CATALASE ACTIVITY IN RELATION TO THE GROWTH CURVE OF BARLEY

BY

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CATALASE ACTIVITY IN RELATION TO THE GROWTH CURVE OF BARLEY.

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CATALASE ACTIVITY IN RELATION TO THE GROWTH CURVE OF BARLEY

Introduction

Many investigators have studied the growth curve of living organisms. In all cases, when the measure of size of an individual taken at successive intervals is plotted against time, the resulting curve is sigmoid. It is often possible to more or less accurately explain such a curve upon a mathematical basis, and this has been done in a number of different ways. However, the formulation of any definite and accurate mathematical statement of growth is so beset with difficulties that there is little agreement between zoologists and botanists or even between individual plant physiologists.

Phenomena necessarily concomitant with growth may logically be suspected of possessing a "grand period" following closely that of growth. In respiration, as in growth, we find that the factors water, heat, and oxygen are essential to the process. In fact, the occurrence of respiration with growth is universaal, and certain authors have stated that the grand period of respiration follows closely the grand period of growth. There are many difficulties connected with the investigation of the problem directly, but it was thought possible to attack it through an easily determined activity of the plant: one presumably directly correlated with metabolism. Ever since Loew first studied catalase activity, such

a direct relation has been suspected between that phenomenon and metabolic activity and in many instances seems to have been proven.

The economic importance of the cereal plants lends a particular interest to any research into their fundamental processes. Of the cereals, barley is particularly adapted to such investigations. The plant is easily and quickly grown, and is small enough to be readily sampled, measured, and weighed.

Review of Literature

Early in the seventies Sachs (1874) observed an orderly and invariable procession of changes in length growth of plant which is followed not only by the whole stalk but by each internode and even by each cross-section (Querschnitt) of an internode. He found that this series of events starts with small daily increments, increases to a maximum and then gradually drops off again until growth ceases altogether. This he called the "grand period of growth". Each internode area has its actively-growing period a little later than the portion adjoining, which is a trifle older. Thus the grand period of growth of any plant part is made up of the summed-up total or the resultant of the grand periods of each small subdivision of that part.

In the graphs drawn by Sachs increments in growth were plotted against time and the resulting curve is roughly symmetrical with the peak in the middle portion where growth is most active. If, however, the total lengths instead of increments, are plotted against time, the curve becomes sigmoid in character. Such a curve illustrates also the progress of events in a monomolecular autocatalytic reaction. Robertson (1923) emphasizes the resemblance between these two curves and argues that "the fact that each growth cycle begins slowly and progressively increases in velocity until the moment of maximal growth velocity is attained at the center of the cycle, is sufficient in itself to show that the process of growth is autocatalyzed." The assumption is made that one of the products of the reaction is a catalyst which reacts on the substrate and produces an accelerated growth. In stating the reaction mathematically the usual monomolecular reaction $\frac{dx}{dt} = K\ (a-x)$ must be modified to include the effect of the catalyzer. "The velocity constant is multiplied by the mass (or concentration) of a catalyzer which is proportional to the mass (or concentration) of the products of the reaction. It thus assumes the form

$$dx = k_1\ x\ (a-x)$$ which when integrated yields the relation

$$\log \frac{x}{a-x} = k_1\ (t-t_1)$$

where $k = k_1 a$, and t_1 is the time at which the reaction is half completed, that is when $x = \frac{1}{2}\ a$".

He gives a possible explanation of the inflection of the curve by assuming that the reaction is a reversible one, a negative acceleration being produced by the accumulation of the products of the reaction in the reverse

direction, to the same degree as it went forward. In answer to the objection that growth is not a single chemical reaction but involves "multitudinous parallel, successive and independent reactions" he asserts the principles of the "master reaction" where in a system of chemical reactions of which each one derives its substrate from the preceding and in turn supplies the substrate for the reaction which follows it. "The specifically slowest reaction in the series will become the master reaction governing the time-relations of the whole.

Reed and his co-workers (1919, 1924, 1927) and Gaines and Nevens (1925), Popp (1926), Monnier (1905), and Crozier (1926), also believe that the growth curve can be mathematically represented by the autocatalytic formula, although the last adds to the dubiety of the theory when, although he accepts the autocatalytic nature of growth, he objects to Robertson's explanation of the process as being due to first order master reactions. He prefers to postulate not one velocity constant but at least two and probably more.

In possible support of the existence of such a growth catalyst, may be cited the findings of Appleman (1918) that small seed pieces of potato tubers produce smaller sprouts than do large ones. The amounts of the "food reserves" are greatly in excess of those needed by the young sprouts in small as well as in large pieces.

Gericke (1924) grew successive 6 weeks crops of wheat in the same culture solution and found crop 2 injured and crop 3 benefitted thereby. He concludes that "plants may be similar to animals in that they have a physiology which is subject to growth inhibitory, growth stimulating and sensityzing agencies produced by the organism itself."

Enriques (1909) severely critcizes the autocatalytic theory especially as applied to the latter half of the growth curve. He says very pertinently (p. 347). "So ist es möglich zu sagen, dass die benutzte Formel in keiner Weise eine privilegierte Stellung in bezug auf die Nachahmung zwischen den anderen von demselben Typus besitzt, sondern nur einen Grad besserer Approximation darstellt im Verhaltnis zu den Formeln, die aus einer algebraischen Funktion ersten Grades nach x herstammen. Es ware aber nooh besser, eine algebraische Funktion dritten, vierten u.s.w. Grades zu benutzen, and hätten wir es gemacht, so hätten wir uns in keiner Weise mehr von der richtigen Interpretation der biologischen Tatsachen entfernt; nur hätten wir auch die Moglichkeit vor uns, eine bessere Approximation zu gewinnen and mehrere Inflexionspunkte nachzuahman."

In a paper presented before the Physiological section of the Botanical Society of America (December 30, 1926) Brody showed a series of 25 charts which pointed to the following conclusions:

1. The period of growth in animals and plants consists of two phases: (1), A self-accelerating phase during which the time-rate of growth increases with the increase in size of the organism, and (2), a self-inhibiting phase during which the time-rate of growth decreases with the increase in size of the organism.

2. The junction between the self-accelerating and the self-inhibiting phases of growth occurs at puberty in higher animals, and flowering in higher plants.

3. In the case of warm-blooded animals, the self-accelerating phase of growth is made up of several (probably five) stages. During each of these stages the percentage-rate of growth is constant. Each of these stages of constant percentage-rate of growth passes abruptly into the succeeding stage of constant, but lower, percentage-rate of growth. The abruptness with which one stage passes into the succeeding stage is of the order of abruptness of metamorphosis in cold-blooded animals. The higher plants also pass through a similar series of stages, but the data are not sufficiently adequate to give full confidence to this conclusion.

4. The self-inhibiting phase of growth is uneventful. The time-rate of growth during this phase declines at a constant percentage-rate.

5. As to cycles, the charts do not indicate the presence of symmetrical cycles in the sense in which Robertson defined them.

Brody (1927) finds several distinct stages of growth in warm-blooded animals each of which possesses its own percentage rate of growth and which are separated from each other by distinct and abrupt breaks which he believes corresponds to metamorphosis in cold-blooded animals.

On account of these breaks in the curve he introduces the "principles of discontinuity into the consideration of the growth process." However, he argues that while each of the several ("perhaps five") epochs which preceded the inflection at puberty has its own growth constant, none can be quantitively represented by the equation employed by Robertson.

$$\frac{dw}{dt} = k\,w\,(a - w)$$

but by the equation $\frac{dw}{dt} = k\,w$

However, after the inflection at puberty the usual monomolecular reaction

$$\frac{dw}{dt} = k\,(a - w)$$ will operate

where $\frac{dw}{dt}$ = instantaneous gain in weight; w= weight at the given instant; (a - w) growth yet to be made and k = proportionality constant or relative rate of growth. It is understood, of course, that the value of k in the above equations will differ numerically for the different growth epochs. In conclusion he says :

"Now that we have equations for every phase of the growth curve, then, if one considers it desirable, andif the needed mathematical knowledge is available,

one can combine the equations of the several phases into one equation to represent the whole curve of growth in a manner analogous to the equations at present available to represent the time curves of consecutive radioactive transformations. But this is a mathematical problem, which, as far as the writer can see, is of no particular interest at the present moment to the biologist. The biologist is interested in reducing the phenomenon to its simplest elements; a complex equation to represent the whole curve of growth cannot, as the writer sees the situation, simplify the phenomenon. Such an equation may only be desirable for interpolation purposes; but practically, one can interpolate with greater safety from the curves themselves."

Gregory (1926) is convinced that only the latter 60 per cent of the growth curve can be represented by an autocatalytic equation. On the other hand Bakhuyzen (1926) states that "the first part of the growth cycle can not be represented by an autocatalytic reaction" and "the latter part of the cycle is only approximated by such an equation."

In noting Robertson's change of position in regard to a growth catalyst Priestly and Pearsall (1922) remark:

"Because the quantitative data of growth obtained under uniform external conditions can be represented by a single curve, it by no means follows that throughout the whole period of growth, the same internal factors alone remain operative. It is probably significant that Robertson's continued study of the problem led him (1) to assume first a greater complexity of internal conditions and (2) to consider separately the different regions of his curves."

To this writer, the rough resemblance of the curve of a monomolecular autocatalytic reaction to the curve of growth seems to be not causal but merely fortuitous.

V. H. Blackman (1919) has a much simpler method of expressing the growth curve mathematically. As a growing individual increases in size, the number of cells capable of multiplication also progressively increases and this increase is continuous. He would therefore apply the compound interest law, using the formula $W = W_o E^{rt}$ where W = final weight, W_o = principal or initial weigl (of seed). E is the base of the natural logarithm, r = interest or percentage increase in dry matter per unit of time or "the efficiency of the plant as a producer of new material" and t = the time. The difference in "r" may be due to (1) rate of assimilation per unit area of leaf, (2) difference in respiration rate, (3) difference in rate of distribution of material to leaves and axis and (4) mechanical relations of larger size. He notes that "r" falls markedly at the time of the formation of the inflorescence. Gregory (1921)and (1926) in his studies on Cucumis and barley, accepts the compound interest principle. This method, however, is criticized by West, Briggs, and Kidd (1920) who correctly say that the "rate depends on various external factors" and upon "the amount of the growing material," but they argue that the amount of this growing material is unknown on account of the deposition of inactive structures, and furthermore that the growing material is not all equally active. Consequently Blackman's W (dry weight) is not a measure of the growing material and his "r" (rate of growth per unit dry weight) is not a constant. These authors while realizing the difficulties in determining precisely the internal and external factors affecting growth and evaluating them, express the hope that "after these factors are known and their values expressed in appropriate units, we may be in a position to state constants for the growth rates of particular plants

applicable to the whole life cycle and such constants will not only be of use from an economic point of view in comparing plants but also will have physiologic significance."

Buchanan (1918) in the "life phases of a bacterial culture" studies multicellular growth in probably its simplest (least complex) form. In this "simple" growth study he finds evidence of 7 stages as follows:

1. Initial stationary phase probably comparable with seed dormancy.
2. Lag or phase of positive growth acceleration.
3. Logarithmic growth phase.
4. Phase of negatively accelerated growth.
5. Maximum stationary stage.
6. Phase of accelerated death rate.
7. Logarithmic death phase.

Probably the only complication existing in the problem which is present to a more potent degree than in annual plants is the phenomenon of cell death in a medium incapable of removing from the environment of living cells the possibly deleterious substances produced thereby. This may be the cause, (but in part only) of the existence of that part of our curve after the major inflection. We may probably neglect phases 6 and 7 as our annual plant reaches maturity in Buchanan's fifth stage. Buchanan has endeavored to fit mathematical formulae to each of his seven stages, but only in the third where Blackman's compound interest law seems to apply, do the mathematical relationships seem seem certain and "relatively simple."

Pearl and Surface (1915) divide the growth period of maize into four cycles which overlap to a certain extent: first, that of root growth in which increase in size is rapid; second, the leaf cycle up to tasseling where the growth is

steady but with a moderate increase in height; third, tassel stage characterized by rapid elongation of upper internode until blooming (dehiscence of anthers) occurs;and fourth, the ear cycle from fertilization of the ovule until the kernel is mature. During this fourth stage there is no increase in height.

Miss Hicks (1928-I)(1928-II) divides the growth cycle of wheat into three stages: (1) active assimilation of first leaf and exhaustion of endosperm of seed, (2) vegetative cycle up to ear formation, and (3) the fruiting stage. In studying carbon and nitrogen content in the growing plant, she finds that the composition follows the development of the plant irrespective of its age in days. Balls (1912) believes that the ability of the roots to absorb soil moisture is a factor limiting growth in the cotton plant in Egypt and cites as evidence of his contention that while the "shedding" of many of the bolls is a normal occurrence soon after flowering, it is "disproportionately severe" after an early Nile flood which so raises the water table that the lower roots are asphyxiated.

In criticising this assumption, Pearsall (1923) concludes from a study of root development that "It is shown from these results that in cotton, the decreased rate of growth of the stem can be attributed to flowering and that subsequently the decreased flowering rate can be attributed to the development of fruits nearer the source of supply of the presumed limiting food factors."

Briggs, Kidd and West (1920) analyzed the old data of Kreusler (maize) and found the relative growth rate to decrease markedly at two points corresponding to the appearance of staminate and pistillate flowers.

Priestly and Evershed (1922) have found deviations from the perfect sigmoid curve to occur at the time of root development. They made a quantitative study of root growth in cuttings of tradescantia and tomato and found that while roots

are not so directly influenced by environment as are other parts of the plant there was a flattening of this curve at the times of secondary and tertiary root formation followed by an accelerated growth as soon as the roots became functional. This they ascribe to the effect of diversion of food material to form secondary and tertiary roots and oxidation of this material at the point of formation of these secondary and tertiary root meristems.

Fernald (1925) found that developing buds have an osmotic concentration of sap varying in degree directly with the vigor of growth and suggests that a correlation exists between the osmotic concentration of plant tissues and the tendency to inhibit growth or be inhibited. Appleman (in conversation) emphasizes the idea that osmotic concentration may be a result and not a cause of growth inhibition. Should there be competition for soil nutrients between different parts of the plant, differences should appear in the osmotic concentrations as well as in the growth rates of those parts.

Murneek (1925) states that "vegetative growth in the tomato diminishes at the exact time and in exact proportion to the amount of flowers formed and fruit set and the rate of vegetative growth appears to be controlled by the developing fruit." He believes that the phenomenon may be the outcome of different stages of localized nutrition and of C/N relations.

Davidson and LeClerc (1924) found that total yield as well as composition of the kernel depends upon the amount of nitrogen available to the plant at definite stages of growth, and Miss Hicks (1928 I) found that the composition of the wheat embryo was affected by nitrogen shortage.

Pearsall (1923) believes that food supply is a limiting factor in the early period of root growth from seeds and cuttings. In his experiments he assumed that the stems competed successfully for the cotyledonary food supply, causing a decrease in root growth rate.

That there is competetion between different parts of the plant is indicated by other authors. Murneek (1926) grew tomatoes under high nitrogen conditions and found that the growth curve showed an inflection at flowering. He defruited the plants and they immediately greened up and started vegetative growth again and the curve resumed its former direction. As soon as fruit again set, another flattening of the curve occurred which, too, disappeared when the plant was again defruited. He even localized this phenomenon by using a part of the plant as a check.

Miss Hicks (1928 I, 1928 II) has studied the C/N ratio in the wheat plant. By a careful and cleverly devised chemical technique she has been able to determine the carbon and nitrogen contents, not only of the whole plant and its major parts but also of the individual leaves and flower parts even to the extent of analyzing the anthers. She, too, finds that the fruit competes successfully with the vegetative organs for nitrogen and (1928 II) states the belief that "Nitrogen is deliberately withdrawn from the leaves into the developing ovaries and embryos and death thus follows as the result of the greatly increased C/N ratio which is inhibiting to both growth and reproduction."

Turner (1922) finds that the growth ratio of tops and roots in barley and corn show significant increases as the NO_3 concentration is increased and explains on the basis of increased rise of carbohydrates in the tops because the greater nitrogen supply makes for greater growth. This results in a decrease in the supply of carbohydrates for the roots which may bring about an absolute or a relative reduction in root growth.

Gustavson (1927) from his studies on the growth of cucumbers, summer squash, muskmelon and tomato, is inclined to believe that growth in fruits is mainly a matter of nutrition. This is much the same idea as that of Priestley and Pearsall. He would explain the fact that cell formation ceases by saying that there is a lack of nutrient material and mentions the fact that the conducting vessels of the plant

do not enlarge in proportion with the pulpy part. Toward maturity, then, the conducting system becomes inadequate. Bakhuysen and Alsberg (1927) have made a critical examination of the mathematical formulae by Robertson, Blackman, Kidd, West and Briggs, and reject them all. They also recalculated the data of Kreusler on maize and believe that growth is tied up internally with the water relations of the plant. In speaking of Murneek's work they say the fact that fruit is able to develop in the tomato at a time when no vegetative growth takes place "is not explained by the fact that the fruit monopolizes the nitrogen of the plant at the expense of the rest of the plant. It proves only that the conditions of the cells in the fruit are such that they can utilize the nitrogen, whereas the vegetative cells can not."

Porterfield (1928) found close agreement between the curve that was based on actual daily growth measurements of bamboo shoots and the curve as computed from Robertson's formula. However, as he states, the bamboo shoot is a growing part of the plant which elongates on the basis of the stored foods in the original bud. Consequently, there will be no complications because of developing roots and growth can be expected to conform to the sigmoid curve. The compound interest law operates in early growth and the major inflection can be due to the inactive materials laid down in the shoot and to progressive nutritional shortage.

A number of investigators have noted the general similarity between the "grand period of growth" and the "grand period of respiration" and logically the inference that the two are interdependent seems a reasonable assumption. In text books we commonly find statements made concerning the relations between the two but without citing the evidence. For example:

Raber (1928) states that "The respiration curve carried through the life of the plant runs parallel to the curve (i.e. of growth) with the result that the grand period of growth and the grand period of respiration as measured both by the amount of oxygen consumed and the amount of carbon dioxide given off are very similar."

Palladin (1923) cites investigations dating back as far as 1875 and 1876 when he says "Thus may be constructed a grand period of respiration, the form of which is practically identical with that of the grand curve of growth. This grand curve of respiration was first shown by A. Mayer who measured the oxygen absorbed. Like results were obtained by Borodin and Rischavi who determined the amount of carbon dioxide eliminated."

Kostychew (1927) also cites the work of A. Mayer and of Rischavi and states that the grand curve of respiration is similar throughout to that of the grand period of growth in germinating seeds.

Warburg (1928) makes a much more inclusive statement when he says that "Respiration furnishes the driving force for everything that happens in living matter."

Stålfelt (1926) states that "From the older as well as from newer researches it is known that intensity of respiration in seeds and in young plants experiences a kind of "grand period" which takes the same course as that which characterizes the growth increase of these objects and that the respiration and growth periods, in general, go hand in hand."

By inference, Miss Hicks (1928 III) in her studies on regeneration of willow cuttings associates respiration and growth. She says "The respiratory activity of the leaf appears to create a suction upon all the tissues below it. Thus the ratio of carbon to nitrogen in these tissues is reduced by carbon loss. The pull of transpiration, too, causes the nitrogen to rise in the stem. Thus the C/N gradient (i.e. low at apex and high at base) is maintained, while the ratio itself below the leaf is lowered, so that growth of roots is established."

There are, however, comparatively few investigations which show a definite relation between respiration and growth. Kidd, West, and Briggs (1921) in their study of Helianthus found that "The fall in the value of the respiratory index (respiration per gram dry weight per hour) with age follows closely that in the value of the relative growth rate, thus indicating a close connection between the "internal" factor of respiration and the "internal" factor for growth." These determinations were made in the field on uncut plants by enclosing the plant in an air tight fabric bag and drawing a current of atmospheric air through and determining the carbon dioxide in the outgoing air by means of caustic soda in two Reiset towers. This operation was carried out on five different occasions scattered throughout the life cycle of the plant.

Inamdar, Singh and Pande (1925) say that the course of the respiratory index runs parallel to the growth rate curve in the cotton plant also. They conclude, however, that the course of the respiratory index merely expresses the intensity of the series of protoplasmic activities which influence growth rate.

Stålfelt (1926) found a definite grand period of oxygen uptake per unit of fresh weight and per organ in seedlings of Sinapis alba. This was most marked in the cotyledonary leaves. For different parts of the plant the maximum of the period falls at different time points and furthermore the duration of the period if unequal. He gives no data to show a parallelism between the grand periods of oxygen consumption and growth.

Hover and Gustafson (1926) followed another method of attack in studying respiration in the leaves of corn, sorghum, wheat and oats. As the leaves of these plants are youngest at the top and are progressively older toward the crown, they removed all the leaves of a plant and determined the CO_2 per unit weight of leaf given off from each one during a period of about 22 hours. Since the leaves were all removed at the same time, were placed in their respective respiration chambers the same time, and the CO_2 given off was determined for the same time under the same temperature and other environmental conditions, the differences in rates of respiration which appeared were due to differences in the leaves themselves. Their results show that as the leaves of these plants increase in age there is a decrease in rate of respiration, but as the leaves become still older, past about middle age, the rate gradually increases. They conclude, therefore, that the respiratory cycle does not correspond with the grand period of growth.

Hafenrichter (1928) in his studies in respiration of soybean seedlings studied two varieties, one "low oil-high protein" and the other "high oil-low protein". After the seeds were germinated and the seed coats removed, the seedlings were grown in the dark in sterilized tap water, consequently the food reserves of the cotyledons presumably were the sole source of the carbon utilized in respiration and the experiment was not complicated by photosynthesis. Respiration rates were determined at 24 hour intervals. The greatest intensity in respiration rate occurred during the early stages of development and an upward trend occurred again just before the seedlings succumbed to starvation. In each variety there was much variation in these rates from day to day and without any indication of periodicity. There was no similarity between the variations of the two varieties. Furthermore, the variations were different in degree and in point of time (except for those at the early stage) at different temperatures. It is conceivable that differences in the shape of the curve would occur between two seedlings or two batches of

seedlings of the same variety. It is to be regretted that the series of determinations was not repeated. During the time that the soybean seedlings were losing weight on account of respiration both hypocotyl and epicotyl were growing at the expense of the food reserves in the cotyledon. This growth he measured in terms of length extension of the organ. He found no direct relation between respiration and growth. Nor is there such a relation between the periodicity of growth and that of respiration. "The maximum rate of respiration is reached earlier in the development of the plants than is the maximum rate of growth" and "the functional interactions which determine growth on the one hand and respiration on the other are affected differently by the same change in environmental conditions. The difficulties in the way of studying both respiration and growth on the same plants or upon similar plants in exactly the same environment are so great that the result must be inconclusive, and a method more nearly accurate and more easily followed is needed before the problem can be solved.

Catalase Activity

A correlation between catalase activity and respiratory activity was first found by Appleman (1910) in the potato tuber after he had discovered a way to prevent the destruction of the catalase by grinding the plant tissue with calcium carbonate.

Catalase activity may be defined as the ability of a product of living material to rapidly decompose hydrogen peroxide with the evolution of molecular oxygen as an end product. The reaction follows the mass action law. If the substrate (hydrogen peroxide)is in excess the monomolecular formula holds for catalase. When catalase is in excess the action is monomolecular for the peroxide, but when there is exactly enough catalase to decompose the H_2O_2 present the action is bimolecular (Northrop 1925). When there is a limited excess of

H_2O_2, (Morgulis (1928) defines as the enzyme unit the quantity of catalase sufficient to decompose 70 per cent of H_2O_2 under standard conditions at 2° C.) the amount of catalase activity is proportional to the amount of oxygen liberated since catalase activity is destroyed in the reaction. The reaction quite generally has been thought to be enzymatic. Loew (1901) and Yamasaki (1920) have made catalase preparations from plant material and Morgulis (1921, I) etc. and his co-workers have separated it from liver and kidney tissues. There are, however, two facts which are contrary to our present definition of an enzyme; first, proportionality exists between catalase activity and the oxygen evolved and second, catalase activity is destroyed in the process. The writer prefers to speak of the phenomenon as "catalase activity" leaving the question of the character of the process in abeyance.

The mechanism of the catalase reaction is unknown but Morgulis et al (1926 II) suspect that catalase exists in two forms, the inactive by dissociation producing the active form which may react with H_2O_2 in two different ways; first, by oxidation producing "poisoned" catalase, and then by reduction producing an inactive form plus molecular oxygen. If atomic oxygen is produced during the process the destruction of catalase might result and there would still be rough proportionality between catalase and oxygen produced.

Cole (1926) says that catalase "is apparently present in nearly all living cells and also in the blood. It seems to be a special form of peroxidase able to transfer oxygen from one molecule of hydrogen peroxide to another. The ordinary peroxidases can only transfer oxygen from hydrogen peroxide to some oxygen acceptor and are not necessarily catalases." He cites Mrs. Callow (1923) as having discovered that catalase is present in all aerobic bacteria but absent from all strictly anaerobic organisms and states that it is also absent from certain streptococci and pneumococci which can, however, grow to a certain extent in the presence of oxygen. He also cites McLeod and Gordon (1923) as

having shown that hydrogen peroxide accumulates in these organisms in sufficient quantity to inhibit growth. If catalase be added the peroxide is destroyed and more luxuriant growth occurs and the fact that oxygen uptake of aerobic bacteria is so very much greater than that of anaerobes may be related to the presence or absence of the catalase.

Dixon (1925) found that when the purine bases were oxidized by molecular oxygen to uric acid in the presence of xanthic oxidase as a catalyst, the oxidase is progressively destroyed during the course of the reaction by the hydrogen peroxide known to be produced in the reaction, but if the hydrogen peroxide is destroyed by the catalase the oxidase can do its work in the reaction, resulting in the production of uric acid.

While purine metabolism is a typically animal phenomenon and uric acid does not occur in plants [Haas and Hill (1921)] Fosse (cited by Haas and Hill) has observed small amounts of urea in higher plants and it is not unlikely that the purines are waste material in plants as well as in animals.

Williams (1928) regards the decomposition of hydrogen peroxide by catalase as involving two reactions; namely, the catalytic decomposition of the peroxide which is at a maximum at the optimum pH 6.8 - 7.0 and the "indirect inactivation" of catalase by the "nascent" oxygen produced by the hydrogen peroxide and still adhering to the catalase surface.

Although the function and mechanism of the catalase reaction is almost entirely speculative there has ever been the opinion among investigators that this phenomenon is in some way linked up with metabolism and with respiration in particular.

The respiration of potato tubers may be increased by such methods as treating with ethyl bromide; storing at a low temperature and then bringing them to room temperature; and by placing them in the light to "green up".

Appleman (1916) made catalase determinations upon treated and untreated tubers which showed that changes in respiratory intensity and catalase activity are parallel. Determinations of the catalase activity in stem and seed ends of the same potato showed these values to be propontional to the amount of respiration in the part tested. Later (1918) he found that respiration and catalase activity decrease at almost the same rate in the expressed juice of sweet corn in storage and concludes that catalase activity is a fair index of the comparative intensity of respiration in the tissues of sweet corn and potato.

Crocker and Harrington (1918) found that respiratory intensity parallels catalase activity in seeds of Johnson grass and probably in Avena fatua but not in Amaranthus seeds. Burge (1918) found that catalase in the blood of animals increases with feeding and the production is greater with foods capable of rapid absorption and much greater when digested foods (meat digest greatest of all) are fed. Moderate exercise also increases catalase content but fatigue decreases it. He concludes that "food and exercise produce an increase in catalase with resulting increase in oxidation by stimulating the liver to an increased output of this enzyme".

Weiss and Harvey (1921) found catalase activity to be strongly correlated with growth, in the proliferation produced by the potato wart disease in spite of the high acidity of the growing tissues.

There is much better agreement among plant than among animal physiologists. Becht's (1919) results seem to indicate that the catalase reaction is unreliable as a measure of metabolism in animals as he found a variation of as much as 1000 per cent in the bloods of different dogs under "identical conditions". Another dissentor on the animal side is Morgulis (1921, II) who placed frogs under temperature conditions which "it was estimated" caused a change of 300 to 400 per cent in the metabolic rate and found that this changed metabolic rate had no influence on the catalase content of the frogs. He concluded that "it is certain that it (catalase activity) is certainly not a measure of metabolic activity".

Heinicke (1923) found more catalase activity in the leaves of apple trees fertilized with sodium nitrate than in the checks and, furthermore, that when but half the plant was given nitrate, that portion directly above gave an increased catalase reaction indicating that the unfertilized half of the plant could be used as a check. He suggests that, since catalase activity shows the nitrogen effect in the apple where chemical analysis gives no significant difference in nitrogen content, "it is very probable that the ability to decompose hydrogen peroxide is a more sensitive measure of the metabolic status of the tissue than the usual chemical analysis". He makes an interesting inference when he says: "Many of the preparations of apple leaf tissue show greater power to decompose hydrogen peroxide than is reported in the literature for tissue from organs more actively engaged in growth processes". Respiration is especially active in leaves. However, it does not seem justifiable to compare results on catalase activity from different tissues when the determinations are made by different investigators using different methods. But in a later paper (Heinicke, 1924) he says that the indications are that there is "no consistent relation between respiratory intensity and catalase activity".

Auchter (1923) confirmed the findings of Heinicke on apple leaf tissue and found that catalase activity was greatly increased in the leaves wherever nitrate of soda was applied to plants of privet, oak and peach. In all these the growth became more vigorous and the plant contained a significantly greater amount of nitrogen than in the checks to which no nitrate was added.

Shull and Davis (1923) state that in the dimorphic seeds of Xanthium, the upper seed, which shows a delayed germination, exhibits constantly less catalase per unit of dry matter than does the lower seed.

Miss Rhine (1924) believes that catalase activity is somehow related to the presence of oxygen and oxidation, but found that during germination, as respiration rapidly increased, catalase activity decreased, consequently the latter cannot be a part of the respiratory mechanism. She concludes that catalase

activity can be a measure of metabolism only when there is no rapid change in respiration.

Morinaga (1925) found that aerobically grown rice seedlings with high catalase use much more oxygen than do anaerobically grown seedlings with low catalase activity and therefore believes that catalase has a close positive connection with the normal respiration.

Davis (1926) has shown that the degree of viability of seeds cannot be tested by the presence of catalase in them, but "if seeds were soaked for a time in warm water the catalase of dead seeds is disorganized very rapidly while that of viable seeds is not affected".

Lantz (1927) does not find a close correlation between catalase activity and respiration in germinating corn seeds of different chemical compositions (high and low oil, high and low protein).

Davis (1927) states that catalase activity parallels after ripening very closely. "It may be taken as an indication of the direction of change whether toward deeper dormancy or toward germination". She has also used it to detect dead seeds. She found great variability in catalase activity of seeds of equal age.

Gracaňin (1927) states that "catalase activity of seeds is not a useful index of their vitality since dead seeds may still possess it, but seeds containing no catalase will not germinate".

Knott (1926) found that the younger and older leaves of spinach are usually low in catalase activity, while those intermediate in age have higher and approximately equal activity. But his results may be criticised since the young leaves probably contained a much lower dry matter content which should be corrected for, and the oldest leaves were yellowing and dying. On the basis of the work of Hover and Gustafson (cited above) these results are at variance with the conclusion that catalase activity and respiration are associated.

Ezell and Crist (1927) found a significantly negative correlation between the activity of catalase and growth or size of the plants of lettuce, radishes, and spinach.

Burge and Burge (1928) determined the catalase activity of pine needles and of rabbits' blood in both winter and summer. They found a higher content in pine needles during the summer and in the blood during the winter, or in both cases during the season when respiratory metabolism is at its highest point.

Materials and Methods

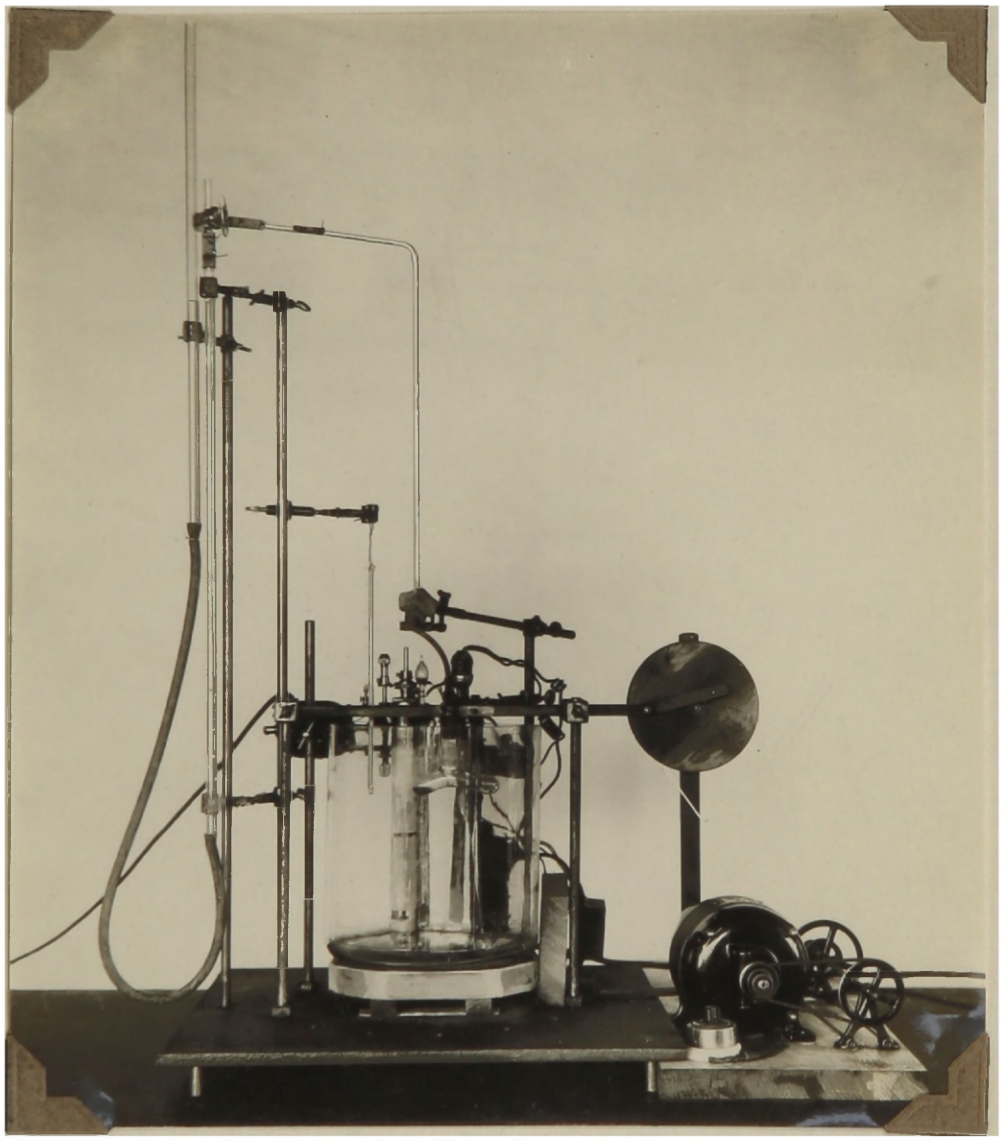
Standard method for determining catalase activity

Since catalase activity is used merely as a test of metabolic activity the various conditions affecting its action are not of great moment here, except in so far as they produce abnormal results. Efforts have been made, therefore, to determine the most nearly optimum conditions for the phenomenon which are practicable and then to keep those conditions as nearly uniform as possible throughout the investigation.

Description of the apparatus

The apparatus used (Plate I) is a modification of the one described by Appleman (1915). It consists of a square wooden motor-driven arm sliding through supports at either end and carrying a Bunzel tube 22 mm. inside diameter, each arm of which has ample capacity for 4 cc. of liquid. A flexible rubber tube of sufficient length to allow a full excursion of the shaker arm connects the Bunzel tube with a small bore glass tube which is in direct connection through a three-way glass stop cock with the upper end of a 50 cc. glass burette, the lower end of which is connected by a thick-walled rubber tube with the lower end of a second burette of the same capacity which may be raised or lowered at will to equalize the water levels in the two burettes. The gas-conducting portions of the apparatus are purposely made of small bore material to minimize the volume of gas subject to temperature and pressure changes. No attempt is made to determine N. P. T. volumes since, as will be seen later, such accuracy is nullified by other variable factors which are not easily controlled. The Bunzel tube alone is bathed in a water thermostat electrically heated and controlled. A knife type heater is used and the thermoregulator is of the mercury type sensitive to about .02 $\pm$ °C, working through a mercury relay in which both poles are permanently bathed in mercury. The

Plate I. Apparatus used for measuring catalase activity



water temperature is equalized by a motor-driven stirrer of the turbine type. The Bunzel tube is shaken at the rate of approximately 204 complete excursions per minute and the thermostat kept constant at 24.5 ± °C. In starting the shaker motor, the switch is thrown one second before the minute which allows the first mixture to occur approximately on the minute. Readings are taken at the end of each successive minute for periods of 3, 5, or 10 minutes.

Standard Method adopted

From June 7 on, five plants selected at random were clipped finely with shears and mixed thoroughly. A 5-gram (wet weight) sample was then weighed out. This clipped material was treated with powdered $CaCO_3$ so that the cut end of the pieces were covered to prevent any acidity developing at those points. A small amount of water, and, where necessary, a little quartz sand which had been carefully cleaned and thoroughly washed, were added and the mixture reduced in the mortar to a thin paste. Distilled water was then added to make 250 cc. The mixture was thoroughly stirred and a 2 cc. sample of this mixture was pipetted into one arm of a Bunzel tube. In the other arm of the tube were placed 2 cc. of 12 volume Dioxygen and the tube was attached to the shaker cork in the thermostat and left three minutes to come to temperature equilibrium. The three way cock in the top of the burette was opened and water levelled to 0.00 cc. At the end of the three minute period the cock was turned so that passage from the Bunzel tube was open to the burette. One second before the minute the switch was thrown and the shaking began practically on the minute. Gas immediately began to be evolved on the mixture of the H_2O_2 and the diluted plant material and the displaced air was collected in the burette. The water surfaces in the burette and the storage tube were levelled off and the number of cc. of

gas evolved was read, estimating to 0.01 cc. at the end of each minute for 10 successive minutes. These were recorded and a duplicate determination made. If the total amounts differed by approximately 1 cc., a third sample was run, the determination farthest off being rejected. The average of the total amounts of gas evolved in 10 minutes was corrected on the basis of Dry Matter. Dry Matter determinations were made by clipping 15 plants and mixing thoroughly after which duplicate 5-gram samples were weighed out and placed in Petri dishes on top of the drying oven over night. The next day the material was transferred to weighed drying bottles and dried to constant weight at 80° C. in a vacuum. When the kernels began to grow rapidly spikes and stalks were dried in separate samples and per cent Dry Matter for total culms was figured from the two determinations by the formula, $\frac{(\text{wet weights of stalks} \times \% \text{ dry matter of stalks}) - (\text{wet weight of spikes} \times \% \text{ Dry Matter spikes})}{\text{Wet weight of culms}} =$ % Dry Matter of culms.

The dilution of the ground plant material was made on the basis of 50 times wet weight. The catalase activity determination desired was on the basis of 250 times Dry Weight. No correction would be necessary if the per cent Dry Matter were 20 % but it was never exactly that, so the actual number of cubic centimeters of oxygen evolved in 10 minutes was multiplied by the fraction $\frac{\% \text{ Dry Matter figured}}{\% \text{ Dry Matter actual}}$. This value was taken as the "catalase activity" of the sample. Since, within the limits used, the amount of evolved gas was very nearly proportional to the dilution of the plant material (appendix, Figure 11), the correction seems valid. The corrected amounts were then plotted against age of plant.

Osterhout (1918) advises in the study of the rate of life processes the comparison up to "half-time" of the lengths of time required to do a given amount of work rather than using the amount of work done in a given time. From a physico-chemical standpoint his method would probably have been preferable to the one followed since the reaction is, of course, much more active at the beginning than after half-time when k is inversely proportional to t. From the following considerations, however, it has seemed better to use the number of cc. of oxygen evolved in 10 minutes.

1. The hydrogen peroxide was always in sufficient excess and the actual amounts of oxygen evolved from the suspensions used did not, in general, vary widely.
2. Most of the oxygen has been evolved in a 10-minute run.
3. Total catalase activity was not desired; relative amounts are sufficient.
4. In order to measure the time taken for the evolution of a definite amount of oxygen it would be necessary to correct the values experimentally obtained for dry matter, plot a curve of these corrected values against time for each day's results and interpolate time in seconds for the volume of oxygen chosen as a unit of work. The labor involved would be unjustified, since, in this investigation, there is a considerable variation from the curve in the individual results obtained which indicates various sources of error of much greater importance than can be offset by the more nearly exact method of Osterhout.

In that part of the study before June 7 certain phases of the above method had to be varied, e. g., the amount of material taken for sample and the degree of dilution, but never in such a way as to invalidate the results.

Use of Weather Data

Before this investigation was begun consideration was given to the difficulties which would probably interfere with the production of reliable data. These difficulties may be classed under two headings. First, the soil at Arlington Farm, Virginia, where the work was done, is extremely heterogeneous. The accurate yield tests on field crops must be very carefully made with adequate attention given to replications and checks. In addition infertile areas, due probably to toxic manures, often appear in plots and nurseries. Second, the climate of Northern Virginia is not favorable to the crop used. If seeding is done in the fall a poor stand may result from dry weather, soil washing or early cold weather. During the winter more or less winter killing normally occurs which leaves vacant areas bordered by plants very favorably located, while further away from the bare spot the competition is keen and only the fittest survive and then with a smaller degree of stooling than is possible where there is more room and available fertility. If the seeding is done in the spring, frost may kill back the seedlings and retard the growth and humid weather may accelerate plant disease. Furthermore, the weather during the growing season varies greatly from year to year and from day to day in sunlight, temperature, and rainfall. Actually the growing conditions are as nearly inconstant as one could wish.

Consequently it seemed wise to make three plantings at two different times with the hope that all three would yield normal plants which,

maturing at different times during the year, would be subject to different weather conditions during any definite growth stage. What barley there is grown in this locality is, for the most part, fall-sown. Seeding is normally done the last week in September and the hardiest winter types are used. The plants germinate and, under favorable conditions, make a strong root growth, but owing to the decumbent habit of the winter types only a luxuriant leafy growth appears above ground remaining more or less dormant over winter. Lack of snow and alternating periods of wet and freezing weather kill many plants during this stage. However, when growth starts in the spring, tillers develop much more freely where the plants are widely spaced than where no killing has occurred and the crop often recovers remarkably from the ill effect of winterkilling. Through this recovery the fall-sown seed produces much more grain in the average year than can be gotten from spring-sown grain, since the latter must germinate and make its active vegetative growth in the relatively unfavorable warm weather of spring. It seemed necessary, then, in studying plants seeded both in fall and spring, to keep an accurate weather record, so as to judge as to the normality of the season and to be able to determine whether the weather conditions obtaining at any one stage of the plant could reasonably explain the events of growth and the attendant metabolism.

Temperature and rainfall records were gotten from the observer at Arlington Farm. Maximum and minimum thermometer records for each day were averaged to find the mean daily temperature. As the readings were taken at approximately 8 A.M., the measurements, as recorded, are those reached in the subsequent 24 hours, i.e., during the day ending at 8 A.M. the next calendar day. Evidently the readings were not taken at <u>exactly</u> 8 A.M. since, in many instances, the set minimum should have shown a temperature

lower than was actually recorded. Another error of this method of obtaining mean daily temperature is, of course, due to utilization of but two points on the daily curve of temperature. For these reasons actual mean daily temperatures were computed from the weekly thermograph record sheets by integrating the curve by means of a #4212 K & E planimeter. The readings were made in duplicate for each day ending at 8 A.M. and using the 10^{0} line for a base. Thirteen determinations on seven different areas on a record sheet gave an average reading of .4719 for 2,160 degree hours (i.e., 90^{0} for 24 hours or an average of 90^{0} for 24 hours). Therefore, X:90:: Reading for the day: .4719. Then $\frac{90}{.4719}$ or 190.718 is the factor to be multiplied by the planimeter reading of the curve to find the average number of degrees Fahrenheit above 10^{0} for the 24 hours. Since the planimeter reading was made on the 10^{0} line as a base, that product must be added to 10^{0} to give the actual mean temperature.

The daily hours or sunlight and relative humidities were obtained from the U. S. Weather Bureau. Those instruments are located 2 miles from the plots at Arlington Farm and while the values shown probably are not exactly those at the Farm the error must be very slight.

Varieties and Methods of Sampling

Two varieties of barley were used. One, Hordeum distichon palmella, var. Hamnchen, C.I. No. 531, is a two-rowed, abundantly stooling, spring sort, and the other, a form of Hordeum vulgare pallidum, var. Tennessee Winter, (Sel. 66) C.I. No. 3546, bears six-rowed spikes and stools less freely. These varieties were grown as follows:

Fall-sown barley

Winter barley was seeded the last week in September and early in the following spring, when it had begun to grow, four small plots of Tennessee Winter were selected for study material. Harvesting was begun on March 17.

From March 26 to maturity samples were taken every day. The plots sloped to the west and there was a marked difference in the soil and moisture content, although no suffering due to drought was observed. Winterkilling in the 2 plots sampled up to June 5, inclusive, was severe, resulting in a patchy stand, which made it very difficult to obtain a continuous series of comparable samples. After June 5 the samples were obtained from the other 2 plots, where the stand was more nearly uniform.

Spring-sown barley

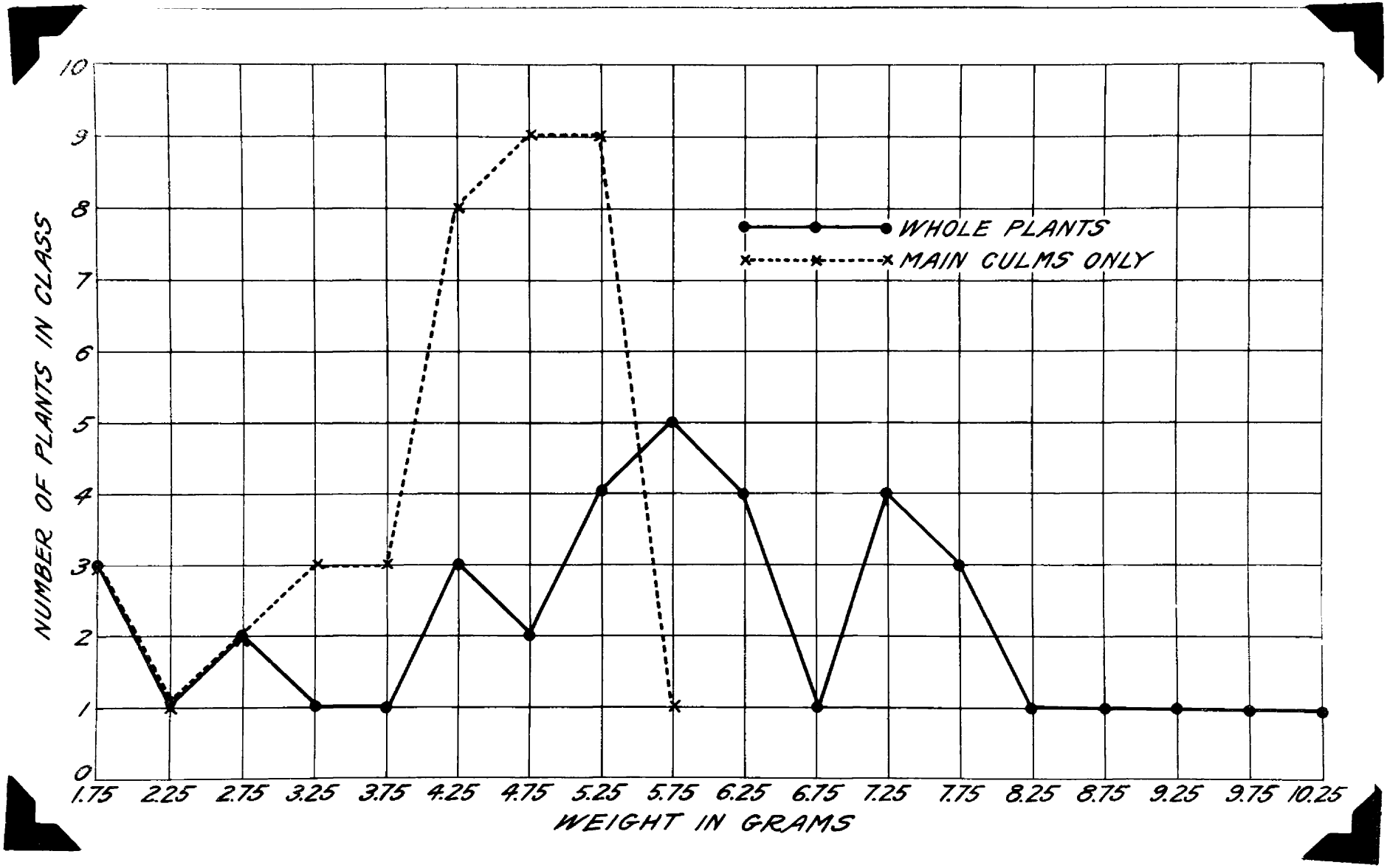
On March 14, two plots were planted, one each of Tennessee Winter and Hannchen. These two plots were upon fairly good soil, sloping to the west and subjected to considerable soil wash. Daily samplings were begun with Hannchen on March 28, and with Tennessee Winter March 30, and continued to June 4, when the interval between sampling dates was lengthened to two or more days because of shortage of suitable material.

Growth Measurements

At the beginning the daily populations consisting of 20 plants were all taken from the same end of the plot working back to the other end on the following days. It was not long, however, before all the plots showed considerable variability as to size, height, and color of the plants. The most nearly uniform part of each plot was then divided transversely into six parts of approximately equal area and the same number of plants were taken, as they came, from each division to make up the sample for the day. On April 17 the variability of the plants in the spring-sown plots was still further reduced by thinning the plants to 2-3" apart in the drill row and by rejecting the abnormally small ones.

The plants were either dug or pulled in such a manner as to be sure of getting the crown. They were then wrapped in moist cheesecloth and taken to the laboratory where the soil was removed.

Figure 1. Frequency distribution comparing the variability in wet weight of whole plants with that of their main culms. Data from Greenhouse population of Jan.23.


NUMBER OF PLANTS IN CLASS
0
1
2
3
4
5
6
7
8
9
10
1.75
2.25
2.75
3.25
3.75
4.25
4.75
5.25
5.75
6.25
6.75
7.25
7.75
8.25
8.75
9.25
9.75
10.25
WEIGHT IN GRAMS
WHOLE PLANTS
MAIN CULMS ONLY

Since it is almost impossible to obtain all of the roots of a maturing barley plant this investigation was conducted upon that portion of the plant above the seed. Later when the permanent roots appeared that portion of the stem above them was used. The tops varied greatly in weight due to the great difference in the number of tillers per plant. It is thought that the main culm is a much better subject for this investigation, since it seems likely that the main culms are comparable provided the plant has at least one vigorous tiller, and consequently, only the main culm was studied. The real variation between the weights of individual plants and the weights of their main culms is shown in Figure I which is taken from the data of greenhouse material harvested January 23 and is typical of much of the material used. After the main culms were removed from the plants they were replaced in moist cheesecloth until measured and weighed. Since the plants pulled from the same plot on any one day were quite variable, it seemed wise to discard the extremes, particularly the abnormally small plants having no vigorous tillers.

The following data were obtained on all plants at harvest: length in centimeters of culm from seed (or crown in older plants) to extreme leaf tip and wet weight of culm to 0.01 g. Early in the study representative plants were bulked and dried to constant weight in a vacuum oven at 80° C. to get percentage of dry matter. The average dry weight per plant was thus calculated from the average wet weight. As soon as enough material was available, all the single day's plants were clipped into approximately 3/4-inch pieces and mixed thoroughly. Dry matter determinations were made on duplicate 5 g. samples (10 g. samples of fall-sown were used). These were averaged for use

in determining average dry matter per plant. From this clipped material the samples for catalase activity were quickly weighed out and immediately coated with pulverized calcium carbonate.

Measurements of the lengths of culm to base of spike and of spike to awn tip and of the wet weight of the spike were begun on the 85th day of the spring-sown plants. This was very near the time when the kernels in general started to develop and makes possible a study of the approximate growth of the culm exclusive of the kernels.

In addition to these data such notes were taken generally on each plant as would indicate the stage of development. These notes include condition of reserve foods in endosperm of seed, leaf stage, presence and stage of development of seminal and permanent roots, presence of tillers, appearance of boot-leaf, appearance and length of awns, emergence of spike, color and dehiscence of anthers, age of developing kernel, browning of awn tips, browning of glumes, and drying of kernels.

Reliability of the growth measurements

In the field the weather conditions varied greatly during the growing season. From March 14, when the spring-sown grain was planted, until May 1, 28 out of the 48 days had a minimum temperature of 40° F. or below. The mean daily minimum of April was about 42°, that of May about 52°, of June 62°, and of July about 69°. The possible hours of sunshine increased from 11.9 on March 14 to 15.0 on June 20, and then dropped to 14.8 on July 9. It is evident that the growth was much less rapid at the beginning. This is shown by the fact that on March 30, 16 days after planting, the acrospires of Selection 66 averaged slightly less than 3 cm. while in the greenhouse the plant length on the 16th day was nearly 16 cm. It is not expected, therefore, that the growth curves obtained are the result solely of the innate ability

of the plant to grow. Gregory (1926) has studied the effect of the environment on the growth of barley by means of partial correlations between seven climatic factors and leaf area, total dry weight and net assimilation rate. He found a high positive correlation between day temperature and both net assimilation rate and efficiency index, and a significantly positive one between day temperature and relative leaf growth. The correlation between night temperature and these three indicators of growth was significantly negative in each case. Relative leaf growth was highly and negatively correlated with radiation, and efficiency index was independent of radiation.

Total height or the length in centimeters from crown to the farthest leaf tip extension indicates fairly well the growth stage of a plant. It is, however, subject to certain sources of error. First: the leaves of a grass are alternate and the growing point is the base of the leaf. Each leaf has its own grand period of growth and, therefore, the grand period of growth as indicated by the curve of the height of the plant varies with the growing activity of the leaf extending the greatest distance distally from the crown. The younger leaf will be past its early slow growing stage when it pushes out beyond the tip of the next older leaf which has just about finished its period of rapid growth. If the height of a single plant were measured at intervals short enough, the curve of growth would show a succession of accelerations corresponding to the appearance at the plant tip of young, actively growing leaves. This error is greatly reduced by averaging the measurements of a plant population which varys as to number and stage of growing leaves. Second: neither absolute nor relative height of a plant variety is constant for different environmental conditions. This was shown by Harlan (1914). In Table I Abyssinian barley was tall at Chico, Calif., and very short at St.Paul, Minn., Williston, N. Dak., and Moccasin, Mont. Odessa barley was tall at

Williston and Moccasin, and very short at St. Paul, and stood eighth on the list of thirteen varieties at Chico. Since the environment seems responsible for this disparity in total heights it seems entirely possible that varying weather conditions may act differently on the two varieties grown for this study. This error is at least partially taken care of by the use of two types of barley sown in 3 plantings. Third: the weather conditions varying from day to day during the growing period have doubtless given a curve different from one produced under a constant weather environment.

According to McDougall (1919) the absorption of water and resulting hydration is an essential part of the enlargement and growth of the cell. However, the use of the wet weight as a measurement of stage of growth is subject to several sources of error. At the stage of the plant characterized by rapid increase of dry matter there may be an actual loss of water causing the wet weight to remain approximately stationary. As the plant nears maturity, the wet weight decreases to a fraction of its maximum value and approaches the dry weight in value. The wet weight will indicate little or no growth when actually that process is progressing rapidly. Then, too, the wet weight varies according to the turgidity of the tissues which in turn depend on the soil and air moisture available. Moreover, the occurrence of rain or dew on the plant at sampling time is corrected with difficulty. On the other hand, deposition, or at least translocation, of solids in the plant continues until growth ceases and the plant parts die or become dormant. The dry weight then is the most reliable measure of growth and is the one emphasized in this study. It is not, however,

free from error. At all stages of development and growth, parts of the plant are liable to mechanical and pathological injury which is not always recognizable. This is especially true during the maturing phases when the leaves lose their turgidity, become brown, and are wind whipped and leached by rain. The situation is still further complicated by the fact that from flowering on to maturity the developing seeds, which are new plants parasitic on the parent, were weighed with the spike. It is not only tedious but impracticable to separate the grain from the rest of the spike before weighing. Moreover, the seed coats are maternal tissues and practically can not be taken off the caryopsis. An approximation of the dry weight of the plant without the seeds was reached by adding to the average dry weight of the culm the average dry weight of the spike on the day of flowering. This is only an approximation, of course, but rather close, since the growth of the maternal tissues in the spike is probably very nearly completed.

Experimental Results

Weather conditions during the growing period.

Thermograph records for the period March 12 to July 8 are reproduced in Plates II, III, and IV, and the planimeter means computed from them are shown in a graph (Figure 2). The distribution of the rainfall is shown in Figure 3, and a summary of weather conditions from March 1 to July 9 is given in Table 1.

The following statements will summarize the conditions under which the plants developed.

March is seen to have been warmer; April, May, and June cooler;and the first 9 days of July much warmer than normal.

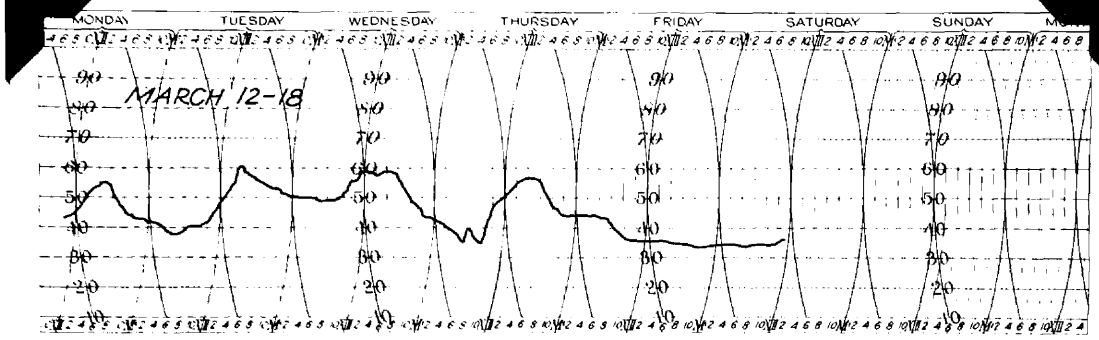
Relative humidities were in general in excess of normal,especially in May and June.

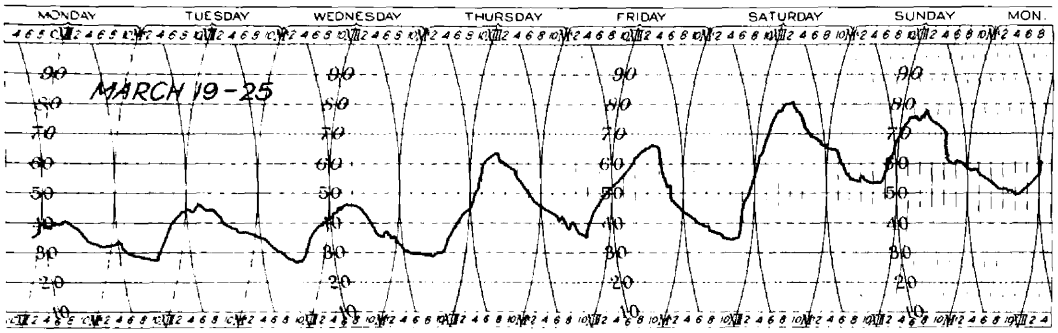
Rainfall was below normal in March and June but the precipitation was, as can be seen in Figure 3 (graph of rainfall), well distributed and at no time did the plant show evidence of droughty conditions.

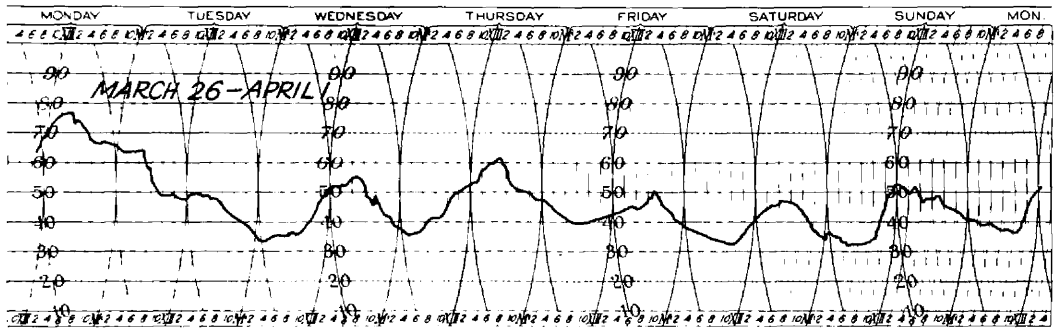
The per cent sunshine was above normal in March and May, slightly under in April, considerably under in June, and much above normal for the first 9 days of July.

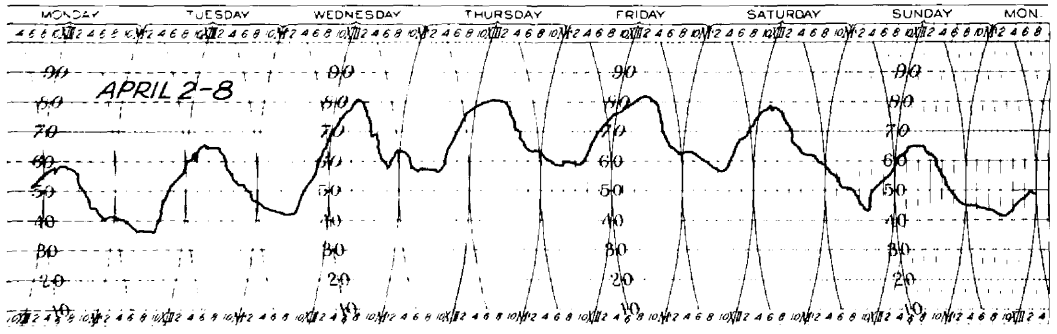
Weather conditions were particularly favorable for spring-sown barley in 1928. The cool weather, especially in June, allowed normal development of the crop and together with a deficiency in rainfall was unfavorable to such plant diseases as Helminthosporium and rust which often result in serious injury to spring-sown grains in Virginia.

Plate II. Thermograph records March 12-April 15, Arlington Farm, Va.


MONDAY
TUESDAY
WEDNESDAY
THURSDAY
FRIDAY
SATURDAY
SUNDAY
MARCH 12-18


MONDAY
TUESDAY
WEDNESDAY
THURSDAY
FRIDAY
SATURDAY
SUNDAY
MON.
MARCH 19-25


MONDAY
TUESDAY
WEDNESDAY
THURSDAY
FRIDAY
SATURDAY
SUNDAY
MON.
MARCH 26-APRIL 1


MONDAY
TUESDAY
WEDNESDAY
THURSDAY
FRIDAY
SATURDAY
SUNDAY
MON.
APRIL 2-8

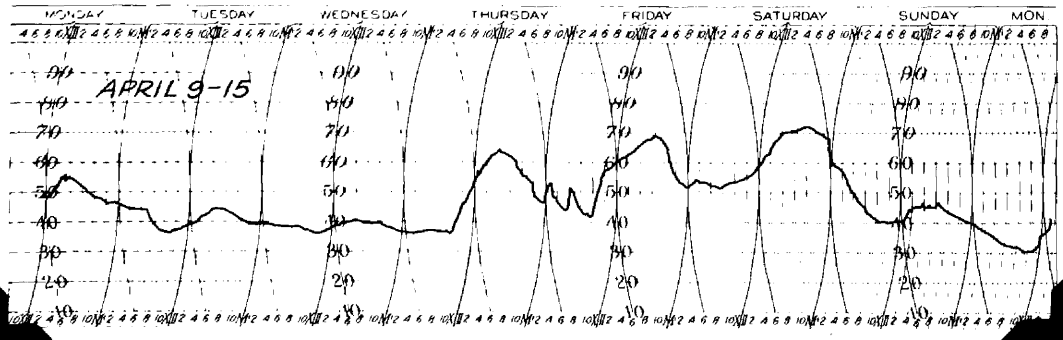
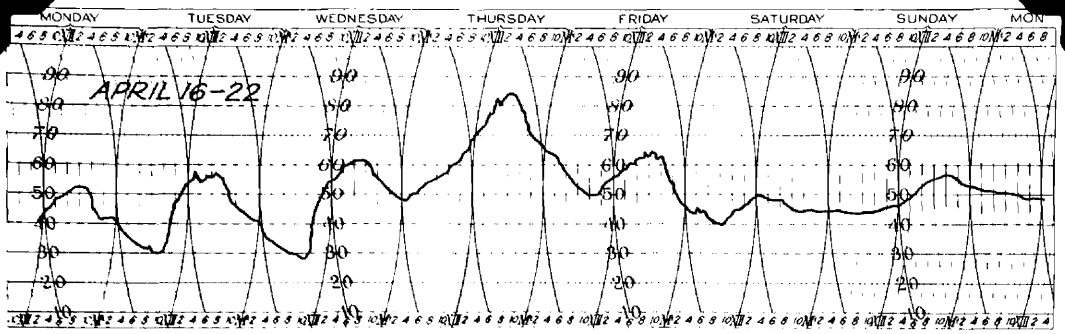
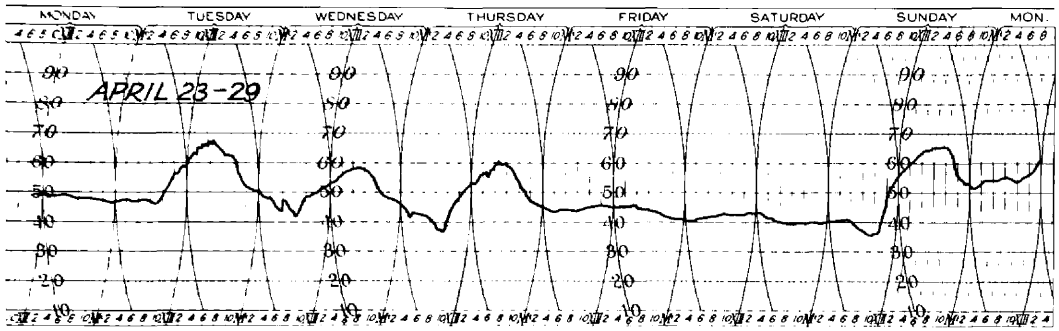
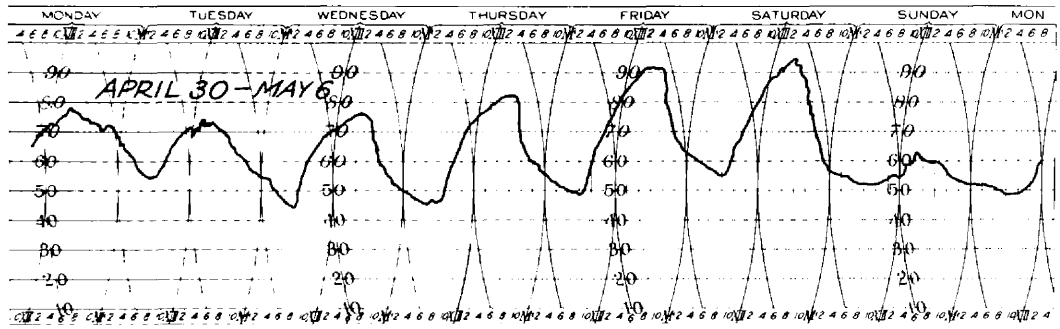

MONDAY
TUESDAY
WEDNESDAY
THURSDAY
FRIDAY
SATURDAY
SUNDAY
MON.
APRIL 9-15

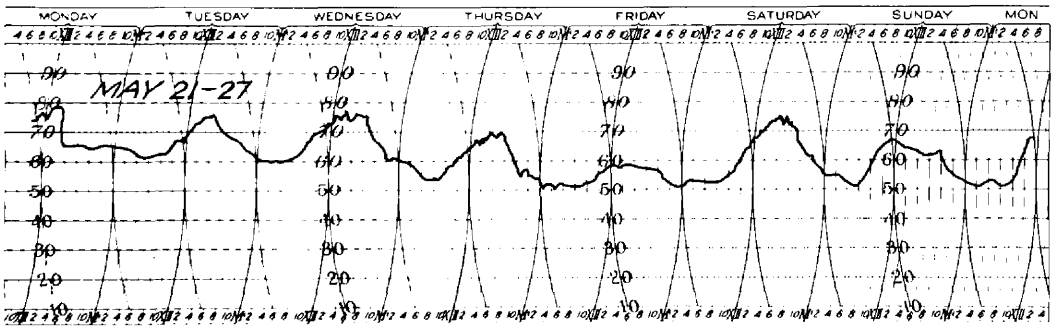
Plate III. Thermograph records April 16-June 3, Arlington Farm, Va.







MAY 7 TO MAY 21 NOT AVAILABLE



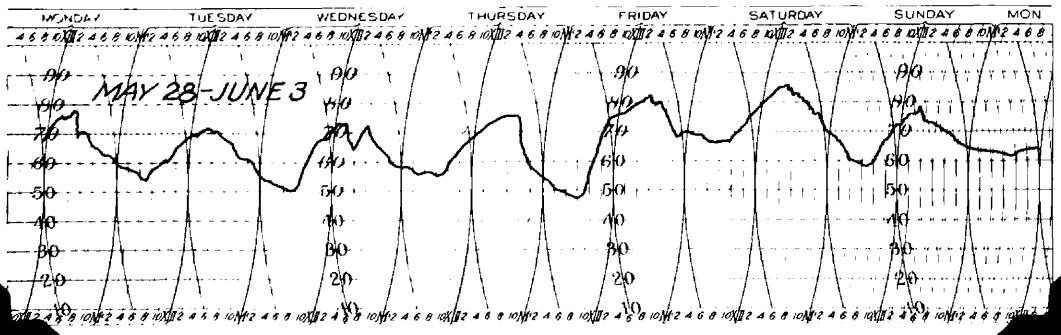
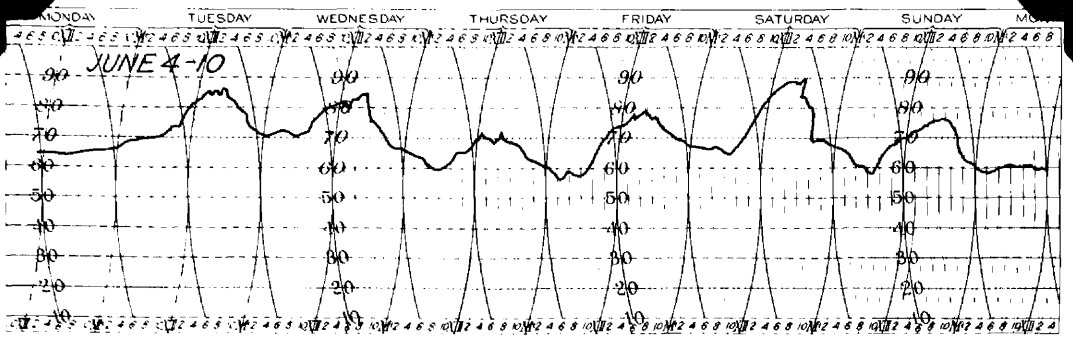
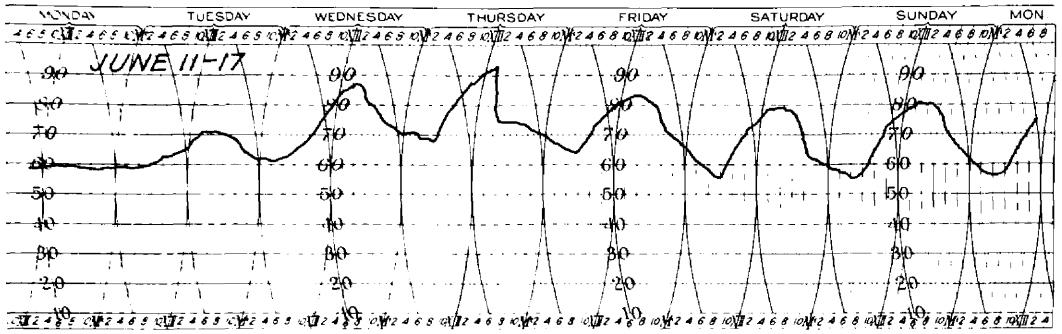
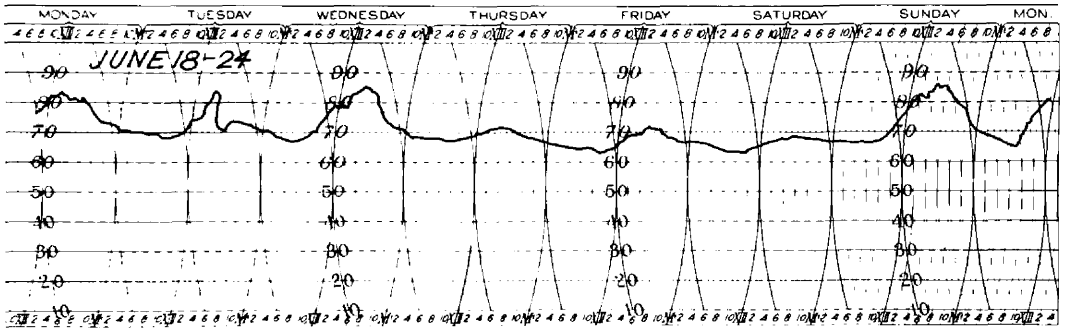
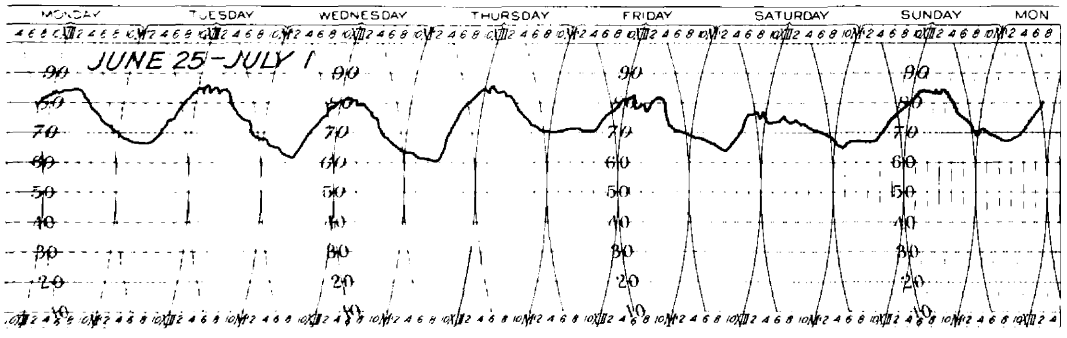


Plate IV. Thermograph records June 4–July 8, Arlington Farm, Va.


MONDAY
TUESDAY
WEDNESDAY
THURSDAY
FRIDAY
SATURDAY
SUNDAY
JUNE 4-10


MONDAY
TUESDAY
WEDNESDAY
THURSDAY
FRIDAY
SATURDAY
SUNDAY
MON
JUNE 11-17


MONDAY
TUESDAY
WEDNESDAY
THURSDAY
FRIDAY
SATURDAY
SUNDAY
MON.
JUNE 18-24


MONDAY
TUESDAY
WEDNESDAY
THURSDAY
FRIDAY
SATURDAY
SUNDAY
MON
JUNE 25-JULY 1

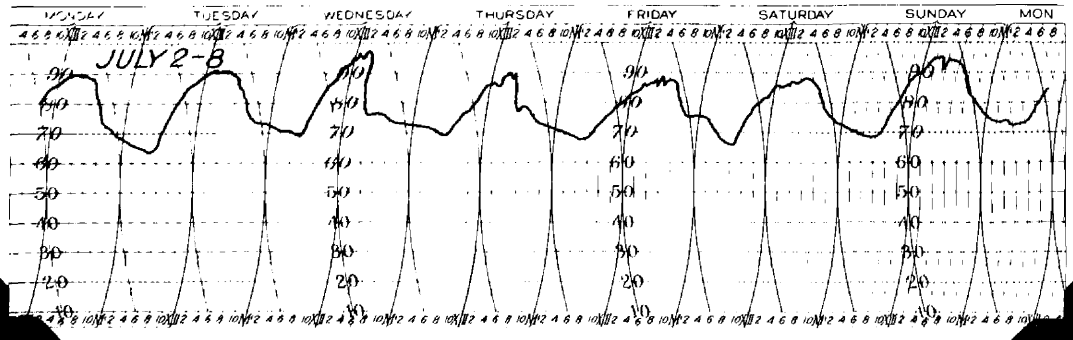

TUESDAY
WEDNESDAY
THURSDAY
FRIDAY
SATURDAY
SUNDAY
MON
JULY 2-8

Figure 2. Mean daily temperatures from March 1 to July 9, 1928, at Arlington Farm, Va., as computed from planimeter readings from thermograph records.

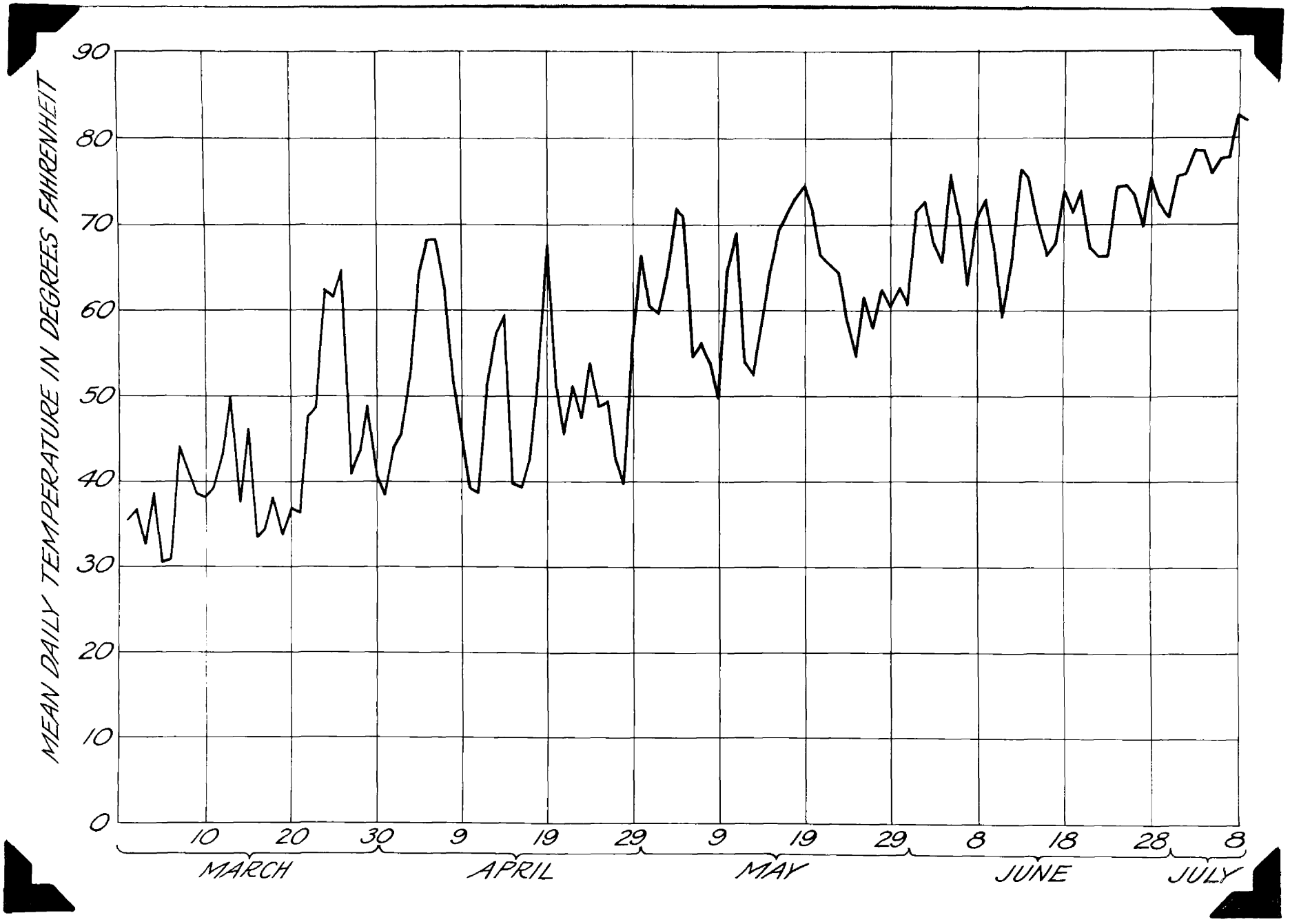

MEAN DAILY TEMPERATURE IN DEGREES FAHRENHEIT
90
80
70
60
50
40
30
20
10
0
10
20
30
9
19
29
9
19
29
8
18
28
8
MARCH
APRIL
MAY
JUNE
JULY

Figure 3. Amount and distribution of rainfall at Arlington Farm, Va., March 1 to July 9, 1928.

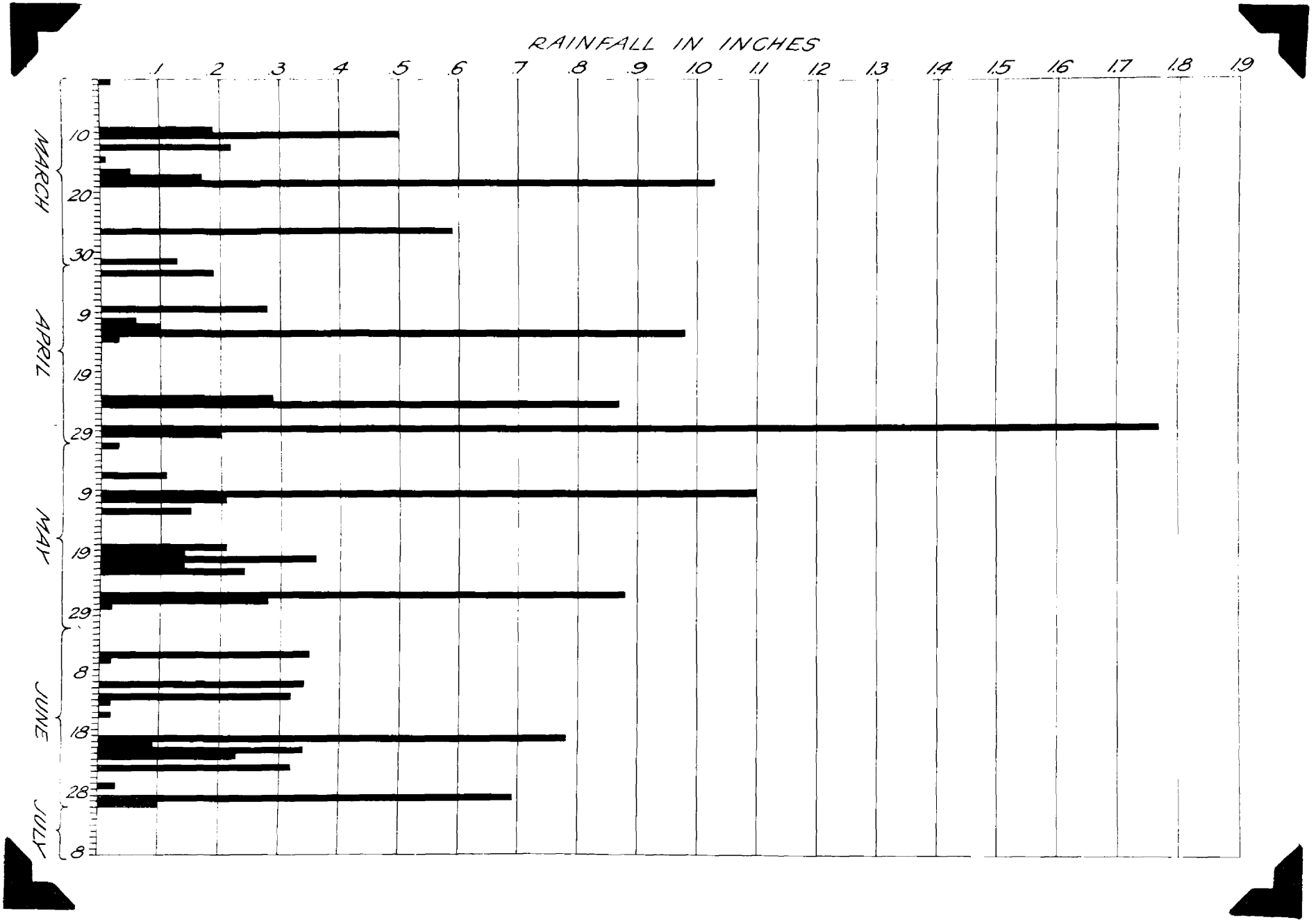

RAINFALL IN INCHES
.1
.2
.3
.4
.5
.6
.7
.8
.9
1.0
1.1
1.2
1.3
1.4
1.5
1.6
1.7
1.8
1.9
MARCH
10
20
30
APRIL
9
19
29
MAY
9
19
29
JUNE
8
18
28
JULY
8

Table 1 . Summary of weather conditions March 1 to July 9, 1928

	March	April	May	June	July (1-9)
Dates of killing frosts	19,20,31	16,17,18	None	None	None
Accumulated excess (+) or deficiency (-) in temperature since first day of month	+49°	-42°	-23°	-40°	+28°
Excess (+) or deficiency (-) in precipitation this month as compared with normal	-1.58 in.	+1.25 in.	+0.31	-1.47	-1.16
Accumulated excess (+) or deficiency (-) since January 1	-2.82 in.	-1.57	-1.26	-2.47	-3.63
Mean % relative humidity					
8 A.M.	74	67	74	79	72
Local noon	55	49	51	62	52
8 P.M.	54	54	60	70	63
Normal % relative humidity					
8 A.M.	68	68	69	74	78*
Local noon	51	48	50	56	57*
8 P.M.	55	54	58	66	73*
Mean % sunshine	60	55	62	53	85
Normal % sunshine	51	58	61	62	64*
Total hours possible sunshine	371.1	397.4	444.0	446.0	133.3

* For the month

Growth in length.

As the Fall- sown Tennessee winter seed was planted the previous fall, the permanent roots and at least some of the tillers were well formed long before the first measurements of the plant were made in the spring. The leaves had been killed nearly to the crown but early in March they began growth at the base and showed green under the brown and dry tips.

The variability of the fall-sown barley has been mentioned. This is particularly noticeable before April 29 when rapid elongation began (Fig. 4). At this time the boot leaf showed in many plants and the spike was slightly developed inside it. Awns began to emerge on May 5 and the first anthers dehisced ("flowering") on May 9. Increasingly rapid growth is shown at two places on the curve, one from March 30 to April 12 and the other beginning at April 29. The first is probably a weather reaction since the temperature curve (Fig. 2) shows a period of about 8 days (April 2-9) of considerably higher temperature with no serious drops during the time. This corresponds to the early part of the rather rapid growth shown on the temperature curve for those dates. Rapid growth, however, continued for a few days after the drop in temperature of April 10 and 11. This may also of course, be an after-effect of the warm spell. The two spring-sown varieties may be treated together. The growth curves of length are shown in Figure 5 (Tennessee Winter) and 6 (Hannchen). As will be seen from these figures the plants have had a preliminary rapid growth in length up to April 8. A retardation in growth then occurred notwithstanding the higher temperatures recorded on April 12 to 14. The permanent roots are just started on April 17 at which time rapid growth again begins, coming, however, after 3 days of a maximum temperature of over 64.

Table 2 . Average length in centimeters of main culm

Day of harvest		Spring sown				Fall sown	
		Hannchen		Tennessee Winter			
Date*	Days from planting**	To tip of distal leaf	To tip of awns	To tip of distal leaf	To tip of awns	Tennessee Winter	
March 14							
15	1						
16	2						
17	3					5.680	
18	4						
19	5						
20	6						
21	7						
22	8						
23	9					7.950	
24	10						
25	11						
26	12					11.225	
27	13					9.390	
28	14	3.040				11.980	
29	15	3.295				11.705	
30	16	3.840		2.995		11.155	
31	17	4.700		3.360		9.850	
April 1	18	4.410		3.710		14.330	
2	19	4.585		4.540		10.235	
3	20	5.515		4.085		14.005	
4	21	5.765		4.450		12.495	
5	22	6.190		5.210		18.170	
6	23	7.655		6.430		16.700	
7	24	6.760		6.640		17.450	
8	25	7.435		7.580		21.255	
9	26	7.560		8.365		22.845	
10	27	7.675		8.360		20.370	
11	28	7.530		8.010		21.790	
12	29	8.115		7.015		25.865	
13	30	7.355		8.295		21.805	
14	31	8.140		7.385		23.570	
15	32	8.255		7.560		26.755	
16	33	9.080		8.410		23.180	
17	34	9.775		10.845		28.405	
18	35	9.250		10.985		23.955	
19	36	10.365		10.015		27.770	
20	37	11.165		10.945		30.460	
21	38	10.805		11.260		30.675	
22	39	11.230		9.385		29.965	
23	40	10.965		10.660		29.050	
24	41	11.000		11.755		25.335	
25	42	12.155		12.315		30.090	
26	43	12.775		11.265		27.500	
27	44	11.070		10.740		29.620	
28	45	12.190		12.430		30.400	
29	46	13.060		11.870		28.580	
30	47	13.075		12.100		29.940	
May 1	48	14.090		12.095		35.580	
2	49	14.160		12.985		37.240	
3	50	15.700		14.165		43.535	
4	51	17.290		13.825		48.585	
5	52	16.110		15.250		49.730	
6	53	16.585		14.320		57.575	
7	54	17.840		15.370		54.530	
8	55	16.980		17.095		61.695	
9	56	16.475		16.315		63.710	
10	57	19.150		17.430		68.415	awns emerged
11	58	18.335		14.975		67.830	
12	59	17.090		19.880		75.165	
13	60	17.910		18.100		76.640	
14	61	20.395		18.510		84.405	
15	62	20.245		19.520		84.980	
16	63	19.560		20.930		88.650	
17	64	21.770		21.610		96.830	
18	65	23.945		24.265		96.900	
19	66	23.315		24.555		101.175	
20	67	25.550		28.715		102.775	
21	68	28.040		31.395		101.875	
22	69	29.790		29.925		100.525	
23	70	31.920		32.205		106.230	
24	71	31.705		34.825		101.450	
25	72	32.735		33.890		108.200	
26	73	31.575		35.735		102.775	
27	74	36.015		37.135		102.050	
28	75	34.595		40.095		103.025	
29	76	39.880		40.175		99.925	
30	77	42.515		46.575		100.575	
31	78	44.210		46.790		104.200	
June 1	79	45.180		52.385		108.150	
2	80	52.530		58.840		108.150	
3	81	53.350		56.375		106.550	
4	82	51.890		66.700		113.450	
5	83	56.500		64.590		108.850	
6	84					98.100	another plot
7	85	58.685	47.620	70.950	69.060	96.775	
8	86					96.300	
9	87	59.830	55.955	73.225	78.350	97.050	
10	88					96.925	
11	89	63.665	64.545	73.670	81.430	95.800	
12	90					100.985	
13	91	64.005	63.515	73.750	83.695	97.100	
14	92					101.475	
15	93	65.485	69.345		89.605	100.325	
16	94					100.175	
17	95	68.565	75.190		88.690	99.675	
18	96						
19	97	62.150	71.290		89.325		
20	98						
21	99	66.995	74.090		86.850		
22	100						
23	101	64.705	71.945		87.650		
24	102						
25	103	64.890	71.870		87.125		
26	104						
27	105						
28	106	70.150	78.690		88.250		
29	107						
30	108						
July 1	109						
2	110	68.500	75.040		75.850		
3	111						
4	112						
5	113				84.366		
6	114	69.800	77.560				
7	115						
8	116						
9	117	71.475	78.100				

* Not applicable to greenhouse material

** Not applicable to fall-sown material

Figure 4. Curve showing growth in length of fall-sown Tennessee Winter barley from the beginning of active growth in the spring until maturity. No distinction is made as to whether distal leaf tip or awn tip furnished the farthest extension of the plant.

Notice the period of rather rapid growth from March 30 to April 12 followed by a slight inflection up to April 29. Also no major inflection occurs until May 19 or 10 days after flowering.

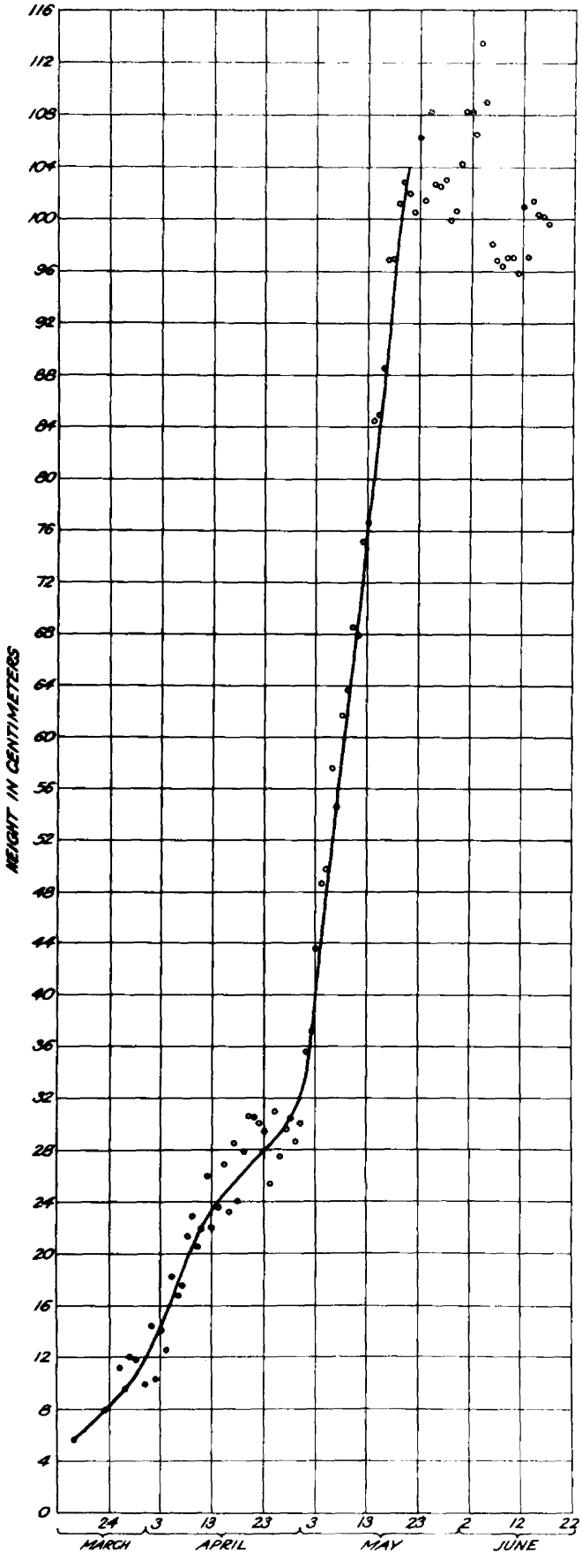

HEIGHT IN CENTIMETERS
116
112
108
104
100
96
92
88
84
80
76
72
68
64
60
56
52
48
44
40
36
32
28
24
20
16
12
8
4
0
24
3
13
23
3
13
23
2
12
22
MARCH
APRIL
MAY
JUNE

Figure 5. Curve showing growth in length of spring-sown Tennessee Winter barley from the emergence of the plumule above the soil until maturity.

Note that two minor inflections occur, the first begins at about the time of exhaustion of the endosperm the first week in April and again trends upward about April 15 when permanent roots were starting, and the second begins as the tillers appear about April 20. When the tiller roots were developing well the growth again becomes more rapid.

While a major inflection occurs in greatest leaf length at flowering the awn tip length continues to increase with undiminished rapidity until June 12 or 5 days after flowering.

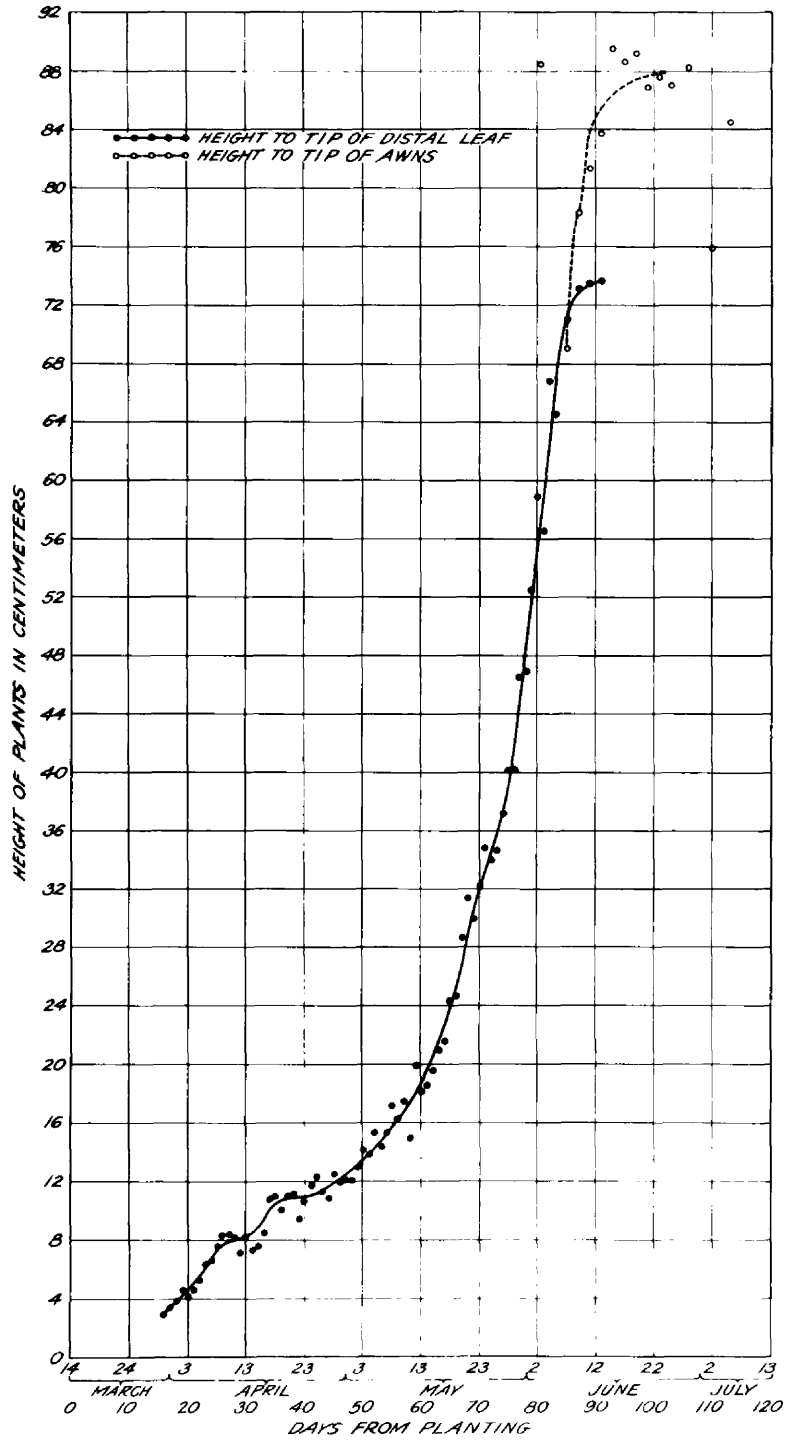
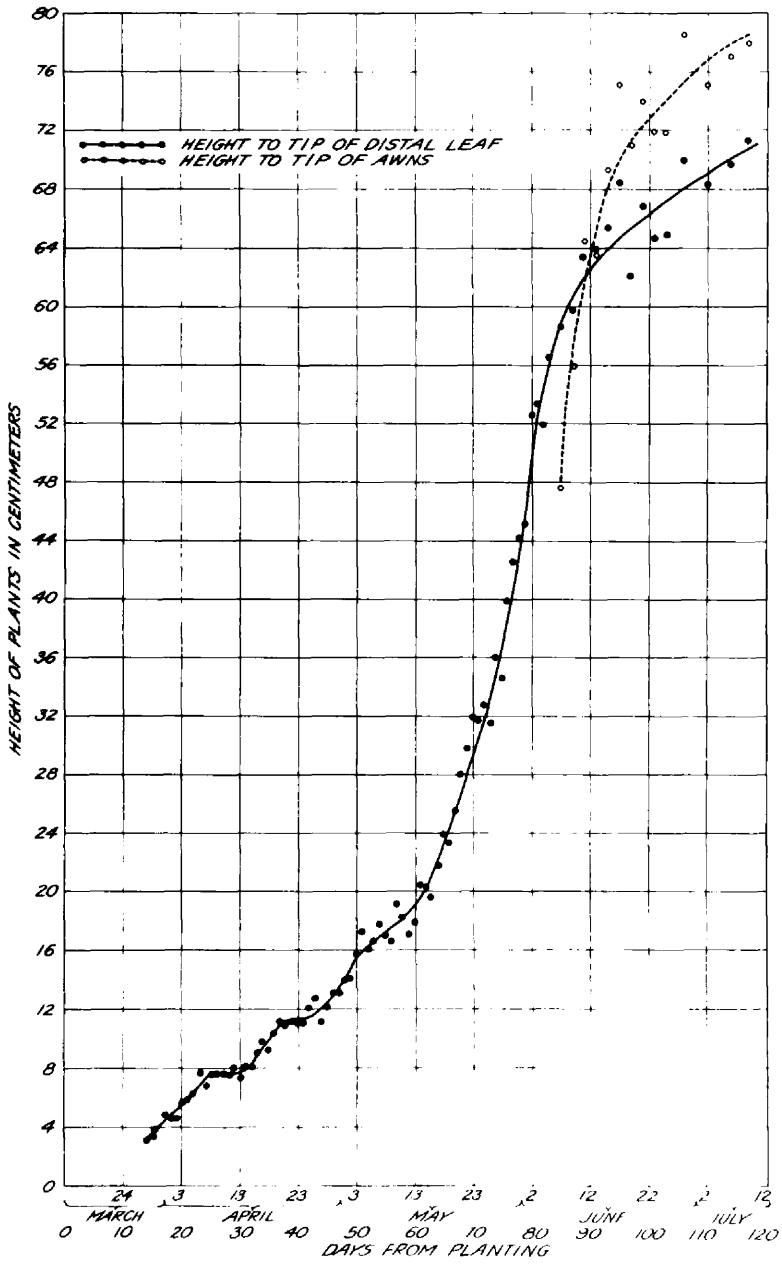

HEIGHT TO TIP OF DISTAL LEAF
HEIGHT TO TIP OF AWNS
HEIGHT OF PLANTS IN CENTIMETERS
0
4
8
12
16
20
24
28
32
36
40
44
48
52
56
60
64
68
72
76
80
84
88
92
14 24 3 13 23 3 13 23 2 12 22 2 13
MARCH
APRIL
MAY
JUNE
JULY
0 10 20 30 40 50 60 70 80 90 100 110 120
DAYS FROM PLANTING

Figure 6. Curve showing growth in length of spring-sown Hannchen barley from the emergence of the plumule above the soil until maturity.

Exhaustion of the endosperm occurred in this variety about April 5 and a minor inflection begins at that time and extends to about April 14. Permanent roots were started on April 17 and the trend of the curve is rapidly upward until April 20 when a second inflection occurs during the development of tillers and gradually disappears during the development of tiller roots.

Here, as in Tennessee Winter (Fig.5) flowering occurred on June 7 when the leaf length was tapering off. The awn tip length, however, shows no inflection until 8 days later.


HEIGHT TO TIP OF DISTAL LEAF
HEIGHT TO TIP OF AWNS
HEIGHT OF PLANTS IN CENTIMETERS
0
4
8
12
16
20
24
28
32
36
40
44
48
52
56
60
64
68
72
76
80
24
3
13
23
3
13
23
2
12
22
2
12
MARCH
APRIL
MAY
JUNE
JULY
0
10
20
30
40
50
60
10
80
90
100
110
120
DAYS FROM PLANTING

About April 21 there begins another period of slowing up. The temperatures are neither very high nor very low and the first tillers have appeared and have not yet developed good roots. By May 2 the tiller roots are developing well and the curve has started upward again as has also the temperature.

Leaf length begins to taper off rapidly in Tennessee on June 7 when first flowering occurs, the awn tip length tapering off on June 17. In Hannchen, flowering occurs on June 7 but the leaf length tapers off much more slowly and the awn tip length grows rapidly until June 17.

In both of the spring-sown varieties these results are at variance with those of Bakhuysen and Alsberg (1927) who found the inflection of the curve of length in wheat to occur at flowering.

Growth in dry weight.

Wet and dry weights of Fall-sown Tennessee winter barley are given in Table 3 and the growth curves according to both wet and dry weights are shown in Figure 7. The curves are far from smooth, due mainly to variability in the plots on account of winter killing and also to unavoidable error in sampling.

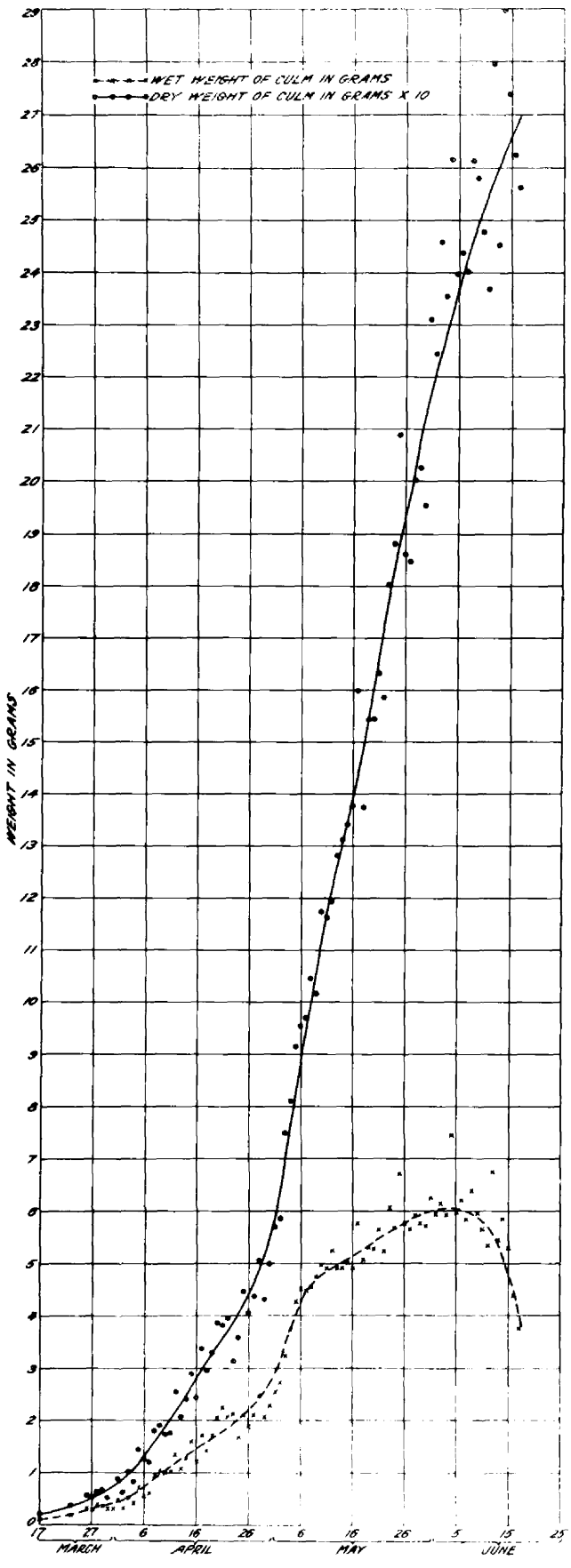
From June 5 on, the daily samples were obtained from another part of the farm and while more nearly uniform, were shorter (see Table 2).

The general shape of the curve is similar to that of the other two seedings but no conclusions can be reached from it as to the relation of the stage of maturity to different portions of the curve. Separate weights were obtained from stalk and spike beginning June 6. However, this data was considerably after the kernels had begun to form and consequently while the figures show the same general trend as in the spring-sown plantings, they are incomplete and will not be discussed further.

Table 3 . Average wet weight, per cent of dry matter and dry weights of the main culm of fall-sown Tennessee Winter barley

Date of harvest	Wet weight			Per cent of dry matter			Dry weight		
	stalk	spike	culm	stalk	spike	culm	stalk	spike	culm
March 17			.11520			19.09			.02199
23			.17690			21.54			.03810
26			.33300			17.28			.05754
27			.27605			19.45			.05369
28			.39140			16.51			.06462
29			.37150			17.95			.06668
30			.30970			17.15			.05311
31			.30850			lost			- - -
April 1			.49255			18.10			.08915
2			.33475			19.16			.06414
3			.53425			18.87			.10081
4			.43425			19.19			.08333
5			.70925			20.45			.14504
6			.56650			22.36			.12667
7			.61650			19.61			.12090
8			.96075			18.86			.18120
9			1.05225			18.23			.19183
10			1.00675			17.27			.17387
11			1.03025			17.18			.17700
12			1.36225			18.80			.25610
13			1.08150			19.29			.20862
14			1.22800			19.69			.24179
15			1.60775			18.21			.29277
16			1.23150			19.87			.24470
17			1.73300			19.57			.33915
18			1.40375			21.17			.29717
19			1.72000			19.31			.33213
20			2.06850			18.83			.38950
21			2.25800			17.02			.38431
22			2.09450			18.98			.39754
23			2.12850			14.76			.31417
24			1.69850			21.21			.36025
25			2.11325			21.23			.44864
26			1.87950			21.68			.40748
27			2.11000			20.76			.43804
28			2.49225			20.32			.50643
29			2.08925			20.76			.43373
30			2.29025			21.86			.50065
May 1			2.55425			22.32			.57011
2			2.73375			21.16			.57846
3			3.25175			23.04			.74920
4			3.83300			21.14			.81030
5			4.28900			21.36			.91613
6			4.51425			21.15			.95476
7			4.49150			21.62			.97106
8			4.56400			22.94			1.04698
9			4.76900			21.36			1.01866
10			4.98100			23.58			1.17420
11			4.93150			23.58			1.16285
12			5.25225			22.75			1.19489
13			4.92100			26.08			1.28340
14			4.93550			26.61			1.31333
15			5.03925			26.67			1.34397
16			4.91425			28.05			1.37845
17			5.77770			27.72			1.60158
18			5.09175			27.04			1.37681
19			5.31325			29.08			1.54509
20			5.28725			29.36			1.55234
21			5.64950			28.92			1.63384
22		1.27325	5.23600			30.33			1.58808
23		1.55525	6.06200			29.74			1.80284
24		1.67275	5.68375			33.12			1.88246
25		1.92775	6.70775			31.16			2.09013
26		1.73025	5.75450			32.33			1.86043
27		1.79550	5.67300			32.57			1.84770
28		2.02450	5.92700			33.76			2.00096
29		1.98475	5.77250			35.11			2.02672
30		2.04750	5.70125			34.32			1.95667
31		2.36975	6.26275			36.89			2.31033
June 1		2.36125	5.94400			37.76			2.24445
2		2.58700	6.15250			39.96			2.45854
3		2.48400	5.94350			39.63			2.35541
4		3.03600	7.46925			35.00			2.61424
5		2.59525	5.98025			40.08			2.39688
6	3.44125	2.77550	6.21675	35.11	44.33		1.20822	1.23038	2.43860
7	3.08025	2.77600	5.85625	35.54	47.08		1.09472	1.30694	2.40166
8	3.34550	3.05525	6.40075	34.41	47.75		1.15119	1.45888	2.61007
9	3.04225	2.92400	5.96625	35.88	50.89		1.09156	1.48802	2.57958
10	2.96475	2.71200	5.67675	34.96	53.22		1.03648	1.44333	2.47981
11	2.78675	2.57750	5.36425	34.87	54.13		.97174	1.39520	2.36694
12	3.61125	3.14725	6.75850	31.22	53.00		1.12743	1.66804	2.79547
13	2.95300	2.51175	5.46475	34.75	56.81		1.02617	1.42693	2.45310
14	3.19675	2.65975	5.85650	36.41	65.07		1.16394	1.73070	2.89464
15	3.01975	2.30475	5.32450	36.79	70.61		1.11097	1.62738	2.73835
16	2.54975	1.85800	4.40775	43.92	80.92		1.11985	1.50349	2.62334
17	2.05150	1.73875	3.79025	53.31	84.39		1.09365	1.46733	2.56098

Figure 7. Curve showing average wet and dry weights of the main culm of fall-sown Tennessee Winter barley.


WET WEIGHT OF CULM IN GRAMS
DRY WEIGHT OF CULM IN GRAMS X 10
WEIGHT IN GRAMS
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MARCH
APRIL
MAY
JUNE

The daily growth in weight of the spring-sown Tennessee winter is shown in Table 4, and Fig. 8. The growth curve is comparatively smooth except at the very end where material had become scarce. Previous to May 2 the curves of both wet and dry weights have risen very slightly above the baseline. During this time the culm length varies with stage of development much more noticeably than does the weight. On May 2 when roots were well started on the tillers the curve shows an accelerated growth. It is true that there was an attendant increase in temperature beginning on this date, but no similar acceleration of growth attended the previous warm spell during the first ten days of April. Furthermore the weight curve keeps on rising notwithstanding the cool period following May2. There is noticeable a further increase in growth rate on May 24 or shortly after jointing began. On the 7th of June first flowering occurred and the wet weights of stalk and spike were taken separately and dry weight was found for each. The sum of these two weights was the weight of the culm. As was found by Burd (1919) the total weight of the culm increased with about no change in the curve to June 19 (97 days) when the awns commenced to turn brown at the tips. From this time on the plants rapidly turned brown and the kernels finally dried out being almost dry and waxy on July 2.

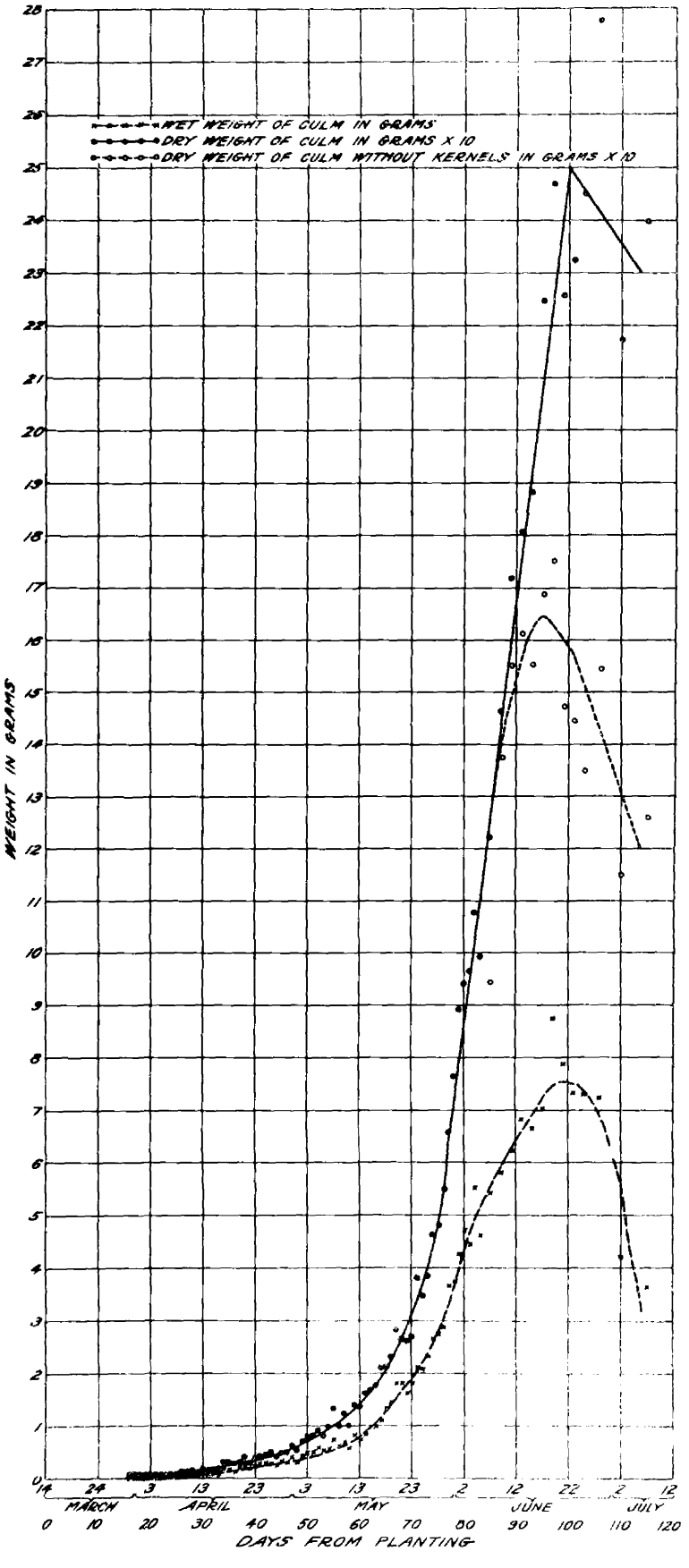
In order to correct for kernel weight the dry weight of the spike on June 7 was added to the dry weight of the stalk on each of the days following that date. This procedure is believed to be justified since the spike tissue, excluding the kernels, had very nearly reached its maximum mass at that time. Any error occurring would be probably on the light side. It will be seen from the curve that when weights of these culms minus the kernels are plotted as a continuation of the culm curve before June 7, no perceptible

Table 4 . Average wet weight, per cent of dry matter, and dry weight of the main culm of spring-sown Tennessee Winter barley

Day of harvest		Wet weight			Per cent dry matter			Dry weight			
Date	Days from planting	stalk	spike	culm	stalk	spike	culm	stalk	spike	culm	culm and spike as of June 7
March 30	16			.04675			11.77			.00550	
31	17			.04550			12.23			.00556	
April 1	18			.05750			11.98			.00689	
2	19			.06875			11.05			.00760	
3	20			.06650			11.68			.00777	
4	21			.05550			13.89			.00771	
5	22			.05750			14.57			.00838	
6	23			.07325			13.99			.01025	
7	24			.07350			15.92			.01170	
8	25			.08700			15.75			.01370	
9	26			.10950			16.00			.01752	
10	27			.10275			15.24			.01566	
11	28			.11350			15.71			.01783	
12	29			.09125			17.97			.01640	
13	30			.13100			16.96			.02222	
14	31			.11025			16.43			.01811	
15	32			.12100			16.45			.01990	
16	33			.12125			18.00			.02183	
17	34			.20650			16.70			.03449	
18	35			.19450			17.21			.03347	
19	36			.17200			17.78			.03058	
20	37			.23225			15.00			.03484	
21	38			.26750			15.91			.04256	
22	39			.20525			15.14			.03107	
23	40			.24800			17.34			.04300	
24	41			.30425			15.31			.04658	
25	42			.31150			15.04			.04685	
26	43			.29725			17.05			.05068	
27	44			.26875			16.14			.04338	
28	45			.35175			14.35			.05048	
29	46			.32075			15.95			.05116	
30	47			.41800			15.51			.06483	
May 1	48			.33475			16.24			.05438	
2	49			.44925			15.70			.07053	
3	50			.49600			16.21			.08040	
4	51			.52175			16.05			.08374	
5	52			.60300			15.49			.09340	
6	53			.55175			14.71			.08116	
7	54			.58475			16.75			.09795	
8	55			.74375			18.03			.13410	
9	56			.64525			15.48			.09988	
10	57			.70825			17.66			.12508	
11	58			.59250			16.97			.10055	
12	59			.82725			16.84			.13931	
13	60			.75750			18.17			.13764	
14	61			.84100			19.25			.16189	
15	62			.97325			17.43			.16964	
16	63			.99075			17.85			.17685	
17	64			1.10200			19.10			.21048	
18	65			1.34375			15.83			.21272	
19	66			1.44700			15.91			.23022	
20	67			1.80775			15.62			.28237	
21	68			1.81550			14.65			.26597	
22	69			1.62650			16.02			.26057	
23	70			1.82450			14.81			.27021	
24	71			2.11500			17.98			.38028	
25	72			2.08300			16.66			.34703	
26	73			2.32525			16.52			.38413	
27	74			2.66200			17.40			.46319	
28	75			2.72900			17.59			.48003	
29	76			2.87100			19.13			.54922	
30	71			3.68050			17.88			.65807	
31	78			3.75450			20.33			.76329	
June 1	79			4.28375			20.86			.89359	
2	80			4.72775			19.93			.94224	
3	81			4.45750			21.65			.96505	
4	82			5.52525			19.54			1.07963	
5	83			4.63325			21.46			.99430	
7	85	4.37150	1.02950	5.40100	21.63	26.76		.94556	.27549	1.22105	1.22105
9	87	4.67700	1.15250	5.82950	23.51	31.49		1.09956	.36292	1.46248	1.37505
11	89	4.90900	1.29450	6.20350	26.01	34.01		1.27683	.44026	1.71709	1.55232
13	91	5.33350	1.51000	6.84350	25.07	31.05		1.33711	.46886	1.80597	1.61260
15	93	4.69000	1.80975	6.49975	27.23	33.34		1.27709	.60337	1.88046	1.55258
17	95	4.65000	2.39600	7.04600	30.37	34.74		1.41221	.83237	2.24458	1.68770
19	97	5.68500	3.08050	8.76550	26.00	32.16		1.47810	.99069	2.46879	1.75359
21	99	4.79050	3.10325	7.89375	25.02	34.07		1.19858	1.05728	2.25586	1.47407
23	101	4.26825	3.06825	7.33650	27.51	37.43		1.17420	1.14845	2.32265	1.44969
25	103	4.03100	3.27125	7.30225	26.65	42.02		1.07426	1.37458	2.44884	1.34975
28	106	3.95425	3.29075	7.24500	32.09	45.82		1.26892	1.50782	2.77774	1.54441
July 2	110	2.19275	2.02125	4.21400	40.05	63.96		.87820	1.29279	2.17099	1.15369
7	115	1.86567	1.74933	3.61500	52.77	80.62		.98451	1.41031	2.39482	1.26000

Figure 8. Curve showing wet weight of main culm, dry weight of main culm and dry weight of main culm with kernels excluded of spring-sown Tennessee Winter barley by days from seedling emergence to maturity.

The curve starts in as typically sigmoid but continues to ascend at a constant rate until the plant is practically mature, due to deposition of dry matter in the kernels. When the weight of the kernels is excluded there is a more clearly marked inflection a few days after flowering with loss in dry weight beginning June 17 due to leaching and loss of dead leaves.


WET WEIGHT OF CULM IN GRAMS
DRY WEIGHT OF CULM IN GRAMS X 10
DRY WEIGHT OF CULM WITHOUT KERNELS IN GRAMS X 10
WEIGHT IN GRAMS
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MARCH APRIL MAY JUNE JULY
0 10 20 30 40 50 60 70 80 90 100 110 120
DAYS FROM PLANTING

inflection occurs until June 11 at the earliest. This is certainly not at flowering as was found by Bakhuysen and Alsberg (1927) in the growth curve of length. The weight of the spike alone increased abruptly from June 7 as we would expect, due to the rapid increase in kernel mass. The weight of the stalk alone increased at an equally rapid rate until June 11 and taperedoff slightly on July 13 and after a drop on June 15 climbed again to June 19. After this date, leaching by rain and leaf breakage caused a decided drop.

The growth in weight of Hannchen (Table 5) differs somewhat from that of Tennessee. In the early part of the growth period (up to April 7) Hannchen was generally heavier in both wet and dry weights; from April 8 to 16 almost the same; and from April 18 to the end was outstripped by Tennessee winter. Final wet and dry weights were much less for Hannchen. However, the same changes in the growth curve (Fig. 9) occurred at very nearly the same points as in Tennessee, i.e., increased rate of growth after May 2 when tillering became active and a further acceleration beginning about May 22 shortly after jointing had been initiated. Here also the curve of the culm without the kernels ran almost parallel with that of the culm with kernels until June 15 when a slight inflection occurred which became marked 10 days after flowering.

Growth as indicated by stage of development of the plant.

Morphologically there are certain definite stages in the development of the barley plant. These stages and the plant age at which they occur are noted in Table 6. From these observations it was hoped that it would be possible to link up the identifiable portions of the growth curve with recognizable stages in development.

Table 5. AVERAGE WET WEIGHT, PER CENT DRY MATTER, AND DRY WEIGHT OF THE MAIN CULM OF HANNCHEN BARLEY

Day of Harvest		Wet Weight			Per Cent Dry Matter			Dry Weight			
Date	Days from Planting	Stalk	Spike	Culm	Stalk	Spike	Culm	Stalk	Spike	Culm	Culm as of June 7
Mar. 28	14			.04533			11.25			.00510	
29	15			.4995			11.25			.00562	
30	16			.05125			11.31			.00580	
31	17			.06325			11.57			.00732	
April 1	18			.06275			11.49			.00721	
2	19			.08050			10.69			.00861	
3	20			.07225			12.56			.00907	
4	21			.07700			13.17			.01014	
5	22			.07375			14.41			.01063	
6	23			.08575			14.91			.01279	
7	24			.08375			17.91			.01500	
8	25			.08650			17.15			.01483	
9	26			.08925			18.21			.01625	
10	27			.09625			17.02			.01638	
11	28			.09725			17.78			.01729	
12	29			.09300			17.68			.01644	
13	30			.10600			17.68			.01874	
14	31			.10500			17.54			.01842	
15	32			.11675			17.88			.02087	
16	33			.13250			18.49			.02450	
17	34			.13825			20.84			.02881	
18	35			.13875			18.92			.02625	
19	36			.14925			20.76			.03098	
20	37			.16300			17.78			.02898	
21	38			.17475			19.10			.03338	
22	39			.16925			16.76			.02837	
23	40			.17850			16.21			.02893	
24	41			.18950			18.03			.03417	
25	42			.22950			17.79			.04083	
26	43			.25500			17.14			.04371	
27	44			.20300			16.21			.03291	
28	45			.25675			15.82			.04062	
29	46			.28725			17.57			.05047	
30	47			.32125			16.65			.05349	
May 1	48			.30500			17.42			.04313	
2	49			.32175			17.65			.05679	
3	50			.33750			17.97			.06065	
4	51			.47625			15.78			.07515	
5	52			.40150			17.24			.06922	
6	53			.48950			15.92			.07793	
7	54			.50825			17.18			.08732	
8	55			.42850			18.88			.08090	
9	56			.43825			16.67			.07306	
10	57			.54800			18.68			.10237	
11	58			.51300			18.07			.09270	
12	59			.43150			18.36			.07922	
13	60			.49175			19.31			.09496	
14	61			.63425			19.30			.12241	
15	62			.58375			18.41			.10747	
16	63			.56900			19.73			.11226	
17	64			.70175			19.57			.13733	
18	65			.83550			18.35			.15331	
19	66			.81300			17.87			.14528	
20	67			.84575			17.93			.15164	
21	68			1.05125			16.94			.17808	
22	69			1.29850			17.39			.22581	
23	70			1.42625			16.74			.23875	
24	71			1.41375			19.73			.27893	
25	72			1.83250			18.74			.34341	
26	73			1.35250			18.15			.24548	
27	74			1.69125			19.65			.33233	
28	75			1.50775			19.31			.29115	
29	76			1.90750			20.70			.39485	
30	77			2.24375			19.31			.43327	
31	78			2.18375			23.04			.50314	
June 1	79			2.21675			22.66			.50232	
2	80			3.09400			23.17			.71688	
3	81			3.02425			23.03			.69648	
4	82			3.20350			20.40			.65351	
5	83			2.97650			22.80			.67864	
June 7	85	2.93625	.65325	3.58950	23.06	23.58		.67710	.15404	.83114	.83114
9	87	2.56225	.63800	3.20025	25.47	27.60		.65261	.17609	.82870	.80665
11	89	2.99400	.81275	3.80675	26.91	30.30		.80569	.24626	1.05195	.95973
13	91	3.26300	.82800	4.09100	24.03	26.44		.78410	.21892	1.00302	.93814
15	93	3.42800	.98100	4.40900	26.80	30.30		.91870	.29724	1.21594	1.07274
17	95	3.30875	1.19125	4.50000	28.79	32.45		.95259	.38656	1.33915	1.10663
19	97	2.75275	1.34125	4.09400	26.82	31.18		.73829	.41820	1.15649	.89234
21	99	3.55500	1.70225	5.25725	26.28	31.51		.93425	.53638	1.47063	1.08829
23	101	2.86625	1.67775	4.54400	30.21	36.04		.86589	.60466	1.47055	1.01993
25	103	2.52775	1.70800	4.23575	32.24	39.95		.81495	.68235	1.49730	.96899
28	106	3.18950	2.06550	5.25500	33.39	42.63		1.06497	.88052	1.94549	1.21901
July 2	110	1.79675	1.75125	3.54800	41.65	52.13		.74835	.91293	1.66128	.90239
6	114	2.16600	1.91725	4.08325	45.51	58.02		.98575	1.11249	2.09699	1.13979
9	117	1.51375	1.33800	2.85175	62.64	81.40		.94821	1.08913	2.03734	1.10225

Figure 9. Curve showing wet weight of main culm, dry weight of main culm and dry weight of main culm with kernels excluded, of spring-sown Hannchen barley by days from seedling emergence to maturity.

Like spring-sown Tennessee Winter(Fig.8) the curve of dry weight continues to ascend until the plant is practically mature. When the kernel weight is disregarded an inflection here also occurs shortly after flowering.

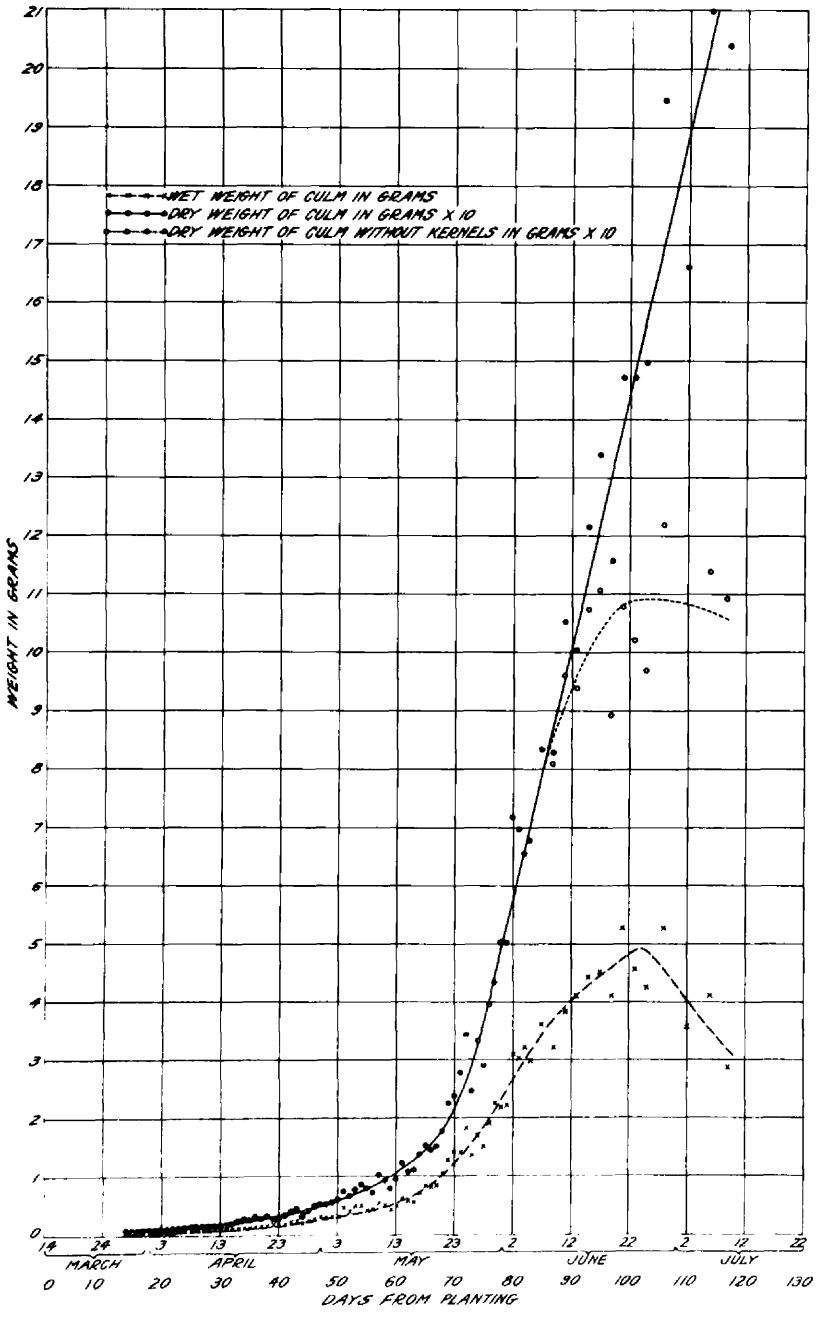

WET WEIGHT OF CULM IN GRAMS
DRY WEIGHT OF CULM IN GRAMS X 10
DRY WEIGHT OF CULM WITHOUT KERNELS IN GRAMS X 10
WEIGHT IN GRAMS
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21
14 24 3 13 23 3 13 23 2 12 22 2 12 22
MARCH APRIL MAY JUNE JULY
0 10 20 30 40 50 60 70 80 90 100 110 120 130
DAYS FROM PLANTING

Table 6. Plant ages at which certain stages of development were reached.

Stages of development	Hannchen		Spring Tennessee		Fall Tennessee
	Date	Plant age	Date	Plant age	
First leaf	March 29	15 days	March 31	17 days	
Second leaf	April 5	22			
Endosperm all matter nearly used		22	March 30	16	
Scutellum shrivelled	April 10	27	April 7	24	
Third leaf	April 16	33	April 17	34	
Perm. roots started	April 17	34	April 17	34	
Tillers first appear	April 22	39	April 20	37	
Perm.roots generally present	April 21	38			
Fourth leaf	April 24	41	April 21	38	
Tillers well developed	May 2	49	May 2	49	
Roots developing on tillers			May 2	49	
Jointing begun(length curves show jointing. Began about 65 days in both)			May 21	68	
Basal leaves mostly dead	May 21	68	May 21	68	
Boot leaf showing	May 31	78	May 30	77	May 1
First awns emerging	June 2	80	June 2	80	May 5
First flowering	June 7	85	June 7	85	May 9
Tip of boot leaf dying	June 17	95			June 3
Awns brown at tip			June 19	97	June 6
Kernels hard dough stage					June 6
Spikes nearly dried out	July 2	110	July 2	110	

The Curve of Catalase Activity in the Barley Plant

The standard method adopted was followed with but few modifications and these were unimportant. Care was taken to give all samples as nearly identical treatment as possible so that, while certain operations may be criticized and different parts of the apparatus might be improved, the results, it is believed, are consistent. For convenience in study there is collected in Table 7 certain data previously presented, namely, length in centimeters, and dry weight in grams, for Hannchen barley. The table also gives the actual catalase activity found for each sample and the catalase activity corrected for actual dry matter present in the sample tested. In the last column is given the catalase activity calculated for the total culm on the basis of its dry weight. This value is obviously a mere approximation since any error in obtaining the catalase activity of but 2 cc of a 1-250 dilution of dry weight would be multiplied by a factor varying from about 1.5 in the first days sample to over 500 in the last.

In Figure 10 the total amount of catalase activity in the culm of the Hannchen barley plant as well as the value per unit dry weight, is plotted against time. On the same sheet are shown the curves of total length and total dry weight of main culm. The stages of development of the plant are also indicated on the dates on which they were noted.

While there is a rather wide variation in catalase activity per unit dry weight from day to day, there is a distinct trend apparent as is indicated by the smoothed curve drawn through the points. There are 4 clearly defined elevations in the first 70 days of growth. During this time the plant is composed mainly of succulent tissue which has been actively growing. The dry matter does not reach 20% until day 76. After day 70

Table 7 . Average length, dry weight and catalase activity in spring-sown Hannchen barley (1928)

Day of harvest		Length in cm.	Dry weight in grams	Catalase activity ($cc O_2$ evolved in 10 min.)	Per cent D.M. figures	Catalase activity corrected for actual Dry Matter	
Date	Days after planting				Per cent D.M. actual	Relative	Per plant
March 28	14	3.040	.00510	---	---	---	
					11.29		
29	15	3.295	.00562	17.930	11.25	17.99	12.64
					11.29		
30	16	3.840	.00580	20.875	11.31	20.84	15.11
					11.31		
31	17	4.700	.00732	21.510	11.57	21.03	19.24
					11.57		
April 1	18	4.410	.00721	17.910	11.49	18.03	16.25
					11.46		
2	19	4.585	.00861	20.540	10.69	22.02	23.70
					10.69		
3	20	5.515	.00907	24.370	12.56	20.74	23.51
					12.56		
4	21	5.765	.01014	23.510	13.17	22.42	28.42
					12.56		
5	22	6.190	.01063	30.640	14.41	26.71	35.49
					14.41		
6	23	7.655	.01279	25.465	14.91	24.61	39.35
					14.91		
7	24	6.760	.01500	28.640	17.91	23.84	53.70
					17.91		
8	25	7.435	.01483	19.210	17.15	20.06	37.19
					17.15		
9	26	7.560	.01625	15.910	18.21	14.98	30.43
					18.21		
10	27	7.675	.01638	13.290	17.02	14.22	29.12
					17.02		
11	28	7.530	.01729	17.280	17.78	16.54	35.75
					17.84		
12	29	8.115	.01644	17.260	17.68	17.42	35.80
					17.68		
13	30	7.355	.01874	14.330	17.68	14.33	33.57
					17.68		
14	31	8.140	.01842	13.320	17.54	13.43	30.92
					17.54		
15	32	8.255	.02087	12.920	17.88	12.67	33.05
					17.88		
16	33	9.080	.02450	11.320	18.49	10.95	33.53
					18.00		
17	34	9.775	.02881	10.100	20.84	8.72	31.40
					18.00		
18	35	9.250	.02625	10.730	18.92	10.21	33.50
					18.00		
19	36	10.365	.03098	13.900	20.76	12.05	46.66
					20.76		
20	37	11.165	.02898	12.200	17.78	14.24	51.58
					17.80		
21	38	10.805	.03338	15.050	19.10	14.03	58.54
					20.00		
22	39	11.230	.02837	13.350	16.76	15.93	56.49
					20.00		
23	40	10.965	.02893	13.290	16.21	16.40	59.31
					20.00		
24	41	11.000	.03417	14.180	18.03	15.73	67.19
					20.00		
25	42	12.155	.04083	14.500	17.79	16.30	83.19
					20.00		
26	43	12.775	.04371	13.400	17.14	15.64	85.45
					20.00		
27	44	11.070	.03291	11.250	16.21	13.88	57.10
					18.00		
28	45	12.190	.04062	14.060	15.82	16.00	81.24
					18.00		
29	46	13.060	.05047	13.570	17.57	13.90	87.69
					18.00		
30	47	13.075	.05349	17.070	16.65	18.45	123.37
					20.00		
May 1	48	14.090	.05313	16.820	17.42	19.31	128.24
					20.00		
2	49	14.160	.05679	16.890	17.65	19.14	135.87
					20.00		
3	50	15.700	.06065	16.720	17.97	18.61	141.09
					20.00		
4	51	17.290	.07515	17.740	15.78	22.48	211.17
					20.00		
5	52	16.110	.06922	21.800	17.24	25.29	218.82
					20.00		
6	53	16.585	.07793	18.120	15.92	22.76	221.71
					20.00		
7	54	17.840	.08732	22.630	17.18	26.34	287.50
					20.00		
8	55	16.980	.08090	16.470	18.88	17.45	176.46
					20.00		
9	56	16.475	.07306	13.500	16.67	16.20	147.95
					20.00		
10	57	19.150	.10237	13.600	18.68	14.56	186.31
					20.00		
11	58	18.335	.09270	15.500	18.07	17.16	196.70
					20.00		
12	59	17.090	.07922	16.650	18.36	18.14	179.63
					20.00		
13	60	17.910	.09496	14.750	19.30	15.28	181.37
					20.00		
14	61	20.395	.12241	15.640	19.30	16.21	248.03
					20.00		
15	62	20.245	.10747	14.230	18.41	15.46	207.69
					20.00		
16	63	19.560	.11226	14.160	19.73	14.35	201.37
					20.00		
17	64	21.770	.13733	15.73	19.57	16.08	276.03
					20.00		
18	65	23.945	.15331	18.65	18.35	20.33	389.60
					20.00		
19	66	23.315	.14528	17.15	17.87	19.19	348.49
					20.00		
20	67	25.550	.15164	18.91	17.93	21.09	399.76
					20.00		
21	68	28.040	.17808	17.96	16.94	21.20	471.91
					20.00		
22	69	29.790	.22581	17.57	17.39	20.21	570.45
					20.00		
23	70	31.920	.23875	18.24	16.74	21.79	650.30
					20.00		
24	71	31.705	.27893	14.93	19.73	15.13	527.53
					20.00		
25	72	32.735	.34341	12.54	18.74	13.38	574.35
					20.00		
26	73	31.575	.24548	14.27	18.15	15.72	482.37
					20.00		
27	74	36.015	.33233	12.58	19.65	12.80	531.73
					20.00		
28	75	34.595	.29115	13.52	19.31	14.00	509.51
					20.00		
29	76	39.880	.39485	12.54	20.70	12.12	598.20
					20.00		
30	77	42.515	.43327	11.94	19.31	12.37	669.94
					20.00		
31	78	44.210	.50314	13.74	23.04	11.93	750.31
					20.00		
June 1	79	45.180	.50232	14.39	22.66	12.70	797.43
					20.00		
2	80	52.55	.71688	12.62	25.17	10.89	975.85
					20.00		
3	81	53.35	.69648	14.03	23.03	12.18	1060.39
					20.00		
4	82	51.89	.65351	13.24	20.40	12.98	1060.31
					20.00		
5	83	56.50	.67864	15.78	22.80	13.84	1174.05
					20.00		
7	85	58.685	.83114	14.585	23.16	12.59	1308.01
					20.00		
9	87	59.830	.82870	14.99	25.90	11.58	1199.54
					20.00		
11	89	63.665	1.05195	16.38	27.57	11.88	1576.25
					20.00		
13	91	64.005	1.00302	13.725	24.51	11.20	1404.23
					20.00		
15	93	65.485	1.21594	17.86	27.62	12.93	1965.26
					20.00		
17	95	68.565	1.33915	12.785	29.84	8.57	1434.56
					20.00		
19	97	62.150	1.15649	13.005	28.32	9.18	1327.07
					20.00		
21	99	66.995	1.47063	18.105	28.01	12.93	2376.91
					20.00		
23	101	64.705	1.47055	16.895	32.39	10.43	1917.23
					20.00		
25	103	64.890	1.49730	17.11	35.43	9.66	1807.99
					20.00		
28	106	70.150	1.94549	11.93	37.66	6.34	1541.80
					20.00		
July 2	110	68.500	1.66128	7.23	46.62	3.10	643.75
					20.00		
6	114	69.800	2.09699	5.765	51.49	2.24	587.16
					20.00		
9	117	71.475	2.03734	5.095	71.16	1.43	364.17

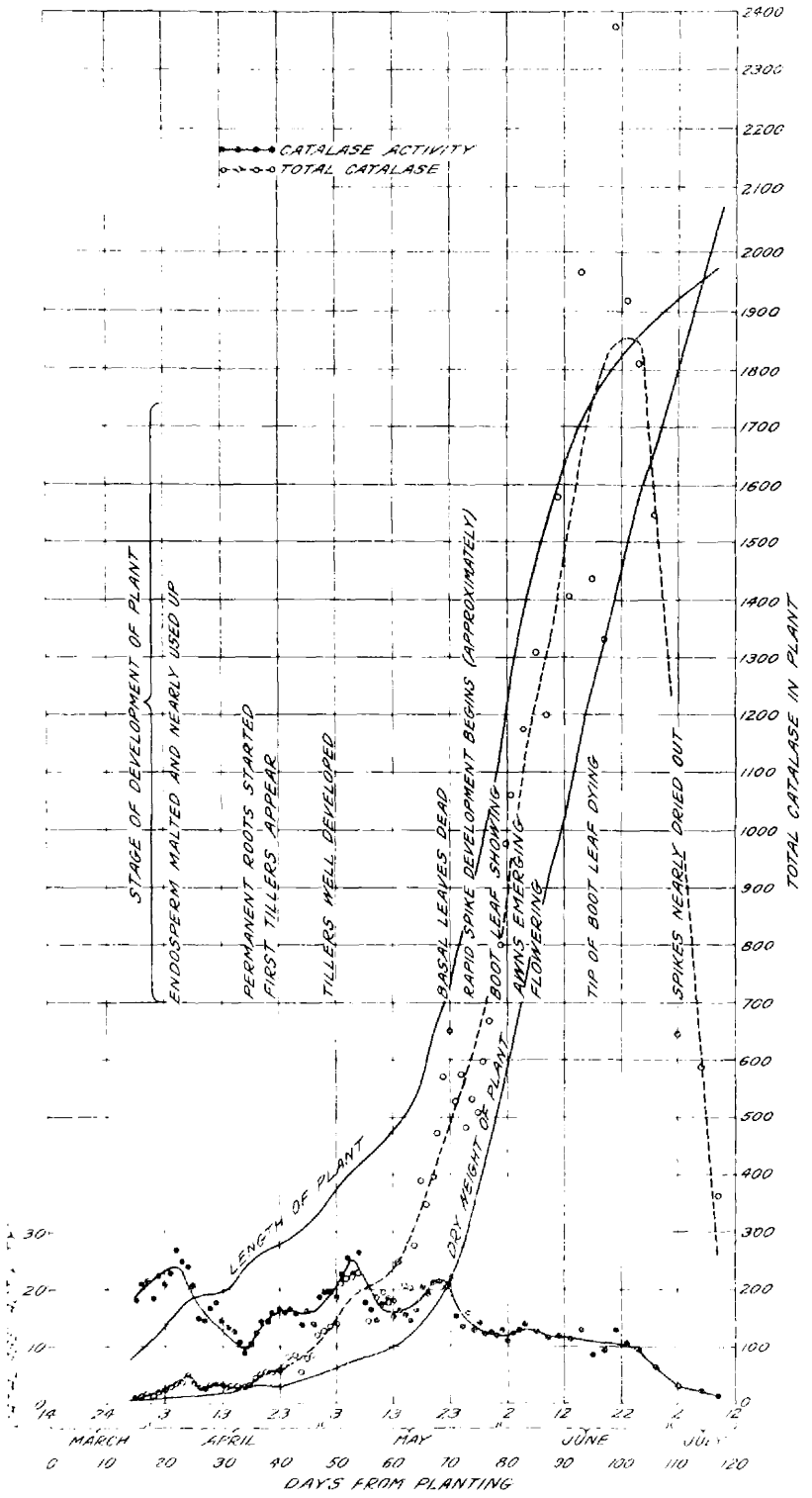
(May 23) the dry matter increases gradually at first and then very rapidly as the seeds dry out. On May 23 the basal leaves are dead. The culm leaves soon follow and on June 17 the boot leaf is dying, the maximum moisture remaining longest at and in the nodes and in the kernels. From May 23 the catalase activity decreases quite uniformly until June 25 when final drying out occurs and the curve drops to 1.43 cc. at the final determination. The period up to day 70 is clearly that of maximum metabolism or at least anabolism and consequently it seems definitely shown that high metabolism is associated with high catalase activity.

One may go still farther and say that periods of rapid building up of tissue such as (1) utilization of endosperm, (2) development of tillers, (3) active growth of tillers, and (4) sex cell development, are directly reflected in elevations in the catalase curve. The two most noticeable depressions in the curve are located at (1) the beginning of permanent root development (day 34, April 17) and (2) around May 28 (day 45) when tillers and crown roots have been developing but have not reached the active growing stage. At both of these periods the plant has outgrown the plant parts previously supplying it with soil nutrients and is under the strain of supplying a vigorously growing organism through insufficient sources of supply. Another depression well marked on the curve appears just before the culm begins to joing actively. The anlagen of the floral parts have been slowly differentiating into a spike since about the first of May. This spike now begins to grow more rapidly as jointing occurs and in about 12 days has almost reached full size.

Figure 10. Graph showing the stages of development of the plant and the curves of length of culm, dry weight of culm, total catalase per plant, and catalase activity per unit of dry weight, from emergence of plumule to maturity in Hannchen barley.

Note that elevations in the curve of catalase activity are associated with those stages of development which are presumably periods of increased metabolic activity. The total catalase seems to vary with the amount of living protoplasm in the plant.

The graph also shows the relation between the amount of growth and developmental stage of the plant. Notice that no inflection of the curve either of length or of dry weight occurs at flowering.


CATALASE ACTIVITY
TOTAL CATALASE
STAGE OF DEVELOPMENT OF PLANT
ENDOSPERM MALTED AND NEARLY USED UP
PERMANENT ROOTS STARTED
FIRST TILLERS APPEAR
TILLERS WELL DEVELOPED
BASAL LEAVES DEAD
RAPID SPIKE DEVELOPMENT BEGINS (APPROXIMATELY)
BOOT LEAF SHOWING
AWNS EMERGING
FLOWERING
TIP OF BOOT LEAF DYING
SPIKES NEARLY DRIED OUT
LENGTH OF PLANT
DRY WEIGHT OF PLANT
TOTAL CATALASE IN PLANT
MARCH
APRIL
MAY
JUNE
DAYS FROM PLANTING

Discussion

Growth curve

The difficulty of reducing the growth curve to an exact mathematical law can not but be readily apparent. If one were to take small definite portions of the curve or work from data apparently insufficient in amount, it is probable that either the monomolecular autocatalytic, or the compound interest formula might apply. The autocatalytic reaction may actually occur in plant growth. On the surface that assumption simplifies matters since it reduces a life process to a question of a chemical reaction. But there are a number of objections.

If we accept this theory of growth we must assume (1) the existence of a growth catalyst, (2) a "master reaction" to which this curve applies and (3) that inflection of the curve is due to the accumulation of products of growth presumably reacting in the opposite direction under the influence of the growth catalyst. In criticism it may be said first, that the existence of a growth catalyst has not been proven, although some evidence seems to point in that direction. Second, while a single "master reaction" may control the velocity of the process throughout the whole growth period, it is probable that different "master reactions" are in the ascendant under different environments and during different stages in the life cycle. Third, many of the products of the growth process, in plants particularly, are materials which seem to be chemically inactive, for example, cellulose, lignin, and the stored foods. This fact argues against the existence of a reversed reaction of enough strength to inflect the curve and finally to kill the plant. There is not entire agreement among the exponents of this theory. One (Reed) explains the inflection by assuming the inactivation of the

catalyst and the rise of growth inhibiting substances. These assumptions seem to need as much proof as does the main thesis itself. Another (Crozier) while believing in the autocatalytic nature of growth can not explain his difficulties by the monomolecular formula but must postulate at least two reactions. This also rapidly complicates matters.

The compound interest law certainly is applicable in part, at least, but so many extraneous factors enter in to complicate its action that it is of doubtful utility.

It is possible to study such external factors as light, temperature, moisture, effect of respiration, and the nature and amount of available nutrients, including the ease and rate of supplying them, but there are possible many other environing factors not now recognized as affecting growth. For example, it is conceivable that electrical charge conditions, as believed by Blackman (1924), and mechanical effect of crowding may influence growth rate. As for the amount of growing material, it is probable that even in the single cell, all parts do not grow with equal rapidity. Then, too, our extremely inadequate knowledge of the complex colloidal system which we call protoplasm precludes the possibility of recognizing, much less evaluating the internal factors at work within a single cell. When one considers the fact that the growth of an organ or plant part depends on the growth of its individual cells, the complexity increases enormously. In this study, not the growth of a single cell nor even of a single plant part is studied, but some 2000 variable individuals are measured utilizing the whole development period, carrying the plant from germinating seed to seed-bearing maturity. With germination we have an actively growing seedling. Soon the seedling parts have grown to full size and died or at best have been transformed into inactive organs of conduction or support. New, vigorously growing parts then

arise to supply the needs of nutrition and upright stability. These soon fulfill their mission and the plant, almost full-sized, becomes a machine for absorbing soil nutrients and manufacturing and storing in the seed the protoplasm, carbohydrates, proteins, fats, enzyms, etc., necessary for the young plant existing in the seed. Several writers have pointed out that the deposition of such dormant or non-growing material during the cycle of growth can not easily be accounted for by a growth formula. As Farr (1924) states, "Dry weight is augmented by photo-synthesis, salt absorption and assimilation: diminished by respiration, digestion and secretion. If we measure the dry weight increase of only a part of the plant, then translocation is also a modifying factor. Growth becomes a balance between other processes and its integrity is lost."

Brody's assertion (cited previously) that the growth curve of an animal consists of probably five definite stages each possessing its own growth constant, indicates the futility of attempting to reduce the curve to a mathematical formula useful or exact in its application. Theoretically it would appear that if one had infinite knowledge of what happens in the growing multicellular organism, an infinite number of "k's" might be shown. Practically it seems that only when these five stages are definitely correlated with easily recognized stages in the growth history may they have a utilitarian value.

In a discussion of the mathematics of the growth curve, Sir Edward Russell (1927) has made several very pertinent observations:

"An equation has a satisfying look of completeness and accuracy: like a camera, it can not lie but it can mislead."

"But its (the growth equation) widely general nature makes it too indefinite to be particularly helpful to the biologist or to the soil in-

vestigator. It has to be simplified before it can be used, and the process of simplification necessitates the making of assumptions. Now the danger of mathematical equations lies in the assumptions they conceal. It would not be difficult to find examples in scientific literature where assumptions have been accepted in a mathematical equation that would have caused some surprise had they been put forward as independent statements."

"The criterion of the value of the mathematical expression is the purely pragmatic test whether or not it serves any useful purpose. Time alone will show this."

A number of investigators have shown the existence of correlations between portions of the growth curve and definite stages in the development of the plant. Some believe that the water or nutrient supply is the limiting factor in certain growth stages and that competetion for this material occurs between different parts of the plant.

The data herein presented indicate that lack of sufficient root tissue is a factor which inhibits length growth and possibly increase in dry weight in the barley plant. Another flattening of the length curve occurs at the time of rapid tiller development and is accompanied by the enhanced growth of permanent roots. The development and growth of the spike anlage into the full-sized flowering head is accompanied by a rapid elongation and increase in dry weight of the whole culm. Up to the beginning of this period of rapid elongation ("jointing" or "shooting") the amount of absorptive tissue has evidently been a factor limiting the growth of the plant. At the time of most rapid tiller growth, these young parts, growing much more actively than the main culm, compete successfully for the nutrients. The rapid growth of the tillers is accompanied by an increased root growth until the tillers are nearly the size of the main culm when the root tissue is sufficient in

amount to supply both. Active jointing then follows. Another factor now enters to complicate the process. For an upright posture the jointing culm must be strong enough not only to support its own weight but to successfully resist wind action and at maturity to carry a heavy load of starch-laden seeds. This function is supplied by the deposition of seemingly inactive materials such as cellulose, lignin, silica, etc., in the stalk. This, of course, rapidly takes much tissue out of the realm of active anabolism, thereby decreasing the "principal." There is for some time, however, enough actively growing tissue to more than counter balance this defection. But barley is a plant of determinate growth. Normally there is no branching at the upper nodes with the exception of the modified branches and leaves which constitute the floral organs in the spike. The flowering head is the terminus of the culm. If this does not proliferate, length growth must cease and the inflections of the curve of awn tip length will be the inflection of the curve of culm growth. Contrary to the results of a number of workers there appears in barley no major inflection in total length nor in total dry weight at flowering. There is, however, an inflection of the leaf length curve at or shortly after flowering. But this is not the height of the plant. We must consider the growth habits of a plant in making such generalizations. Both maize and wheat extrude their flower heads above the leaf tips before flowering takes place. At anthesis the upward growth is about ready to stop. It is very different in barley where flowering quite generally occurs while the spike is still enclosed in the boot leaf. The last internode of the culm, bearing the spike, is still young and composed of soft growing tissue and continues to elongate until the spike emerges from the sheath and extends, in most varieties, above the end of the distal culm leaf. The basal leaves are practically dead at the time jointing begins, and necrosis proceeds

distally, first appearing in the leaf tips and working back through the blade to the leaf sheath. The top or boot leaf begins to die at the tip when the kernels are about one-half or two-thirds their mature age and the awn tips begin to brown a few days later. The culm axis or stalk loses its chlorophyl rapidly at this time. However, the leaf sheaths protect the stalk from excessive drying for a considerable time and the vessels are still capable of conducting soil water to the still actively growing kernels. After the chlorophyllous tissue has ceased to function there probably is still translocation of dry matter from the upper part of the stalk and the spike into the kernels even after the culm has been harvested. This is certainly true when the kernels are immature when harvested (Harlan and Pope, 1926).

If now we turn to a consideration of the growth in dry weight we find a state of affairs entirely different from growth in length. Not only does the inflection of the curve not occur at flowering, but there seems to be no true inflection at all. This is due to the fact that the growing seeds present in the spike serve as storage organs for an amount of dry matter which may equal or exceed the total dry matter contained in the remainder of the culm. The deposition of this material begins actively a few days after fertilization of the egg cell and continues for nearly a month when structural difficulties abruptly halt growth.

"Endosperm cells mature abruptly; the proteid content probably reaches a density beyond which it can not function. The Jet is a naked variety in which a black pigment is formed in the pericarp whenever this tissue ceases to be active. The appearance of color indicates that the first region to mature is on the dorsal surface near the tip. The region of the embryo on the dorsal surface is still later, and the cells adjacent to the furrow on

the ventral surface are the last to mature. The first cells on the dorsal surface are affected when the moisture content of the kernel has reached 60 to 62 per cent. The kernels are fully mature when the water has fallen to 46 per cent and have carried on only a limited amount of translocation for some time.

The date of final maturation can be postponed and the size of the kernel increased where kernels are protected by leaf sheaths or other shade and by cool weather at ripening." (Harlan and Pope, 1923).

After considering the evidence presented herein and also that of investigations already cited, the writer advances what to him is a reasonable and relatively simple explanation of the growth curve in plants. Postulating favorable conditions of soil and weather environment, rate of growth in the plant is largely, if not entirely, conditioned by the maximum nutritional opportunities in different parts of the plant and the ability of the plant from a physical and chemical standpoint to take advantage of those opportunities.

According to this hypothesis the aerial portions of the young plant will grow with increasing rapidity according to the compound interest formula provided the roots have an absorbing and carrying capacity sufficient to satisfy the nutritional needs of the plant. As plant parts mature, more and more inactive substances such as cellulose and lignin are laid down as is evidenced by a greater $^{C}/_{N}$ ratio. There is consequently less tissue capable of growth as such inactive tissue is no longer "principal" and, therefore, this "principal" increases more slowly or at a rate directly proportional to the amount of actively growing tissue. If a sufficient amount of this actively growing tissue is or can be maintained the growth rate will continue constant. Such a condition exists in plants of indeterminate growth such as the tomato.

Here increase in size will continue until the production of organs such as flowers and fruits robs the vegetative growing point of the $^{C}/_{N}$ ratio necessary for active growth. In a plant of determinate growth, such as barley, where branching of the culm does not occur normally and the spike is produced at the apex, growth continues in the culm and spike until inactive materials are laid down in all parts in sufficient amount to hinder enlargement. Growth will then stop.

In the barley kernels this limitation to growth is largely due to starch congestion. Such large amounts of this material are stored up in the endosperm cells that mitotic division ceases and the available space is soon taken. The cell protoplasm is crowded into an elaborate mesh work of fine strands. This has been demonstrated by Cobb (1905) in beautiful preparations made from the kernels of wheat. This deposition of starch, in amounts sufficiently large to hinder cell division, places a limit on this maximum size of the kernel. Starch deposition will then proceed until prevented by dehydration, lack of necessary materials, or the tension of the cell walls.

It seems possible to harmonize the theses of a number of investigators on the basis of the above hypothesis.

The slackening of growth during root formation (Priestley, Pearsall, Evershed) is due to the inability of the available root surface to absorb water soluble nutrients in sufficient amount to balance the maximum photosynthetic activity possible in the leaves and stem.

Osmotic concentration is higher in actively growing portions (Fernald) possibly partly because more evaporation is possible from soft unhardened tissues.

The C/N ratio is low since the tissues are young and have not yet been clogged up with inactive material composed largely of carbohydrates. ("The younger the tissue the lower the C/N ratio." - Hicks) The "metabolic gradient" of Child is explainable in plants by the deposition of inactive material as age progresses, thereby producing a gradient of "nutritional opportunity".

Miss Hicks (1928 III) explains Loeb's hormone theory on the basis of C/N ratio which in turn is associated with "nutritional opportunity". The disappearance of water from the plant at flowering (Bakhuyzen 1926) seems to be merely the deposition of inactive material and consequent drying out of tissues which are approaching senescence.

As soon as the tomato flower is fertilized the ovary becomes the youngest and most actively growing tissue in the plant. It is capable of enormous enlargement due to its lack of fibrous cellulose tissue. Therefore, it continues to compete successfully with the vegetative parts of the plant for nutrients. (Murneek)

Respiration and Catalase Activity

The evidence heretofore advanced on the relation of catalase activity to metabolism and particularly to respiration, is somewhat contradictory with the balance in favor of an association between the two. The evidence against such a relation comes chiefly from some of the animal men although Lantz did not find a close correlation between the two in germinating corn. Burge is a stanch defender of the affirmative. W. E. Davis and Gracanin state that

catalase activity is a neeessary adjunct to germination and Appleman has shown clearly that the catalase activity and respiration vary together in the expressed juice of the potato tuber and sweet corn. Altogether the writer feels safe in accepting the thesis that catalase activity is necessary to some phase of metabolism and his data point strongly to respiration as being that phase. A low C/N ratio is generally accepted as necessary to active metabolism. Auchter's data (1923) on woody plants indicate that an increase in carbohydrates in proportion to the nitrogen present is accompanied by a decreased catalase activity. In wheat Miss Hicks (1928 II) found the lowest C/N ratio in the distal end of an organ, for instance, the leaf tips have a lower C/N ratio than the bases even though the meristem of the leaves is at the base. In the writer's determination of catalase activity in different parts of the barley plant (see appendix) the flag of the boot leaf contained an amount greater than any other part tested and the sheath of the boot leaf held second place in one variety and third in another variety. The awns held third and second place respectively in these experiments. All the parts of the stalk, including the kernels, proximal to the awns were significantly lower in catalase activity. It is interesting to compare the curves of respiration in the soybean seedling obtained by Hofenrichter with those of catalase activity in barley. Both are quite variable but both have a high value in the early stages.

Catalase activity per unit dry weight has in every series of tests shown a high point during germination and early seedling stages of barley growth. In the data herein presented other high points occur at definite developmental stages where presumably metabolic activity is at a maximum, although rate of growth is not. Rapidity of growth as evidenced by extension in length or increase in dry matter, is not necessarily a measure of metabolic activity. For example, the early development of the sex cells occurs before the most rapid elongation of the spike. May not this <u>differentiation</u> of tissue, like

that in germination and tiller bud and crown root bud development, be the really crucial phase of metabolic activity rather than the more obvious increase in size? It would be very interesting to know exactly how much protoplasmic differentiation and cell division there is in the most rapid stage of growth.

Growth and respiration

From a review of the literature the relation between growth and respiration seems to be an open question. Theoretically, intensity of respiration seemingly should run parallel with activity of growth and most of the earlier investigations agree that such is the case. Among later writers, Kidd, West and Briggs; and Inamdar, Singh and Pande also agree, but Hover and Gustafson, and Hofenrichter present evidence to the contrary.

The data presented in this paper show no parallelism existing between catalase activity and growth, but the writer believes that it does show an association between catalase activity and intensity of metabolism.

Summary

1. The growth curve of the barley culm without kernels is typically sigmoid with certain slight variations from a perfectly smooth curve in both early and rapidly maturing stages.

2. Such variations as occur in the curve of the early growth are associated with definite stages of development.

3. Retardations in length growth rate occur at about the time of permanent root inception and again when the first tillers are appearing and before their roots are established. When the roots have become functional the curve rises more sharply.

4. The curve of "leaf length" reaches its inflection at about first flowering, but neither the curve of "awn tip length" nor total length of culm reaches inflection until 5 days later in the Tennessee variety and 8 to 10 days later in Hannchen.

5. In the curve of growth in weight no marked retardation is evident until practically mature. However, at the period of rapid growth of tillers, the growth of the main culm becomes more rapid and when jointing begins the acceleration is still more marked.

6. The curve of culm weight excluding kernels shows inflection occurring not at flowering but 4 days later in the case of Tennessee and 10 days after flowering in Hannchen.

7. The total weight of the culm (including kernels) increases at a regular rate until about the hard dough stage of the kernel at which time the leaves and awns have all begun to die.

8. Beyond the hard dough stage the culm loses weight on account of mechanical breakage and leaching by water.

9. The grand curve of total catalase activity per plant runs roughly parallel with that of growth until the plant nears maturity, when a decided drop occurs.

10. The curve of catalase activity per unit dry weight shows three peaks corresponding to stages in the growth of the plant when metabolic activity is presumably at a maximum.

Conclusions

The entire growth curve is sigmoid. However, since this curve is a composite picture of the grand periods of growth of not only all the organs but also of each individual cell in the measured individual, any event, external or internal, which can affect the development, either favorably or unfavorably, of a cell or group of cells, must be able to modify the shape of such a curve.

It seems futile to attempt to fit a definite and accurate mathematical formula to a procession of events varying so much with the forces actuating it and so much affected by unfavorable and intermittant conditions as is the growth curve. Such a formula must be an average to the same degree that the grand growth curve is the resultant or the average of all the growth curves comprising it.

Definite alterations in the growth curve have been shown to occur at well-defined stages in the growth cycle of the plant.

In the early part of growth the structural inability of the existing roots to absorb nutrients rapidly enough is the factor believed to inhibit the growth in length of the culm of barley. This inhibition is removed as soon as new roots are formed and become functional to a degree sufficient to supply the necessary nutrient materials.

No marked change in the growth curve of barley occurs at the flowering stage either in length or in dry weight.

No major inflection is evident in the curve of dry weight of culm until practically mature. If, however, the weight of the seed in the spike is excluded, a sigmoid curve results, the major inflection of which is several days after flowering in barley.

The following statement is suggested as a theory to explain the growth curve in plants: "Postulating favorable conditions of soil and weather environments, rate of growth in plants is largely, if not entirely, conditioned by the maximum nutritional opportunities in different parts of the plant and the ability of the plant, from a physical and chemical standpoint, to take advantage of those opportunities".

It is apparent that total catalase activity varies directly with the total amount of actively growing tissues in the plant.

In this investigation the periods of enhanced catalase activity are associated with stages in the development of the plant where metabolic activity is presumably at a maximum. This is not necessarily during most rapid growth.

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Appendix.

NOTES ON THE CATALASE REACTION

1. Strength and amount of hydrogen peroxide used.
2. Freshness of the tissue.
3. Acidity of the suspension.
4. Methods of triturating the sample.
5. Effect of materials added.
6. Effect of different degrees of dilution.
7. Effect of stirring before sampling.
8. Preservation agents.
9. Storage of the suspension.
10. Variation in catalase activity in different plants of the same age.
11. Catalase activity in different parts of the plant.
12. Localization of catalase activity in the suspension.
13. Effect of heat on catalase activity.

Summary and conclusions.

In the process of developing a standard method for the determination of catalase activity in barley it was necessary to do a considerable amount of preliminary work in order to find out the conditions favorable for maximum oxygen evolution and the effect of deviations from this standard method which became necessary during certain stages of the investigation. During the progress of the daily determinations, various phenomena occurred which suggested various lines of work on the catalase reaction itself. Insofar as time permitted, these lines of work were followed up and are herein given, not at all as a completed piece of research but merely as isolated and oft times incomplete observations which point the way to further lines of investigation.

In all the work presented, the apparatus described in the body of the paper was used and all deviations from the standard method are noted in the particular experiment described.

Strength and Amount of Hydrogen Peroxide Used

The H_2O_2 used was 12 Volume Dioxygen and has always been found sufficiently strong. It was frequently tested and always exceeded the value ascribed to it. Typical tests are as follows:

Date	Sample	O_2 evolved in 10 min.	2 cc more extract added and run, gave an additional	Total from 2 cc H_2O_2
March 28	#1	18.48	8.35 cc (5 min.)	26.83 cc
31	#2	21.32	4.54 (3 min.)	25.86 cc
May 2	#2	16.08	9.64 (3 min.)	25.72 cc

The exact amount of H_2O_2 does not matter so long as (1) it is sufficiently in excess, (2) there is enough to be easily available and (3) not so much as to produce appreciable poisoning.

Results from 2 cc H_2O_2 were gotten in all cases except April 3, 4, 5, 6, 7, 8, 9, when 3 cc was used. On some other days the gas evolved exceeded 20 cc and the results might seem low. On days when the gas evolved was less than 10 cc it probably would have been better to use but 1 cc of H_2O_2 as there probably was some poisoning of catalase.

Poisoning. On December 13, a greenhouse-grown plant 13 days old, including roots and seed weighing .3028 grams, was ground with $CaCO_3$ and diluted. Two cc samples were run against different amounts of H_2O_2 neutralized with $CaCO_3$.

Run number	Start	No. cc H_2O_2	1	2	3	4	5 minutes	
1	9:15	2	2.11	3.38	4.35	5.15	5.80	(
2	9:23	2	2.07	3.30	4.23	5.00	5.60	(2 cc evidently not too much
3	10:00	4	1.88	2.94	3.84	4.51	5.03	(poisoned)
4	10:08	1	2.08	3.35	4.32	5.05	5.62	

There is a slight error introduced when the H_2O_2 has not had sufficient time after addition of $CaCO_3$ to evolve and get rid of the CO_2 produced in the neutralization process. Overholser, (1928) takes care of this by introducing a U-tube of soda-lime to absorb the CO_2 but it seems to be a needless precaution since neutralized H_2O_2 run against a water blank has been tried a number of time, with an insignificant amount of gas being evolved.

Date	Sample number	Number minutes run	cc gas evolved
March 7	1	10	0.18
	2	10	0.09
June 23	1	3	0.09
	2	10	0.09
June 26	1	3	0.20
	2 (3 hrs. 24 min. later)	3	0.05

When unneutralized H_2O_2 was run against a water blank containing $CaCO_3$ the following evolution of gas occurred:

Date	Sample number	Start	1	2	3	4	5	6	7	8	9	10
May 7	1	3:04	0.05	0.06	0.06	0.06+	0.05	0.06	0.06+	0.06	0.06+	0.06+
	2	3:18	0.04	0.04+	0.03	0.02	0.01	0.01	0.00	0.00	0.00	0.00

The acid of the dioxygen seems incapable of causing evolution of enough Co_2 to show an appreciable error.

Freshness of the Material

The catalase activity of barley tissue is greatly decreased by drying before suspending in water.

Date	Sample number	Treatment	O_2 evolved in 5 min.	In 10 Min.
Febr. 1	a_2 1	Standard	6.30	
	2	"	6.11	
4	a_{1a} 3	dried @ less than $<35^o$	0.11	
	4	before standard treatment.	-0.10	gas absorbed
	a_{1b} 5	" "	0.55	0.76
	6	" "	0.14	0.20

Acidity of the Suspension

As acidity develops in freshly ground plant tissue, catalase activity decreases. This acidity shows an effect as soon as the catalase activity of an unneutralized sample can be measured.

a. On February 1, 1928, a plant 75 days old, grown in the greenhouse, was divided into two parts by splitting culm and each leaf. The two parts were then weighed and ground in the mortar one with and the other without $CaCO_3$ and diluted 1-50. The latter was then immediately tested for catalase activity.

Treatment	Run #	Time Ground	Start	1	2	3	4	5	10
With $CaCO_3$	1	?	11.46	2.34	3.74	4.91	5.84	6.61	
	2		11.55	2.40	3.90	5.10?	6.03	6.81	8.73
	3		12.37	2.43	3.96	5.16	6.17	6.96	9.05
Without $CaCO_3$	1	11:08:10	11:19	2.11	3.46	4.62	5.63	6.43	
	2		12.19	2.10	3.45	4.63	5.54	6.31	8.20
	3		1:19	1.74	2.98	4.03	4.87	5.56	7.22

b. On February 24, the primary culm on a 98 day plant with a young spike 14 mm. long was split lengthwise (culm, leaves and spike) into two parts. Part 2 weighed 1 gram less than part 1 and contained about .8 grams less leaf tissue.

Part	Grinding Treatment	Time Ground	Start	1	5	
1	4 g. sand	1:10	1.40	1.45	5.12	(
			1.49	1.46	5.20	(neut. H_2O_2 used.
2	4 g. sand+ 2 g. $CaCO_3$	1:15	1.58	2.73	7.68	(
			2.07	2.73	7.68	(neut. H_2O_2 used.
			2.16	2.50	7.01	(
			2.25	2.53	7.15	(unneut. H_2O_2 used.
			3.31	2.62	7.30	(
			3.40	2.70 ?	7.62	(neut. H_2O_2 used.

b. On February 25, several whole plants were cut up with shears and ground in a Nixtamal mill.

Time schedule

Cut with shears at 10:13 A.M.

Ground 10:17½

Sample A $CaCO_3$ added 10:20 within 7 minutes of cutting

Sample B reground with sand 10:26½

Sample B diluted 10:30½

Sample B sub sample 1 $CaCO_3$ added 10:32 within 19 minutes of cutting

Sample B sub sample 2 " " 10:41½ " 29 " " "

" " " " 3 " " 10:47½ " 35 " " "

" " " " 4 " " 11:32½ " 80 " " "

Sample	treatment	Start	1	5	Notes
A1	Neut. within 7 min.	11:10	2.15	6.00	
A2	" " " "	11:19	2.10	5.90	
A4	" " " "	2:50	2.06	5.94	
A5	" " " "	3:18	1.97	6.10	Av. 5.985 cc
B1a	" " 19 "	11:29	1.98	5.62	
b	" " " "	11:38	1.94	5.55	Av. 5.585 cc
B2a	" " 29 "	11:47	2.00	5.64	
b	" " " "	11:53	1.90	5.46	Av. 5.55 cc
B3a	" " 35 "	1:26	1.97	5.76	
	" " " "	1:35	1.95	5.70	Av. 5.73 cc
B4a	" " 80 "	1:44	1.85	5.50	
		1:53	?	5.53	Av. 5.515 cc
Ba	Unneutralized	2:03	1.40	4.75	
b	"	2:12	1.40	4.60	Av. 4.675 cc
Bc	Neutralized within 4 hr. 20 min. (2:33 p.m.)	3:27	1.85	5.28	
		3:38	1.87	5.20	Av. 5.24 cc

There was some recovery in catalase activity if neutralized and let stand, but the experiment should be repeated, using "clipped" samples, also the CO_2 given off with $CaCO_3$ should be measured when shaken one hour at $24\frac{1}{2}^{o}$ C.

pH of the dilutions was found on March 3 and March 24 but the results were inconclusive as the amount of buffering is unknown.

Trituration of the Sample

The simplest and most efficient method of preparing the tissue is by grinding the plants in mortar with a pestle as was described under "Standard Method" (A). While the nixtamal mill reduced the labor of grinding a great deal, acidity developed so rapidly that the results are not reliable. When $CaCO_3$ was ground with the tissues the difficulty of obtaining duplicate samples of a definite weight of tissues so greatly increased that the method was discarded.

Effect of Materials Added.

The materials added before grinding have very little effect on the results.

a. Quartz sand used for sand culture work (98 % Sio_2 from Ottawa, Illinois) was treated with glassware cleaning solution and thoroughly washed. About 3 grams of this was used with 5 gram samples of plant tissue and 2cc samples of 1-50 dilution run against 3cc H_2O_2.

Date	Sample	Grinding Treatment	Start	1	2	3	4	5	Aver.
April 27	I	+$CaCO_3$ (2g)	10:50 a.m.	8.65	13.92	18.53	22.30	25.30	
		"	10:59	8.95	14.43	19.0±	22.83	25.82	25.56
	II	+$CaCO_3$+sand	11:10	7.82	12.45	16.30	19.50	22.11	
			11:19	7.80	12.30	16.10	19.10±	21.70	21.91
	III	50cc of sample I reground with sand.	11:28	8.60	14.06	18.44	22.00	25.03	
			11:37	8.50	13.55	18.05	21.60	24.50	24.77

b. The volume of the insoluble plant tissues, quartz sand and $CaCO_3$ is insignificant in a dilution of 1-50.

Dry residue from duplicate 5 grams were placed individually into a 50cc cylindrical graduate with approximately 2 gram $CaCO_3$ and 4 gram quartz sand. Water was then run in to the 50cc mark from a burette and the volume of the solids found by subtraction.

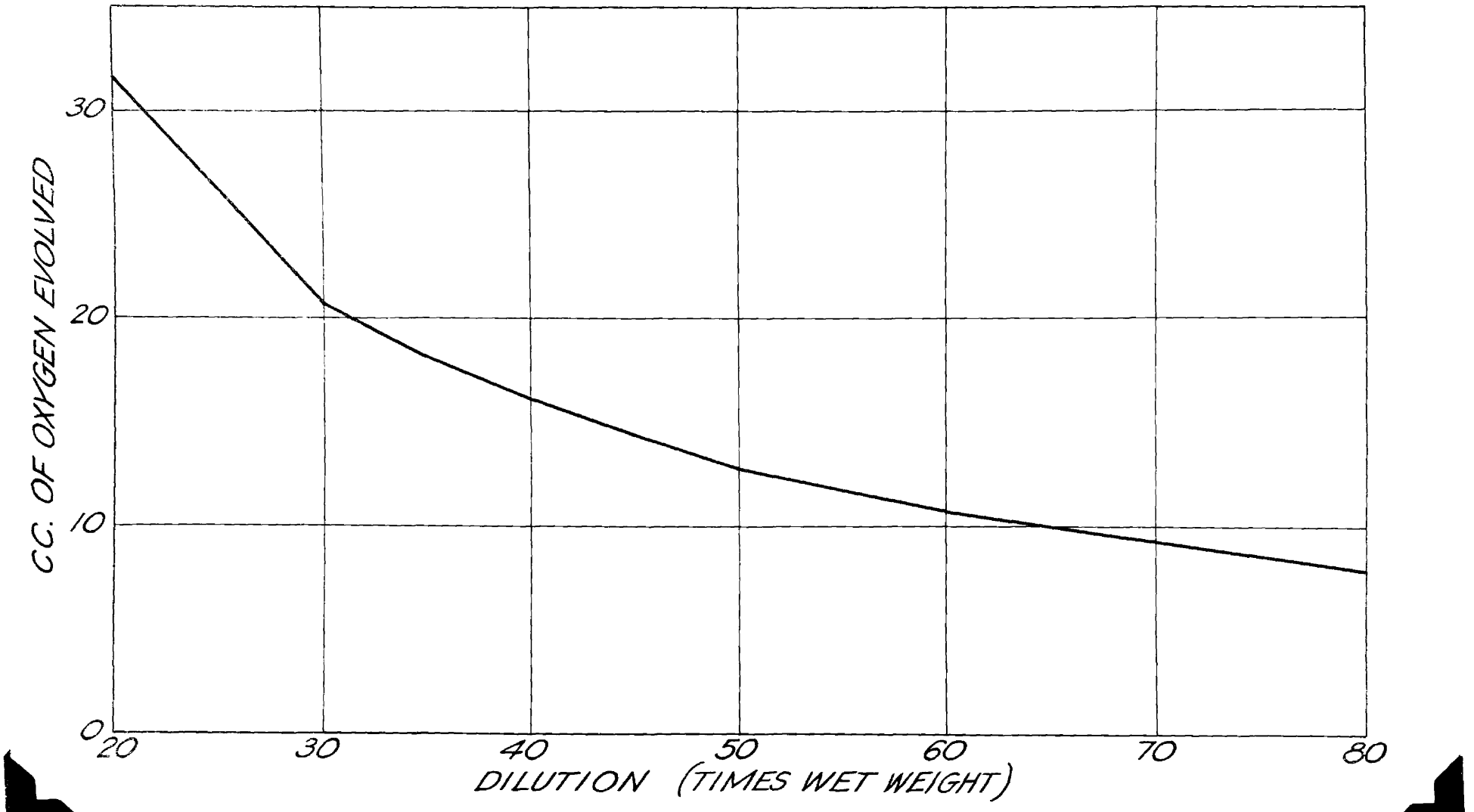
Sample Number	Capacity of graduate	Final reading of burette	Volume of solids in 500cc of a 1-50 dilution
A	50.00	46.3	3.7cc
B	50.00	46.2	3.8cc

or an average of 3.75cc solids in 500cc of mixture or .75% which would produce a much smaller error than is commonly found between duplicate samples of the same suspension.

Effect of Different Degrees of Dilution on Catalase Activity

Dilution of the macerated tissue is necessary on account of the large amount of catalase activity present in the plant. The first method used was that of dilution with distilled water at the rate of 1 gram green weight up to 50cc of mixture. But the per cent of dry matter in the green plant varied from day to day with the turgidity of the plant and the amount of dew or rain on it when harvested. Then, too, as the moisture content varies in the different parts of the plant, the dry matter content is a much more reliable basis upon which to work. Consequently, while it was necessary to determine the catalase activity before the dry matter content was known the results were later corrected to a dilution basis of one part dry matter to 250 parts suspension. In order to measure the reliability of this method, an experiment

Figure 11. Curve showing relation between the volume of oxygen liberated from hydrogen peroxide by 2cc. of a tissue suspension and the dilution of that suspension.


CC. OF OXYGEN EVOLVED
0
10
20
30
20
30
40
50
60
70
80
DILUTION (TIMES WET WEIGHT)

was performed with a basic sample, portions of which were diluted to make suspensions covering the range of dilutions most likely to be encountered in the work.

A ten gram sample of barley was ground with $CaCO_3$ and diluted to make 100cc. 10cc aliquots of this suspension were then diluted to make the dilutions tested. 2cc samples were run against 3cc H_2O_2 for 5 minutes.

Dilution	Start	1	2	3	4	5	Av.
1-20	1:18	10.00	17.20	23.07	27.65	31.00	
	1:27	10.65	18.13	24.30	28.90	32.20 ±	31.60
1-30	9:55	6.66	11.20 ±	14.95	18. ±	20.60	
	10:04	6.68	11.40	15.17	18.22	20.76	20.68
1-35	10:13	6.02	10.02	13.30	16.01	18.14	
	10:22	6.03	10.05	13.28	15.97	18.17	18.16
1-40	10:31	5.25	8.72	11.63	13.95	15.80	
	11:06	5.58	9.30 ±	12.30	14.70	16.60	16.20
1-45	10:48	5.02	8.30	10.88	12.95	14.56	
	10:57	4.85	8.04	10.62	12.71	14.41	14.49
1-50	11:15	4.26	7.11	9.45	11.30	12.83	
	11:25	4.28	7.15	9.47	11.30	12.74	12.79
1-60	11:34	3.70 ±	6.05	7.99	9.55	10.79	
	11:43	3.65	6.05	7.98	9.46	10.64	10.72
1-80	11:52	2.72	4.51	5.95 ±	7.06	7.89	
	12:07	2.68	4.46	5.92	7.05	7.92	7.91
Check	12:17	5.38	8.96	11.84	14.20	16.11	
1-40	12:27	5.40	8.97	11.88	14.24	16.13	16.12

When these averages are plotted against the dilutions it will be seen that the curve (Figure 13) between the dilutions 60 and 30 is very nearly a straight line, i.e., catalase activity is proportioned to dilution.

Since very few of the tests made were of dilutions outside of this range the method seems valid.

Effect of Stirring the Suspension Before Sampling

In all tests for catalase activity the suspension was vigorously stirred and the sample withdrawn with a pipette while the liquor was still in motion. It was desired to learn, however, how stirring affected the catalase activity. Two experiments were tried.

On February 29:

Treatment	Run Number	Start	1	5
Settled	1	11:59	3.10	8.60
	2	12:17	2.95	8.02
	3	12:35	3.00	8.15
Stirred	4	1:27	3.18	9.00
	5	1:41	3.10	8.90

Here the results are inconclusive: the seemingly greater activity of the stirred may de due to greater extraction on standing or to experimental error.

On April 27: (3cc H_2O_2 used)

Treatment	Run Number	Start	1	2	3	4	5	Av.
Stirred	1	10:50	8.65	13.92	18.53	22.30	25.30	
	2	10:59	8.95	14.43	19.0±	22.83	25.82	25.56
Supernatant liquid 10 min.after stirring	3	11:59	8.90	14.25	18.80	22.48	25.47	
	4	12:11	8.70	14.20	18.85	22.60	25.67	25.57

Further extraction may have taken place during the time elapsed between the"stirred" and "unstirred" tests to make up for a possible precipitation of catalase with the solids. The final results, however, indicate that from a practical standpoint the supernatant liquid may be used.

The Effect of Temperature

Temperature (together with storage) has an appreciable effect on catalase activity.

On May 5: With room temperature at 29° C,,the following tests were made with barley plants clipped with shears, sprinkled with pv. $CaCO_3$ and ground in a Nixtamal Mill. The macerated tissue was then extracted with a hydraulic press at a percent of approximately 8000 pounds per square inch, and the juice diluted 25 times.

Treatment	Run Number	Start	1	2	3	Av.
A. tested at once	1	9:56	3.53	6.05±	8.28	
	2	10:04	3.44	5.81	8.00	8.14 cc
B. 1½ hrs. at 29° C	3	11:31	4.35	7.30	9.97	
	4	11:38	4.40	7.40	10.10	10.065 cc
C. Sample "B" above stored in ice box 6½ hrs.	5	5:54	4.70	7.90	10.70	
after 1½ hrs. at 29° C	6	6:00	4.54	7.60	10.38	10.54 cc
D. Sample "C" above heated at 40° C for 15 min.	7	10:40	2.42	4.20	5.81	
	8	10:50	2.50	4.32	5.97	5.89 cc

Further extraction of catalase occurs in 90 minutes at 29° C and is held approximately stationary by storage for 6½ hours in the ice box (temperature unknown). Heating to 40° C for 15 minutes decreases the catalase activity markedly.

The Effect of Preservative Agents

a. On March 21 the effect of ethyl alcohol on catalase activity was tested.

Grinding Treatment	Start	1	2	5	10	
Standard	12:16	2.68		7.6		
"	11:35	2.60		7.3 +		
Standard + .3cc alcohol	12:05	2.28		6.03		
ditto	12:48	2.43		6.60		
In alcohol + $CaCO_3$ diluted 1-5 with alcohol	11:07	1.03	1.37	1.60	2.08	sudden evol. of gas on start of motor

Even a small amount (0.3 cc) of alcohol caused some inactivation of catalase while grinding and diluting with alcohol reduced it more than 75 %.

b. On April 13 Toluol was tried as a preservative agent.

Treatment	Start	1	2	3	4	5	Av.
Standard	12:17	5.38	8.96	11.84	14.20	16.11	
"	12:27	5.40	8.97	11.88	14.24	16.13	16.12
" + toluol	12:31	5.56	9.0 ±	10.94	12.43	13.44	
" "	4:52	3.40	4.90	5.83	6.40	6.74	

There is probably an immediately detrimental effect due to toluol. After storage the catalase activity of the sample covered with the thin film of toluol was reduced about 60 per cent.

Storage of Suspension

On March 24, the effect of storage on catalase activity and acidity was investigated but should be repeated, using standard methods (omitting $CaCO_3$ in 1 sample) and obtaining pH of dilution at time of test.

On January 16, two different plants were tested for catalase activity and the extract stored until the next morning and again tested showing a loss of about 30 per cent after standing 23 hours.

Plant	Date	Run Number	Start	1	2	3	4	5	Av.
8	January 16	1	11.43	2.17	3.43	4.42	5.25	5.95	
		2	11:57	2.30	3.55	4.60	5.40 ±	6.10	6.075
	January 17	1	10:26	1.10	1.98	2.81	3.55	4.16	
		2	10:35	1.23	2.20	3.02	3.80	4.43	4.295 (loss 29.3 %)
9	January 16	1	12:11	2.40	3.80	4.93	5.82	6.57	
		2	12:25	2.32	3.67	4.80	5.66	6.41	6.49
	January 17	1	10:44	1.20	2.13	?	3.85 ±	4.54	
		2	10:53	1.23	2.20	3.10	3.90	4.54	4.54 (loss 30.1 %)

Variation in Catalase Activity Between Two Plants of Same Age

During a part of the preliminary work, two entire plants were taken from the day's population for determination of catalase activity. Each plant was ground separately with sand and $CaCO_3$ and diluted 50 times the wet weight. Duplicate determinations were then made upon each plant suspension. The following data are the results from 10 successive samplings from the same greenhouse planting. Each catalase value is the average of two determinations.

Age of plant in days	35	39	43	48	53	59	66	73	82	87
Plant No.1	932	9.285	10.735	8.43	10.635	7.93	6.78	7.52	8.765	8.085
Plant No.2	10.00	10.385	10.125	6.99	9.40	8.47	7.17	9.405	9.305	9.10

It will be seen that in 5 out of the 10 samplings the catalase activity of the two plants studied differed by more than 1 cc, the widest being on day 73 when the difference was 1.885 cc. It is possible, assuming that catalase activity is a measure of metabolic activity, that the plants differed widely in metabolic activity even though of the same age. After the last sampling enumerated (Day 87) a number of plants were always bulked for the catalase sample in order to get an average for the stage reached.

Catalase Activity in Different Parts of the Plant

The main culms and the tillers of two plants were contrasted for catalase activity on January 16, and it was found that in one plant the tillers and in the other the main culms were greater in catalase activity.

Plant	Plant part	Run Number	Start	1	2	3	4	5	Av.
2	Main culm	1	12:39	2.32	3.65	4.70	5.55	6.25	
		2	12:48	2.42	3.85	4.95	5.83	6.55	6.40
	Tillers	1	12:57	2.05	3.20	4.10	4.85	5.45	
		2	1:06	2.10	3.31	4.30	5.03	5.65	5.55
15	Main culm	1	1:15	2.43	3.88	5.00	5.95	6.72	
		2	1:24	2.54	4.00	5.17	6.07	6.81	6.765
	Tillers	1	1:35	2.79	4.30	5.53	6.55	7.36	
		2	1:44	2.70	4.25	5.50	6.45	7.30	7.33

On March 19 and March 21 preliminary tests for catalase were made on awn material. These showed very high amounts. This was particularly surprising since it has been assumed by those working with plants that catalase activity is correlated with actively growing or anabolic tissue and it seemed certain that the awns of that stage had long since attainedtheir full size and moreover contained a rather high percentage of ash. (Harlan and Pope, 1921). Therefore the experiment of contrasting the catalase activity of the different parts of the plant became interesting.

On June 26, from each of 10 culms of Hannchen barley, age 104 days, were taken tissue samples contrasting greatly in character. Part of each sample was used for dry matter determinations and part for catalase activity diluting each sample 50 times its green weight and correcting later to 50 times dry weight by dividing the value obtained by the percent of dry matter.

Plant Part	% D.M.	Run Number	Start	1	2	3	Av.	Corrected value
Awns	31.562	1	2:38	7.25	12.16	16.23		
		2	2:45	7.42	12.49	16.55	16.39	51.929
Kernels	29.860	1	2:19	3.77	5.48	6.69		
		2	2:26	3.56	5.09	6.24	6.465	21.651
Rachis	38.846	1	1:54	2.53	4.20	5.84		
		2	2:11	3.37	5.16	6.70	6.27	16.140
Culm under spike	31.957	1	1:40	6.37	10.30	13.55	13.55	42.400
Culm: base of top internode	16.703	1	1:22	1.60	2.40	3.06		
		2	1:29	1.60	2.40	3.06	3.06	18.320
Top Node	18.837	1	1:03	2.23	3.36	4.27		
		2	1:10	2.23	3.32	4.19	4.23	22.456
Culm: top of second internode	27.143	1	12:43	2.83	4.48	5.84		
		2	12:50	2.70	4.25	5.58	5.71	21.036

Plant Part	% D.M.	Run Number	Start	1	2	3	Av. (50 x W.W.)	CcO % D.M. (50 x D.M.)
Culm: base of 2nd internode	18.985	1	12:25	1.58	2.40	3.09		
		2	12:32	1.55	2.34	3.02	3.055	16.092
Flag of Boot leaf	32.831	1	12:04	13.45	21.75	26.10		
		2	12:11	12.90	21.10	28.05	27.075	82.468
Base of boot sheath	26.786	1	11:44	7.36 $\pm$	11.95 $\pm$	15.75		
		2	11:52	7.45	12.15	15.97	15.86	59.210

On July 10, the experiment on the catalase activity of plant parts was repeated with a 6-rowed barley (variety unknown). As before, the corrected value is for dry matter (diluted 50 times).

Plant Part	% D.M.	Run No.	Start	1	2	3	Av.	Corrected value
Awns	38.709	1	2:05	6.40±	10.58	13.90		
		2	2:12	6.19	10.35	13.64	13.77	35.573
Kernels	32.682	1	2:25	5.25	7.54	9.17		
		2	2:32	4.85	6.90	8.45	8.81	26.957
Rachis	44.612	1	2:42	2.90	4.39	5.63		
		2	2:48	2.86	4.32	5.60	(5.615†) 6.567	14.720
Culm under spike	42.909	1	2:59	3.51	5.49	7.08		
		2	3:06	3.45	5.42	7.00	7.04	16.407
Culm, base of top internode	28.209	1	3:16	2.96	4.41	5.63±		
		2	3:23	2.96	4.43	5.64	5.635	19.976
Top node	33.333	1	3:31	2.38	3.51	4.45		
		2	3:38	2.30±	3.43	4.33	4.39	13.170
Top of 2nd internode	37.998	1	3:49	1.20	1.88	2.46		
		2	3:56	1.23	1.96	2.53	2.495	6.566
Base of 2nd internode	36.183	1	4:06	1.30	1.98	2.58		
		2	4:13	1.29	1.96	2.54	2.56	7.075
Flag of Boot leaf	38.477	1	4:23	12.95	22.40	26.60	discard	
		2	4:31	10.8±	18.45	24.90		
		3	4:41	10.7±	18.20	24.52	(24.71*) 31.65	82.257
Sheath of boot leaf	32.356	1	4:54	5.12	8.10	10.60		
		2	5:03	5.25	8.31	10.84	10.72	33.131

Plant Part	% D. M.	Run No.	Start	1	2	3	Av.	Correct Value
Flag 4th leaf from top(mostly dry & brown)	76.605	1	5:12	2.13	3.46	4.39		
		2	5:19	2.17	3.47	4.42	4.405	5.750
Sheath 4th leaf from top 5 cm at base	32.697	1	5:30	3.10	4.97	6.50		
		2	5:37	3.04	4.92	6.43	6.465	19.772

+By error this sample was diluted to approximately 17 cc instead of 14.535

correction applied by multiplying 5.615 by $\frac{17000}{14535} = 6.567$

*By error this sample was diluted to 44 cc instead of 34.035 correction applied

by multiplying 24.71 by $\frac{44.000}{34.035} = 31.65$

Catalase activity seems to vary with the amount of chlorophyl present.

Localization of catalase activity in the suspension.

a. Filtration.

On April 27 a 5-gram sample was ground with $CaCO_3$ and diluted 50 times. One portion was stirred thoroughly before testing while a second was centrifuged with a hand machine for 2 minutes and the supernatant liquid tested. This supernatant liquid filtered through No. 2 Whatman paper was much lighter green than before filtering and was also tested, using in all cases 3 cc H_2O_2.

Treatment	Run No.	Start	1	2	3	4	5	Av.
Ground and stirred	1	10:50	8.65	13.92	18.53	22.30	25.30	
	2	10:59	8.95	14.43	19.0 ±	22.83	25.82	25.56
Centrifuged supernatant	1	12:21	8.50	13.90	18.24	21.88	24.74	
	2	12:30	8.42	13.60	18.18	21.77	24.62	24.68
Supernatant filtered	1	1:16	7.82	12.72	16.93	20.16	22.33	
	2	1:26	7.70 ±	12.70	16.70	19.92	22.30	22.32

It, therefore, appears that neither hand centrifugation nor filtration with Whatman No. 2 paper separates out the substances associated with catalase activity in amounts at all large.

On May 5, plants of Hannchen barley were clipped, sprinkled with powdered $CaCO_3$ ground in the Nixtamal Mill and the sap pressed out under 8,000 lbs. per square inch. A portion of the stirred expressed sap was filtered through a Berkefeld "V" filter, the water capacity of which was about 5 cc. The first 5 cc. was accordingly discarded and the next $3\frac{1}{2}$ cc. diluted 25 times and put into the ice box. The next 7 cc. was discarded and the last 2 cc. diluted 25 times and tested. During the filtration the remainder of the expressed sap stood by the filter.

Treatment	Run No.	Start	1	2	3	Av.
First filtrate	1	5:31	0.00	0.01	0.03	
	2	5:37	0.03	0.04	0.07	0.05
Last filtrate	1	5:18	0.28	0.47	0.69	
	2	5:23	0.23	0.47	0.67	0.68
Unfiltered check	1	5:42	1.35	2.52	3.73	
	2	5:47	1.42	2.65	3.83	3.78

It is evident that the Berkefeld V filter separates out most of the material associated with catalase activity.

b. Centrifugation.

In an experiment noted above (April 27) it was found that separation by hand centrifugation did not materially affect catalase activity.

1. On May 5 a portion of the expressed sap from experiment described above was diluted 25 times and tested for catalase activity at the beginning and the end of the experiment. Another portion was centrifuged with a force of 70.000 x gravity for 10 minutes.

Treatment	Run No.1	Start	1	2	3	Av.	Total for treatment
1.Check	1	9:56	3.53	6.05 ±	8.28		
	2	10:04	3.44	5.81	8.00	8.14	8.14
2a Centrifuged solids made up to volume	1	10:11	0.48	0.72	0.90		
	2	10:18	0.53	0.80	0.97	0.935	
2b Centrifuged supernatant liquid weak "humus" color	1	10:26	3.18	5.36	7.36		
	2	10:33	3.75	6.38	8.60	7.98	8.915
3 Check	1	11:31	4.35	7.30	9.97		
	2	11:38	4.40	7.40	10.10	10.035	10.035

No. 3 (check) determined about $1\frac{1}{2}$ hours after No. 1 shows further extraction catalase.

2. On May 14, sap of Hannchen barley was expressed and diluted 25 times and portions centrifuged for varying lengths of time.

Treatment	Run No.	Start	1	2	3	Av.	Total for treatment
Check uncentrifuged	1	11:34	2.62	4.75±	6.77		
	2	11:41	3.00	5.31	7.60	7.185	7.185
I.Centrifuged 3 minutes solids	1	12:08	0.73	1.11	1.40		
	2	12:15	0.74	1.11	1.42	1.41	
Do Supernatant	1	11:48	2.85	5.00	7.07		
	2	11:55	3.07	5.31	7.47	7.27	8.68
II.Centrifuged 6 minutes solids	1	12:36	0.76	1.18	1.50		
	2	12:43	0.78	1.18	1.49	1.495	
Do Supernatant	1	12:22	3.09	5.39	7.58		
	2	12:29	3.07	5.40	7.60	7.59	9.085
III.Centrifuged 9 min. solids	1	1:04	0.80	1.26	1.60		
	2	1:13	0.84	1.27	1.60	1.60	
Do Supernatant	1	12:50	3.23	5.63	7.95		
	2	12:57	3.16	5.44	7.60	7.775	9.375
IV. Centrifuged 12 min. solids	1	1:34	0.90	1.36	1.70		
	2	1:41	0.87	1.31	1.66	1.68	
Do Supernatant	1	1:20	2.86	4.98	6.99		
	2	1:27	3.02	5.20	7.29	7.14	8.82
V.Centrifuged 15 min. solids	1	2:03	0.82	1.28	1.62		
	2	2:10	0.98	1.48	1.79	1.705	
DoSupernatant	1	1:49	2.98	5.12	7.21		
	2	1:56	3.08	5.35	7.50	7.355	9.06
VI.Centrifuged 3 min.* solids	1	2:45	0.82	1.23	1.50		
	2	2:51	0.79	1.21	1.44	1.47	
Do Supernatant	1	2:31	2.99	5.20	7.30		
	2	2:38	3.20	5.56	7.80	7.55	9.02
Check uncentrifuged	1	2:17	3.65	6.30	8.77		
	2	2:21	3.65	6.25	8.76	8.765	8.765

*Powdered $CaCO_3$ was added to all centrifuged samples at 10:28 soon after IV was centrifuged and to V and VI immediately after centrifugation.

D. Effect of heat on catalase activity and separation by centrifugation.

a. On May 5 a portion of diluted sap expressed from ground tissue under a pressure of 8000 pounds per square inch was heated to 40° C. for 15 minutes, tested for catalase activity and a portion supercentrifuged and the fraction tested.

Treatment	Run No.	Start	1	2	3	Av.	Total for treatment
1. Check unheated	1	9:56	3.53	6.05±	8.28		
	2	10:04	3.44	5.81	8.00	8.14	8.14
2. Unheated solids	1	10:11	0.48	0.72	0.90		
	2	10:18	0.53	0.80	0.97	0.935	
Supernatant	1	10:26	3.18	5.36	7.36		
	2	10:33	3.75	6.38	8.60	7.98	8.915
3. Heated 40° for 15 minutes	1	10:40	2.42	4.20	5.81		
	2	10:50	2.50	4.32	5.97	5.87	5.87
4. Centrifugate from heated dilution	1	11:02	0.67	0.90	1.15		
	2	11:09	0.65	0.90	1.09	1.24	
Supernatant from heated dilution	1	11:17	1.92	3.43	4.80		
	2	11:24	1.99	3.52	4.94	4.87	6.11
5 Check unheated	1	11:31	4.35	7.30	9.97		
	2	11:38	4.40	7.40	10.10	10.035	10.035

Heating inactivated a portion of the catalase and a larger proportion was thrown out by centrifugation than in the unheated.

Summary

1. The strength of the H_2O_2 used has always been up to specification (i.e. 12 Volume).

2. An amount of H_2O_2 in excess of 50 per cent more than is necessary poisons the "catalase activity," thereby decreasing the amount of O_2 liberated.

3. Acidity of either H_2O_2 or suspension, even to a very slight degree, decreases the amount of O_2 liberated. Acidity seems to develop in the suspension on standing.

4. Attempts to determine "catalase activity" from dried material have failed.

5. Individual plants vary more or less greatly in "catalase activity."

6. The best results are obtained with the addition of powdered $CaCO_3$ before the sample is ground.

7. The amounts of quartz sand and $CaCO_3$ used in grinding have no appreciable effect on the amount of O_2 liberated.

8. There is further extraction of "catalase activity" in the substrate when allowed to stand. Storage in ice box (temperature unknown) for periods up to 6 hours does not appreciably alter the amount of O_2 liberated.

9. Stirring the substrate suspension before sampling does not increase the amount of O_2 obtainable.

10. Alcohol and toluol added to the substrate immediately decrease the amount of O_2 obtainable.

11. On keeping suspension over night the "catalase activity" was decreased about 30 per cent.

12. There is no consistent difference between the catalase activity of the main culm and that of the tillers.

13. The different portions of the culm (including leaves, stalk and spike) vary greatly in amount of "catalase activity." This quantity seems to vary almost directly with the amount of chlorophyll present.

14. Hand centrifugation had no effect on amount of "catalase activity" found.

15. "Supercentrifugation" caused a decrease in amount of "catalase activity" of the supernatant liquid by about 10%-18%, the remaining "catalase activity" appearing when the centrifugate was diluted up to volume and tested.

16. Filtration through Whatman No.2 paper had no effect on the amount of O_2 liberated.

17. Filtration through a Berkefeld "V" filter separates out most of the material associated with catalase activity.

18. Heating the substrate for 15 minutes at 40° C. inactivates about 30% of the "catalase activity" and supercentrifugation after heating throws out about 25% of the "catalase."