NOVEMBER, 1950 RESEARCH BULLETIN 466

UNIVERSITY OF MISSOURI COLLEGE OF AGRICULTURE

AGRICULTURAL EXPERIMENT STATION

J. H. LONGWELL, *Director*

SOME FACTORS INFLUENCING POD SET AND YIELD OF THE LIMA BEAN

Victor N. Lambeth

(Publication authorized November 14, 1950)

COLUMBIA, MISSOURI

TABLE OF CONTENTS

SOME FACTORS INFLUENCING POD SET AND YIELD OF THE LIMA BEAN

Victor N. Lambeth

INTRODUCTION

The problem of securing consistent, satisfactory yields in varieties of the large-seeded lima type is one of great practical importance to the vegetable industry. Even in sections possessing ideal climatic conditions the yields are uncertain. Low yields are always associated with profuse dropping of the buds, flowers, and developing pods. It has not been established whether the environmental conditions concurrent with this abscission affect pod set directly through interruption of the fertilization mechanism or by affecting other internal physiological processes within the plant.

Most of the earlier investigations considered the inconsistent yields solely from the standpoint of unfavorable weather with little consideration being given to the physiological responses of the plant, and of the specific plant characteristics involved in the breeding and adaptation of new varieties. Only recently, investigators have focused attention on and have made considerable progress in correlating the differential fruiting responses with specific physiological characteristics of the plant.

In spite of increased interest in the physiology of plant reproduction in recent years, research of this nature is yet far from adequate in providing the plant breeder with the specific information required in controlled breeding of types which are adapted to exacting environmental conditions. Obtaining varieties and strains better adapted to adverse environmental conditions is then a problem of finding breeding stock carrying germplasm expressing the necessary specific physiological characteristics.

The southeastern section of Missouri has for many years produced annually several thousand acres of lima beans, principally the small-seeded varieties, for canning. The problem now arises as to whether the more finicky large-seeded varieties can be made to produce satisfactory yields in this section, and if so, what cultural practices are to be recommended.

REVIEW OF LITERATURE

Great advances have been made in plant improvement through breeding and selection. One of the major problems still remaining with many of our economic plants is unfruitfulness. The frequency of fruit failing to set suggests that the underlying causes are numerous and complex.

One of the most comprehensive reviews on unfruitfulness of cultivated fruits was given by Gardner, Bradford, and Hooker (31)* who classified unfruitfulness as associated with various internal and external factors. In addition to the internal factors mentioned in this review, it has now been established that certain other physiological factors such as internal nutritive conditions, hormone effects, and chemical composition influence fruitfulness by affecting the differentiation of flower primordia.

In contradistinction to general unfruitfulness, sterility is important in any economic seed-producing plant. Self-sterility, as herein referred to, is restricted to cases where viable seed is produced. A rather complete survey on self-sterility in plants for the period up to 1929 was given by East (26). This survey has very recently been supplemented by the work of Giles (32).

Fruiting in Relation to Certain Climatic Factors

Temperature and Humidity.—The low yields of the lima bean, particularly the large-seeded type, have long been correlated with excessive dropping of the flowers and young pods. This difficulty has, furthermore, most frequently been attributed to high temperature, low relative humidity, and soil moisture deficiencies.

Shaw and Sherwin (72) in 1911 and Hendry (39) in 1918 pointed out the climatic factors favoring pod set of the lima bean in the coastal area of southern California. These authors emphasized the great value of the night and early morning fog drifting in from the Pacific in "lessening the evaporation" and in "tempering the atmosphere." They observed that the vines grew vigorously and produced blossoms in profusion, but yield was poor when the humidity was low. If no fogs occurred for a week or ten days during the blossoming period, the small pods just forming abscissed without making seed.

According to Cordner (22), blossom abscission of the Henderson Bush lima is associated with high air temperature and dry atmosphere. However, he states that the detrimental effect of these conditions will depend upon the time of occurrence and the duration of adverse weather. That the critical period for pod set is the early part of the blooming period was further substantiated by Blinkley (10) who worked with the snap bean.

From extensive experiments covering a period of several years Andrews (3) showed that with high temperatures and low relative humidity the plants fail to set pods and mature a normal crop of seed, but that under such conditions the Henderson (a small-seeded type) produces a greater yield than does the Fordhook (a large-seeded type). However, when grown under relatively cool or moderate temperatures and relatively high humidity, the Fordhook often out-yields the Henderson. Andrews suggested, therefore, that the inconsistent fruiting habit of the Fordhook is due to its adaptation to a relatively narrow range of weather conditions particularly during the

*See list of references beginning on page 57.

fruiting period. He further implied that this adaptation is best explained by the larger root system and the more efficient leaves of the Henderson variety.

In a study of the effect of some environmental factors on the set of pods and yield of white pea beans (*Phaseolus vulgaris L.*) in Michigan, Davis (24) concluded that maximum temperature influences the set of pods more than any other of the factors studied and that the per cent set of pods can be predicted from maximum temperature with a fair degree of accuracy.

The length of the growing season also has been stressed as an important factor which influences the yield of lima beans. Allard and Zaumeyer (2) pointed out that there is considerable difference among the lima types as to the length of time required between planting and maturity of the pods. Whereas under field conditions the earliest bush varieties of the lima bean assemblage cannot mature sooner than 62 to 65 days, the earliest dwarf bush forms of Phaseolus vulgaris will mature in 45 to 50 days. The pole varieties of lima beans are as much as 15 to 20 days later than the latest pole varieties of Phaseolus vulgaris.

Light.—In a study to determine the cause of the lack of uniformity in maturity of the Henderson bush lima, Havis (37) demonstrated the importance of light upon the fruiting habit. He showed that if the plants are shaded for a few days in the critical stage about 23 to 25 days after seedling emergence, there is an elongation of the seventh and eighth internodes with a consequent twining of the main axis of the plant. The effect upon fruiting habit is to speed the maturity of the pods developing from the terminal inflorescence.

The importance of light in regulating the phototropic orientation of plant leaves has been described by Lundegardh (48).

Andrews (3) showed this phototropic orientation to be a factor in the adaptation of certain lima bean varieties to varying light conditions. He found that the Henderson bush lima (a small-seeded variety) was adapted to a wider variation in light intensity than Fordhook (a large-seeded variety) for two reasons. First, with an open type of plant, light was able to penetrate to and uniformly illuminate most of the leaves of the plant; secondly, due to greater phototropic orientation, the outer leaves were partially protected from extremely intense sunlight.

Subsequent to the important discovery of photoperiodism in plants, the varietal relationship to photoperiod effects in the genus *Phaseolus* has been summarized by Allard and Zaumeyer (2). They reported that considerable variability of response to definite photoperiod exists within the genus *Phaseolus*. Most beans of the species *Phaseolus vulgaris L.* and *Phaseolus lunatus L.* are day-neutral. This particular behavior allows them to be grown over a great range of latitudes, wherever other conditions are suitable.

Another effect of photoperiod is that of determining whether a variety or species is bushy or twining in its growth behavior. The pole varieties of Phaseolus *vulgaris* were found to be twining and the bush varieties were bushy regardless of length of day but some of the semipole varieties became bushy on the shorter photoperiods and twining on the longer ones. Most of the lima beans had a rather fixed growth form regardless of the length of the photoperiod, but some of the bush sorts showed tendencies to develop the indeterminate habit of growth as the photoperiods were lengthened.

The characteristic of dwarf habit of growth of the lima bean is in part a response to certain conditions of environment. Bailey (6) in 1895 stated that the Burpee bush lima variety, normally behaving as a very dwarf sort and showing little tendency to climb in the field, developed an excessive vine habit, with stems becoming 5 to 7 feet in height when it was forced under glass in wintertime.

Edaphic Factors in Relation to Pod Set With Particular Reference to Phaseolus

Soil Reaction.—Hester (40) has shown that the acidity of the soil has an important influence on the growth of the lima bean plant. A pH of approximately 5.0 provided the desirable combination of nutrients for the growth of lima beans on the Portsmouth soil type (Virginia) on which the plants were grown.

Fertilizer Treatment.—The bean has proven no exception to other plants, both leguminous and non-leguminous, in the fact that excessive nitrogen fertilization serves to stimulate vegetative growth at the expense of fruitfulness. However, with respect to a species comparison, Thompson (78) states that the fertilizer requirement of the lima bean is similar to that of the snap bean, but it is probable that the lima bean plant can use more nitrogen to advantage.

As would be expected, the results obtained with fertilizer application to increase yield of seed are inconsistent, depending upon various other factors which may become limiting. Davis (24), working in Michigan with the white pea bean (Phaseolus vulgaris L.), found that fertilizer had no effect on the set of pods. He explains the inconsistency of annual response to fertilizer as a result of the limiting influence of high temperature at the blossoming period.

In general as with other crops, the fertilizer placement may require as much consideration as the rate of application and the fertilizer analysis. In a study of the effect of fertilizer placement on snap beans, lima beans, and peas in Virginia, Parker (61) obtained the highest yields by placement of the fertilizer in bands, two inches to each side and two inches below the level of the seed at the time of planting.

In a study of the effect of split applications of nitrogen and phosphorus on the yield of the large seeded lima bean, Emmert (28) found that double drilling of 500 pounds of 20 per cent superphosphate at planting time and before pod set gave the best results of any method of application. Furthermore, he found that the only highly significant increase for nitrogen on lima beans was when the split application was used. Applications broadcast after pod set gave high plot responses.

Comparative studies in New York by White-Stevens and Hartman (89) over a three-year period on the effect of fertilizer placement and spacing upon yield of the Fordhook Bush Lima bean show that there was no advantage from applying the fertilizer in bands on either side of the seed row, either at planting or after "come-up" over the usual broadcast application, where the same analysis and amount of fertilizer was employed throughout.

Yields were increased by increasing the concentration in the row of seed at planting up to two beans per 9 inches or one bean per 4½ inches which were equal in yield.

The work of Tiedjens and Schermerhorn (79) on fertilizer requirements for lima beans in New Jersey indicates that the Fordhook variety has a relatively high requirement for nitrogen—higher than the small-seeded varieties. They obtained a large increase in yield of Fordhook by increasing the nitrogen from 32 to 90 and 160 pounds per acre. However, there was no increase in yield of Jackson Wonder and Henderson from increasing the nitrogen above 32 pounds per acre.

Boron application as a soil treatment has frequently been reported to have increased seed yield of several economic species. Thus Grizzard and Matthews (34) reported that an application of borax (15 pounds per acre) to Cecil sandy loam soil at Chatham, Virginia in 1941, increased the yield of alfalfa hay from 289 to 743 pounds per acre and resulted in yields of 82-184 pounds of seed per acre, whereas the plants on the plots receiving no borax failed to set any seed.

That boron treatments in the form of field applications may increase the dry seed yield of the lima bean is shown by the work of Wester and Magruder (86). They found that the addition of 5, 10, and 15 pounds of H_3BO_3 per acre to Elsinboro sandy loam soil in Maryland during the 1940 season one week before blooming caused a significant increase in the yield of dry lima bean seed from a large-seeded bush lima bean selection No. 23-6-3. No significant increase in the total fresh weight of the plant was obtained. The H_3BO_3 was diluted with sand and applied to triplicate plots 25 x 30 feet arranged randomly in each block, by spreading the sand-H_3BO_3 mixture on both sides of each row 8-10 inches from the plants. Control, 5, 10, and 15 pounds per acre of H_3BO_3 produced a mean yield of dry seed per plot of 1860, 2034, 2236, and 2629 grams, respectively.

Radspinner (64) and Smith (75) reported that blossom drop in the tomato is greatly increased by low soil moisture as well as by hot dry winds and low humidity. A lag of approximately three days was found between the time the temperature exerts an effect and the time that the effect becomes visible.

Cochran (20) associated the dropping of buds, blossoms, and immature fruits of peppers with unfavorable water relations due to an insufficient water supply to the plant, or to excessive water loss as a result of high temperature, low humidity, excessive transpiration, or low soil moisture.

Davis (24), working with the white pea bean in Michigan, found that the set of pods was not associated to any appreciable degree with soil moisture changes. However, he pointed out that this data was applicable within rather narrow limits of soil moisture as the amount of moisture in the soil did not reach a critical point either from the standpoint of an excess or a too-limited supply.

Maximov and Zernova (54), in a study of the stomatal activity of plants grown with and without irrigation, showed that the stomata of non-irrigated plants opened but little and only early in the morning while stomata of plants with a sufficient moisture supply were fully open throughout the day. From this study they proposed that the degree of opening of the stomata during the day could be used as an index of the amount of water available to the plant.

In summarizing one phase of his work, Andrews (3) stated that recent studies indicate that one of the chief reasons for the greater dropping of blossoms and pods and the inconsistent yield of the Fordhook may be attributed to its relatively small root system in proportion to top. The Henderson and other high-yielding varieties possessed more numerous, and more extensive roots and a greater root-top ratio, obtained more water from the soil, maintained greater leaf turgor and more open stomates, and therefore maintained a higher rate of photosynthesis. As the evaporative power of the air increased and available soil water decreased (during drought) particularly during the blossoming period, the extent of the root system became increasingly important as indicated by stomatal movement and pod drop.

Plant Spacing.—Considerable attention has been given to the problem of correct plant spacing in the commercial production of the lima bean. While there is some evidence (52, 89) that close planting within the row is associated with high yields, other work (49) is contradictory. However, these reports indicate that the plant spacing factor may be more important with the large seeded sorts than with the small-seeded varieties. Obviously, the weather conditions prevailing during the growing season are important factors in plant spacing. Thus, Odland, Larson, and Li-Paang Fi (60) report on inverse relationship between spacing within the row and the total yields per acre obtained in good weather for the Fordhook 242 and Thorogreen varieties on three different soil types in different counties of Pennsylvania.

Crop Rotations.—In many respects, the principles which apply to crop rotations with other economic agricultural crops should also apply to the lima bean. However, according to Smith, Holland, and MacGillivray (73) long continuous cropping of lima beans on fertile soil does not result in a decrease in yield. They pointed out that after forty-five years of continuous cropping with lima beans on the same soil in the lima bean area of California, the yield has not declined. The explanation offered is that the available nitrogen supply is replenished by the action of the lima bean nodules, providing, of course, that the other essential elements are present in abundance.

Morphological Characteristics in Relation to Reproduction

Leaf Area, Leaf Structure and Stomatal Frequency.—Numerous investigators including Zalenski (94), Yapp (93), Timmerman (80), Salisbury (66), and Smith (74) have studied the leaf size, structure, and position on the plant in relation to the physiological responses of the plant. As a result of this work it has been established in general that shaded leaves are thinner, less dense in structure, and have a lower stomatal frequency per unit area than sun leaves.

Working with the tomato, Gustafson and Stoldt (35) found that the efficiency of the plant in setting fruit is highest when the leaf area per fruit is small. After setting, however, the size of the fruit is increased by a larger leaf area.

With respect to the relationship in the lima bean, Cordner (22) found a direct correlation between the size of the plant of the Henderson variety and the yields.

Andrews (3) in a comparison of the morphological and physiological plant characteristics of large and small lima types and from results of defoliation and depodding experiments, concluded that the products of photosynthesis frequently, and probably usually limited pod set after the first two weeks of blossoming and thereafter the development and final yield of pods. However, the relation did not hold true for the early pod set, during which time pod set was governed more by water relations than by leaf area or the products of photosynthesis. Likewise, in cases of crowding and overvegetativeness of the large-lima type, pod set and yield were inversely proportional to leaf area. In addition, by means of shading experiments, Andrews demonstrated that an open type of plant (such as the Henderson variety) was capable of protective phototropic orientation in intense sunlight but at the same time allowed light to penetrate to the inner leaves of the plant, and thereby provided for a wider adaptation with respect to light.

In a recent paper Davis (24) concluded that the correlation between leaf area and yield of the white pea bean is not constant, and may be either positive or negative depending upon the weather conditions at the time of pod formation.

Size and Distribution of the Root System.—According to Pearsall (63), root weight is proportional to top weight for any species under constant environmental conditions. However, Weaver and Read (84), and Crist and Stout (23) showed that the growth rate of roots relative to tops can be modified by edaphic factors, temperature, light, and other environmental factors which influence the rate of top growth.

Several investigators, including Maximov (53), have stressed the desirability of an extensive root system as a means of drought escape.

Andrews (3) established the root-top ratio as an index of the adaptability of certain lima bean varieties to various ecological conditions. He attributed the inconsistent yields of varieties of the large lima type under unfavorable environmental conditions to an unfavorable root-top ratio. Under conditions where moisture supply to the plant is limiting, the cells are flaccid and the stomates closed. He further found these conditions to be favorable to a lowered photosynthetic rate.

Other Morphological Factors.—Katz (43) working with various flowering plants such as petunia and snapdragon, observed that under natural conditions the stigmatic fluid is indispensable to successful pollination, its function being protection of the pistil and pollen from dessication rather than any chemical stimulus. Through its content of oil the stigmatic fluid decreases transpiration and holds the necessary moisture for the growth of the pollen tube. Secretion was stimulated by light and heat. Where secretion was inhibited by darkness and low temperature, no pollen germination occurred on the dry stigma. Removal of the stigma did not prevent pollination provided secretion was present on the cut surface of the style. In many species almond oil was found a satisfactory substitute for stigmatic fluid when placed on the stigma, but in artificial cultures it was found necessary to have water present with the oil to insure pollen development. Tubes in this artificial medium were short and quickly flattened at the growing tip. A piece of stigma of the same species added to the oil culture increased germination in most cases.

The course of development of the embryo sac and of the embryo in Phaseolus vulgaris has been described in detail by Brown (16).

Giles (32) in a critical study of the morphological aspects of self-sterility in Lotus corniculatus L., demonstrated the presence of a stigmatic film which acted as a barrier to cross- and self-pollination. He further describes the detailed anatomical features of the floral organs which play an important role in the pollination mechanism. The structure of the keel in this species favors autogamy by making the stigma receptive by causing rupture of the stigmatic film and by stimulating secretion of stigmatic fluid.

Internal Physiological Factors

Vegetative-Reproductive Relationships.—Investigators of the relationship existing between vegetative growth and reproduction in plants have generally concerned themselves with the effect of various internal factors on fruit bud differentiation. This review, however, is limited to certain relationships found subsequent to fruit bud differentiation, since an abundance of flowers is normally produced on the lima bean plant.

In general, fruit set and development of seed and fruit have been found to retard vegetative growth and may also limit further reproductive activity. Murneek (57) pointed out a case of intermittent sterility in the spider flower as a direct effect of fruit set. Periodicity of setting of fruit has also been reported in other plants (18). The bean plant has been shown to be no exception in this respect. Cordner (22) found that bean pods on the base of racemes reduced fruit set toward the apical end and that apical set was increased by basal defloration. Furthermore, he found that early blooming racemes were more fruitful than later blooming ones, and stressed the importance of securing an early set. He states that fruit setting follows until a "capacity set" is attained; the remaining reproductive structures are disposed of by abscission.

McCollum (5) noted the inhibitory influence of growing fruits was not characteristic of parthenocarpic fruits. He believed this inhibitory effect to be due to growth regulating substances produced by the fertilized ovules.

Austin (4), in a study of the effects of defloration on the metabolism of the soybean plant, found that growth stopped about the time the fruits developed. However, vegetative development was not increased by defloration, as growth ceased simultaneously in deflorated and control plants.

In an attempt to correlate pod set with the chemical composition of the plant extract, Wolf (92) compared the concentration of various mineral elements in the tissue of poorly-set plants with that of well-set plants. He found that well-set plants had substantially higher concentrations of nitrate nitrogen, potassium, available calcium, and magnesium but less available phosphorus than poorly-set plants. The concentration of nitrate and of available calcium in the main stems were closely associated with seed set. In general, a nitrate concentration of 2,250 to 4,000 parts per million and a calcium concentration above 9,000 parts per million in the main stems were associated with good set. Considering both soil and plant tests, it was shown that plants containing a good supply of calcium and grown on soil with a fair to good supply of organic matter had a good set in all cases.

Andrews (3), in a comparison of the chlorophyll content of the leaves of Henderson and Fordhook varieties, found the concentration of chlorophyll, expressed on both fresh weight and leaf area basis, of the Henderson leaves after the early seeding stage to be consistently higher than that of the Fordhook. While the chlorophyll content may have some bearing on the efficiency of light utilization, the physiological effect on pod set is not clearly established.

Effects of Growth-Regulating Substances on Ovary and Seed Development.—There now exists an abundance of literature pertaining to the effect of growth-regulating substances on fruit development. In recent years the use of the synthetic growth-regulating substances has become widespread in effecting "set" on several economic plants. This review is limited largely to the specific effects on ovary and seed development in a relatively few plant species.

The time of application of the growth-regulating substances has been found to be very important. In general, application one or two days prior to pollination has resulted in the production of seedless or partially seeded fruits and reduced yields (14, 19, 82, 65) whereas application at the time of pollination or shortly thereafter, results in parthenocarpic development with increased yield but fewer seed in a number of plant species (41, 17, 38).

The effect on ovary and seed development also varies with the species, even within the genus Phaseolus. Application of growth-regulating substances as aqueous sprays and dusts to green bush bean plants after the flowers begin to open often increase the yield of pods, apparently by reducing flower bud, flower, and pod drop and by stimulating pod development (30, 58, 91). The average number of seeds per pod, however, is less in treated pods than in untreated pods, indicating a depressive effect on seed formation.

It is evident from the preceding statements that the application of growth-promoting substances cannot be expected to increase the seed yield of a plant unless it prevents abscission of the flower buds, flowers, or immature pods. In no instance can these substances substitute for fertilization but they may favor fertilization indirectly by retarding abscission. Another indirect beneficial effect is exemplified in the case of self-sterile plants of Petunia. Eyster (29) reported that self-sterile plants of Petunia produced viable seed following the application of a solution of alpha-naphthalene acetamide at a concentration of ten parts per million to the open flowers. He believed that the growth substance neutralized the inhibitory action of an ovarian secretion responsible for retarding pollen tube growth in the style.

Since excessive dropping of blossoms and small pods of the lima bean is often directly responsible for reduced yields, several investigators have tried growth-promoting substances on limas with the hope of delaying abscission and increasing yield (36, 87, 88, 91). However, in only one instance was the yield of bean seed reported increased by hormone treatment, and in this case a very unique technique of application was employed. Wester and Marth (88) reported that when the base of the lima bean flower was scratched with a dissecting needle and the growth-regulator mixture (indolebutyric and parachlorophenoxyacetic acids) in lanolin paste was then pressed into this area, the growth regulator entered the flower and the pedicel and affected their development. The majority of abscissed treated flowers remained attached 2 to 3 days longer than abscissed untreated flowers. Under the conditions of these experiments, the growth regulator treatment caused a significant increase in pedicel diameter of mature pods with an increase in the number of successful crosses from 18.7 per cent to 28.8 per cent. It also caused a significant increase in the average number of seed per pod, from 1.95 to 2.43, and doubled the number of seeds per pollination. A procedure employing this technique would, of course, be impractical in commercial lima bean production.

In a recent paper, Whiting and Murray (90) pointed out that the availability of a source of auxin or growth substance is associated with the delay in abscission. They suggested that inasmuch as anti-auxinic activity has been attributed to 2, 3, 5-triiodobenzoic acid and this substance induces abscission in bean plants that reduction in auxin levels may be associated with the induction of abscission.

Pollen Germination

A comprehensive review of the literature on pollen germination revealed very little work on the germination of Leguminosae pollen until the eighteenth century. However, it is to be noted that even the earliest references attested to the specificity of bean pollen to very exacting germination requirements.

Sandsten (68), working with numerous species reported germination in sucrose, glucose, lactose, levulose, maltose, dextrin, glycerine, olive oil, gum, and decoctions of styles and stigmas. However, he found the rubber bean to be very specific in its germination requirements as it germinated successfully only in olive oil. The optimum medium for pollen germination was found to vary with the species and each species was quite specific in its optimum.

Although many techniques have been developed for the study of pollen viability and pollen tube growth in vitro, no reference was found to work in which consistent germination has been obtained with lima bean pollen.

Andrews (3), after trying various media by several different techniques, concluded that lima bean pollen requires a certain stage of maturity before removal from the blossom if germination occurs at all. No germination was found by the end of 48 hours where the pollen was taken from unopened white blossoms.

Jacob (42) made a very extensive study of artificial germination of lima bean pollen, using combinations of several varieties, media, and techniques. Although he did not in any case report entirely satisfactory germination, the best results were obtained with a 50 per cent honey and water solution using the hanging drop technique in a van Tieghem cell. Light (photoperiod) had no significant effect on pollen germination. Jacob further stressed the importance of accurate temperature control in these germination experiments.

It has been accepted by most workers that the physical properties of the basal medium, particularly the moisture supply, is a very important factor in pollen germination. Thus Martin (50), after many unsuccessful attempts to germinate the pollen of Trifolium pratense in different sugar and acid solutions, discovered that this pollen germinated readily on moistened hog bladder or parchment paper. Consequently, he concluded that pollen germination in this case was delicately adjusted to water absorption and that the stigma functioned only to control the water supply.

The intricate and extremely variable growth responses of pollen has been described by several workers of pollen physiology. In his extensive investigations, Brink (11, 13) found that germination and growth of the pollen of many species occurred on widely different concentrations of sugar, the tubes apparently being permeable to sugar solutions. Sugar was suggested as the source of carbon for growing tubes, the stored material in the grain being too small to account for the volume of the tube and the contents. Inorganic nitrogen supplied in the form of soluble salts was found to check the growth of tubes. Food stored in Vinca minor pollen was in the form of fats. As the tube elongated this material was carried forward in the tip region.

In many cases it has been found necessary to add stimulants to the basal medium to obtain satisfactory pollen germination; in other cases the results with stimulants are either negative or inconclusive. The most common and effective stimulant added by the earlier workers was either the gynoecium or an extract of the gynoecium of the same plant. Brink (12) found that the addition of raw potato juice and extracts of gynoecium parts to artificial media regularly increases the growth of pollen tubes. He attributed the bursting of tubes in artificial cultures to osmotic action. Pollen tube growth was markedly depressed in the presence of small amounts of various inorganic salts or when sea water is added to the culture medium in concentrations as low as 12 per cent. Moreover, he attributed the difference in form of the growth curves of pollen tubes growing in vitro and in vivo to be the result of the difference in the water relations in the two cases. Increased growth on culturing pollen on yeast and in yeast free media is interpreted as the result of the more complete utilization by the groups of some diffusible growth-promoting substance or substances. Tips of pollen tubes cut off from the older portions by callose plugs were found capable of independent growth.

The chemotropic effect of stigmas and other flower parts on the growth of pollen was observed by Molisch (56) as early as 1893. Subsequent investigations have shown many cases of similar responses, although they are not universal. Brink (12) noted that growth of pollen tubes is enhanced by clustering of the grains. This grouping he observed with many species; in fact, none of those tried failed to respond in this way. Brink reported, furthermore, that germination is quite frequently much more profuse when the grains are grouped closely together.

There is little information available concerning the effect of weak concentrations of growth-regulating substances on the development of the male gametophyte. High concentrations, however, appear to arrest development and prevent pollen shedding (33, 81).

Several workers have reported stimulatory effects upon germination of mature pollen grains when these substances were added to the media. Addicott (1) and Smith (76, 77) presented data which indicated that pollen germination on sugar agar medium was favored by the addition of growth promoting substances. Concentrations of indole-3-acetic, indole-butyric and naphthalene-acetic acids weaker than 20 ppm. were favorably simulative, but all stronger concentrations were toxic, as indicated by decreased germination, bursting and distortion of tubes.

In addition to the growth promoting substances, pollen germination is often favored by the addition of inorganic salts, especially of the minor elements. However, as compared to the growth-promoting substances, the action of these salts appears to be more specific. Of the trace elements, boron has most frequently been reported as affecting pollen viability both in vivio and in vitro.

Babko and Tserling (5) found the concentration of boron to be higher in the stigma and pollen. Application of boron in the form of H_3BO_3 and borax in the hanging drops of sucrose resulted in a considerable increase of pollen germination with many species. The highest percentage germination occurred in the .001% solution of boron. Other minor elements tried were less effective than boron. The pollen of red clover plants fertilized with boron showed under field conditions increased germination as compared with the pollen of plants which had not received such treatment. The treatment of plants with boron fertilizer resulted in a greater effect upon the yield of their seed than of their vegetative mass.

Blaha and Schmidt (9) report a great stimulatory effect of boron on the germination of pollen in fruit trees. Sugar media plus 0.0005% of borax is said to have increased the percentage germination of pollen of cherries and pears several thousand times on some occasions. Furthermore, the pollen tubes were found to be much longer in the boron media.

A possible explanation of the way by which boron increases the yield of seed plants may be revealed by the work of Schmucher (71) on the physiology of pollen germination. He found in general that concentrations of H_3BO_3 between 0.001% and 0.01% were favorable for increased germination percentage or pollen tube growth, generally both. The author inferred that boron in some manner provides better water relationships and prevents bursting of the pollen tubes during germination.

Much of the variation in pollen growth can apparently be eliminated by careful selection of techniques and by giving special attention to the selection of pollen. Although many of the physiological pollen studies have previously been made by the hanging-drop technique (12, 83, 21, 76) later experiments show that much better results can be obtained with the sugar-agar technique of Eigsti (27).

In order to eliminate variations in the physiological response of pollen from different flowers and from different anthers of the same flower in studying the effect of test substances in the media, Smith (77) used only a single flower and combined the pollen from all the anthers of this flower to form a mixture from which the pollen in a test series was taken. In this manner, consistent germination responses were obtained.

Some Applications of Phenology to Agriculture

The science of phenology has important practical applications to agriculture since it provides a thorough understanding of the degrees of harmony between climate and the vegetative rhythms of plants and enables the grower to better control the phasic development of the plants and to shift the "critical periods" (van de Sande-Bakhuyzen) (67) of plant development to a time when better climatic conditions prevail.

Although the climates of certain areas have their optimum and erratic periods, it is possible to establish an average sequence of climatic phenomena —the so-called phenological mean. This phenological mean places on a statistical basis the probabilities of the availability of the ecological factors such as moisture, temperature, and light at given intervals throughout the year. Of most importance, agriculturally speaking, are the months of the growing season, especially that portion in which economic seed-producing plants blossom.

In a general way, Werneek (85) suggests the application of phenology to aid agriculture in the following problems:

(1) in avoiding unfavorable or erratic climatic periods by shifting the planting date (and hence the blossoming date)
(2) in conserving a limiting factor by the initiation of special cropping systems
(3) in providing a basis for variety adaptation to existing climatic conditions.

THE PROBLEM FOR INVESTIGATION

The objectives in this investigation were as follows:

(1) to determine more specifically the morphological and physiological factors associated with pod set in the lima bean with particular reference to the newer varieties of the large-seeded type.
(2) to compare adaptation to controlled environmental factors of newer variety introductions with the long-established varieties.
(3) to find some practical treatment contributing to satisfactory, consistent yields of large-seeded lima bean varieties.
(4) to apply available climatological data to culture practices in growing lima beans in Southeastern Missouri.

To accomplish these objectives an intensive investigation of the morphological and physiological characteristics of representative lima varieties was required together with a consideration of their interactions with climatic factors. In order to comprehend more completely the problems in pod set in the genus Phaseolus, it was desirable that some work be done with the bush snap bean.

MATERIALS AND METHODS

General

During the two-year period of this investigation, several varieties of lima beans were grown both in greenhouse culture and under field conditions. In this manner floral abscission and pod set was observed over a wide range of climatic and edaphic conditions. The external factors included in this study were temperature, relative humidity, light intensity, soil fertility, and available soil moisture.

Field studies were made at the Vegetable Research Farm, Campbell, Missouri and at the South Farms and Midway Farms at Columbia, Missouri. This provided a wide range of soil types and diverse climatic conditions.

The field work was carefully correlated with controlled experiments in the greenhouse ranges. For the controlled temperature studies, a specifically designed greenhouse was used. This equipment has been described in detail by Brown (15). In general, it consists of a small greenhouse whose longitudinal axis lies east and west, and which contains three thermo-regulated growth chambers designed to operate simultaneously and provided with independent control of air and soil temperatures. The temperature control consisted of maintaining constant air temperatures around the culture vessels. Maximum variations in temperature of ±3° F. from the desired values occurred due to the mechanical limitations of the equipment. However, these fluctuations were always of very short duration. Natural light was admitted to the plants in the usual manner, and the light conditions were fully comparable in the three chambers. No supplemental light was applied.

The temperature and humidity measurements for the individual experiments were secured from Friez recording hygro-thermographs placed at the level at which the plants were growing both in the greenhouse and in the field. The hygrometers were calibrated by comparison with readings of a sling psychrometer. Continuous readings of the available soil moisture were obtained by the use of soil moisture tensiometers purchased from a reputable irrigation supply company. An official Weather Bureau pyrheliometer located in the greenhouse area furnished continuous light intensity records.

Commercial seed stock was used in all experiments with the exception of the controlled temperature experiments. In this case pure line seed was obtained from Dr. R. E. Wester of the United States Department of Agriculture.

Histological Technique

The material for examination and sectioning was killed, fixed, and stored in FAA (formaldehyde, acetic acid, ethyl alcohol).

The preparation of temporary mounts for examination of the young pods for pollen tube growth and fertilization was facilitated by sectioning longitudinally with a freezing microtome.

For the more detailed studies and for the preparation of permanent slide mounts, the pistils were embedded in paraffin. Dehydration for embedding was accomplished by a graded series of alcohol solutions. After dehydrating, the material was run up in absolute alcohol-chloroform mixtures to pure chloroform and embedded in Tissuemat (mp. 52-54° C.), employing the method described by Saas (69). Longitudinal sections, 16 microns in thickness, were cut on a rotary microtome and mounted on clean slides. These sections were then stained with lacmoid-martius yellow stain by the technique described by Nebel (59). Slight modifications in technique, however, were required to adapt the method to the material being studied.

Lacmoid was made up in a one per cent aqueous solution and martius yellow in one-half per cent aqueous solution. To prevent chemical breakdown the solutions were kept separate in tightly stoppered bottles and fresh mixtures were prepared each time immediately before use. The stain was mixed by filling a staining jar approximately four-fifths full with martius yellow solution and adding lacmoid solution until a dark green shade developed.

After removing the paraffin with two changes of xylol, the slides were run down through an alcohol series to tap water, stained in the lacmoid-martius yellow mixture for 2 to 3 minutes, destained with tap water, and mounted in distilled water. The pollen tube walls were stained yellow and the callose plugs were stained a brilliant blue. Observations were made of pollen tube growth in the lower style and in the ovarian tissue and a written record kept of these observations for the various treatments.

The study of fertilization and early embryo development in these same sections necessitated a second staining technique. After the observations described above were completed in the water mounts, the cover glasses were removed by standing the slides in jars of tap water. After destaining completely with a 50 per cent alcohol solution and washing with tap water the sections were stained with Delafield's haematoxylin and safranin.

The slides were dipped in and out of the haematoxylin stain for approximately one minute, washed in a large volume of tap water (pH approximately 8.0) and placed in 50 per cent alcohol for 5 minutes. From alcohol the sections were counterstained with safranin, destained in 95 per cent alcohol, washed with absolute alcohol, cleared in carbolxylol and mounted in balsam.

EXPERIMENTAL RESULTS

Field Experiments

Correlations of Pod Set with Environmental Factors Tendergreen Snap Bean, Campbell, Mo., 1949.—Green snap beans of the Tendergreen variety were grown during the summer of 1949 on a Lintonia fine sandy loam at the Vegetable Research Farm at Campbell, Mo. The fertilizer application was made on the basis of the degree of base saturation as previously determined by chemical tests on several representative soil samples. For this particular experiment the only nutrient varied was nitrogen. The following experimental levels of nitrogen were established: 100, 200, and 400 pounds per acre. Since the soil tested 20 pounds of nitrogen per acre, 80, 180, and 380 pounds of actual nitrogen was added (per acre basis) as NH_4NO_3 (33⅓%) to establish these levels. A randomized block arrangement was used in the experimental design, the plots (35' x 14') appearing in replica five times for each of the three nitrogen treatments. The plots in all three nitrogen treatments received 80 pounds of nitrogen as a plow-down application. In addition, the 200 pound nitrogen level received three applications of 33⅓ pounds nitrogen, and the 400 pound nitrogen level three applications of 100 pounds nitrogen as side dressings on the following dates: June 20, July 7, and July 25, 1949. Other fertilizers were added to give an experimental level of 265 pound P_2O_5 per acre, and to provide saturation levels of the other principal nutrients as follows: calcium 70.7 per cent, magnesium 8.8 per cent, and potassium 5.4 per cent. Sulfur was applied with the magnesium as magnesium sulfate. Minor elements were added in the form of Es-Min-El at the rate of 50 pounds per acre.

In order to determine accurately the number of flowers produced and the set of pods, a daily record was kept of the development of each flower for 40 plants in each treatment. In collecting this experimental data, the individual plant records were always taken from one of the three center rows with a check row to either side in order to minimize the possibility of fertilizer effects from adjoining plots. The task of record keeping was facilitated by the use of jeweler's tags to mark the flowering racemes on the individual plant and also by a chart record of each flower on each receme. By such a procedure it was possible to determine whether a flower had set or dropped by the third day after anthesis. This record was maintained throughout most of the blossoming period (July 3 to July 21).

From the data taken the following correlations were made on a statistical basis: percentage set at the three nitrogen levels with mean daily temperature and with mean daily relative humidity. In addition, anthesis of the flowers was determined for the three nitrogen levels for the recorded flowering period. This data is shown in Tables 1 and 2.

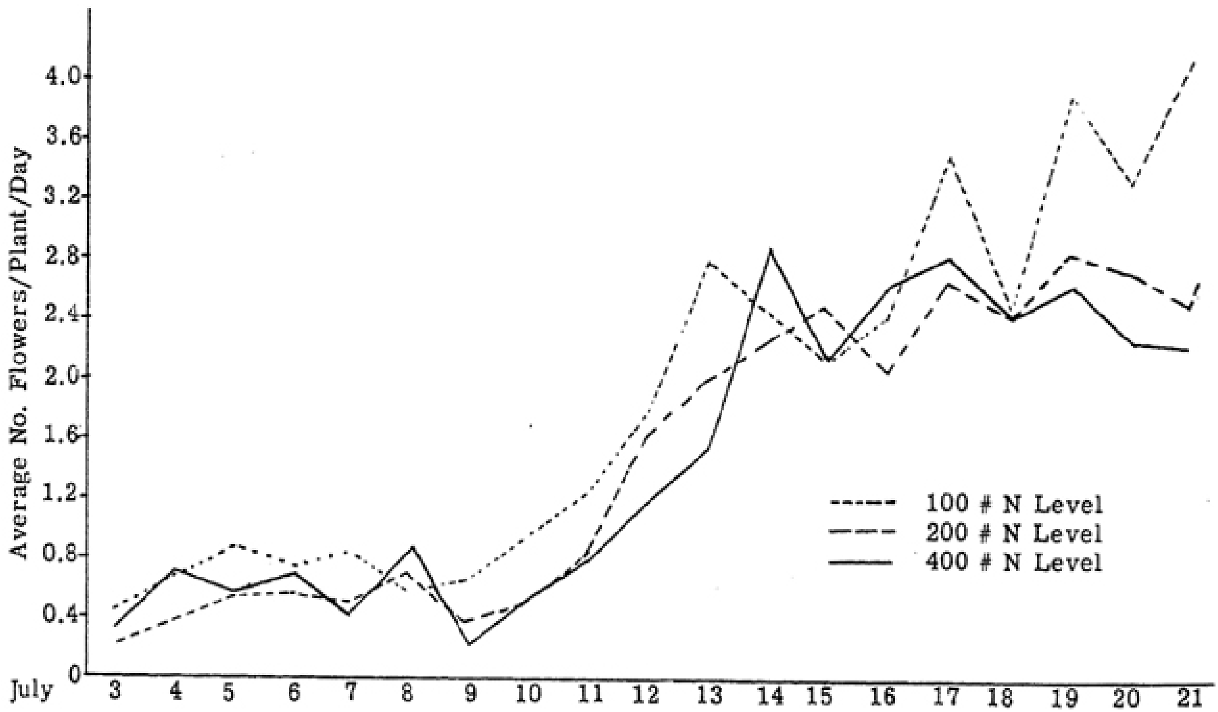
Discussion of Results.—Correlation of pod set in the snap bean with air temperature and relative humidity. The correlation coefficients calculated between per cent set of pods and mean temperature, per cent set of pods and mean relative humidity, are reported in Table 2. The mean temperature was taken for the 24-hour period following anthesis of the flower. Mean

TABLE 1--DAILY ANTHESIS OF FLOWERS AND PERCENTAGE POD SET OF THE TENDERGREEN SNAP BEAN IN RELATION TO ATMOSPHERIC TEMPERATURE AND RELATIVE HUMIDITY. VEGETABLE RESEARCH FARM, CAMPBELL, MO., 1949

Date July 1949	Days from Planting	Mean Air Temp. (°F.)	Mean Relative Humidity (%)	Average No. of Flowers per Plant Opening on			Per Cent of Total Flowers Setting Pods		
				100#N	200#N	400#N	100#N	200#N	400#N
7/3/49	27	----	----	0.48	0.24	0.36	----	----	----
7/4/49	28	81.6	70.6	0.68	0.40	0.72	----	----	----
7/5/49	29	80.5	79.2	0.88	0.56	0.60	25.0	----	----
7/6/49	30	77.3	91.1	0.76	0.60	0.72	31.0	50.0	40.0
7/7/49	31	77.6	89.0	0.84	0.52	0.48	20.0	22.2	30.7
7/8/49	32	79.0	86.2	0.60	0.72	0.88	15.0	6.2	<1
7/9/49	33	84.8	75.5	0.68	0.40	0.24	15.5	14.3	7.7
7/10/49	34	79.4	85.0	0.96	0.52	0.56	11.7	33.3	<1
7/11/49	35	77.7	86.6	1.24	0.84	0.80	13.6	33.3	22.2
7/12/49	36	79.8	80.1	1.80	1.64	1.20	4.0	<1	<1
7/13/49	37	79.4	81.0	2.80	2.04	1.56	13.6	<1	7.1
7/14/49	38	83.0	No Record	2.48	2.28	2.92	11.1	3.5	<1
7/15/49	39	78.0	82.5	2.16	2.52	2.16	9.6	15.0	2.2
7/16/49	40	75.0	86.0	2.44	2.08	2.64	37.9	47.5	20.0
7/17/49	41	76.0	83.1	3.52	2.68	2.80	40.0	39.3	17.6
7/18/49	42	75.7	83.5	2.52	2.44	2.44	34.6	42.8	39.2
7/19/49	43	76.5	87.0	3.88	2.88	2.64	49.3	53.5	45.4
7/20/49	44	81.2	79.0	3.36	2.76	2.28	23.6	23.0	30.8
7/21/49	45	81.2	74.5	4.12	2.56	2.24	30.6	44.7	44.3

TABLE 2--CORRELATION COEFFICIENTS CALCULATED BETWEEN PER CENT SET OF PODS AND MEAN TEMPERATURE, PER CENT SET OF PODS AND MEAN RELATIVE HUMIDITY, OF THE TENDERGREEN BUSH BEAN, VEGETABLE RESEARCH FARM, CAMPBELL, MO., 1949

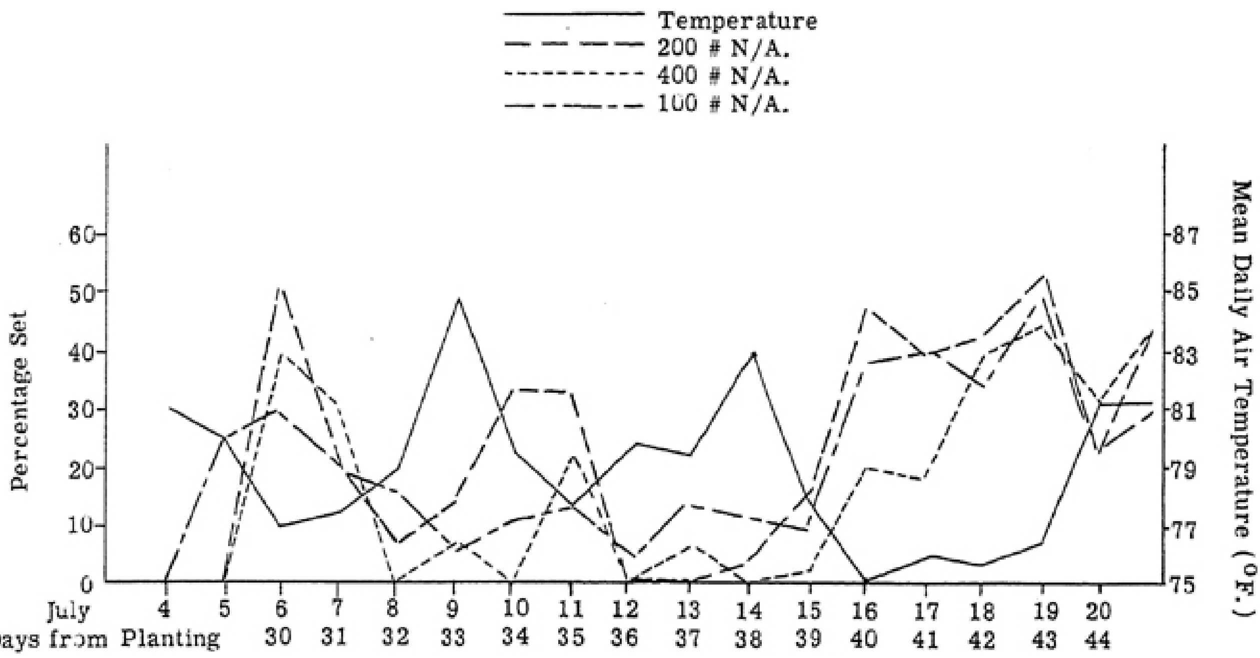
Item	r Mean Daily Temperature	r Mean Daily Relative Humidity
Per cent Set of Pods 100#/A N level	-0.6241**	0.2799
d.f.	15	15
Per cent Set of Pods 200#/ A N level	-0.5728*	0.3290
d.f.	14	14
Per cent Set of Pods 400#/A. N. level	-0.3699	0.1645
d.f.	14	14
r values required for significance	p* = .05	p** = .01
n-2 = 14	.497	.623
n-2 = 15	.482	.606



Graph 1. Daily anthesis flowers Tendergreen bush green bean. Campbell vegetable research farm, summer 1949.

temperature rather than the maximum temperature was used on the assumption that the mean represented a better index of the intensity heat factor and therefore of the physiological reactions of the plant providing, of course, that critical temperatures were not reached. The results would appear more striking had the maximum temperature been used.

The data in Table 1 show a significant relation between the per cent



Graph 2. Pod set Tendergreen bush green bean at three nitrogen levels in relation to air temperature. Campbell, Mo., July 3-July 21, 1949.

set of pods and the mean daily temperature. It appears that when the mean temperature for the 24-hour period following anthesis is above 78° F., the pod set is materially reduced. A mean temperature of 78° F. usually represents several daylight hours in which the air temperature is greater than 90° F. This inverse relationship between temperature and pod set is clearly shown in Graph 2. The relationship varied also with the nitrogen level, being significantly different only at the 100 and 200 pound per acre nitrogen levels. Furthermore, significance for the lower nitrogen level is at the 1% level as compared to the 5% level for the medium nitrogen level. The failure to correlate with temperature at the high nitrogen level is more adequately explained in the next section of the dissertation in which an immediate detrimental effect on pod set is shown by side-dressing applications at the highest nitrogen level. It may be that this detrimental effect masks the effect of high temperature.

The data shows no significant correlation between pod set and the mean daily relative humidity. However, it should be realized that the humidity during this period was in a favorable range (70-91%) and was probably in no case limiting pod set.

From a comparison of anthesis at the three nitrogen levels, it is apparent that more flowers opened at the 100 pound nitrogen level than for the other levels. As seen in Graph 1, this tendency increased as the blossoming season advanced. Small variations in anthesis rate occurred, however, at the higher nitrogen levels. Most rapid flowering for this period commenced about 38 days from seeding date at which time the average number of flowers opening per plant per day was 2.48 for the 100 pound nitrogen level, 2.28 for the 200 pound nitrogen level, and 2.92 for the 400 pound nitrogen level. Although the anthesis rate did not increase at the 200 pound and 400 pound nitrogen levels, the rate remained practically constant for the remainder of the blossoming period for which records are available. There appeared to be little effect on the anthesis rate as a result of side dressing with the nitrogen fertilizer.

Pod Set of the Snap Bean in Relation to Nitrogen Fertilization.—The results in Table 1 show there was as much as 8 per cent difference in the percentage pod set for the three nitrogen treatments. The average daily percentage set for that portion of the blossoming period recorded (approximately 3 weeks) was as follows: 100 pound nitrogen level 22.7 per cent, 200 pound nitrogen level 26.9 per cent, and 19.4 per cent for the 400 pound nitrogen level. The most favorable level for these experimental conditions was the 200 pound nitrogen level, half of which was applied as a side dressing in three different applications. The final side dressing application was not made until July 25, 1949 which is after the termination of the flowering records. The additional nitrogen furnished by the 400 pound nitrogen level showed a detrimental effect on pod set as compared to the other levels. This detrimental effect was very serious for a period approximately one week following the side dressing applications. The only side dressing application during this part of the blossoming period was made on July 7, 1949 at which time the average daily percentage set was approximately 35 per cent. The average daily percentage set for the following week dropped to approximately five. This injurious effect on pod set can most likely be attributed to a high concentration of nitrogeneous substances in the plant extracts. These results emphasize the importance of timely application in fertilizer practice.

Henderson and Fordhook 242 Bush Limas, South Farms, Columbia, Mo., 1949.—During the summer of 1949, lima beans of the Henderson and Fordhook 242 varieties were grown at the South Farms, University of Missouri, Columbia, Mo., for the purpose of correlating pod set of the two varieties with specific climatic factors, namely, air temperature and relative humidity. The beans were grown on a Lindley silt loam soil which had been cropped the previous year with soybeans. Fertilizer treatment consisted of drilling into the surface after spring plowing a fertilizer mixture which provided per acre 200 pounds NH_4NO_3, 160 pounds KCl (60%), and 320 pounds of a 6-18-6 analysis fertilizer. The beans were planted on May 14, 1949 in 42 inch rows at a heavy rate of seeding to provide a good stand. Upon reaching a height of 8-10 inches, the plants were thinned to give a uniform spacing of 6 inches within the row. At the beginning of the blossoming stage 25 representative plants of each variety were chosen from which to collect data. A daily record of the floral development and pod set was kept by the method described earlier for the snap bean. Continuous air

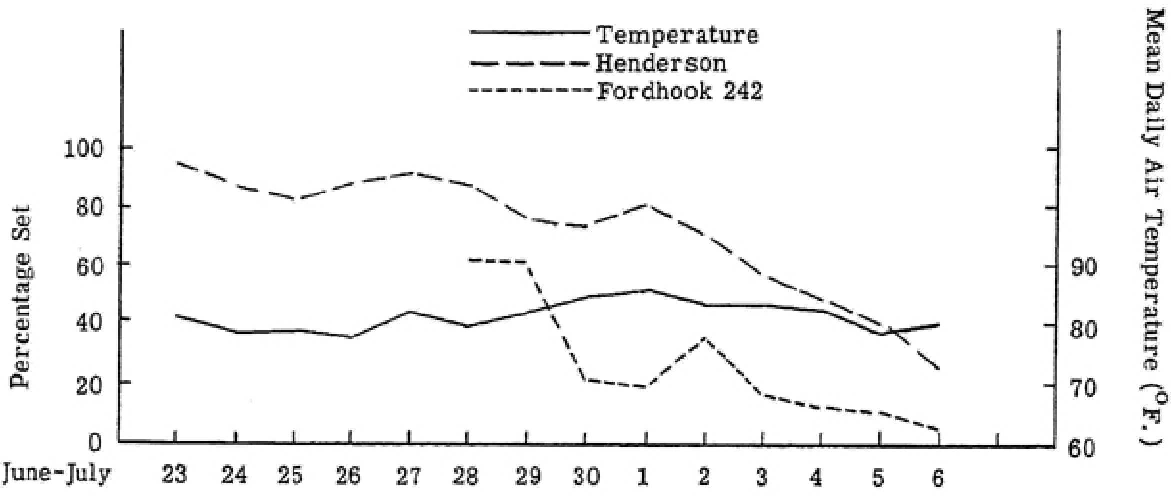
TABLE 3--DAILY ANTHESIS OF FLOWERS AND PERCENTAGE POD SET OF HENDERSON'S AND FORDHOOK 242 BUSH LIMA IN RELATION TO ATMOSPHERIC TEMPERATURE AND RELATIVE HUMIDITY. SOUTH FARMS, COLUMBIA, MO., 1949

Date	Days from Planting	Mean Air Temp. (Degrees F.)	Mean Relative Humidity (%)	Average No. of Flowers per Plant Opening on		Per Cent of Total Flowers Setting Pods	
				Henderson	Fordhook 242	Henderson	Fordhook 242
June 23	40	81	79	0.80	----	95.0	----
24	41	79	81	1.56	----	87.1	----
25	42	79	87	0.68	----	82.3	----
26	43	78	83	2.60	0.44	87.6	27.2
27	44	82	81	2.84	0.40	91.5	30.0
28	45	80	79	6.36	1.04	87.4	16.5
29	46	82	80	6.35	2.52	75.9	61.9
30	47	85	76	7.44	2.04	66.6	21.5
July 1	48	86	71	12.48	3.56	80.7	19.1
2	49	84	71	8.52	3.56	70.4	35.9
3	50	84	74	15.04	7.08	56.1	16.3
4	51	83	78	17.96	8.12	48.9	12.8
5	52	79	89	15.96	8.24	39.8	10.1
6	53	80	84	15.28	6.52	25.6	6.1
7	54	81	86	13.64	7.52	41.3	7.9
8	55	81	89	----	6.44	----	11.1
Mean for blossoming period				8.5	4.42	69.08	21.26

temperature and relative humidity readings were obtained by use of a Friez recording hygro-thermograph placed in the field at the level at which the plants were growing. The sunshine readings were obtained from the Weather Bureau located at Columbia, Mo. Light intensity readings were also made comparing full exposure with that obtained in the plant interior. The instrument used in this case was a Weston Illumination Meter, Model 603.

A comparison was made of the percentage set of the Henderson and Fordhook 242 varieties under the conditions of the experiment, and an attempt was made to correlate set with air temperature and relative humidity (Table 3).

Discussion of Results.—Daily pod set in relation to certain climatic factors. Upon the basic assumption that abscission of blossoms and subsequent poor set is associated with high temperature and low relative humidity, the daily percentage pod set was compared with the mean daily values for these climatic factors. As is readily noted from a comparison of Tables 1 and 3 or Graphs 2 and 3, the degree of correlation is poor in comparison



Graph 3. Pod set of Henderson's and Fordhook 242 bush limas in relation to air temperature. Columbia, Mo., June 23-July 6, 1949.

to that obtained in the Tendergreen snap bean experiment previously cited. The poor correlation obtained does not necessarily discount the effects of temperature and relative humidity, since there was a very narrow range of these factors during the period of the experiment. Furthermore, the lima bean is generally regarded as being able to set satisfactorily at considerable higher temperatures than the snap bean. Yet it is apparent from previous work that these values for air temperature and relative humidity are favorable for pod set. These facts would lead one to believe that the extremely low set for the Fordhook 242 variety must, in this case, be attributed to other factors of the environmental complex which became limiting during the blossoming period and for which this variety is particularly sensitive. Low light intensity may have been a contributing factor to low set since dull cloudy skies and frequent rains characterized much of the weather during the blossoming period. According to the Official United States Weather Bureau records, the average percentage of possible sunshine for the area during this period was only 65.5. The effect of low light intensity was accentuated by the vigorous vegetative condition of the plants, very likely stimulated by the abundant nitrogen supply. Practically all of the pods which were set on the Fordhook 242 variety occurred on the terminal racemes. This characteristic contrasts sharply with the usual high basal pod set for the variety. That this light relationship also varies greatly with the plant type is shown by a comparison of light penetration to the plant interior for the two varieties. (Table 4.) In general, the light intensity readings on the plant interior were approximately 1/80 of the full exposure value for the Fordhook 242 variety and ¼ of this value in the case of the Henderson variety. The Fordhook 242 plant being larger in size and more compact, with larger leaves and shorter petioles, allows more shading of the inner and lower leaves by the outer and upper leaves. Furthermore, the leaf arrangement of Fordhook 242 is usually opposite whereas that of the Henderson is usually alternate. These

TABLE 4--A COMPARISON OF THE LIGHT INTENSITY READINGS ON THE INTERIOR OF THE HENDERSON AND FORDHOOK 242 BUSH LIMA BEAN PLANT

Light Intensity (Foot-Candles)
(Average of four separate determinations)

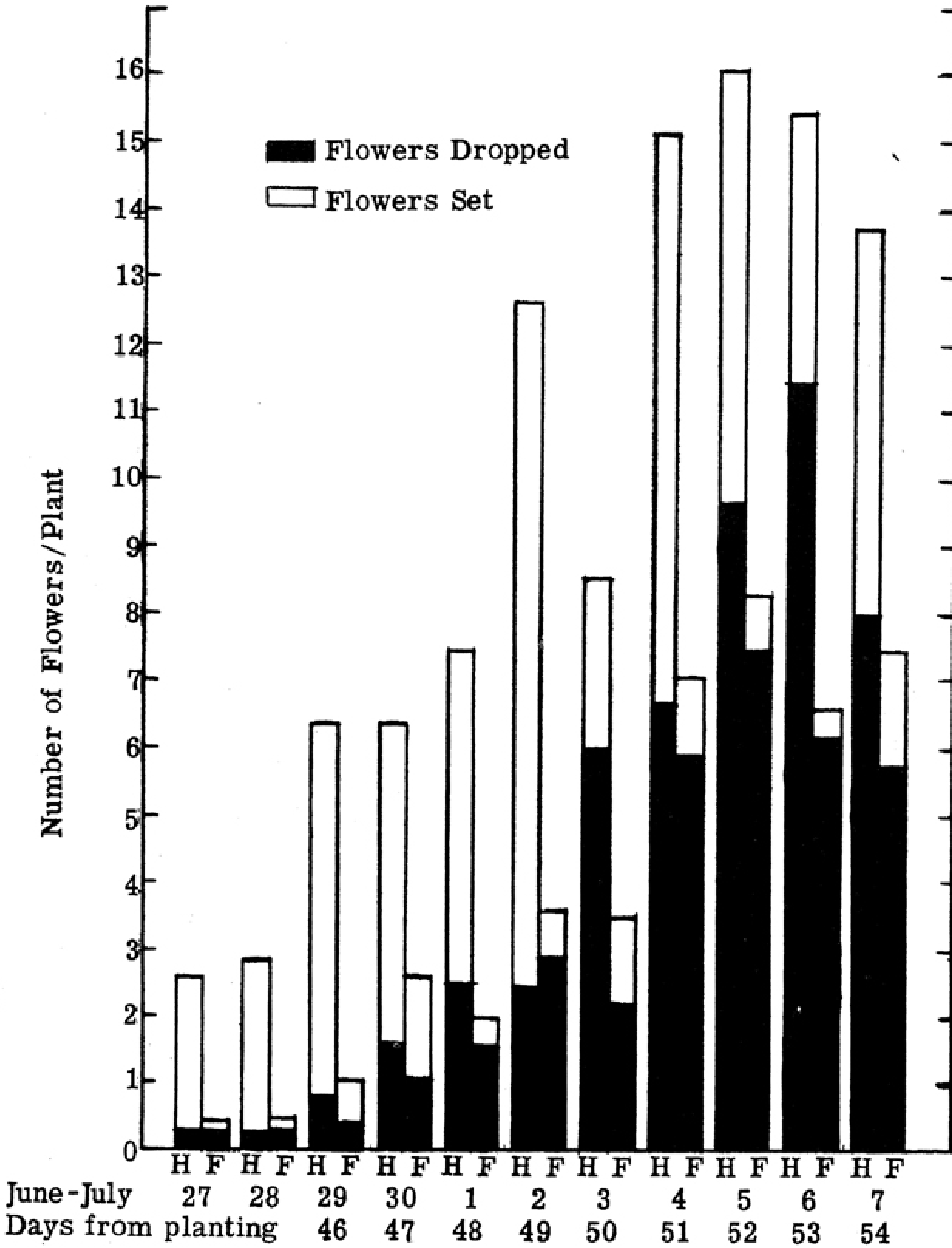
Full Light Exposure	In Plant Interior Henderson	Fordhook 242
360	50	08
800	80	10
1000	290	10
1100	400	12
1200	430	16

plant characteristics result in a more open type of plant for the Henderson variety and permit greater light penetration and air movement to the plant interior.

The mode or modes by which the physiological significance of plant type may be manifested can only be mentioned in a general way as no conclusive evidence was given by this experiment. Low light intensities, particularly to the plant interior, could severely limit the photosynthetic activity of the inner shaded leaves perhaps even to the point where they would be saprophytic. This factor could affect the reproductive capacity indirectly through an effect on the supply of photosynthetic products. With poor metabolic efficiency of the leaves under these conditions, there exists insufficient food reserves to adequately supply both the growing points and the flowers.

Closely associated with low light intensity in compact plants is the restricted air movement to the plant interior. This limited air movement favors the maintenance of a high relative humidity around the basal blossoms during rainy periods and could conceivably interfere with pollen germination and proper fertilization of the ovules. Limited air movement may have been a contributing factor to poor set in this experiment. A careful histological study showed that practically without exception the pollen on dropped blossoms was ungerminated. In many cases residues of pollen grains were apparent, indicating perhaps that the pollen grains had burst prior to or in the process of germination. The critical osmotic requirements of lima bean pollen will be considered in greater detail in connection with pollen germination experiments in artificial media.

Differential Response of the Henderson and Fordhook 242 to Climatic Factors. It is very evident from Table 3 that there was a marked differential response of the two varieties in both flowering and pod set during the course of the experiment. Plants of the Henderson variety consistently produced more flowers throughout the blossoming period than did the Fordhook 242 variety, the daily averages for the recorded blooming period being, respectively, 8.50 and 4.42 flowers per plant. This response can not be explained simply on the basis of a shorter maturity requirement for the Henderson's Bush lima since the flowering peak was reached for both varieties on the fifty-first and fifty-second days from seeding (Graph 4). The Henderson variety



Graph 4. A comparison of anthesis, pod set, and blossom drop for Henderson's and Fordhook 242 bush limas. Columbia, Mo., June 27-July 7, 1949.

was, however, about 10 days earlier in flowering than the Fordhook 242 variety. There can be no doubt that more blossoms are normally produced by both varieties than can be developed into mature seed-bearing pods. Therefore, the problem is obviously not one of insufficient flower bud differen-

Figure 1. Lima bean plants of Fordhook 242 variety (left) and Henderson variety (right), stripped of foliage, and showing extent of pod set for the two plant types. Note also barrenness of the terminal portions of the racemes on Henderson.

tiation. Still more striking differences between the two varieties are apparent in the percentage set (Fig. 1). The percentage of the total flowers setting pods for the two week period was 69 for Henderson and only 21 for Fordhook 242. Furthermore, the daily per cent set was in every case significantly greater for the Henderson variety than for the Fordhook 242 variety.

The fact that the percentage set of the Henderson variety (and to a lesser degree the Fordhook 242 variety) continually decreased as the blossoming period progressed is not difficult to understand. This response is characteristic in many fruiting plants with prolonged fruiting seasons. It is primarily for this reason that most of the experimental work on pod set was performed on plants at the initial stages of flowering. From a practical standpoint, growers consider basal pod set as the most important.

Greenhouse Experiments

Controlled Temperature Studies in Thermo-Regulated Growth Chambers. —In order to isolate the specific influence of temperature from other factors of the environmental complex and better determine its effect on pod set, carefully controlled temperature experiments were conducted in the greenhouses during the fall and winter of 1949 and the spring of 1950. In all the temperature studies, representative plants of the Fordhook and Fordhook 242 varieties were transferred upon reaching the embryonic bud stage to the thermo-regulated growth chambers (Fig. 2) described earlier in the section Materials and Methods. The Fordhook and Fordhook 242 bush limas were selected as being standard, widely-grown varieties of the large, thick-seeded lima type which, in general, has a narrow "margin of safety" in its pod-setting characteristics.

Several series of plants of both varieties were grown. Other than in planting date, each series differed from the others only in the size and type of the containers. This difference in containers permitted greater variation and better control over the available soil moisture, which was also a part of the investigation. The soil mixture for all series consisted of equal parts of loam soil, sand, and leaf mold to which was added sufficient fertilizer (8-16-8 analysis) to promote good growth. All the plants were grown at the same temperature prior to their transfer to the thermo-regulated growth chambers.

Records were kept of blossom drop and pod set and the percentage pod set was calculated for intervals of one week. In order to keep at a minimum the adverse effect of developing pods on subsequent set, the plants were depodded weekly.

A critical histological study was made in an attempt to provide a concrete explanation for differential pod set for the various experimental conditions and for any observed differential response of the two varieties to pod set with identical experimental conditions. This study included bud and flower developmental stages in relation to pollen maturity, germination of pollen on the stigmatic surface, growth of pollen tubes in the style and ovary following pollination, and the early stages of embryonic development following fertilization. A time schedule was developed for the successive stages at several constant temperature levels.

Figure 2. The thermo-regulated growth chambers in which the controlled temperature studies were conducted.

Series I.—This experimental series included 6 plants of each variety grown in five inch porous clay pots and transferred to thermo-regulated chambers on October 25, 1949. The chambers were set to maintain constant temperatures of 62° F., 72° F., and 86° F. No satisfactory method of controlling the humidity was found; however, the variation at these temperature levels was quite low ranging from 45 to 65 per cent during the day and 55 to 70 per cent during the night. Records were taken for the following weekly intervals: October 25 to November 1, November 5 to November 12, and November 12 to November 19.

Series II.—Eight plants of each variety were grown in six inch clay pots which were imbedded in gravel-filled one-gallon crocks. The water supply to the plant roots was accurately controlled at the desired levels by varying the depth at which the clay pots were embedded in the gravel and by varying the water level in the gravel medium. The holes in the bottom of the clay pots were stoppered to prevent root growth downward into the gravel medium. Transfer to the thermo-regulated chambers was made on November 23. Two of the chambers were set for a constant temperature of 86° F.; the third chamber was set at 72° F. Two different soil moisture levels were maintained in the 86° F. chambers as follows:

Medium available soil moisture (Tensiometer reading 50-60)

Very low available soil moisture (Tensiometer reading 68-75)

The latter level was just above the wilting coefficient of the soil.

Records for these plants were taken for the following weekly intervals: November 23 to November 30, December 1 to December 7, December 8 to December 14.

Discussion of Results.—As shown in Table 5, there is a differential varietal response in pod set which is closely correlated to temperature. Over a three-week blossoming period with a constant air temperature of 62° F., the Fordhook 242 plants set pods on 91 per cent of the blossoms as compared to only 37 per cent for regular Fordhook plants. At 72° F., however, the set of Fordhook 242 was but little different from that at 62° F. but the set of the Fordhook plants increased from 37 per cent to 68 per cent.

The percentage set of the two varieties was almost identical at 86° F. As compared to set at 72°, the set at 86° F. for Fordhook was 15% lower and for Fordhook 242 was 34% lower for comparable soil moisture levels. This data indicates that the Fordhook 242 variety is adapted to a wider temperature range than regular Fordhook as indicated by better pod set for these experimental conditions. The latter variety appears to be particularly sensitive to temperatures near the lower limit of the growing range. With increases in temperature above 62° F., set improves and approaches that of Fordhook 242. Set in both varieties is satisfactory (over 50%) even at constant temperatures of 86° F. provided there is abundant available soil moisture. These facts might indicate that the frequent failures of pod set attributed to high temperature and low humidity may more specifically be

TABLE V--A COMPARISON OF ANTHESIS AND POD SET OF FORKHOOK AND FORDHOOK 242 BUSH LIMAS AT CONTROLLED TEMPERATURE AND SOIL MOISTURE LEVELS

Period of Blossoming	62°F. Medium Moisture				72°F. Medium Moisture			
	Fordhook		Fordhook 242		Fordhook		Fordhook 242	
	Total Flowers	Per Cent Set	Total Flowers	Per Cent Set	Total Flowers	Per Cent Set	Total Flowers	Per Cent Set
Series I (6 plants)								
Oct. 25 - Nov. 1	32	21.9	19	89.4	110	78.1	72	87.5
Nov. 5 - Nov. 12	65	44.6	51	86.2	106	60.3	48	89.5
Nov. 12 - Nov. 19	40	45	50	98	92	66.3	51	90.2
Mean		37.1		91.2		68.2		89.0
Series II (8 plants)								
Nov. 23 - Nov. 30	---	----	---	----	---	----	---	----
Dec. 1 - Dec. 7	---	----	---	----	90	73.3	107	73.8
Dec. 8 - Dec. 14	---	----	---	----	61	60.6	59	74.5
Mean						66.9		74.1

Period of Blossoming	86°F. Medium Moisture				86°F. Low Moisture			
	Fordhook		Fordhook 242		Fordhook		Fordhook 242	
	Total Flowers	Per Cent Set	Total Flowers	Per Cent Set	Total Flowers	Per Cent Set	Total Flowers	Per Cent Set
Series I (6 plants)								
Oct. 25 - Nov. 1	---	----	---	----	97	39.1	35	22.9
Nov. 5 - Nov. 12	---	----	---	----	---	----	---	----
Nov. 12 - Nov. 19	---	----	---	----	---	----	---	----
Mean								
Series II (8 plants)								
Nov. 23 - Nov. 30	72	61	100	58	---	----	---	----
Dec. 1 - Dec. 7	120	45	117	50.4	185	26.4	109	28.3
Dec. 8 - Dec. 14	---	----	---	----	173	31.7	142	11.2
Mean		53.0		54.2		29.0		19.7

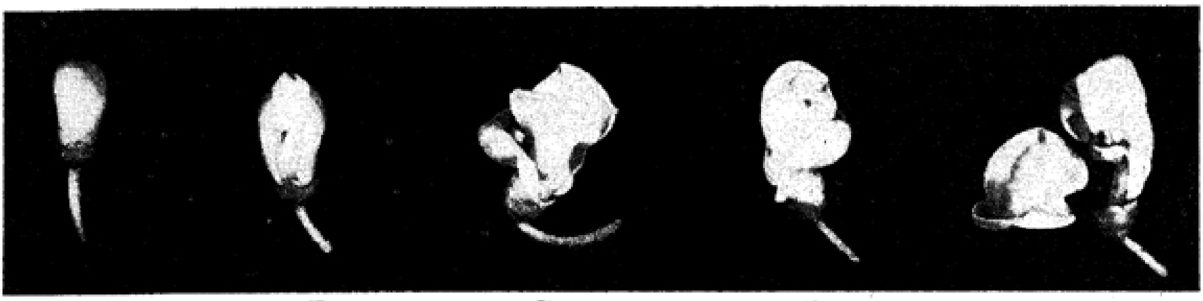
attributed to a low water supply to the plant. Obviously, these factors are normally associated in the field.

The importance of adequate soil moisture in obtaining good pod set was demonstrated first in the Series I plants. In these plants because of the limited soil volume of the five inch pods, it was found very difficult to control a favorable and constant water supply to the plants at the high temperature level (86° F.). Consequently, the moisture level was observed at times to approach the wilting coefficient. With these conditions, entirely comparable for the two varieties, Fordhook set approximately 39% of the blossoms as compared to only 23% for plants of the Fordhook 242 variety. After a period of one week, the soil moisture was allowed to decrease until the plants wilted, and to remain in this condition for approximately 6 hours. Water was then added in sufficient quantities to permit the plants to regain their turgidity. Pod set for these plants for the next two weeks was in all cases less than 5 per cent. Much of this low set may be attributed to floral abscission in the bud stage. These facts emphasize the detrimental effect to pod set when the soil moisture reaches the wilting coefficient even for a short period of time, even though the plants do not remain permanently wilted. Furthermore, this effect was to some degree permanent, persisting for the remainder of the blossoming period. Microscopic examination revealed that these buds and flowers became dessicated prior to their abscission. Apparently, when the soil moisture becomes critically low, water is readily withdrawn from the floral organs thereby initiating a series of reactions which ultimately leads to forced abscission. In the case of young buds not abscissed, the essential organs were permanently injured and failed to function normally.

Additional experimental evidence as to the importance of the available soil moisture is given by the Series II plants (Table 5) under conditions of better control of the soil water supply.

Thus over a two-week flowering period at 86° F. significantly different responses for the two varieties were shown, depending on the level of available soil moisture. The regular Fordhook plants set 53% of the blossoms with average available soil moisture as compared to 29% at a low available soil moisture level. Similarly, plants of the Fordhook 242 variety set 54% and 20% respectively, for the same conditions. The tensiometer reading for "average available soil moisture" ranged from 50-60 as compared to 68-75 for "low available soil moisture." The latter range represents a soil moisture content just above the wilting point of the plant. This data indicates a difference in pod set of 20-35 per cent which can be attributed to a difference in the soil moisture supply at the medium to low range. Under these conditions (high temperature, low soil moisture) the set for the Fordhook plants was approximately 10 per cent greater than that for the Fordhook 242 variety.

Effect of Air Temperature on Rate of Floral and Pod Development.— A better understanding of the specific manner by which temperature affects pod set may be gained by a consideration of the effect of temperature on the rate of the various physiological processes involved; for example, pollen germination, pollen tube growth, fertilization, and post-fertilization development of the pod. An investigation of this type necessarily demands a careful association of these internal changes with the outward appearance of the buds and flowers, and required, in this case, the collection, dissection, and examination of hundreds of flowers at various stages of development. A classification of flower bud stages was developed for the lima bean



A B C D E

Figure 3. Bud and flower stages of the lima bean
A—Close bud enclosing immature pollen grains
B—Loose or hooded bud enclosing mature pollen at or just previous to dehiscence of the anthers.
C—White full open flower (erect standard of Coffman)—pollen on the stigma.
D—Fading whitish-yellow flower, pollen germinating.
E—Yellow flower with corolla dropping, post-fertilization.

which is similar to that described for alfalfa by Coffman (20a). These bud and flower stages are represented in Figure 3.

The stage at which the pollen is mature and ready to be released from the anther can be distinguished by noting the bursting of the calyx and the showing of the white carolla through the slit of the bud (stage B). From this stage the period of time required for the flower to open fully is 12-18 hours at 62° F., as compared to 3-5 hours at 72° F. or 86° F. This variation in anthesis rate with temperature is also shown in Table 6 which gives the average number of flowers opening weekly over a three-week blossoming ing period for the Fordhook and Fordhook 242 varieties at three temperature levels. The values at each temperature are based upon an average of six representative plants of each variety and were taken for the first three weeks of the blossoming period.

Anthesis was very slow at 62° F., the rate being on the average of about one blossom per day per plant for the three-week period. At 72° F. the anthesis rate was approximately twice as rapid as at 62° F. The rate at 86° F., however, was not materially different from that at 72° F. in both plant series.

The fact that more flowers opened on the Fordhook plants than on the Fordhook 242 plants, especially at the higher temperature levels, is explained by a difference in the stage of maturity of the two varieties, the Fordhook 242 plants being a few days later. Soil moisture had little effect on the anthesis rate.

Very marked differences in the rate of the physiological processes are also shown by a comparison of the maximum pod lengths attained in one week's development from the hooded bud stage (Figure 4). Since there was very little varietal difference, the following values are representative for both varieties: 62° F.-1.5 cm.; 72° F.-3 cm.; 86° F.-5 cm. This differential rate of physiological development is also shown on the intact plants in Figure 5.

Morphological and Histological Observations.—Morphological and histo-

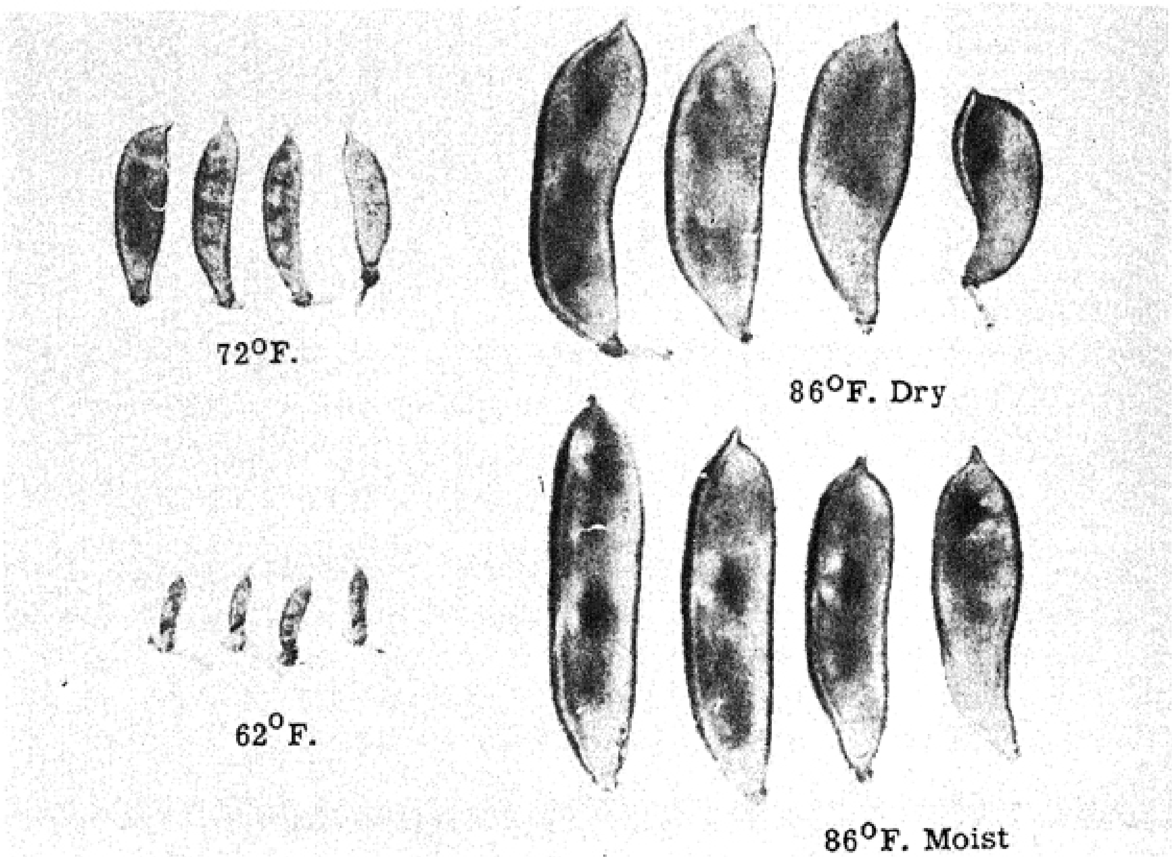


Figure 4. Rate of pod development in relation to air temperature. The photograph shows actual pod size attained in a period of one week from the pollination stage (loose or pointed bud) under the conditions indicated.

logical observations were made a regular and important part of the study because it was believed that structural abnormalities might be revealed which would explain in part the inconsistent and variable performances of the two lima varieties.

Gross Morphology of the Flower.—The lima bean flower represented in Figure 6 was reproduced from the book "Beans of New York." The flower has been described in detail by Hedrick (37a). The calyx is five-lobed, the upper two lobes often connate; the standard is usually more or less orbicular and emarginate; wings are oblong to obovate; the keel is coiled in one or more close turns; stamens are diadelphous 9 and 1; pistils are on a short cup-shaped disk; the style is coiled within the keels, bearded within, the stigma oblique or lateral.

Because of this structure, the flower is normally almost entirely self-pollinated. Mechanical tripping of the flower is not necessary to insure adequate pollination.

Morphology of the Microspores.—The pollen grains of the lima bean are normally about 15 microns in diameter, rounded-triangular in outline, with rather thick walls. The pores present at each of the three angles appear identical morphologically and are apparently all capable of germination. Morphologically speaking, no abnormal anatomical features were noted which

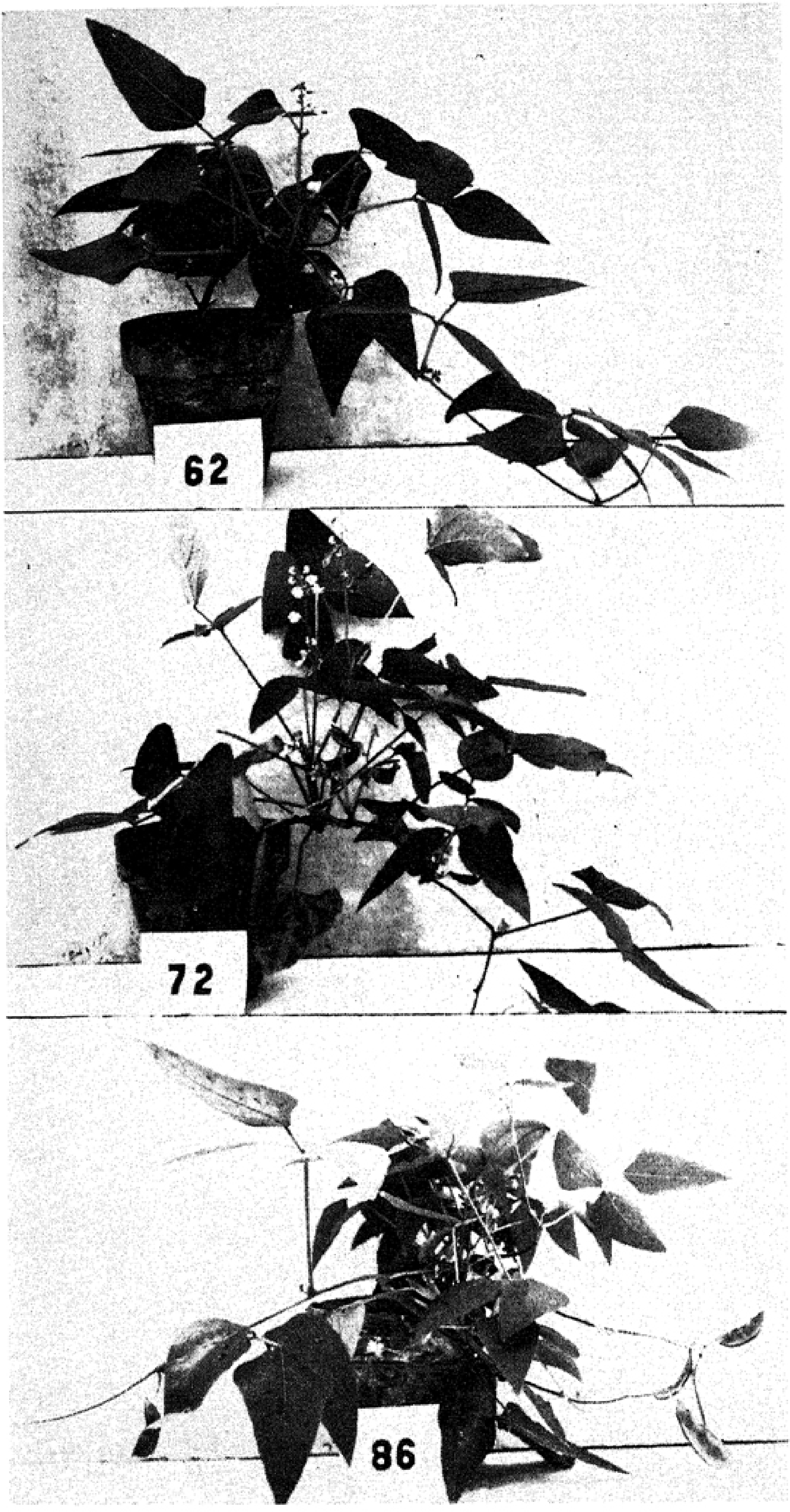


Figure 5. Fordhook 242 bush lima bean plants showing a week's pod development from the hooded bud stage at a controlled temperature of 62° F. (above), 72° F. (middle), and 86° F. (below).

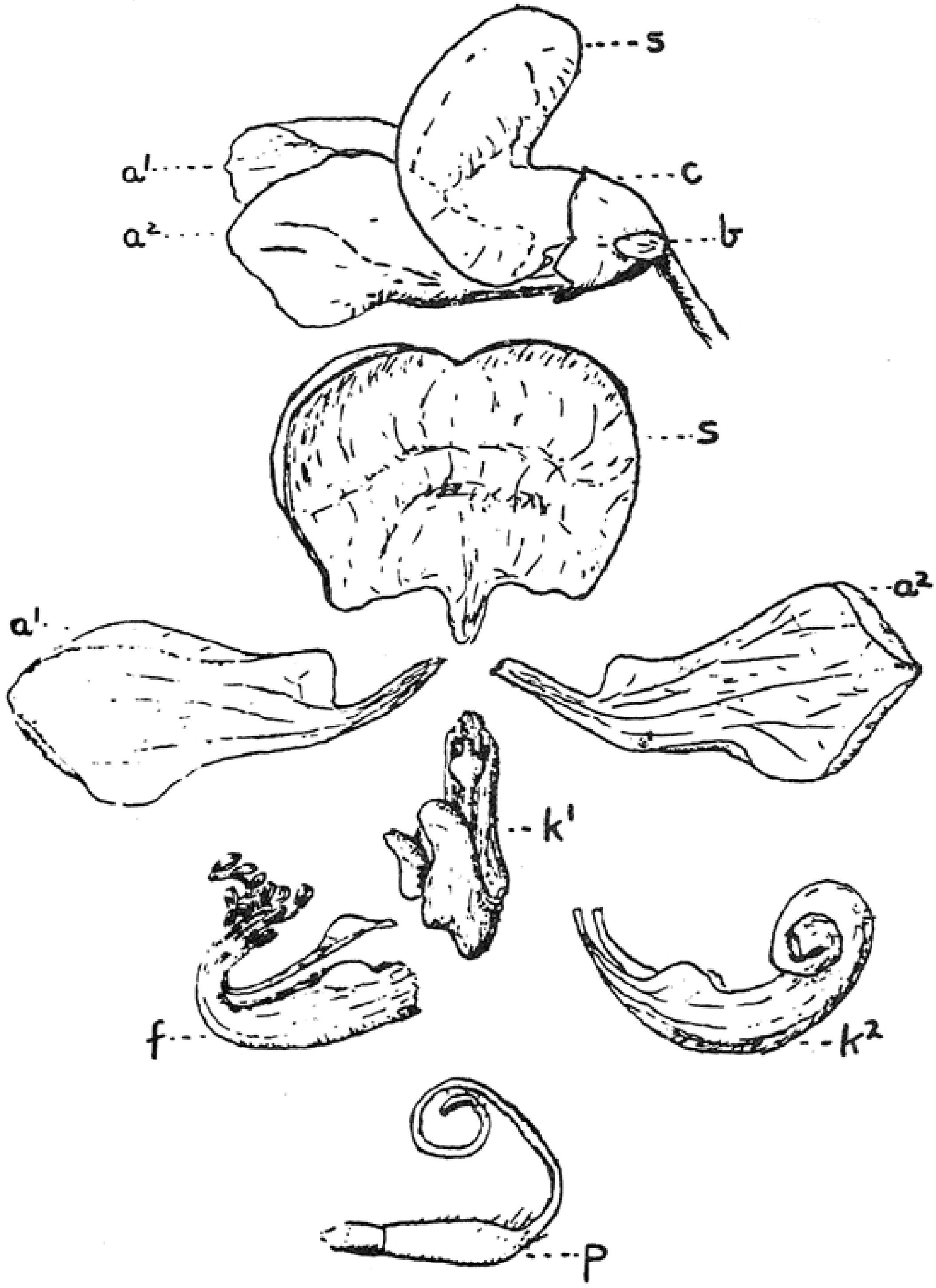


Figure 6. A flower of the lima bean, King of the Garden, (side view above, and dissected to show parts below) b—bracteole. c—calyx. a^1 and a^2—wings or alae. s—standard. k—keel (k^1—with essential organs included: k^2—keel alone). f—stamens, showing filament tube and free stamen. p—pistil. (Adapted from *Beans of New York*)

might be associated with the very specific germination requirements of the pollen grains.

The Macrospores and the Embryo-Sac.—Although a critical cytological study was not attempted, the course of development of the macrospores and the embryo-sac appears to be very similar to that of Phaseolus vulgaris as reported by Brown (16). The egg after formation lengthens, broadens at the base, and projects into the embryo-sac beyond the synergids. The pollen tube was frequently observed entering the micropylar end of the sac. Also, the division of the primary endosperm nucleus frequently precedes that of the fertilized egg, the endosperm nuclei placing themselves in the peripheral region of the embryo-sac.

Pollen Behavior on the Stigma under Controlled Conditions.—Due to the difficulties encountered in pollen germination in vitro and the question of applicability to the intact plant, a study was made of the pollen behavior on the stigmatic surface within the keel of flowers of the controlled temperature plant series. In order to effect the study and to provide a time schedule for dehiscence of the anthers, time and method of pollination, pollen germination, pollen tube growth and fertilization, it was necessary to tag and systematically study hundreds of flowers in various stages of development under specific controlled environmental conditions.

Normally at anthesis the stigmatic surface within the keel is covered with an abundance of fluid on which is massed the pollen grains. As the style is bearded, most of the microspores lodge among the papillae at the base of the stigma. The pollen is not massed into the tip of the keel as in the case of the garden pea (*Pisum sativum*). Under favorable conditions for pod set (as in the 72° F. treatment) an abundance of both pollen and stigmatic fluid was produced, (Fig. 7). Normally, then, poor pollination is not an important consideration. With drought conditions (as in the 86° F., low soil moisture series) less stigmatic fluid was produced, the fluid was more viscous and relatively few pollen grains were found on the stigma. This fact alone may partially explain the difference in pod set obtained in this experiment. As pointed out by Andrews (3), the size of the keel may be a factor in the retention of the pollen on the stigmatic surface. In the large-seeded varieties the keel is less compactly arranged around the pistil than in the small-seeded varieties. This anatomical difference permits much of the pollen mass to fall to the base of the pistil out of contact with the stigmatic surface and doubtless may explain the difference in pod set of the two types under drought conditions. Germination of the pollen within the anther prior to dehiscence was observed on several occasions (Fig. 8). Just what physiological importance, if any, this function plays in pod set is not definitely known. It may possibly provide a mechanism for retaining the pollen in contact with the stigmatic surface during drought conditions until the pollen tube can establish itself in the stigmatic and stylar tissues. Regardless, it serves to further substantiate the importance that moisture plays in the microspore-stigma relationships.

Rate of Pollen Germination and Pollen Tube Growth in Vivo.— It was practically impossible to follow the growth of the pollen tube from the stigma to the ovary because of the close coiling of the style within the keel. The other alternative of sectioning the ovary at variable time intervals after pollination was found to be entirely satisfactory.

Comparing the two varieties, the greatest difference in the rate of pollen

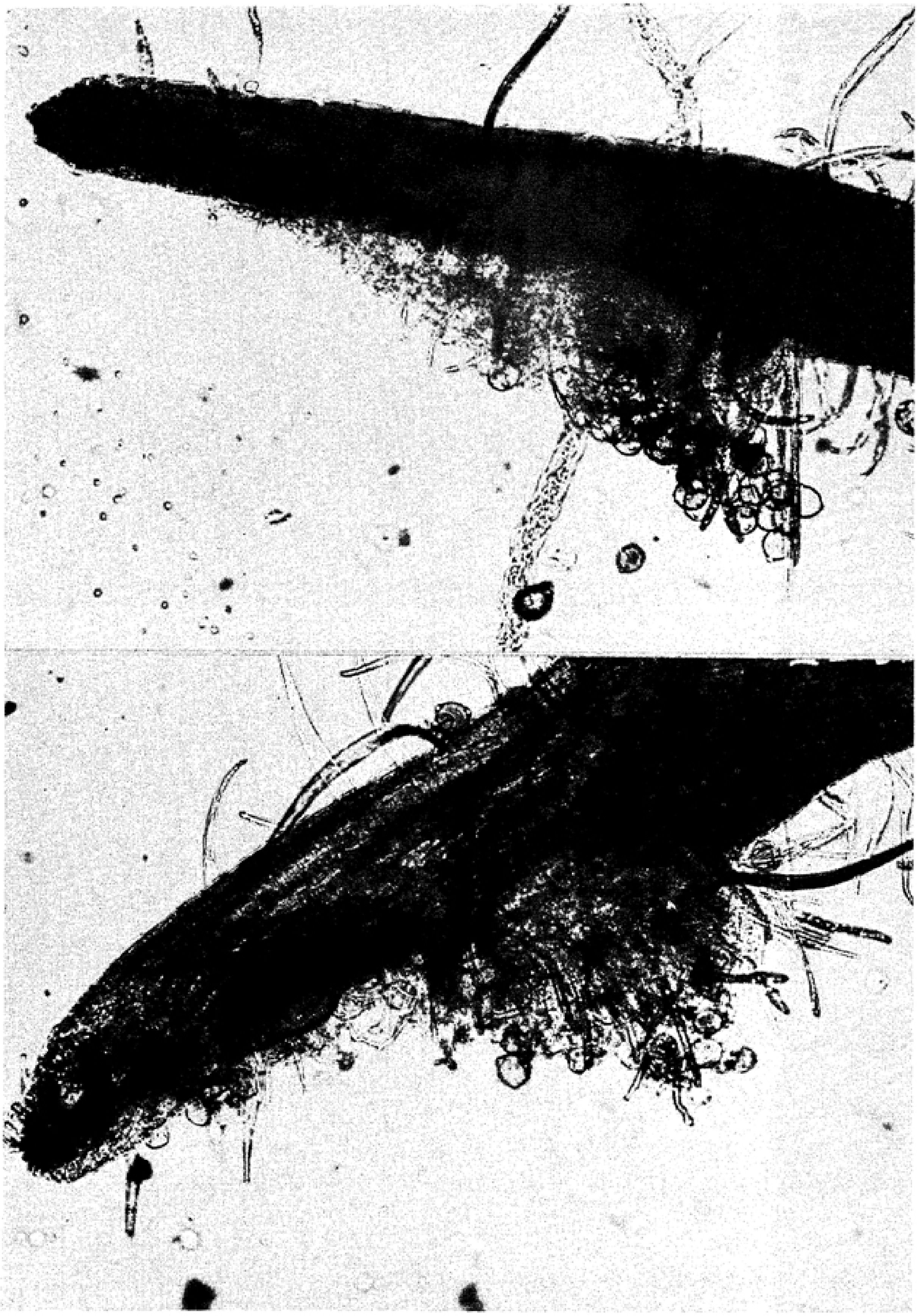


Figure 7. A comparison of the gross anatomical characteristics of the stigmas of Fordhook 242 bush lima (above) and Henderson's bush lima (below).

TABLE 6--ANTHESIS RATE OF FORDHOOK AND FORDHOOK 242 BUSH LIMAS AS RELATED TO THE ATMOSPHERIC TEMPERATURE
Average Number of Flowers Opening/Plant/Week

Period of Blossoming	62°F. Ford.	62°F. Ford. 242	72°F. Ford.	72°F. Ford. 242	86°F. Ford.	86°F. Ford. 242
Series I Plants						
Oct. 25-Nov. 1	5.3	3.1	18.3	12.0	16.1	5.8
Nov. 5-Nov. 12	10.8	8.5	17.6	8.0	19.3	7.6
Nov. 12-Nov. 19	6.6	8.3	15.3	8.5	--	--
Series II Plants	86°F. Dry Ford.	86°F. Dry Ford. 242	72°F. Ford.	72°F. Ford. 242	86°F. Moist Ford.	86°F. Moist Ford. 242
Nov. 23-Nov. 30	9.0	12.5	1.0	1.6	7.2	11.2
Dec. 1-Dec. 7	23.1	13.6	11.2	13.3	15.0	14.6

germination and tube growth occurred at the 62° F. temperature level. At this temperature, both pollen germination and pollen tube growth was faster in the Fordhook 242 variety. Moreover, a greater proportion of the flowers studied were observed to have germinated pollen on the stigma. These facts provide a possible explanation for the much higher set obtained for the Fordhook 242 variety at a constant temperature of 62° F., and may indicate that low night temperature as well as maximum day temperature should be considered in pod set. While there is some difference in the time required to effect fertilization at a given temperature, an equally important consideration appears to be whether or not the pollen germinates at all within the short period of time (approximately 48 hours) that the egg is receptive.

Where germination of the pollen occurred at all, the approximate time required to effect fertilization after pollination for the experimental conditions was as follows: Fordhook, 62° F., 42-48 hours; Fordhook 242, 62° F., 36-40 hours; both varieties, 72° F., 34-38 hours; both varieties, 86° F. moist, 32-36 hours; both varieties, 86° F. dry, 38-42 hours.

No morphological evidence was found in any part of the study of gross abnormalities which would suggest any specific form of sterility. That the microspores were viable in all these treatments was demonstrated by their ready germination in vitro when the specific requirements for germination were supplied. The mature megagametophyte with the egg visible is shown in Figure 8.

Nature of the Stigmatic Fluid.—A histochemical analysis of the stigmatic substance did not reveal the presence of a tough pellicle. Much of the substance of the fluid was readily soluble in the fat solvents and gave positive fat stain reactions. The stigmatic fluid appeared to be a type of emulsion with the oil globules suspended in a jelly-like matrix. No striking physical or chemical differences in the fluid was noted between varieties.

Relationship Between the Number of Fertilized Ovules and Floral Abscission.—It was also very evident from these experiments that at least one ovule in each pistil must be fertilized and start development in order to prevent abscission of the flower. This observation may indicate a hormone

Figure 8. Above (left)—Mature megagametophyte of Fordhook 242 showing two endosperm nuclei and the egg apparatus. Above (right) Mature megagametophyte of Fordhook 242 with unfertilized egg. Below—Anther smear Henderson's bush lima showing germination of the pollen prior to its release from the anther sac.

relationship between the developing ovule and the initiation and development of the abscission layer.

High Available Soil Moisture Series.—In the controlled temperature series, no study was made of the effect of soil moisture at the higher levels. It was for this purpose that pot experiments were conducted in the greenhouses during the fall of 1949.

Nine plants each of the Fordhook and Fordhook 242 varieties were grown at two soil moisture levels under otherwise comparable growing conditions and the percentage basal pod set determined for two one-week intervals, November 14-21 and November 23-30. The plants were depodded at the end of the first week. The soil moisture was maintained at the following levels:

"medium" soil moisture; tensiometer reading 50-60

"high" soil moisture; tensiometer reading 0-10

The latter level represents a soil moisture level approaching field capacity. In no case did water-logging of the soil occur, and there were no visual symptoms of reduced plant activity as a result of high soil moisture. The variation in soil moisture was carefully controlled at the desired levels by filling one-gallon jars to a depth of five inches with gravel and topping off with a sandy loam soil of good fertility. The water table in the gravel was then maintained at the required height by frequent water additions through a glass tube inserted through the soil layer to the gravel level.

The data obtained in this experiment is given in Table 7.

Results.—On the basis of these results, the percentage pod set of the Fordhook bush lima was decreased as much as 16 to 18 per cent by soil moisture levels approaching field capacity. The fact that no significant difference in pod set occurred for the Fordhook 242 variety for these conditions may indicate a greater degree of tolerance of this variety to high soil moisture. At both soil moisture levels, the percentage pod set for the Fordhook 242 variety was equal to or better than that of the Fordhook variety. Noteworthy also is the fact that the average pod set of all plants for both varieties was almost identical for the two weekly intervals indicating that the results can be duplicated providing the plants are depodded between each series.

It should be realized that these experimental conditions duplicate very inadequately the natural conditions which prevail in the field during cloudy rainy weather. With proper consideration being given to the associated factors of wet foliage and flowers, high relative humidity, restricted air movement, and low light intensity during cloudy weather, one may find herein another important reason for the frequent failures of lima bean plants to reach the "capacity set."

Application of Soluble Boron Sprays to the Blossoms and Leaves.—*Leaf Area and Pod Yield Relationships.*—The results from preliminary field tests during the summer of 1949 indicated that pod set may possibly be in-

TABLE 7--POD SET OF FORDHOOK AND FORDHOOK 242 BUSH LIMA BEAN AT MEDIUM AND HIGH AVAILABLE SOIL MOISTURE LEVELS

Individual Plants	Blossoming Period November 14-21								Blossoming Period November 23-30							
	Medium Soil Moisture				High Soil Moisture				Medium Soil Moisture				High Soil Moisture			
	Fordhook		Ford. 242		Fordhook		Ford. 242		Fordhook		Ford. 242		Fordhook		Ford 242	
	Set	Drop	Set	Drop	Set	Drop	Set	Drop	Set	Drop	Set	Drop	Set	Drop	Set	Drop
1	8	2	1	2	8	11	11	3	11	2	18	5	18	10	13	4
2	11	8	5	0	4	8	9	5	21	3	18	9	15	13	29	7
3	12	10	15	6	9	9	13	1	15	4	14	4	15	17	11	4
4	12	7	8	2	12	12	12	4	11	3	15	3	22	13	18	7
5	13	4	4	1	--	--	10	4	22	4	19	2	6	5	15	8
6	11	3	4	2	4	1	12	3	20	6	13	5	15	12	12	3
7	10	7	11	4	9	8	8	5	14	7	22	2	17	8	17	4
8	18	5	12	7	6	8	5	2	15	7	19	5	14	5	7	2
9	--	--	6	0	14	6	6	2	--	--	23	7	23	8	22	4
Totals	95	46	66	24	66	63	86	29	129	36	161	42	145	91	144	43
Average Percentage Set	67		70		51		74		78		79		60		76	

creased through the application of soluble boron sprays to the above-ground portions of the plant. In order to investigate this possibility under more exacting experimental conditions, plants of the Fordhook 242 variety were grown in a fertile loam soil in a greenhouse ground bed during the fall and winter of 1949. The beans were seeded October 8, 1949 in 36 inch rows and later thinned to give a spacing of 18 inches within the row. The plants were trained upward from suspended strings. Applications of aqueous solutions of boric acid were made to the foliage, buds, and flowers with a hand atomizer when the terminal racemes commenced flowering (November 23, 1949) and again on December 3, 1949. The concentrations used were 100, 250, and 500 parts per million. The rate of application was sufficient to wet the plants thoroughly. Six plants were used in each treatment and the treatments replicated eight times.

Results.—No definite correlation was found between the boron applications and pod set or bean yield. There was considerable variation in pod set between individual plants in each treatment, indicating perhaps the influence of other factors. It is very likely that the high boron requirement of the bean plants was satisfied even on the control plants by a favorable supply in the soil.

Aside from the effects of boron, several observations were made during the course of the experiment which may contribute valuable information to the problem of pod set and bean yield.

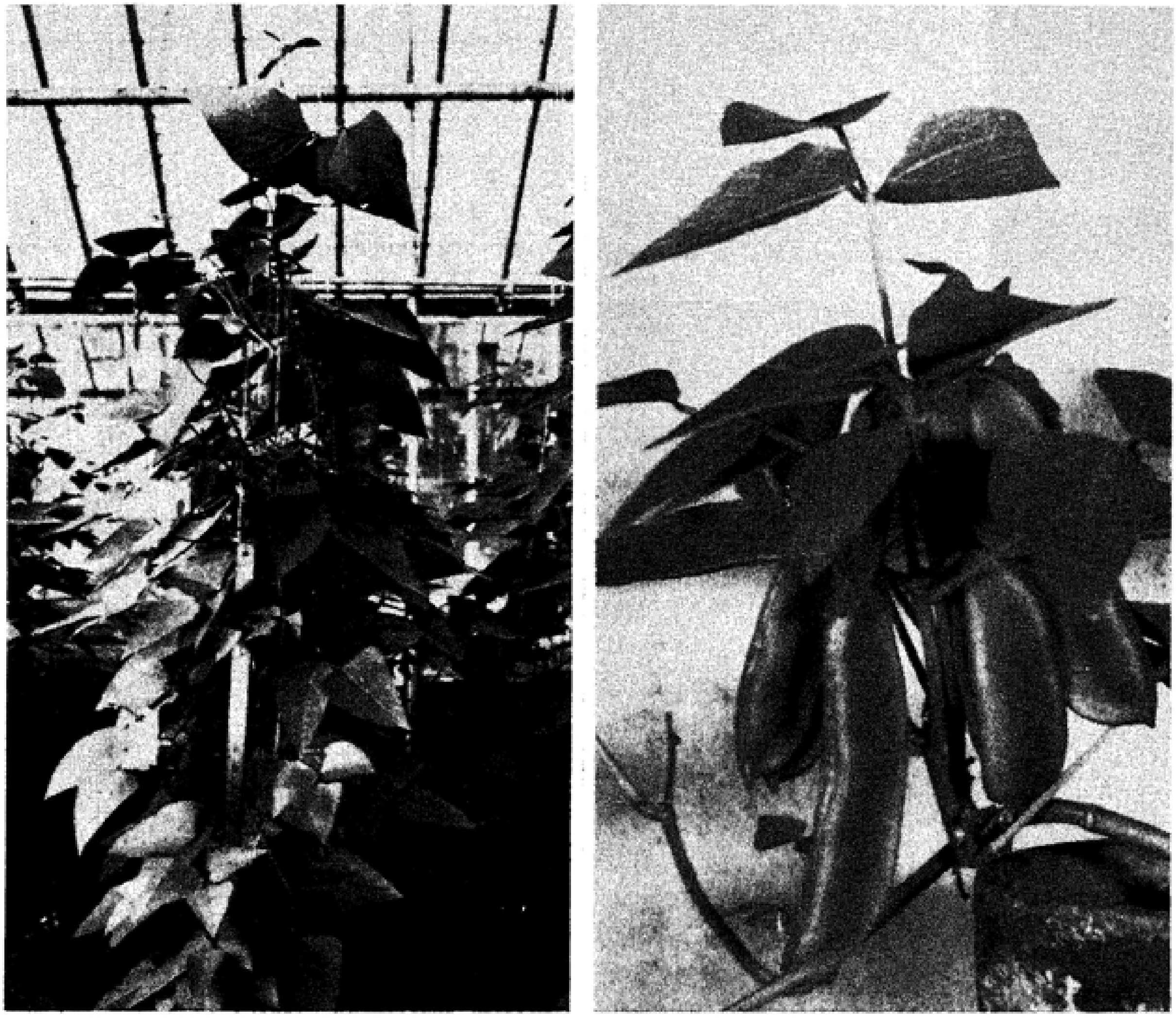


Figure 9. Fordhook 242 plants with indeterminate (left) and determinate (right) habit of growth. Note the concentration of mature pods in the terminal part of the indeterminate plant and the basal concentration in the determinate plant.

One striking observation was the exceptional habit of growth of the plants. While the Fordhook 242 bush lima normally behaves as a dwarf sort showing little tendency to climb, these plants showed definite tendencies towards developing an indeterminate habit of growth (Figure 9). Furthermore, the pod set was concentrated on the terminal racemes and at the expense of the basal flowers. The lower racemes later flowered and set profusely but abscission followed before any appreciable pod size was attained. It should be realized that this plant response is the opposite of that normally observed for this variety. In fact, the ability to set basal pods is a widely recognized and highly desirable trait of the variety. It is now known that this vining habit of growth results from excessive elongation of the internodes and is enhanced by high temperature, high soil fertility, and low light intensities.

Leaf Area and Pod Yield Relationships.—In this experiment an interesting relationship was found between the total leaf area and the number of pods

TABLE 8--RELATIONSHIP OF PLANT LEAF AREA AND POD YIELD OF FORDHOOK 242 BUSH LIMA UNDER GREENHOUSE GROUND BED CULTURE

Mean Daily Light Intensity 325 Gr. Cal./Day

Successive Plant No.	Total Plant Leaf Area (Cm.^{2})	Number Mature Pods/Plant
1	3,661	6
2	5,866	10
3	6,170	10
4	6,904	17
5	7,045	16
6	7,248	18

TABLE 9--RELATIONSHIP OF PLANT LEAF AREA AND POD YIELD OF FORDHOUSE 242 BUSH LIMA UNDER GREENHOUSE POTTED CULTURE

Mean Daily Light Intensity 232 Gr. Cal./Day

Successive Plant No.	Total Plant Leaf Area (Cm.^{2})	Number Mature Pods/Plant
1	828	10
2	842	11
3	912	10
4	743	9
5	701	10

maturing per plant. The leaf area was determined for representative control (that is, untreated) plants by the use of a planimeter, and is presented along with the pod yield in Table 8.

The data shows a definite relationship between the leaf area and the yield of fully developed pods. As the leaf area increased, the yield increased, though the relationship is not strictly linear. With a mean daily total light intensity of 325 Gr. Calories for the month of fruiting, approximately 600 square centimeters of leaf area was required per pod. That such is not always the case is shown from the results of potted lima bean plants grown in the greenhouse during the spring of 1950. As shown in Table 9 plants with approximately one-eighth the leaf area produced the same yield even at a lower light intensity.

Quite obviously, other factors than leaf area and light intensity must be considered in interpreting these results. In the case of the plants in ground-bed culture, vegetative growth was stimulated at the expense of fruitfulness. With the indeterminate habit of growth, very large leaves, and vertical training, the basal portion of the plants was shaded sufficiently that much of the leaf area was possibly photosynthetically ineffective. Under these conditions, leaf area determinations do not give a true index of the pod producing capacity of the plant. Also, under field conditions positive correlations between leaf area and yield are dependent, in addition, upon such factors as the weather conditions prevailing during the blossoming period.

Pollen Germination Experiments

Intensive preliminary pollen germination tests proved sucrose solutions to be a poor media for the germination of lima pollen in vitro as the tubes ruptured over a very wide range in concentration. In this study the sucrose concentration was varied from 5% to 60% at 5% levels. The hanging drop technique was used with a controlled temperature ranging from 22-25° C. Pollen of the Fordhook 242 variety was taken from carefully selected flowers at the loose or hooded bud stage (stage B) so as to provide as nearly a uniform maturity stage as possible. At all concentrations below 40%, rapid rupture of the tubes occurred; above a 50% sucrose concentration, plasmolysis of the grains was quite evident. Germination at concentrations of 40, 45, and 50% were better but still unsatisfactory.

Attempts to Find a Favorable Medium.—Considering that unfavorable osmotic pressure was contributing to the bursting of the tubes, agar (Bacto-Agar) was added to the sugar solutions for the purpose of providing better water relationships to the delicate pollen cells. A series of sugar-agar solutions was then prepared in which the agar concentration was kept constant at 1% and the sucrose varied at 20, 30, 40 and 50 grams per 100 cc. of the medium. The technique and conditions were identical to those of the sugar solution experiments except that it was found necessary to pool the pollen from the anthers of a flower and from several flowers in order to eliminate variationability in the pollen itself. The germination in sugar-agar solutions did prove to be much better than in the sugar solutions. However, germination at the 20 and 30 gram sugar levels in the sugar-agar solutions was still unsatisfactory as the tubes burst soon after extending from the pore. Germination in the sugar-agar solution containing 40 and 50 grams sugar was fairly dependable and satisfactory, the tubes reaching good length, and only occasionally failing to grow. The results at the 40 gram sugar level were, on the average, more consistent but the tubes were generally shorter than those obtained at the 50 gram sugar level.

Effect of Temperature on Pollen Germination in Vitro.—The data in Table 10 shows the effect of temperature at four different levels on the percentage germination of pollen of the Henderson and Fordhook 242 varieties. The values given represent an average of duplicate samples obtained by the hanging drop technique in a 40% sucrose-1% agar solution. The readings were taken at the end of a 24 hour period.

Considering a considerable degree of variation which occurred between the series in spite of the precautions taken, there appeared little difference in the percentage germination of the pollen of either variety at 68, 77, or 86° F. This indicates that lima pollen can germinate satisfactorily over a fairly wide temperature range provided that other conditions are favorable. Germination at 98° F. was always poor, indicating, perhaps, the approach of a critical temperature for germination.

TABLE 10--PERCENTAGE GERMINATION OF HENDERSON AND FORDHOOK 242 POLLEN IN 40% SUCROSE-1% AGAR AT FOUR TEMPERATURE LEVELS

Date	Temperature							
	68°F.		77°F.		86°F.		98°F.	
	Hend.	Ford. 242	Hend.	Ford. 242	Hend.	Ford. 242	Hend.	Ford. 242
8/30/49	--	--	26	16	11	28	4	1
8/31/49	--	--	43	32	17	17	10	6
9/1/49	55	73	24	52	33	41	5	5
9/2/49	44	40	18	45	24	30	--	--
9/6/49	--	26	24	23	32	37	--	--
9/7/49	46	47	22	50	--	--	--	--

Effect of Boron Additions on Pollen Germination in Vitro.—In an attempt to secure more consistent pollen germination and better pollen tube growth, boric acid was added to the sugar-agar medium at concentrations of 0.003 and 0.006 per cent. The results were negative in all cases.

Application of Phenology

From an analysis of the preceding part of the dissertation it is apparent that the practical problem of securing satisfactory pod set and yields in the lima bean is not a simple one. Since there are no satisfactory substitutes for fertilization itself, the grower can best aid the plant by providing the conditions most favorable for proper pollination and fertilization of the ovules. But many of the practices which are effective under controlled greenhouse conditions are not practical in the field. It would appear, therefore, that the application of phenology might provide a practical approach to the problem.

The basis for this project was a survey of the climatological data in the Southeastern Missouri area (Cairo, Illinois) for the forty-year period 1908-1947. The mean maximum temperature, mean minimum temperature, and the total precipitation by days was considered for weekly intervals during the growing season. Then by setting up arbitrary limits for these factors, it was possible to determine the per cent frequency of "hot" or "cold," "wet" or "dry" weeks during the growing season based on the phenological mean for the forty-year period. Theoretically, by this method it should be possible to predict with a fair degree of accuracy those planting dates which would permit the plants to blossom in periods of favorable pod-setting weather.

The per cent frequency figures were based on the following limits:

mean maximum temperature $>90°$ F.
mean minimum temperature $<69°$ F.
"Dry" week: None, trace or $<0.25"$ of rainfall on 1 or 2 days of the week
"Wet" week: 5-6 days of week with rain

These limits were carefully chosen on the basis of the information obtained in the controlled experiments (Greenhouse Controlled Temperature Section). The "week numbers" herein referred to run consecutively from

TABLE 11--PER CENT FREQUENCY OF DAYS WITH SPECIFIC WEATHER CONDITIONS FOR THE PERIOD 1908-1947 AT CAIRO, ILLINOIS

Date	Week No.	Mean Maximum Temperature (Degrees Fahrenheit) <60	60-69	70-79	80-89	≥90	Mean Minimum Temperature (Degrees Fahrenheit) <60	60-69	70-79	80-89	≥90
5/31 6/6	14	0.0	0.0	25.0	62.5	12.5	10.0	80.0	10.0	0.0	0.0
6/7 6/13	15	0.0	0.0	22.5	70.0	7.5	7.5	75.0	17.5	0.0	0.0
6/14 6/20	16	0.0	0.0	10.0	67.5	22.5	2.5	67.5	30.0	0.0	0.0
6/21 6/27	17	0.0	0.0	5.0	65.0	30.0	0.0	47.5	52.5	0.0	0.0
6/28 7/4	18	0.0	0.0	7.5	65.0	27.5	2.5	50.0	47.5	0.0	0.0
7/5 7/11	19	0.0	0.0	0.0	65.0	35.0	0.0	27.5	70.0	2.5	0.0
7/12 7/18	20	0.0	0.0	0.0	55.0	45.0	0.0	22.5	77.5	0.0	0.0
7/19 7/25	21	0.0	0.0	0.0	65.0	35.0	0.0	25.0	75.0	0.0	0.0
7/26 8/1	22	0.0	0.0	0.0	47.5	52.5	0.0	22.5	77.5	0.0	0.0
8/2 8/8	23	0.0	0.0	2.5	62.5	35.0	0.0	30.0	70.0	0.0	0.0
8/9 8/15	24	0.0	0.0	0.0	72.5	27.5	0.0	40.0	60.0	0.0	0.0
8/16 8/22	25	0.0	0.0	5.0	75.0	20.0	0.0	50.0	50.0	0.0	0.0
8/23 8/29	26	0.0	0.0	12.5	70.0	17.5	0.0	70.0	30.0	0.0	0.0
8/30 9/5	27	0.0	0.0	10.0	77.5	12.5	5.0	67.5	27.5	0.0	0.0
9/6 9/12	28	0.0	0.0	27.5	57.5	15.0	15.0	62.5	22.5	0.0	0.0
9/13 9/19	29	0.0	0.0	27.5	65.0	7.5	27.5	50.0	22.5	0.0	0.0

Total Precipitation

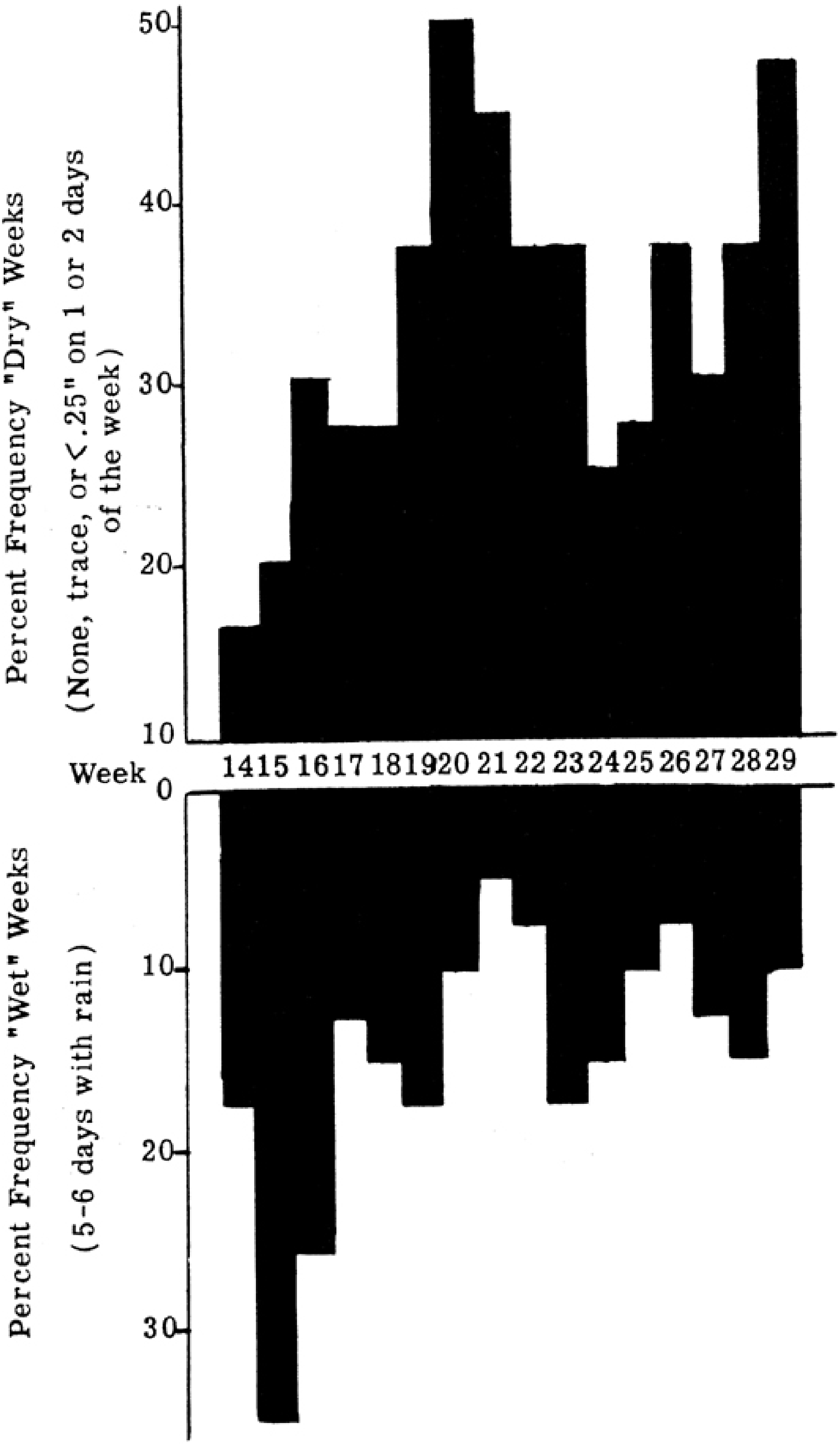
Trace or None	On 1 or 2 Days 0" .25"	.25" .99"	≥1.00"	On 3 or 4 Days 0" .25"	.25" .99"	≥1.00"	On 5 or 6 Days 0" .25"	.25" .99"	≥1.00"
10.0	2.5	10.0	5.0	7.5	30.0	17.5	2.5	5.0	10.0
12.5	7.5	2.5	5.0	7.5	17.5	12.5	0.0	12.5	15.0
20.0	10.0	7.5	2.5	10.0	15.0	10.0	2.5	10.0	10.0
5.0	22.5	10.0	0.0	20.0	22.5	7.5	0.0	2.5	10.0
12.5	15.0	10.0	5.0	5.0	25.0	12.5	2.5	10.0	2.5
10.0	27.5	7.5	12.5	10.0	10.0	5.0	0.0	10.0	7.5
20.0	30.0	10.0	5.0	10.0	7.5	7.5	0.0	2.5	7.5
20.0	25.0	17.5	0.0	12.5	12.5	7.5	0.0	0.0	5.0
22.5	15.0	10.0	0.0	12.5	15.0	17.5	0.0	2.5	5.0
20.0	17.5	15.0	2.5	7.5	12.5	7.5	0.0	5.0	12.5
15.0	10.0	5.0	7.5	17.5	20.0	10.0	2.5	0.0	12.5
20.0	7.5	5.0	12.5	17.5	15.0	12.5	0.0	7.5	2.5
22.5	15.0	15.0	2.5	10.0	12.5	15.0	0.0	0.0	7.5
22.5	7.5	10.0	5.0	10.0	17.5	15.0	0.0	5.0	7.5
22.5	15.0	15.0	0.0	5.0	22.5	5.0	0.0	2.5	10.0
30.0	17.5	7.5	2.5	2.5	12.5	17.5	0.0	5.0	5.0

March 1, the period March 1-7 inclusive representing week number one.

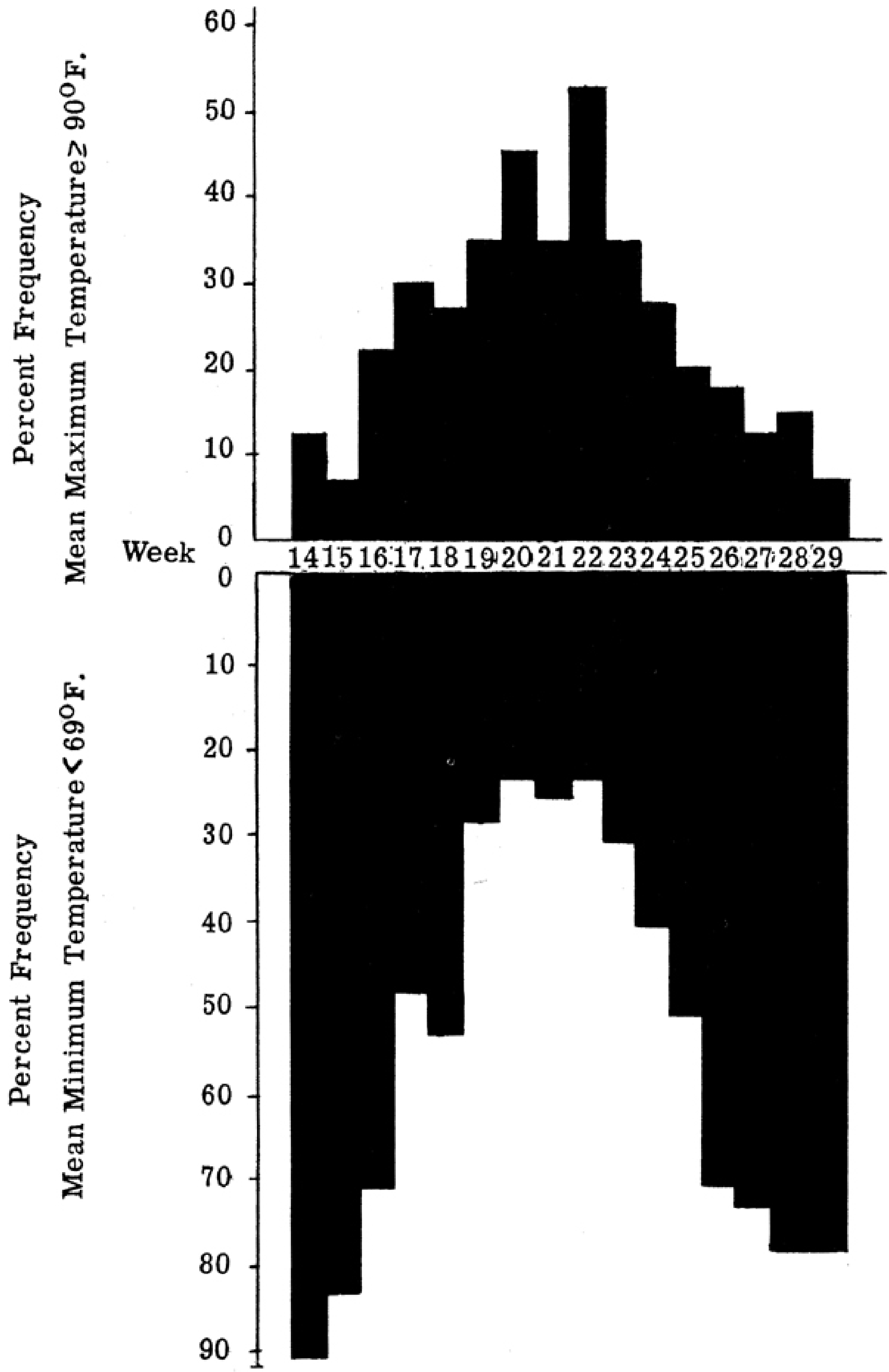
Analysis of the Climatological Data for Southeastern Missouri.—The climatological data obtained from the 40-year survey at Cairo, Illinois is represented in Table 11 and in Graphs 5 and 6. This information was taken from the Official Weather Records of the United States Department of Commerce, and the analysis was facilitated by the use of the International Business Machine punch-card system available at the University of Missouri.

On the basis of the rainfall distribution pattern represented by Graph 5, the following facts are evident:

1. After the sixteenth week (June 14-June 20 the likelihood of pod set being limited by wet weather is much less than that for dry weather.
2. As implied above, pod set during the fifteenth and sixteenth week may frequently be limited by excessive rainfall. In fact, judging



Graph 5. Percentage frequency of "dry" weeks and "wet" weeks for the 40-year period 1908-47 at Cairo, Illinois.



Graph 6. Percentage frequency of mean maximum and mean minimum temperatures for weekly intervals over the 40-year period 1908-1947. Cairo, Illinois.

from the data, with the per cent frequency averaging twenty-five, one would expect critical limitation to pod set one year in four from excessive rainfall.

3. There is a very great likelihood (approaching 50 per cent frequency) that pod set on plants blossoming during the twentieth to twenty-third week will be limited as a result of lack of rainfall.
4. Considering only rainfall distribution, the period from the seventeenth to nineteenth and the twenty-fourth to twenty-sixth weeks would provide the best moisture relationships for pod set.

Temperature Data.—Minimum night temperature. An analysis of the data using a mean minimum temperature below 69° F. as the limit (Graph 6), points out the following facts:

Up to the seventeenth week (June 21-June 27) and after the twenty-sixth week (August 23-August 29) one can expect the mean minimum temperature to be less than the required minimum greater than fifty per cent of the time, or one year out of every two years.

Maximum daytime temperature. The mean maximum temperature graph with the limit set at 90° F. or greater shows in general that pod set is not likely to be limited severely (that is, greater than 50 per cent frequency) during any part of the growing season. The greatest likelihood for limited pod set as a result of high daytime temperature would be in the period of the twentieth to twenty-third week.

Application of the Data to the Problem.—Considering the climatological data in its entirety, the optimum weather conditions for pod set and high yield in Southeastern Missouri would normally occur during the following weekly periods: June 21-27, June 28-July 4, July 5-11, August 2-8, August 9-15, and August 16-22. Consequently, the cultural practices should be modified so as to promote flowering during these periods.

DISCUSSION

The evidence obtained in these studies indicates that the frequent failure to obtain satisfactory pod set with the large-seeded lima varieties can be attributed directly to inadequate fertilization of the ovules. In order to prevent abscission of the flower within the forty-eight hour period following pollination, it was found necessary that at least one ovule in the ovary be fertilized.

The histological evidence from this study supports the contention that poor fertilization most frequently results from a failure of the microgamete to reach the egg while the latter is receptive. The following observations attested to the very sensitive nature of the pollen grains of lima species:

(1) very exacting osmotic requirements for pollen germination in vitro
(2) remnants of ruptured pollen grains frequently found on the stigmatic surface
(3) germination of pollen prior to release from the anther

(4) frequent occurrence of partially-filled pods.

It would appear, therefore, that lima species, especially the large-seeded types, are limited ecologically to a narrow environmental complex by reason of their sensitive pollen. Since the expression of the climatic and edaphic effects is directly through the pollen, the results on pod set can now be interpreted on this basis.

The results, however, do not support the widespread belief that the failure to set is indirectly due to high temperatures. In fact, in carefully controlled greenhouse experiments, the environmental factor found limiting pod set to the greatest degree was the available soil moisture. Pod set was markedly lowered at soil moisture levels exceeding field capacity or at those approaching the wilting coefficient. Under field conditions, frequent heavy rains at the beginning of the blossoming period may seriously limit and sometimes almost completely prevent basal pod set, apparently by favoring rupture of the pollen grains prior to or during germination. The difficulty is aggravated by close plant spacing and by vigorous vegetative growth.

Air temperature was found to be an important factor limiting pod set markedly only at the upper and lower temperature extremes for satisfactory vegetative growth of warm season crops. The controlled temperature experiments indicate that the minimum night temperature may be as important in limiting set as high daytime temperatures. However, considerable varietal difference was found in this respect; the Fordhook 242 variety being considerably more tolerant of low temperature than regular Fordhook. Other than the temperature factor, differential response of the large-seeded varieties to the environmental complex may be attributed largely to the relative ability of the plants to maintain a favorable moisture relationship on the stigmatic surface. This conclusion supports that of Andrews (3) who attributed the larger yield of the small-seeded varieties under unfavorable conditions to their relatively greater root system and more efficient leaves. In addition, the small-seeded varieties such as Henderson possess a smaller keel which is more compactly arranged around the pistil—a feature which may retain the pollen in contact with the stigmatic surface for longer periods of time and favor pollen germination under drought conditions.

The results from various fertilizer treatments emphasize the importance of timely application as well as carefully controlled rates. Heavy side-dressing applications of soluble nitrogeneous fertilizers during the blossoming period inhibit pod set. Excessive nitrogen fertilization on low phosphorus soils may also affect pod set by delaying anthesis and shifting the pollination date.

Pod set and yield are not necessarily comparable, even though the "capacity set" for the plant is reached, since floral and pod abscission may occur as a result of competition among the developing pods for essential metabolites. It is apparent from these results that satisfactory yields depend first upon obtaining the "capacity set" of pods during the first two weeks of the blossoming period. With this provision, good yields are then dependent primarily upon the metabolic activity of the plant. Sunlight duration and intensity, and the "active" leaf area of the plant then become very important factors. In this respect, the final yield of the bush varieties was found to be closely associated with the habit of growth of the plant. Excessive internode elongation as a result of low light intensity, high temperature, and high soil fertility gave an indeterminate type of growth with the terminal racemes maturing pods at the expense of the basal flowers.

Since pod set and yields are so closely dependent upon the environmental conditions, the most logical practical approach in obtaining satisfactory yields with a particular variety in a given area appears to be the application of the principles of phenology. An analysis of the weather pattern to determine the most favorable blossoming periods together with the necessary modification of culture practices to promote flowering during these periods should prove most helpful.

SUMMARY

The fruiting behavior of several varieties of bush lima beans was investigated in the greenhouse and in the field under widely variable conditions of air temperature, relative humidity, available soil moisture, soil fertility, and light. From these studies the following conclusions were reached:

(1) The failure to obtain a "capacity set" with large-seeded lima varieties is most frequently due directly to inadequate fertilization of the ovules.

(2) The expression of climatic and edaphic factors on pod set is primarily through pollen tube growth and fertilization.

(3) Floral abscission during the initial stages of flowering is largely independent of the leaf area, and generally occurs within forty-eight hours following the hooded bud stage in the case of unfertilized flowers. A minimum of one ovule per pod must be fertilized and start development to prevent abscission.

(4) Pod set and yield are not necessarily comparable, since flower, bud, and pod abscission may occur later in the blossoming period as a result of their competition for essential metabolites. If the "capacity set" is obtained during the first two weeks of flowering, the yield is dependent primarily on the "active" leaf area and light conditions.

(5) Several lines of evidence are presented which indicate that the narrow ecological adaptation of large-seeded lima varieties is due to the very sensitive nature of the microspores.

(6) The outstanding morphological characteristics of the varieties are discussed together with their possible correlation with pod set.

(7) There was no histological evidence of structural abnormalities which would suggest any specific form of sterility in any of the varieties or treatments.

(8) Within the experimental limits, the water relations of the soil-plant complex were found to have a greater effect on pod set than high temperature. In all cases, satisfactory pod set was dependent upon the maintenance of a favorable moisture supply to the stigmatic surface.

(9) Controlled temperature experiments indicated that the minimum night temperature is an important consideration as well as the maximum day temperature.

(10) In comparing adaptability of varieties, the Fordhook 242 was found to be more tolerant than regular Fordhook to low temperature and excessive soil moisture.

(11) Conditions contributing to rapid vegetative growth and excessive internode elongation promote an indeterminate type of growth with the terminal racemes maturing pods at the expense of the basal flowers. Under these circumstances, leaf area is not a true index of the pod-carrying capacity of the plant.

(12) The application of phenological principles to cultural practices appears to afford the most practical means for providing satisfactory, consistent yields with a particular variety or strain within a given area.

BIBLIOGRAPHY

1. Addicott, F. T., "Pollen Germination and Pollen Tube Growth, as Influenced by Pure Growth Substances," *Plant Physiology* 18: 270-279, 1943.
2. Allard, H. A. and W. J. Zaumeyer, "Responses of Beans (Phaseolus) and Other Legumes to Length of Day," *U. S. D. A. Technical Bulletin* 867, 1944.
3. Andrews, F. S., "Morphological, Physiological, and Environmental Factors Affecting the Fruiting of Bush Lima Beans," Unpublished Thesis, Cornell, 1935.
4. Austin, Stanley, "Effects of Exfloration on Plant Metabolism," *Plant Physiology* 10: 225-243, 1938.
5. Babko, E. V., i V. V. Tserling (Zerling), "Vliianie bora na reproduktivnoe razvitie rastenii (The Effect of Boron on Reproduction in Plants," *Botanicheskii Zhurnal SSSR* (*Jour. Bot. USSR*) 23 (1): 3-11, 1938.
6. Bailey, L. H., "The Dwarf Lima Beans," *N. Y.* (*Cornell*) *Agr. Exp. Sta. Bul.* 87: 83-101, 1895.
7. Balls, W. L., "The Effect of Sub-soil Water on the Cotton Crop," *Suppl. Yearbook Khed. Agr. Soc.*, 1910. Cited by Lloyd.
8. Barre, H. W., "Cotton Physiological Experiments," *South Carolina Exp. Sta. 28th Annual Report*, 1915.
9. Blaha, J., and J. Schmidt, "Effects of Boron on the Germination of Pollen in Fruit Trees" (with English summ.) *Sbor. Cesk. Akad. Zem.* 14 (2): 186-192, 1939.
10. Blinkley, A. M., "The Amount of Blossom and Pod Drop on Six Varieties of Garden Beans," *Proc. Amer. Soc. Hort. Sci.* 29: 489-492, 1932.
11. Brink, R. A., "Preliminary Study of the Role of Salts in Pollen Tube Growth," *Bot. Gaz.* 78: 361-377, 1924a.
12. ...., "The Physiology of Pollen IV. Chemotropism; Effects on Growth of Grouping Grains; Formation and Function of Callose Plugs; Summary and Conclusion," *Amer. Jour. Bot.* 11: 218, 283, 351, 417, 1924b.
13. ...., "The Influence of Hydrogen-ion Concentration on the Development of the Pollen Tube of the Sweet Pea (Lathyrus odoratum)," *Amer. Jour. Bot.* 12: 146-162, 1925.
14. Britten, E. J., "The Effect of Naphthalene Acetic Acid on the Developing Maize Caryopsis," *Amer. Jour. Bot.* 34: 211-218, 1947.
15. Brown, E. Marion, "Equipment for the Growing of Plants at Controlled Temperatures," *Plant Physiology* 14: 517-526, 1939.
16. Brown, M. M., "The Development of the Embryo Sac and of the Embryo in Phaseolus vulgaris," *Bul. Torr. Club.* 44: 535-544, 1917.
17. Burrell, P. C., and Whitaker, T. W., "The Effect of Indoleacetic Acid on Fruit Setting in Muskmelons," *Proc. Am. Soc. Hort. Sci.* 37: 829-830, 1940.
18. Bushnell, J. W., "The Fertility and Fruiting Habit in Cucurbitae," *Proc Amer. Soc. Hort. Sci.* 17: 47-52, 1920.
19. Clark, H. E., and Kerns, K. R., "Effects of Growth-Regulating Substances on Parthenocarpic Fruit," *Bot. Gaz.* 104: 639-644, 1943.
20. Cochran, H. L., "Some Factors Influencing Growth and Fruit Setting in the Pepper (Capsicum Frutescens L.)," *Cornell Univ. Agr. Expt. Sta. Memoir* 190, 1936.
20a. Coffman, F. A., "Pollination in Alfalfa," *Bot. Gaz.* 74: 197-203, 1922.
21. Cooper, W. C., "Vitamins and the Germination of Pollen Grains and Fungus Spores," *Bot. Gaz.* 100: 844-852, 1939.
22. Cordner, H. B., "External and Internal Factors Affecting Blossom Drop and Set of Pods in Lima Beans," *Proc. Amer. Soc. Hort. Sci.* 30: 571-576, 1933.
23. Crist, J. W. and Stout, A. J., "Relation between Root and Top Size in Herbaceous Plants," *Plant Physiology* 1: 63-86, 1929.
24. Davis, J. F., "The Effect of Some Environmental Factors on the Set of Pods and Yield of White Pea Beans," *Jour. of Agr. Res.* 70 (No. 7): 237-249, 1945.
25. Dutt, Charles P., "Abscission in Cotton Flowers," *Bot. Gaz.* 85: 208-220, 1928.
26. East, E. M., "Self-Sterility," Biblio. *Genetica*, 5: 331-370, 1929.
27. Eigsti, O. J., "Methods for Growing Pollen Tubes for Physiological and Cytological Studies," *Proc. Okla. Acad. Sci.* 20: 45-47, 1940a.
28. Emmert, E. M., "The Effect of 'Split Applications' of Nitrogen and Phosphorus on the Yields of Tomatoes and Large Seeded Lima Beans," *Proc. Am. Soc. Hort. Sci.* 44: 433-440, 1944.
29. Eyster, W. H., "The Induction of Fertility in Genetically Self-Sterile Plants," *Science*, 94: 144, 1941.
30. Fisher, E. H., Riker, A. J., and Allen, T. C., "Bud, Blossom, and Pod Drop of Canning String Beans Reduced by Plant Hormones," *Phytopath.* 36: 504-523, 1946.

31. Gardner, V. R., Bradford, F. C., and Hooker, H. D., *Fundamentals of Fruit Production*, McGraw-Hill Book Co., 1922.
32. Giles, W. L., "The Morphological Aspects of Self-Sterility in *Lotus Corniculatus L.*" Unpublished Thesis, University of Missouri, 1949.
33. Grigsby, Buford H., "Some Effects of 2,4-D on Ragweed and Certain Woody Plants," *Mich. Agr. Exp. Sta. Quar. Bul.* 28: 304-310, 1946.
34. Grizzard, A. L., and E. M. Matthews, "The Effect of Boron on Seed Production of Alfalfa," *Jour. Amer. Soc. Agron.* 34 (4): 365-368, 1942.
35. Gustafson, F. G., and Stoldt, E., "Some Relations Between Leaf Area and Fruit Size in Tomatoes," *Plant Physiology* 11: 445-451, 1936.
36. Hardenburg, E. V., "Effect of Hormone Dust on Pod Set and Yield in Beans," *Proc. Am. Soc. Hort. Sci.* 45: 367-370, 1944.
37. Havis, Leon, "Some Factors which Influence the Fruiting Habit of Henderson's Bush Lima Bean (Phaseolus lunatus)", *Ohio Agr. Exp. Sta. Bul.* 535, 1934.
37a. Hedrick, U. P., *The Vegetables of New York*, J. B. Lyon Co., 1931.
38. Hemphill, D. D., "The Effects of Certain Plant Growth Regulating Substances on Flower Bud Development and Fruit Set," *Mo. Res. Bul.* 434, 1949.
39. Hendry, G. W., "Bean Culture in California," *Cal. Agr. Expt. Sta. Bul.* 294, 1918.
40. Hester, J. B. "The Influence of Soil Acidity and Soil Type upon the Growth and Composition of the Lima Bean Plant," *Proc. Amer. Soc. Hort. Sci.* 32: 600-603, 1934.
41. Howlett, F. S., "Effects of Indolebutyric Acid upon Tomato Fruit Set and Development," *Proc. Amer. Soc. Hort. Sci.* 39: 217-227, 1941.
42. Jacob, W. C., "A Study of Artificial Pollen Germination in the Lima Bean," Unpublished Thesis, University of Maryland, 1937.
43. Katz, E., "The Stigma as a Factor in Pollination," (Trans. title) *Flora (Jena) N. Ser.* 120: 243-281, 1926.
44. Kendall, J. N., "Abscission of Flowers and Fruits in the Solonaceae with Special Reference to Nicotiana," *Calif. Univ. Pubs. Bot.* 5:347-428, 1918; Thesis, University of California, 1917.
45. Lloyd, F. E., "The Physiology of Stomata," *Carnegie Institute of Washington*, Pub. 82, 1908.
46. ...., "The Abscission of Flower Buds and Fruits in Gossypium and its Relation to Environmental Changes," *Trans. Roy. Soc. Canada*, Section LV: 55-61, 1916.
47. ...., "Environmental Changes and their Effect upon Boll Shedding in Cotton (Gossypium herbaceum)," *Ann. New York Acad. Sci.* XXXIV: 1-131, 1931.
48. Lundegardh, H., *Environment and Plant Development* (Translated and edited by E. Ashby), Edward Arnold and Co., London, 1931.
49. Mahoney, C. H., Hunter, H. A., and White, Albert, "Performance Trials of New "Baby" Bush Lima Beans Grown for Canning—A Progress Report," *Proc. Amer. Soc. Hort. Sci.* 38: 541-545, 1941.
50. Martin, J. N., "The Physiology of Trifolium pratense," *Bot. Gaz.* 56: 112-126, 1913.
51. Mason, T. G., "Growth and Abscission in Sea Island Cotton," *Ann. Bot.* 36: 457-484, 1922.
52. Matthews, W. A., "The Influence of Planting Distances on the Yield of Snap and Lima Beans," *Proc. Amer. Soc. Hort. Sci.* 30: 567-570, 1934.
53. Maximov, N. A., *The Plant in Relation to Water*, MacMillan Publ. Co., 1929.
54. ...., and Zernova, Lydia K. (Summary of a Brief Paper on Stomatal Behavior), *Plant Physiology* 11, No. 3: 654, 1936.
55. McCollum, J. P., "Vegetative and Reproductive Responses Associated with Fruit Development in the Cucumber," *Cornell Univ. Agr. Exp. Sta. Memoir* 163, 1934.
56. Molisch, H., "Zur Physiologie des Pollens, mit besonderer Rucksicht auf die chemotropischen Bewengungen der Pollenschlauche," *Sitzungsher, d. kais. Akad. d. Wissl, Math-Naturw. Cl.*, 102: 423-448, 1893.
57. Murneek, A. E. "The Physiological Basis for Intermittent Sterility with Special Reference to the Spider Flower," *Mo. Res. Bul.* 106, 1927.
58. ...., Wittwer, S. H., and Hemphill, D. D., "Hormone Sprays for Snap Beans," *Proc. Amer. Soc. Hort. Sci.* 44: 428-432, 1944.
59. Nebel, B. R., "Lacmoid-Martius Yellow for Staining Pollen Tubes in the Style," *Stain Tech.*, 6: 27-29, 1931.
60. Odland, M. L., R. E. Larson, and Li-Paang Fi, "Thick Planting of Lima Beans Improves Yields in Good Weather," *Science For The Farmer*, 61st Annual Report Pa. Agr. Exp. Sta. Bul. 502, Supplement No. 2, 1949.

61. Parker, M. M., "The Interrelation Between the Effect of Fertilizer Composition and Method of Application on the Germination and Growth of Lima Beans and Snap Beans," *Proc. Amer. Soc. Hort. Sci.*, 37: 737-742, 1940.
62. Passecker, F., "The Value of Sugar Solutions for Determining the Usefulness of Pollen," *Gartenbauwissenschaft* 3: 201-236, 1930.
63. Pearsall, W. H., "Studies in Growth. IV. Correlation in Development," *Ann. Bot.* 37: 261-275, 1923.
64. Radspinner, W. A., "Effects of Certain Physiological Factors in Blossom Drop and Yield of Tomatoes," *Proc. Amer. Soc. Hort. Sci.* 19: 71-75, 1923.
65. Roberts, R. H., and Struckmeyer, B. Esther, "The Use of Sprays to Set Greenhouse Tomatoes," *Proc. Amer. Soc. Hort. Sci.* 44: 417-427, 1944.
66. Salisbury, E. J., "On the Causes and Ecological Significance of Stomatal Frequency with Special Reference to the Woodland Flora," *Phil. Trans. Roy. Soc.*, Ser. B., Vol. 216: 1-65, 1928.
67. Sande-Bakhuyzen, van de H. L., "Studies Upon Wheat Grown under Constant Conditions," *Plant Physiology* 3: 1-30, 1928.
68. Sandsten, E. P., "Some Conditions which Influence the Germination and Fertility of Pollen," *Wisc. Agr. Exp. Sta. Res. Bul.* 4, 1909.
69. Sass, John E., *Elements of Botanical Micro-technique*, McGraw-Hill, New York, 1940.
70. Schoch-Bodmer, H., "Zur Physiologie der Pollenkeimung bei Corylus avellana: Pollen- und Narben Sangkrafte, Quellungserscheinungen der Kolloide des Pollens," *Protoplasma* 25 (3): 337-371, 1936.
71. Schmucker, Theodor, ""Uber den Einfluss von Borsaure auf Pflanzen insbesondere keimende Pollenkorner," *Planta* 23 (½): 264-283, 1934.
72. Shaw, G. W. and Sherwin, M. E., "Production of the Lima Bean," *Univ. Calif. Agr. Exp. Sta. Bul.* 224, 1911.
73. Smith, F. L., A. H. Holland, and J. H. MacGillivray, "Forty-five Years of Continuous Cropping with Lima Beans," *Science* 105 (No. 2720): 179-180.
74. Smith, Hugh B., "Stomata Index and Transpiration," *Science* 89: 268-269, 1939.
75. Smith, Ora, "Relationship of Temperature to Anthesis and Blossom Drop of the Tomato, together with a Histological Study of the Pistils," *Jour. Agr. Res.* 44: 183-190, 1932.
76. Smith, P. F., "The Influence of 3-Indole acetic Acid on Pollen Germination," *Science* 90: 163-164, 1939.
77. ...., "Studies of the Growth of Pollen with Respect to Temperature, Auxins, Colchicine, and Vitamin B_1," *Amer. Jour. Bot.* 29: 56-66, 1942.
78. Thompson, H. C., *Vegetable Crops*, McGraw-Hill Book Co., Inc., 1949.
79. Tiedjens, V. A., and Schermerhorn, L. G., "Fertilizer Requirements for Lima Beans," *Proc. Amer. Soc. Hort. Sci.* 37: 743-746, 1940.
80. Timmerman, H. A., "Stomatal Numbers; their Value for Distinguishing Species," *Pharmaceutical Jour. Reprint* 118, 1927.
81. Tukey, H. B., Hamner, C. L., and Imhofe, B., "Histological Changes in Bindweed and Sow Thistle following Applications of 2,4-Dichlorophenoxyacetic Acid in Herbicidal Concentrations," *Bot. Gaz.* 107: 62-73, 1945.
82. Varrelman, F. A., "Anent Parthenocarpic Apples," *Science* 87: 414, 1938.
83. Von Berg, H., "Beitrage zur Keuntnis der Pollenphysiologie," *Planta* 9: 105-143, 1929.
84. Weaver, J. E., and Read, M., "Investigations on the Root Habits of Plants," *Amer. Jour. Bot.* 12: 502-509, 1925.
85. Werneek, H. L., "Phenology in its Application to Agriculture," *Int. Rev. Sci. and Pract. of Agric.* 2: 13-21, 1924.
86. Wester, R. E., and R. Magruder, 'Effect of Boron on Plant Growth and Dry Seed Yield in Lima Bean (Phaseolus lunatus L.)," *Proc. Amer. Soc. Hort. Sci.* 38: 472-474, 1941.
87. Wester, R. E., and Marth, P. C., "Effect of Some Growth Regulators on Yield of Bush Lima Beans," *Proc. Amer. Soc. Hort. Sci.* 49: 315-319, 1947.
88. ...., "Some Effects of a Growth Regulator Mixture in Controlled Cross-Pollination of Lima Bean," *Proc. Amer. Soc. Hort. Sci.* 53: 315-318, 1949.
89. White-Stevens, R. H., and Hartman, J. D. "Lima Bean Production in Relation to Fertilizer Placement and Seed Spacing Under Long Island Conditions," *Proc. Amer. Soc. Hort. Sci.* 36: 517, 1939.
90. Whiting, A. G., and M. A. Murray, "Abscission and Other Responses Induced by 2, 3, 5 triiodobenzoic Acid in Bean Plants," *Bot. Gaz.* 109: 447-473, 1948.
91. Wittwer, S. H., and Murneek, A. E., "Further Investigations on the Value of "Hormone" Sprays and Dusts for Green Bush Snap Beans,' *Proc. Amer. Soc. Hort. Sci.* 47: 285-293, 1946.

92. Wolf, B., "Chemical Factors Influencing the Set of Henderson Lima Beans," *Journal of the American Society or Agronomy* 34: 646-650.
93. Yapp, R. H., "Spirea Ulmaria, L., and its Bearing on the Problem of Xeomorphy in Marsh Plants," *Ann. Bot.* 26: 815-871, 1912.
94. Zalenski, V., "The Intensity of Transpiration in the Upper and Lower Leaves," *Bul Agr. Inst. Saratov.* 1: 13-17, 1923. *Bot. Abst.* 15: 165-166, 1926.