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Size and Structure of Leaves of Cereals in Relation to Climate

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I.

SIZE AND STRUCTURE OF LEAVES OF CEREALS IN RELATION TO CLIMATE

A STUDY IN CROP ECOLOGY

BY W. E. BRUNER AND J. E. WEAVER

INTRODUCTION

Although a very large amount of work has been done on the variation in form, size, and anatomical structure of native plants grown under different conditions, relatively little attention has been given to similar studies of crop plants. As far as we are able to ascertain, no investigations have been made of variation in the leaves of cereal crops grown under widely different, measured environments.

During the past few years (1920-23), in connection with studies on the development and activities of the roots of crop plants (Weaver, Jean, and Crist, 1922), plant production as a measure of environment (Weaver, 1924), and experimental vegetation (Clements and Weaver, 1924), crops of oats, wheat, and barley, among others, have been grown under measured environmental conditions of air and soil at stations from near the Missouri River to the Great Plains of Colorado. This offered an excellent opportunity for an intensive study of the development of the plants and their leaf characters as influenced by different environments, a line of inquiry that has thrown much light on crop adaptation.

Location of Stations

Stations at which the crops were grown were located at Lincoln, in eastern Nebraska (altitude 1100 feet), Phillipsburg, in north central Kansas (1935 feet), and at Burlington, in eastern Colorado (4160 feet), the stations being approximately 200 miles apart. The differences in mean annual precipitation (28, 23, and 17 inches at the three stations respectively) are clearly indicated by the type of native vegetation, viz., tall-grass prairies at Lincoln, mixed tall- and short-grasses at Phillipsburg, and short-grasses on the Great Plains of Colorado at Burlington. The cumulative effects of unfavorable growth conditions westward, lower temperatures of early spring in part, but chiefly a deficiency of soil and air moisture, are also expressed in decreased stature and yield of crops (Weaver, 1924). Owing to differences in altitude the growing season usually begins about 10 days earlier at Lincoln than at Phillipsburg and fully two weeks later at Burlington than at the Kansas station. The early portion of the growing season is quite mesophytic even on the Great Plains, where drought begins first. Summer drought occurs somewhat later at Phillipsburg, and usually it is still later and less severe at Lincoln.

At all the stations the crops were grown in thirtieth-acre plats under field conditions and under methods of tillage common to the region, each plat being surrounded at a little distance with other crops. Manchuria barley, Marquis spring wheat, and White Kherson oats were planted from the same lot of seed and at the same rate (72, 90, and 64 pounds per acre respectively) at all of the stations on March 24-30. Details of the physical and chemical composition of the soil need not be repeated here (*cf.* Weaver, et al., 1922). It is sufficient for our purpose to know that all of the soils were very fertile and that water-content and not nutrients or unbalanced soil conditions was the limiting factor in growth. The crops at Burlington, owing to a dry subsoil with a hard-pan (Weaver and Crist, 1922), were

rooted almost entirely in the surface two feet of soil. Those at the other stations were deeper-seated, much absorption occurring in the third and fourth foot of soil.

Methods of Selecting and Preparing Materials

Plant materials for detailed study were selected at two periods in the development of the crop in 1921. The first collections were made during the third week in May when the plants each had four or five leaves, and the second during the last ten days of June when they had headed but while the upper leaves were still green. In 1923 all the collecting of materials was done when the crops were in this latter stage of development. After careful examination and measurements had been made to determine the number of leaves, tillers, height, etc., twelve plants of average size, each with two tillers, were finally selected and cut off at the ground line at each station. These were put into dry flasks of known weight which were closed with air-tight stoppers. The flasks were again weighed on a portable balance sensitive to one-hundredth of a gram, and the wet weight determined. Records of the humidity, air and soil temperature, and water-content of the soil were secured at this time, in addition to the usual continuous factor records for the season. The plants were then removed from the containers and blue prints made of the flattened leaves, from which leaf areas were ascertained by means of a planimeter. The average lengths and diameters of the stems were also determined. Finally, the dry weights of stems and leaves were ascertained after they had undergone a sufficient period of desiccation at 100° C. to reach a constant weight.

For the study of ecological anatomy, twelve leaves of average length and width and the third or fourth in the sequence of development were selected from each plat at each station, at the first examination. At the later period, as during 1923, only the upper leaves were used. The first

2.5 cm. of leaf above the ligule were discarded, the next centimeter was cut into appropriate sections for killing and fixing, while the two centimeters above were preserved for stomatal counts and measurements. Careful selection of leaves for comparison as regards the distance from the base of the stalk (i. e. numerical sequence in development) and the part of the leaf used for sectioning and stomatal counts, etc., is very important. Kolkunov (1905) has shown that in size of cells the leaves of cereals are not uniform throughout, those at the base of the leaf being smaller than those in the upper third. Sorauer (1878:19) in comparing the structure of cereal leaves in the sequence of their development on the stalk, showed that the number of stomata per unit surface increased and the size of the stomata decreased, the greater the distance of the leaf from the soil surface. Similarly, the number of bundles per unit area and the number of epidermal cells increased while the epidermal cells decreased in size and the mesophyll cells in diameter.

The leaf sections were killed either in chromo-acetic acid or in a saturated solution of picric acid in 70 per cent alcohol. They were imbedded in paraffin and cut into sections 20 microns thick. The cutting was done in such a manner that entire cross-sections of the leaves were secured as well as longisections. After trying several stains, the most satisfactory was found to be carbol-fuchsin or safranin counterstained with Delafield's haematoxylin. The epidermis was stripped from both sides of the leaves and preserved separately in 95 per cent alcohol.

Results of First Examination, May 18-24

Leaf material at Lincoln was collected at 2:30 P. M., about two hours after a light shower. The temperature was 74°F. The wind had dried the plants fairly well but the sky was still overcast with clouds and the humidity was 78 per cent. The soil had a good water-content. At Phil-

lipsburg the material was gathered at 3:00 P. M., following a shower, but after an hour of bright sunshine and strong wind. The temperature was 80°F., the humidity 76 per cent, and the surface foot of soil had 12 per cent available moisture. The first leaves were dead and decaying, so these were discarded. The second leaves were dead in many cases, due to dry weather, but they were kept for their dry weight. Due to dry, windy weather, the dead tips on many of the leaves gave a brownish color to both the wheat and oats plats. At Burlington the material was collected on a warm day (85°F.) while a strong dry wind was blowing and the humidity was only 40 per cent. The leaves were twisted and somewhat shriveled, although the soil had a fair water-content to a depth of two feet.

Data on the measurements of 12 plants from each plat at each station are shown in table 1. As the height and diameter decreased westward, the leaf area also showed a marked decrease. The leaves at Phillipsburg (except those of oats) had only slightly more than half as much area as those at Lincoln, while that of the Burlington cereals was only about one-fourth as great. The number of stomata increased westward with the decrease of leaf area. There was a direct relation between the size of the leaf and the number of stomata per unit area. It is of interest to note that single plants taken from each of the three stations showed difference quite as constant and marked as the average of the twelve. Barley, at all the stations, made the greatest growth (with two exceptions) whether measured in height, leaf area, or dry weight. But as with the other cereals, both wet and dry weights decreased rapidly westward. Plants grown at Lincoln weighed three to nine times as much as those grown at Burlington.

Microscopic examination showed that the leaves of oats, wheat, and barley are typical grass leaves, although showing less marked xerophytic structures than native species. The stomata are in rows at the ends of the long, narrow,

TABLE 1
Data on size, weight, and structure of cereals on May 18-24, 1921.

Crop and station	Average height	Average diameter of stalk	Average leaf area (one side)	Average No. of stomata per sq. mm.	Total wet weight	Total dry weight	Water-content based on dry weight
	Cm.	*Mm.*	*Sq. cm.*		*Grams*	*Grams*	*Per cent*
Barley							
Lincoln	45.7	5.0	250.1	27.1	67.9	15.2	346.7
Phillipsburg	25.5	3.7	127.1	38.8	33.5	5.1	556.8
Burlington	9.0	2.7	53.6	43.6	7.2	1.6	350.0
Wheat							
Lincoln	45.7	3.0	133.5	23.8	31.1	3.7	740.5
Phillipsburg	20.3	2.8	73.1	49.7	15.7	2.7	481.5
Burlington	7.6	2.3	31.6	49.3	5.4	1.1	390.9
Oats							
Lincoln	43.2	4.2	138.1	26.2	36.1	4.2	759.5
Phillipsburg	7.8	4.1	131.7	38.0	26.4	3.4	676.5
Burlington	7.6	2.0	38.1	38.5	6.8	1.1	518.1

epidermal cells. They are always found between the veins directly above or below the bulk of the chlorenchyma tissue of the leaf. In the case of the upper epidermis, they do not occur in connection with the bulliform cells, but are found immediately on either side of them. This point is well illustrated in barley (fig. 3 c.). The large bulliform cells which comprise part of the upper epidermis are the structures which cause the rolling of the leaves when transpiration is excessive, being very characteristic of grasses. The cell walls of the midribs are heavily lignified. The other veins are of two sizes. Usually there is one large vein alternating with three smaller ones. The small veins are typically separated from the epidermis by parenchyma cells, while the larger ones are connected with the epidermis by lignified cells. The bundles are surrounded by fibrous tissue which becomes thicker-walled as the leaf develops xerophytic characters. The bundle is surrounded by a layer of large elongated cells which appear to contain little except water and a few scattered chloroplasts. These cells are thin-walled and are in contact with a large number of the chlorenchyma cells. The chlorenchyma is of the sponge type and even in the more compact regions there is some intercellular space.

Drawings and measurements were made in the same region of all of the sections. The cross-section drawings were made from typical leaves and the region shown is half way between the midrib and the margin of the blade. The longisections were selected from a plane passing through the leaf at the margin of the bulliform cells. By cutting in this way it was possible to show the stomata and longisections of some of the bulliform cells in the same drawing. There was an advantage in this for the chlorenchyma between the stomata was loose, while that below the bulliform cells was compact, if any compact tissue occurred. All measurements were taken from cross-sections in the region from which the drawings were made.

At this examination the morphological variations were not found to be great. The epidermis was not cutinized and the chlorenchyma showed no marked difference at the several stations. It was evident, however, that the leaves from the western stations were in a younger stage of development than the ones from Lincoln. Those from Burlington had the veins poorly developed. Leaf sections of the Lincoln cereals showed well-developed veins which were beginning to become lignified and the wheat leaves showed a slight cutinization. These variations were evidently due to differences in the degree of development. Externally, however, the leaves showed marked differences. All of the leaves varied in thickness, being thicker at the veins and thinner in the chlorenchyma region between them.

Although externally the leaves varied markedly in size, area, and number of stomata per unit area, internally little differentiation in response to environment had occurred. The absence of the lignification, cutinization, and the lack of striking differentiation of the individual tissues in general, so pronounced later, showed that the plants had only begun to respond anatomically to the environmental conditions, which up to this time, aside from temperature, had been quite favorable at all the stations.

The Environment

At the time of planting the soil was in good tilth at all the stations. An examination of table 2 shows that while the water-content decreased rather uniformly westward, up to May 21 no deficiency occurred even at Burlington, at least in the first two feet of soil. The total precipitation since the time of planting had been 7 inches at Lincoln, 4.2 inches at Phillipsburg, and 4.6 inches at Burlington. However, runoff was much greater at the western stations and evaporation somewhat higher.[1]

[1] A detailed record and comparison of all the climatic factors at the three stations from 1920 to 1923 inclusive may be found in "Experimental Vegetation" (Clements and Weaver, 1924) where it may be seen that 1921 was not an exceptional year in any way.

TABLE 2

Water-content in excess of the hygroscopic coefficient, 1921.

Depth in feet	April 28 to 30			May 19 to 21			June 9 to 10			June 22		June 30
	Lincoln	Phillipsburg	Burlington	Lincoln	Phillipsburg	Burlington	Lincoln	Phillipsburg	Burlington	Lincoln	Phillipsburg	Burlington
0 to 0.5	15.6	13.9	11.2	11.9	13.1	6.6	17.5	20.7	2.7	7.6	1.0	—2.6
0.5 to 1	16.5	12.3	9.7	15.7	12.2	7.4	16.2	14.9	1.9	9.4	5.1	—1.9
1 to 2	16.6	7.7	8.8	17.1	9.9	3.5	12.5	3.9	0.3	10.8	3.6	—2.1
2 to 3	15.9	2.7	2.4	14.9	5.4	—0.7[1]	12.9	4.3	—0.4	10.2	1.0	—1.0
3 to 4	14.0	3.6	0.9	14.7	5.2	0.2	13.9	5.2	—0.2	12.0	5.0	—1.3

[1]The fluctuations of soil moisture at depths below 2.5 feet slightly above or below the hygroscopic coefficient are due largely to variations in the texture of the soil which occur even at the short distances apart at which the samples were taken and not to actual changes in the water-content.

Owing to a late spring with severe freezes and snow during the first half of April, the crops developed rather slowly, particularly those at the stations with the higher altitudes. For example, by April 30, wheat was 7, 4, and 2 inches high at the several stations, respectively.

Temperature was the limiting factor to growth. At Lincoln the mean temperature for April was 53.9°F., at Phillipsburg, 54.2°F., and at Burlington, 50.4°F. The temperature at Lincoln fell below freezing on 6 days during April (latest April 17), at Phillipsburg on 7 days (latest April 26), and at Burlington on 13 days, including April 28. The crops at the Great Plains station had been damaged somewhat by frost. During the first three weeks of May the temperature was highest at Lincoln, intermediate at Phillipsburg, and lowest at Burlington (fig. 1). The low night temperatures (ave. 45°–56°F.) at the Great Plains station were not conducive to rapid growth. Soil temperatures were also much lower here.

At the time of examination the crops had reached the stage of development shown in table 3. The decrease in average height and number of leaves as well as number of tillers from east westward is very marked and consistent among all of the crops.

Marked environmental differences occurred at the several stations during the interval between the first examination (May 20) and the second, when the crops ripened (June 22-30). Rainfall at Lincoln (May 20 to June 10) was 4 inches, 5.7 inches at Phillipsburg, but only 1.7 inches at Burlington. Between June 10 and 22 when the plants were nearly ripe and materials were again collected, the rainfall at both Lincoln and Phillipsburg was very light, 0.4 inch or less. At Burlington 2.3 inches of rain fell on June 18-21, but by June 30 no moisture was available for growth at any level (table 2). An examination of table 2 shows well-watered soil at Lincoln (13 per cent or more on June 10) which contrasts strikingly with the drier subsoil at Phillips-

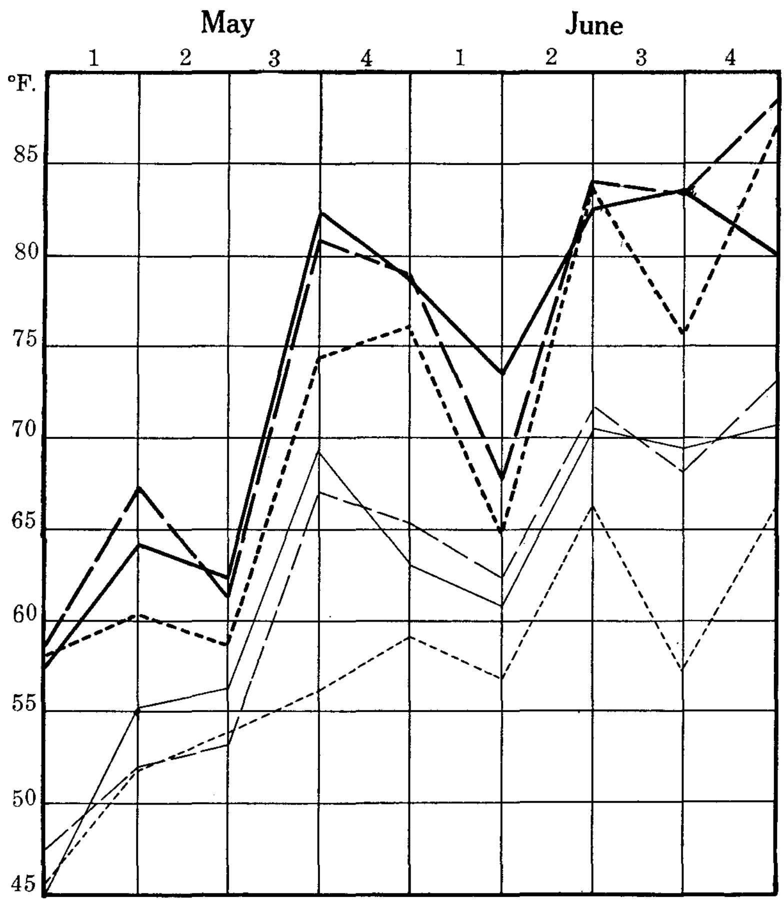

Figure 1. Average day air-temperatures by weeks (heavy lines) and average night temperatures (light lines) at Lincoln (solid lines), and Phillipsburg (long broken lines), and Burlington (short broken lines), 1921. Numbers indicate the first, second, etc., week of the month.

burg (5 per cent or less), while at Burlington scarcely 2 per cent was available in the first foot and none deeper. However, during the intervening period (May 28), conditions were slightly better, with 4 to 7 per cent available moisture in the surface foot at Burlington, while water relations at Phillipsburg were similar to those indicated in

the table. At no time at Lincoln was there a period of drought, well-distributed and ample rainfall keeping the soil moist. The light rainfall after June 10 is reflected in the low available water-content on June 22-30, where it may be seen to be very low at Phillipsburg and lacking entirely at Burlington.

Air temperatures at all the stations were very much higher than during the preceding interval, but the same general relations with the highest temperatures at Lincoln and the lowest at Burlington prevailed. An exception to this was the slightly warmer nights at Phillipsburg during a part of the period (fig. 1).

Continuous records of soil temperatures obtained at the several stations (Weaver, et. al., 1922:85) showed that differences were so small that they probably exerted little effect upon crop growth, especially after the seedling stage

TABLE 3

Crop development at the several stations May 18 to 21, 1921.

Crop	Station	Average height in inches	Average number of leaves	Average number of tillers	Remarks
Barley..	Lincoln.......	18.0	6	5	Thick, even growth.
	Phillipsburg...	10.0	5	4	Crop thinner than at Lincoln.
	Burlington....	3.5	4	2	Crop uniform but thin.
Oats....	Lincoln.......	17.0	6	5	Thick, even growth.
	Phillipsburg...	7.0	5	4	Crop thinner than at Lincoln.
	Burlington....	3.0	4	2	Crop uniform but thin.
Wheat..	Lincoln.......	18.0	5	6	Thick, even growth.
	Phillipsburg...	8.0	4	5	Crop uneven, thinner than at Lincoln.
	Burlington....	3.0	3	3	Crop uniform but very thin.

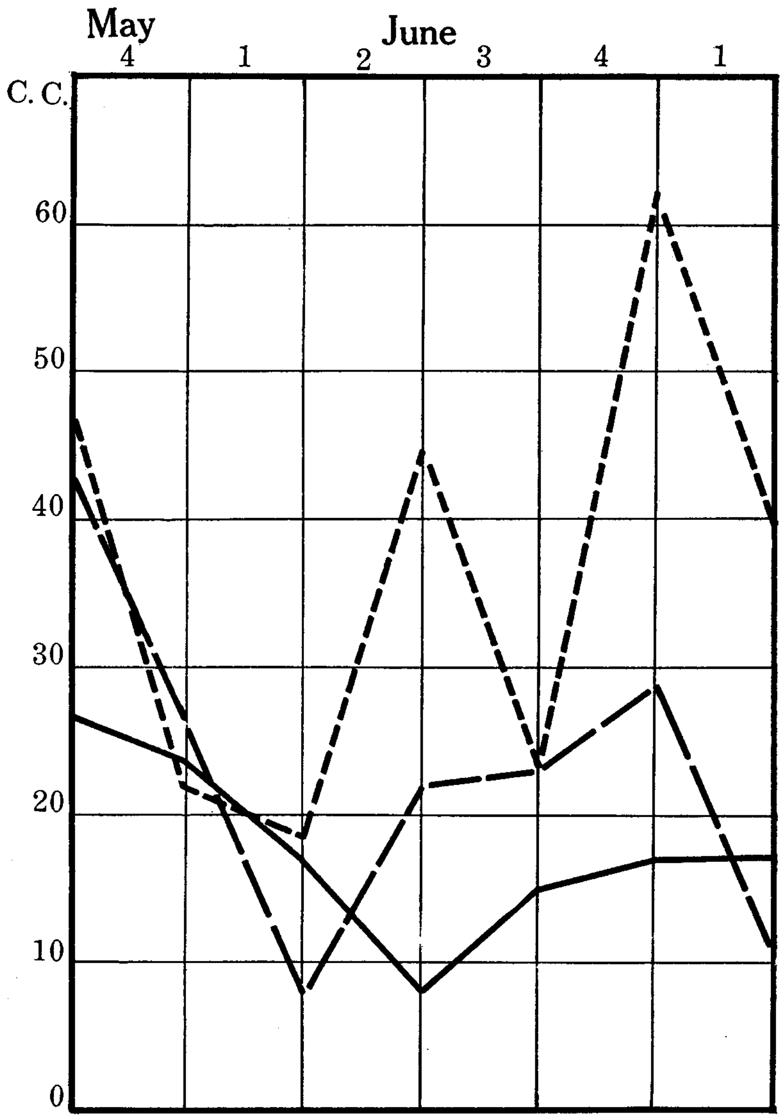

Figure 2. Average daily exaporation by weeks at Lincoln (solid line), Phillipsburg (long broken lines), and Burlington (short broken lines), 1921.

had been past. The evaporating power of the air as measured by standardized, non-absorbent, white, cylindrical atmometers was practically the same at all stations during the last week in May. But otherwise it was much higher at Burlington (Fig. 2). Transpiration at the western stations is increased also by a more constant air movement, the winds often blowing steadily for many days and sometimes reaching high velocities. The great range of temperature and humidity at the Great Plains station especially from day to night and the low night temperatures were not conducive to rapid growth.

Development and Leaf Structure of Cereals

The crops at Lincoln developed rapidly and by June 1 all were fairly well headed with an average height of three feet. They had been blown down badly. At Phillipsburg the crops headed about a week later than at Lincoln and were more irregular in their development. Owing to drought, the wheat, especially, was badly damaged, all but the upper two or three leaves being discolored or dead. At Burlington none of the cereals exceeded a foot in average height on June 10 and they did not begin heading until June 20. The effect of a low water supply was shown by the many dry and dead leaf tips, this being especially marked on the first four or five leaves. It is interesting to note that these shorter plants with less extensive foliage had almost as many leaves as the taller ones at the other stations.

When the crops were nearly ripe and the grain was in the dough stage, collections of leaves were again made. At Lincoln this was done on a warm humid day (June 22) when the temperature was 89°F., the humidity 71 per cent, and the water-content of the soil 8 to 12 per cent above the hygroscopic coefficient to a depth of 4 feet. The crop was badly lodged as a result of wind and heavy rain, so that in the wheat and oats plats only the two uppermost leaves were

used. But from the barley, some of which was standing, selections were made of the five uppermost leaves. At Phillipsburg collecting was done on June 23, a bright, calm day, at a temperature of 87°F. and a humidity of 43 per cent. At this time the soil moisture was very near the hygroscopic coefficient in the surface 6 inches and only 1 to 5 per cent above at any depth to which the roots penetrated (table 2). The crops were nearly ripe and beginning to dry. Only the three upper leaves of the oats were used. The wheat was badly rusted and the two upper leaves alone were collected. Barley alone had four good upper leaves. In every case all of the dried lower leaves were clinging to the plants, and these were saved for dry weight determinations. The crops at Burlington did not reach the dough stage until June 30. The stalks and heads were green but most of the leaves had been eaten by grasshoppers. At the time of collecting the temperature was 80°F., the humidity 35 per cent and practically no water was available for growth at any depth. The best plants only

TABLE 4
Relative development of crops, June 22-30, 1921.

Crop	Station	Average height in inches	Average number stalks per sq. m.	Average number stalks per plant	Average number of heads per sq. m.	Average length of heads or panicles in inches
Barley...	Lincoln.......	37	384	3.7	306	3.5[2]
	Phillipsburg...	34	253	2.2	201	3.3
	Burlington....	16	255[1]	1.6	197	2.0
Oats.....	Lincoln.......	39	375	3.3	283	10.5
	Phillipsburg...	34	353	2.9	269	9.0
	Burlington....	18	414[1]	2.5	171	5.0
Wheat...	Lincoln.......	39	648	2.8	365	4.0
	Phillipsburg...	31	475	1.8	211	3.5
	Burlington....	19	419[1]	1.6	277	2.5

[1]Many stalks were only 2 to 4 inches tall and had been dead for some time.
[2]Average length of heads without awns.

were selected for study, and of those chosen the oats had two usable leaves, the wheat three, but the barley only one. In case of barley it was necessary to use the upper leaf for sectioning and stomatal counts. In all other cases the specimens were taken from the leaf second from the top.

At the time of collecting, several representative meter-quadrats of each plat were studied and the data obtained recorded in table 4. There was a striking decrease in height of the crops from the more humid to the more arid stations. The same general relation held for the average number of stalks per square meter, except at Burlington where many tiny stalks, only 2 to 4 inches high, started growth early and soon dried out. The average number of stalks per plant was in direct relation to the water-content of the soil and other factors favorable to plant growth. The average number of heads per square meter and the average length of heads or panicles decreased from Lincoln to Phillipsburg and Burlington, respectively. An exception to this occurred in the case of the number of heads of wheat at Burlington, when compared with Phillipsburg, while the difference in this respect in the case of barley was small.

A comparison of the wet and dry weights of the twelve plants selected at each station (table 5) showed that the per cent of water, based on the dry weight was largest at Lincoln, intermediate at Phillipsburg, and lowest at Burlington, the ratio for oats, for example, being 100:75:72. The total dry weight decreased rapidly to the westward. Wheat, which was quite representative for the whole lot, had in the above sequence of stations a dry weight ratio of 100:52:41. The stem length and diameter, and the length of head or panicle showed the same correlations with decrease of moisture westward. The leaf area was found to be surprisingly small. In most cases it was smaller than at the earlier period (May 18 to 21) when the plants were not fully grown. This was due largely to the fact that the older leaves had died. However, the leaf area was reduced

TABLE 5

Data on size, weight, and anatomical structure of cereals on June 22-30, 1921.

Crop and station	Average height	Average diameter of stalk	Average length of head or panicle	Total wet weight	Total dry weight	Water-content based on dry weight	Area of individual leaf (one side)	Average leaf area of plant (one side)	Average number of stomata per sq. mm.	Average thickness upper outer epidermal wall	Average thickness lower outer epidermal wall	Average total thickness of leaf	Average diameter of tracheary tubes
	Cm.	*Mm.*	*Cm.*	*Grams*	*Grams*	*Per cent*	*Sq. cm.*	*Sq. cm.*		*Microns*	*Microns*	*Microns*	*Microns*
Barley													
Lincoln............	94.0	5.8	8.6	170.0	42.4	301.3	70.2	351.0	53.7	0.8	0.9	191.5	32.4
Phillipsburg.........	86.4	5.1	8.0	106.1	37.7	181.6	38.6	114.4	88.4	2.1	3.1	163.1	29.5
Burlington..........	40.7	4.3	5.0	41.3	17.1	141.5	7.1	7.1	74.2	3.4	4.9	147.9	26.3
Wheat													
Lincoln............	99.1	4.8	10.0	97.7	28.2	253.5	49.0	98.0	49.8	1.1	2.3	194.4	34.9
Phillipsburg.........	78.7	3.6	8.0	42.6	14.8	187.5	19.6	39.4	60.5	1.9	3.6	187.6	31.7
Burlington..........	48.3	3.1	5.0	32.9	11.6	183.6	10.3	21.3	79.2	3.8	5.4	176.4	20.9
Oats													
Lincoln............	99.1	5.1	25.5	184.9	49.1	276.5	45.2	135.5	37.9	1.1	1.7	198.0	38.5
Phillipsburg.........	86.4	4.5	21.5	101.4	32.9	207.5	26.9	80.8	47.7	1.1	1.8	194.4	36.4
Burlington..........	45.7	3.6	12.5	42.2	14.2	197.2	12.9	25.8	55.2	1.8	2.5	165.6	33.5

still more at Lincoln by lodging, at Phillipsburg by rust, and at Burlington by grasshoppers. These were not abnormal conditions, but recur to a greater or lesser extent every year. The number of stomata per unit area was in all cases much higher than at the time of the first examination but the general sequence was the same, that is, they increased markedly in numbers westward. An exception occurred in the case of barley which had fewer stomata at Burlington than at Phillipsburg. A comparison of these data showed that the number of stomata per unit area was determined by the size the leaf attained.

The cross-sections of barley leaves from the three stations showed differences in xerophytic characters that correlated well with the differences in the several habitats. The leaves from Lincoln varied greatly in thickness averaging 191.5 microns (fig. 3a). They had the greatest thickness at the veins and were thinnest in the chlorenchyma regions between the veins. The outer epidermal walls were thinly cutinized. There was lignification in the fibrous layer around the bundle and the fibrous tissue between the veins and the epidermis showed a thickening of the walls. The outer wall of the lower epidermis was 0.9 micron in thickness, while the upper wall was 0.8 micron. The chlorenchyma was loose and had large intercellular spaces.

The Phillipsburg barley (fig. 3b) had a leaf more uniform in thickness (average 163.1 microns). The outer walls of the lower epidermis varied from 2.0 to 4.5 microns, with an average of 3.1 microns. The upper epidermis showed similar variations with an average of 2.1 microns. The chlorenchyma was more compact than at Lincoln. The fibrous tissue around the bundle was decidedly more lignified. More cells within the bundle and between the bundle and epidermis showed lignification. The cells comprising the fibrous sheath around the bundle were more heavily lignified on the side next to the vascular tissues than on the side adjacent to the chlorenchyma. This condition was

conspicuous in all leaves where the cells were becoming lignified.

The leaves from Burlington (fig. 3c) showed the least variation in thickness averaging 147.9 microns. The epidermal cell walls were heavy and well cutinized. The outer walls of the lower epidermal cells averaged 4.9 microns in thickness, while the corresponding measurements for the upper epidermis averaged 3.4 microns. The chlorenchyma was much more compact than at the other stations, and there was a relatively larger amount of fibrous tissue within and around the bundle. The fibrous cells of the sheath around the bundle were completely lignified. The lignification of the cell walls next to the bundle, so conspicuous in the sections from Phillipsburg, was here extended around the entire cell making the lignification more complete.

A study of the longisections is essential to the understanding of the leaf. It was apparent from the cross sections that the chlorenchyma was of a spongy nature but the degree of sponginess could only be appreciated by an examination of the longisections. The upper epidermis is composed of two kinds of cells; the one, thin, heavily cutinized, typical epidermal cells among which the stomata are found, and the other large bulliform cells which make up the rolling device of the leaf. These sections showed the chlorenchyma to be actually more spongy in nature than the cross-sections indicated (fig. 4). In the barley from Lincoln all of the cells were separated by large intercellular spaces. Sections of Phillipsburg barley showed spongy tissue between the stomata of the upper and lower leaf surfaces but more compact tissue in other parts of the leaf. There were relatively very few intercellular spaces of any considerable size in the leaf sections of the barley from Burlington.

Cross-sections of the wheat leaves in general showed the same variations as those of barley. The average thickness at the three stations, proceeding from east to west, was

194, 187, and 176 microns, respectively (fig. 5). However, the wheat leaves were more heavily lignified and cutinized at the eastern stations, than were those of barley. The outer walls of the lower epidermis were 2.3, 3.6, and 5.4 microns thick at Lincoln, Phillipsburg, and Burlington, respectively, while the measurements of the outer upper epidermal walls were 1.1, 1.9, and 3.8 microns. The fibrous tissue of the bundles was large-celled and not heavily lignified at Lincoln. But the fact that lignification and cutinization were greater than in the case of barley, coupled with the fact that it began to show earlier, indicated that wheat was able to adapt itself structurally to xerophytic conditions more readily than barley. Amount of lignification of the fibrous layer around the bundle, and the fibrous tissue between the veins increased from the more humid to the less humid stations in about the same manner as in barley.

The longisections of the wheat presented an altogether different aspect than those of barley. The cells of the chlorenchyma were much more lobed and elongated. The chlorenchyma in the Lincoln wheat was composed of three, with occasionally four or five tiers of cells, in the thicker parts of the leaf (fig. 6a). The cells were long and lobed in a palisade-like manner with considerable intercellular space. The leaf sections from Phillipsburg and Burlington had typically four or five tiers of chlorenchyma and occasionally as many as six or seven tiers in the thicker parts of the leaf. The leaves of Lincoln wheat, with the three tiers of chlorenchyma, were the thinnest. The Phillipsburg and Burlington leaf sections, each with the same number of tiers of chlorenchyma, were more compact. However, the Phillipsburg leaves were thicker than those from Burlington. The fact that both had more tiers of chlorenchyma than the Lincoln leaves indicated more xerophytic conditions, while the fact that the Phillipsburg leaves showed a greater thickness indicated better growing conditions than at the Burlington station.

Two types of response were shown in barley and wheat. Barley responded by an adjustment of the intercellular spaces and wheat more largely by the thickening of the chlorenchyma. As already indicated, there were also many similar responses such as modifications of the epidermis and of the leaf area.

Sections of oat leaves were measured in the same manner as the other cereals. They varied in average thickness from 198 microns at Lincoln, to 194 at Phillipsburg and 166 at Burlington. Differences in thickness of epidermal walls and diameter of tracheary tubes are shown in table 5. These leaves showed less lignification and cutinization at all stations. The spongy tissue did not show much differentiation. The veins were somewhat smaller and closer together in the Burlington leaves, but the tracheary tubes did not vary greatly in size. The size of the leaf and the number of stomata per unit area bore the same relation to habitat factors as was shown by the other cereals. This indicated that the oat plant responded less readily than wheat and barley to habitat changes. Longisections showed that the leaf structure was similar to that of wheat. Both had elongated, much lobed chlorenchyma cells.

A comparison of the leaves indicates that wheat responded most readily and oats least readily in the way of anatomical adjustments. The size of the leaf and the number of veins are worthy of notice. Although the leaves were many times larger at Lincoln than at Burlington, there were approximately the same number of veins per leaf. The tracheary tubes were somewhat smaller at Burlington but the veins were so much closer together that they more than offset this difference.

It is evident that the results of a study of the mature plants are of much greater value than those obtained earlier in their development. At maturity the plant had completed its response to environmental conditions while during its earlier growth this had just begun. The difference in the

mature plants correlated closely with the marked changes in environment, which were chiefly differences in water relations.

Studies During 1923

Environmental conditions at any station vary from year to year. In order to ascertain anatomical changes due to these differences in local environment as well as to study again the wider differences occuring at the several stations, the experiment was repeated in 1923. Methods of seed bed preparation, rate of planting, etc., were the same as during 1921. Since it had been determined that the plant completes its response to environment only when nearing maturity, materials for study were not collected until the crops were beginning to ripen.

Conditions for plant growth were somewhat less favorable at Lincoln than during 1921, but the soils at the western stations were unusually well supplied with mois-

Table 6

Relative development of crops, 1923.

Crop	Station	Date of collecting materials	Height of plants in inches	Stage of development
Barley....	Lincoln........	June 14	43	Well headed, not quite in bloom.
	Phillipsburg....	June 22	37	Do.
	Burlington.....	July 5	26	Kernel in dough.
Oats......	Lincoln........	June 14	40	Well headed, not yet in bloom.
	Phillipsburg....	June 22	32	Do.
	Burlington.....	July 5	18	Kernel in dough.
Wheat....	Lincoln........	June 14	37	Well headed, starting to bloom.
	Phillipsburg....	June 22	29	Do.
	Burlington.....	July 5	24	Kernel in dough.

ture. Not only was the rainfall of April, May, and June several inches above normal (7 inches above at Phillipsburg) but the rains were well distributed and drought periods westward were fewer and of shorter duration than usual. At Lincoln there was a deficit of one-half inch in April and an inch in May, but June had an excess of one inch.

In response to these conditions growth was more luxuriant at the western stations and somewhat less so at Lincoln than in 1921. However, the progressively later growing season at the higher altitudes, the low night temperatures and colder soil, with low afternoon humidities retarded growth. Here the cereals, forced into early maturity by the June drought, although unusually luxuriant for these semi-arid stations were decidedly smaller and more xerophytic in nature than those at Lincoln. Table 6 shows the relative heights and stages of development when materials were collected for study as the crops were approaching maturity. The average height of the wheat, 37, 29, and 24 inches, from east to west, is representative of the sequence for the other cereals.

Materials for study were collected at Lincoln on June 14, at Phillipsburg on June 22, and on July 5 at Burlington. On these dates the plants were all fully grown or quite mature, as indicated in table 6. The second leaf from the top was used in all cases. Entire sections were cut across the middle of the leaf. Epidermis for stomatal counts was stripped from the leaf in approximately the same regions and preserved in absolute alcohol. In addition to paraffin, celloidin was also used as an imbedding material, the latter yielding the best sections.

Drawings of typical unit areas of portions of the lower epidermis of oats and barley from the several stations are shown in fig. 7. While the number of stomata per unit area varied considerably at any station, the average number of stomata per sq. mm. was found in all cases for

TABLE 7

Comparative structure of mature leaves, 1923.

Crop and station	Average number of stomata per sq. mm.	Average length of guard cells	Thickness of upper epidermal cell wall and cuticle	Thickness of lower cell wall and cuticle	Width of epidermal cells	Number of veins	Number of epidermal cells between veins	Average thickness of the leaf
		Microns	*Microns*	*Microns*	*Microns*			*Microns*
Barley								
Lincoln	64.6 (53.7)[1]	66.5	2.4 (0.8)	2.9 (0.9)	35.4	23.3	17.6	193.0 (191.5)
Phillipsburg	84.1 (88.4)	57.4	2.6 (2.1)	3.2 (3.1)	35.1	21.6	16.7	185.0 (163.1)
Burlington	91.2 (74.2)	50.9	3.1 (3.4)	3.9 (4.9)	32.6	19.8	15.8	182.0 (148.0)
Wheat								
Lincoln	34.1 (49.8)	83.9	1.8 (1.1)	2.1 (2.3)	38.7	27.3	14.8	192.0 (194.4)
Phillipsburg	36.0 (60.5)	77.1	2.5 (1.9)	2.7 (3.6)	37.7	24.4	14.8	186.0 (187.6)
Burlington	49.8 (79.2)	69.5	2.9 (3.8)	3.6 (5.4)	34.1	19.8	14.7	184.0 (176.4)
Oats								
Lincoln	28.5 (37.9)	84.7	1.7 (1.1)	1.9 (1.7)	39.3	28.1	16.6	194.0 (198.0)
Phillipsburg	41.0 (47.7)	82.8	1.9 (1.1)	2.3 (1.8)	36.2	26.1	16.8	189.0 (194.4)
Burlington	43.3 (55.2)	72.6	2.2 (1.8)	2.9 (2.5)	33.7	20.4	17.1	185.0 (165.6)

[1]Numbers in parenthesis are for the year 1921.

all three cereals to be lowest at Lincoln and highest at Burlington (table 7). Moreover, the average was lower than in the drier year in all cases excepting two. Both of these points bear out the theory that the number of stomata per unit area is influenced by the amount of expansion which takes place in the leaves. The leaves of the Lincoln cereals were more expanded than those at Burlington, and the leaves of the cereals grown in the drier year of 1921 were less expanded than those grown during the more moist season of 1923.

Study of cross-sections of barley leaves from the three stations showed that those from Lincoln exhibited more xerophytic characters than in 1921. The chlorenchyma was somewhat more compact and the fibrous tissue about the veins heavier-walled than was previously found. The average thickness of the outer epidermal wall and cuticle was found to be greater than when the previous examination was made, i. e., 2.4 and 2.9 microns for upper and lower epidermis, respectively, as compared with 0.8 and 0.9 microns in 1921.

In contrast to this the Phillipsburg and Burlington leaf sections were in most respects less xerophytic histologically than during the previous year, although distinctly more xerophytic than those at the Lincoln station of the current year. The leaves were slightly thinner and more compact in cross-section than at Lincoln and they had a decidedly xerophytic appearance, although this was not so extreme as in 1921. The chlorenchyma was more compact than at the Lincoln station, the fibrous tissue around the veins was thicker-walled and the thickness of the epidermal wall and cuticle increased to the westward, but the change in this respect was less marked than in 1921 (table 7). A comparison of the longisections of barley leaves for the three stations showed the leaves from the Lincoln station to have a larger amount of spongy chlorenchyma than those from the Phillipsburg and Burlington stations. None of the leaves from the Lincoln station showed the large amount of

intercellular space characteristic of the leaves in 1921, and none of the leaves from the Burlington station were so compact as in 1921.

In both cross- and longisections, wheat and oats showed the same variations in general that are recorded for the barley leaves (table 7). Wheat, as in 1921, in most cases at least, showed more response to habitat differences than oats. Thus a very clear correlation between crop and climate was again evident.

Summarizing, leaves of all the cereals, from the Lincoln, Phillipsburg, and Burlington stations were slightly thinner and the chlorenchyma more compact as one proceeded westward. Moreover, the heavy-walled fibrous tissue about the bundle was more conspicuous and the epidermal cell walls more decidedly thickened. The length of the guard cells, the width of the epidermal cells, and the amount of intercellular space decreased to the west, responses being in accord with the fact that conditions at the western stations were progressively more xerophytic. Compared with 1921, leaves from Lincoln were found in 1923 to have more compact chlorenchyma, to be more uniform in thickness, to have heavier-walled fibrous tissue about the bundle, and also a heavier epidermis and cuticle. Leaves of the cereals from the western stations were found in 1923 to be less xerophytic in the same respect than in 1921.

As regards the anatomical cause of the variation in the size of the leaves at the different stations, it was found that the average width of the epidermal cells decreased as the habitat became drier as also did the number of veins per leaf. The number of epidermal cells between any two veins was found to be nearly the same for all stations. Table 7 shows that the smaller leaves had on an average 4 to 8 veins less than the larger ones. These leaves were often 100 cells or more narrower than the leaves from the Lincoln station. Moreover, each cell of the leaf was smaller, therefore, the size of the leaf was due in part to the number of cells and in part to the size of the individual cells.

Discussion

These results in general are quite in accord with similar variations in size, area, and leaf anatomy of native species grown under different environments ranging from mesophytic to xerophytic (Clements, 1905; Clements and Weaver, 1924; and many others).

The findings reported by Duggar (1916: 92) as regards number of stomata on corn and wheat leaves are quite the reverse of those just discussed. More stomata were found per unit area of the leaves of these plants where the water-content was high, other conditions being the same, than where it was low. Wheat grown in soil with a low water-content (11 per cent) had only 57 per cent as many stomata as that grown in soil with a high water-content (38 per cent). However, these counts were made on plants only 17 days old and apparently grown under green house conditions.

Sorauer (1878:19) found that the stomata of barley plants grown in moist air were larger than those grown in drier air and that the same relation held for soil moisture.

Harter (1908) made a study of wheat, oats, and barley grown in soils containing various amounts of soluble salts, principally sodium chloride. He found that the cereals grown in soils containing salts had, along with other variations, smaller epidermal cells and a thicker cuticle than those grown in a non-saline soil. It is evident that the physiological drought caused by the presence of soluble salts produced essentially the same changes as were found in the leaves of cereals grown in habitats physically dry.

Kiesselbach and Keim (1921:56) in their extensive work with corn grown in various environments ranging from eastern to western Nebraska and under conditions very similar to those of our experiments, obtained results which are in essential agreement with those from the smaller cereals. "The immediate result of growing corn under more adverse climatic conditions, namely, shortage of heat

units and moisture, is to reduce the vegetative development in all its phases. Stalks become shorter, and bear the ear closer to the ground; leaf development and production of plant substances are markedly reduced. The general effect is to reduce the opportunity for transpiration and photosynthesis. Associated with this reduction in vegetative growth is found a reduction in the size of the vegetative unit—the cell. Accordingly, under more adverse conditions the leaves are thinner, as are also the epidermis and the cuticle. The stomata are more numerous in a given area of leaf surface. The stomata as well as the stomatal apertures are somewhat reduced in size. Furthermore, the cells being smaller, more vascular bundles occur in a unit of cross-section."

Although the anatomical responses in corn to increased xerophytism are similar to those of the cereals, they are much less pronounced, no important histological leaf characters exhibiting more than 15 per cent deviation. This suggests one explanation why the smaller cereals from the same lot of seed can be successfully grown over a wide range of climatic conditions without a few years of acclimatization, while corn similarly grown is almost sure to be a failure especially in a less favorable habitat.

Kolkunov (1905) reached the conclusion, after the study of a great variety of cereals in Russia, including barley, wheat, oats, corn, and millet, that lowering of the transpiration rate in cultivated cereals is obtained mainly by a lowering of the anatomical coefficients which are most conveniently measured by the decrease in the stomatal lengths of cereals. Undoubtedly however, physiological changes such as higher concentration of cell-sap, greater colloidal content, etc. (MacDougal and Spoehr, 1918) are responses quite as pronounced and perhaps even more effective than anatomical change. J. Arthur Harris (unpublished data) has found not only an increasing density of cell sap as cereals grown on unirrigated land in the arid west ad-

vanced in maturity but also increased density of cell sap in cereals grown on dry land as compared with irrigated crops under the same climatic conditions. However, this was a phase of the work into which, because of the pressure of other field studies, we were unable to enter.

SUMMARY

Manchuria barley, Marquis spring wheat, and White Kherson oats were grown at Lincoln in eastern Nebraska, at Phillipsburg in north central Kansas, and at Burlington in eastern Colorado. These stations afforded a wide range of environmental conditions, chiefly differences in soil and air moisture, becoming progressively more xerophytic westward. Each crop was grown from the same lot of seed, planted at about the same time in a well prepared seed bed and in fertile soil of measured physical and chemical composition. Complete records of the environmental factors affecting plant growth were obtained,

The Lincoln station was characterized by having an abundance of moisture throughout the growing season. At Phillipsburg the latter part of the growing season was quite dry, while at Burlington the rich moist soil promoted a luxuriant growth during early spring, but the extremely dry summer caused the death of a large number of the tillers and resulted in a small vegetative growth. Here low soil and air temperatures delayed growth earlier in the season while later it was further retarded by drought.

Examination of the half-grown crops showed a lack of histological characters common to xerophytes. Cutinization of the epidermis and lignification of the bundle sheath were practically absent. Up to this time (May 20) moisture relations of both soil and air had been quite favorable for growth at all the stations. Temperature, however, had been more fa rable at the lower altitudes eastward and this correlated with the greater leaf area and dry weight of plants going from west to east. The number of stomata per

unit area was least on the larger fully expanded leaves at Lincoln.

At maturity, the water-content, as well as the dry weight of the crops, was highest at Lincoln and lowest at Burlington. A marked change in the structure of the leaves was brought about with the drier conditions of the advancing season. Cutinization of the epidermis and lignification of the bundle sheath were much more pronounced at Burlington than at Phillipsburg, and least so at Lincoln. The intercellular space in the leaf decreased and the number of stomata per unit area increased progressively westward. The number of stomata was greater at all stations than earlier in the season.

The cereals exhibited a difference in the degree of response to the factors of the habitat. Wheat showed the greatest, barley somewhat less, and oats by far the least. A variation in the manner in which plants respond was shown in the case of the barley as compared with wheat and oats. In wheat the leaves became thicker as a result of the addition of more tiers of chlorenchyma cells, while in barley a great reduction of intercellular space and some reduction in the size of the cells resulted in the leaves being more compact in the drier habitats. Longisections showed the barley leaves to be very different from those of wheat or oats. The chlorenchyma in the former was made up of unlobed cells arranged in a palisade-like manner. In wheat and oats the chlorenchyma was composed of much-lobed cells. The lobes were all in a vertical plane and produced a palisade-like appearance.

The size of the cells decreased with increasing drought, sufficient water not being available to hydrate the cell colloids completely. Moreover, low water-content of cells, especially at Burlington, resulted in semi-wilting during the hottest part of the day and a decrease in photosynthesis. Thus vigorous growth, earlier delayed by unfavorable temperatures, was later impossible because of drought, both

factors contributing to produce plants of low stature. In mature plants the degree of lignification and cutinization at the several stations was in direct relation to decrease in water-content of soil and humidity.

The experiment was repeated during 1923, during which season water relations were relatively much more favorable for growth at Burlington and Phillipsburg but less favorable at Lincoln than during 1921. As during 1921, cereals grown under conditions of drought when compared with those under more favorable environment had shorter and smaller stalks, shorter heads or panicles, much smaller leaves, and a smaller percentage of water in the green plants. These differences were again marked in 1923, but the crops at the western stations were somewhat better developed and those at Lincoln slightly less so than in 1921.

Histological studies showed for plants at the progressively more xerophytic stations, more stomata per unit area, thicker epidermal cell walls, smaller epidermal cells, shorter guard cells, less intercellular space, more compact chlorenchyma, and fewer vascular bundles in the cross-section of the leaf, and also a smaller size of the leaf. The latter is due to the fact that the cells of the leaf are both fewer in number as well as smaller.

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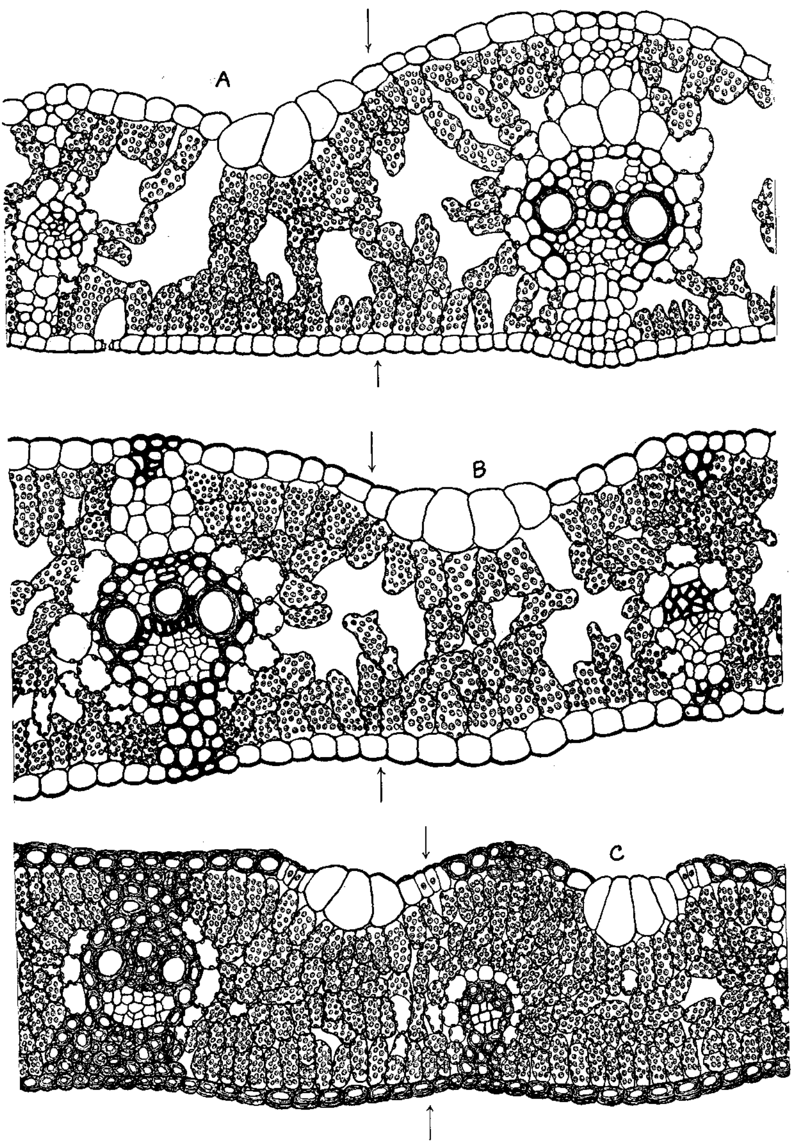

Figure 3. Cross-sections of barley leaves from (a) Lincoln, (b) Phillipsburg, and (c) Burlington.

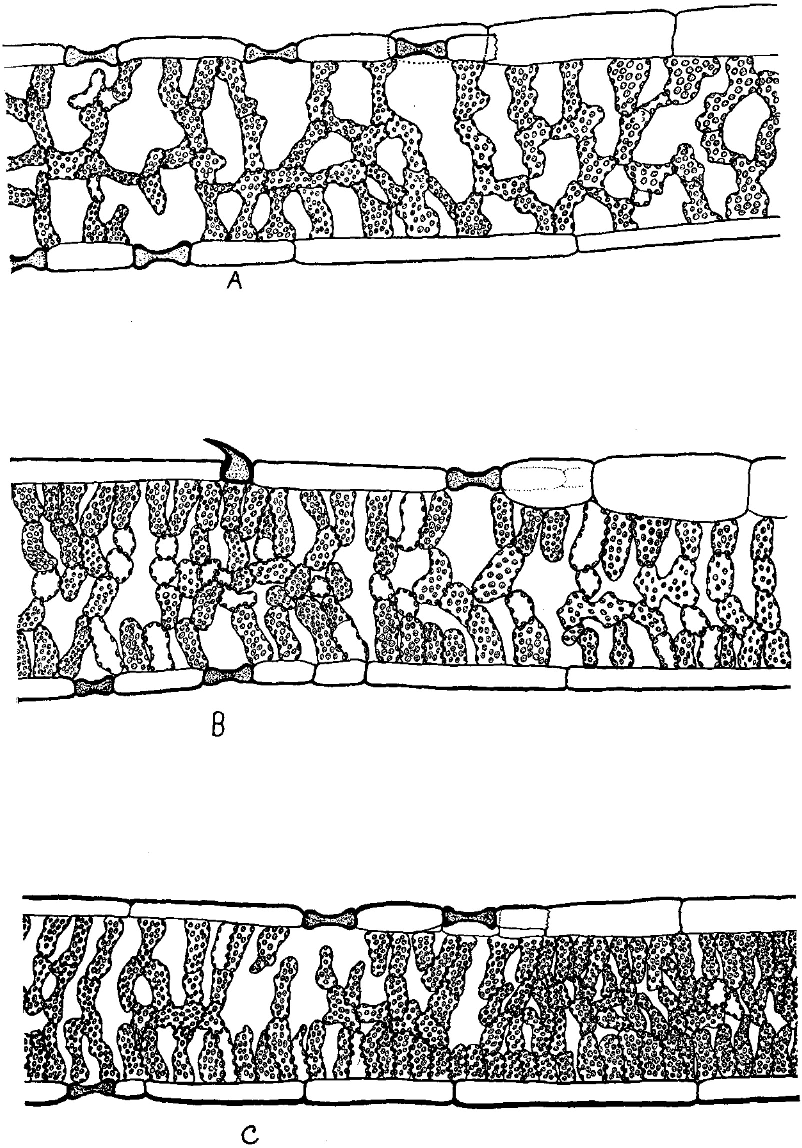

Figure 4. Longisections of barley leaves from (a) Lincoln, (b) Phillipsburg, and (c) Burlington. Cut along lines indicated by arrows in figure 3.

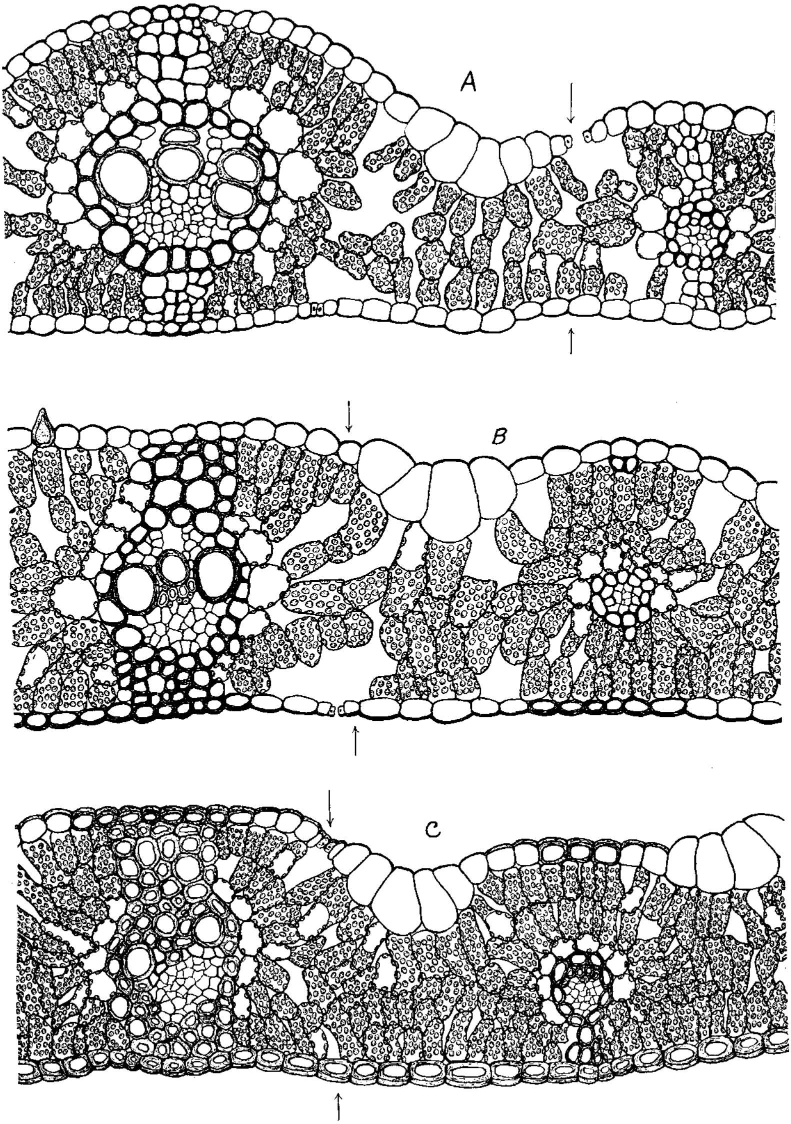

Figure 5. Cross-sections of wheat leaves from (a) Lincoln, (b) Phillipsburg, and (c) Burlington.

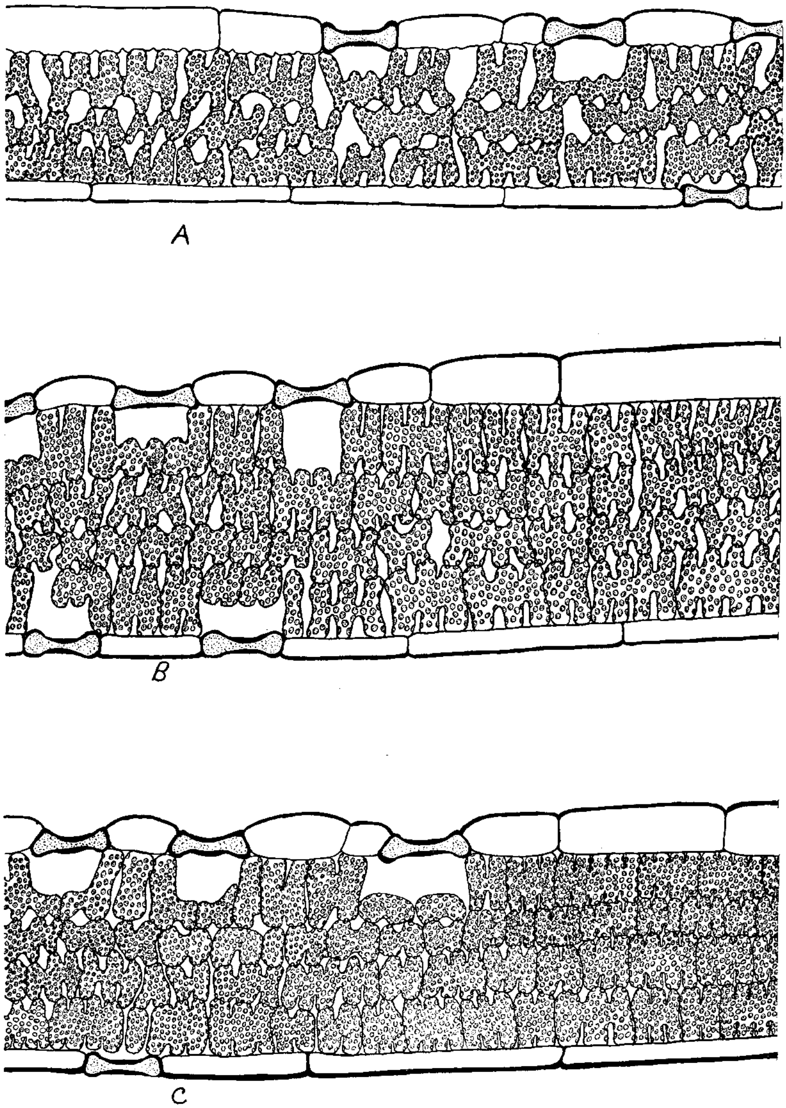

Figure 6. Longisections of wheat leaves from (a) Lincoln, (b) Phillipsburg, and (c) Burlington. Cut along lines indicated by arrows in figure 5.

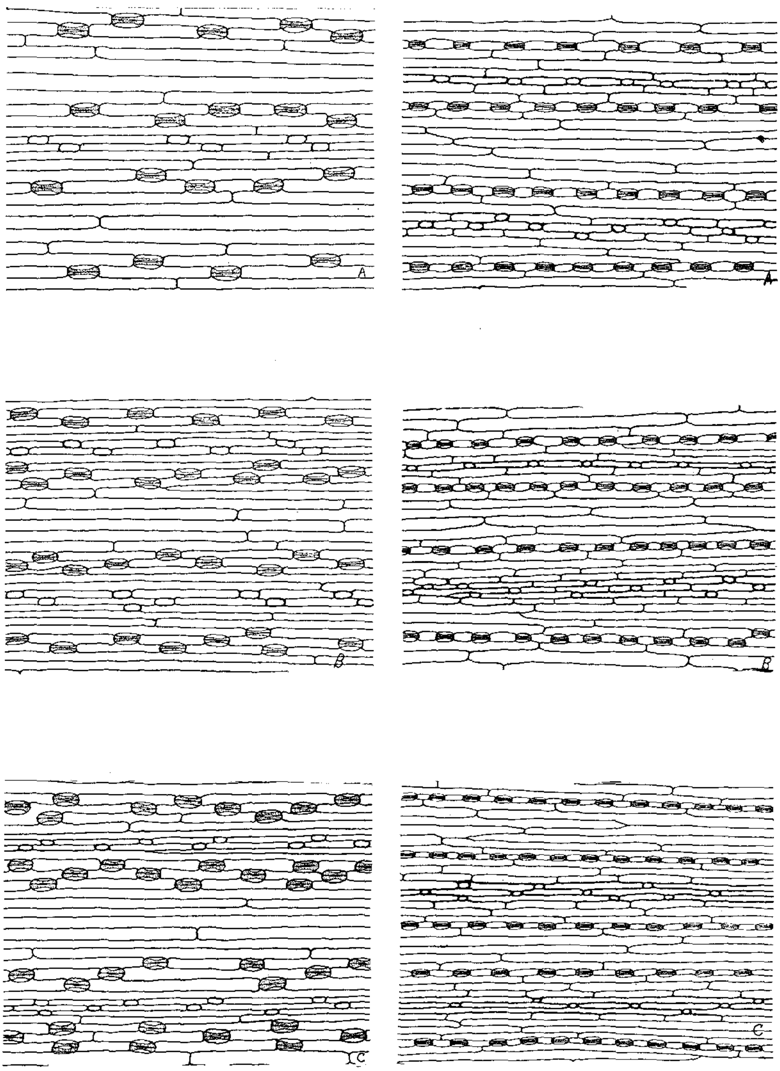

Figure 7. Micro-projection drawings of unit areas of epidermis of oats (left) and barley (right), from (a) Lincoln, (b) Phillipsburg, and (c) Burlington.