AMERICAN
JOURNAL OF BOTANY

VOL. VI APRIL, 1919 No. 4

THE INFLUENCE OF LIGHT UPON THE ACTION OF STOMATA AND ITS RELATION TO THE TRANSPIRATION OF CERTAIN GRAINS

JOHN GRAY AND GEORGE J. PEIRCE

The stomata of leaves are the passages through which the necessary exchange of gases takes place between the interior of the land plant and its leaves and the outside world. They are the organs through which CO_2 and O_2 are taken from the air and given back to it. And it is through them that water vapor passes out into the air. The stomata are the exterior openings of intercellular spaces which may extend for great distances in the body of the plant. These openings are bounded by paired guard cells possessing in most plants a markedly greater degree of elasticity than the other epidermal cells. The mechanism and the behavior of stomata have been the object of study of many botanists for decades. For the older literature of the subject one may refer to Pfeffer (1). Only the few more recent papers bearing on the specific question which we asked ourselves will be referred to here.

In general the behavior of the stomata of the Gramineae is as follows. When the opposite ends of the guard cells of a pair are in contact with each other the stoma is thereby closed. These opposed ends are thin-walled, the rest of the wall being thick and stiff. Opening begins with the expansion of the guard cells. This results in the separation of the guard cells, at first at the ends of the stoma, and later in the middle as well, the guard cells straightening and giving to the pore its familiar oblong shape and its uniform width for nearly its whole extent. Closing appears to be the reverse of this process. This action of the guard cells seems to be a mechanical process dependent upon the character of the environment in which the plant lives.

The times, conditions, and significance of the opening and closing of stomata have interested many plant physiologists, and there are certain general conceptions current, as indicated by the concise statements of the textbooks. But the work of one of us in the field, in which the possible effect of sulphur dioxide fumes upon the stomata, as well as upon the other cells of the leaf, became a matter of importance, made it necessary to ascer-

[The Journal for March (6: 87–130) was issued March 28, 1919.]

tain the conditions of opening and closing and the connection of these phenomena with transpiration, a process the importance of which is inversely proportioned to the amounts of moisture in soil and air. Thus in the far west, where "dry farming" is resorted to, it is of the utmost importance to the plant to be able to control its transpiration; and, at the times of most rapid evaporation from all wet masses, to be able to hold its water in spite of the temperature and dryness of the air. It is obvious that water vapor will escape more rapidly along a continuous volume of air, for example through an open stoma, than across one or more membranes, no matter how permeable, interposed between two volumes of air of different humidities, areas and other things being equal. What then causes the opening and closing of stomata? And what are the consequences? On these questions the studies of Darwin and Lloyd bear directly.

Darwin (2), working under the well known climatic conditions of Cambridge, England, which are the general conditions in which the plant physiology of today has been developed, and Lloyd (3), experimenting at the Desert Botanical Laboratory of the Carnegie Institution, at Tucson, Arizona, with climatic conditions not altogether easy for the plant physiologist of other so-called temperate climates to grasp, came to conclusions sufficiently divergent to justify us in reëxamining the subject, especially as we live in a region the climatic conditions of which are near the mean between these two. Darwin, studying the leaves of ivy (*Hedera helix* L.) and of laurel (*Prunus laurocerasus* L.), claims that transpiration is regulated mainly by the stomatal aperture. His results on individual leaves do not clearly show this; but examining the series as a whole, it is seen that the transpiration and the condition of the stomata appear to be related, usually with the relative transpiration somewhat exceeding the stomatal ratio. Lloyd worked on the ocotillo (*Fouquieria splendens* Engelm.) and a showy verbena (*Verbena ciliata* Benth.), both desert plants growing near his laboratory. The study of these extreme types led him to conclude that the "regulatory function of the stomata is almost *nil*." During the day the outgo of water in the ocotillo is greater than the intake. The reverse is true at night. The stomata are open during the day, in which time also there is the greatest decrease in leaf weight. At no time is this effectively regulated by the movements of the guard cells. The stomata do not appear, therefore, to afford the plant any protection against evaporation during the times when evaporation is most dangerous.

The opening and closing of the stomata have been attributed to the changes in the turgidity of the guard cells, their particular shape and their relations to the adjoining epidermal cells causing the changes in turgor to open and close the stomata. Von Mohl (4) and Schwendener (5) were the first to demonstrate this. The latter showed that the convex walls of the two guard cells, facing each other, are more extensible than the flat or concave walls adjoining the next cells of the epidermis. Increasing turgidity stretches the guard cells, their walls flatten or remain unchanged according to their thickness, and the slit between the two opens and widens. Decreasing turgidity has the opposite effect. Thus the direct connection between turgor changes and stomatal movements has been generally accepted, and Darwin and Loyd seem to assume, in their investigations, that this is the case.

Our study of these papers has led us to make certain experiments which cause us to conclude *first*, that the turgidity of the guard cells is a necessary factor in producing and maintaining their elasticity, but *second*, that the direct and indispensable agent in controlling the opening and closing of the stomata is sunlight, which acts as a stimulus upon the guard cells themselves. Their peculiar form and position give them a freedom of movement, in consequence of differences in turgidity, quite unlike that possessed by any other cells of the body of land plants.

Before any reasonable comparison of the results of Darwin, Lloyd, and ourselves may be made we should note the conditions under which the particular plants studied were grown, as well as the methods used in securing the different results. The conditions of the three sets of experiments present two extremes and a favorable mean. The ivy and laurel which Darwin used were growing in the moist climate of eastern England: the ocotillo and verbena of Lloyd are typical xerophytes of the Arizona desert. Ocotillo, or "coach-whip cactus," is lithe, slender, unbranched, and in appearance and structure like the cactus among which it grows. Verbena is a hardy perennial, persisting through the annual period of drought by means of its deep roots and withering stems. Intermediate between the extremes of England and Arizona are the climatic conditions at Stanford University, California (6), where the humidity is fairly high at all times, and where the temperature, while varying considerably, very rarely reaches an extreme of heat or cold. At times the vegetation is subjected to conditions as moist as those of England. This is true of certain rainy seasons particularly, and we find here a rainy season flora, typified by *Montia perfoliata* (Donn.) Howell, very different from that of the dry season, of which the Hemizonias may be taken as characteristic.

It is well known that plants develop more or less different structural characters in different environments. Where the humidity is highest, the cuticular covering of the epidermis is thinnest, other things being equal; and where, as in the deserts of Arizona, the humidity is low and the temperature high, the cuticula is thickest. It is important to bear this in mind, since it is obviously possible that, in certain circumstances, cuticular transpiration or evaporation may take place. While it is true that the cuticula protects the underlying tissues from evaporation, it does so only incompletely. Stahl's cobalt chloride test (7), which is purely qualitative, is not delicate enough to demonstrate cuticular evaporation, or cuticular transpiration: but the method of Buscalioni and Polacci (8) may be employed to show that cuticular transpiration may take place in addition to that through the stomata. Thus on applying a film of collodion to the epidermis of a leaf, through which the details of the leaf could be seen, it was found that clouding took place between the stomata as well as over them, though of course not necessarily at the same rate. Applying this fact to the plants with which Darwin experimented, it must be seen that a part of Darwin's error in recording transpiration was due to the cuticular evaporation of which the plants of his region are certainly capable. The cuticular evaporation of Lloyd's plants, on the other hand, would be very slight, while the Gramineae studied at Stanford University, with their moderately cutinized epidermis, exhibit a certain amount of cuticular transpiration. The regulation of transpiration seen to be effected by the stomata would lead one to conclude that they directly and mainly control the exchange of gases and the outgo of water. Only under exceptionally dry conditions would evaporation of water and the transfusion of gases through the outer walls of the epidermal cells be of any considerable importance. This we shall show shortly.

Furthermore, the density and the humidity of the air determine the intensity and the composition of the light reaching the earth's surface in different regions. Unfortunately it is still impossible to express these differences in definite terms, there being no single instrument or set of instruments which will give us all the data involved; but we know that on a hazy or cloudy day there may still be enough light to affect plants noticeably, though the sun's rays do not reach them directly. We are forced to conclude that the quantity of light reaching the earth's surface is less in Cambridge, England, because of the higher humidity of the air, than at Stanford University, and still less than in Tucson, Arizona. Some of the bearings of this inequality of light distribution have been already discussed by one of us elsewhere (6); it must be distinctly borne in mind in connection with the study of stomatal behavior. For example, the occurrence of high fogs at night and in the early forenoon in the San Francisco Bay region marks an important difference in the illumination as compared with that where Lloyd worked.

Darwin measured the amount of transpiration and the corresponding changes in the stomata by means of a "poremeter" fitted directly upon the surface of the leaf. The variations were estimated from the rate of flow of a current of air drawn through the stomata of an uninjured leaf under a given pressure. The leaf was supported upon a glass plate and the poremeter placed on a washer or perforated disc fastened to the leaf by means of gelatine. Others used paraffine, and also illuminated the leaf from below. Obviously, stomata enclosed within a poremeter are shut off from both light and air; CO_2 necessary for food manufacture is shut off during the progress of the experiment, and the leaf undergoes a sweating process in which water is given off but nothing is taken in, which would very rarely, if ever, take place in nature.

Lloyd, on the other hand, used two methods of experimentation, of which one was more natural and satisfactory than the other. His early method involved the measurement of stomata in strips of epidermis torn from leaves and fixed in absolute alcohol. Although there are many evident advantages in this method, even its convenience was never sufficient to convince everyone that there was no change whatever in the stomata by the most deft stripping of the epidermis from the surface of a leaf and the dropping of the strip into absolute alcohol. These objections are all avoided, though others may be raised, by making direct observation and measurement of the stomata in position on the living leaf, still attached to the plant which bears it. By attaching a Soyka flask, filled with water or an aqueous solution of suitable composition, to the under side of the stage of the microscope so that the light is cooled before it reaches the leaf to be examined, one may directly observe the condition and the changes in the position of the guard cells as the illumination changes.

We used this latter one of Lloyd's methods, but only on hot days did it seem necessary to us to apply the cooling cell. Measurements of the stomata on the leaves of plants of wheat, oats, rye, and barley, growing out of doors or in pots in the greenhouse, were made by bending over a leaf and gently applying and fastening it to the stage of a horizontal or other microscope for a minute, or a minute and a half, during which time the part of the leaf in the field was subjected to observation. The measurements were made by eye-piece micrometer, and made and recorded as rapidly as possible, so that no injury to the leaf and no change in position of the guard cells followed the slight darkening under the microscope. Most of the readings were made by daylight; but the few night readings showed the stomata to be closed in darkness. Measurements were made from two or three leaves on each plant for each period of time, and the average mean was taken in plotting each point; for in a group of plants there would always be some variation in the amount of light received by each plant and each leaf, both as to the times at which the most light would reach them and as to the quantities to which each would be exposed. In choosing the parts to be examined care was taken to select those leaves and those parts of leaves which were most isolated and most subject to variation at the particular times of examination. When the illumination is fairly equal for all the plants and all the leaves in a pot or box of plants of the same species, the condition of all the stomata is similar. This is not the case when the exposure is not similar. In cases in which the plants and their leaves were not similarly exposed all around, the turning of the plants so that they were similarly exposed was followed by the corresponding changes in the guard cells. No attempt was made to secure a series of readings for the early morning hours, or after sunset, because we were concerned primarily with the behavior of the stomata in relation to natural light; and we believe that intense artificial light disturbs and perhaps interferes with the natural action of the guard cells.

Temperature and humidity readings for each examination of the stomata were made. The latter readings were on a Mason hygrometer, which consists of a wet and dry bulb thermometer and is supplied with tables indicating the humidity at the different readings. Soil samples were taken from each box of plants, weighed, dried to constant weight, and the percentage of soil moisture was calculated from these figures. Darwin and Lloyd appear to have worked with mature plants and to have had them under fairly constant conditions. We have used the domestic grains, wheat (*Triticum vulgare* Vill.), oats (*Avena sativa* L.), rye (*Secale cereale* L.), barley (*Hordeum sativum* Jess.); and the wild oats of this region (*Avena fatua* L.). Plants of each species have been studied under three sets of soil conditions, namely (1) with the soil moist, (2) with soil saturated by watering, and (3) with the soil dry and often caked. They have all been examined under such different atmospheric conditions as hot, bright days, cloudy and very dark days, and days of light rain. Young, mature, and older plants have been studied, each being subjected to the same environmental factors whenever that was possible. The degree of illumination and the percentage of soil moisture have been recorded for each reading. Most of the experiments were conducted in a greenhouse with frosted roof but clear end. Sunlight is reduced, but only slightly, by these means which, in our climate, are indispensable. As a check upon this work in the greenhouse, we cultivated the same domestic grains in the Experimental Garden, and examined them there, under conditions as nearly as possible like those of the greenhouse. The wild oats were studied in the field and also in the greenhouse, into which plants of various ages had been removed from time to time in order to permit them to adjust themselves to the new conditions.

These four grains react similarly under like conditions; but each species displays individual differences which may be distinguished, and which partly explain the differences in needs and behavior which are familiar to the practical farmer. We shall discuss these differences after each species has been reported upon. Barley, wheat, and oats live under conditions of soil and of moisture essentially similar, while rye is best grown in drier and warmer localities. When planted in a fairly humid greenhouse it presents a somewhat different appearance from the normal and from that of the other grains. These things are indicated by the details which follow.

Barley

Figure 1 indicates the behavior of stomata of barley seedlings eighteen days old and grown in the greenhouse. The soil was very wet in the early morning, and no more water was added during the readings. The temperature was fairly high, but increased, with the increase in light, to a maximum of 34.5° C. at 1 P.M. The humidity reached its lowest point at 3:20 P.M., when it was 33%. The stomata opened gradually in the morning until 11:50 A.M., when they reached their maximum width for the day.

At the same time the sunlight attained its maximum brightness. In this figure it is indicated by curves that the light intensity and the opening and closing of the stomata vary similarly, whereas the humidity curve goes in the reverse direction. The curves are made of broken lines, as follows: the *continuous* lines connecting the small circles form the curve indicating the opening and closing of the stomata; the *broken* lines of pieces of *equal* length between the small circles form the temperature curve; the *broken* lines of *two unequal* lengths connecting the small circles form the humidity curves. The figures at the bottom of the diagram indicate the hours from 8 o'clock in the morning to 6 o'clock in the evening. The figures at the left indicate the temperature in degrees Fahrenheit because our Mason hygrometer was supplied with Fahrenheit thermometers. In words we have stated the temperature in degrees Centigrade. In the graphs the difference is *nil.*

Figure 2 indicates the behavior of the stomata of barley seedlings twenty-four days old, with 11.3% of moisture in the soil at 9:25 A.M. and 10% at 4:30 P.M. The loss of water from the soil during this time from the surface of the soil and from the plants, through their stomata and otherwise, was 1.3%. The temperature of the air decreased slowly from 84° F., 28.9° C., at 2:40 P.M., and the humidity rose from 62% at 9 A.M. to 72% at 2:40 P.M., from which time the two curves converged somewhat. Thus the atmospheric conditions of this day were moderate, and represent a mean average condition as compared with those of the previous day. At 9 A.M. there was a heavy fog but nevertheless enough light to cause the stomata to open slightly. At 11 A.M. there was a short time of fairly bright light, but a time of comparative darkness followed, and at 2:40 P.M. the stomata were all but closed. At 4:15 P.M., when it was almost too dark for further readings, the stomata were all closed.

Figure 3 presents a contrast to the case just described. This is the record of a similar box of barley seedlings, thirty-seven days old. The soil was moist at 9 A.M. but was watered. At 3:40 P.M. it showed 21.7% of moisture. In the last case a heavy fog prevailed during the early hours of the morning, but there was a fair amount of light. In the present instance the day was cloudy and, for this country, very dark until 2 P.M., when there was a brief interval of light, but by 3 P.M. the dusk of evening had begun. The stomata remained closed throughout the day, as the straight line shows. The soil was moist and the plant cells were turgid, but with light insufficient to stimulate the guard cells the stomata remained closed. The slight amount of light at about 2 P.M. was not enough to affect them. The temperature reached 28.9° C. at 11.55 A.M., and the humidity fell to 47% at 2 P.M., the curves of temperature and humidity showing a rather wide divergence in the early afternoon hours and a close convergence, as indicated before, at 4:55 P.M.

Wheat

Similar measurements were made of wheat stomata, and the data follow. Thus figure 4 shows stomatal and other measurements taken in a box of wheat seedlings twenty-three days old. The conditions were nearly the same as those described in connection with figure 1, and there is much similarity in the diagrams. The light was bright from the beginning of the day, and was intense between 12:30 and 2:30 P.M. The maximum temperature was 38° C. at 1:15 P.M., the maximum humidity 57%. The humidity fell to 46% at 2:15 P.M., but rose slightly from then on with the decrease in warmth. The soil moisture was 20.4% at 8:25 A.M. More water was added and the soil kept very wet all day. With this abundance of moisture and light the stomata opened, but they did not respond as rapidly as the barley, as shown by the slower start. The period of greatest expansion of the stomata was shorter than in barley, and the period of closing was also shorter. The temperature and the humidity both remained high as late as 4:15 P.M., when the stomata almost closed.

The degree of turgidity of the cells of a plant depends, other things being equal, upon the percentage of moisture in the soil in which they grow. Without considerable turgidity the guard cells shrink or collapse. In this condition light does not so stimulate them that they open. This fact is indicated by wheat seedlings, the minimum water requirements of which, under the conditions of our experiments, were found to lie between 16.7% and 17.8% of soil moisture.

Another set of wheat seedlings thirty-seven days old, grown in 17.8% of soil moisture, illustrate the intimate relation between light and the opening of the stomata. The morning of observation was dark and cloudy until 10:30 A.M.; then there followed an interval of weak light to about 1:30 P.M., a cloudy period at 2 P.M., a clearing of the clouds at 3:00 P.M., and the dusk of evening coming on at about 4:45 P.M. The stomata opened slightly during each light period and closed with each recurring darkness, and thus opened twice during the single day. The temperature and the humidity were high throughout the period of the readings.

In figure 5 no such sensitive response to light is recorded. Here are shown the records of wheat seedlings fifteen days old, with soil moisture at 16.7%. The plants were erect and showed no outward signs of wilting, but the guard cells appeared to be somewhat collapsed. The temperature rose to 34° C. at 2:05 P.M., and the humidity rose slightly in the forenoon and fell in the afternoon to 42% at 3:15 P.M. The sun shone brightly at 8:30 A.M. and the sky was cloudless throughout the day. External stimuli were such in this instance as to indicate a wide opening of the stomata; but, because of the shortage of soil water, they remained tightly closed throughout the day, protecting the leaf from evaporation and thus conserving such moisture as it contained. In the preceding case, as in this one, there is a striking similarity between the humidity curve and the stomatal curve.

As the humidity began to fall the stomata began to open: but as the curves show, the stomata did not begin to fluctuate with the changes in humidity, but rather were guided in their movements by the amount of light. Fall of humidity, therefore, is probably not a factor influencing the behavior of the guard cells.

Figure 6 is representative of many sets of wheat seedlings examined on moderately bright days with varying light intensity. In this case the light was brighter than in the two preceding. The plants were ten days old, and the soil moisture in the box varied from 20.9% at 9:25 A.M. to 22.2% at 4:30 P.M. The temperature and the humidity both remained within the limits of 76° and 64%, converging very closely at 4:15 P.M. Conditions were such as to keep the stomata open all day, although a dark period about 11:30 A.M. caused a partial closing, as indicated by the drop in the line in the figure. By 2 o'clock the light was strong enough to cause the maximum opening for the day, as shown by the stomatal curve.

Oats

The reactions of the stomata of oat seedlings very closely resembled those of barley and wheat. That there are special differences, however, is revealed by the accompanying graphs. On examining an extensive series of stomatal reactions, figure 7 was chosen as indicating the optimum condition for oats. The soil was comparatively dry at 8:30 A.M., there being only 9.9% of soil moisture, and no water was added during the day. The temperature rose to 35.5° C. at 1:30 P.M., and the minimum humidity, 44–46%, prevailed between 12:45 and 2:55 P.M. The seedlings were fifteen days old and well developed. The sun was unclouded from 8 A.M. on the day of these readings, and the light reached its greatest brightness between 12 o'clock noon and 2 P.M. The first readings showed the stomata open. They continued to open still wider until the maximum was reached at 12:45 P.M. The maximum width was maintained for a few minutes only, after which they began to close. The closing was very gradual, however, as they were still slightly open at the last reading, which was at 4:30 P.M.

When one compares this curve with that in figure 1 for barley and that in figure 4 for wheat, all representing optimum conditions for their respectives species, it is obvious that the stomata of oats, when they have reached their maximum expansion, take longer to close, as well as to open, than do those of the other two plants. Their guard cells react less promptly to light stimuli than the guard cells of the stomata of barley and wheat. Of all the grains examined, wheat stomata are the ones most sensitive to light, if one may judge by the speed of reaction of the guard cells.

To determine the amount of soil moisture necessary for oats, two boxes of plants, each sixty-three days old and very similar in character, are used. At 9 A.M. both contained 8% soil moisture, and each was abundantly watered. The soil was slightly caked and the plants showed some wilting. Figure 8 represents box A which, after watering, was placed out of doors in very bright sunlight. The humidity fell rapidly and remained low for the day, varying only 4% from 27% at 11 A.M. to 4:10 P.M. The temperature reached 32° C. at 11 A.M. and gradually fell to 61 at 4:10 P.M. By 1 P.M. the wilted plants had become erect and the cells had assumed a more nearly normal turgescence. By 4:10 P.M. twilight had come on, and the stomata remained closed thereafter.

Figure 9 represents the similar plants in box B, treated in like manner except that they were left in the greenhouse. Although the plants in this box did not exhibit such extreme wilting as those in box A, nevertheless the guard cells proved, on examination, to be completely collapsed. Light was abundant throughout the day. By 1:40 P.M. the guard cells had recovered the necessary degree of turgidity and began to open steadily until 4:10 P.M. After this hour the light was too dim for readings.

These two cases, therefore, show that both conditions must be present together in order that the guard cells may open. The cells must have reached their full degree of turgidity before light will affect their action; and the more turgid the cells are at the time of exposure, the more responsive they will be to the different degrees of light which reach them. Comparing the soil moistures of boxes A and B with that of figure 7, it is found that the minimum moisture requirements of these oats lay between 9.9%, a favorable condition, and 8%, a wilting condition.

Rye

Rye seedlings of different ages exhibited differences in the stomatal reactions of the young and the old plants. In the three grain species already described there is no difference in the behavior of the stomata of the plants of different ages; all reacted in the same way under the same stimuli. As already mentioned, rye thrives best, for economic purposes, where the soi and air are relatively drier than where the other grains thrive best. Thus our rye grew poorly in the humid air of the greenhouse, but finally matured into somewhat stunted plants.

Figure 10 gives the stomatal curve for rye seedlings fourteen days old, on a very bright day, with soil moisture at 10% at 9:45 A.M., and with no more water added during the day. The temperature reached 36° C. at 1:40 P.M., and the humidity remained high throughout the day. The stomata opened at 8:50 A.M. and remained but slightly open until 1:30 P.M., when they closed for the day. Sunlight continued until after 4 P.M. This and other similar cases showed that the stomata of young rye seedlings open to a very slight degree only.

Rye plants seventy-two days old and beginning to bloom are indicated by figure 11. At this time the soil moisture was 20.9% and the temperature reached 32° C. at 1:30 P.M. The humidity remained stationary at 31%

from 11:50 A.M. to 2:30 P.M. The light was bright from 8:30 A.M. till 1:30 P.M. From that hour it waned until twilight, which came on shortly after 4 P.M. The stomata of these older plants more noticeably responded to the light than did those of the younger plants described immediately above. They opened at 9:30 A.M. and remained open until 1:30 P.M.; but though their width was uniform throughout this time, they were not as widely open as the stomata of the other grain plants. Nevertheless, in the case of rye also, the width of the stomatal opening corresponded to the intensity of the sunlight. The fact that the stomata never opened to their full width may be an important and significant adaptation, or reaction, to their environment. If, for example, the stomata opened wide where the air is dry, transpiration might be so excessive as to dry out the plant and destroy it. One of us has shown that when the guard cells are killed or paralyzed by sulphur dioxide fumes, the rate of transpiration goes up, other things being equal, and the plant, the organ, or the part may dry out and die, because the plant has lost control, locally or generally, of its water.

The minimum water requirements of rye were not especially studied; but our observations indicate that they are below 10%. Rye plants appeared to develop quite favorably on soil containing 10% or less of water, whereas if the amount reached 20% or more the plants did poorly.

To provide a check upon the previous work we conducted the following experiments upon these four species of grain grown in pots. One pot of each species was placed in bright sunlight and one pot of each was put into the dark. The temperature and the humidity were kept the same for both sets by means of an electric fan. In all cases the stomata were slightly open when the experiment began. The results were so similar in all four species that only those obtained from the wheat plants will be reported here. Wheat is selected for this because we were able to reverse the positions of the pots, in the case of the wheat, on the following day, thus giving us a check on the check furnished by the first set of experiments. Figure 12 gives the two very dissimilar curves constructed from the records of the behavior of the stomata of wheat in light and in darkness. Temperature and humidity were both high, and there was an abundance of moisture in the soil. At 8:30 A.M. both sets of plants were examined. The stomata were open. Each set was watered and one, A, placed in the light, the other, B, in darkness. The rise and fall of the line A in figure 12 corresponds with and indicates the increase and decrease of sunlight during the day. B shows that the stomata closed at 10 A.M. and so remained as long as they were in darkness. Figure 13 records the behavior of the same two pots of plants,. B put into the light, and A into the dark. Conditions of light and of soil moisture were very similar to those of the day preceding, but the plants were subjected to the opposite effects of light and darkness. Hence the stomata of the plant placed in the dark closed promptly and remained closed, whereas those of the plant which was brightly lighted continued to open. Hence, the behavior on one day, in one set of conditions, was the opposite of the behavior on the following day, under conditions exactly the reverse so far as light was concerned but similar in every other respect.

To answer the question what effect, if any, the conditions in the greenhouse exerted upon the behavior of the stomata of these four species of grain plants, we grew another set in the open, in the Experimental Garden which now forms part of the equipment of this laboratory. When fifty-five days old, on December 29, they were examined. The results are indicated in figure 14. The day followed a cold frosty night, but was very clear from early dawn. The sun appeared at 8:30 A.M.[1] The humidity fell to 57% at 1:15 P.M., but it remained much higher during the rest of the day, both earlier and later. The temperature was about 15.5° C., ranging somewhat above and below this figure. It is obvious too that, at this season of the year, even in a latitude no further north, the position of the sun results in a light intensity considerably less than at other seasons. Nevertheless, it will be seen, on comparing the stomatal curves in this figure, 14, with those of the same species previously reported and experimented upon under glass, that there was little or no difference in the general behavior of the stomata. At 2 P.M. the light began to fade and all the stomata began to close. Of the four, the stomata of wheat closed soonest, the other three closing at about the same time and rate, as the line Y-Y′ shows. These results indicate plainly that, so far as stomatal action in response to light is concerned, there is little or no difference in behavior due to the effect of the greenhouse.

Wild Oats

While the experiments above described were in progress it was suggested that we ascertain the behavior of the native wild oats (*Avena fatua* L.), which were growing in the Experimental Garden. Several plants were potted and taken into the greenhouse. Figure 15 indicates the stomatal movements on a young and on an old plant on the day following transplanting. The soil was kept very moist, and the light was moderately bright. The stomata on the young plants remained open during the brightest part of the day, from 9:15 A.M. till 2:05 P.M., but on the old plant they failed to open.

Mature plants of wild oats tend to open their stomata somewhat during the early morning hours, and then to close them for the remainder, and the hottest part of the day. Figure 16 shows this action on a very clear, bright day, with the temperature at 23° C. and the humidity at 43% during the middle of the day with the soil moisture at 12%. Similar plants

[1] It should be stated that, owing to the two mountain ranges bounding the Santa Clara Valley to the eastward and the westward, sunrise and sunset are respectively later and earlier than they would be on a wide plain in the same latitude. Sunset and darkness come especially earlier in our laboratories because Stanford University is built at the foot of the hills which rise to the mountains to the west.

examined on days with high clouds, or with light rain, the soil moisture being about the same as before, namely 12%, showed that the stomata remained open throughout the day. In this respect there was more variety of action among the older plants than among the younger ones. Thus figure 17 records the behavior of two such plants, *a* young, 4–8 inches high, and *b* a plant in bloom, under the same conditions and on the same day. The temperature reached 34° C. at 1:40 P.M., and the humidity remained almost constant, namely at about 60%. There was no direct sunlight during the day, but at times there were very bright intervals. Wild oats, therefore, are able to absorb CO_2 and to manufacture food on days when the other plants here reported upon would keep their stomata closed, because the stimulus required for their opening would be too weak to produce the needed effect. On the other hand, figure 16 indicates the behavior of a mature wild oat plant growing out of doors in the Experimental Garden. After a day of rain the moisture in the soil amounted to 12%, the sky was cloudless, and the sunshine correspondingly bright. Nevertheless, the stomata closed at the time of maximum illumination. This time was also that of maximum temperature and minimum humidity. Apparently we have, in this behavior of the wild oat out of doors, a decided contrast with the behavior of the cultivated grains. Its behavior in the greenhouse is similar, however, to the others. Out of doors the conditions of its existence are somewhat different from those ordinarily prevailing for the cultivated varieties, as the following description will show. The wild oat of California grows and fruits throughout most of the year, naturally reaching its best development during the wet weather of early winter and of spring. The leaves are slender, tough, thick, and hairy, well adapted to withstand severe drying of the soil; for this species grows commonly along dry roadsides and in open fields and pastures, where it is subjected to pronounced drying, perhaps more than once during its life. The specimens which we studied were well developed and in bloom during the early part of December. In midsummer and later the plant would not thrive, and only where there was some moisture would it hold out at all. On the other hand, the cultivated grains can be grown at any time of the year, providing there is sufficient warmth, moisture, and light. They are naturally spring species, however, and grow best with the warm rains, and fruit in the early summer. In these differences in habitats, and in the corresponding differences in habits, we see reasons for the behavior of the two sets of plants.

That the behavior of *Avena fatua*, in the respects in which it differs from that of the four cultivated grain plants which we studied, is the product of circumstances is indicated by figure 18, in which the movements of the stomata of wild and cultivated oat plants are recorded. In this experiment in which wild oats, transplanted from a field and kept in the greenhouse for thirty-five days, were compared with cultivated oats, we find the following circumstances. The temperature was moderate throughout the period of observation, the maximum being 27° C. at 1:10 P.M.; the humidity was lowest at the same hour, namely 49%. In the figure line (*a*) represents the behavior of stomata of cultivated oats, mature plants seventy days old, in soil containing 24.5% of moisture. Line (*b*) indicates the behavior of the stomata of a large plant of *Avena fatua*, then in bloom, which had been in the greenhouse for thirty-five days, in soil containing 15–20% of moisture at the time of observation. No water was added to either box during the day. The sky was clear and the light bright from 8:00 A.M., reaching its maximum brightness between 11:00 A.M. and 1:45 P.M. At the latter hour the sky clouded, but by three o'clock it was lighter again. The sun set behind our mountains at four o'clock, and the plants were in shadow from that time on. The two curves show that the stomata remained open as long as there was bright light; that, on the dimming of the light soon after one o'clock, the stomata of the cultivated species closed and did not open again during the rest of the day, although the light later on became somewhat brighter again. On the other hand, the stomata of the wild oat closed very gradually, and they were completely closed only at sun-down. The stomata of the wild oat reacted to the dimming light, but only much more slowly than those of the cultivated species.

Wild oats growing in hard, dry soil were examined on various days. The plants were usually erect and exhibited no outward microscopic signs of wilting, but the stomata were shrunken and showed no movement. These wild plants survive in such dry surface soil, and on hot days on which the cultivated species wilt or burn, even though abundantly supplied with water in the soil. In the behavior of the stomata of these two species of oats we see one reason for the differences in their occurrence and in their requirements. Thus, the wild species can and does maintain itself in changing conditions in which the temperatures, humidity, soil moisture, and illumination may cover a very wide range; whereas the cultivated species requires a fairly high proportion of soil moisture, and it can withstand only moderate dryness of the air and moderate heat.

The Significance of Stomatal Movements

The foregoing experiments show that, in the species studied, there are definite times during which the stomata are open and other times during which they are closed. While the stomata are open carbon dioxide enters the leaves of these plants more rapidly than while they are closed, other things being equal. Furthermore, carbon dioxide will enter a green leaf not only in accordance with the openness of its stomata, but also in accordance with the rate at which it is being used in the leaf. This use constitutes the photosynthetic process resulting in the production of sugar, starch, etc., a process which goes on at rates proportioned, among other factors, to the intensity of the light. We see, therefore, that, so far as these five species of annual plants are concerned, a factor which regulates the rate of food

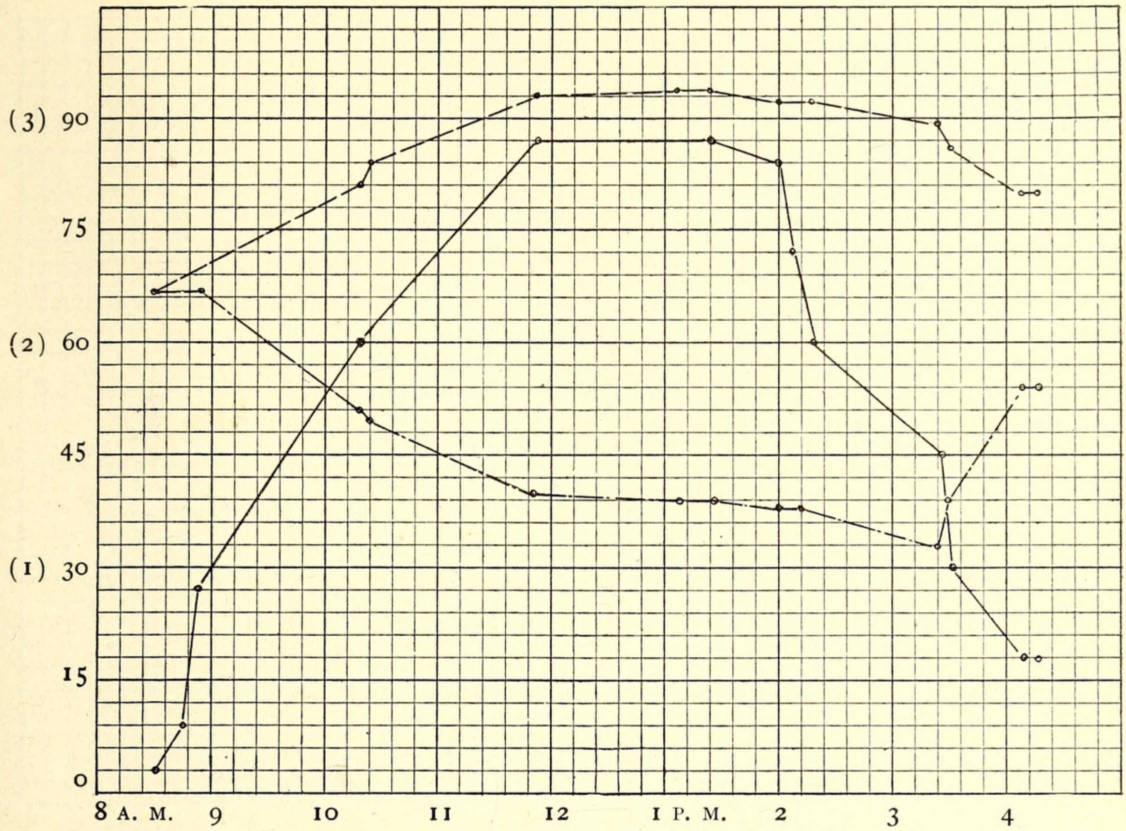

FIG. 1 (For explanation, see p. 155).

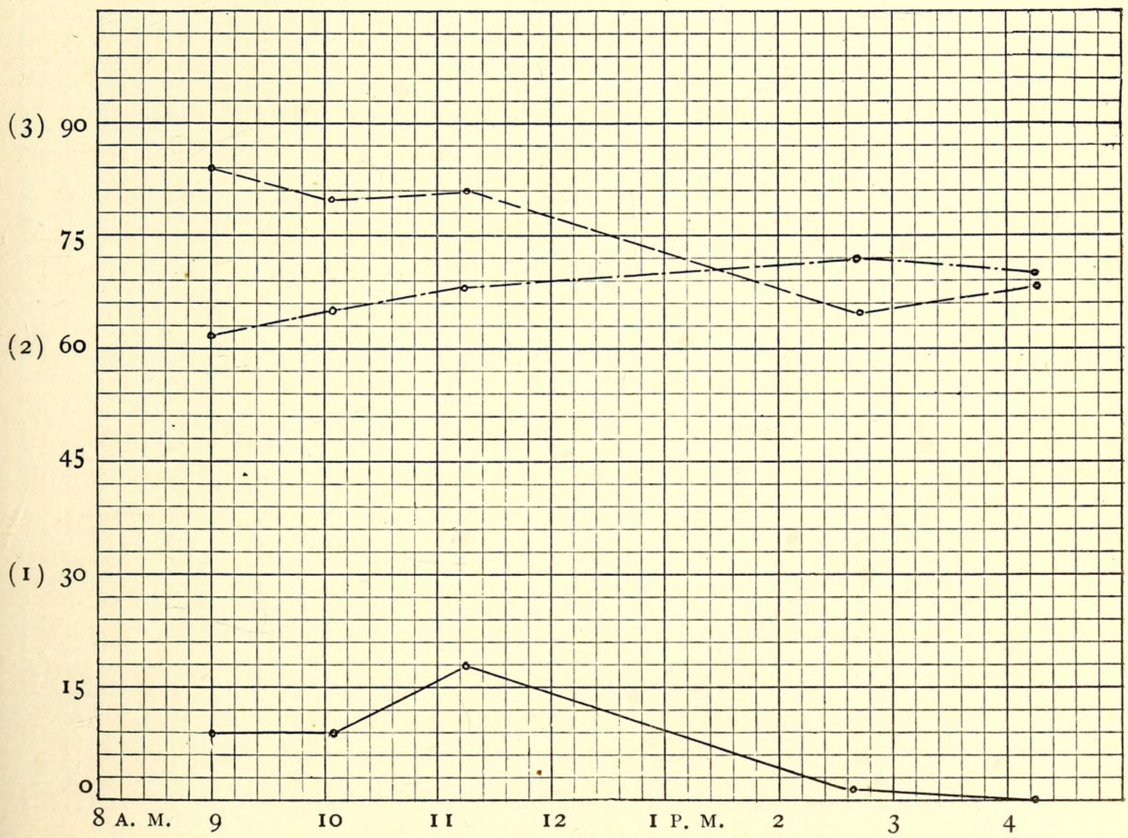

FIG. 2.

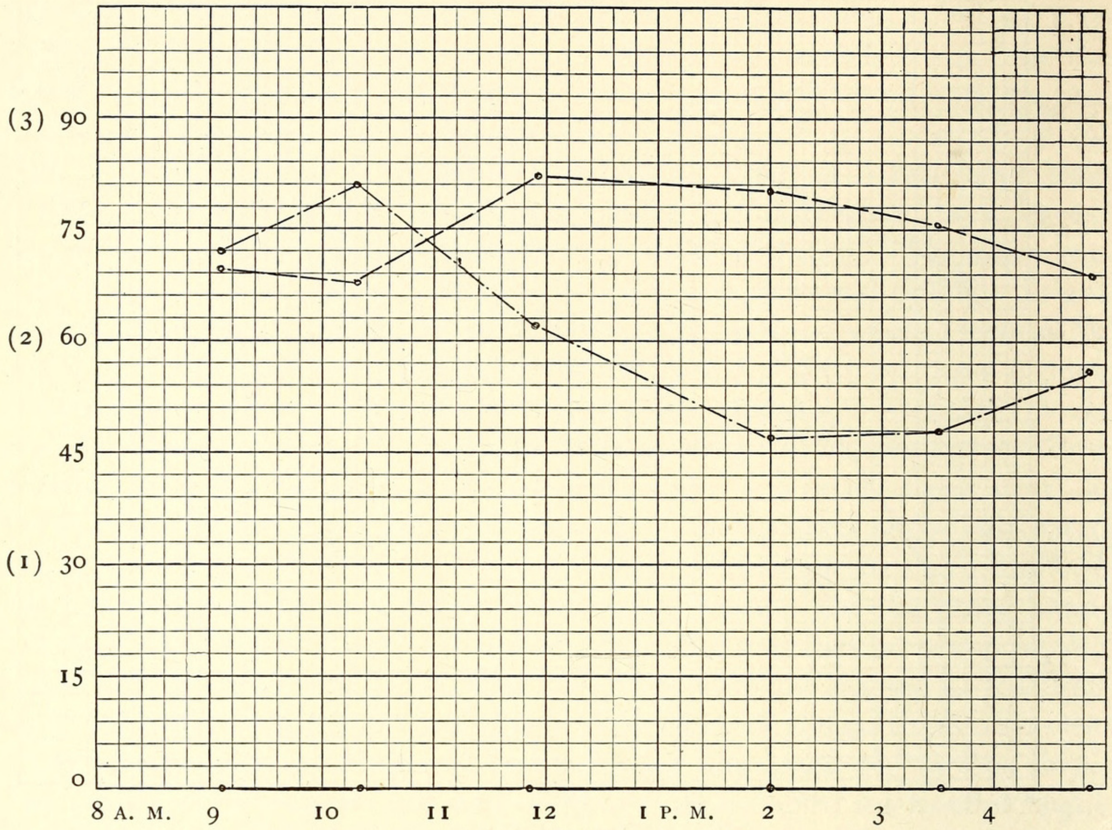
(3) 90
75
(2) 60
45
(1) 30
15
0
8 A. M.
9
10
11
12
1 P. M.
2
3
4

FIG. 3.

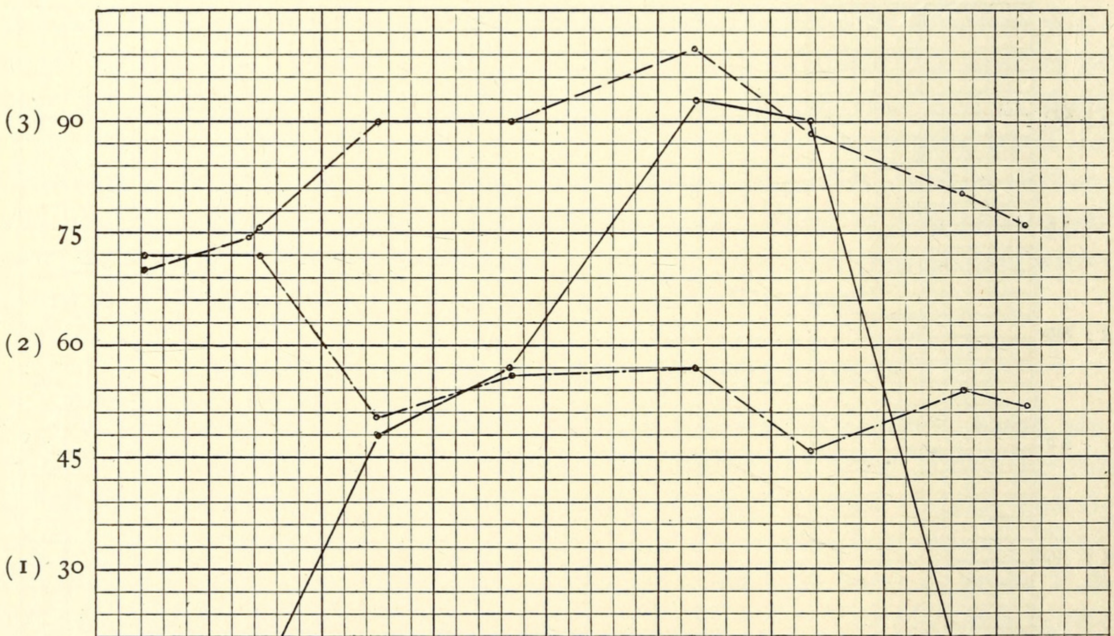
(3) 90
75
(2) 60
45
(1) 30
15
0
8 A. M.
9
10
11
12
1 P. M.
2
3
4

FIG. 4.

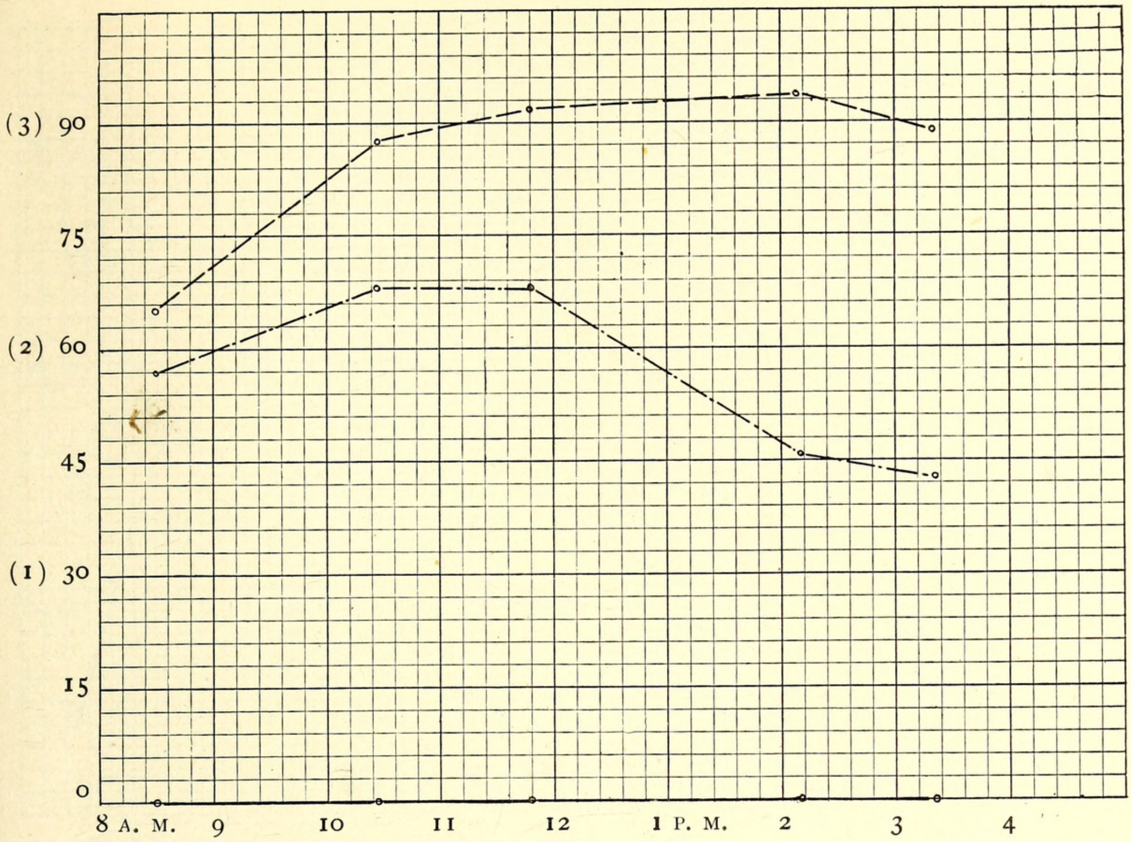
(3) 90
75
(2) 60
45
(1) 30
15
0
8 A. M. 9 10 11 12 1 P. M. 2 3 4

FIG. 5.

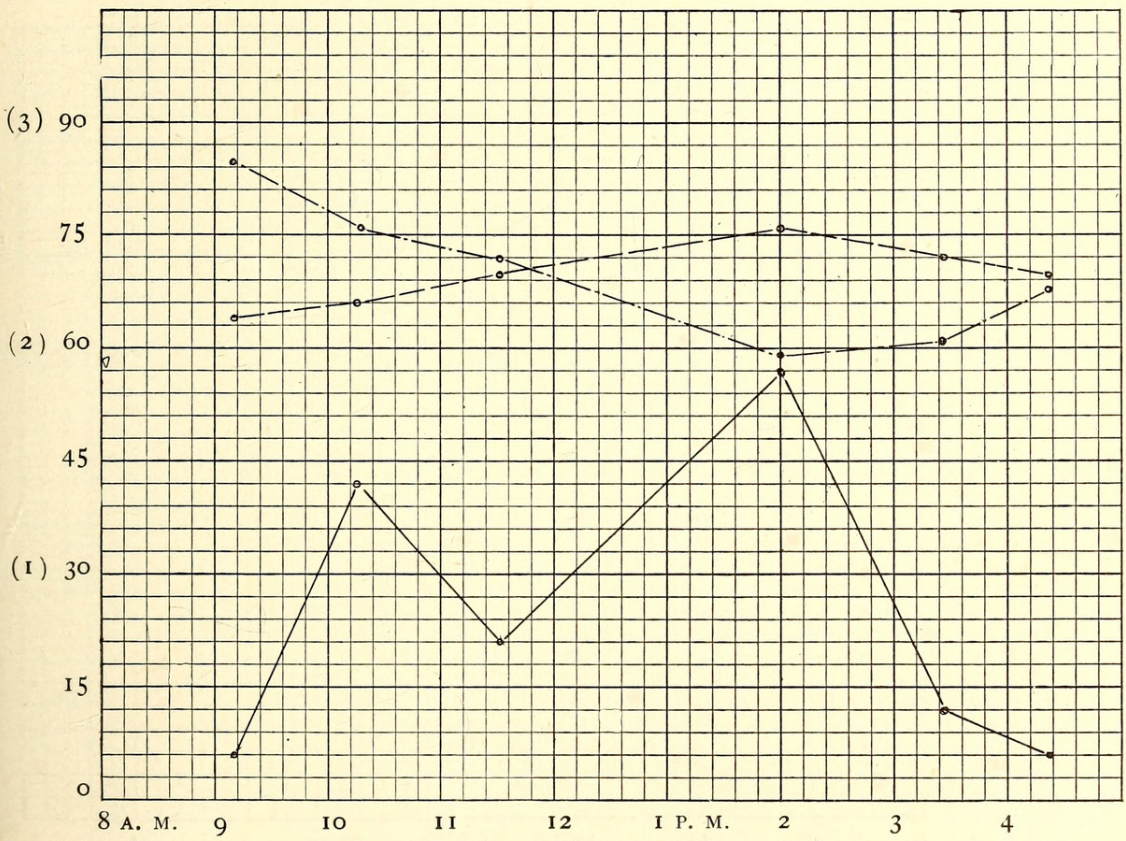
(3) 90
75
(2) 60
45
(1) 30
15
0
8 A. M. 9 10 11 12 1 P. M. 2 3 4

FIG. 6.

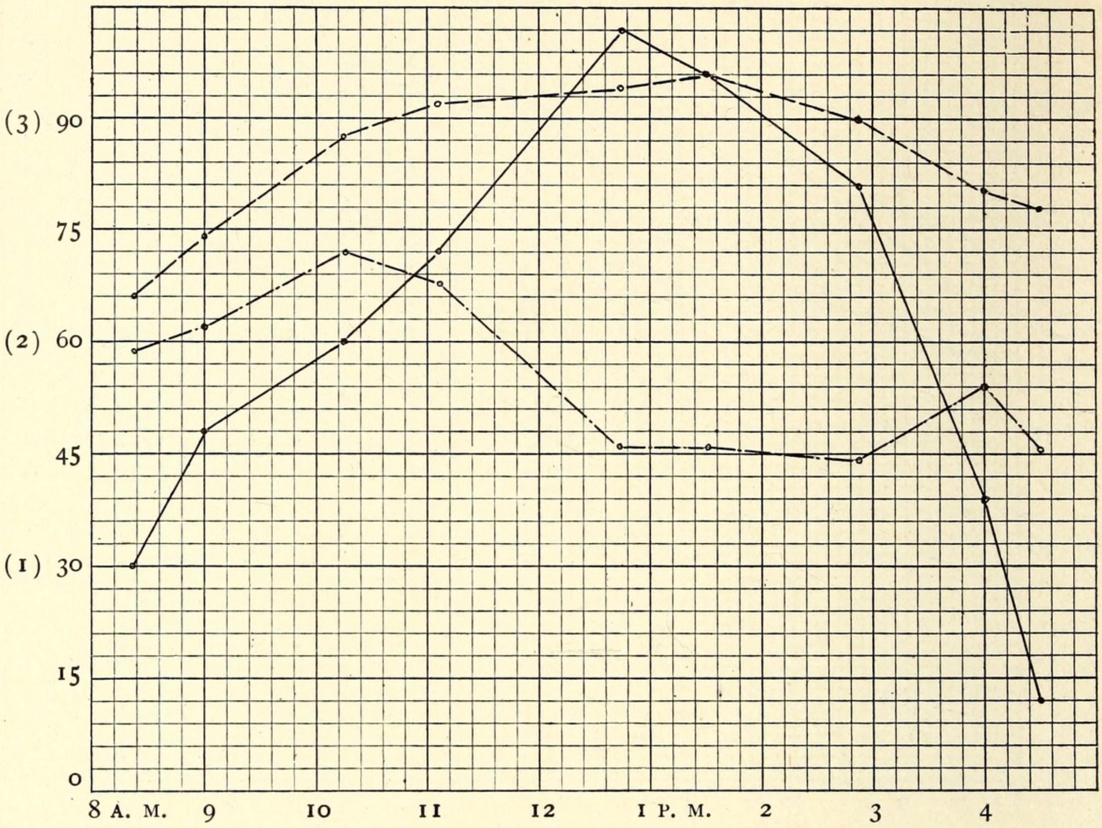
(3) 90
75
(2) 60
45
(1) 30
15
0
8 A. M.
9
10
11
12
1 P. M.
2
3
4

FIG. 7.

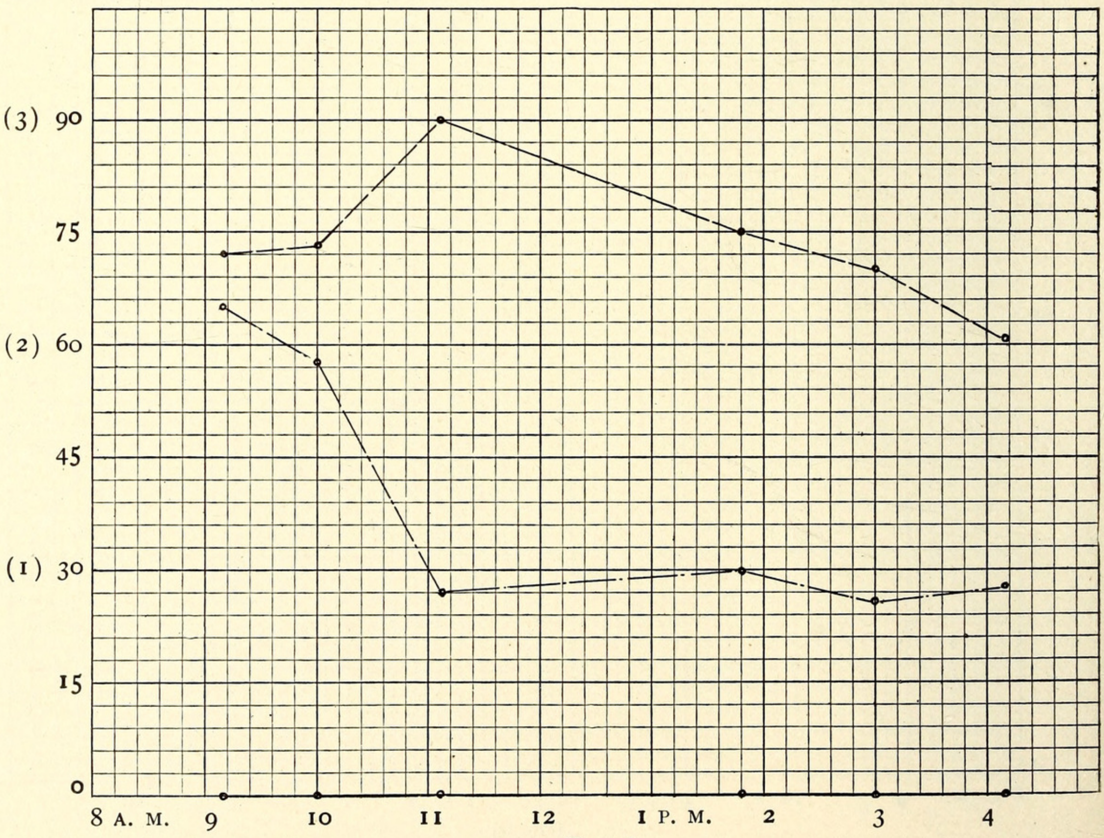
(3) 90
75
(2) 60
45
(1) 30
15
0
8 A. M.
9
10
11
12
1 P. M.
2
3
4

FIG. 8.

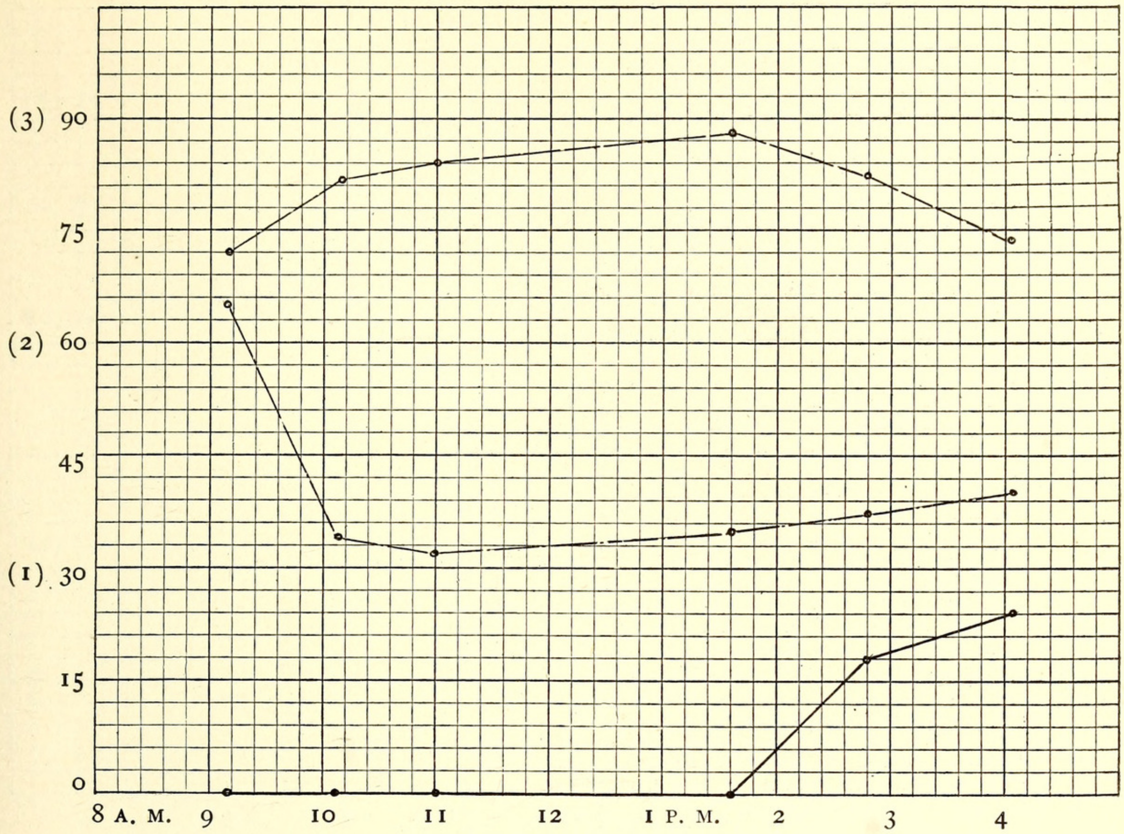
(3) 90
75
(2) 60
45
(1) 30
15
0
8 A. M. 9 10 11 12 1 P. M. 2 3 4

FIG. 9.

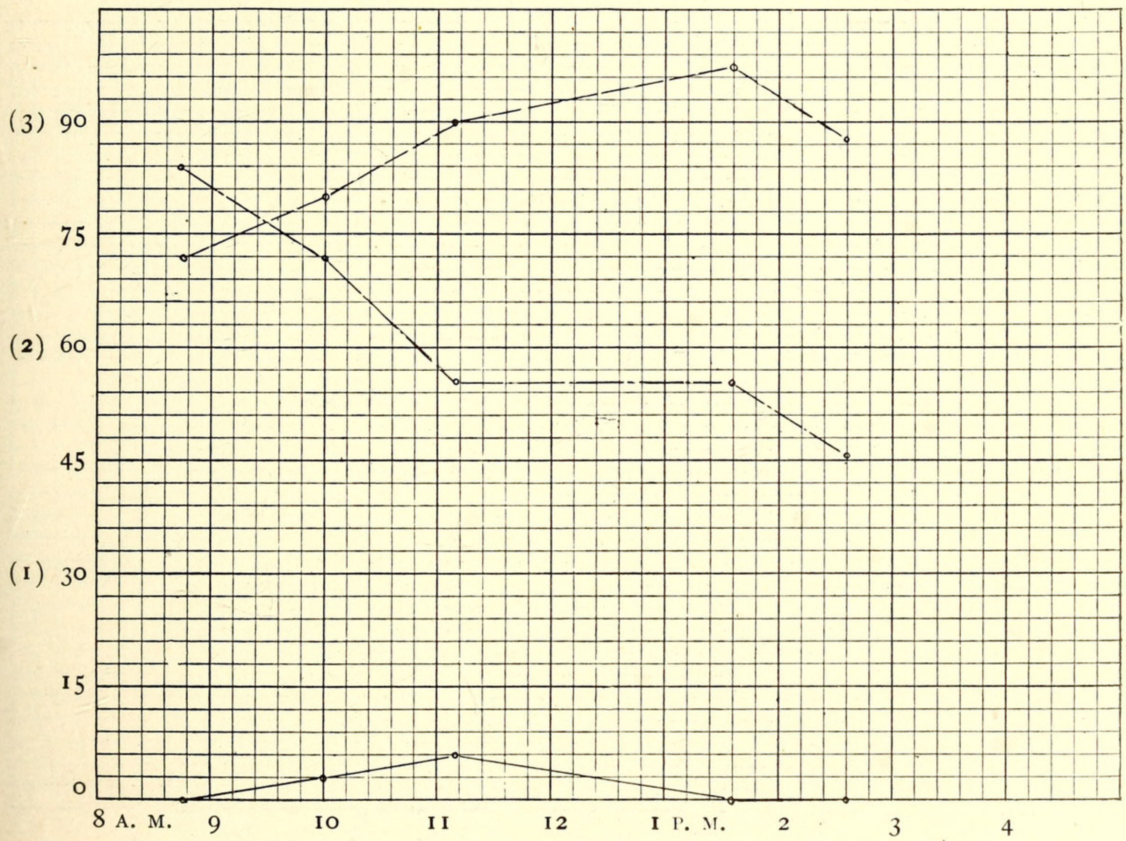
(3) 90
75
(2) 60
45
(1) 30
15
0
8 A. M. 9 10 11 12 1 P. M. 2 3 4

FIG. 10.

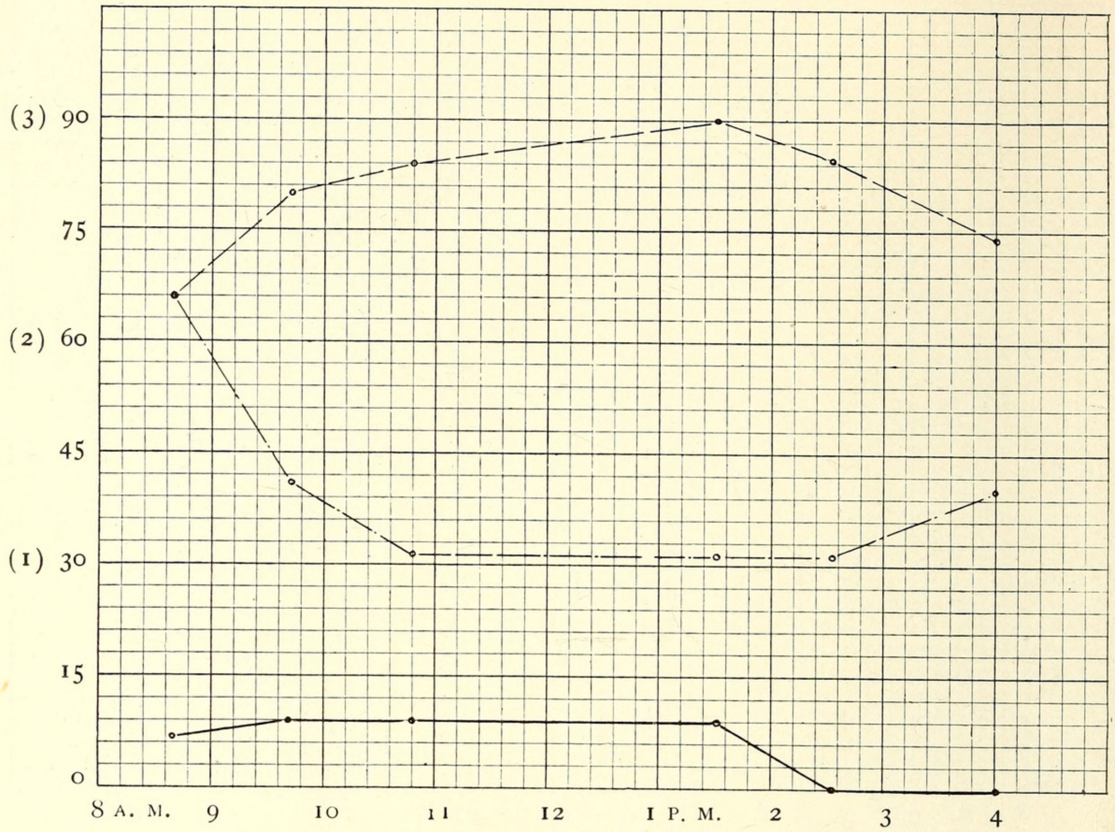
(3) 90
75
(2) 60
45
(1) 30
15
0
8 A. M.
9
10
11
12
1 P. M.
2
3
4

FIG. 11.

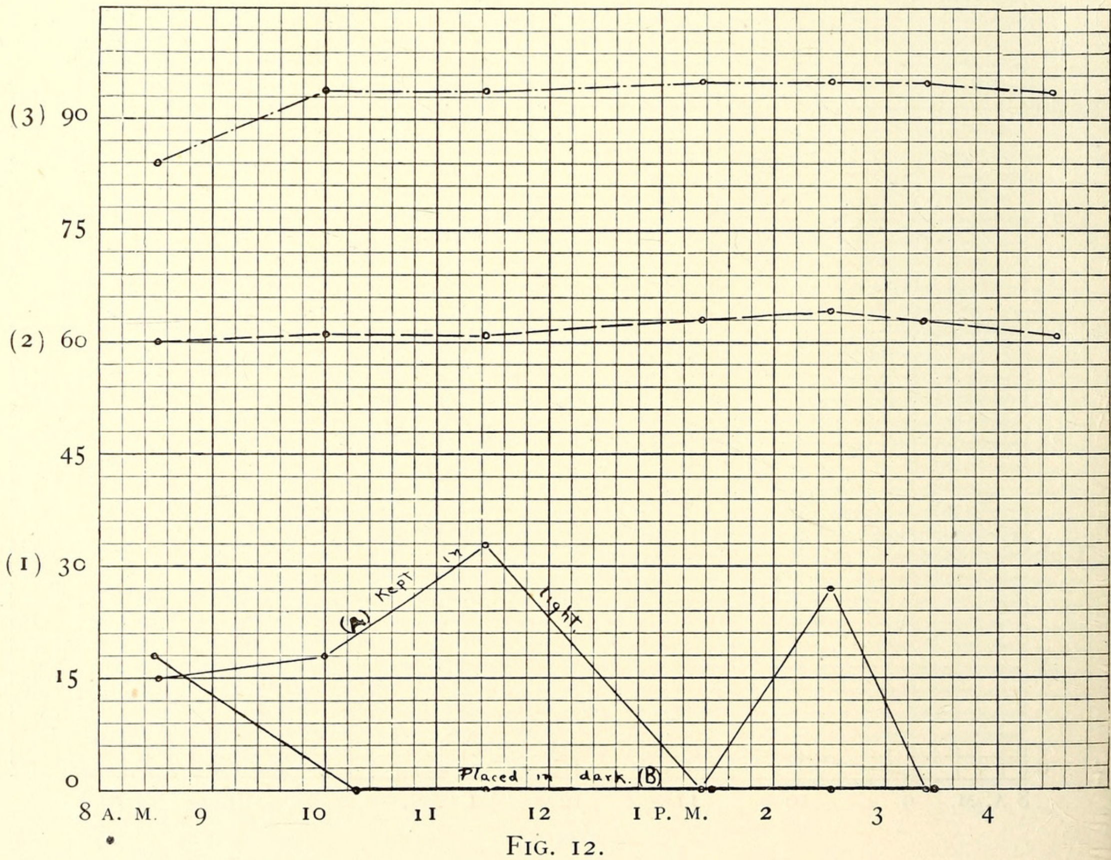
(3) 90
75
(2) 60
45
(1) 30
15
0
(A) Kept in light.
Placed in dark. (B)
8 A. M.
9
10
11
12
1 P. M.
2
3
4

FIG. 12.

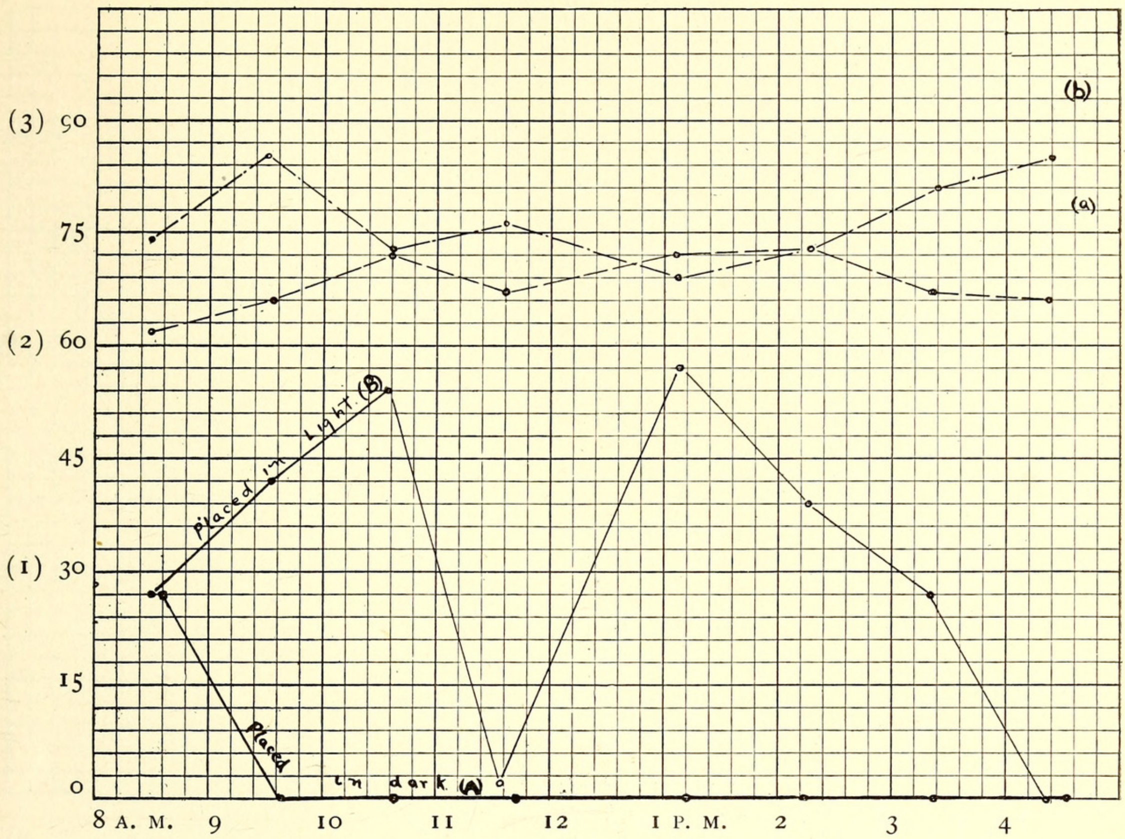
(b)
(a)
(3) 90
75
(2) 60
45
(1) 30
15
0
placed in light (B)
Placed in dark (A)
8 A. M.
9
10
11
12
1 P. M.
2
3
4

FIG. 13.

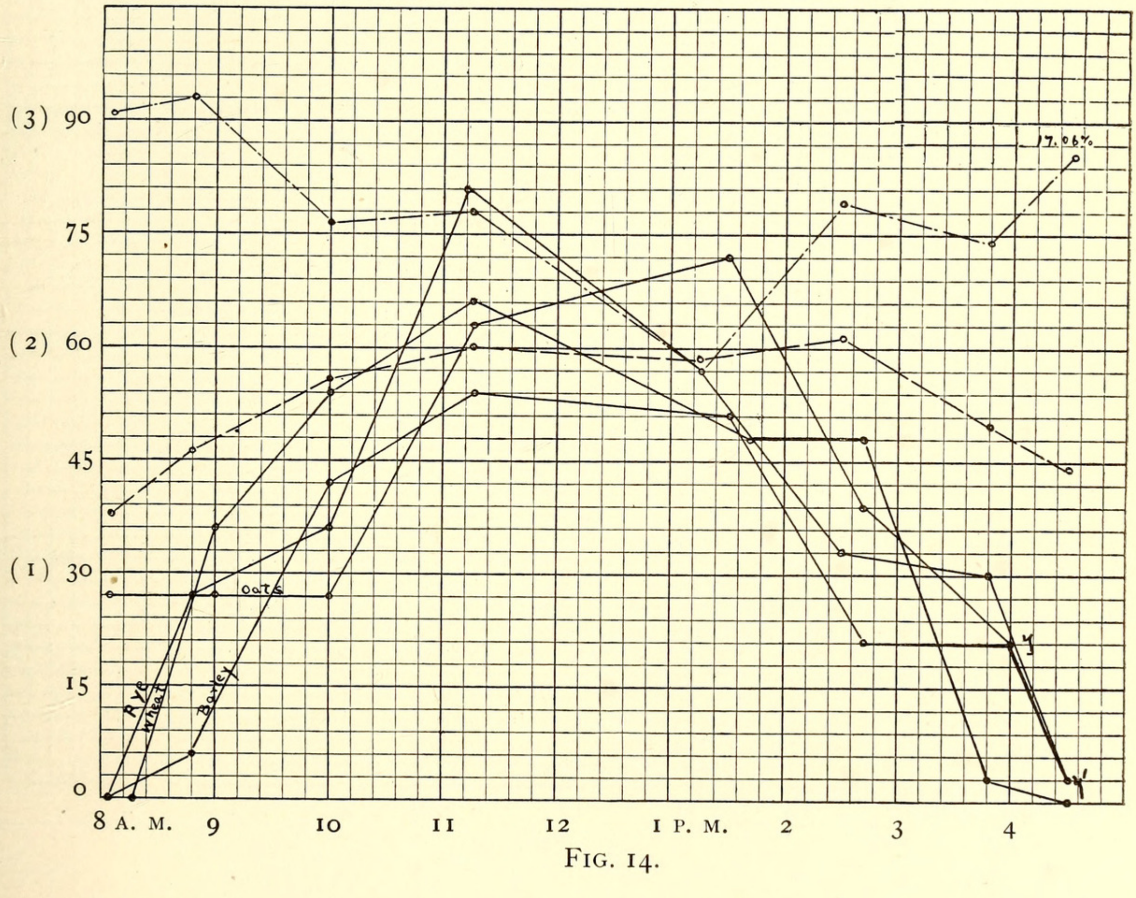
17.06%
(3) 90
75
(2) 60
45
(1) 30
15
0
oats
Rye
wheat
Barley
y
y'
8 A. M.
9
10
11
12
1 P. M.
2
3
4

FIG. 14.

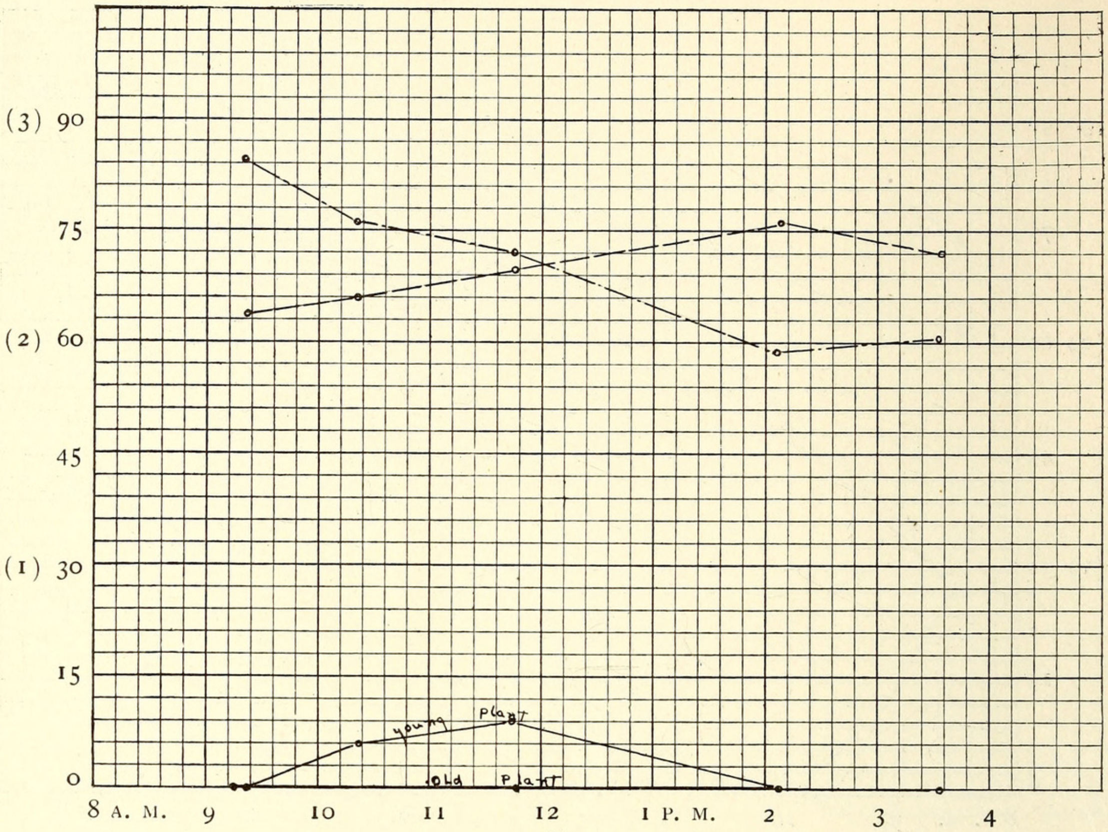
(3) 90
75
(2) 60
45
(1) 30
15
0
young plant
old plant
8 A. M.
9
10
11
12
1 P. M.
2
3
4

FIG. 15.

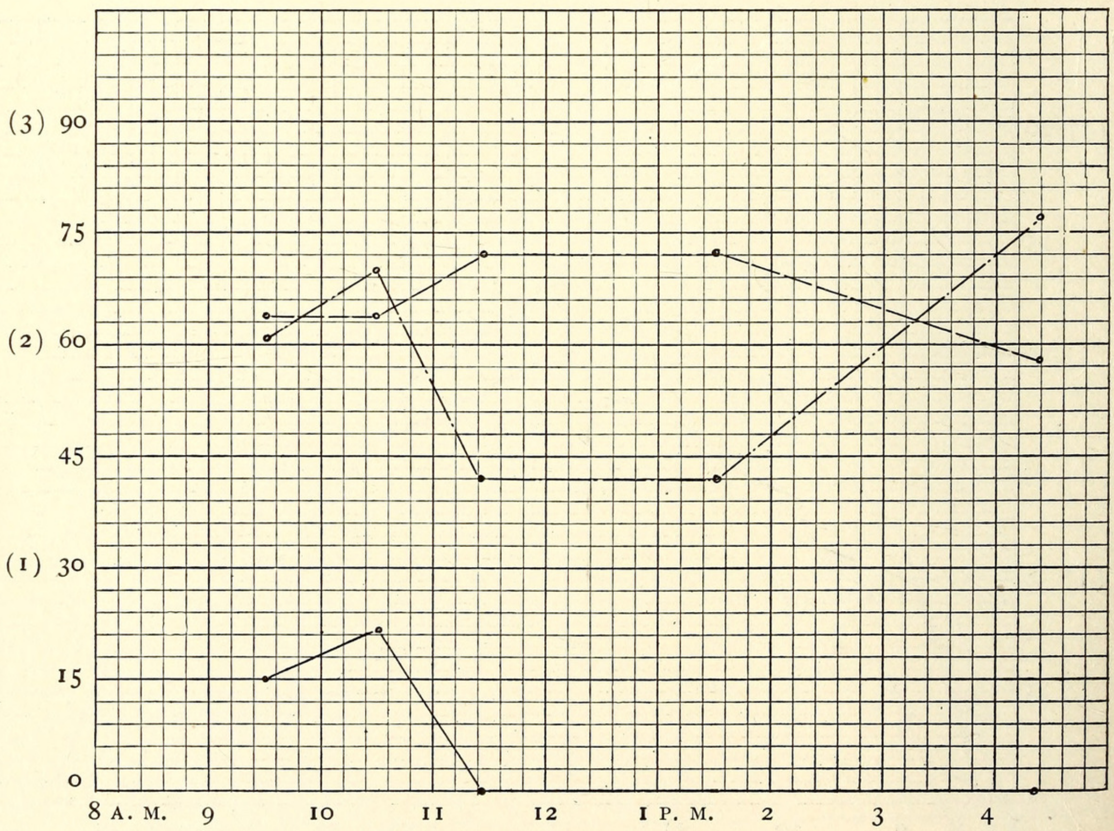
(3) 90
75
(2) 60
45
(1) 30
15
0
8 A. M.
9
10
11
12
1 P. M.
2
3
4

FIG. 16.

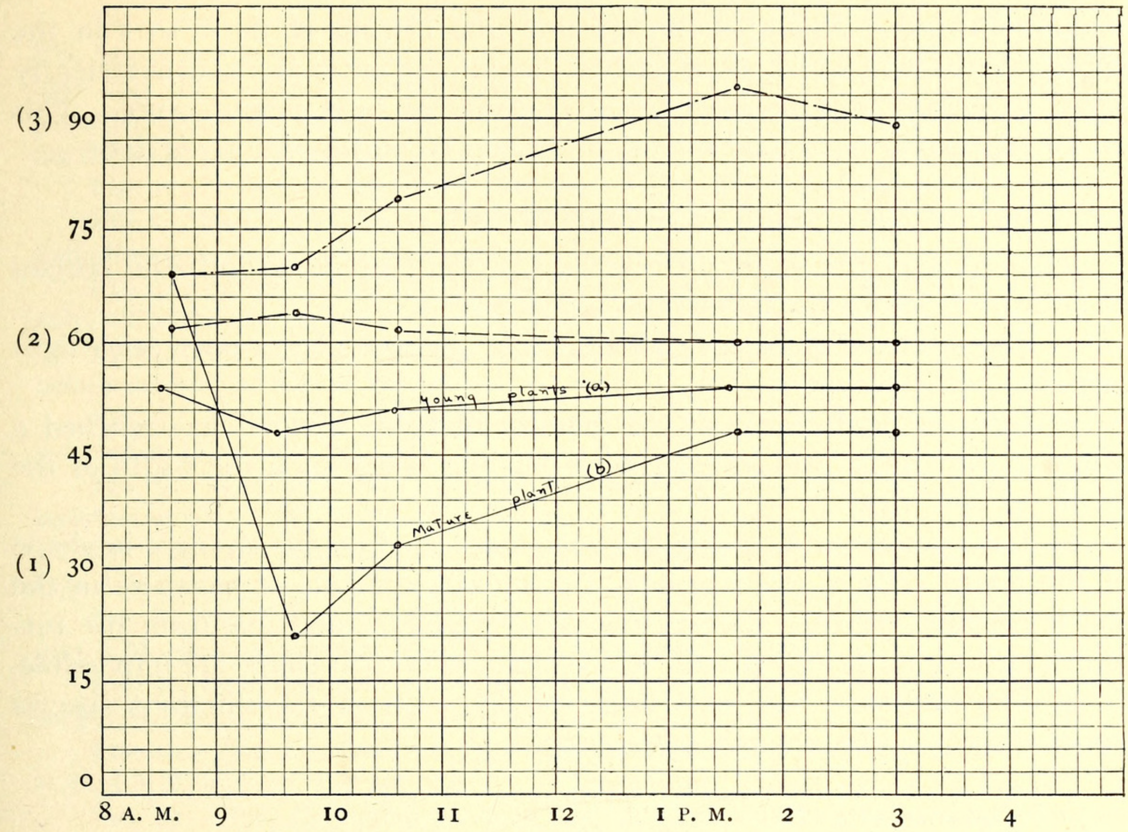
(3) 90
75
(2) 60
45
(1) 30
15
0
8 A. M.
9
10
11
12
1 P. M.
2
3
4
young plants (a)
Mature plant (b)

FIG. 17.

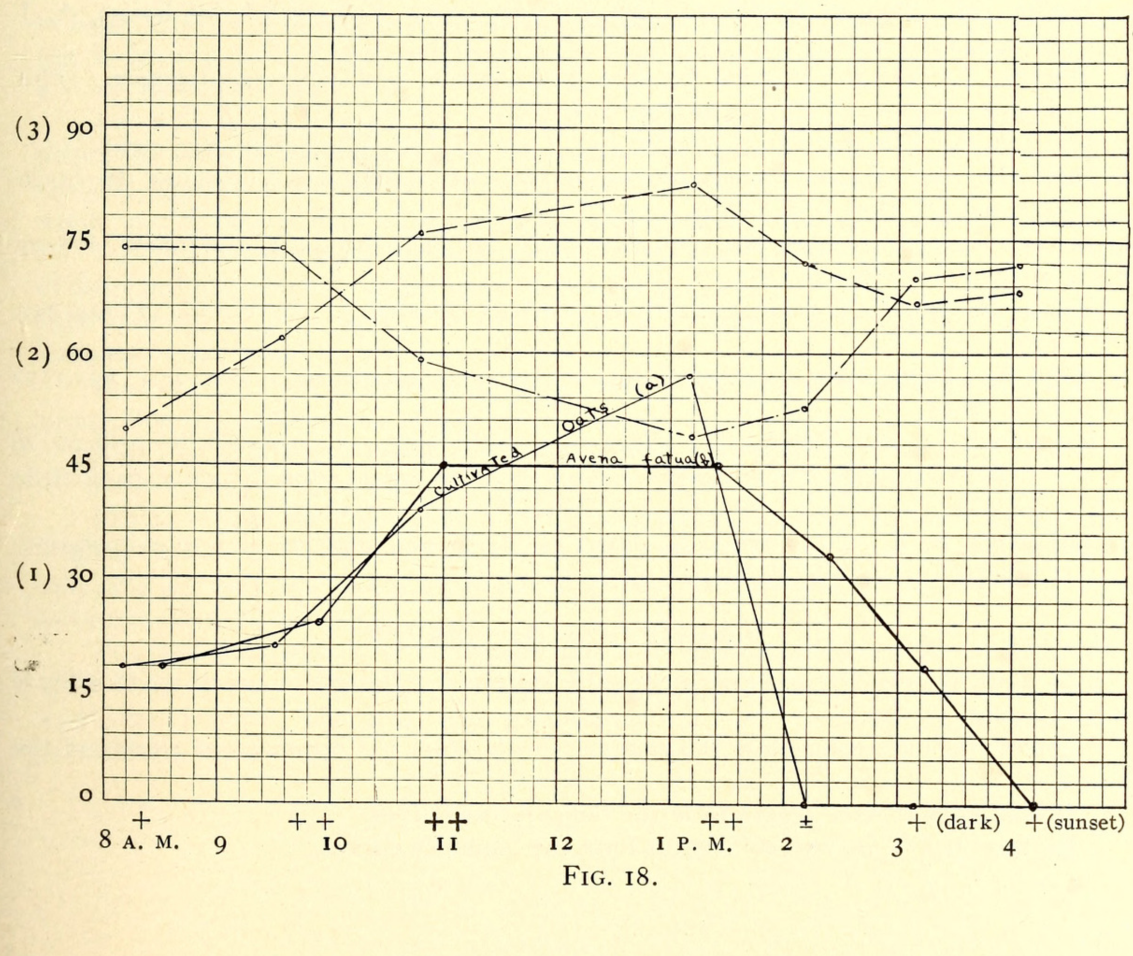
(3) 90
75
(2) 60
45
(1) 30
15
0
+
+ +
++
++
±
+ (dark)
+(sunset)
8 A. M.
9
10
11
12
1 P. M.
2
3
4
cultivated Oats (a)
Avena fatua (b)

FIG. 18.

manufacture also regulates the opening and closing of those slits in the epidermis through which alone carbon dioxide can enter at a rate sufficiently rapid to supply this necessary raw material of food manufacture. This factor regulating both food manufacture and stomatal opening is light.

Conclusions

The study of the stomatal reactions of the cultivated and wild species of grains has led to the following conclusions:

1. The stomata of barley, wheat, oats, and rye plants open with light and close with darkness.

2. Increase or decrease in the amount of light, when it has reached a minimum intensity, will have a corresponding effect upon the width of the stomatal openings.

3. The opening and closing being accomplished by the changes in shape of the guard cells of the stomata, a minimum amount of moisture in the soil is required by each species in order to produce and maintain the turgidity of the guard cells without which changes in their shape are impossible.

4. The moisture, soil, and light requirements of the different species are essentially alike, though not identical.

Leland Stanford Junior University,
California

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DESCRIPTION OF GRAPHS

In all the graphs the curves are made of broken lines. The different sorts are to indicate the following records:

Continuous lines connecting the small circles indicate the opening and closing of the stomata.

Broken lines of *equal* length form the temperature curves.

Broken lines of *two unequal* lengths form the humidity curves.

The figures at the bottom of the diagrams indicate the hours from 8 A.M. to 6 P.M. The figures at the left are those of the Fahrenheit scale, because our Mason hygrometers have only the Fahrenheit thermometers. The other figures to the left of the graphs (in parentheses) are the purely arbitrary numerals of the eye-piece micrometer used in measuring the widths of the stomata.

FIG. 1. Barley, September 16, 1916, age 18 days, bright sunlight, soil very moist.

FIG. 2. Barley, October 14, 1916, age 24 days, dark foggy morning, light toward noon, dull afternoon, 11.3% soil moisture at 9:25 A.M., 10% at 4:30 P.M.

FIG. 3. Barley, October 7, 1916, age 37 days, dark, cloudy day, light about 2 o'clock only, soil very moist at 9:05 A.M., then watered, 21.7% soil moisture at 3:40 P.M.

FIG. 4. Wheat, September 23, 1916, age 23 days, very bright day, 20.4% soil moisture before watering at 8:25 A.M.

FIG. 5. Wheat, September 24, 1916, age 15 days, bright day, 16.7% at 8:30 A.M., watered at 12:25 noon.

FIG. 6. Wheat, October 14, 1916, age 10 days, dark early, light later, 20.9% soil moisture at 9:25 A.M., 22.2% at 4:30 P.M.

FIG. 7. Oats, September 24, 1916, age 15 days, brilliant day, 9.9% soil moisture at 8:30 A.M., no water added.

FIG. 8. Oats, November 11, 1916, age 63 days, bright clear day, 8% soil moisture at 9:15 A.M., watered and put out of doors in very bright sunshine.

FIG. 9. Oats, November 11, 1916, age 63 days, bright clear day, 8% soil moisture at 9:15 A.M., watered and pot left in greenhouse in bright light.

FIG. 10. Rye, September 23, 1916, age 14 days, bright clear day, 10% soil moisture at 9:45 A.M. and not watered later.

FIG. 11. Rye, November 11, 1916, age 72 days, very bright clear day, 20.9% soil moisture, not watered afterwards, plant beginning to bloom.

FIG. 12. Wheat, October 21, 1916, age 17 days, foggy early, later bright; pot A, kept in light, 17.5% soil moisture at 8:45 A.M., watered, 23.7% at 4:00 P.M., difference 6.2%; pot B, kept in dark, 30.8% soil moisture at 8:40 A.M., watered at 8:45 A.M., 39.1% at 4 P.M., difference 8.3%.

FIG. 13. Wheat, October 22, 1916, age 18 days, light similar to that of preceding day, plants the same as preceding (figure 12), treatment exactly reversed, thus: pot B put into sunlight, 23.9% soil moisture at 8:40 A.M., watered at 8:40 A.M., very moist all day afterwards; pot A, kept in dark after 8:40 A.M., 21.7% soil moisture at 8:40 and then watered.

FIG. 14. Wheat, oats, rye, and barley, as indicated, December 29, 1916, growing out of doors in Experimental Garden since November 4; clear night with heavy frost at 8 A.M., light from daylight, sun up at 8:00, light brightest between 11 and 12, following by gradual dimming, 17.06% soil moisture.

FIG. 15. Wild oats, October 14, 1916, bright day, soil very moist; two plants, one young, first day after transplanting; one old enough for blooming.

FIG. 16. Wild oats, November 8, 1916, clear, very bright day with no clouds, 12% soil moisture, large plant in full bloom growing in Experimental Garden.

FIG. 17. Wild oats, November 4, 1916, cloudy with slight rain at intervals, light much brighter than could be expected with clouds, etc.; two sets of plants, one young, 4–8 inches tall, 12% soil moisture; one large, in bloom, growing in Experimental Garden out of doors, soil moisture 12% before rain.

FIG. 18. Comparison of cultivated and wild oats, changes in light as indicated by symbols at bottom of graph; two sets of plants: cultivated oats, large, about 70 days old, 24.5% soil moisture; wild oats, large, transplanted from field to greenhouse 35 days earlier, 15–20% soil moisture.