It is recognized that physiological reactions, and therefore photoreactions, must interact with temperature. Toole et al. (45, p. 477) and Evenari (15) point out that as a rule, photoreaction in seeds is best displayed at temperatures most favorable for germination.

Evenari has pointed out that photosensitivity shows up from wave lengths well within the visible spectrum, normally in the red range. However, Toole et al. (45) reported germination inhibition of lettuce seeds occurring from wave lengths in the blue range.

METHODS AND MATERIALS

This study consists of four principal parts: (1) a survey of the prevalence of hard seed from bindweed infestations in diverse environments in the western United States; (2) the mechanism of impermeability; (3) methods of inducing imbibition in hard seed; and (4) the influence of environmental factors upon imbibition and germination of bindweed seed. Seed for parts (2), (3), and (4) were collected at Corvallis, Oregon.

A six-pound bulk sample of seed was harvested from a 360 square foot area in Avery Park at Corvallis. This was obtained by mowing the prostrate plants close to the ground ($\frac{1}{4}$ inch) with a power scythette, allowing the vines and capsules to dry, and threshing the material in a small experimental rub-bar plot thresher at a cylinder speed of 1000 rpm.

Impermeable seed were obtained by soaking seed from the bulk sample for one week and then separating the permeable from the impermeable seed. Since a large portion of the bulk sample was permeable, a rough separation was made by flotation in a pan of water. The slight difference in specific gravity between imbibed and impermeable seed provided a means for good separation of fully imbibed

seed, which were less dense than the dry seed. Since imbibition is a gradual process, some seed were intermediate between the fully imbibed and the "hard" or impermeable stage, and thus the density difference was smaller. These were separated out by hand as completely as possible, the separation criteria being color intensity, softness, or resistance to pressure applied by forceps, and resiliency, as determined by the tendency of imbibing seeds to bounce when dropped on a hard surface.

The Prevalence of Hard Seed

Ten-gram samples of mature seed were carefully hand harvested from four different locations representing varied environments in the western United States; Genesee, Idaho; Corvallis, Oregon; Lone Pine, Oregon; and Sacramento, California. Table 1 gives climatic and edaphic data for each location.

The seeds in each sample were counted with a vacuum-counter and the average weight was determined. Fifty seeds were randomly selected from each lot, and the gross morphology was observed with the unaided eye and with a microscope.

To determine the germination and viability of the seed lots from the four locations, four replicates of 100

Table 1. CLIMATE AND SOIL DATA

Location	Average Frost-Free Days	Ave. Temperatures July Ave.	Ave. Temperatures January Ave.	Long-Time Temp. Extremes Max.	Long-Time Temp. Extremes Min.	Ave. Precipitation	Soil Type
Corvallis	191	65	39	106	-14	40"	Gray-brown Podzolic
Lone Pine	92	60	22	118	-37	9"	Azonal lithosol
Genesee	153	67	28	105	-30	22"	Nez Perce loam
Sacramento	307	74	46	114	17	16"	Azonal alluvial fan

seeds from each lot were placed on moist germination blotters in plastic sandwich boxes and incubated in a germinator at 35° C. for two weeks. Total germination was recorded at the end of two weeks. Each ungerminated but not imbibed seed was considered "hard" and was then scarified on a file, returned to the appropriate box and incubated for one additional week, after which germination was again counted. The final total germination after scarification was designated as live seed percentage.

The Mechanism of Hard Seed

Hyde's work (22) with leguminous seeds showed that the hilum is a primary site of water loss and uptake due to a moisture-sensitive seed coat layer at the hilum which acts as a valve for opening and closing the opening. External morphological similarities between bindweed seeds and legume seeds indicated the possibility of a parallelism in bindweed seed. An exploratory experiment was conducted to determine whether this is so.

Three replications of 200 hand-threshed seeds containing approximately 95 percent impermeable seeds were divided into two 100-seed portions. One was soaked in concentrated sulfuric acid for one hour; the other was not treated with acid. The hilum ends of half (50 seeds)

of each portion were dipped in petroleum jelly, and the four 50-seed groups were placed in a 20-30° C. germinator. Imbibition was recorded after 3 days.

To determine the effect of concentrated sulfuric acid upon the seed coat in order to further investigate the significance of the hilum in water uptake, seed were scarified in concentrated sulfuric acid for varying lengths of time. Approximately 500 seeds were soaked in concentrated sulfuric acid; groups of 50 seeds were removed, washed, and examined after 15 minutes, 30 minutes, and 1, 4, 8, 16, 32, and 48 hours of acid treatment.

Three hundred twenty impermeable seeds were used in another experiment to observe the effect of relative humidity upon the hilum aperture. Wax islands were placed in eight glass petri dishes, and sulfuric acid solutions of a range of known concentrations varying from 8 percent to 28 percent were poured into the dishes, so that when the petri dish covers were replaced, each dish would be a chamber of known relative humidity. Twenty hard seeds with noticeably open hilum apertures were mounted on each wax island with the basal (hilum) end protruding from the wax so it could be observed by microscope through the glass petri dish cover. A ten-minute lapse was allowed for the seed to come to equilibrium with

or adjust to the relative humidity to which they were exposed. Then the number of seeds with closed hilums were noted.

Some experience was necessary to determine whether a hilar fissure was actually closed. Since the hilum is microscopic in width, proper focusing of a binocular microscope was essential. If the focal point is above or below the level where the valve action occurs in the testa, the determination of closure became difficult and appeared to be rather subjective and doubtful. Careful focusing prior to hilum closure made the change easy to detect. The hilum aperture would open within one minute after being removed from a high relative humidity, so it was necessary to keep them in the chamber while making observations.

To reveal the seed coat structure, particularly in the hilum and micropyle area, seeds were microtome-sectioned and permanent slides were prepared. Hard seeds were scarified with a razor blade and allowed to imbibe water until perceptibly soft so that they could be cut without shattering. They were placed in a killing and fixing solution (40 percent formalin-glacial acetic-70 percent alcohol, 15-15-70 percent by volume), subjected to vacuum aspiration, and left in the solution for 48 hours.

They were then dehydrated by the tertiary butyl alcohol-paraffin oil series (25, p. 130-131), infiltrated with three changes of paraffin, and embedded in Tissuemat. The bindweed seed thus embedded was sectioned with a rotary microtome in 12 to 20 micron thicknesses.

The sections were then dewaxed with two changes of xylene, hydrated by passing back through the ethyl alcohol series, and stained with safranin or with a safranin-crystal violet-fast green triple stain.

Inducation of Imbibition

Since surface tension reduction facilitates water penetration into interfaces, an attempt was made to cause water penetration through the hilum aperture by using detergents to decrease the surface tension of water. Three common household detergents were used in 15 percent solution, each on groups of 100 seeds. The seeds were allowed to soak in the detergent solutions and a water control for one week, and imbibed-seed counts were made.

Ethyl alcohol treatment was used to determine whether a dehydrating agent might inactivate the hygroscopic hilum. An experiment designed to determine whether a pathway for imbibition might be opened by that means.

Two hundred seeds were divided into two groups; 100 were soaked in 100 percent ethyl alcohol for ten hours and 100 were untreated. After the ten-hour soaking period, the hilum end of 50 seeds from each 100-seed group were dipped in petroleum jelly. The four 50-seed groups were then placed in a germinating environment, and imbibition was noted after three days. This was repeated twice.

To determine whether powerful fumigants might affect seed imbibition by inactivating the counter-palisade tissue either alone or in a penetrating carrier such as alcohol, the following experiment on impermeable seed was conducted.

One hundred twenty thousand impermeable seeds were counted by vacuum counter in lots of 100. These were randomly assigned to treatments in a 2 x 2 x 2 x 5 completely randomized experiment consisting of forty treatments repeated three times. The treatments were as follows: there were two pretreatments in which seeds were soaked in 100 percent ethyl alcohol for 16 hours versus no alcohol; two prefumigating states where seeds were fumigated after removal from the alcohol and dried versus seeds fumigated after removal from the alcohol but not dried; two fumigating conditions in which seeds were fumigated in a germinating environment or in dry flasks;

and five fumigants, Chloropicrin, formaldehyde, carbon disulfide, sodium N-methyl dithiocarbamate (Vapam), and no fumigant, as a control.

After the seeds were fumigated for twelve hours, all seeds were placed in germinating boxes and allowed to incubate at room temperature for one week, after which, imbibition and germination were recorded.

The Influence of Temperature, Light and Moisture

Machine-scarified and unscarified seeds were used to estimate the response of bindweed seed to a range of germination temperatures. The seeds were placed in germinators held at four different temperatures: 20-30° C., 5-15° C., 15-25° C. and 5° C. The percentages of imbibition and germination were recorded each day for the first week after initiation of the experiment, then at ten and fourteen days.

To test for a possible response to light, a two-by-four factorial experiment in a split-treatment design replicated four times was conducted. The temperature variants used were the alternating temperatures 20-30° C., 15-25° C., 5-15° C., and a constant temperature of 5° C. Each treatment was imposed upon one 100-seed sample in a plastic germination box and was replicated in germinator

levels four times. Within each temperature treatment, half of the samples were kept in a light-proof box and half were subjected to illumination during the day. Seed imbibition and germination were observed after ten days of incubation. Criteria used to estimate growth rate were radicle elongation as determined by measurement, and advancement to the hypocotylar arch, or crook, stage as determined by visual judgment.

In order to estimate seed imbibition and germination under moisture stress conditions, a test was designed to observe the effect of moisture tension created by osmotic pressure, or moisture tension, simulated by mannitol concentrations. The molar concentrations applied were 0.0 molar (distilled water) as a control; 0.015 molar, representing 1/3 atmosphere, corresponding to the generalized "field capacity" of a soil; 0.15 molar, or ten times the moisture tension at field capacity; 0.67 molar, representing the 15-atmosphere "wilting coefficient" of a soil; and 1.38 molar, representing 31 atmospheres, the generalized "hygroscopic coefficient" of a soil (28, p. 216-218).

The treatments were imposed upon replicated sample groups of 100 seeds, each group on a standard germinating blotter in a plastic sandwich box to minimize concentration

of the medium by evaporation. The seeds were incubated for two weeks in a 20-30° C. alternating temperature germinator, and observations were made each day for one week, then every two or three days for the second week. Criteria used in evaluating the response of bindweed seed to these treatments were percent imbibition and germination.

The reaction of bindweed seeds to submergence in water was tested. Eight boxes of 100 seeds each were filled with water, the seeds were allowed to sink, and the boxes were placed in a 20-30° C. germinator. This was replicated three times. Water was removed at two-day intervals, and the seed was allowed to remain on moist germinating blotters. Germination and mortality were observed three days after removal of the water.

Statistical Analysis of Data

Analysis of variance and Duncan's multiple range test were made where applicable for those experiments designed for such tests. The analyses are summarized in tabular form in the appendix. The multiple range tests are shown graphically, where applicable, in figures accompanying the description of the observations.

OBSERVATIONS

The Prevalence of Hard Seed

Impermeability was uniformly high among the samples collected from the four widely separated locations in Oregon, Idaho, and California. Seed viability after scarification of impermeable seed was also uniformly high. The average percentages of permeable seed and live seed for the four locations were:

Location	Permeable Seed	Germination	Live Seed
Corvallis	8%	7%	99%
Lone Pine	7%	7%	100%
Genesee	8%	5%	97%
Sacramento	8%	6%	98%

One percent of the permeable seed in the Corvallis sample and three percent of those in the Genesee sample were non-viable due to unknown causes. Two percent of such seed in the Sacramento sample were non-viable, and these two percent were found to harbor nematodes. The occurrence of nematodes was noted in several instances in Corvallis seed samples.

The Morphology of Bindweed Seed

Seed shape varied according to the completeness of development or the number of seeds per capsule in which

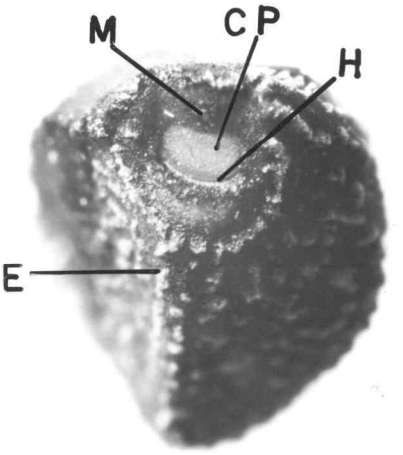
four is the normal number. The seed (figure 1) is sectoroid and rather plump, tending to become spherical, narrower at the basal or hilar end than at the upper end. It has a convex dorsal surface, with flat ventral surfaces which form at an angle of from 90 to 180 degrees to each other.

Externally, the seed coat is brown or black, papillose and rather granular and roughened, with a scurfy appearance when magnified.

The hilum area lies at an oblique angle to the axis of the seed. The hilum is a curved cleft approximately 40 microns wide by 1 millimeter long and lies in the center of this area, where the dark-colored roughened portion of the seed coat is not present. The location of the micropyle is represented by a circular area almost microscopic in size, and is 2-4 times its own diameter (60μ) from the hilum area.

The internal morphology of the seed is shown in the median-longitudinal section in figure 2. The short, straight radicle which lies in the basal half of the seed is pointed downward and lies against the dorsal surface. The thin, laterally redoubled cotyledons fold over a septum which originates at the apex of the angle of the ventral surfaces and extends about half way to the dorsal surface throughout the length of the seed.

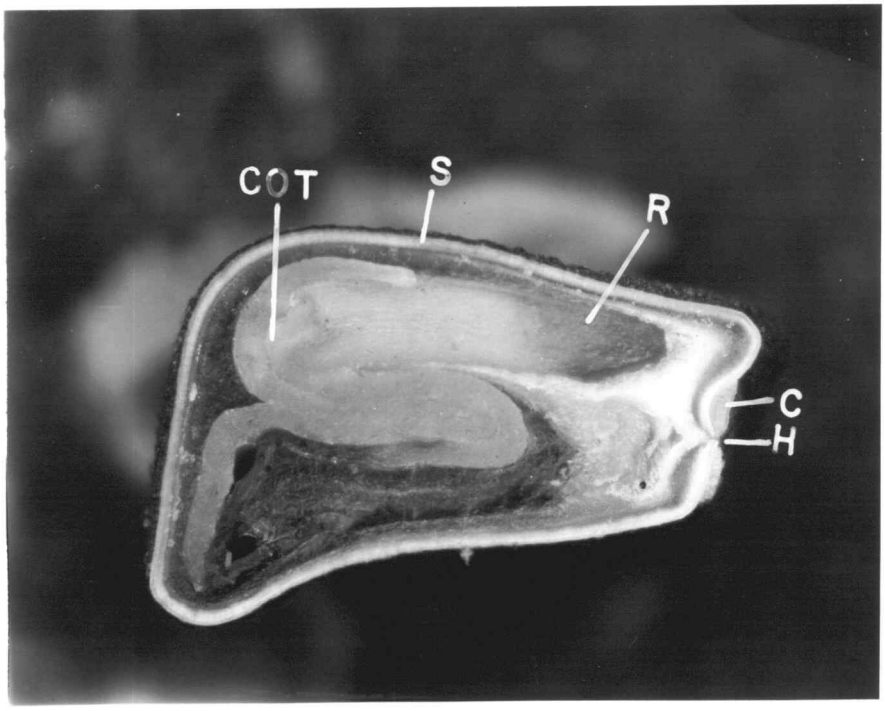
FIGURE 1



Bindweed Seed
20X

M - Micropyle
CP - Counter Palisade Layer
H - Hilum
E - Epidermis

FIGURE 2



Longitudinal Section of a Mature
Bindweed Seed

22X

C - Counter Palisade
H - Hilum
R - Radicle
COT - Cotyledons
S - Seed Coat

The average seed weight is approximately 10.5 milligrams, so there are approximately 43,600 seeds per pound.

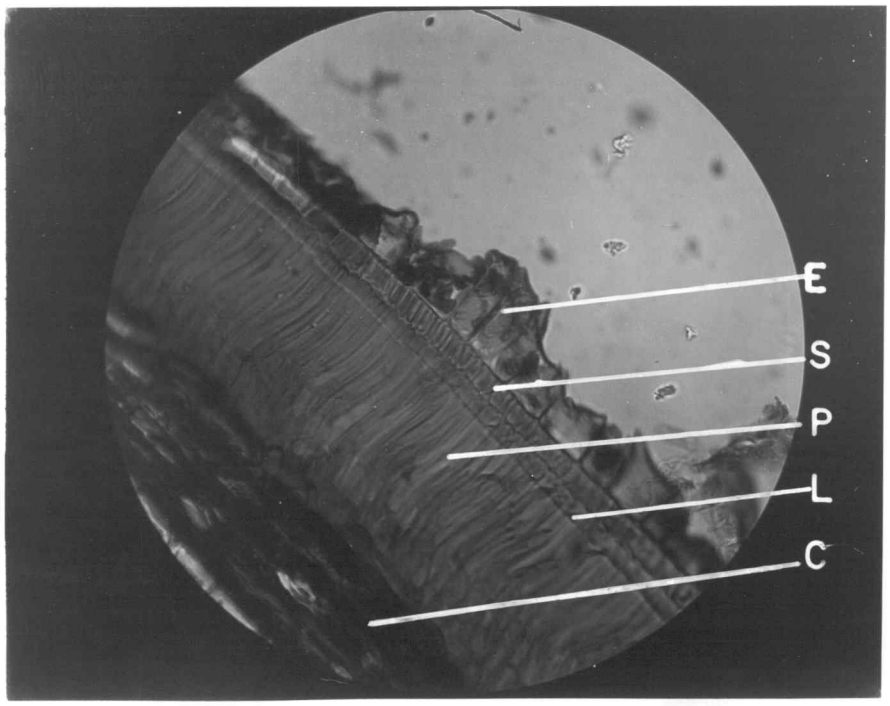
Seed Coat Anatomy

In figure 2, the white palisade sclerenchyma is seen as a continuous layer except for the hilar fissure which dips beneath a thicker layer of palisade sclerenchyma on either side of the hilum.

Figure 3 shows the anatomical features of the seed coat. Immediately beneath the palisade layer is a layer of crushed parenchyma which possibly originated from the nucellus and endosperm. Just above the palisade layer is a subepidermal layer of small compact cubical sclereids. Above these sclereids is a layer of epidermal cells which contains the pigments and gives the characteristic black or dark brown color.

Figure 4 shows a longitudinal section of the hilum of a maturing seed coat at the time of separation from the funiculus. The thick secondary epidermis on the left side of the hilum is almost identical in structure to the rest of the testa, but the malphigian cells are somewhat longer, causing it to be twice as thick as the main part of the palisade layer. This is called the

FIGURE 3

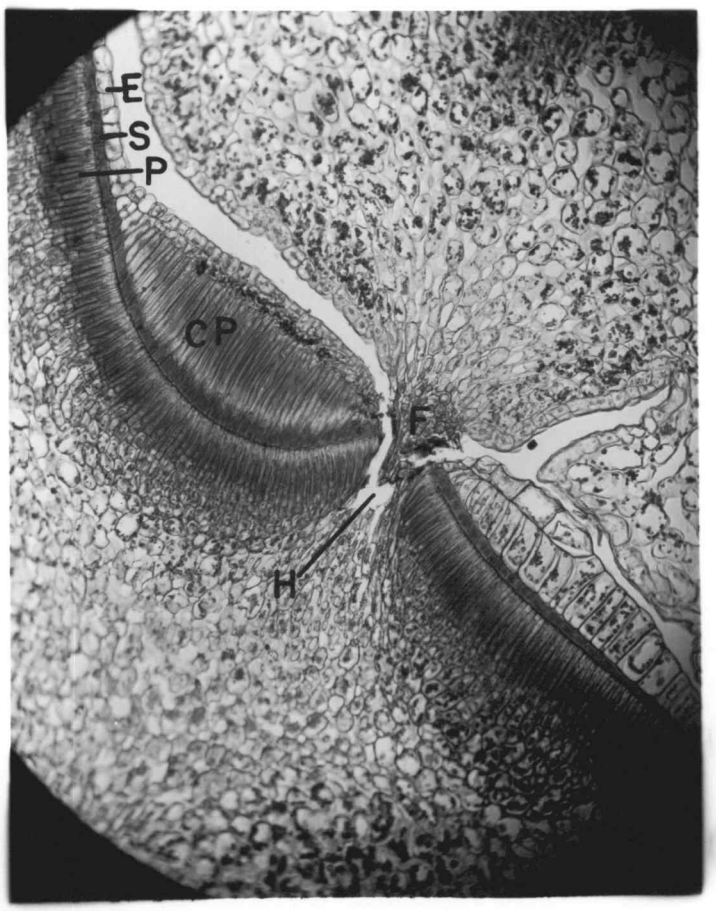


Section of a Mature Bindweed Seed Coat

400X

E - Epidermis
S - Subepidermis
P - Palisade Layer
L - Light Line
C - Crushed Parenchyma

FIGURE 4



Longitudinal section of the hilum area of a maturing bindweed seed still in the capsule, showing the seed coat at the hilum at the time of separation from the funiculus. The light line is not well defined at this stage.

150X

F - Funiculus
E - Epidermis
CP - Counter Palisade Layer
S - Subepidermis
P - Palisade Layer
H - Hilum Opening

counter-palisade layer by virtue of its similarity and position in relation to the main testa.

It is this counter-palisade layer that is thought to be highly hygroscopic and sensitive to changes in relative humidity. This assumption was verified by later experiments in this study.

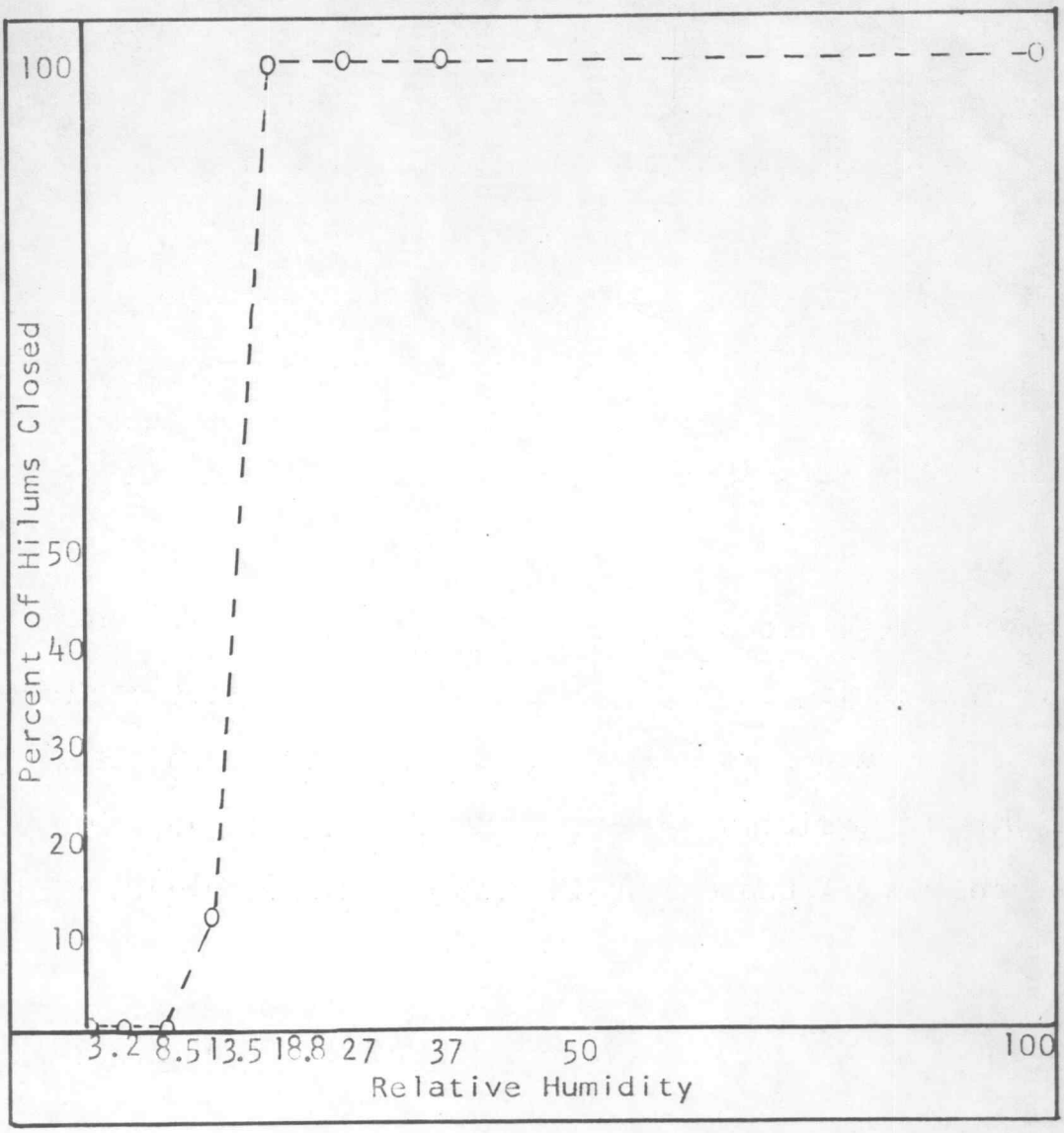
The Effect of Atmospheric Relative Humidity on the Hilum Aperture

At those relative humidities used above 13.5 percent, the hilar fissures were closed without exception, and at those below 13.5 percent, they were all open (figure 5). At 13.5 percent relative humidity, 88 percent of the hilums remained open. Figures 6 and 7 show the hilum of one seed in these two states.

Sulfuric Acid Scarification

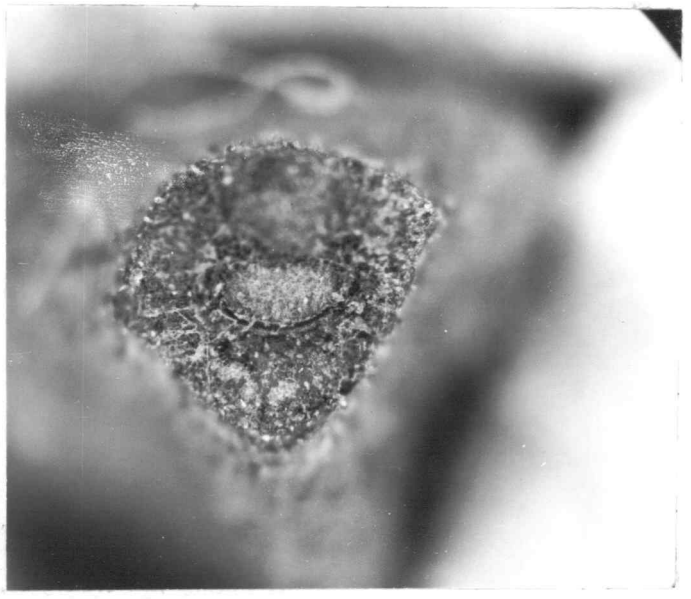
Figure 8 illustrates the effect of concentrated sulfuric acid on percent imbibition, or percent permeable seed. The sample contained about 87 percent hard seed; treatment with acid for 45 minutes reduced this to 55 percent. When the hilum ends of untreated seed were covered with petroleum jelly, percent imbibition was reduced to 5 percent; when the hilum ends of acid-treated

FIGURE 5



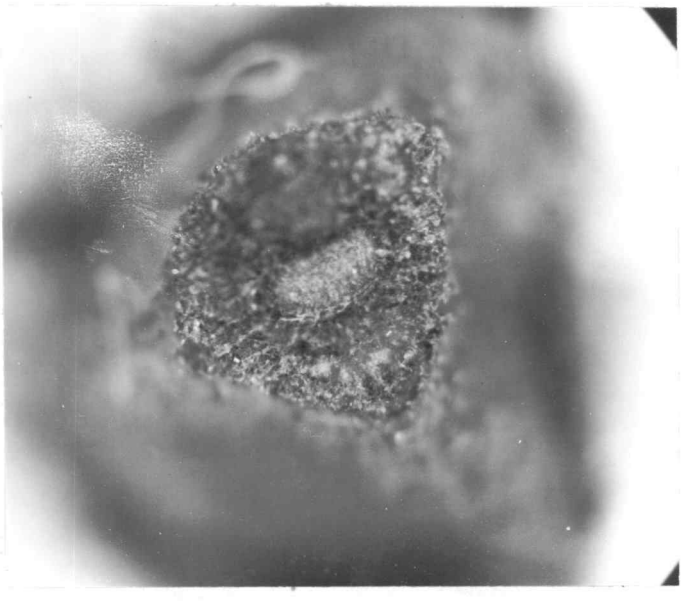
The Influence of Relative Humidity
Upon Hilum Aperture

FIGURE 6



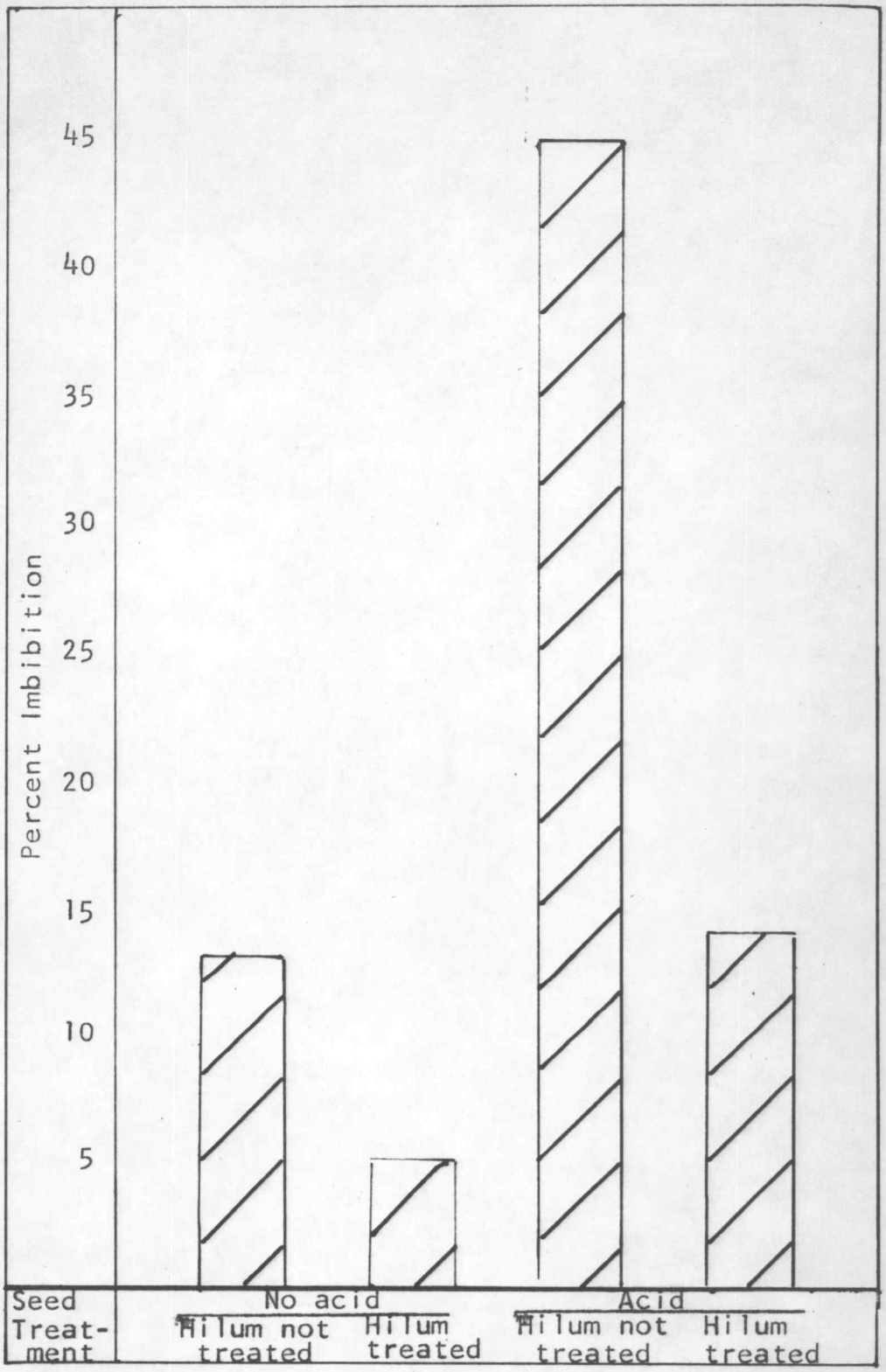
Hilum Open - Relative Humidity: 8%
40X

FIGURE 7



Hilum Closed - Relative Humidity: 18.8%
40X

FIGURE 8



Bindweed Seed Imbibition as Influenced by 45 Minutes of Soaking in Concentrated H_2SO_4 and Blocking of the Hilum

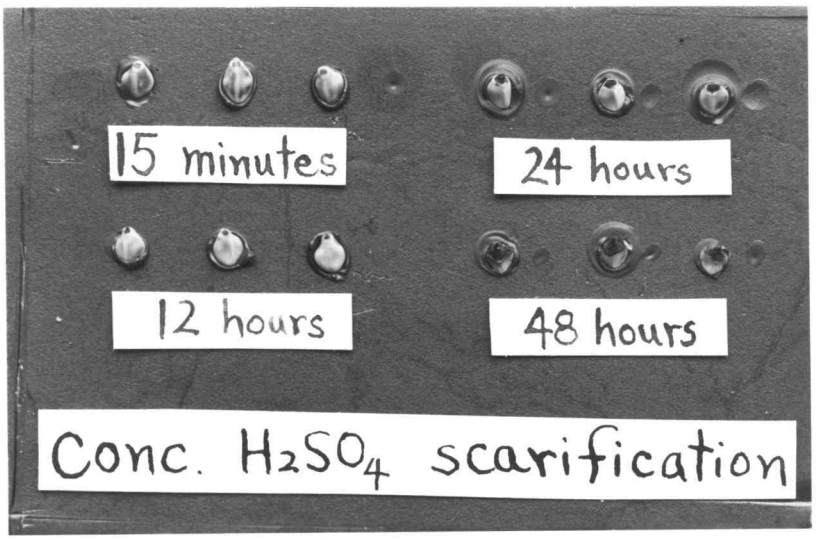
seed were coated with petroleum jelly, it was reduced to 14 percent.

Sulfuric acid immersion was decidedly selective in dissolving the tissue around the hilum area (figure 9). Immersion for fifteen minutes in concentrated sulfuric acid removed all of the pigmented epidermis, leaving much of the subepidermal layer and the entire palisade layer intact. After about thirty minutes of immersion, a few impermeable seeds became permeable, but at least one hour of immersion was required in order to cause 90 percent of the seed to become permeable. After one hour, the counter-palisade layer began to separate from the main testa. Figure 9 shows the dissolution of the seed occurring progressively away from the hilum. Even after two days the palisade layers on the end opposite the hilum had withstood the attack of the acid. Figure 10 shows a seed which had been submerged for 15 minutes to outline the extent of the counter-palisade layers and the white palisade layer.

Treatments to Induce Permeability

Reduction of surface tension through the use of detergents was not effective in causing water uptake.

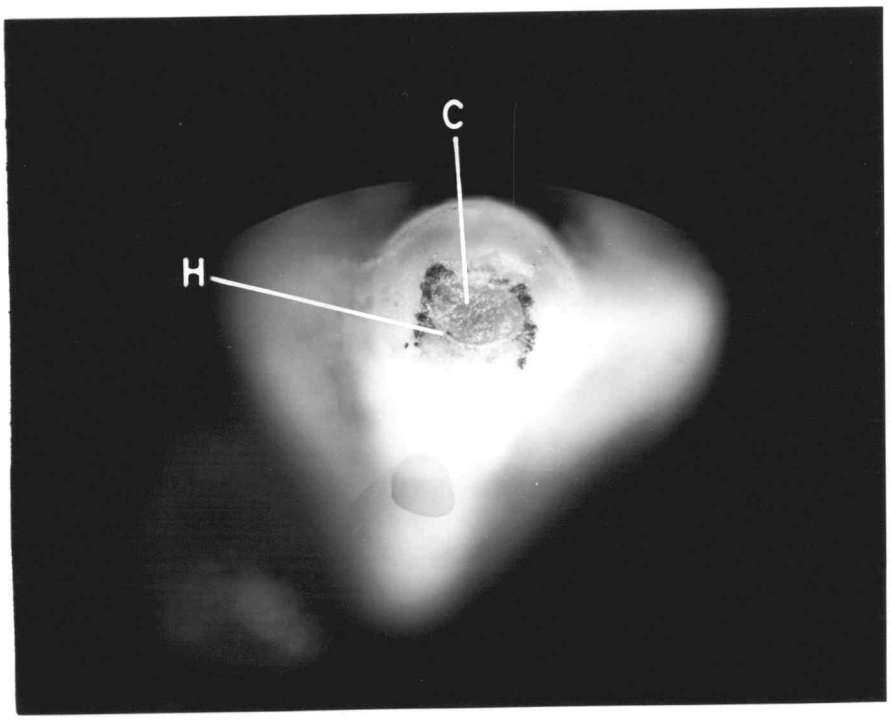
FIGURE 9



The effect of soaking in concentrated H_2SO_4 for varying lengths of time, showing acid decomposition beginning at the hilum and moving progressively toward the chalazal end.

1.5X

FIGURE 10



Hilum area of a bindweed seed after a 15-minute immersion in concentrated sulfuric acid.

22X

C - Counter Palisade
H - Hilum

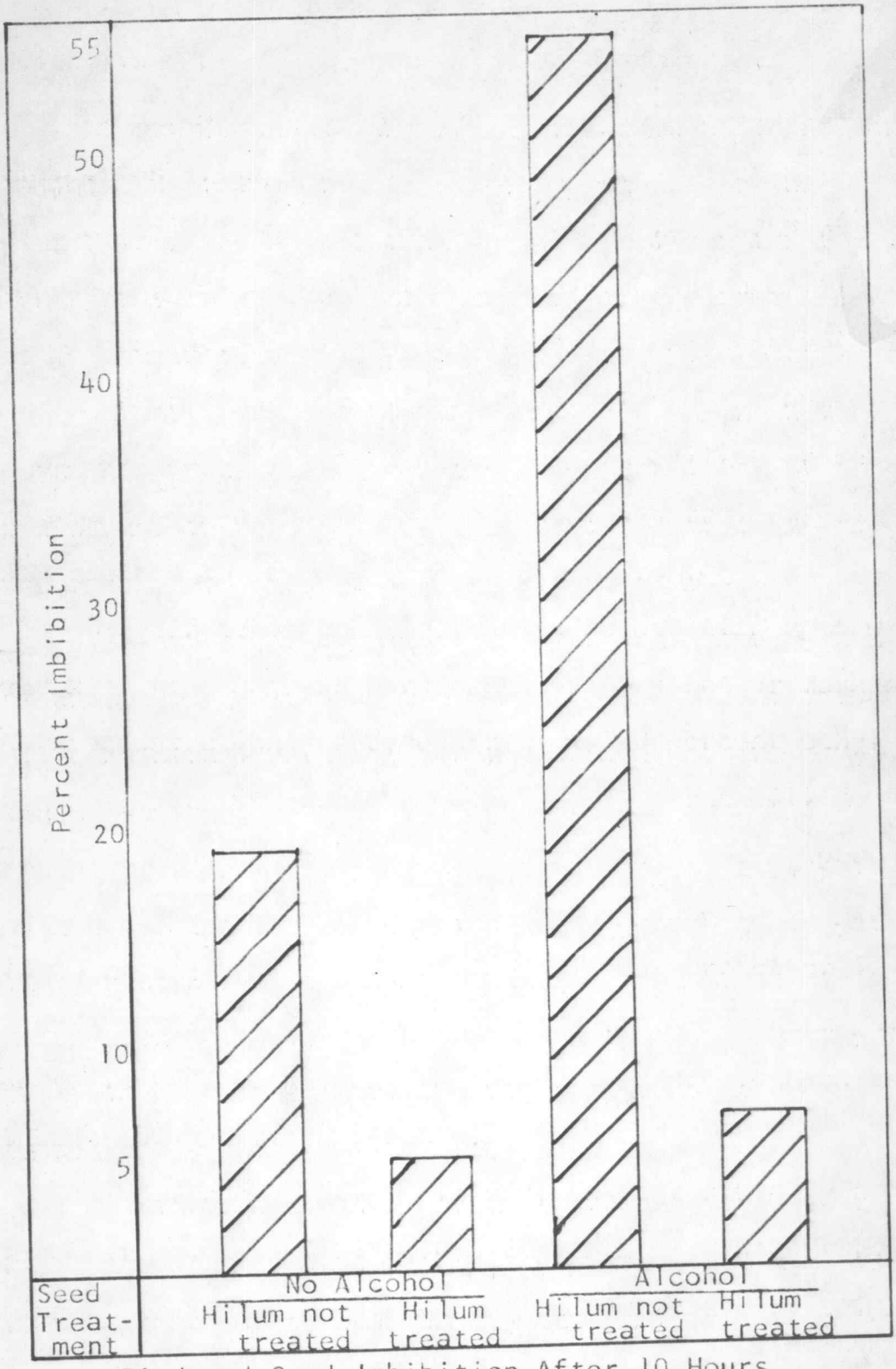
None of the detergents used were observed to cause even slight increases in percentages of impermeable seed.

Figure 11 shows the effect of absolute alcohol on imbibition of impermeable seed. About 55 percent permeable seed was obtained by alcohol immersion for ten hours, as compared with the original permeability of 19 percent. Blocking the hilum area with petroleum jelly after treatment with alcohol reduced the number of permeable seed to 7 percent. The permeability of seed not soaked in alcohol but treated with petroleum jelly was reduced to essentially the same level.

The effect of ethyl alcohol concentration upon water uptake in seeds treated for 20 hours was rather marked. All concentrations used, other than 100 percent ethyl alcohol, were ineffective in producing permeability, but 100 percent ethyl alcohol caused at least 90 percent of the seeds to become permeable as opposed to 15 percent in untreated seeds. Figure 12 shows the effect of 10 hours of soaking in absolute alcohol. The counter palisade layer is contracted, and the hilum aperture is open as in naturally permeable seeds.

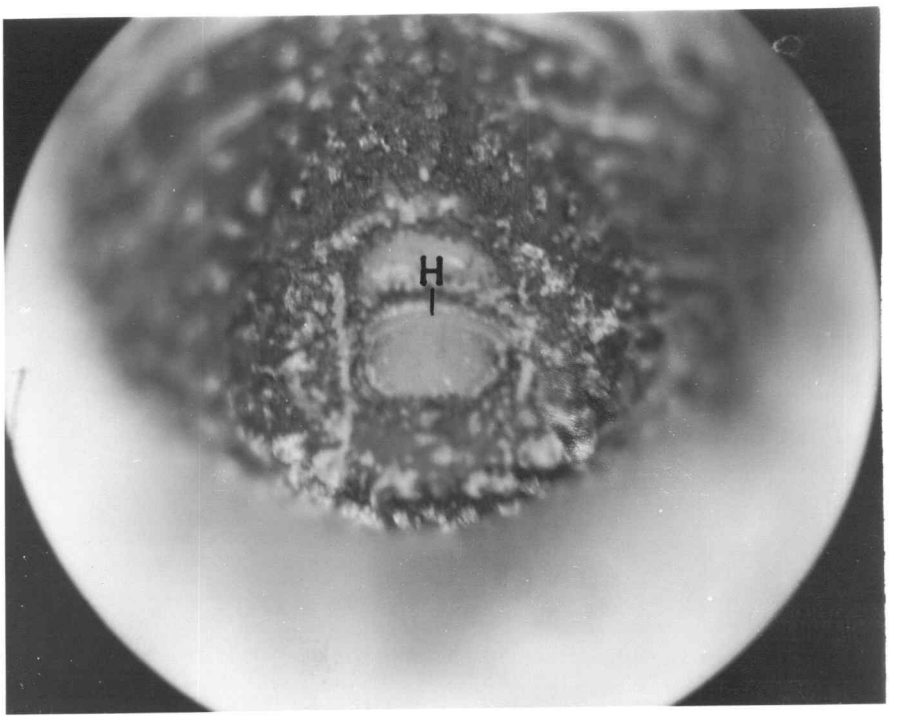
Considerable radicle injury, characterized by a blackening and an inhibition of growth, was noted after alcohol treatment. This injury ranged from very slight to

FIGURE 11



Bindweed Seed Imbibition After 10 Hours of Soaking in Absolute Alcohol

FIGURE 12



The hilum area of a bindweed seed in which permeability has been induced by absolute alcohol, showing the opening of the hilum fissure.

40X

H - Hilum

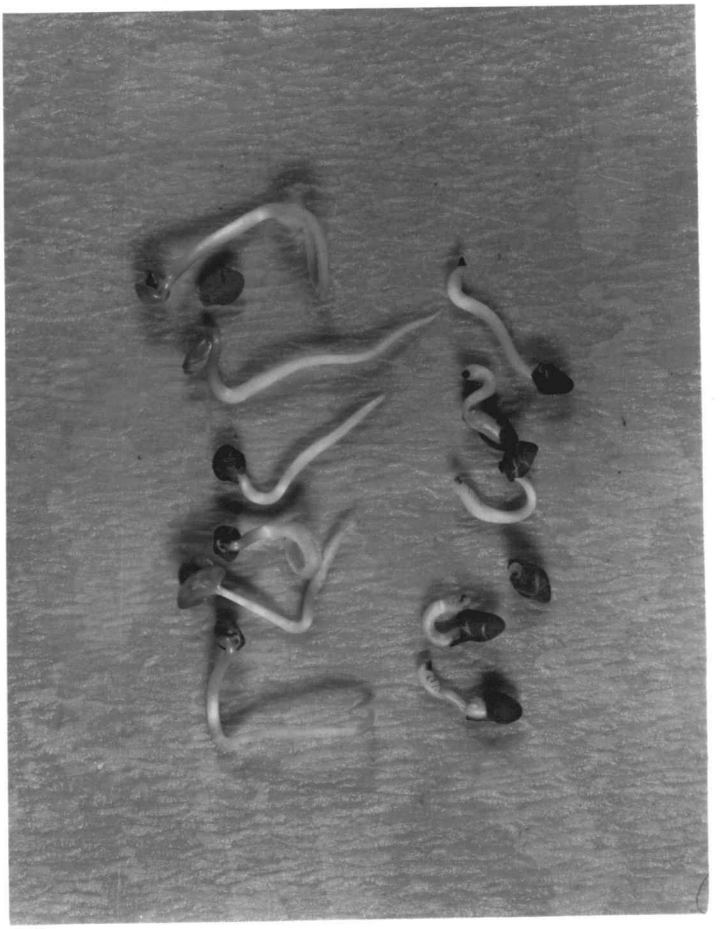
very severe. Several severe cases are shown in comparison with normally developing seedlings in Figure 13. In the severely affected seedlings, no radicle growth occurred, but the hypocotyl often developed normally or somewhat thickened even after cotyledon emergence.

On treating seeds with the four fumigants in all combinations with and without ethyl alcohol (fumigated both dry and wet with alcohol) and with and without water during fumigation, the only effective treatment was absolute ethyl alcohol, which increased the percentage of permeable seeds from 10 percent to 82 percent.

Fumigants, in the manner used, were not effective in impairing the hilar valve mechanism. Vapam and carbon disulfide dissipated rapidly enough that no toxic effect upon seedlings were observed. Chloropicrin and formaldehyde had a residual effect in the germination medium and inhibited all seed germination and all bacterial and fungal growth for at least 30 days after termination of the imbibition experiment.

In Figure 14, chloropicrin fumigation after ethyl alcohol soaking is compared with alcohol treatment alone, chloropicrin fumigation alone, and no treatment. Chloropicrin and formaldehyde were indistinguishable in their effect upon seedling growth and subsequent fungal

FIGURE 13



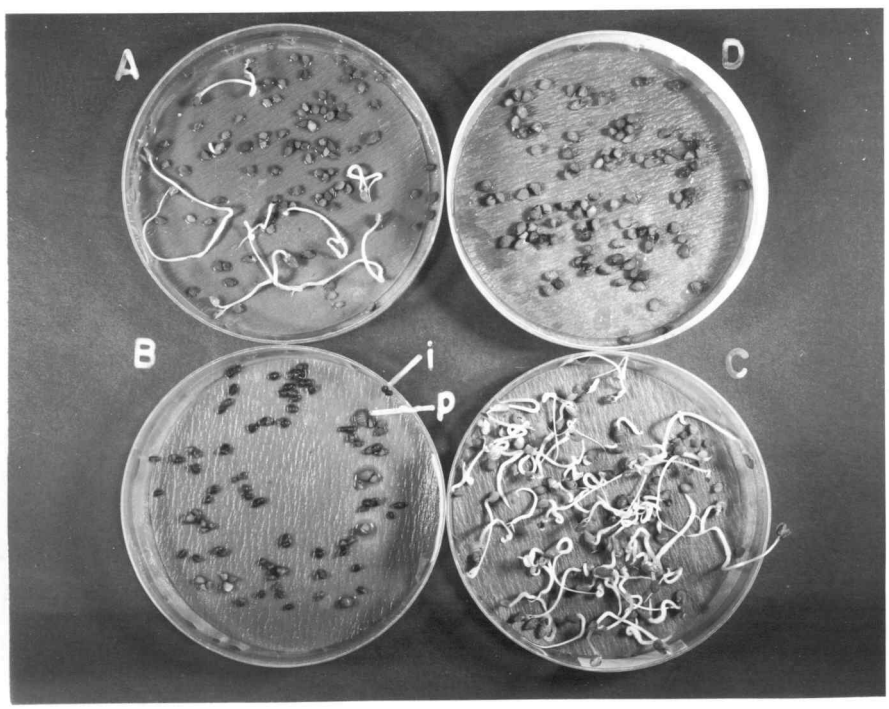
Normal Seedlings

Alcohol Injury

Radicle injury caused by soaking in absolute alcohol, showing hypocotyl elongation without radicle development.

1.25X

FIGURE 14



The effect of alcohol and chloropicrin upon impermeable bindweed seed, showing hard seed, imbibed seed, and germinated seed ten days after treatment.

1/4X

A - No Treatment
B - Chloropicrin Alone
C - Alcohol Alone
D - Alcohol plus Chloropicrin
i - Impermeable (Hard) Seed
p - permeable (Imbibed) Seed

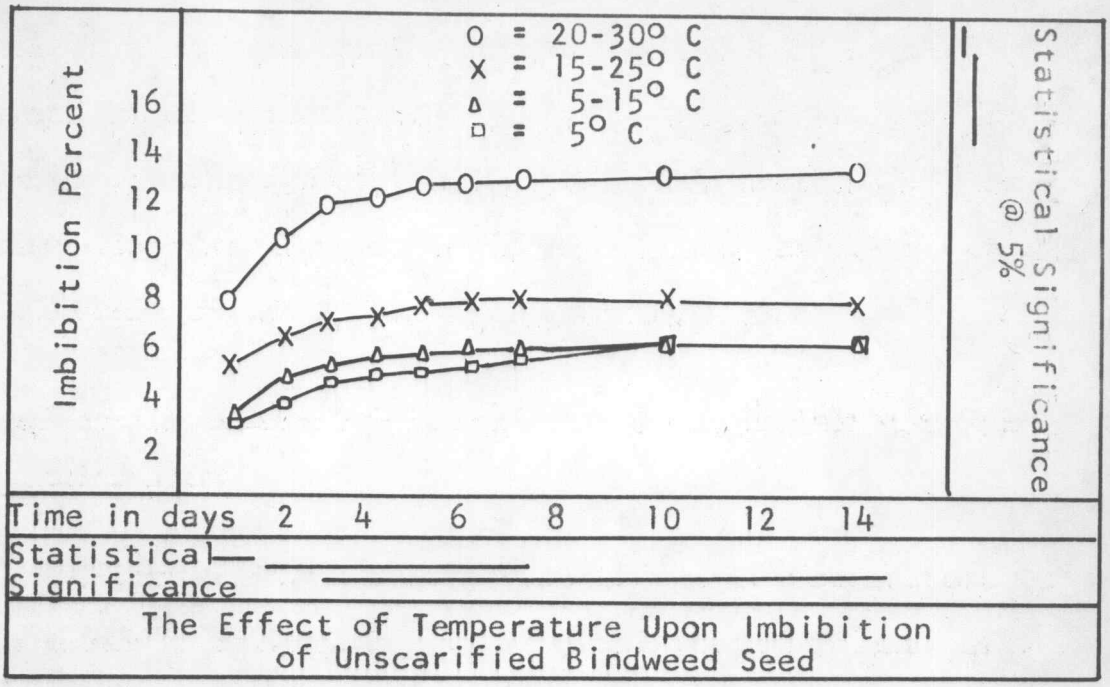
and bacterial growth because inhibition was so nearly complete in both cases. Discoloration of the plastic boxes by chloropicrin indicated that reaction with or solubility in the plastic may have been the cause of the residual effect of chloropicrin.

The Effect of Temperature and Light on Imbibition and Germination

Figure 15 illustrates the effect of temperature on the number of imbibing seeds (imbibition percent) of unscarified bindweed seed. At 20-30° C., imbibition percent was greater than at the three lower temperatures, but there were no differences among the three lower temperatures. The time course of imbibition and the significance test show that imbibition by the unscarified but permeable seed was accomplished by the third day, with a few seeds becoming permeable from time to time after that.

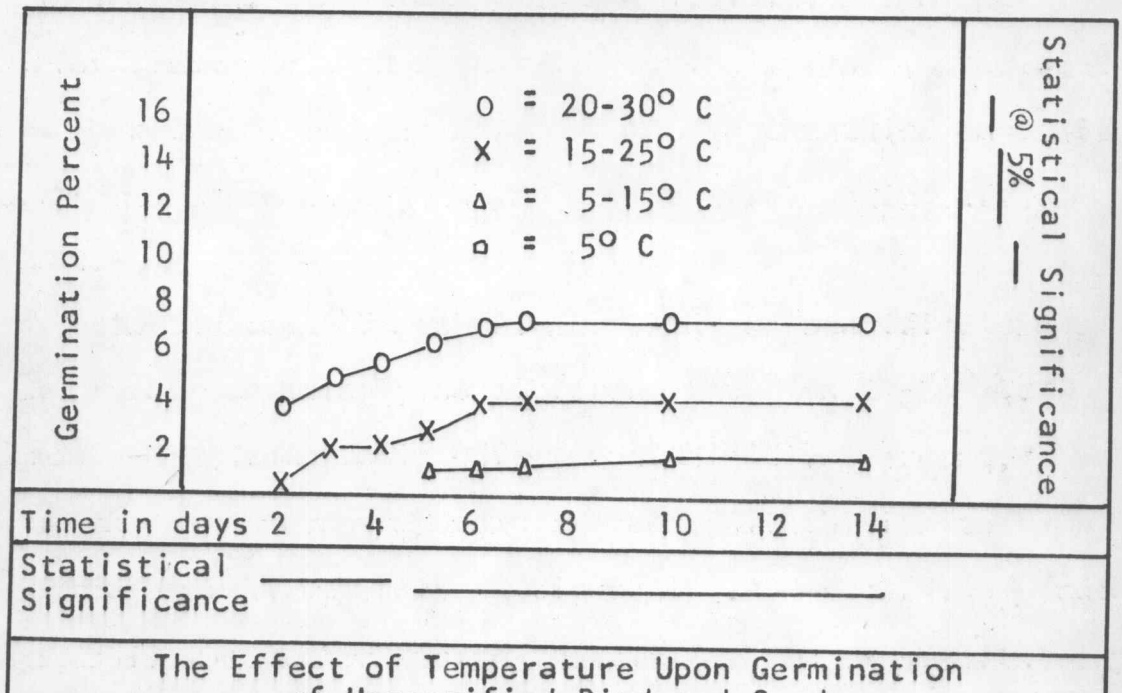
In the case of machine-threshed, or scarified seed, there was essentially no difference between 20-30° C. and 15-25° C. in their effect on imbibition percent, but the two lower temperatures caused imbibition to decrease with temperature (figure 17). Most of the permeable seeds imbibed water during the first three days, with very few becoming permeable after that.

FIGURE 15



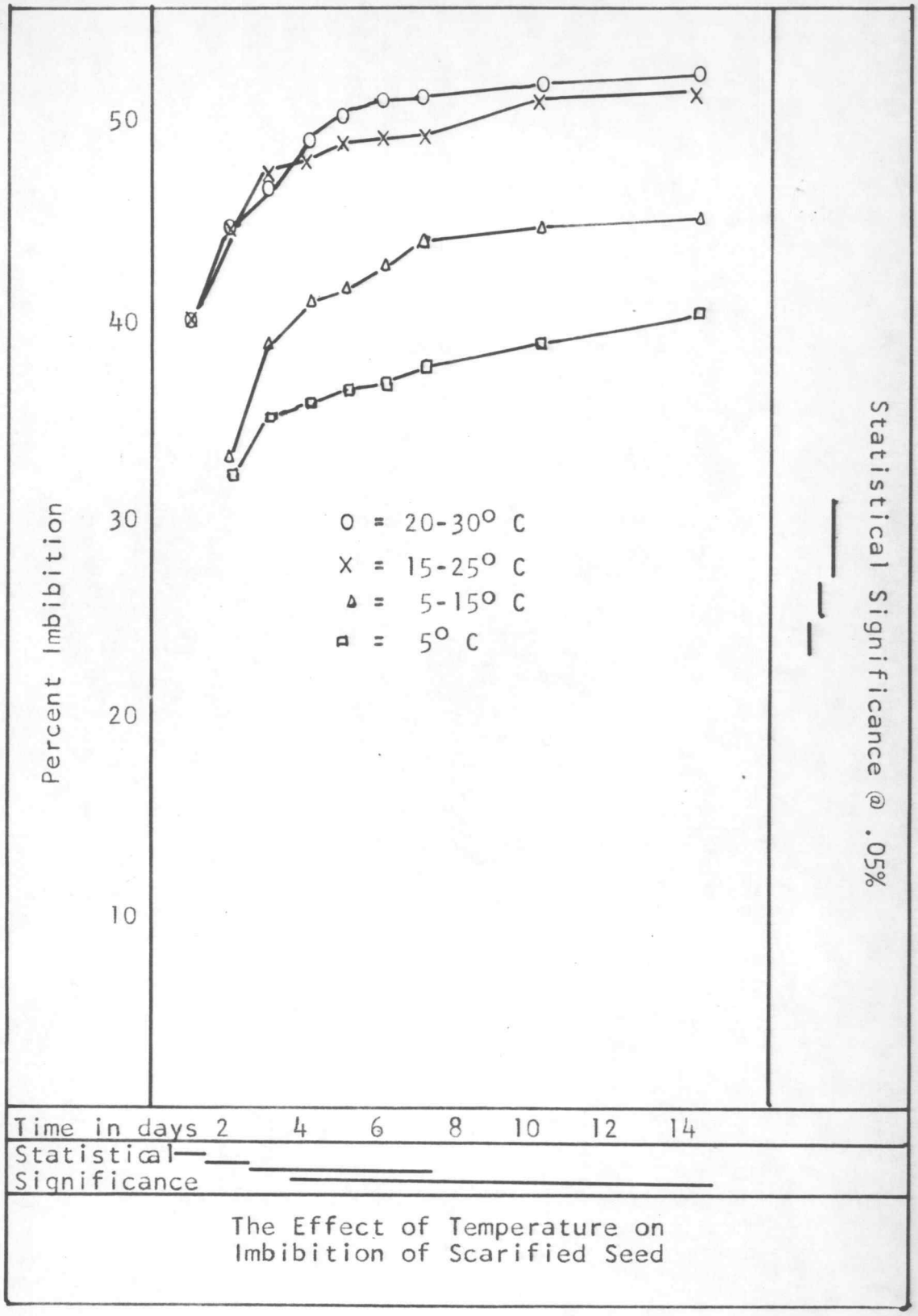
The Effect of Temperature Upon Imbibition of Unscarified Bindweed Seed

FIGURE 16



The Effect of Temperature Upon Germination of Unscarified Bindweed Seed

FIGURE 17



The Effect of Temperature on Imbibition of Scarified Seed

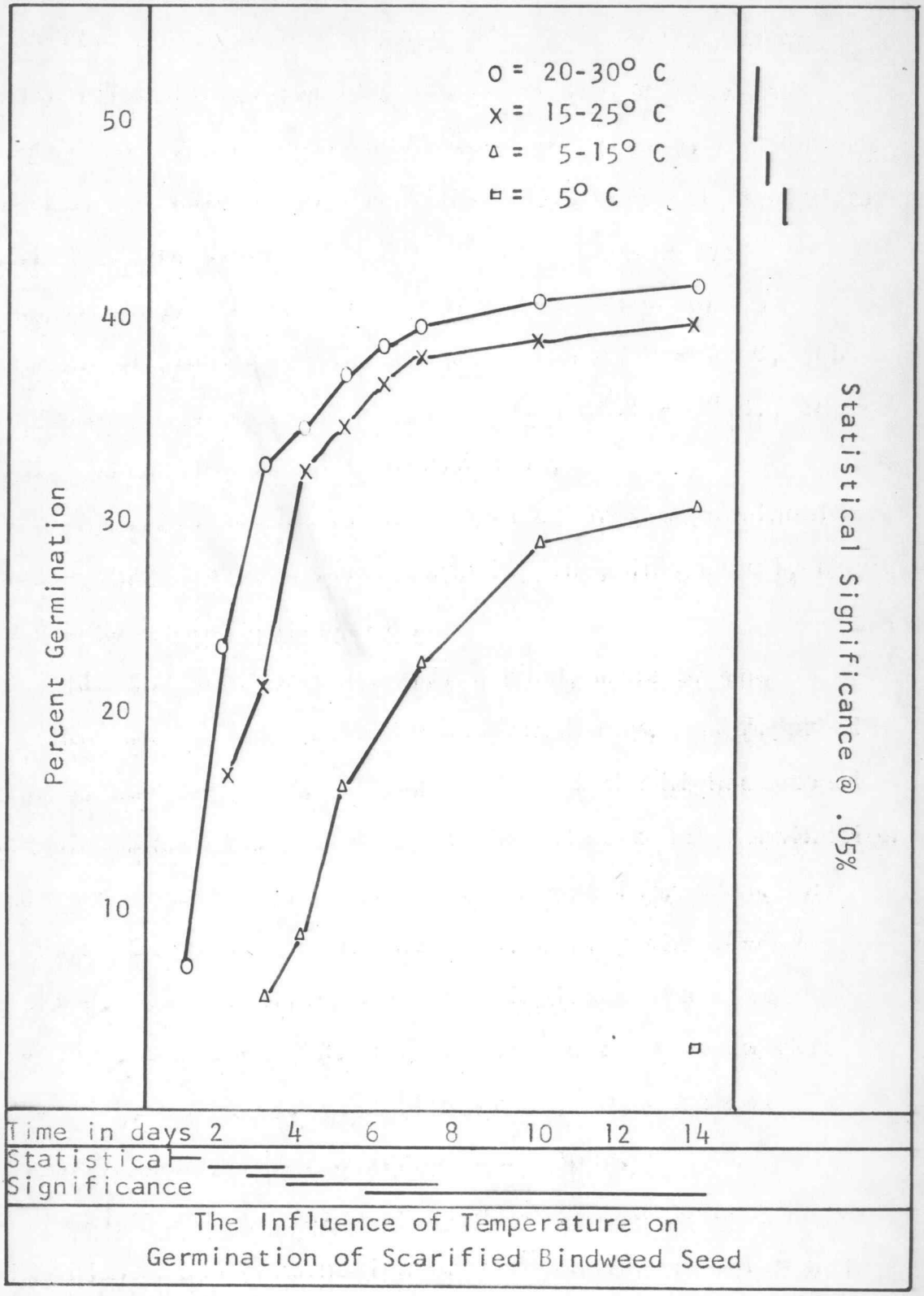
The temperature effect on total germination of acid-scarified seed (figure 18) was the same as it was on imbibition of scarified seed; that is, 15-25° C. and 20-30° C. were not different in their effect, but caused much more rapid germination than the lower temperatures, while germination was delayed more at each lower temperature. Observation during the course of the experiment indicated that germination at 20-30° C. was advancing somewhat more rapidly than at 15-25° C., but this difference was not significant.

As indicated in figure 18, most of the germination had occurred by the fifth day, lagging, in general, about two days behind imbibition.

In the case of unscarified seed, most of the germination had occurred by the sixth day. As figure 16 shows, the 20-30° C. treatment caused the highest total germination rate. The graphs show that total germination increased progressively as temperature increased. No germination was observed at 5° C. during the 14-day period. The significance tests indicate that the 5-15° C. and 15-25° C. temperatures were between the 20-30° C. and 5° C. in their effect, but were not significantly different from each other.

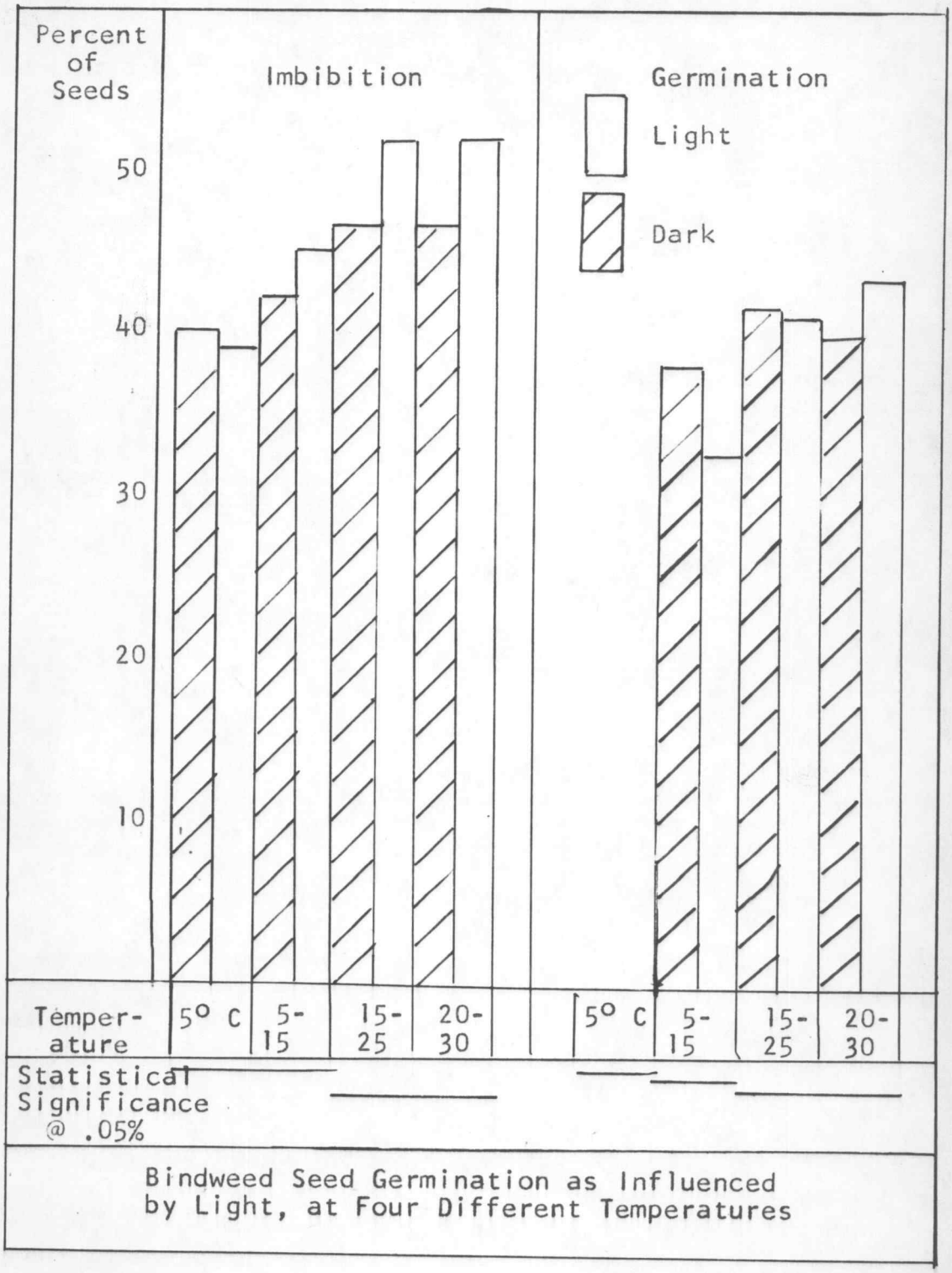
Figure 19 graphically illustrates the average imbibition and germination of seed under the influence

FIGURE 18



The Influence of Temperature on Germination of Scarified Bindweed Seed

FIGURE 19



Bindweed Seed Germination as Influenced by Light, at Four Different Temperatures

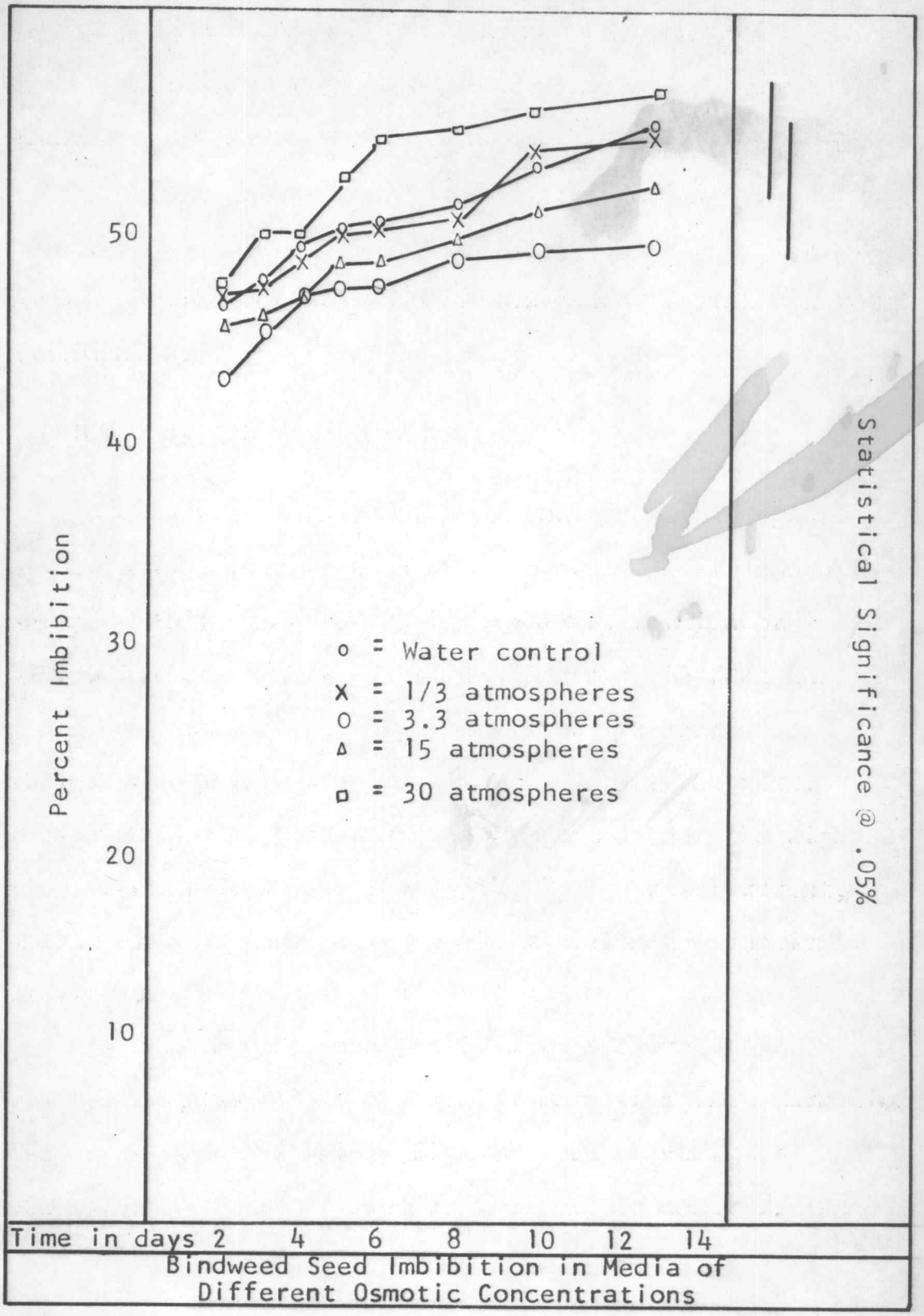
of the four temperatures in combination with a test for photoreaction. The bar graphs show no marked effect of light at 5° C. and 5-15° C., but at 15-25° C. and 20-30° C., there is an apparent increase in total imbibition due to light. However, this apparent increase was not significant. There was likewise no observed influence of light on total germination.

The Influence of Osmotic Pressure

The effect of increasing osmotic pressures upon imbibition and germination of scarified seed is shown in figures 20, 21 and 22. There was no inhibition of imbibition due to high osmotic pressures; in fact the seeds subjected to the highest osmotic pressure had a higher mean imbibition percentage than all the rest. Although the test of significance showed that the highest and lowest mean imbibition percentages were different, there was no consistent trend in relation to osmotic pressure.

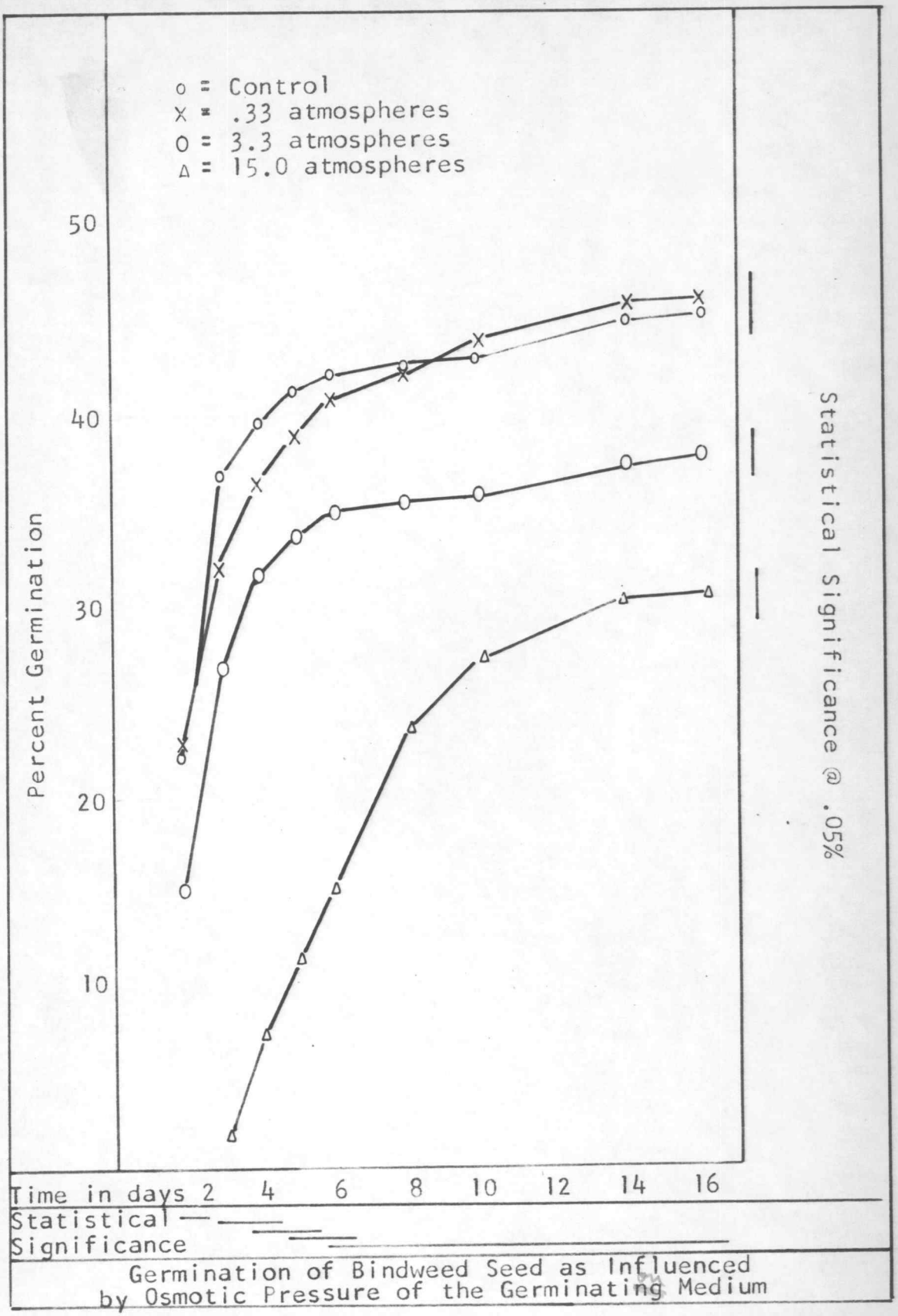
As osmotic pressure was increased above 1/3 atmospheres, there was a definite reduction in germination with each higher tension (figure 21). One-third atmospheres did not differ from the water control. At

FIGURE 20



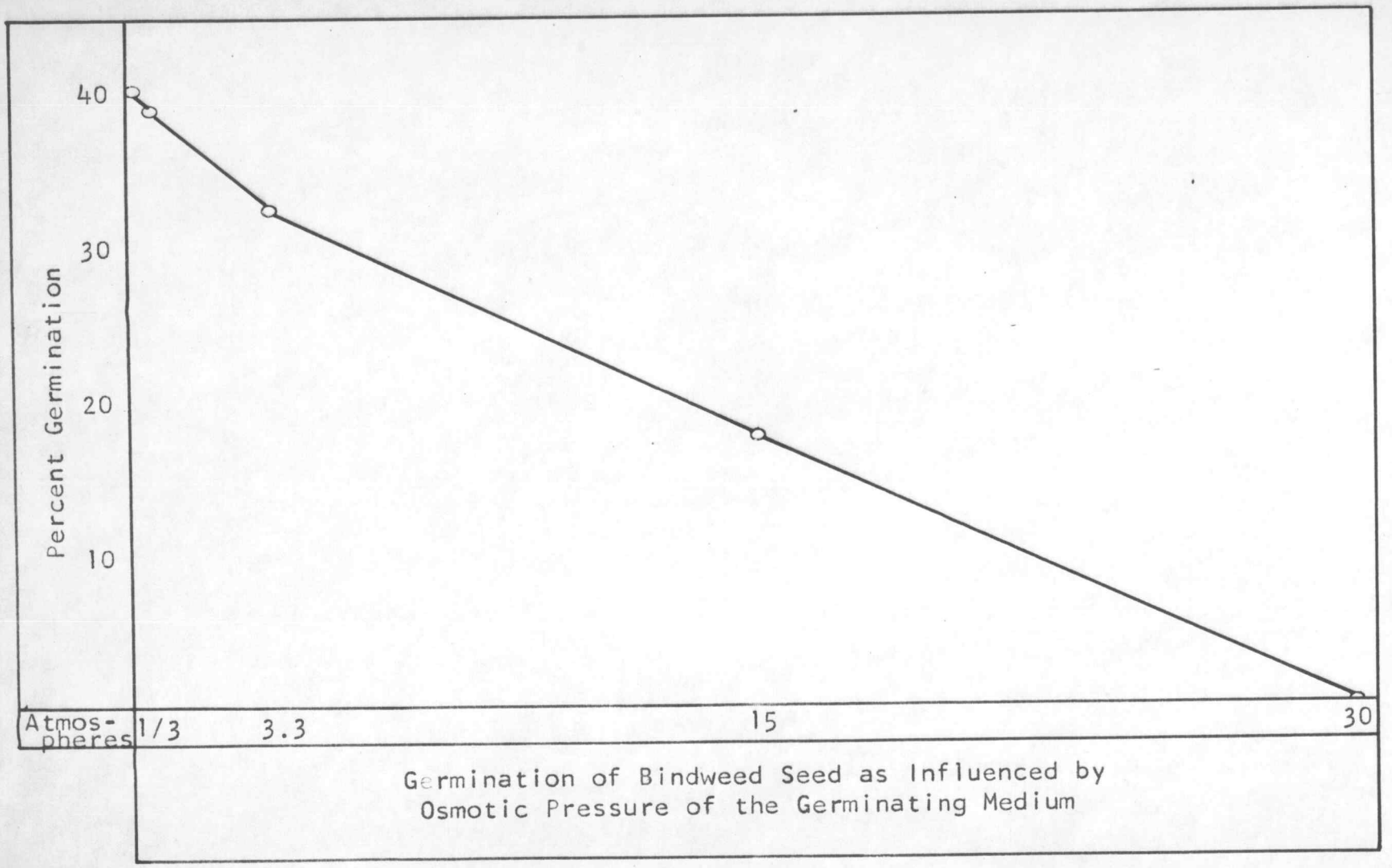
Bindweed Seed Imbibition in Media of Different Osmotic Concentrations

FIGURE 21



Germination of Bindweed Seed as Influenced by Osmotic Pressure of the Germinating Medium

FIGURE 22



Germination of Bindweed Seed as Influenced by Osmotic Pressure of the Germinating Medium

3-1/3 atmospheres, the germination curve was almost congruent with that for 0 atmospheres (the water control) but germination was considerably lower. There was no apparent lag in the time course of germination, however. At 15 atmospheres, there was a pronounced lag in germination as shown by the gradually sloping curve in figure 21, and total germination was considerably lower than for lower tensions. By the tenth day, germination had begun to level off. At 30 atmospheres, no germination took place during the first sixteen days, but germination began to occur at a slow rate after eighteen days. At the end of twenty-seven days, approximately twelve percent of the seeds had germinated, and the cumulative germination percentage was still increasing at a slow rate, thus displaying considerable lag as compared to the lower tensions.

Mold growth was present only to a minor extent in the germination medium, more being present at 0 and 1/3 atmospheres than at 3.3, 15, or 30 atmospheres. Progressively less was noted from low to high tensions. Molds did not begin active growth until the tenth day at the lower tensions but very little growth occurred at any time at 30 atmospheres.

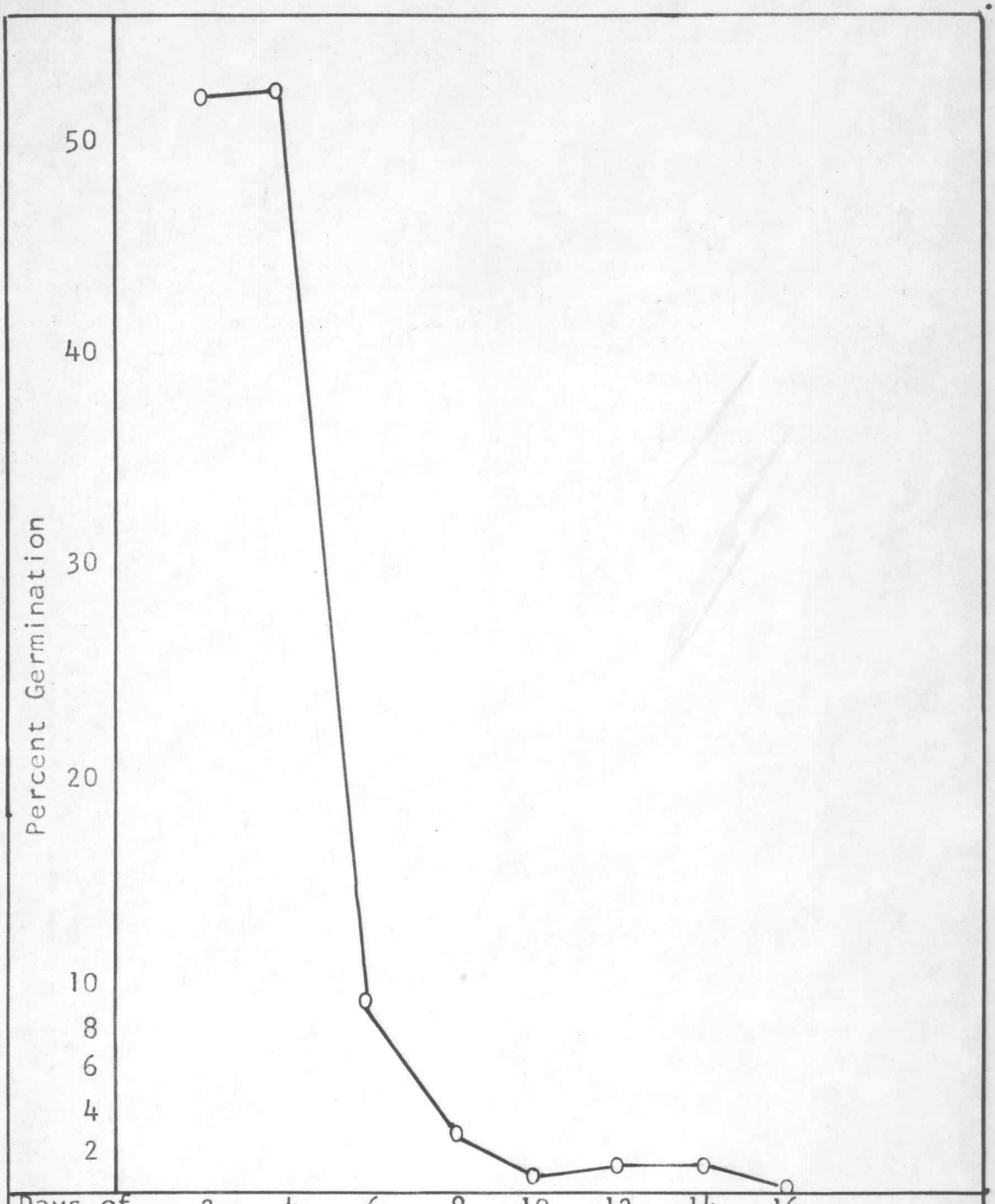
Figure 22 summarizes the relationship of simulated osmotic pressure and total germination of scarified

seeds after a seven-day incubation period.

Submergence

Submerged permeable seeds were found to be injured by water after soaking for four days (figure 23). There was a sharp decrease in viability after four days, and a slower drop after six days. After eight days of soaking, there was very low survival until the sixteenth day of soaking, when no survival was recorded.

FIGURE 23



The Viability of Bindweed Seed After Distilled-Water Soaking for Varying Lengths of Time

DISCUSSION AND CONCLUSIONS

Seed is a material which lends itself readily to a number of areas of scientific investigation. It is usually available in large quantities; it is relatively uniform; and is easily handled with respect to size, physiological and morphological characteristics. It could be adapted for either large-scale field studies or laboratory projects where space is limited and controlled environment and more elaborate procedures are needed. The application of seed research to agronomic problems has occurred widely only within recent years. It is not surprising that so little is understood of the basic problems of weed control through eradication of seed and other production and breeding problems affected by the characteristics of this important phase of the life cycle of spermatophytes.

Seed dormancy is a primary consideration in this study, due to its prevalence in seed of field bindweed. Several scientists have commented on dormancy in bindweed seed, but apparently the subject has stimulated little research since it falls into the category of "hard seed." Until recently, hard seed has been taken for granted as being a rather simple matter; or a matter with which little could be or need be done. Rampton (32) discusses the current

status of work with hard seed and the recent impetus given this topic.

This thesis includes a brief look at some of the effects of a few basic environmental factors upon the initiation of germination, a survey of dormancy and its prevalence, and a study of the mechanism of impermeability.

Seed production by bindweed was found to be much higher than previously reported. Kiesselbach et al. (24, p. 24) found a maximum of 1,419 seeds per square yard sample, or the equivalent of 160 pounds per acre. From the 360 square foot area at Corvallis, the equivalent of 726 pounds per acre was harvested late in the fall after an estimated 30 percent shattering loss had occurred.

In the survey of the prevalence of hard seed, it was noted in all cases that seed which was impermeable was germinable after scarification. This indicates that the seed coat plays a vital role in the process of growth resumption in a germinating environment. The seed coat is generally considered to be dead tissue which has a rather simple protective function. This is apparently not entirely true in the case of bindweed.

Seed Coat Anatomy

From the anatomical study of the mature seed, comparisons show some similarity between functional structures of bindweed seed and leguminous seed (red clover, white clover, and tree lupine) with which Hyde (22) worked. The basal end, although different in shape, appears to have a similar structure, particularly in the hilum opening, the micropyle, and the area surrounding the hilar fissure. The hilar cleft is U-shaped, whereas in most Leguminosae it is straight. Otherwise little difference is noted between the two in this respect. The micropyle is obscure as in many legumes, and the surface tissue surrounding the hilar fissure is markedly different in color and texture from the rest of the seed coat surface, as is the case in many legumes. The surface of the seed coat itself, however, is rougher and more pappilose than most legumes.

The seed coat structure in bindweed differs in some respects from that of the legume seeds. In bindweed, the seed coat develops from a single integument, rather than two integuments, and the seed coat layers are arranged differently than in many legumes. The palisade layer in legumes is the epidermal layer (50, p. 386-388), whereas in bindweed the outermost layer is a layer of apparently

tanniniferous parenchyma cells at maturity. The subepidermal layer is composed of very compactly arranged stone cells. Beneath these stone cells lies the palisade layer, which stains with Sudan III, indicating the presence of one of the plant waxes, probably cutin. A "light line" is obviously localized in the palisade as shown in figure 19. Unlike most legumes (10) there is no layer of stone cells beneath the palisade layer. There are only crushed parenchyma cells from the interior portion of the integument.

The counter-palisade layer in bindweed arises from periclinal divisions of epidermal cells. The divided cells next to the subepidermal layer elongate, their cell walls thicken, and they take on the resemblance of the rest of the true palisade layer, separated from it only by the subepidermal sclereids. Exterior to this counter-palisade layer is an outermost layer of epidermal cells which are more compactly arranged and which do not have the black pigments as present in the epidermal layer over the rest of the seed.

This counter-palisade layer has been called the hilum, or the "scar" which marks the point of attachment to, or abscission from, the funiculus. Even botany textbooks give it this useage. By definition, the hilum is, indeed, the

scar left by the funiculus, but as figure 19 shows, the funiculus is not directly attached to the counter-palisade epidermis, but is attached to the tissue between the two counter-palisade tissues which lie above the subepidermis on both sides of the cleft. Thus, the "hilum" on bindweed seed is not protruded from the seed coat, and the term should not be applied to this epidermal area which is adjacent to the hilum. The light colored counter-palisade tissue might be more logically termed a hilum area. The term "hilum" when applied to bindweed, would more correctly refer to the cleft itself, including the walls of the cleft.

The Hard Seed Mechanism

Hyde's well-substantiated proposal is based on the fact that as the legume seeds dry down to about fourteen percent moisture, water is lost both through the hilum opening and through the testa in general. Below fourteen percent, the hilar valve is the only site of water loss. Each time the outside relative humidity falls below the lowest relative humidity with which the palisade layer has come to equilibrium, the counter-palisade shrinks and opens the hilar aperture allowing free exchange of gasses and water vapor. Thus the inside of the seed and the palisade layer comes to equilibrium with the lower

relative humidity, and the seed becomes drier. When the relative humidity rises, the counter-palisade layer swells enough to force together the edges of the palisade tissue bordering the hilar fissure, closing the hilum and forming a virtually air-tight seal where the two sides meet at the "light line" of the palisade layer. This "light line" is apparently a dense translucent band near the top of the palisade epidermal cells, (figure 19), and has been referred to by some workers as the impermeable layer in the seed coat (8; 18, p. 755-757). The maintenance of hard seed in moist environment is made possible by this closing mechanism of the counter-palisade. The effect of relative humidity fluctuations on the expanding and contracting of the counter-palisade layers of field bindweed seeds (figure 14) verify that the mechanism described by Hyde is characteristic of bindweed seeds. The relative humidity level of 13.5 percent in figure 14 was critical in this case only because the particular seeds used had not come to equilibrium with a lower relative humidity.

This then, resolves the apparent anomaly in which desiccation of the interior of the seed occurs simultaneosly with the development of an intensely impermeable seed coat. Evidently, Hyde's findings are applicable not only to

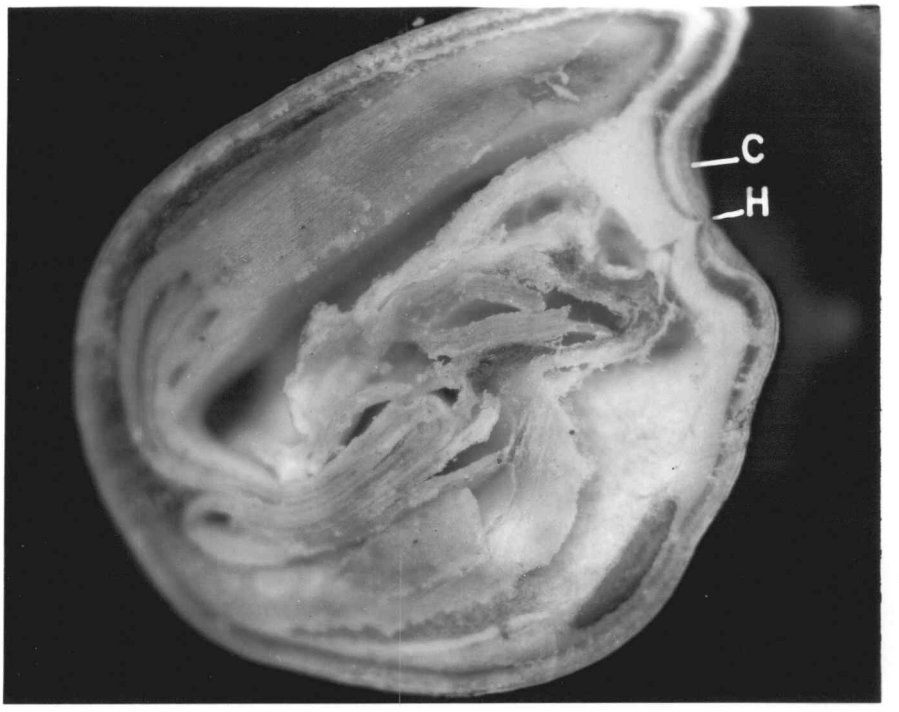
leguminous seeds, but also to field bindweed and very likely also to hedge bindweed, which has a similar seed coat structure (figure 24).

The Effect of H_2SO_4

Soaking seed in concentrated sulfuric acid for periods up to 30 minutes did not materially reduce the percent of impermeable seed. This indicated a resistance of the seed coat to dehydration or corrosive action of the acid. Seed thus treated for 45 minutes lost much of the initial impermeability; the hilar aperture was blackened by the acid (figure 12), and the epidermal and subepidermal layers of the seed coat were dissolved. When the hilar aperture of such seed was blocked, the number of permeable seed was greatly reduced in a germination environment. This shows several things: (1) the palisade layer of the seed coat is the impermeable layer, (2) the site of action of the acid which causes permeability is around the hilum, and (3) the site of water absorption is the hilum. Longer periods of acid treatment substantiated this idea.

The reduction of imbibition in both acid-treated and untreated seed samples by blocking the hilum opening with petroleum jelly further indicated that the natural site of water absorption is the hilum.

FIGURE 24



Hedge Bindweed (Convolvulus sepium)

22X

C - Counter Palisade
H - Hilum

Induction of Permeability

The tendency of field bindweed to produce large amounts of impermeable seed presents a serious problem of bindweed control due to reinfestation by seedlings from seed which has lain dormant in the soil. This difficulty would be obviated if the dormant seeds could be induced to take up water and sprout or die when the environment is unfavorable. Dead seeds, of course, are harmless, and germinating seedlings are very simply controlled before they become established as perennials by ordinary cultural practices, such as mechanical cultivation or herbicide application. For these reasons, it would be desirable to determine whether it is possible to induce water uptake.

Surfactants. The failure of a detergent to facilitate water uptake is not too surprising when the hygroscopic nature of the counter-palisade layer is considered. Burns (4, p. 108) also reported failure to cause water penetration by using a surfactant. Rather than cause water uptake through the hilum aperture, reduction of water surface tension probably only succeeded in facilitating water uptake by the counter-palisade layer, causing perhaps even more rapid closure of the fissure. This approach to induce permeability does not appear to offer hope thus far.

Absolute ethyl alcohol. Immersion in absolute alcohol for several hours was effective in inducing permeability in much of the impermeable seed, but 16 hours of immersion is necessary to obtain 100 percent permeability. Since blocking the hilum fissure with petroleum jelly nullified the effect almost completely, the site of action is obviously in that specific area of the seed coat. Therefore, the effect is apparently not one of dissolving waterproof materials over the general surface of the seed coat.

The exact mechanism wherein alcohol causes permeability is a matter of conjecture thus far. This effect has been reported previously (2, 11, 47) but the only explanation that is offered yet is that by Verschaffelt (47), in his studies with honey locust (Glidetsia triacanthos) seeds. He explained that the absolute alcohol makes a path through the open micropyle which water could follow. Since the micropyle is permanently closed in bindweed seeds this explanation would not hold true for bindweed. The fact that water does pass through the micropyle of honey locust seeds was demonstrated by the use of dyes, but this still does not explain the mode of action of absolute alcohol.

Since it seems that dilute ethyl alcohol has no capacity to desensitize the hilum valve, perhaps pure

alcohol causes irreversible dehydration of the counter palisade layer, thus incapacitating the valve action.

Another possibility may be that the alcohol permanently coagulates the highly hygroscopic protein in the counter-palisade layer. If this is the case, other known protein coagulants, such as concentrated urea solutions, trichloro acetic acid, acetone, etc. might cause the same effect. In any case, this avenue offers some promise, and should without doubt be pursued.

None of the fumigants used had any effect in inducing permeability, but chloropicrin and formaldehyde were both very effective in killing the seed which did become permeable. Carbon disulfide and Vapam were effective to a much lesser degree. The latter two had no residual effect, whereas the chloropicrin and formaldehyde were effective for at least a month. This residual effect was probably due to the solubility of formaldehyde in water and of chloropicrin in the plastic germination boxes.

Although the solution to the dormancy problem in bindweed seed is not completely solved, considerable insight into the mechanism of impermeability has been gained from this research. From the data and conclusions herein, it seems that active investigation into the physiology of hygroscopicity of the counter-palisade layer is the next step. When this is done, a way of

practical control of seedling reinvasion may be intelligently pursued. It appears, at present, that even empirical investigations into the means of inducing complete permeability in bindweed seeds in the soil would be in order. If this can be attained, effective extended control of field bindweed may be a reality.

The Influence of Environmental Variables

The seed of many plants are rather specific in their germinating requirements, germinating within rather narrow limits of temperature, light, moisture, and other environmental factors. Upon investigating the effects of some of these factors, it was found that field bindweed was rather non-specific in its requirement in comparison with those plants more demanding in their developmental environment. This may help to explain its wide adaptation.

Temperature. Considerable variation in germination temperature optima has been reported to occur among plant species (43). However, temperatures used in such tests are not always as readily translated to natural conditions as they might appear. Some workers have used constant temperatures in such studies (43) while others, especially more recently, have used alternating temperatures (11). It is known that many species require daily temperature alterations (11) corresponding roughly to natural diurnal

alterations during the growing season. It is difficult to imitate natural diurnal fluctuations without rather intricate and expensive growth chambers, so those values used in most temperature studies are not absolute, but only good guides in drawing conclusions.

Since the Convolvulaceae is primarily a tropical family (12, p. 307-308), it is not surprising that the higher temperatures resulted in higher germination percentage. It was noted, however, that slow germination did occur at 5° C., and that seedlings produced were not injured by the low temperatures but continued growing until food reserves were exhausted in the germinator. Many seedlings were observed to stay in an apparently healthy condition at 5° C. on a wet blotter for as long as three months after cotyledon emergence. Under field conditions, natural soil fertility would probably provide sufficient nutrients for the seedling to develop to an established plant. This would indicate considerable ability of seedlings to establish themselves under cool conditions early or late in the growing season and in cold climates, although field bindweed is commonly known to be a "slow starter" in the spring.

Very rapid imbibition and germination of permeable seed was noted, especially at the higher temperature, contributing to the rapid seedling development which occurs in bindweed.

This rapid seedling establishment contributes to quicker plant maturation which enables the plant to overwinter and establish the perennial characteristic of the species. Again, this may be attributable to the tropic adaptation of the Convolvulaceae.

It may be observed from the time course of imbibition that development of permeability is a constant process, one percent or so imbibing and germinating every few days after the initial "flush" of the bulk of the readily permeable seeds. This indicates that bindweed seeds do not exhibit a periodicity of germination during the year as some weed seeds do, such as lady's-mantle (Alchemilla vulgaris) and rough pigweed (Amaranthus retroflexus) (11, p. 90). In bindweed seed-infested areas, seeds are constantly imbibing water and germinating. As comparisons between figures 2 and 3 and figures 4 and 5 show, germination occurs for the most cases only a couple of days after imbibition. So germination too, is a continuing process throughout the growing season, rather than in seasonal flushes as occurs in rough pigweed and lady's-mantle. Thus, frequent tillage in bindweed-infested land serves not only to control perennial plants by root reserve depletion, but it also kills summer seedlings.

Light. The indication of a response to light by increased imbibition at the higher temperatures in figure 6 was not statistically significant at the 5 percent level. The present research data may be too limited to reveal such interactions; further research might do this. Toole et al. (45) reported enhancement of reaction to red light in Lepidium virginicum and other species by raising the incubation temperature from 15° C. to 25 or 30° C. The results so far obtained indicated that light is not necessary for the germination of bindweed seed.

Osmotic Pressure. Rather unexpectedly, imbibition followed no definite trend in response to increasing moisture tensions. This corroborates Shull's classical work with Xanthium pennsylvanicum, (28, p. 98-99), in that he also found that dry Xanthium seed would extract water from a medium whose osmotic pressure was much above the wilting percentage for plants. However, the criteria in determining imbibition by bindweed seeds was by observing the number of swollen seeds rather than determining moisture content of the seed as Shull did. Had the latter analysis been conducted, proportional decreasing moisture content to increasing osmotic pressure would very likely have been found.

Germination did bear an inverse relation to osmotic pressure (figure 8). Germination of bindweed seed is apparently possible within a week at the wilting point of plants. Some slow germination was found to occur at 30 atmospheres after approximately 25 days, indicating that even at tension considerably above the wilting percentage some germination may occur. Due to the lack of enough reference points beyond 15 atmospheres, the curve presented in figure 9 is probably inaccurate beyong the 15 atmosphere point.

Apparently as a bindweed seed becomes permeable, it would begin the imbibition process immediately even in a fairly low-moisture environment. As moisture tension decreases, the seed would be in a much better position to germinate and establish seedlings rapidly. This sequence would be conditioned by the water holding capacity of soil, however. Moisture availability in a soil is influenced by the total amount of water in the soil in addition to the very important physical binding forces of the soil colloids and the soil solution concentration (1, p. 82-84).

Such a sequence also could conceivably work to the detriment of the plant because it favors germination of seeds in a short period with favorable conditions such as often occur in mid-summer in dry areas. When seedlings

germinate in such conditions, they would likely succumb when the moisture availability falls to the wilting point again, for they would not have time to accumulate root reserves.

From these data, it would seem that investigation beyond 15 atmospheres would be pertinent in estimating the ability of bindweed (or other) seeds to germinate under low moisture conditions.

Submergence. Seeds of many plants are not injured by prolonged soaking. This is particularly (and reasonably) so in the case of hydrophytes, but the seeds of many land plants survive soaking for extended periods of time also, indicating that soaking is not as harmful as is often thought, especially in the case of impermeable seeds such as bindweeds.

The rapid decomposition of imbibed bindweed seed after a week's soaking indicates that in this case, injury may have been due to an alteration of metabolism. An example of such an alteration might be alcohol production caused by anaerobic respiration which could cause inhibition of enzymatic processes. This may be followed with an attack by microorganisms while the seed germination was slowed by the low level of oxygen in the distilled water.

There was slight survival even after two weeks of soaking, however, which is explained by the characteristic slow continuing development of permeability, thus illustrating the effectiveness of this phenomenon as a survival mechanism.

SUMMARY

Field bindweed seed samples from four locations in three western states in the United States were found to contain 92 percent impermeable seeds. Machine-threshed seed from one location contained approximately 50 percent permeable seed.

The seed coat of bindweed is composed of an outer layer of permeable epidermal cells, a subepidermal layer of sclereids, and a probably cutinized impermeable palisade layer. The testa is interrupted at the basal end of the seed by the hilum, which is a long fissure at the point of separation of the funiculus. A hygroscopic counter-palisade layer on either side of this fissure expanded and contracted rapidly with changes in surrounding relative humidity, thus opening and closing the hilum, making it a moisture-sensitive valve on the impermeable seed coat. This valve maintained seed dry and hard.

Scarification with concentrated sulfuric acid and immersion in absolute ethyl alcohol were two methods found to inactivate the hilar valve and permit water uptake through that structure. These findings indicate that the hilum is the natural site of water absorption and that destruction of the integrity or the function of the counter-palisade layer will cause the hard seed to

become permeable. Dilute ethyl alcohol had no effect on the valve mechanism, nor did the fumigants chloropicrin, formaldehyde, carbon disulfide, and Vapam. Surfactants did not induce water uptake.

This study showed that disrupting the hilar valve mechanism may be a solution to control of bindweed seed infestation. If hard seed dormancy can be broken, the seeds should imbibe water and germinate under favorable conditions or deteriorate in an adverse environment. Once the seed germinates, the young seedlings are easily killed by conventional weed control methods.

Bindweed seed imbibed water and germinated best at the highest temperatures tested (15-25° C. and 20-30° C.), thus demonstrating the plant's basically warm-weather adaptation. The slow growth observed at 5° C. indicated an ability to establish plants in cold environments. Artificial light did not significantly influence imbibition or germination at these temperatures. Small percentages of hard seed were continually becoming permeable under moist conditions throughout the experiments, and germination occurred within two days after imbibition.

Imbibition was not affected by osmotic pressure simulated by mannitol solutions up to 30 atmospheres; however, as osmotic pressure increased to 30 atmospheres,

germination within a two-week period remained zero. Considerable germination at 15 atmospheres and slight, delayed germination at 30 atmospheres indicated an ability to germinate to a certain extent under adverse conditions of high moisture stress.

Soaking in water for more than a week caused nearly 100 percent mortality of permeable seeds, probably because of altered metabolism under anaerobic conditions, and subsequent attack by microorganisms.

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APPENDIX

Analysis of Variance Tables

Table 1

The Effect of Temperature on Imbibition of Unscarified Hard Seed.

Source of Variation	Degrees of Freedom	Mean Square
Replications	3	9.77*
Treatments	35	38.48**
Temperatures	3	279.16**
Days	8	58.36**
Interaction	24	1.76
Error	105	3.54

Table 2

The Effect of Temperature on Germination of Unscarified Hard Seed. (Lowest temperature was excluded from the analysis)

Source of Variation	Degrees of Freedom	Mean Square
Replications	3	53.25**
Treatments	26	115.14**
Temperatures	2	895.44**
Days	8	116.75**
Interaction	16	16.8
Error	78	12.13

Table 3

The Effect of Temperature on Imbibition of Scarified Seed

Source of Variation	Degrees of Freedom	Mean Square
Replications	3	274 **
Treatments	35	221 **
Temperatures	3	1552 **
Days	8	362 **
Interaction	24	7.5
Error	105	8.4

Table 4

The Effect of Temperature on Germination of Scarified Seed

Source of Variation	Degrees of Freedom	Mean Square
Replications	3	200 **
Treatments	35	1095 **
Temperatures	3	7916 **
Days	8	1295 **
Interaction	24	176 **
Error	105	16

Table 5

The Influence of Light and Temperature on Imbibition of Scarified Seed

Source of Variation	Degrees of Freedom	Mean Square
Replications	3	43
Treatments	7	91 *
Light	1	69
2 high temp. vs. 2 low temp.	1	402 *
Within high & low temp.	2	58
Light x temp.	3	18
Reps x Temperatures (error a)	9	45.6
Error (b)	12	14.4

Table 6

The Effect of Light and Temperature on Germination of Scarified Seed

Source of Variation	Degrees of Freedom	Mean Square
Replications	3	38.5
Treatments	7	1153.2 **
Temperatures	3	2675.1 **
Light	1	1.5
Interaction	3	13.1
Reps x Temperatures (error a)	9	43
Error (b)	12	3.8

Table 7

The Influence of Osmotic Concentration on Imbibition of Scarified Seed

Source of Variation	Degrees of Freedom	Mean Square
Replications	3	33.73 **
Treatments	44	46.6 **
Osmotic Pressures	4	175.5 **
Days	8	156.7 **
Interaction	32	2.9
Error	132	7.58

Table 8

The Influence of Osmotic Concentration on Germination of Scarified Seed (30 atmospheres omitted)

Source of Variation	Degrees of Freedom	Mean Square
Replications	3	73.5 **
Treatments	35	660 **
Osmotic Pressures	3	383.6 **
Days	8	1186.6 **
Interaction	24	519.0 **
Error	105	9.8

Table 9

The Effect of Soaking Concentrated Sulfuric Acid and Blocking the Hilum with Petroleum Jelly upon Imbibition of Impermeable Seeds

Source of Variation	Degrees of Freedom	Mean Square
Replications	3	10.4
Treatments	3	1230.8 **
Acid	1	1681.0 **
Petroleum Jelly	1	1482.2 **
Interaction	1	529.0 **
Reps x Acid (error a)	3	5.2
Error (b)	6	1.1

Table 10

The Influence of Soaking in Absolute Alcohol and Blocking the Hilum with Petroleum Jelly upon Imbibition of Hard Seeds

Source of Variation	Degrees of Freedom	Mean Square
Replications	5	58 **
Treatments	3	3270 **
Alcohol	1	2185 **
Reps x Alcohol (error a)	5	7.3
Petroleum Jelly	1	5735 **
Reps x Petroleum Jelly (error b)	5	8.5
Interaction	1	1890.0**
Error (c)	5	7.5

Table 11

(Completely Randomized Experiment)

The Effect of Four Fumigants Applied in the Presence and Absence of Water and Absolute Alcohol

Source of Variation	Degrees of Freedom	Mean Square
Alcohol (A)	1	66552.0**
Fumigation after Drying (D)	1	2
Water (W)	1	8
Fumigants (F)	4	79
A x D	1	9
A x W	1	15
A x F	4	30
D x F	4	7
W x F	4	12
D x W	1	21
A x D x W	1	8
A x D x F	4	25
A x W x F	4	19
D x W x F	4	4
A x D x W x F	4	48
Within Samples	80	57