

FACTORS CAUSING VARIETAL DIFFERENCES IN GEEMINATION
OF THE COMMON BEAN (PHASEOLUS VULGARIS)

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INTRODUCTION

The problem of growing beans successfully has engaged the attention of both growers and research workers for a considerable period of time. Germination studies in relation to different environmental conditions have been conducted but little is known about the physiological conditions, existing in the seed, during maturation, dormancy and germination, which might influence the subsequent growth and development periods of the plant.

During recent years the production areas have changed, from the humid eastern, to the semi-arid western states. This relocation was necessitated to combat the seed-borne diseases prevalent in humid areas and for greater acreage (3). At the same time it has increased the interest of the bean growers for the germination qualities of the seed.

It has been pointed out by Morris (41) that some black seed-coated varieties have a much higher percentage of germination in comparison to some light or white colored varieties.

Because of the possible value to the bean growers and seed producers, a study of the factors influencing these differences in germination was undertaken. Environmental factors, disease, soil organisms, mechanical injury

incurred in threshing and other possible causes, which might contribute towards this marked difference in germination, were investigated.

Such a study may further indicate whether better germination of dark seed-coated varieties is associated with color or structure of the seed coat, or some anti-microbial agents which might be present in such seeds.

REVIEW OF LITERATURE

Several prerequisites for the germination of seeds of all species of plants are (1) water, (2) a suitable temperature, and (3) oxygen. A fourth factor, light, appears to influence the germination of seeds of some species and to be essential for the germination of the seeds of a few species. Other factors such as the size of seed, aeration, chemical composition, structure of the seed-coat and growth inhibitors in the seeds have been found to influence germination. The literature on these subjects has been reviewed in this paper.

Absorption of water is a primary and essential step in the germination of a seed. The water passes through a protective seed-coat to reach the embryo and endosperm and initiates a series of physical and chemical processes which in the absence of any limiting factor results in the emergence of the embryo from the seed.

Stiles (57) compared the rate and amount of water intake in several species of beans and concluded that soybeans are xeric (dry land) types and lima beans are hydric (wet land) types. She also compared the rate of intake of water into various parts of cotton and corn seed (58), and pointed out that different varieties had distinctly different characteristics that had been brought

about by selection, either natural or deliberate. Corn with a rapid rate of intake of water was better adapted as a dry land type than other varieties having a less rapid rate of absorption.

A wide range of vegetable species was tested by Doneen and MacGillivray (19) in two types of soils; they found that the rate of germination increases and reaches its maximum at higher moisture levels. Fuhr (22) working on the moisture requirement of various crops reports that beans require about one and one-half percent additional moisture above the permanent wilting percentage of the seedlings for development. Toole, Miles and Toole (61) used varying amounts of moisture in their germination experiments, and report that germination was significantly poorer at 60 percent moisture than at lower moisture levels.

Whetzel (67) found that in saturated soils there was a greater amount of infection by damping-off organisms than in unsaturated soils. Kidd and West (32, 33) found that soaking seeds of Phaseolus vulgaris (dwarf bean) prior to germination had a marked effect upon the subsequent course and vigor of development. The striking effects of soaking the seed are not visible in first observations, but show up later in the course of development. They found no decrease in the rate of germination in seeds soaked for six hours, little decrease from soaking for twenty-four hours, but complete failure from treatment for seventy-two hours. However, a deleterious effect of temperature

was visible even after shortest periods of soaking (6 hours) and the effect progressively increased with longer periods of soaking at all temperatures used, ranging from 10 to 30° C.

Results obtained with Broad beans Vicia Faba gave an absolute reversal in the effects following soaking compared to the dwarf beans. Soaking for periods up to three days gave increasingly beneficial results, not only in percentage germination, but also in the size of the plant produced. They concluded that the effect of soaking is strongly specific; quite different with similar treatment in closely allied species of plants. Tilford, Abel and Hibbard (60) report that soaked bean seeds that were sterilized and well aerated during soaking, germinate well under water; provided that there is sufficient oxygen for respiration, that carbon dioxide and other by-products are removed and bacteria eliminated.

Bailey (5) found a decrease in germination after eight hours of soaking with aeration, and it progressively decreased as the length of soaking period increased. These results do not agree with the previous authors mentioned because they did not change the water in which the beans were soaked and also did not grow them in soil. Bailey (5) further pointed out the fact that there was an increase in the rate of production of respiratory carbon dioxide in sprouted seed that had been soaked in comparison with those sprouted without previous soaking. This is due to

the fact that soaked seeds contained more water than the untreated ones. This higher water content in the seed was favorable to a more rapid rate of respiration.

Soaking seeds without aeration resulted in a regular decrease in catalase activity. Soaking with aeration resulted at first in a small decrease in catalase activity, but this was soon followed by a considerable increase. This modification of the result is evidently due to the fact that the seeds when soaked with aeration pass through the early stages of germination and consequently there is an increase in catalase activity -- characteristic of seed germination.

Aeration of the soil has an important influence on germination and is closely related to the soil texture, moisture content of the soil, and the oxygen requirement for germination for different kinds of plants.

Crocker (15) reports that seed-coats which exclude oxygen caused a delay in germination. In nature delayed growth was brought about by the disintegration of the seed-coat structures by a longer or shorter exposure to germinative conditions of temperature and moisture. The length of delay depends on the persistence of the structure of the seed-coat. A rise in temperature lowered the minimum oxygen required for Xanthium seeds to germinate, and Shull(53) suggested that this might be due to the increase in anaerobic respiration at higher temperatures.

The temperature requirements for optimum growth and

maximum germination are better understood than any other factor in the process of germination. Sub-optimum temperatures during the germinating period are common causes for poor stands. Planting of spinach or beet seed in soil at a high temperature will result in a low emergence due to the much more favorable conditions for the pathogens, primarily Rhizoctonia sp., which causes pre-emergence damping-off in vegetable seedlings (67).

Kotowski (34) has pointed out that the optimum temperature for germination was in the range of 70 to 80° F. He also pointed out the fact that the rate of production of seedlings at different levels of temperature was in accordance with van't Hoff's law. Harrington (27) using alternating temperatures on vegetable seed found that they germinated well. The temperature changes giving best germination results, with a large number of kinds of seed, correspond rather closely with soil temperatures in the field under conditions which induce the most prompt and vigorous production of seedlings. Field tests suggest the use of an alternation between 18° and 32° C for optimum germination. The minimum number of days required for producing bean seedlings in the soil was four days; and the first seedlings are produced in the smallest number of days in moderately warm weather in the months of May and June. Reynolds (47) indicated that beans require a warm soil for their best development. In a cold soil, presumably, bean plants would not have as great vitality and might

prove particularly susceptible to species of Fusarium. According to Morris (41), bean varieties vary in their ability to withstand low temperature. In some varieties this was partly inherent, while in all varieties tested it was related to a reduction in their ability to withstand the attack of soil pathogens. Gilman (23) observed that at higher temperatures the plants may possess a lower degree of vitality and hence should be more susceptible to facultative parasites.

Axentjev (4) found that the light inhibiting effect on germination of a large variety of seeds was entirely dependent on the presence and integrity of the seed-coats. The coats evidently act by restricting the oxygen available to the embryos. In some cases, light apparently interferes with oxidation processes within the seed, while in others it favors these processes. Rudolfs (49) studied the rate of growth of bean seeds from large and small seeds in the dark, and concluded that large seeds gave by far the better growth.

Seed-coats differ in their structure from seed to seed and may also differ in the same variety of seed (45); and some of these differences result in "hard" seeds. Barton (6) describes these modifications in the legumes as a cutinization of the ends of the palisade cells or the formation of a water impermeable cuticle. Gloyer (24) and Lebedeff (37) point out that the tendency to develop "hard" seeds in field beans is a genetic character, but

the degree to which this is developed is a function of environment.

Swanson and Hunter (59) correlated field stands of Sorghum with the thickness of the starchy mesocarp layer, and pointed out that soft grain types with a thick mesocarp averaged thirty-nine percent field stands, whereas Sorghum types with a thin mesocarp averaged fifty-seven percent. They state that water intake was one-third greater in the soft grain types but molds and rots accounted for the decreased stands. Watson's (66) work on the structure of the testa in relation to germination in several species of Papilionaceae tribes indicates that there is much variation in the structure and chemical nature of the seed-coats of different species. The amount of suberized and cuticularized sub-cuticular and Malpighian thickening found in individual species of the group seems to justify a high general rate of impermeability and difference in germination.

Ott and Ball(43) working with Red Kidney beans suggest that there is an indication that polyurenides and "true pentosans" are involved in water retention in the dried seed-coats. Schulze and Godet (50) point out that seed-coats contain a large percentage of hemicellulose, also some brown unrecognizable substances which they termed "Holzgummi".

Mechanical injury in seed beans has attracted considerable attention of research workers in recent years.

Asgrew* (3) research workers began an intensive research program in 1940 to determine the causes and means of prevention of mechanical damage to bean seed. Injury is brought about by mechanical harvesting, threshing, processing and undue handling or improper storage. The Asgrow workers have divided seed injury into two categories: (1) visible or external injury, and (2) internal injury to the embryo, detectable only by a viability test. The first type of injury ranges from slightly cracked seed-coats, hardly discernible to the naked eye, to severely cracked or chipped seed. Chipping and cracking can occur anywhere on the seed-coat, but are more commonly found on the ends. A sample of seed which at first appears to be of normal viability reveals upon closer examination these external injuries which are indicative of broken embryos and consequent lowering of germination. Internal injuries give rise to abnormal seedlings which are consequently weak and, if lucky to survive, produce a poor plant of little or no value to the grower (3). Goss (25) indicates that the poor germination of Fordhook lima beans is due to cracking injury of the seed-coat. Fungus pathogens are more apt to cause infection of the seeds and cause a lower percentage of germination. According to Morris (41), under optimum conditions for germination and with a minimum of mechanical injury, there is no significant varietal

*Asgrow - abbreviation for Associated Seed Growers Inc. This abbreviation will be used throughout the paper.

difference in germination. Low germination of some lima varieties he attributes to mechanically injured seed obtained from some sources.

Virgin (62) has reported that bacteria may be found underneath the seed-coats of large wrinkled type peas, finding entrance during threshing through cracks in the seed-coats.

Morris (41) further points out that seed-coat color in certain varieties is associated with the ability to withstand soil infection. This resistance is considerably greater in dark seeds than those with white or light colored seeds.

Soil pathogens take a big toll of seeds and young seedlings. Among the most important soil pathogens causing pre-emergence damping-off are Rhizoctonia solani and Pythium debaryanum. Excessive soil moisture, poor aeration and favorable temperatures are ideal conditions for development of soil pathogens, especially fungi which cause damping-off.

Burkholder (10) found that the bean plant appears to be little affected by the pH of the soil and thrives well in an acid or alkaline soil. Severe infection by Fusarium martii may be produced in a soil of fairly low pH, 5.0, and in an alkaline soil of pH 8.0 as well. Generally speaking, fungi thrive in a fairly acid soil, while bacterial pathogens favor a neutral or slightly alkaline medium. There are a large number of microorganisms present in the soil, and most of them are saprophytes or facultative parasites

causing secondary infection. Fawcett (21) points out that mixtures of pathogenic organisms, i.e., the presence of two or more of them, cause the greatest amount of damage in seeds and seedlings.

Likewise, according to Christensen (14) association of microorganisms are the cause of seedling injury especially when the seeds are infected. When the seeds are planted in the soil, the seed-borne pathogens first cause infection and later on secondary organisms come in and cause death of the seedlings.

Walker (63) states that no effective seed treatment has been perfected for seed-borne diseases on beans, especially bacterial pathogens. Arason and Spergon have proved beneficial on some varieties of beans and other vegetable seeds.

McGuffy (39) reports that lima beans treated with a fungicide failed to germinate. This he attributed to injury received during the treatment.

Hay (28) found that infection by the common soil fungi and bacteria lead to decreased germination and pointed out the fact that chemical seed treatment with the fungicide Uspulun tends to reduce the amount of injury both under laboratory and field conditions.

Morris (41) concluded from his experiments that light colored or white bean seed planted in soils are significantly benefited by treatment with Spergon.

Evanari (20), in his review on "germination inhibitors",

states that many plants produce substances which inhibit or delay the germination of seeds of the same or different species. It is doubtful whether germination itself or merely the first phase of subsequent growth is inhibited. Therefore, he defines them as substances which, from observation, have been found to inhibit germination. Germination and growth inhibition are nearly always associated with one another (20). When seeds in different stages of germination were transferred from water to tomato juice, their growth was inhibited.

Many authors (20) have found auxins in seed and some have shown that the auxin content of seed decreases germination. If the auxin in seeds were to function as a "germination hormone", the action of inhibitors could be explained by auxin destruction or inactivation. Evanari (20) doubts whether auxins can be considered germination hormones.

Siegel (54), using Red Kidney beans, obtained extracts which showed an inhibitory effect on the germination of oat seed. Barton and Solt (7) found inhibitors of both germination and enzyme activity in a number of seeds. Using water extracts of the seed-coats, cotyledons and embryos of Phaseolus vulgaris, they found that it inhibited root growth of wheat seedlings.

The presence of antibiotics in seeds has been reported. It is presumed that these substances provide them with a measure of protection against plant pathogens.

In an intensive study of resistant and susceptible strains of black mustard and turnip, Stahmann et al (56) refute the claims that allyl isothiocyanate (mustard oil) in black mustard seed was responsible for resistance against the clubroot organism. Quantitative estimation of the amount of mustard oil in the roots of these plants failed to show a correlation of isothiocyanate content to resistance or susceptibility to clubroot.

Lucas et al (38) in their search for antibiotic substances have found that extracts from some varieties of Brassica showed a marked degree of inhibitory action against many bacteria and some fungi.

Berriss (9) concludes in a discussion on germination by saying "that the germination promoting effect of the soil is due not to the presence of any stimulating factor, but rather to the removal of an inhibiting substance from the seeds by the absorptive power of the soil complex".

EXPERIMENTAL PART

Commercial stock of beans of several varieties were obtained from seed companies, which will be referred to as A, B, C, and D, whenever the source of the seed is mentioned. Incidentally, some of the varieties were the same as those used by Morris (41) in his investigation on the factors influencing the germination of snap, and lima beans in 1949. Of the ten varieties selected six, namely Lazy Wife, Dixie White, Brittle Wax, Black Valentine, Plentiful and Pencil Pod Wax, were further selected for more detailed study.

The seed when received from the seed companies had been treated with a fungicide for protection against soil pathogens. Since untreated seeds were to be used in the investigation, the treated seeds were washed with a detergent solution, rinsed with distilled water, and quickly dried on cheesecloth. This washed seed was used throughout the investigation unless specified. Experiments were carried out in the laboratory, greenhouse, and in the field.

Moisture Absorption Capacity of Six Varieties of Beans

Absorption of water is a primary and initial step in the germination process of a seed. In order for the

moisture to reach the dormant embryo, it has to pass through the protective layers of the seed-coat. Barton (6), Gloyer (24), Lebedeff (37), and Watsen (66) who have made a study of the seed-coats of leguminous seeds point out that there is a difference in the structure of the seed-coats of different varieties, which accounts for their differences in moisture absorption and subsequent germination.

Stiles (57, 58) working with corn and cotton, studied the rate of water intake into various parts of the seed, and pointed out that various varieties had distinctly different characteristics in relation to affinity for moisture absorption. She also compared the rate of amount of water intake in several varieties of beans, and classified soybeans as xeric types, and lima beans as hydric types.

This experiment attempts to make an examination of the moisture absorption capacity of six varieties of garden beans in order to determine whether there is any difference between dark and light varieties.

Procedure. Six varieties of beans, three black-seeded and three white-seeded, were selected for the experiment. Duplicate samples of each variety were tested for their moisture content. Average readings were taken of the moisture content of the sample, by means of a Steinlite Moisture Tester.

Two sets of approximately equal numbers of uniform seeds of each variety were selected and weighed in a beaker. Equal quantities of water were added to each beaker; one group was held at room temperature 77° F, the other in the refrigerator 44° F.

After a period of twenty-four hours had elapsed, the seeds were quickly taken out, dried on cheesecloth to remove excess moisture, and weighed. Increase over the original weight was assumed to express the amount of water absorbed in a twenty-four hour period. This procedure was repeated every twenty-four hours for a period of six days, and the total increase over the original weight was calculated.

Results of water absorption are given in Table 1 and Figures I and II. The initial level of moisture content was higher in all black varieties than in all the white varieties.

Results and discussion. Results indicate that there is a significant difference in the amount of water absorbed by different varieties. Black Valentine absorbed the largest amount of water in the first 24 hours, both at 77° and 44° F, followed by Dixie White, Pencil Pod Wax, Brittle Wax, Plentiful and Lazy Wife. The variety Lazy Wife is inconsistent because of the large percentage of "hard" seeds. Black Valentine and Dixie White reached their peak of absorption on the fourth day. At a temperature of 77° F, the black varieties absorbed a larger

TABLE 1

WEIGHTS OF SIX VARIETIES OF BEANS SOAKED FOR SIX DAYS AT 44° F (a) AND 77° F (b)

Variety		Air-dry weight (grams)	Original moisture content (percent)	Weight in grams after soaking for					
				1 day	2 days	3 days	4 days	5 days	6 days
Black									
Plentiful	(a)	19.0	7.75	22.5	24.5	25.5	28.5	29.5	30.5
	(b)	19.0		28.0	32.5	35.5	38.5	39.5	40.5
Black Valentine	(a)	15.5	10.1	25.9	29.9	31.5	32.0	32.0	31.5
	(b)	15.0		30.5	32.0	32.5	33.0	33.0	32.5
Pencil Pod Wax	(a)	16.0	7.75	19.3	21.5	26.5	29.5	30.1	31.3
	(b)	17.0		28.5	32.5	35.5	37.0	38.0	38.5
White									
Brittle Wax	(a)	16.8	4.75	20.5	23.5	25.2	27.0	28.5	29.5
	(b)	16.5		27.0	31.5	33.5	34.5	35.0	35.5
Dixie White	(a)	9.9	5.35	14.5	16.0	16.1	16.4	16.5	16.5
	(b)	10.0		18.5	19.0	19.0	20.0	20.0	20.0
Lazy Wife	(a)	22.0	5.35	23.8	27.0	27.5	28.0	28.5	28.7
	(b)	22.0		32.1	34.1	35.1	36.5	37.0	37.5

1 BLACK VALENTINE
2 PENCIL POD WAX
3 PLENTIFUL
4 BRITTLE WAX
5 DIXIE WHITE
6 LAZY WIFE

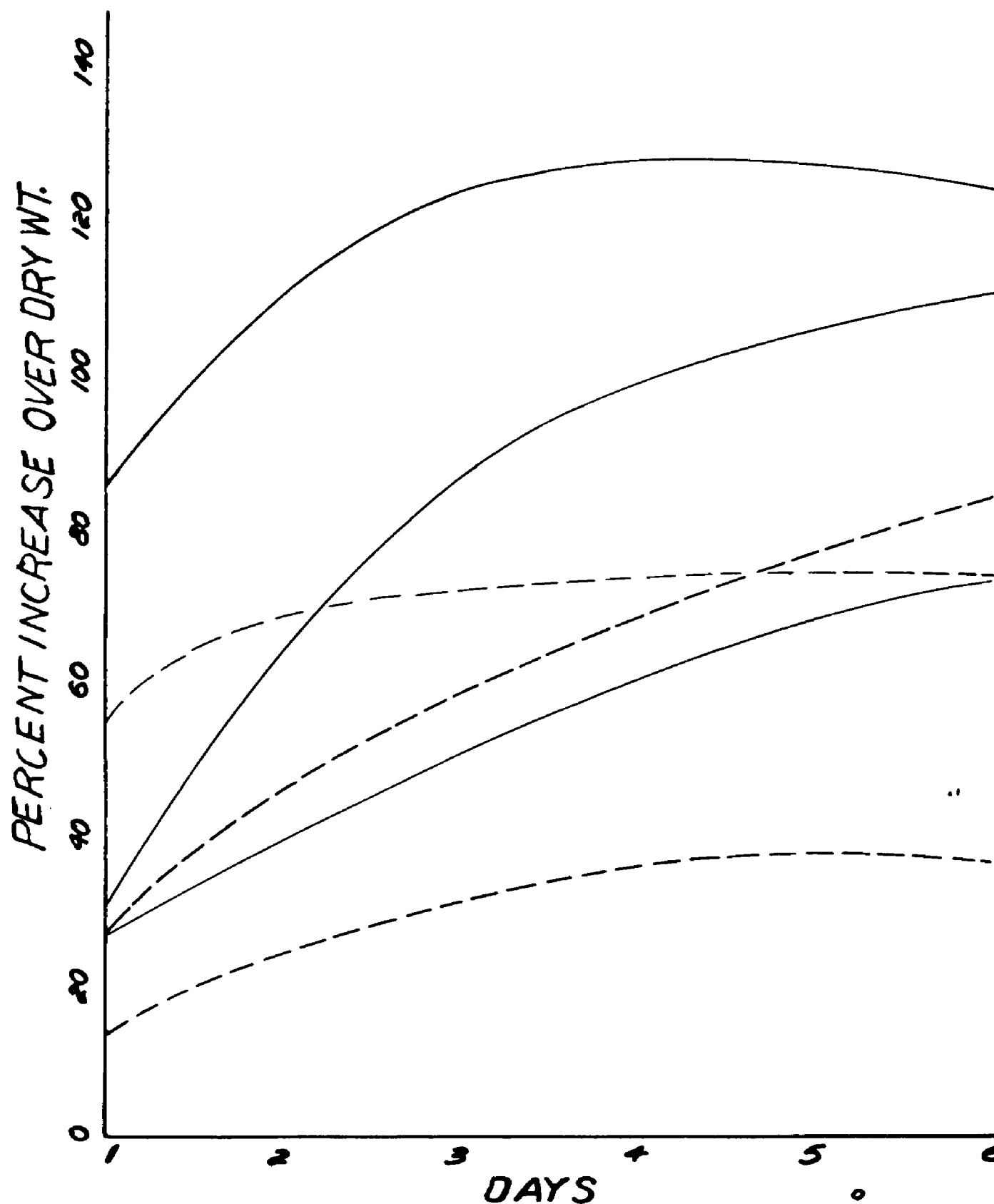


Figure I. *MOISTURE ABSORPTION AT 44°C*

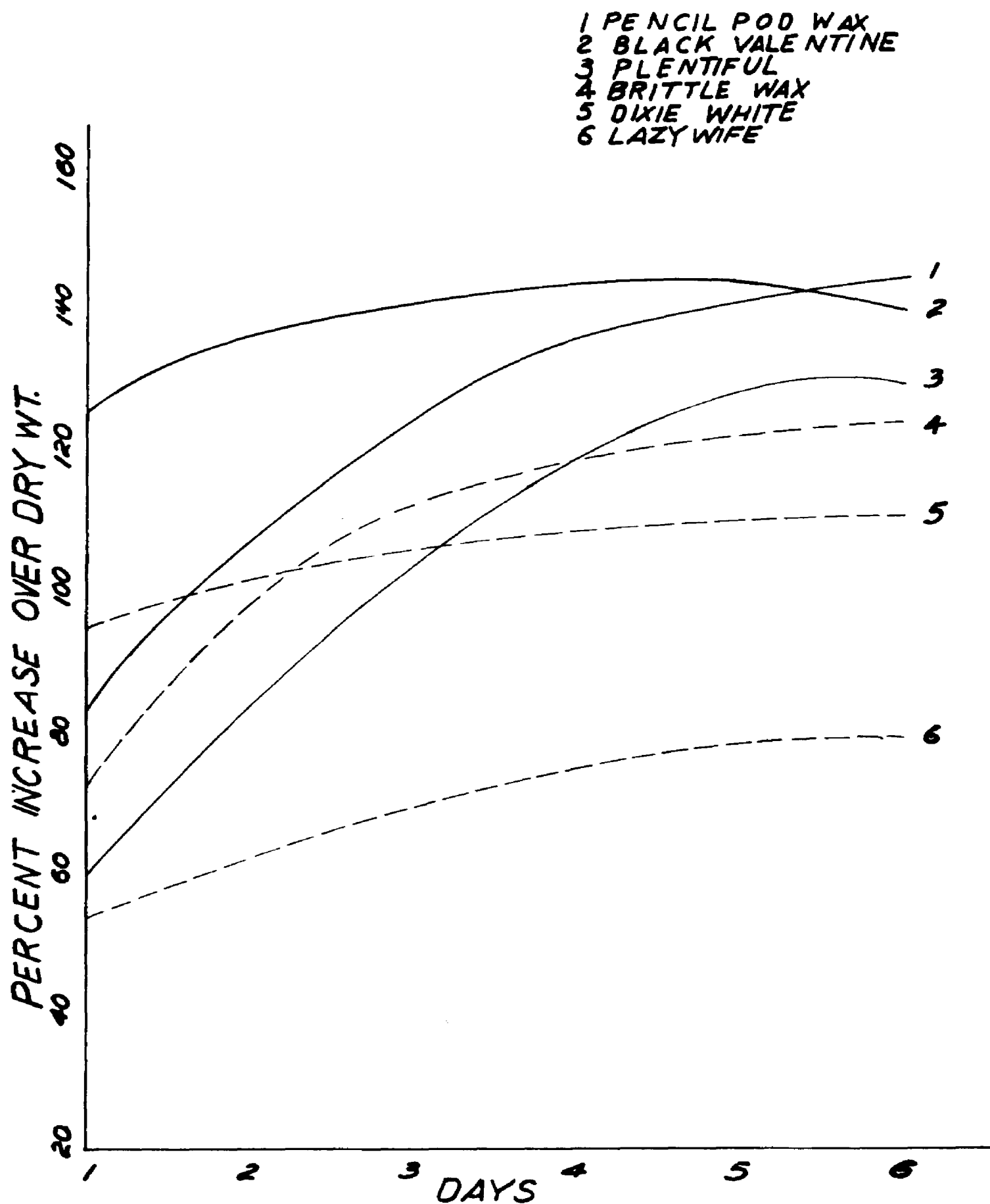


Figure II. MOISTURE ABSORPTION AT 77°C

amount of water in comparison with the white varieties at 44° F. The amount of water absorbed at 77° F is significantly greater than at 44° F in all the varieties. Nevertheless, the results were considered inconclusive. Fermentation set in sooner in the white than in the black varieties. If such were true in the soil it might explain deterioration and consequently poor germination of the white varieties. Similar and confirming results were obtained in experiments conducted to show the effect of soaking on germination and presented subsequently.

The Effect of Temperature and Period of Soaking
Bean Seed as a Factor Influencing Germination

The Effect of Temperature and Period of Soaking bean,
Bean Seed as a Factor Influencing Germination
soaking seeds prior to germination has a marked effect upon the subsequent course and vigor of development. Kidd and West (32, 33) observed that soaking of seeds for only eight hours reduced the total growth considerably as compared to that of seeds sown dry on damp sand. This was true in spite of the fact that those which had been soaked had a considerable start in germination and sprouted earlier. They further pointed out that the period of soaking bears no relation to the percentage germinated. Bailey (5), on the other hand, found a decrease in germination after eight hours of soaking, and it progressively decreased as the length of the soaking period increased.

The object of the present experiments was to examine the effect of temperature and period of soaking on the

germination of some varieties of beans.

Effect of Soaking for 12 and 24 Hours at
3 Different Temperatures on Germination

Procedure. Four varieties of beans, Pencil Pod Wax, Black Valentine, Brittle Wax, and Dixie White were selected for this experiment. A preliminary experiment with one black variety, Pencil Pod Wax, and one white variety, Brittle Wax, was set up in the laboratory. Fifty seeds of each variety were soaked in distilled water at three different temperatures - 44°, 82°, and 96° F; dry seed was used as a control at 82° F. After a period of twenty-four hours the seeds were removed from the water and placed on filter paper, in Petri dishes. Germination counts were made at intervals of three and six days.

Results of germination are given in Table 2.

TABLE 2

GERMINATION OF TWO VARIETIES OF BEANS SUBJECTED TO
TWENTY-FOUR HOURS OF SOAKING AT THREE TEMPERATURES

Fifty seeds of each group were treated

Variety	Period of observation	Control 82° F	Number germinated after soaking 24 hours at		
			44° F	82° F	96° F
<u>Black</u> Pencil Pod Wax	3 days	48	15	48	28
	6 days	50	43	50	41
<u>White</u> Brittle Wax	3 days	40	0*	9*	10*
	6 days	48	13*	9*	12*

*Contaminated by growth of microorganisms

Results. Germination results indicate a clear-cut difference between the black and white varieties. A deleterious effect of soaking was very noticeable in the white variety, whereas the black one was little affected. Brittle Wax was injured at all temperatures. Germination was considerably slowed down in both varieties at very low and high temperatures. These results are in agreement with those of Bailey (5) who worked with Early Valentine beans.

Effect of Soaking for 12 Hours at 3 Temperatures
on the Emergence of Beans Planted in Soil

Procedure. Four sets of 100 seeds of each of the four varieties of beans were soaked in distilled water at three temperatures - 40° , 55° , and 73° F, with one set of dry seed as a control. Temperatures at which the beans were soaked had been selected on the basis of soil temperature levels possible under field conditions during the growing period. After a period of twelve hours, the seeds were taken out of the controlled temperature chambers and planted in flats in the 60° F greenhouse. Emergence counts were made after a period of one week.

Results and discussion. Results (Table 3) indicate that soaking for twelve hours had an injurious effect on all the varieties tested; however, two black varieties were much less injured than the two white varieties. The effect of temperature on soaking did not indicate any

TABLE 3

EMERGENCE OF FOUR VARIETIES OF BEANS SUBJECTED TO
TWELVE HOURS OF SOAKING AT THREE TEMPERATURES*

Variety	Control	Percent emergence after soaking 12 hours at		
		40° F	55° F	73° F
<u>Black</u>				
Black Valentine	100	78	76	72
Pencil Pod Wax	92	79	60	79
<u>White</u>				
Dixie White	88	3	5	1
Brittle Wax	96	20	10	27

*Seeds planted in soil

significant differences among the varieties. It would appear that the period of soaking is more important than the temperature at which the seeds were soaked. The white varieties again responded to the treatment by a far greater decrease in germination than the black ones.

Effect of Soaking at Room Temperature for
3, 6, 12, and 24 Hours Respectively on the Germination
of Bean Seeds

Procedure. Four sets of 100 seeds each, of twelve varieties, were soaked in distilled water at room temperature (78° F) for 3, 6, 12, and 24 hours respectively. The seeds were removed after the above mentioned periods of soaking and placed in moist chambers. Dry seed was used as control. The amount of germination was noted; the data are expressed as percentages.

Results of germination are given in Table 4.

TABLE 4

GERMINATION OF BEANS SOAKED FOR 3, 6, 12, AND 24 HOURS
AT ROOM TEMPERATURE 78° F

Variety	Control	Percent germination after soaking for			
		3 hrs.	6 hrs.	12 hrs.	24 hrs.
Black					
Pencil Pod Wax	96	98	99	96	85
Black Valentine	100	100	100	100	99
Plentiful	86	86	80	82	71
Brown					
Top Crop	96	84	68	48	18
Rival	96	64	72	60	12
Bountiful	94	84	76	60	48
Landreths' Stringless	96	92	88	88	90
Full Measure	98	96	96	76	70
White					
Brittle Wax	90	96	82	66	49
Lazy Wife*	48	16	28	28	32
Dixie White	89	48	32	24	17
Michelite	96	96	68	52	6

*Results of germination in this variety are inconsistent because of the large percentage of "hard" seeds.

All results are the average of 3 experiments performed consecutively. No standard deviation was necessary as the results being close, the simple arithmetic mean served as an average.

Results and discussion. The deleterious effect of soaking is clearly evident in this experiment. Soaking periods affected germination of different varieties to

varying degrees. As previously noted the black varieties germinated better than the white varieties. The brown varieties were intermediate. Besides the difference in germination another fact became evident in this experiment; that is, the black beans were not contaminated by micro-organisms whereas the white beans had a high percentage of affected seeds even after only 6 hours of soaking.

This effect of soaking may be compared to that of a saturating rainfall immediately after planting. The saturated condition of the soil may have the same effect as the immersion in water.

The Influence of Variety, Temperature and Period of Cold Storage on Germination

The investigation was undertaken on the basis of results of Morris (41), as reported in the review of literature.

Seeds of ten varieties of beans were selected at random and planted in nine-inch pots in August, 1950. The experiment was arranged as a two-way classification design with three periods - three, six and nine days - at 40° F in cold storage, and a control. After planting, the pots were watered and covered with wax paper to prevent evaporation and drying out of the soil. The pots were placed in the cold storage at 40° F. Ten control pots were placed outdoors. Ten pots of each of the ten varieties were removed from the cold storage at intervals of three, six and nine days respectively and placed outdoors. From then

on normal watering was done and emergence counts made at regular intervals. Outdoor night temperature during the month of August averaged 67° F.

Results of germination and emergence are given in Table 5.

Results and discussion. The results show a significant difference in the emergence of the ten varieties tested and the effect of the length of the period of cold treatment on germination and emergence.

These results are in agreement with those reported by Morris (41) that "Varieties vary in their ability to withstand low temperatures, due to a reduction in their ability to combat infection by soil pathogens." Seeds that failed to germinate were found on examination to be infected by microorganisms. The organisms were isolated in pure culture and identified.

Seed-coat color seemed to be associated with resistance to infection at low temperatures. There is little varietal difference in emergence in the untreated controls, except in the variety Brittle Wax which had many "hard" seeds.

Reynolds (47) pointed out the fact that beans require a warm soil for their best development. In a cold soil presumably bean plants would not have as great a vitality and might prove susceptible to Fusarium sp. Two different species of Fusarium were isolated from the beans which failed to germinate. Aspects of fungus infection are

TABLE 5

**EMERGENCE OF TEN VARIETIES OF BEANS SUBJECTED TO
COLD TREATMENT PRIOR TO GERMINATION***

Variety	Control	Number emerged after days in cold storage			
		3	6	9	Average
Black					
Plentiful	20	18	20	5	15.75
Pencil Pod Wax	20	20	11	13	16.00
Black Valentine	19	16	15	1	12.75
Average	19.6	18.0	15.3	6.3	14.80
Brown					
Landreths' Stringless	19	15	18	16	17.0
Top Crop	19	16	14	1	12.5
Lows' Champion	16	16	10	1	10.75
Red Valentine	18	12	11	2	10.75
Average	17.5	14.7	13.2	5	12.75
White					
Lazy Wife	18	6	4	4	8.0
Dixie White	20	3	15	3	10.25
Brittle Wax	6	3	0	0	2.25
Average	14.6	4	6.3	2.3	6.80
General average	17.5	12.5	11.8	4.6	

*Twenty seeds planted per pot.

Least significant difference for varieties - 5% = 5.95
 1% = 8.03
 Least significant difference for treatments - 5% = 3.75
 1% = 5.07

discussed in a later section of this paper. The period of cold treatment had a decided effect on the germination of all varieties. As the period of cold treatment increased, the germinating capacity of all the varieties progressively decreased. The longer the beans were in the cold soil, the greater were the chances of infection (41). Leach (35) compared the growth rates of host and pathogen for several crops and found that the relative rates of growth determine, to a considerable degree, the severity of pre-emergence infection at different temperatures.

Seed treatment with fungicides provides a measure of protection against soil pathogens. The degree of protection afforded by fungicides is very important during the critical period of germination; it was absent in this experiment since all the seeds had been washed.

The Effect of the Period of Illumination on the Germination of a Black And a White Wax Bean

Axentjev (4) found that the light inhibiting effect on germination was entirely dependent on the presence and integrity of the seed-coats in Amaranthus retroflexus and other species of plants, but not entirely due to the coats in Cucumis melo and related species. Apparently, in some cases, light interferes with the oxidation processes within the seed, while in others it favors these processes.

Lettuce seeds are frequently light sensitive and the germination of these has attracted much attention. According to Shuck (51) American lettuce seed is in a physio-

logically unstable condition that makes it particularly sensitive during germination to the effects of light, moisture and temperature.

On the basis of these reports it was thought to be of interest to test the influence of illumination on the germination of black and white bean varieties.

Procedure. One black variety, Pencil Pod Wax, and one white variety, Brittle Wax, were selected on the basis of results of the previous experiment. The former had proved to be the best black variety and the latter the poorest white variety in regard to emergence.

Three hundred uniform seeds of each variety, free from external injury, were selected and placed in square glass dishes, each containing one hundred seeds. Another set of dishes was prepared with unselected seed. The filter paper in the dishes was moistened with distilled water, and each set of dishes was exposed to illumination as described below. Seeds of both varieties were grown in the dark as controls.

Either variety was placed on a greenhouse bench under conditions of normal daylight. The second set was placed on another bench with overhead fluorescent lights, which were kept on continually thus extending the period of illumination to 24 hours. The third set was placed in a box completely sealed to prevent the entry of light. Germination counts were made after an interval of four days,

the normal period in which beans should germinate at the temperature of the experiment. Greenhouse temperatures did not fall below 60° F. Results of germination are given in Table 6.

TABLE 6

PERCENTAGE GERMINATION OF TWO VARIETIES OF WAX BEANS
UNDER DIFFERENT PERIODS OF ILLUMINATION

Variety	Normal daylight	Light for 24 hours	Darkness
<u>Black</u>			
Pencil Pod Wax			
(Selected seed)	14	52	92
(Unselected seed)	28	52	96
<u>White</u>			
Brittle Wax			
(Selected seed)	70	88	92
(Unselected seed)	54	92	96

Results and discussion. The germination of the black beans under normal daylight and under continuous light was significantly poorer than that of the white variety. The unselected seed of the white variety did better than that of the black under light. In darkness there was no significant difference, both varieties having a high percentage of germination.

Seedlings of both varieties, in total darkness, had larger roots than those germinated under light. The hypocotyls of the white variety were well developed and had

emerged from the seed-coats, while very few of the black variety had appeared.

The strong inhibition exhibited at exposure to normal daylight followed by darkness, and the less pronounced inhibition caused by continuous illumination, seem to indicate that not light alone but also photoperiod may be involved. The white variety was less subject to this influence than the black variety.

Chemical Analysis

Schulze and Godet (50) found hemicelluloses to be the largest constituent of the bean coats; the content of the hemicellulose reaching a maximum in the ripe bean. Besides hemicellulose, the coats consist of nitrogen-free substances insoluble in water, significant quantities of lignin, and a brown unrecognizable gummy substance which Schulze and Godet called "Holzgummi".

The cell walls consist of pectic substances which are extremely hydrophilic compounds. These pectic substances are composed, for the most part, of uronic acids. Ott and Ball (43) suggest that a knowledge of the polyuronide and pentosan content of the coats points towards an explanation of varietal difference in water absorption and retention. One of the most important components of the hemicelluloses is pentosan, which is closely related to the polyuronides. This investigation sought to determine whether there was any difference in the pentosan content in the seed-coats of different varieties and whether this

difference is related to the water absorbing capacity of these varieties, and has any influence on germination.

Procedure. The method used in this experiment is that of McRary and Slattery (40). Briefly, it involves removing the soluble sugars from the dry material by alcohol extraction, hydrolyzing the residue to make the pentosans soluble, and assaying the resulting solution by a simple colorimetric test.

The colorimetric determination is based upon one of the color reactions of the five-carbon sugars, namely, the interaction of pentose with orcinol in the presence of ferric chloride and concentrated hydrochloric acid (Bials reaction) producing a green color.

Concentration of xylose in the unknowns was read off the xylose standard curve (Figure III) previously worked out from the readings of the standard xylose solution. Concentration of pentosans in the unknown samples was calculated and the values expressed as percentage xylose, presented in Table 7. A graphic presentation of the results is given in Figure IV.

Results and discussion. The percentage of pentosans in the white varieties is slightly higher than in the black varieties.

The results obtained are in disagreement with the expectations, just as Ott and Ball found and reported in their paper (43). The black varieties with a lower pentosan content would normally be expected to have a

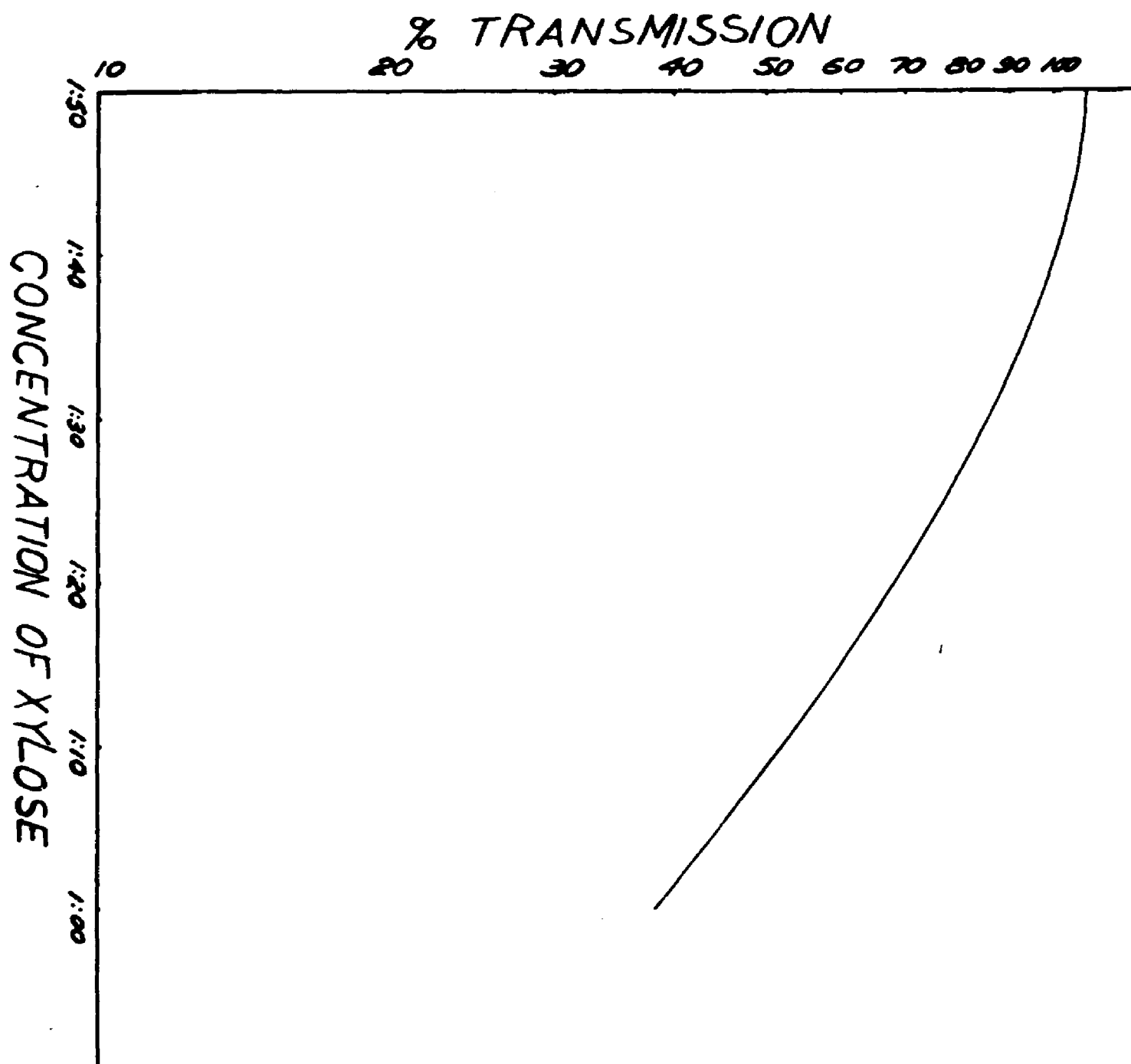


Figure III. STANDARD LOG CURVE FOR XYLOSE

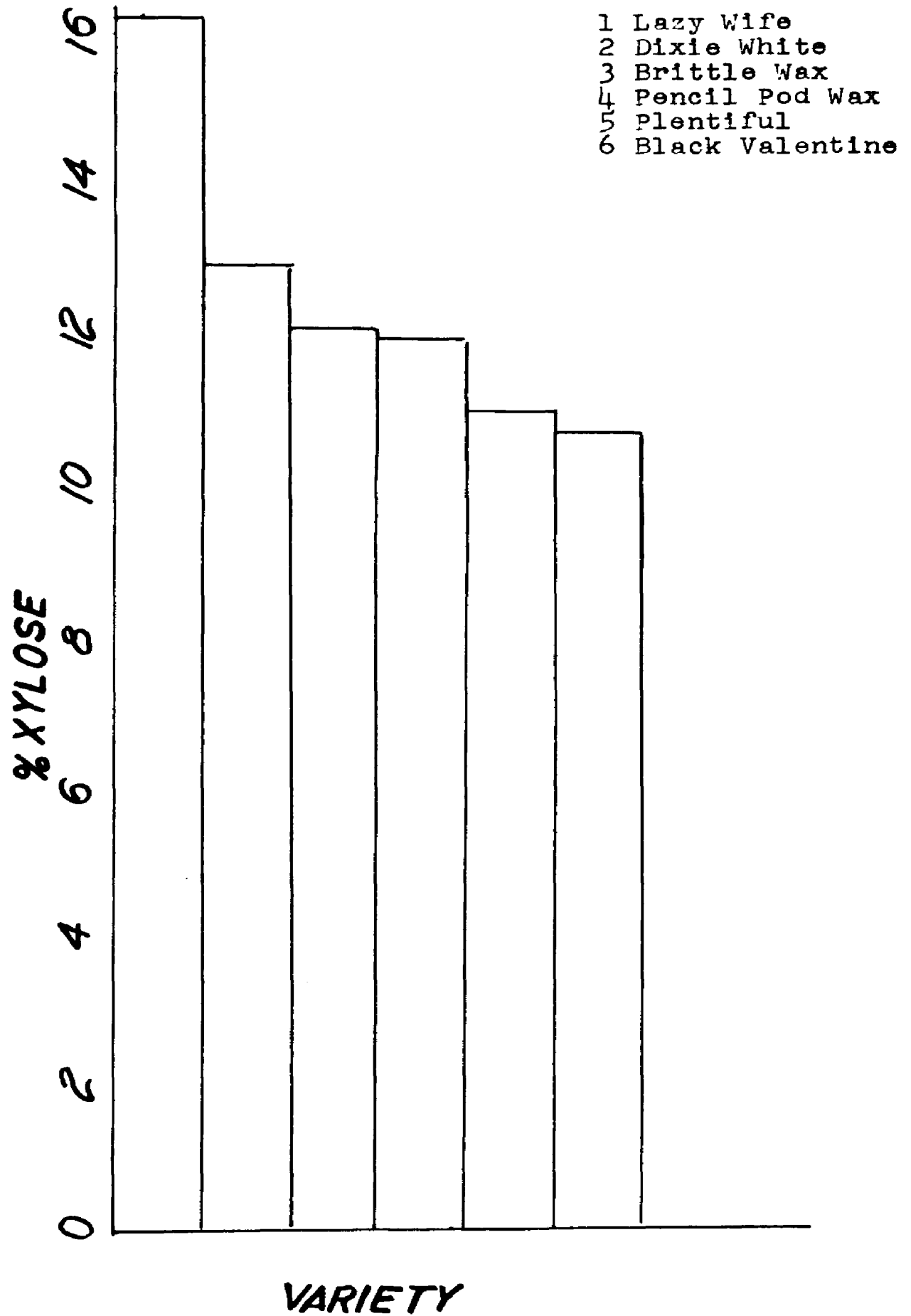


Figure IV. *PENTOSAN CONTENT*

TABLE 7
PENTOSAN DETERMINATIONS IN SIX VARIETIES OF BEANS

Sample number	Variety	Percent transmission	Percent concentration of xylose	Remarks
1	Lazy Wife	78	16.19	White
2	Dixie White	87	12.88	
3	Brittle Wax	91	12.11	
4	Pencil Pod Wax	92	11.92	Black
5	Plentiful	94	10.89	
6	Black Valentine	95	10.70	

lower retentivity for water. This is not the case according to the results obtained in the experiment dealing with water absorption.

An Anatomical Study of the Seed-coats of Beans

Considerable work has been done in the past on the anatomical structures of the seeds of the Leguminosae (24, 37, 44, 66). Most of the work, however, has been concerned with the permeability of the seed-coat and its influence on germination. Watson (66) has correlated impermeability with poor germination in his investigation on the papilionaceous tribes Trifoleae and Loteae.

This investigation is primarily concerned with examining the structure of the seed-coats of some bean varieties in an attempt to correlate thickness with their

ability to withstand mechanical injury, and consequently explain varietal differences in germination.

Procedure. Ten seeds of approximately the same size, color and maturity representative of the variety in question were soaked in water for twelve hours. The seed-coats were then easily removed by hand and small squares were cut out from the same place of each seed-coat to insure uniformity in the sections.

The material was placed in a formalin-glacial acetic acid-50% alcohol killing solution (5:5:90 parts) for eighteen hours and embedded in paraffin for sectioning. Sections were cut twelve microns in thickness on a rotary microtome, stained with Conants' quadruple stain, and mounted permanently in Canada Balsam.

Measurements and camera lucida drawings (Figures V, VI, VII, VIII) were made using a magnification of 430X.

The drawings were enlarged to twice the original size and photographed.

Seed-coat measurements are given in Table 8.

General Description of the Seed-coats

The outermost layer of the coat consists of a row of palisade cells, called Malpighian cells, ranging from forty to sixty microns in length. Their outer ends are pointed and the exposed surfaces are covered by a thin cuticle which forms a continuous layer of unequal thickness and extends between the projections of the Malpighian cells.

SEED-COAT STRUCTURE OF FOUR BEAN VARIETIES

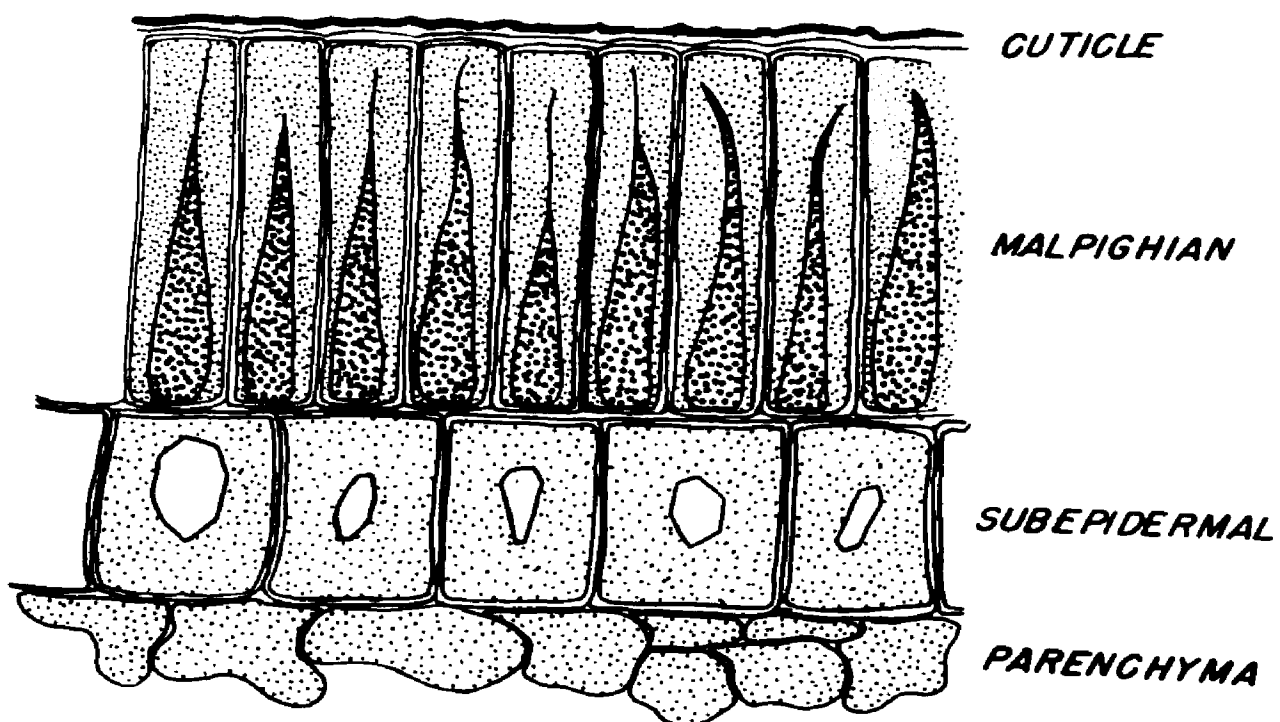


Figure V. Brittle Wax

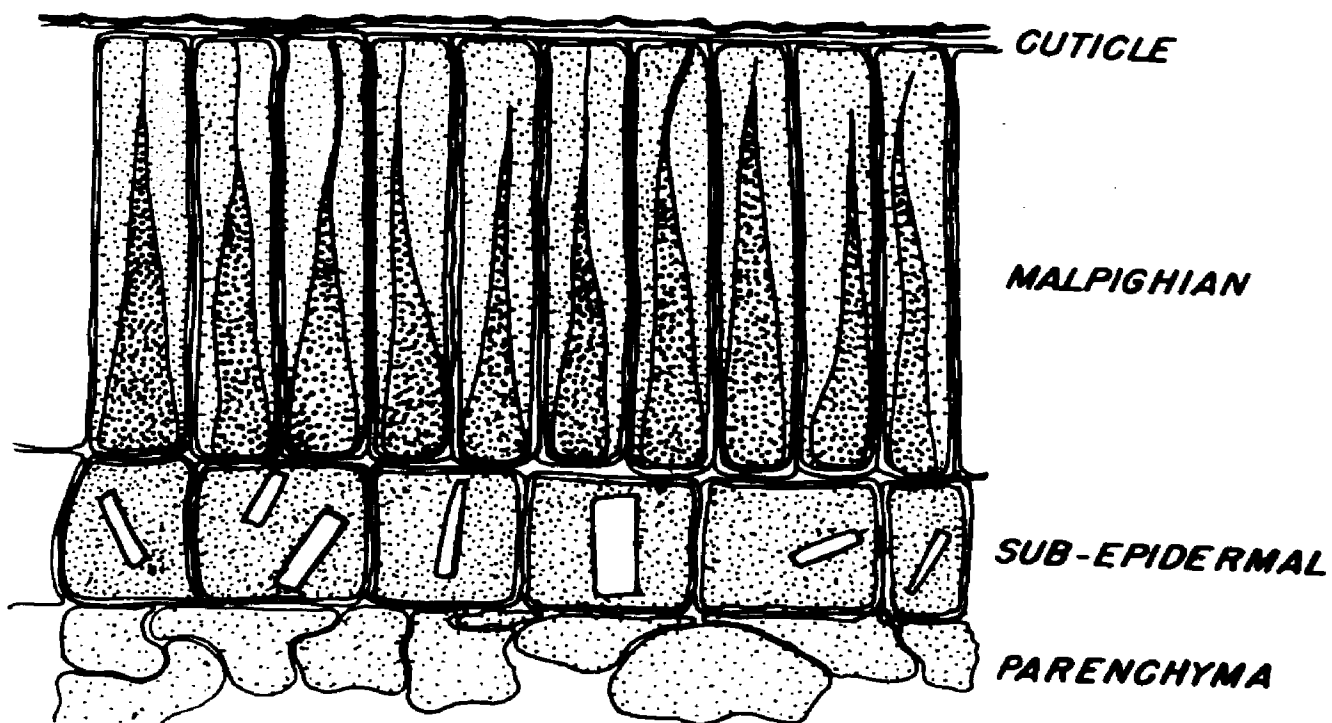


Figure VI. Pencil Pod Wax

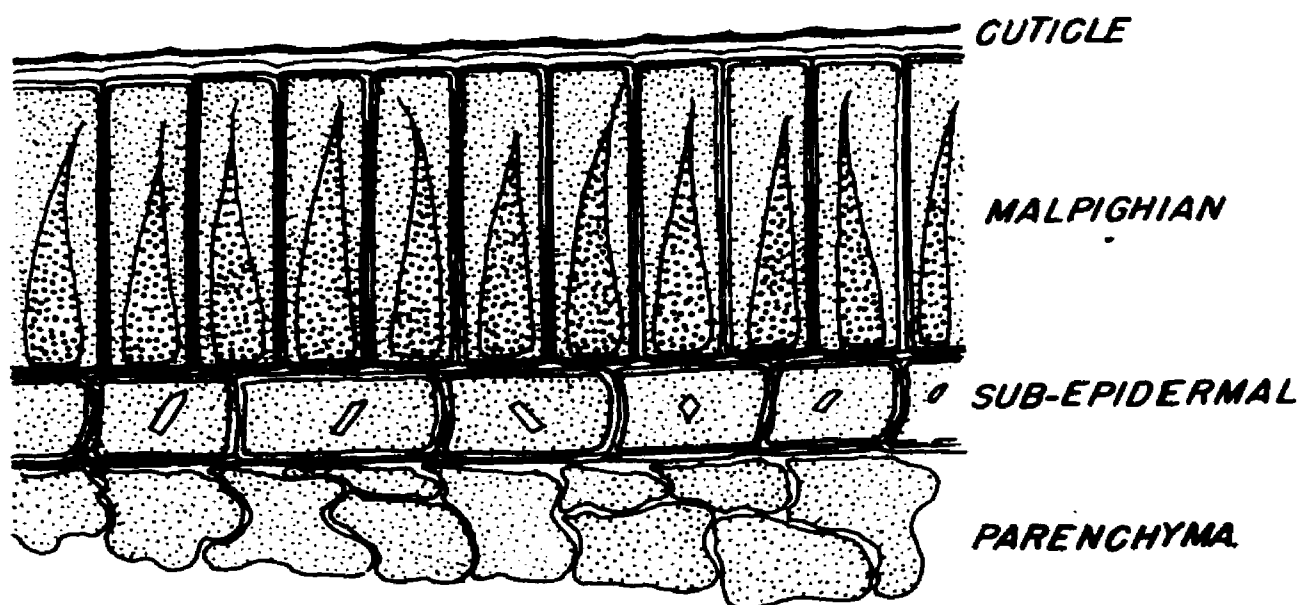


Figure VII. Rival

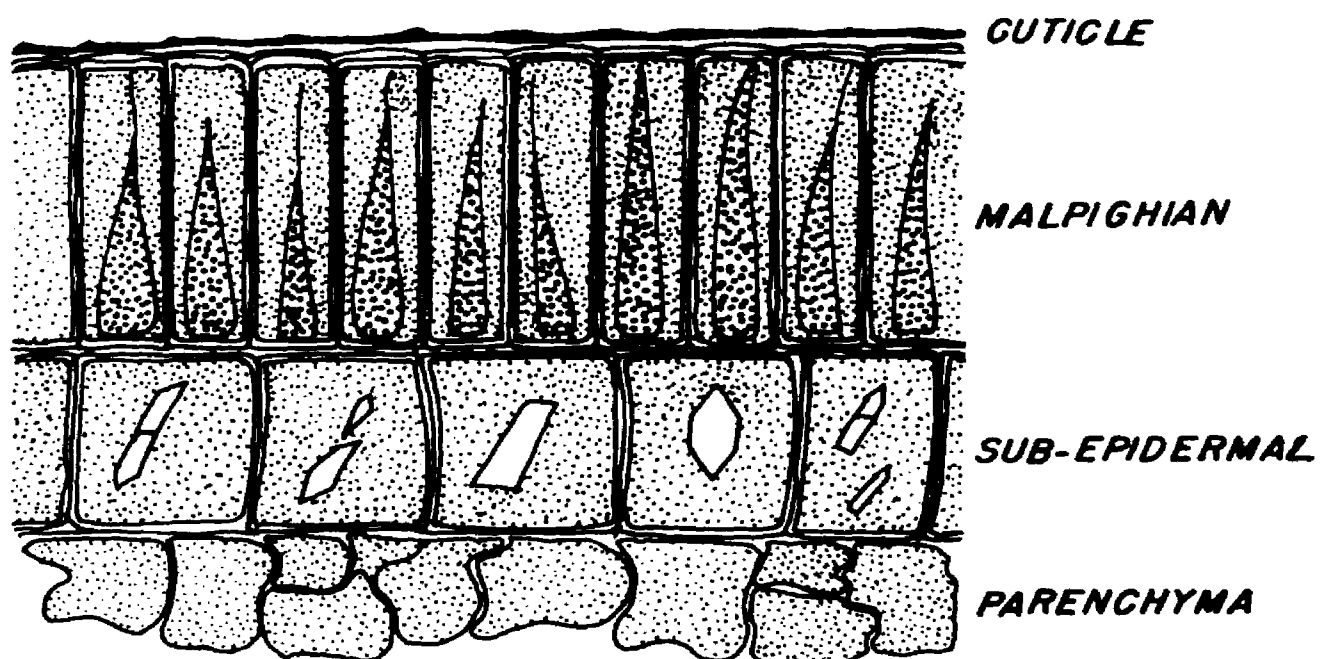


Figure VIII. Lazy Wife

TABLE 8
SEED-COAT MEASUREMENTS OF SEVEN VARIETIES OF BEANS

Variety	Measurement in microns	
	Malpighian layer	Sub-epidermal layer
<u>Black</u>		
Pencil Pod Wax	60.97	14.74
Plentiful	56.28	17.42
Black Valentine	51.59	11.39
<u>Brown</u>		
Top Crop	48.24	14.74
Rival	46.90	12.06
<u>White</u>		
Brittle Wax	46.90	20.10
Lazy Wife	44.22	24.79

Coefficient of correlation between layers within varieties significant at 1% level = -0.54.

The lumen of the Malpighian cells is large at the base and tapers toward the top, being very narrow in the upper part of the cell owing to pronounced thickening of the cell walls.

Beneath the palisade cells is a sub-epidermal layer of closely packed cells, with no intercellular spaces, and greatly thickened walls with little or no lumen. Underneath the sub-epidermal layer is a parenchymatous zone which consists of several layers of much compressed thin-walled cells. The inner layers are spongy in their arrangement, while the outermost layers are flattened and without intercellular spaces. Drawings were made of four varieties

of beans -- Pencil Pod Wax (black) Figure VI, Rival (brown) Figure VII, Brittle Wax (white) Figure V, and Lazy Wife (white) Figure VIII.

Results and discussion. The over-all picture does not indicate any differences in the basic structure of the seed-coats. Individual parts of the different varieties examined showed decided variations in thickness.

The cuticular layer is thin, uneven and continuous. The Malpighian layer varies in depth in the different varieties examined. Pencil Pod Wax has the thickest Malpighian layer measuring 60.97 microns, Figure VI; Plentiful and Black Valentine 56.28 and 51.59 microns respectively. The two brown varieties, Top Crop and Rival (Figure VII), and the white variety Brittle Wax (Figure V), have Malpighian layers intermediate in thickness between the black and white varieties. Lazy Wife (Figure VIII), a white variety, has the thinnest Malpighian layer measuring 44.22 microns with an exceptionally thick sub-epidermal layer 24.79 microns in depth. The sub-epidermal layer in all the varieties examined contain crystals of various shapes and sizes.

The thickness of the Malpighian layer explains to some extent the amount of mechanical injury incurred in threshing and handling. Generally speaking, the black varieties which have a much thicker Malpighian layer appear to be more resistant to mechanical damage. The black variety Plentiful, however, although having a thick palisade layer, had a high percentage of basic injury. This anomaly may

be explained by the fact that the samples examined could have come from lots which were harvested and threshed when too dry, thus making the seeds more brittle. Hand-shelled seeds of this variety showed no signs of mechanical injury and germinated just as well as the two other black varieties.

A study of the variety Lazy Wife was prompted by the fact that the commercial sample contained a large percentage of "hard" or impermeable seeds. The sub-epidermal layer is extremely thick, preventing the absorption of water and accounting for the large number of impermeable seeds.

Within varieties there is a significant negative correlation between the thickness of the Malpighian and sub-epidermal layers.

A Study of Mechanical Injury

The subject of mechanical injury in bean seeds has been investigated in the past decade (3, 17) but specific information on seed-coat color in relation to injury is lacking.

The present studies were undertaken with a view to discover the relative resistance of black and white seeded varieties to mechanical injury.

Basic Injury in Commercial Bean Seed

Procedure. Samples of seed were taken at random from the bags in which they had been shipped, and examined for

basic injury -- external injury incurred in threshing and handling operations prior to sale to the growers. Broken or chipped seeds and those with large cracks were first picked out. The remaining seeds in the sample were further examined for invisible cracks by placing them in a flat dish containing water. Seeds with fine cracks had a wrinkled appearance after 10 minutes in the water.

The results of such studies in six varieties of commercial bean seed are given in Table 9 and graphically represented in Figure IX.

Results and discussion. Basic injury varies between varieties, different lots of the same variety, and different sources.

The white varieties Brittle Wax and Dixie White had a higher percentage of injury than the two black varieties Pencil Pod Wax and Black Valentine. The anatomical study of the seed-coats of these varieties showed that white ones have a thinner seed-coat than the black.

Although there are indications that mechanical injury may be inversely proportional to the thickness of the seed-coat, the exceptions of the black variety Plentiful, which shows a high percentage of injury, and of the white variety Lazy Wife having the least amount of injury, make it impossible to draw definite conclusions.

TABLE 9

BASIC INJURY COUNTS IN SIX VARIETIES OF COMMERCIAL BEAN SEED

Variety	Source of seed	Number of seeds in sample	Percent basic injury (cracked, broken and shrivelled seed)	
<u>Black</u>				
Black Valentine	A	529	4.3	3.7*
	B	426	3.2	
Pencil Pod Wax	A	525	4.9	
	B	617	3.0	3.5*
	C	705	2.5	
Plentiful	A	772	12.9	
	B	663	5.2	9.0*
<u>White</u>				
Brittle Wax	A	529	8.5	
	B	647	6.4	6.7*
	C	820	5.3	
Dixie White	A	557	10.4	
	B	799	6.5	8.4*
Lazy Wife	A	667	4.9	
	C	545	1.0	2.5*

*Average basic injury count.

Mechanical Damage Experimentally Induced

Types of abnormality resulting from mechanical injury have been described by Asgrow (3).

The object of this experiment was an attempt to examine the varietal resistance exhibited by black and white beans to internal injury resulting from undue handling

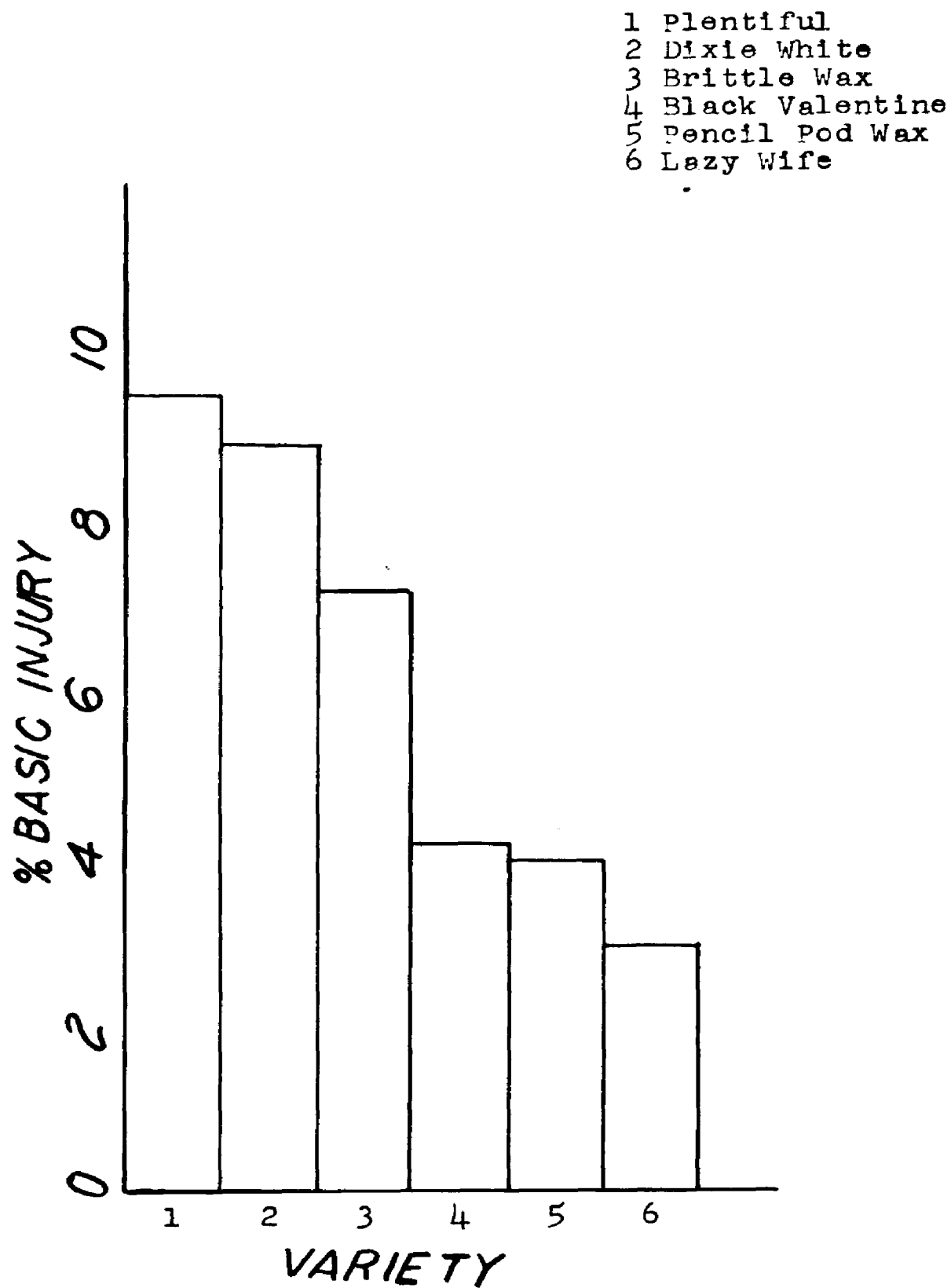


Figure IX. *MECHANICAL INJURY IN COMMERCIAL SEED*

during threshing and shipping. This degree of resistance would explain the varietal differences in germination and show whether this damage is cumulative due to repeated impacts incurred in rough handling and threshing.

Procedure. Apparently uninjured seed, i.e., without evidence of external injury, was selected from each lot of commercial seed. These seeds were divided into two lots of one hundred seeds each, one lot to be treated and the other left as an untreated control. To simulate rough handling and to obtain a cumulative effect of impacts, one hundred seeds of each variety were placed in a metal box with a rough wire gauze base. The box was placed on a mechanical shaker and the seeds shaken for five minutes. Thereafter the seeds were removed and planted in soil in the 60° F greenhouse for testing their viability. This method had an advantage over the rolled towel or "rag doll" test commonly used in the laboratory, in that "bald heads" and other abnormal seedlings could be readily detected. The untreated control of one hundred seeds was planted directly in the soil.

Results of germination and abnormality are given in Table 10.

Results and discussion. All three of the black varieties had a lower percentage of abnormal seedlings in comparison to the three white varieties. The variety Lazy Wife showed very low germination due to the fact that

TABLE 10

PERCENTAGE GERMINATION AND ABNORMAL SEEDLINGS
IN SIX COMMERCIAL VARIETIES OF BEANS

Selected Seed Subjected to Mechanical Injury

Variety	Untreated control		Treated	
	Percent germi- nation ¹	Percent abnor- mality ²	Percent germi- nation	Percent abnor- mality
<u>Black</u>				
Black Valentine	100	0	100	20
Plentiful	92	8	98	17
Pencil Pod Wax	96	8	92	10
<u>White</u>				
Dixie White	88	12	100	28
Lazy Wife	38	62	46	54
Brittle Wax	96	8	97	28

¹Average germination of a number of lots received from two different commercial sources.

²Abnormality here denotes bald heads, broken seeds, "hard" seeds, etc.

it had a large percentage of "hard" seeds, which, being impermeable to water, failed to germinate. Treated seeds had a larger proportion of abnormal seedlings in comparison to the untreated controls. This result is indicative of the cumulative effect of repeated impacts received by the seed prior to and during this experiment. The percentage of abnormality seen in the untreated controls is the result of internal injury incurred in the process of threshing and handling.

These results are in agreement with those reported in the monograph of Asgrow (3) whose authors point out that apparently uninjured seed may have been internally injured which showed up only in viability tests.

The varietal differences in germination and the proportion of abnormal seedlings of black and white varieties indicate more resistance to mechanical injury in black varieties. The better germination of the black varieties may be partly due to this fact.

The Effect of Varying Relative Humidity on Mechanical Injury and Germination

It was presumed that varying degrees of relative humidity at the time of threshing had some influence on the degree of mechanical injury, and consequently on the varietal differences in germination. Asgrow research workers (3) point out that the moisture content of the seed at the time of threshing had a marked effect upon the amount of mechanical injury incurred. Seeds having a higher percentage of moisture are less susceptible to injury than seeds with a lower moisture content.

Procedure. Five varieties of beans, Pencil Pod Wax, Black Valentine, Plentiful, Brittle Wax, and Dixie White, were used in this investigation. Examination of the climatological data of the East Lansing area for a period of ten years revealed that the mean relative humidity during the month of October, when threshing of beans is usually

performed, was 68 percent. Three levels of relative humidity, 25, 75, and 100 percent, were used. In order to obtain these conditions, solutions of sulphuric acid of different molarity were used. Fruit jars containing these solutions were set up and small perforated cans containing the seeds were suspended from the metal covers so as to hang over the solution in the jars without touching it. Sets of one hundred seeds each, of all five varieties, were exposed to the desired relative humidities.

The seeds used in the experiment were harvested and hand-shelled simultaneously; it was assumed that the moisture content of the seeds of all the varieties was approximately the same. Freedom from mechanical injury was insured by hand-shelling the beans.

Each set of seed was held at the desired relative humidity for a period of 48 hours. Thereafter, they were removed from the fruit jars and subjected to rough handling to simulate field conditions. This was achieved by placing them in a metal box with a wire gauze base and placing them on the mechanical shaker for five minutes. After this treatment, the seeds were examined for signs of external mechanical injury; no instance of external injury was recorded. The seeds were planted in flats. The flats were placed in the 40° F chamber for a period of twenty-four hours and later transferred to the greenhouse for germination. Emergence counts were made after one week and abnormal

seedlings counted, as indicative of those having incurred internal injury during the treatment.

Results and discussion. The three black varieties seem to be little affected by the three levels of relative humidity in connection with subsequent shaking, while the two white varieties germinated very poorly at all levels. The variety Dixie White showed an increased percentage of germination at 75 percent relative humidity, as compared with the corresponding figures at 25 and 100 percent relative humidity. No conclusions can be drawn, however, because the experiment was carried out only once.

By comparing the results of Table 11 with those presented in Table 3 there is a similarity between the emergence figures of the soaked white beans, and the white beans of the present experiment.

TABLE 11

EMERGENCE OF FIVE VARIETIES OF BEANS SUBJECTED
TO THREE LEVELS OF RELATIVE HUMIDITY
AND SUBSEQUENT MECHANICAL INJURY

Variety	Untreated	Percent relative humidity		
		25	75	100
<u>Black</u>				
Pencil Pod Wax	100	88	83	92
Black Valentine	100	85	93	96
Plentiful	90	77	75	81
<u>White</u>				
Brittle Wax	91	14	14	23
Dixie White	88	14	44	24

Since germination of white beans was depressed regardless of the level of humidity it must be assumed that shaking produced the deleterious effect, an effect to which the black varieties are obviously resistant.

The Effect of Mechanical Injury on Field Stands
of Six Varieties of Beans

Asgrow research workers (3) have shown that mechanical injury in seed beans is the cause of poor field stands. The abnormal seedlings that are produced from injured embryos are said to be weak and susceptible to diseases and insect attack.

The following experiment was planned to show the relative emergence of selected injured and intact seeds in comparison to that of random samples.

Procedure. Six commercial varieties of beans were used for the experiment and divided into lots of uninjured seeds, injured seeds, and a random sample. Injury was assessed by visual evidence. Forty seeds were planted per row, each row being ten feet long and accommodating one group. Group (1) comprised good selected seed, (2) cracked seed, and (3) a random sample. Thus three rows of forty each were planted in the field for each variety to be tested. The experiment was set up in a randomized block design.

Emergence counts were made after the first and second week. Average emergence counts of the two observations are given in Table 12.

TABLE 12

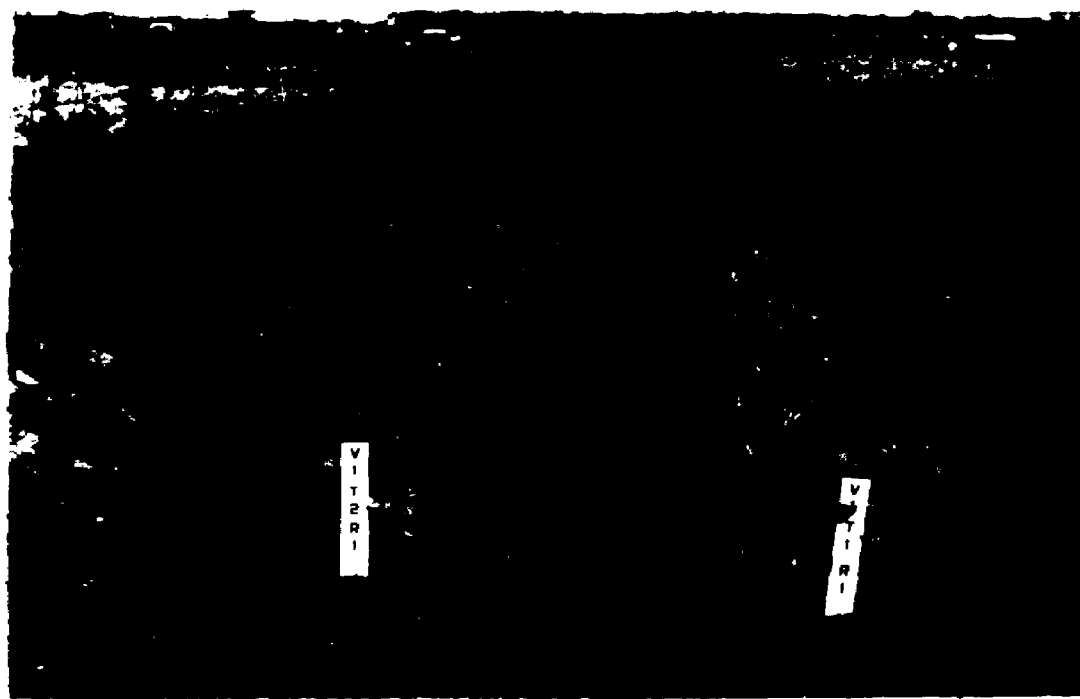
INFLUENCE OF SELECTION ON EMERGENCE OF FIELD PLANTED BEANS

Variety	Percent emergence*			
	Uninjured	Injured	Random	Average
Black				
Pencil Pod Wax	95.0	48.75	93.75	79.16
Black Valentine	90.0	78.75	83.75	84.16
Plentiful	80.0	25.0	85.0	63.33
Average	88.3	50.83	87.5	75.55
White				
Brittle Wax	48.75	16.25	51.25	38.75
Dixie White	78.75	41.25	65.0	61.66
Lazy Wife	23.75	21.25	28.75	24.58
Average	50.41	26.25	48.31	41.66
General average	69.38	38.54	67.91	58.61

*Average of two replications.

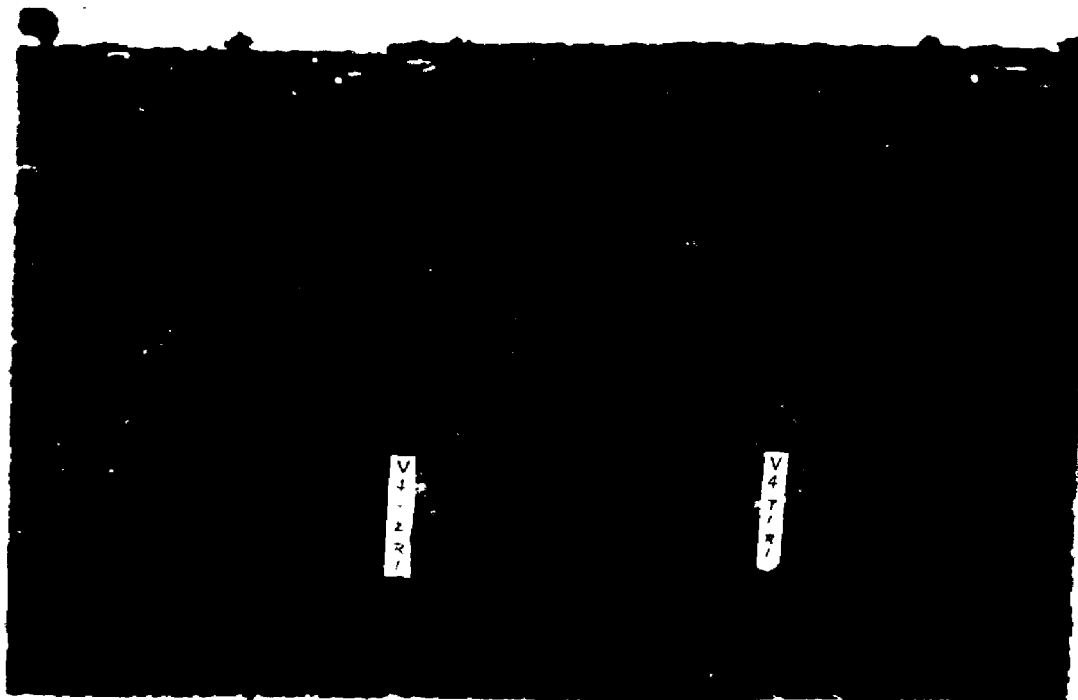
Results and discussion. Selected uninjured seed of all the varieties shewed a higher percentage of emergence than that of the injured group of seed. There is no significant difference between the selected uninjured and the random group of seed. It was evident that the black seeded varieties had a significantly higher percentage of germination regardless of injury in comparison to the white varieties.

FIELD STANDS OF TWO BEAN VARIETIES



T_1 - uninjured group of seed
 T_2 - injured seed

Figure X. Pencil Pod Wax



T_1 - uninjured group of seed
 T_2 - injured seed

Figure XI. Brittle Wax

A Study of Possible Interference with Germination
by Microorganisms

Fungi and bacteria are considered a part of the normal soil flora and the majority of them are non-pathogenic. However, there are some that are pathogens of specific plants, and are then considered host-specific. Among the many fungi causing pre-emergence damping-off, species of Rhizoctonia, Pythium, and Fusarium are the principal ones. Fusarium, although normally a soil saprophyte, can become a facultative parasite by long association with a particular host, as in the case of beans (10).

Temperature and moisture are intimately connected with infection in the soil. Whetzel (67) found greater infection by Pythium debaryanum in saturated soils, and also pointed out that sub-optimum temperatures during germination are common causes of poor stands.

In this investigation a number of fungi were isolated from the beans which failed to germinate and have been recorded below. The beans used in this experiment were commercial samples and had been treated with fungicides. It was assumed therefore that they were reasonably free from contamination when planted.

Procedure. Bean seeds which failed to germinate were removed from the soil of three different plantings, carefully cleaned and placed in sterile moist-chambers. When the fungi and bacteria emerged on the surface of the beans, they were transferred by means of an inoculating needle to

media slants. The colonies that grew from these transfers were mixed cultures and two or more transfers had to be made before the cultures were pure. The fungi were examined and identified on the basis of their asexual spores.

Fungi isolated in pure culture

1. Aspergillus flavus
2. Penicillium brevi-compactum series
3. Rhizopus nigricans
4. Fusarium sp.
5. Trichoderma sp.
6. Monilia sp.
7. Alternaria sp.

Results and discussion. All the fungi were isolated from white beans. No fungus development was observed from the isolates that had been made from the black varieties. However, bacterial cultures were obtained from both groups.

Nine distinctly different types of bacteria were isolated but were not identified.

The fungi that were isolated in pure culture and identified normally are soil saprophytes, causing secondary infection. Some of them, like Trichoderma lignorum and species of Fusarium are capable of becoming facultative parasites.

Christensen (14) points out that the association of two or more soil organisms take a heavy toll of young

seedlings, especially if the seeds were infected by spores prior to planting. Usually the pathogenic species of Rhizoctonia and Pythium cause primary infection and the saprophytes come in later to kill the seedlings.

The results of the experiments are suggestive of a combined effect of bacteria and fungi in which bacterial contaminants may have paved the way for secondary infection by saprophytic fungi. The absence of these saprophytes from black beans seems to support the possibility that some agent in the black seed-coats counteracted the fungi and perhaps also the bacteria.

Antibiotics

In view of the results obtained in the preceding experiment, the question of presence of an antibiotic agent in black beans seemed appropriate. The field of antibiotics, though comparatively new, seems to have some bearing on the problem under study.

Reports have been published that some seeds have shown a high degree of inhibitory action against fungi and bacteria (38, 56).

The possibility of bean seeds possessing such substances was also indicated in the experiments on the effect of soaking.

Procedure. Seed-coats, whole seeds, and young hypocotyls of the varieties Pencil Pod Wax and Brittle Wax were extracted with water, 95 percent alcohol and ether.

Water and alcohol extracts were made in a Waring Blendor; the ether extract was prepared by immersion of the material for one hour. Five times the quantity of liquid was used to the weight of material used. The extracts were sterilized by Seitz filtration. The test organisms used were Rhizoctonia solani, Pythium debaryanum, and Xanthomonas phaseoli, which were the organisms mainly suspected of interfering with germination.

The fungi isolated in the previous experiment were not used because of their primarily saprophytic nature. However, each of the nine bacteria isolated in the previous experiment were also included in this test.

The fungi were inoculated by placing a small piece of mycelial mat in the center of the agar plate. When the colony started to grow two small sterile pads were dipped in the extracts and placed equidistant from the organism in the center of the plate. The bacteria were tested by streaking them radially on plates towards a filter pad containing the extract which was placed in the center of the plate.

Results and discussion. No clear-cut antagonistic action of any of the bean extracts was recorded. There were occasional indications in some of the tests with the fungi that growth in the vicinity of the pads containing extracts of the black seeds was inhibited or retarded. In some cases actual inhibition was observed but duplication

was not obtained. The tests with bacteria were negative in all instances.

Germination Inhibitors

Since tests for the presence of antibiotic substances proved negative, it was postulated that inhibiting substances may be present in the white bean seeds.

Siegel (54) reported finding growth inhibiting substances in Red Kidney beans. Such substances have been found in seeds of a large number of plant species (20).

Procedure. The varieties Pencil Pod Wax (black) and Brittle Wax (white) were used. The seed-coats were removed by soaking them in distilled water for twenty-four hours and dried in the oven at 82° F.

The water in which the beans had been soaked was used for subsequent extraction of the seed-coats in a Waring Blendor. Extractions with ether were also made. In this case the seed-coats were immersed for one hour; the ether was then evaporated and water that had been used for soaking was added.

Seeds of both varieties were surface sterilized with Tetrasan (1:1000) and quickly rinsed with distilled water. Ten seeds of each variety were used in each case. The sterile seeds were placed in Petri dishes on filter paper. Ten milliliters of the extract were pipetted into each dish. Controls were set up similarly, using water.

Results and discussion. No difference in germination was observed between controls and the treated seeds, nor between varieties. It was concluded that no inhibitory substance that can be extracted by simple procedures as the ones used is present in the seed-coat of beans.

The Effect of Artificial Inoculation with
Bacteria on the Germination of Beans

In previous experiments on the effect of soaking on germination and in the study for antibiotic substances it was noted that the black varieties obviously resisted infection. To further substantiate this observation an experiment was set up in which both injured and intact seeds were inoculated with bacterial cultures.

Procedure. Six varieties of beans, three black and three white, were selected for the experiment. The seeds were separated into two categories -- injured and intact. Ten of each category, and each variety, as well as intact and injured seeds to serve as controls were sterilized by immersing them in a solution of Tetrosan (1:1000) for three minutes and rinsed with distilled water. The seeds were then picked up with sterile forceps and placed in Petri dishes. Twenty-four-hour old cultures of Xanthomonas phaseoli, Bacillus subtilis, Escherichia coli and two unknown soil bacteria, W_1 and W_2 , isolated in pure culture from infected white beans, were used in the experiment. Four milliliters of sterile distilled water were first put

into each plate, followed by two ml of bacterial suspension. The plates were rotated to insure even distribution of the bacteria. The controls were set up with six ml of water. Infection and germination counts were made after four days and are given in Table 13.

Results and discussion. A large percentage of injured seed in all the white varieties artificially inoculated with bacteria failed to germinate due to bacterial infection. Infection occurred in the case of every organism used. In the variety Dixie White, injured seed inoculated with Xanthomonas phaseoli and W₂, an unknown bacterium, ten and twenty percent respectively, germinated. Within two days these seedlings died.

It is significant to note that the black varieties remained completely free from infection in all cases. This experiment, like some of the preceding, points to the presence of some antimicrobial substance in the black varieties of beans. No black variety had below fifty percent germination in either injured or uninjured seed.

TABLE 13

PERCENTAGE GERMINATION AND CONTAMINATION IN SIX VARIETIES OF BEANS
ARTIFICIALLY INOCULATED WITH BACTERIA

(Figures in table represent the averages of three experiments)

Variety	Xanthomonas phaseoli				Bacillus subtilis				Escherichia coli			
	T ₁	T ₂	T ₃	T ₄	T ₁	T ₂	T ₃	T ₄	T ₁	T ₂	T ₃	T ₄
Black												
Pencil Pod Wax	100	100	100	100	100	100	100	90	100	100	100	100
Plentiful	80	90	100	100	100	100	90	100	100	100	100	100
Black Valentine	100	100	100	100	100	100	80	100	100	100	70	100
White												
Brittle Wax	95	100	100*	70 30*	70 30*	100	80 20*	70 30*	70 30*	100	60 40*	100
Dixie White	100	100	10 80*	70 30*	90	100	20 80*	80	70 30*	90	100*	80
Lazy Wife	90	100	70 30*	70 30*	80	70	80 20*	60	90 10*	80	90 10*	90

*Percent infected seed.

T₁ - uninjured seed inoculated with bacteria

T₂ - uninjured seed control (untreated)

T₃ - cracked seed inoculated with bacteria

T₄ - cracked seed control (untreated)

TABLE 13 CONTINUED

Variety	W ₁ (Unknown) (Dry rot)				W ₂ (Unknown) (Moist rot)			
	T ₁	T ₂	T ₃	T ₄	T ₁	T ₂	T ₃	T ₄
<u>Black</u>								
Pencil Pod Wax	100	100	100	100	100	100	100	100
Plentiful	100	100	100	100	50	100	90	100
Black Valentine	100	100	100	100	100	100	100	100
<u>White</u>								
Brittle Wax	90	100	60 40*	90	80 10*	100	100*	90
Dixie White	90 10*	100	100*	90	80	100	20 100*	80
Lazy Wife	80	100	40 40*	90	100	100	70 30*	80

*Percent infected seed.

T₁ - uninjured seed inoculated with bacteria

T₂ - uninjured seed control (untreated)

T₃ - cracked seed inoculated with bacteria

T₄ - cracked seed control (untreated)

GENERAL DISCUSSION

The studies on some of the factors effecting varietal differences in germination of beans, the results of which have been reported and briefly discussed, were started as a result of the findings of Morris (41). His observations pointed to a possible relation between seed-coat color of beans and the ability to withstand low soil temperatures during germination. Several theoretical explanations for the difference in the behavior of white and black beans were considered when the present work was undertaken. It was most tempting to speculate about the possibility of the presence of a substance in the black seed-coats that might be antagonistic to potentially harmful microorganisms. Proof that such a specific activity existed in the black, and perhaps other dark varieties, but were not present in white varieties, would have been a simple explanation of the differences encountered. Preliminary tests showed, however, that such relationship, if it existed at all, was quite difficult to ascertain. It was therefore decided that all other factors which could be possibly connected with poor germination should be tested in both black and white varieties in order to establish any basic differences between these groups.

The individual experiments have been discussed when the results were presented. While some of the experiments had inconclusive results it was possible to establish differences between black and white varieties which may lead to an interpretation of the difficulties that have been observed and reported by Morris (41) in the germination of white beans in soil.

It was established in this work that soaking in various forms greatly impaired the germination of white beans. Germination of black beans was not affected when the seeds were germinated under laboratory conditions. It was somewhat lowered when planting took place in soil. The white beans also responded to this difference in condition of germination but in all cases their germination percentages were much lower. This seems to indicate a far greater sensitivity of white varieties to excess of moisture. Bailey (5) has shown that Early Valentine beans germinated increasingly poorly when soaking was extended over eight hours. The present findings are in agreement with these results. In applying the observations recorded to the differences in germination between white and black beans in the field it may be reasoned that a wet condition of the soil would be detrimental to the germination of white beans while it would hardly affect black beans.

Pronounced differences were obtained in the absorption of water by black and white varieties. With one exception the water absorption curves were steeper in the case of the

black varieties (Figures I and II). This would be in agreement with Morris' assumption (41) that poorer water adsorption might be the reason for poorer germination although the present experiments do not fully justify such a point of view.

Significant differences were shown in the experiment in which the beans had been subjected to low temperatures immediately after planting. The results are in favor of the black varieties. All black varieties and also the varieties with red and brown seed-coats which were used in this experiment emerged satisfactorily when the cold treatment was not carried beyond six days. The white varieties uniformly failed to germinate well even after three days. One exception of satisfactory germination of a white variety after six days of cold treatment cannot be explained. However, this result did not interfere with the significance of the superiority of the black and other dark varieties over the white beans. This result would explain poorer germination and emergence of white beans in the field when low temperatures prevail after planting.

Coupled with the visible effects of exposure to low temperatures and excess of moisture as expressed by germination and emergence figures was the observation that contamination by microorganisms definitely prevailed in the white beans. In the experiments in which soaking was used as treatment no contamination of black beans was observed; the degree of microbial growth on black beans in the other

two experiments discussed was much lower than that of the white varieties since in all cases it was restricted to the seeds that had not germinated. The significant difference in germination which has been shown can therefore also serve as a measure of microbial contamination.

Differences in the seed-coat structure have been discussed before. The results obtained, although somewhat indicative of a more favorable condition in the black beans concerning resistance to mechanical injury, are not considered sufficient to warrant any far-reaching conclusions.

In both experiments in which mechanical injury was artificially induced the results were clearly indicative of a greater susceptibility of the white beans. One experiment was conducted mainly to determine the amount of abnormal seedlings resulting from mechanical injury inflicted in addition to injury that might have been received during threshing and handling. These beans were commercial samples. In the other experiment the purpose was to investigate whether varying levels of humidity to which the seed was exposed before the injury was inflicted would affect the results. In this instance the beans had been grown in Experiment Station plots and were hand-shelled. The greater susceptibility of the white beans was shown in both experiments whereas the assumption that low relative humidity might be a contributing factor in threshing injury could not be confirmed under the conditions of the study.

Field plantings of black and white varieties further

confirmed the assumption that not one single cause could be responsible for the generally poorer field germination and emergence of white beans but that a variety of causes contributed to the final results. The experiment under discussion indicates that hidden injuries as inflicted by average commercial handling may play a minor role. This can be concluded from the fact that random samples did not show poorer germination than selected uninjured seed; unless it is assumed that no internal injury was present in the material used, this result would support the view that internal damage did not lower emergence noticeably. There is a seeming contradiction between this result and the one obtained in the experiment in which seed was shaken at different levels of humidity. The only obvious explanation that could be given for this discrepancy may be the difference in the impact; while the experimental shaking deliberately used a rather drastic method, commercial threshing and handling evidently avoids procedures that are liable to damage seed. While this may not have been the case at the time when the previously cited Asgrow study (3) was conducted conditions may have improved since then.

It has been mentioned initially that preliminary experiments failed to give evidence of an effect exerted by black beans on potentially harmful microorganisms. The following experiments which dealt with the presence of bacteria and fungi on or near seeds which were removed from soil, have not served to substantiate any clear-cut conception in this

respect. In all cases where isolation of organisms was attempted from black and white beans fungi were obtained only from white seeds. Bacteria were isolated from both black and white seeds. Although no pathogens were found in any of these instances the possibility of impairment of germination by the presence of fungi or of bacteria cannot be ruled out. Since white beans showed the occurrence of both fungi and bacteria, the suggestion of a combined action of these organisms is put forth. The absence of fungi in the case of black beans may support the initial assumption of an antibiotic agent in the black seeds. Similar ideas have led Walker and his associates to investigate a possible connection between pigmentation of onions and resistance to smudge in a series of studies which were started more than 25 years ago (65) and are still underway.

For this reason experiments were set up in which extraction of a possible antibiotic agent was attempted. The results were negative. They do not disprove, however, that such antibiotic action exists. It is possible that the antibiotic activity is present only in living tissue. Such investigation, however, was not considered promising at the present moment.

Similarly unsuccessful were the experiments designed to detect a possible inhibitory substance in white beans.

Artificial inoculation of black and white beans, however, provided an interesting confirmation of the earlier observations that contamination occurred almost exclusively

in white beans. These experiments, although carried out with organisms which did not include the common pathogens of beans -- with the exception of X. phaseoli -- again strongly emphasize the possibility that black beans possess some mechanism of resistance.

In summarizing the results of the present study one might therefore state that the fact of poorer germination of white beans as compared with black and, generally speaking, dark-colored varieties, which as first pointed out by Morris (41), has been corroborated in several independent experiments. These experiments tend to emphasize that this difference is due to a number of factors. The occurrence of one, two, or more of these contributing factors would be a logical explanation of smaller or greater differences in germination, emergence, and consequently field stands of white and dark bean varieties.

SUMMARY

1. Bean varieties varied in their ability to absorb moisture both at high and low temperatures. Black seeded varieties absorbed more water than the white varieties at a temperature of 77° F.
2. Soaking bean seeds for varying periods prior to germination resulted in a considerable decrease in germination of the white varieties. Their average germination percentage in all experiments was 57.1 percent, that of black beans tested under identical conditions 96.9 percent of the respective controls.
3. Low temperature treatments of beans after planting in flats resulted in a significant difference of emergence between white and dark colored varieties. The average emergence, in all experiments, of white beans was 24 percent, that of brown beans 62.6 percent, and that of black beans 50.3 percent of the respective controls.
4. Light affected the germination of black beans more adversely than that of white beans. Germination of the black variety Pencil Pod Wax was 22.3 percent at a normal day length of approximately 10 hours, and 55.3 percent at continuous illumination when based on germination of the respective control grown in darkness.

Under identical conditions the white variety Brittle Wax showed germination figures of 61.7 and 95.7 percent.

5. Determination of the pentosan content of the seed-coats of six varieties revealed that the white varieties had a slightly higher percentage than the black varieties.
6. The result of a study of the seed-coats of six varieties was that there is a negative correlation between the thickness of the Malpighian and sub-epidermal layers within the varieties. No such correlation was found when varieties were compared with each other. However, the white varieties had the thinnest Malpighian layer and the thickest sub-epidermal layer.
7. Basic mechanical injury in commercial bean samples was not noticeably different in black and white varieties.

Two experiments in which mechanical injury was induced by shaking indicated that white beans were more susceptible to such treatment than black beans. This was evident in one experiment from germination figures, white beans having one-fourth the germination percentage of black beans. The other experiment showed normal germination in both groups but three times the number of abnormal seedlings in the white group.

In field plantings of random commercial bean samples, selected intact and selected injured beans, emergence of the first two groups was identical within the groups. The white beans in these groups emerged somewhat more

than half as well as the black ones. Emergence of the injured beans was only half that of the other two groups in the white beans; it was slightly higher in the black beans as compared with the other two groups of black varieties.

8. Fungi and bacteria were isolated from beans that had failed to germinate in soil. Fungus isolates were obtained only from white varieties while bacteria were isolated from both white and black seeds.
9. Extracts made from whole beans, seed-coats, or hypocotyls of both black and white varieties failed to show evidence of the presence of an antibiotic principle in in vitro tests against two fungi and nine bacteria.
10. No substance inhibitory to the germination of beans could be isolated from either black or white beans.
11. Black and white beans artificially inoculated with five species of bacteria responded differently to inoculation. Germination of intact black beans was 95.7 percent of that of the respective control, of white beans 86.1 percent of the corresponding control. When damaged seeds were subjected to the same conditions, germination of black beans was 95.9 percent of the control, that of white beans 48.8 percent of the corresponding control.

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**FACTORS CAUSING VARIETAL DIFFERENCES IN GERMINATION
OF THE COMMON BEAN (PHASEOLUS VULGARIS)**

By

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AN ABSTRACT

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Some of the basic reasons for varietal differences in the germination of the common bean (Phaseolus vulgaris) have been investigated. Poorer germination of white beans as compared with black and colored varieties first observed by Morris (41) have been corroborated in several independent experiments.

The influence of moisture, soaking of seeds, cold treatments, chemical composition and structure of the seed-coats, in relation to germination were studied. Mechanical injury in commercial seed and the influence of different levels of relative humidity on the amount of injury incurred in black and white varieties were investigated. The influence of selection on emergence of field planted beans was shown.

Experiments determining the presence of antibiotic substances in the seed-coats of a black variety proved negative. Similarly unsuccessful were experiments designed to detect a possible inhibiting substance in white beans. Artificial inoculation of bean seeds with bacteria showed that contamination was limited to white varieties. Differences in germination, emergence and field stands of different varieties are attributed to a number of factors acting singly or in combination.