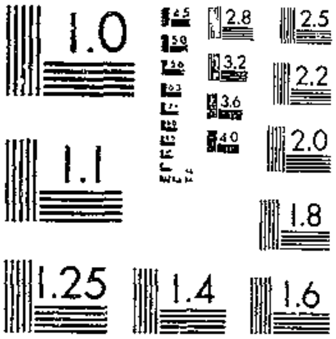
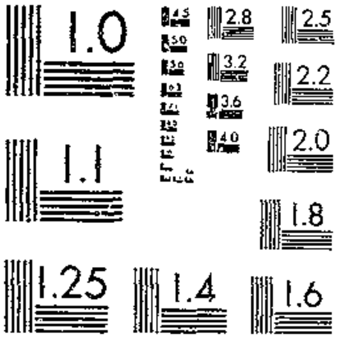
TB 266 (1931) USDA TECHNICAL BULLETINS UPDATA
MORPHOLOGIC AND PHYSIOLOGIC STUDIES ON STEM-RUST RESISTANCE IN CEREALS
HART, H. 1 OF 1

START





MICROCOPY RESOLUTION TEST CHART
NATIONAL BUREAU OF STANDARDS-1963-A

TECHNICAL BULLETIN No. 266 DECEMBER, 1931

UNITED STATES DEPARTMENT OF AGRICULTURE
WASHINGTON, D. C.

MORPHOLOGIC AND PHYSIOLOGIC STUDIES ON STEM-RUST RESISTANCE IN CEREALS[1]

By HELEN HART

Assistant Plant Pathologist, University of Minnesota, and Agent, Division of Cereal Crops and Diseases, Bureau of Plant Industry[2]

In Cooperation with the Minnesota Agricultural Experiment Station

CONTENTS

	Page
Introduction	1
Historical review	3
Host structure and the relation of susceptible host tissue to rust resistance	6
Pustule formation	30
Relation of size of collenchyma strands to formation of pustules	33
Structure of the epidermis and its resistance to rupture	35
Structure of the collenchyma	42
Effect of fertilizers on structure	43
Relation of stomatal behavior to stem-rust resistance in wheat	49
Uneven or one-sided infection of peduncles in the field	59
Effect of other diseases on resistance of wheat to stem rust	60
Discussion	62
Summary	70
Literature cited	72

INTRODUCTION

The nature of resistance of cereals to *Puccinia graminis* Pers. has been a subject for much speculation and investigation. There are probably at least three types of resistance: Two types of physiologic resistance, protoplasmic and functional, and morphologic resistance.

The protoplasmic type of resistance has been investigated more thoroughly than the other types, and it was long assumed that the rust resistance of cereals could be accounted for on a physiological basis. There is a high degree of specificity between host plant and pathogene. A cereal variety may be susceptible to certain physio-

---

[1] The investigations here reported were conducted by the Division of Cereal Crops and Diseases, Bureau of Plant Industry, in cooperation with the Plant Pathology and Botany Division of the Minnesota Agricultural Experiment Station.

Presented as a thesis to the faculty of the Graduate School of the University of Minnesota in partial fulfillment of the requirements for the degree of doctor of philosophy.

[2] The writer gratefully acknowledges her indebtedness to Dr. E. C. Stakman for his supervision and criticism during the course of the investigation, and for his suggestions in the preparation of the report. She is also indebted to Dr. Olaf S. Aamodt for wheat samples from the uniform rust nursery.

logic forms of the pathogene and highly resistant to, or even immune from, other physiologic forms of the same species. In all cases infection begins normally, for the germ tubes of the fungus may enter physiologically resistant as well as physiologically susceptible varieties. However, after the germ tubes enter a host that has protoplasmic resistance, they kill some of the host cells and then they themselves die, so that the fungus is never able to establish itself as an active parasite. The nature of the incompatibility or antagonism between the cereal plant and the fungus is not known. Varieties that have protoplasmic resistance to certain forms of *P. graminis* probably are resistant in all stages of development. In some cases, however, cereal varieties are susceptible in the seedling stage and moderately resistant in later stages of development. This may be due to a morphologic rather than a protoplasmic resistance.

Morphological resistance to *P. graminis* is dependent on the structure of the cereal plant rather than on the interrelations or compatibility of the cereal plant and the fungus. The pathogene can enter and develop normally in a variety with protoplasmic susceptibility, but the structural characters of the host may be such as to prevent an extensive development of the rust. *P. graminis* can develop in almost all tissues of the seedlings, but in older plants its development is possible only in the chlorophyllous, nonlignified tissues. In the stem, the rust can grow only in the chlorophyllous collenchyma bundles, which extend lengthwise of the stem and are partly surrounded by the thick-walled, lignified sclerenchyma. In morphologically resistant varieties the collenchyma bundles are narrow, isolated strands, separated by broad bands of sclerenchyma; hence the rust is restricted to relatively small areas. Long, linear pustules are formed rather than broad confluent ones, and the host is practically resistant. Thus a variety may be physiologically susceptible but morphologically resistant to certain rust forms. The resistance of certain wheat varieties in the field may be explained on this morphological basis. However, there are some varieties that have protoplasmic and morphologic susceptibility in all stages of development when inoculated artificially in the greenhouse, and yet they remain almost free from rust in the field. The reason for the field resistance of these varieties has not hitherto been explained and has constituted one of the principal problems of the present investigation. A third type of resistance was discovered, which, for want of a better term, may be designated as functional resistance.

Functional resistance to stem rust is, in certain cereal varieties, the result of a characteristic stomatal behavior that causes the exclusion of the fungus. There is a tendency for the cereals to carry on photosynthesis with many stomata closed, but it has been found that stomatal behavior differs greatly in different varieties and is very characteristic for a given variety. The stomata of some varieties are closed for long periods. Those of other varieties open early in the day and remain wide open or partly open most of the day. The germ tubes of *P. graminis* enter cereals through the stomata but are unable to penetrate closed stomata. Hence, those varieties whose stomata remain closed for long periods, and especially at critical times for germ-tube entry, are resistant to rust.

HISTORICAL REVIEW

It has long been known that some varieties of wheat are more susceptible to rust than others. The general opinion about 1890 was that the hardy, stiff-straw varieties were the least injured by rust. In 1892 Cobb (*13*)[3] advanced a "mechanical theory" to explain rust resistance, stating that morphological characters, such as a thick cuticle, a waxy covering, small stomata, a large number of leaf hairs, or upright leaves, might be responsible for the resistance of certain varieties. Hitchcock and Carleton (*22*) supported Cobb's theory, for they state that varieties with stiff upright leaves are affected less than varieties with flaccid foliage and that varieties with thick epidermis or with many hairs are less likely to rust than those with thin epidermis and few hairs. Farrer (*16*), in Australia, concurred in the mechanical theory of resistance; and Sappin-Trouffy (*35*), in France, reported and illustrated the localization of *Puccinia graminis* in the chlorenchymatous regions of the stem of Avena.

Eriksson and Henning (*15*), however, were unable to correlate morphological characters and rust resistance, inasmuch as they observed that a wheat variety might be resistant to some species of rust and very susceptible to others. They believed that the rust resistance was to be explained on a chemical and physiological basis rather than a morphological one.

Ward (*47, 48*), in 1901 and 1902, studied the behavior of *Puccinia dispersa* on *Bromus* spp. After investigating the size, number, and distribution of stomata, hairs, and vascular bundles, the chlorophyll-containing tissues, and the sclerenchyma of the brome leaf, he concluded that resistance to infection is not to be accounted for by observable structural peculiarities. Ward (*49*) was of the opinion that resistance is due to internal or intraprotoplasmic properties that are beyond the reach of the microscope. Biffen (*11*) agreed with Ward that rust resistance is independent of discernible morphological characters and believed that it would be practicable to breed wheat varieties morphologically similar but differing in susceptibility to the attacks of certain parasitic fungi.

Ward (*48*) found different degrees of susceptibility when bromes were artificially inoculated with *P. dispersa*. According to Ward, infection in a few cases fails entirely, either because the spores do not germinate or because the germ tubes are unable to enter the stomata and produce a mycelium. At the other extreme the germ tubes enter the leaf, produce an extensive mycelium, and give rise to pustules. In intermediate cases there may be a pale flecking of the leaf, indicating a more or less extensive mycelium which never produces spores. Sometimes the infecting fungus attacks the host cells so rapidly that they are killed at once, and then the fungus dies, for the dead host cells are unsuitable medium for its further growth.

The study of rusts and rust resistance of grasses was continued in Ward's laboratory, and in 1904 Gibson (*18*) reported that germ tubes of rust might easily enter the stomata of plants that are not natural hosts. Thus the germ tubes of a grass rust might enter the stomata

---

[3] Italic numbers in parentheses refer to Literature Cited, p. 72.

of a leaf of Ranunculus. The rust reaches various stages of development, depending on the taxonomic relationship of the inoculated plant to the natural host, but in all cases of infection of a nonhost plant the fungus dies before a mycelium is established.

The infection of an extremely resistant wheat by *Puccinia glumarum* has been described in detail by Marryat (*29*). She found that the germ tubes enter and produce hyphae in the highly resistant host just as in a susceptible host. In the former, however, the progress of the rust is checked by the disintegration and death of the host cells, resulting in starvation of the fungus hyphae. The destructive action of *P. glumarum* is very rapid in a highly resistant host. The reason for such a reaction is unknown, but Marryat supposed that it might be due to the production by parasite and host of certain toxins and antitoxins which are mutually destructive.

A similar phenomenon, the infection of a highly resistant host by *P. graminis*, was investigated by Stakman (*38*) in 1914. The germ tubes of *P. graminis* are able to enter the stomata of the highly resistant Khapli emmer. The substomatal vesicle may or may not send out short club-shaped hyphal branches, which soon become vacuolate and never send haustoria into the host cells. If a hypha comes in contact with a host cell, the protoplast of that cell disintegrates and soon dies. The hypha may continue to grow and attack other cells, but eventually it also disintegrates and dies. The incompatibility of host and fungus is extreme, for the host cells seem to be hypersensitive to the fungus.

Newton (*30*) also studied the behavior of *Puccinia graminis tritici* in a susceptible wheat variety, Marquis, and in a resistant variety, Kanred. Her observations were essentially the same as those of Stakman (*38, 39*). The susceptible host adjusts itself readily to the presence of the fungus and the rust often develops luxuriantly. The resistant host is intolerant of the fungus, and the attacked host cells and the fungus hyphae soon perish. Infection seems to depend on a delicate balance between host and parasite.

Recent cytological investigations on the cereal rusts and the infection of their hosts have been made by Allen (*4, 5, 6, 7, 8, 9*). She studied several physiologic forms of *Puccinia graminis tritici* on susceptible, moderately resistant, and highly resistant wheat varieties (*4, 5, 6*). Allen (*7, 8*) also studied the behavior of leaf rust, *P. triticina*, on the susceptible Little Club and the resistant Malakoff wheats, and lately she (*9*) has reported her observations on the infection of *Bromus marginatus* and *Triticum vulgare* by stripe rust, *P. glumarum*. The reactions of *P. coronata* on Banner and Cowra 35 oats have been studied cytologically by Ruttle and Fraser (*34*), and Wellensiek (*51*) worked with two physiologic forms of *P. sorghi* on corn. All recent cytological findings are in accord with the phenomena reported earlier, although the authors do not agree on the causes and explanations for the phenomena.

It was long supposed that all the rust resistance of cereals could be accounted for on a physiological basis. The theory of a mechanical resistance had been so completely discarded by 1905 that no one seems to have thought of studying the structure of rust-resistant wheats until 1921, when Allen (*3*) suggested that the resistance of Kanred might be due in part to the fact that the stomatal slits were not wide enough to permit the germ tubes to enter. Under the conditions of her experiments the stomata of Kanred excluded 90 per cent of the germ tubes of *Puccinia graminis tritici*, and she suggested (*3* and *4, p. 141*) that the presence of the appressorium might act as a stimulus to the stoma, narrowing still further the naturally small stomatal slit or causing the guard cells to remain closed. She believed (*4, p. 146*) that the resistance in Kanred was due in part to the nature of its stomata.

Hursh (*23*) likewise investigated the possibilities of a morphological basis for resistance. In 1924 he reported that the distribution of sclerenchyma and collenchyma in wheat culms could be correlated with the rust resistance of the variety in question. Hursh found that the stems of some resistant wheats contain large amounts of sclerenchyma, constituting a mechanical limitation to the spread of mycelium and the size of pustules, which are likely to be narrowly linear. On highly susceptible varieties, on the other hand, characterized by little sclerenchyma but extensive strands of collenchyma, the rust pustules are broad and often confluent. Hursh's work reestablished the older idea of a morphological type of resistance, but on a slightly different basis. He pointed out that the number of leaf hairs and the size and number of stomata on the leaves were unimportant in influencing the entrance of germ tubes. The important factors were the distribution of susceptible tissues in the wheat culm and the mechanical restrictions to the spread of the rust after it had established itself within the peduncle of the host.

Although many investigators studied the size and number of stomata in relation to rust resistance in cereals, very little thought was given to the possible bearing of stomatal behavior on the resistance of a variety. As early as 1916 Pool and McKay (*32*) found that infection of sugar-beet leaves by *Cercospora beticola* was dependent on stomatal activity. As germ tubes of the conidia were unable to penetrate closed stomata, infection occurred only when the stomata were open. The possibility that stomatal movements in wheats might influence the entrance of germ tubes of the rust was mentioned by Hursh (*23*) and by Allen (*7*), but no reports of actual studies were made.

The stomatal behavior of several wheat varieties and the effect on rust infection in the field and greenhouse have been investigated by Hart (*20*). The germ tubes of *Puccinia graminis tritici* usually do not penetrate closed stomata; thus infection can occur only when the stomata are open and conditions are favorable for spore germination and growth of germ tubes. The type of stomatal behavior is characteristic for varieties of wheat. In some varieties the stomata respond rapidly to favorable stimuli and open almost immediately after sunrise; in other varieties the stomata open very slowly or not at all. In the first case the fungus enters its host readily, but in the second case many of the germ tubes are excluded, and the host is rarely infected. According to Hart, the field resistance of certain wheat varieties may be accounted for on the basis of stomatal behavior.

The present paper deals with the morphological and functional resistance of wheat varieties. Ever since the extensive studies on physiologic specialization in *Puccinia graminis* by Stakman and his coworkers (*38, 39, 40, 41, 42, 43, 44, 45*), it has been known that there are certain discrepancies in the type of infection on seedlings in the greenhouse and that on mature plants in the field. Many of these perplexing varieties have been studied to determine whether they possess a morphological or functional type of resistance that would modify the physiological reaction to virulent rust forms.

HOST STRUCTURE AND THE RELATION OF SUSCEPTIBLE HOST TISSUE TO RUST RESISTANCE

Many of the early conclusions regarding morphological resistance were based on circumstantial evidence, on a consideration of the gross anatomy of the cereal hosts, and on their reaction to rust in the field. About 1902 Ward (*48*) made more detailed studies of the minute anatomy of the hosts. He investigated the leaf rust of bromes and naturally, but unfortunately, paid particular attention only to seedlings and mature leaves. His conclusion that there was no morphological basis for rust resistance probably was justified as far as his material was concerned, but later studies of the structure of the culms of mature plants have shown that his generalization was too sweeping. The present investigation shows that the structure of wheat seedlings in no way limits rust infection; that the structure of the peduncles of mature wheat plants may have a very great influence on rust infection; and that the structure of leaves, leaf sheaths, glumes, and awns of certain wheat varieties may limit the development of rust.

THE SEEDLINGS

Seedlings are used in most of the greenhouse studies on physiologic specialization in the rusts and in some of the studies on the inheritance of rust reaction in certain wheat crosses. The writer therefore made a thorough study of the structure of seedlings and its possible relation to resistance.

The blunt linear seedling leaf is a modified duplicate of the older leaf blade. (Fig. 1.) The lower surface usually is smooth, with a more or less prominent midrib and parallel veins running the entire length of the leaf, but the upper surface is ridged in almost all varieties. The epidermis consists of thin-walled cells, and epidermal hairs often are present. The stomata are arranged in parallel rows on upper and lower surfaces. The mesophyll tissue comprises a loose, spongy mass, with no compact palisade layer, and the numerous intercellular spaces are sometimes relatively large. The vascular bundles are parallel with the midrib, and large bundles usually alternate with smaller ones.

There is very little sclerenchyma in the seedling leaf, aside from that in the fibrovascular bundles. The midrib may be reenforced by a group of cells whose walls have become thickened and lignified, and there may be a small group of such cells along each leaf margin. Occasionally, just beneath the epidermis and opposite the large vascular bundles, there may be a few lignified cells. As seen in cross section, each of these groups has only three or four cells, while the groups at the margins and the midrib may contain a dozen or more. This scanty development of sclerenchyma is characteristic of seedlings of all varieties, and there seems to be no structural hindrance to rust development. Virtually all tissues of the seedlings are susceptible to virulent forms of rust. (Fig. 1, B.)

There is a possibility that leaf hairs may be so numerous on seedlings that the germ tubes of the fungus are kept from entering the stomata, or that the stomata are so small or so few and scattered that the germ tubes are unable to infect the seedling. Ward (*48*) investigated these problems with respect to the leaf rust of the bromes, and Hursh (*23*) studied them with respect to stem rust of wheat. Both workers concluded that such factors were of no importance in the entrance of germ tubes. Allen (*3*) studied the entrance of *Puccinia graminis tritici* through the stomata of Kanred and Mindum wheats. The spores germinated readily on leaves of both varieties, and many appressoria were formed over the stomata, but only one-tenth of the sporelings entered Kanred, while virtually all entered Mindum. The stomatal apertures of Kanred are extremely long and narrow, and those of Mindum are rather short but about twice as wide as those of Kanred. Allen suggested that the naturally small stomatal opening in Kanred might exclude the sporelings, especially if the opening is still further narrowed by the action of the guard cells when the appressorium is formed.

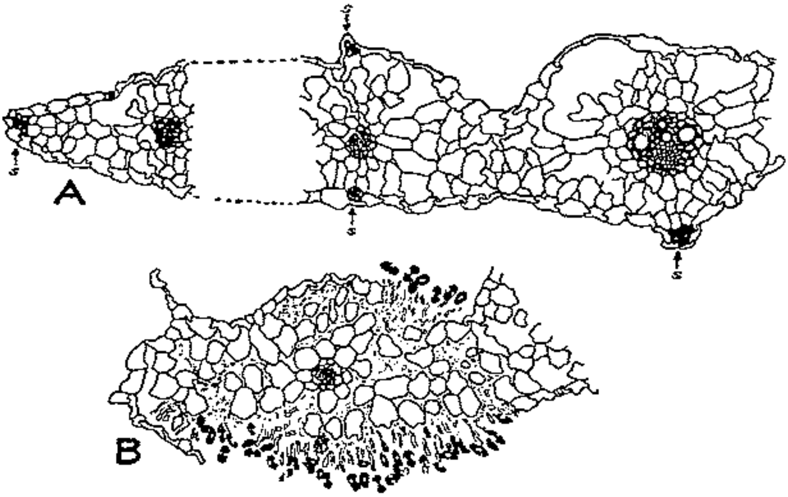


FIGURE 1.—A, Cross section of a seedling leaf blade of wheat, showing the meager amount of sclerenchyma (*s*) at the midrib, at the margin of the blade, and opposite the vascular bundles. A large proportion of the seedling blade is chlorenchyma. B, Cross section of a rusted wheat seedling, showing that there are practically no morphological restrictions to the spread of rust mycelium. × 70

Newton (*30*) also studied rust infection on seedlings of Kanred wheat. She obtained heavy infection with forms 3, 11, 12, 15, 18, and 32 of *Puccinia graminis tritici*. If Allen's suggestion that the narrow stomatal slits of Kanred excluded the germ tubes were true, it was to be assumed that the germ tubes of Newton's rust forms were smaller than those of the form with which Allen worked. Newton measured the average diameters of the germ tubes of form 18, to which Kanred is highly susceptible, and form 17, to which Kanred is highly resistant. There was no appreciable difference, so it is probable that the stomatal size was not the factor responsible for the resistance of Kanred.

Apparently it is justifiable to conclude that wheat seedlings usually possess no morphological resistance to *Puccinia graminis.* Other parts of the plant vary more in structure and contain larger proportions of the kind of tissue that can not be attacked by rusts.

THE PEDUNCLE

Puccinia graminis causes the greatest injury when it attacks the peduncle of the plant—that portion of the stem just below the rachis. The peduncle usually is a hollow cylinder, although it is sometimes solid. The outer part, beneath the epidermis, is composed of strands of chlorophyllous tissue separated by sclerenchyma and vascular bundles, and the inner region is made up of colorless, thin-walled parenchyma. The cellulose walls of the cells in the chlorophyllous tissues may be thin or irregularly thickened, so that Hursh speaks of this tissue as collenchyma. The collenchyma is almost surrounded by the sclerenchyma, the cells of which have thick, lignified walls. The vascular bundles form the inner part of the sclerenchyma sheath and help to separate the strands of collenchyma.

Puccinia graminis attacks only the chlorophyllous collenchyma strands in the peduncles of cereals, entering through the stomata, which are located in single or double rows in that part of the epidermis that covers the collenchyma. The fungus mycelium invades the collenchyma region but is unable to penetrate the surrounding sheath of sclerenchyma. Sappin-Trouffy (*35*), in 1896, presented an excellent drawing showing *P. graminis* confined to the chlorophyllous strands of the stem of Avena. He observed that the mycelial filaments were localized in the parenchymatous regions of a plant, and on the stem of the host the mycelium was extended freely only longitudinally, because it was bordered on all sides and underneath by a hemispherical sheath of sclerenchyma. The mycelium was not found within the sclerenchyma, although some of the haustoria might penetrate the cells of the first layer bordering on the chlorophyllous tissue.

Eriksson and Henning (*15, p. 120–123, figs. 40, 44*) also found that rust was confined to the chlorophyll-containing tissues of the plant and was not found in the mechanical tissues. They studied the structure of uredinia on different parts of the plant and explained the variable aspect of the pustules on the basis of the distribution of chlorophyll tissue, pointing out that the sheath was far richer in this tissue than the stem.

The peduncles of several wheat varieties and the extent of their chlorophyllous collenchyma strands were studied by Hursh (*23*). Varieties differ greatly in the amount and distribution of collenchyma in the peduncle. Little Club and similar varieties have large proportions of collenchyma, and it is distributed in a few very broad strands which may extend one-third or more of the way around the stem. When such strands are attacked by *P. graminis*, the pustules that are formed are very large and broad, so that not many are needed to encircle the stem. Kota and some of the emmers have smaller proportions of collenchyma, and it is distributed in many narrow strands isolated from one another by broad bands of sclerenchyma. When such strands are attacked by rust, the pustules formed are long and narrow and often very distinct from one another, so that a greater number of infections are required to encircle the stem. Varieties with the latter type of peduncle structure are morphologically resistant to stem rust.

The relative proportions of collenchyma and sclerenchyma in the peduncle have been studied in many other varieties of wheat. In addition the writer has studied several other factors that are important in a morphological type of resistance, namely, the size, shape, and disposition of collenchyma strands in the sclerenchyma sheath, the thickness and resistance to rupture of the epidermis of the peduncle, the structure of the collenchyma cells, and the effect of fertilizers on the structure of the peduncle of certain varieties. In some cases one factor is more important than all others in determining the morphological resistance of a wheat variety to stem rust. In other cases resistance may be the result of the interaction of several factors.

PROPORTIONS OF COLLENCHYMA AND SCLERENCHYMA IN THE PEDUNCLE

The structure of the peduncle was studied in a number of standard wheat varieties to determine whether it varied greatly or was reasonably constant in any given variety and whether there was a constant relationship between rust susceptibility and a large proportion of collenchyma. To secure definite, easily comparable results, the proportion of collenchyma was recorded as a percentage of the tissue facing on the periphery of a circle superposed on a cross section of the peduncle. This method of measurement takes into account only the face of the collenchyma strand adjacent to the periphery and does not consider the depth or configuration of the strand. It was, however, the only feasible method for comparing large numbers of peduncles and determining the relative proportions of collenchyma and sclerenchyma in the outer regions of the stem. Hundreds of peduncles, sectioned 1½ inches below the head, have been measured for each wheat variety. All the varieties were grown in the field at University Farm, St. Paul, Minn.

The preliminary work indicated that the structure of a variety is remarkably constant from season to season and also from the beginning to the end of a single season. At different intervals after heading, three different varieties were compared as to the percentage of collenchyma in the peduncle. Twenty samples of each were taken 3 days after heading, 10 days after heading, and 20 days after heading. The percentages of collenchyma for any one variety were approximately the same at all times, never varying more than 1 per cent. Thus the proportions of collenchyma and sclerenchyma in the peduncle do not change after the plants have headed. In spite of this fact, most of the samples of the standard varieties were taken at a fairly uniform stage, about two weeks after heading.

Table 1 gives the percentages of collenchyma in the peduncles of some of the varieties examined. In the common wheats there seems to be a correlation between rust reaction and the proportion of collenchyma. Quality, Marquis, Ruby, and Haynes Bluestem, varieties that are highly susceptible if virulent rust forms are present, have relatively large proportions of collenchyma in their peduncles. Kota and Webster, on the other hand, usually resistant to rust in the field, have less collenchyma in their stems. Ceres, selected from a cross between Marquis and Kota, varies markedly in rust reaction, and its structure seems to be intermediate between that of its parents. The percentage of collenchyma in Ceres is less than in Marquis, the susceptible parent, but greater than in Kota, the semiresistant parent. A number of hybrids between varieties of *Triticum vulgare* were studied, and in all these cases the percentage of collenchyma in the peduncle seemed to be intermediate between the percentages present in the parent varieties.

TABLE 1.—*Percentages of collenchyma in the peduncles of different varieties of wheat grown in the field at University Farm, St. Paul, and at Coon Creek, Minn., each season from 1924 to 1928, inclusive*

Variety	C. I. No.	Collenchyma	Variety	C. I. No.	Collenchyma
		Per cent			*Per cent*
Triticum compactum:			Triticum durum:		
Little Club	4066	63.8	Mindum	5296	77.2
Triticum vulgare:			Acme	5284	70.4
Quality	6607	69.8	Pentad	3322	77.1
Marquis	3641	69.0	Iumillo	1736	76.0
Haynes Bluestem	2874	70.0	Kaaerd	5146	74.1
Ruby	6047	68.8	Triticum dicoccum:		
Marquillo	6887	66.6	Khapli	4013	49.9
Ceres	6900	64.6	Vernal	3686	57.5
Kota	5878	60.8	Sonem	4402	58.0
Webster	3780	60.6			
Hope	8178	61.3			

The differences in percentage of collenchyma in the varieties are only slight and may not be responsible for the marked differences in the appearance of rust pustules. Nevertheless, the more resistant varieties of *Triticum vulgare* have less collenchyma than the highly susceptible varieties.

All of the durum wheats contain large percentages of collenchyma in the peduncles, far more than the most susceptible common wheats. (Table 1.) On the basis of the proportion of collenchyma in the peduncle, none of the durum wheats would seem to have any morphological resistance to stem rust.

The emmers have less collenchyma than the durums or the common wheats. (Table 1.) About half of the circumference of the peduncle of Khapli emmer is composed of sclerenchyma, as compared with less than one-quarter in the durums and about one-third in the common wheats. This certainly points to a morphological resistance in the emmers, if the proportion of collenchyma is a reliable indication on which to base a decision.

It is the writer's opinion that the proportion of collenchyma varies more in different parts of a single peduncle than in the peduncles of different varieties. Percival (*31*) has said that the chlorophyllous tissue is arranged in parallel strands that are wide at the apex, taper gradually as they are traced downwards, and finally disappear before reaching the base of the internode. He also states that the average width and thickness of each individual strand is greatest in the upper internode or peduncle, and the strands are closer together there; consequently, that portion of the straw is more uniformly green than the lower internode. If the peduncle of any wheat variety is cross sectioned just beneath the head or within one-eighth of an inch of the rachis, a great abundance of collenchyma is found. The collenchyma strands coalesce in this region and from 1 to 4 or 5 extensive strands encircle the stem almost completely, leaving only a few narrow bands of sclerenchyma tissue to interrupt the cylinder. (Fig. 2.) This great expanse of collenchyma in the region just below the rachis is characteristic of all wheat varieties, resistant as well as susceptible. It was found also in the emmers examined. The total percentage of collenchyma abutting on the circumference in this region would be between 90 and 98, and a virulent rust form would produce broad confluent pustules on this part of the peduncle, even though it is restricted to narrow isolated collenchyma strands farther down the stem. All varieties are morphologically susceptible in this particular region.

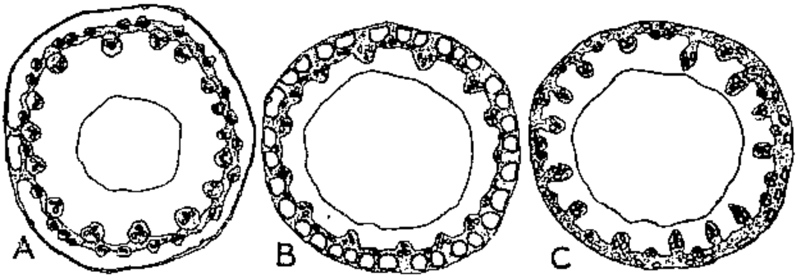


FIGURE 2.—Cross sections of a peduncle of wheat: A, Cut one-sixteenth inch below the head; B, cut 1⅛ inches below the head; C, within the leaf sheath. × 15

The portion of the peduncle from half an inch below the rachis almost to the enveloping leaf sheath is fairly uniform in structure. It is this part of the stem which varies according to the wheat variety and is to be taken as standard for any one variety. (Fig. 2.)

The stem within the leaf sheath and just above it contains a large amount of sclerenchyma and very little of the chlorophyllous collenchyma. Susceptible and resistant varieties both have large amounts of mechanical tissues in this part of the stem. The collenchyma strands usually are extremely narrow and shallow and are separated by wide bands of sclerenchyma, so that only 25 or 35 per cent of the periphery is rust-susceptible tissue; therefore, the pustules are necessarily small and narrow. (Fig. 2.)

Thus it would appear that the morphological resistance of a variety probably is not influenced so much by the proportion of collenchyma in the peduncle as by the disposition of that tissue into collenchyma strands and by the size and shape of these individual strands in the sclerenchyma sheath.

SIZE AND SHAPE OF COLLENCHYMA STRANDS

As Hursh (*23*) has pointed out, the collenchyma strands of one wheat variety may be very broad and confluent while those of another are narrow and distinctly separated by bands of sclerenchyma. The broad and confluent strands seem to be composed of two, three, or more individual strands which have never been divided and separated by sclerenchyma. The collenchyma areas are never so extensive as they are in the region just below the rachis, but they are broad enough to permit the development of large, conspicuous rust pustules all along the peduncle. Not all the collenchyma areas of a single peduncle, however, are of the same size. For example, the peduncle of Quality (fig. 3) may contain several collenchyma areas made up of three or four fused strands, a number composed of double strands, and some of single, isolated strands. The general structure of the peduncle is determined by the most prevalent type of collenchyma area.

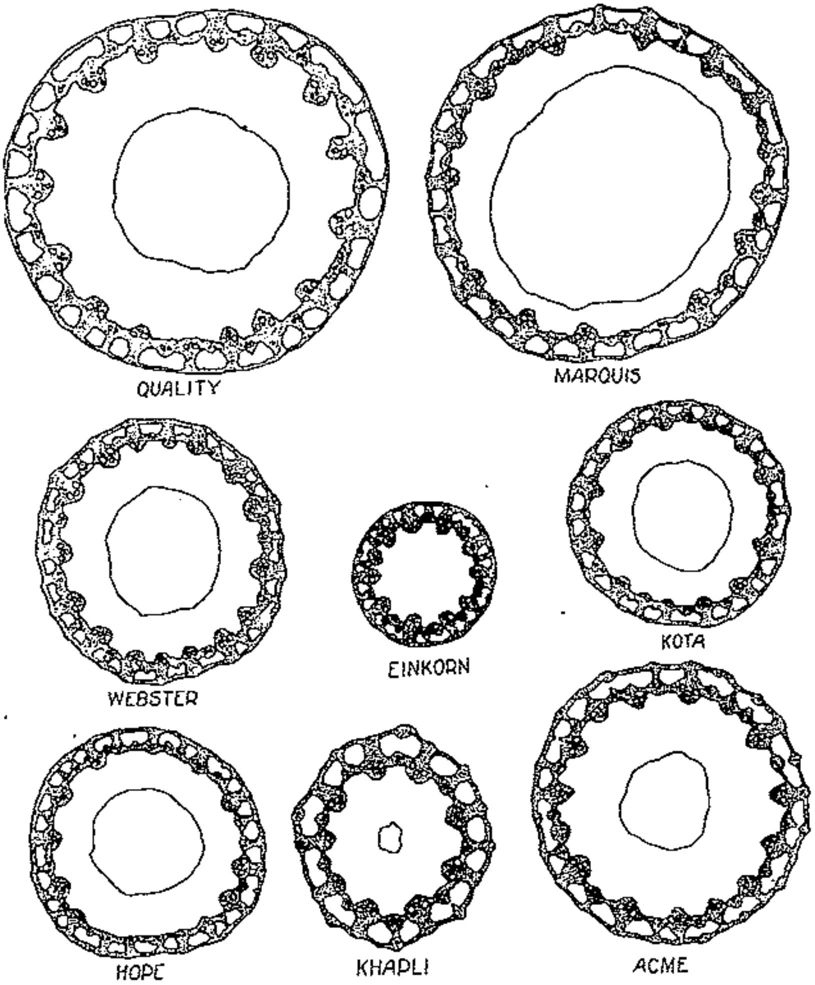


FIGURE 3.—Cross sections of peduncles of seven varieties of wheat and of einkorn, showing the distribution of the collenchyma in strands that are partly surrounded by sclerenchyma. × 24

The broad confluent strands are characteristic of Little Club, Quality, Dicklow (C. I.[4] 3663), Marquis, Haynes Bluestem, Reward (C. I. 8182), Early Baart (C. I. 1697), Axminster (C. I. 8196), and Redsask (Minn. 2193). All these varieties have been highly susceptible in the field at University Farm, St. Paul, Minn., and the rust pustules are always large and prominent. These varieties are physiologically susceptible to a large number of physiologic forms of *Puccinia graminis tritici*, and, in addition, a single germ tube infecting a broad collenchyma strand can produce a large amount of mycelium over a relatively large area. Consequently, a few large pustules cover a considerable surface of the peduncle.

In the peduncles of certain other varieties the strands of collenchyma are almost always single and isolated, with only a few double strands scattered here and there. The three or four extensive collenchyma areas just beneath the rachis are broken up into 30 or 40 narrow and distinct strands farther down the stem. This is the case in Kota, Webster, Hope, Minturki (C. I. 6155), and Sevier (C. I. 6247), usually resistant to stem rust at University Farm. (Fig. 3.) These varieties possess a protoplasmic resistance to a large number of physiologic forms, but, even when they are infected by a form to which they are physiologically susceptible, the development of the rust is restricted by the structure of the peduncle. The mycelium produced by a single infecting germ tube is confined to the limited area of a single narrow collenchyma strand, and the pustules produced usually are small and narrow. Thus, a great number of infections is required to cover the amount of surface that, in a variety like Little Club, is covered by a few pustules. Occasionally, Kota appears to have large confluent pustules, but in almost all cases these are compound pustules, composed of several small narrow ones in which spore production has been so abundant that the margins of the individual pustules can not be determined without microscopic examination.

Some of the other varieties are intermediate in structure. Thus the peduncle of Ceres has about equal proportions of single and double collenchyma strands. Ceres does not have the broad, confluent strands often found in Marquis and it has fewer of the double strands than Marquis, but it has many more double strands than are ever found in Kota. Marquillo also has a large number of double collenchyma strands in the peduncle, but has far fewer than its Marquis parent. The variety Progress (C. I. 6902) also is in the intermediate class and has about equal numbers of narrow single strands and double strands.

Single collenchyma areas of six different varieties are shown in Figure 4. All the tracings were made with the camera lucida and were drawn to the same scale in order that a representative strand of one variety might be compared with that of another. The collenchyma area of Quality is more than four times as broad as that of Kota and about seven times as broad as that of einkorn. It is small wonder that narrow linear pustules are formed on some varieties while broad ones appear on others.

---

[4] C. I. indicates accession number of the Division of Cereal Crops and Diseases, formerly Office of Cereal Investigations.

The collenchyma strands in the peduncles of the common wheats usually are simple and quite regular in shape. They are sometimes rather shallow and have moderately straight radial borders, as though they were set in relatively smooth-faced, even grooves in the sclerenchyma sheath. (Fig. 5.) They are covered by the epidermis, which, in the case of broad confluent collenchyma strands, bears a considerable amount of the pressure exerted by the developing rust mycelium.

Most of the collenchyma areas of the durums are very irregular in shape, for they are made up of from two to seven individual strands, partly coalesced, that never have been completely separated by sclerenchyma. The whole mass forms a broad, continuous strand that is easily penetrated by the fungus hyphae, but its individual parts are more or less distinct. Groups of sclerenchyma cells lie just beneath the epidermis and between the component parts of the collenchyma mass. These groups of sclerenchyma cells may act as mechanical reenforcements to the epidermal membrane over the collenchyma, and the epidermis is therefore more resistant to rupture by the growth pressure of the developing pustule.

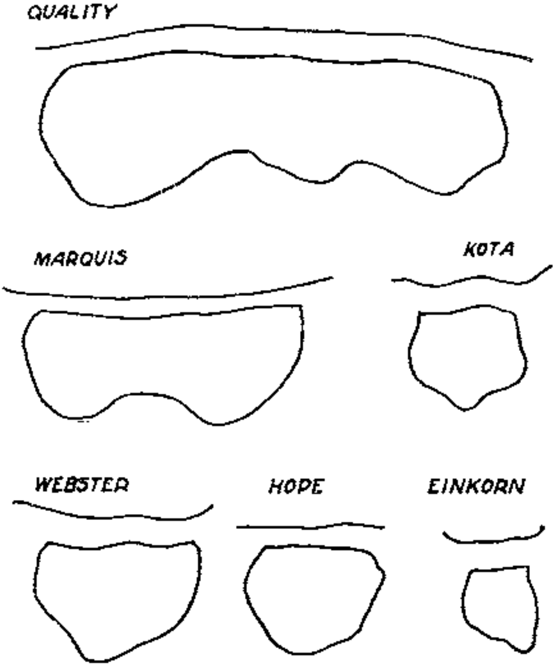


FIGURE 4.—Camera-lucida tracings of collenchyma areas in cross section, showing the relative sizes of strands which are typical of five varieties of wheat and of einkorn. × 115

The individual collenchyma strands of the durum wheats are deeper and more irregular in shape than those of the common wheats. The collenchyma seems to be more firmly embedded in the sclerenchyma sheath (fig. 5); that is, the sclerenchyma surrounds more of the collenchyma than in the common wheats, and the epidermis borders a smaller proportion of the strand. At the periphery of the culm the collenchyma strand is narrower than it is a short distance below the surface. It is as though the collenchyma tissue occupied an undercut groove in the sheath of sclerenchyma. In such cases a large part of the area upon which the infecting rust fungus exerts pressure would be protected by the unyielding sclerenchyma and comparatively less covered only by the more elastic epidermis.

In the peduncles of Khapli and Sonem emmers the collenchyma is in narrow, isolated strands or sometimes in double strands that resemble the fused strands of the durums, with a group of sclerenchyma cells beneath the epidermis and between the individual parts. The culms of these two varieties usually are more slender than those of the common or durum wheats and there is a smaller number of collenchyma strands in each peduncle. Not only are the relative amounts of collenchyma less in these two emmers than in the common wheats but the size of the individual strands is actually smaller. The structure of the peduncle of Vernal emmer is more like that of the common wheats than is that of either Khapli or Sonem. The culm of Vernal has a greater diameter and has a larger number of collenchyma strands, some of which are single and a few double; hence the development of the fungus is less restricted in Vernal than in the other two emmers. Khapli and Sonem have protoplasmic resistance to most of the rust forms, but, when they are inoculated with virulent forms, normal pustules develop in the restricted collenchyma tissues. Such pustules are always small and narrow, especially if two distinct pustules are formed in a double strand that has a particularly large or strong group of sclerenchyma cells between its component parts.

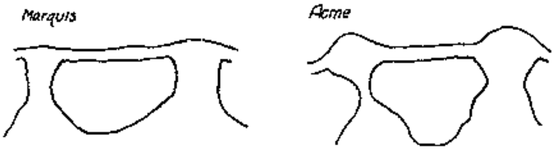


FIGURE 5.—Camera-lucida tracings of collenchyma areas in cross section, showing the straight regular margins that characterize collenchyma strands of common wheats (Marquis) and the irregular margins of the strands of durum wheats (Acme), which seem to be set in deep, undercut grooves in the sclerenchyma sheath. × 75

The shape of the individual collenchyma strands in Khapli and Sonem is very similar to that in the durums. The undercut edges and irregular borders are so arranged that the epidermis covers only a small surface of the strand, which seems to be almost completely buried in the sclerenchyma.

The peduncle of einkorn (C. I. 2433) is extremely slender; consequently the collenchyma strands are fewer and smaller than those in any other wheats. (Figs. 3 and 4.) Some of the strands are single and isolated and some are fused. Even when three or four collenchyma strands have coalesced, the groups of sclerenchyma cells beneath the epidermis are so large that a very small part of the strand actually borders on the epidermis. A virulent rust form could produce only narrow linear pustules, and in many strands pustules would never be formed.

According to these studies, the rust pustules on the peduncle are sharply limited by the size and shape of the collenchyma strands. In addition, the appearance of the pustules depends on the position of the collenchyma in its sclerenchyma sheath.

DISPOSITION OF COLLENCHYMA WITHIN THE SCLERENCHYMA SHEATH

In all varieties there are found occasional collenchyma strands that are entirely embedded in the sclerenchyma sheath, with no part of the strands bordering on the epidermis. These buried strands are characteristic of no particular variety but may occur in almost any of them. They have been found in Marquis, Kota, Ruby, Little Club, Hope, Progress, Webster, Ceres, Acme, Mindum, Pentad, Iumillo, Kanred, Khapli, Sonem, and in einkorn. In nature it is, of course, impossible for *Puccinia graminis* to infect the buried collenchyma unless the strand abuts on the epidermis at some point along the peduncle.

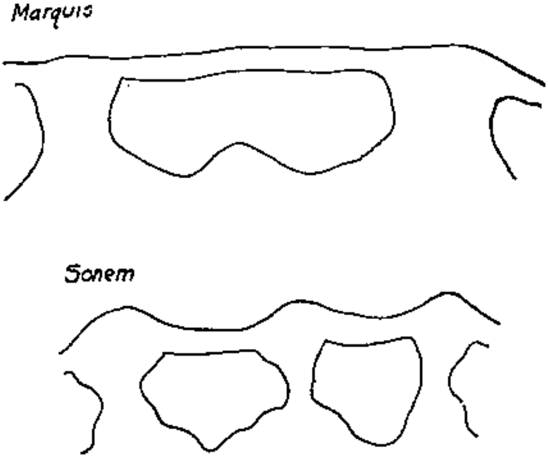


FIGURE 6.—Diagrams showing the contour of the surface of the peduncle in the smooth-stemmed common wheat, Marquis, and the furrowed emmer, Sonem. × 115

The peduncles of most of the common wheats are almost circular and relatively smooth in outline when viewed in cross section. Those of the durums and emmers, however, are rather deeply furrowed and present a wavy outline in cross section. The collenchyma strands in the common wheats usually are almost flush with the intervening sclerenchyma bands, while those of the durums and emmers are flanked by prominent ridges of the sclerenchyma sheath. (Fig. 6.)

The peduncles of Sonem and Khapli emmers probably are more distinctly furrowed than those of any other wheat. Hursh (*23*) noted the unevenness of the surface of Sonem emmer, caused by the bulging of the sclerenchyma bands. In Figure 6, a diagrammatic presentation of a camera-lucida tracing, it is seen that a rust pustule would be approximately hemispheric as soon as it succeeded in breaking the epidermis of Marquis. In the case of Sonem, however, when the epidermis is broken the spore mass still is in the bottom of a furrow and can spread across an area of much less than 180°, unless pushed out beyond the projecting sclerenchyma ridges.

If a virulent rust form infects a highly susceptible common wheat, it is a relatively easy matter for the fungus to force back the torn epidermis until the latter is broken off entirely or lies flattened against the outside of the bordering sclerenchyma band. (Fig. 7, A.) If many spores are formed, the pustule will spread out like a fan and cover the infected collenchyma strand and the neighboring sclerenchyma bands on both sides. (Figs. 7, A, and 8.)

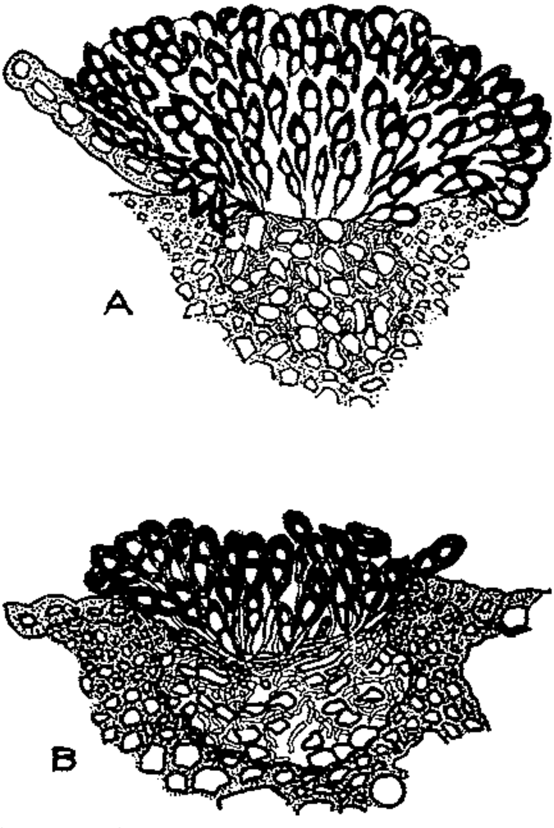


Figure 7.—Diagrams of pustules on a smooth-stem variety of wheat and on the furrowed stem of a spelt. In A the spore bed is flush with the periphery of the peduncle; in B the spore bed is at the bottom of the furrow. × 150

In the cereals with furrowed peduncles, the durums, emmers, spelts, and einkorn, the formation of such fan-shaped pustules is more difficult. The pustule usually lies at the bottom of a furrow and the spore mass must be pushed out a considerable distance before it can spread laterally over the sclerenchyma ridges. Unless the epidermis is torn off entirely and unless spore production is very great, the pustule is confined to its furrow between the sclerenchyma ridges. (Fig. 7, B.)

The peduncles are not smooth in all varieties of common wheat. Some show a tendency toward a decided furrowing, but the furrows are not so deep as those of the durums or the emmers. Rather are they intermediate between those of the durums and those of most of the common wheats. Some of the varieties examined have been roughly grouped into three classes, namely, smooth, intermediate, and furrowed. (Table 2.) Those of the first class usually are considered as rust susceptible in the field and give no indication of a morphological resistance. Those of the second class often have a definite morphological resistance due to the size and shape of the collenchyma strands. The structural resistance of these varieties is enhanced slightly by the furrowing of the stem. In the third class, the emmers have a morphological resistance based on the size and shape of collenchyma strands, this resistance being further increased by the sinking of the collenchyma between prominent sclerenchyma ridges. The durums and spelts, however, have no morphological resistance due to size of collenchyma strands, but they probably have a certain amount of morphological resistance if stem furrowing is taken as the criterion. The position of the collenchyma in the furrows and the reenforcement of the epidermis by sclerenchyma are the important causes of any structural resistance to stem rust which the durums may possess.

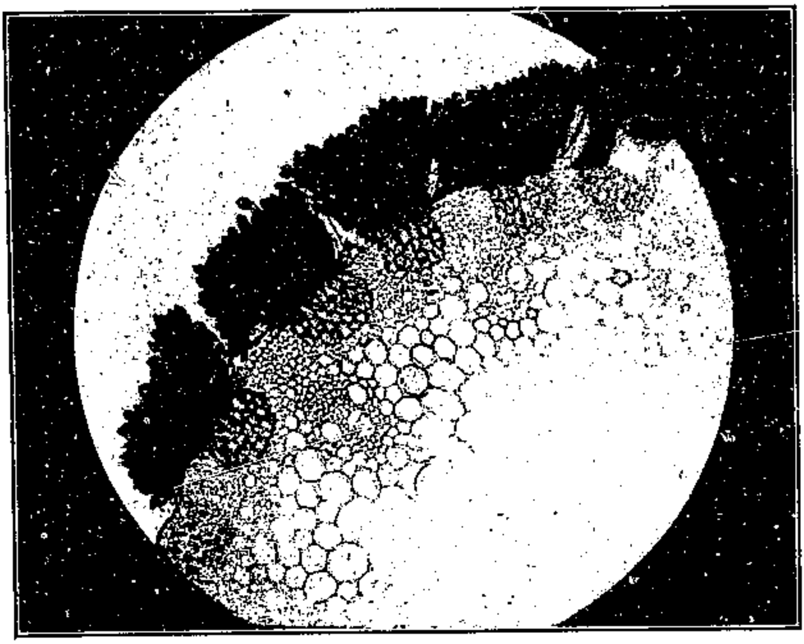


FIGURE 8.—Photomicrograph of a cross section of the rusted peduncle of *Agropyron repens*, showing the large fan-shaped pustules and the abundance of spores produced on the smooth stems of a highly susceptible host. In surface view the pustules appear as a continuous mass of spores, in spite of the fact that the infected collenchyma strands are separated by moderately broad bands of sclerenchyma. × 50

TABLE 2.—*Groups, based on the contour of the peduncle in cross section, of several wheat varieties and hybrids grown in the field at University Farm, St. Paul, Minn., each season from 1924 to 1928, inclusive*

Smooth culms	C. I. No.	Intermediate culms	C. I. No.	Deeply furrowed culms	C. I. No.
Triticum compactum:		Triticum vulgare:		Triticum durum:	
Little Club	4066	Kota	5878	Acme	5284
Triticum vulgare:		Hope	8178	Black Persian	2442
Quality	6607	Webster	3780	Akrona	6881
Dicklow	3663	Progress	6902	Iumillo	1736
Federation	4734	Sevier	6247	Kanred	5146
Hard Federation	4980	Ceres	6900	Kubanka	2094
Reward	8182	Minturki	6155	Mindum	5296
Haynes Bluestem	2874	Hybrids:		Nodak	6519
Garnet	8181	Marquis × Kota II-19-163		Pentad	3322
Axminster	8196	Kota × Ruby I-20-40		Velvet Don	1445
Marquis	3641	Kota × Kanred II-20-43		Triticum dicoccum:	
Parker's Marquis	8020	Kota × Kanred II-20-44		Khapli	4013
Ruby	6047	Kanred × Marquis II-17-40		Sonem	4402
Marquillo	6887			Vernal	3686
Hybrids:				Triticum monococcum:	
Kota × Ruby I-20-39				Einkorn	2433
Kota × Ruby II-20-32				Hybrids:	
Marquis × Kota II-19-162				Marquis × Emmer I-25-2	
				Khapli × Mindum (8 selections)	

THE LEAF

THE LEAF BLADE

The mature leaf blade of wheat has been fully described by Percival (*31, p. 53–61*), and a very good general description of a grass leaf has been given by Eames and MacDaniels (*14, p. 275–277*). The linear, parallel-veined leaf blade is composed of chlorophyllous parenchyma, vascular bundles, and lignified sclerenchyma, and is covered by a rather elaborate epidermis. The epidermis has been carefully described by Percival, so its structure will not be reviewed here.

The leaves of most wheat varieties have a large vascular bundle associated with the prominent midrib of the blade. The other vascular bundles are parallel with the median one, and large bundles usually alternate with smaller ones. A certain amount of sclerenchyma usually is associated with each bundle. The smaller bundles have no enveloping sheath of sclerenchyma, but there are small groups of sclerenchyma cells just beneath the epidermis and opposite the bundles. There are girders of sclerenchyma surrounding the larger bundles and extending to the lower and upper epidermal layers.

The chlorophyllous parenchyma lies between the vascular bundles and their sclerenchyma girders. These mesophyll cells are thin walled and irregular in shape, and so loosely arranged that there are numerous intercellular spaces. Usually there is no definite palisade layer, although the cells just beneath the lower epidermis sometimes are more or less isodiametric and compactly arranged.

Each mesophyll area is definitely separated from the neighboring mesophyll area by the large vascular bundles with their girders of sclerenchyma. (Fig. 9.) The rust fungus infects the mesophyll tissue but can not spread beyond the limits set by the sclerenchymatous girders. Thus the potential size of the rust pustule is determined by the structure of the leaf or by the sclerenchyma girders in the leaf. Although rather broad pustules are possible on the leaves of most wheat varieties, it is true that some leaves permit the formation of broader pustules than others. Figure 9 shows the mesophyll areas in comparable leaves of Marquis and Webster. In the leaf of Marquis the rust-susceptible mesophyll area is about one and one-half times as broad as that in the leaf of Webster.

The mesophyll areas in the leaves of spelt are even narrower than those in the leaves of Webster, because almost all of the vascular bundles in spelt are accompanied by sclerenchymatous girders which, although very narrow, extend to the epidermis and constitute effective barriers to the radial growth of the rust hyphae.

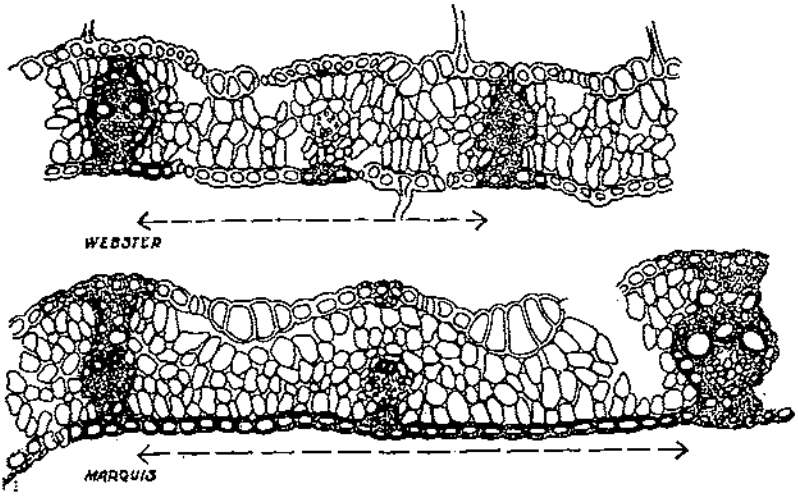


FIGURE 9.—Cross sections of mature leaf blades of Marquis and Webster wheats, showing the possible size of rust pustules as determined by the vascular bundles and their girders of sclerenchyma which separate the chlorophyllous areas of the leaf. × 60

Therefore, it is seen that morphological limitations are set for the rust in the leaves of cereal plants as well as in the peduncle. The practical importance of the leaf-blade structure, however, is not so great as that of the peduncle for several reasons: (1) The leaf blade is not so often infected as the peduncle or the leaf sheath, (2) rust infection on the leaf blade usually is not so destructive as infection on the peduncle, and (3) the mesophyll areas in the leaves of all varieties are so broad that there is ample space for large pustules even though the rust-susceptible tissue may be more extensive in some varieties than in others. The leaf blades of spelt probably have more numerous and more closely placed sclerenchyma girders than the leaf blades of any of the wheats, but even in spelt fairly large pustules can be formed, and the white awnless spelt grown in the rust nursery at University Farm, St. Paul, has been very severely rusted. The structural limitations to rust development in the leaf blade are not very effective if the leaf is infected with virulent rust forms, but they make necessary a larger number of infections in leaves of certain varieties if the rusted area is to be as extensive as it is in varieties with less decided structural limitations.

THE LEAF SHEATH

Stem rust usually appears on the leaf sheath before it is found on any other part of the plant. Sometimes the pustules are numerous just above the swollen base of the leaf sheath before any other part of the plant is infected. In taking field notes on stem rust under conditions of natural inoculation, the estimates of infection often are based largely on the rust on the lower part of the leaf sheath.

In different parts of the leaf sheath there is variation in structure. A cross section of a culm near the leaf blade is illustrated in Figure 10. The leaf sheath encircling the stem is rather thin, and its margins taper to delicate parenchymatous membranes. The epidermis of the outer surface is partially lignified and all of its cells have thickened walls, but the epidermis on the inner surface is composed of thin-walled parenchyma. The vascular bundles of the sheath are continuous with those of the blade, and each one is accompanied by a sheathing strand of sclerenchyma extending from the bundle to the outer epidermis. The chlorophyllous tissue, between the strands of sclerenchyma and just below the outer epidermis, usually is not more than three or four cells deep. The inner part of the sheath is made up of colorless parenchyma, and in the mature sheath of almost every variety there are large lysigenous cavities in the parenchyma, beneath the chlorophyllous cells and between the vascular bundles. The rust enters through the stomata in the outer epidermis and the mycelium ramifies the chlorophyllous tissues. In cases of severe infection great masses of hyphae fill the lysigenous cavities and force the parenchyma cells apart. The spores are formed just beneath the outer epidermis in most cases, but occasionally spores appear in the inner parenchyma region and rupture the inner epidermis. (Fig.

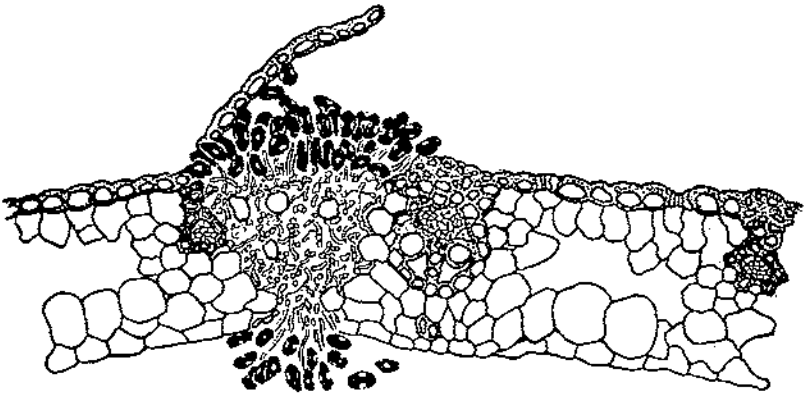


FIGURE 10.—Cross section of the leaf sheath of wheat, cut a short distance below the leaf blade. The rust mycelium develops in the chlorophyllous tissue and the lysigenous space between the vascular bundles and their sheaths of sclerenchyma. Pustules may be formed on the outer surface of the leaf sheath or on its inner surface between the sheath and the inclosed stem. × 70

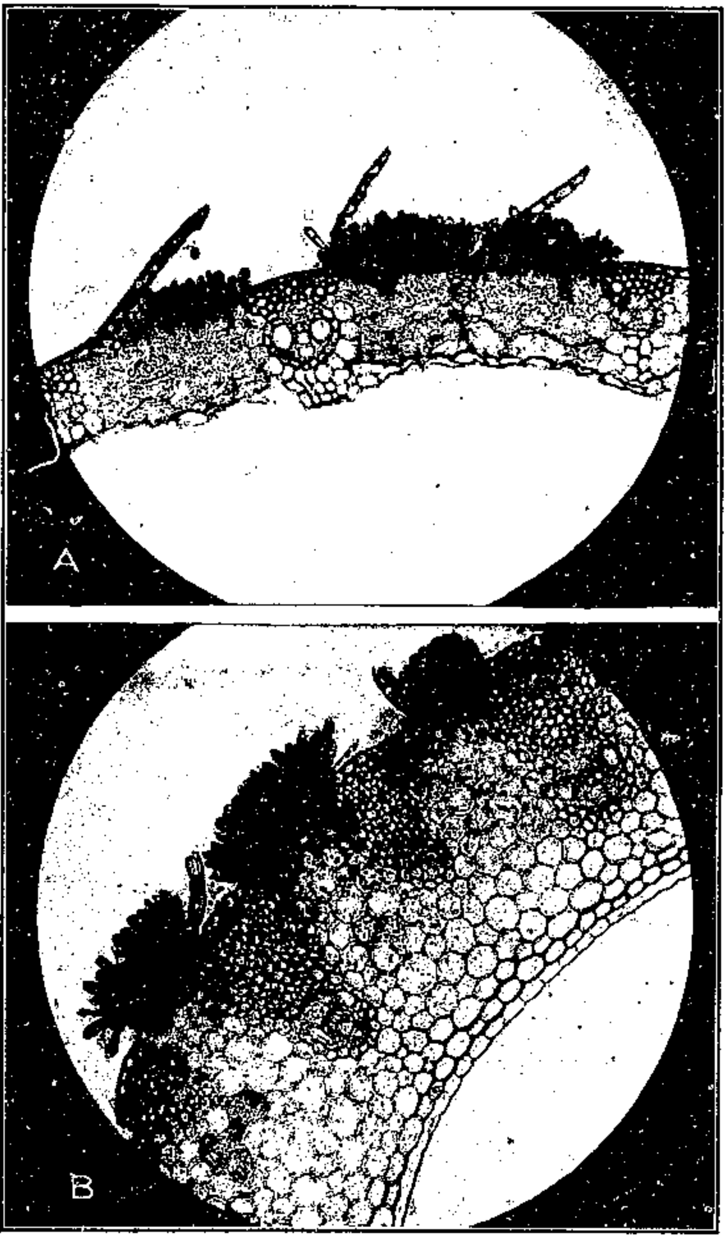


FIGURE 11.—A, Photomicrograph of the rusted leaf sheath of Ceres wheat, showing how the size of pustule is limited by the structure of the leaf sheath; section cut close to the leaf blade. B, Photomicrograph of a rusted leaf sheath cut about 1 inch above the leaf base. × 50

10.) Most of the pustules are formed on the outside of the leaf sheath, however, and the size is limited by the sclerenchyma strands and vascular bundles, just as the size of pustules is limited in the peduncles. (Fig. 11, A.)

About an inch above the node the entire sheath is thicker than it is near the leaf blade, the vascular bundles are farther from the outer epidermis, and there is more sclerenchyma between the vascular bundles and the epidermis. (Fig. 11, B.) Lysigenous cavities seldom occur in this part of the sheath; therefore, the size of the rust pustules is still limited by the sclerenchyma strands.

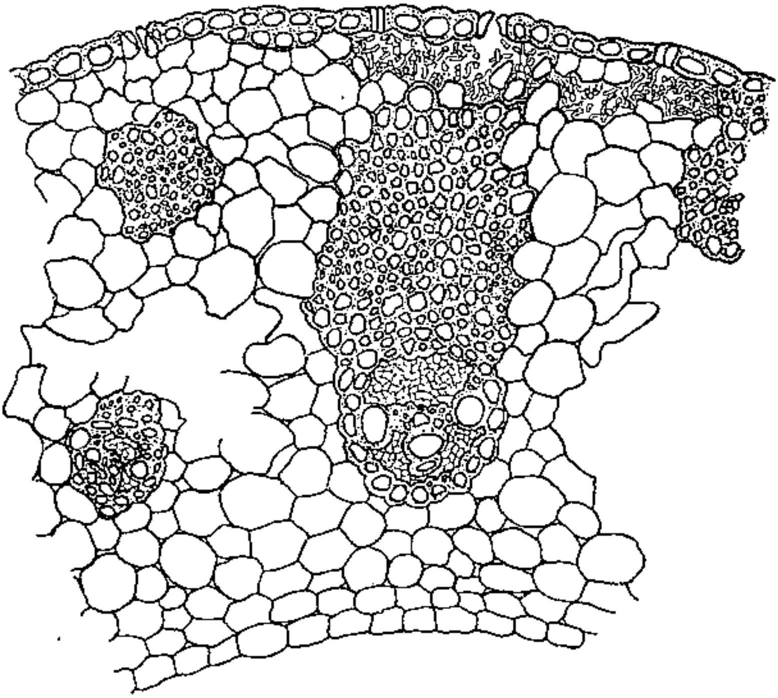


FIGURE 12.—Diagram of a cross section of a leaf sheath cut about one-eighth inch above the leaf base. × 100

Immediately above the swollen leaf base surrounding the node, the vascular bundles are more deeply embedded in the leaf sheath, and the sclerenchyma strands form semielliptical sheaths around the vascular bundles but seldom extend to the outer epidermis. The expansion of the rust mycelium is no longer stopped by a sclerenchyma girder, but may spread in the leaf sheath outside the embedded sclerenchyma sheath (fig. 12); consequently, the pustules may be larger in this part of the sheath than they are in other parts.

The structure of the swollen leaf base is still different from that of the rest of the leaf sheath. (Fig. 13.) This part of the culm is so tough and hard that sectioning is difficult. The vascular bundles are very deeply embedded and are nearer the inner epidermis than the outer one. Their semielliptical sheaths of sclerenchyma are also entirely embedded in the ground tissue of the leaf base, which consists of compact, appressed cells with thickened cellulose walls. The epidermis is composed of thick-walled cells that sometimes take a lignin stain. Epidermal hairs often are present, but there are no stomata on the swollen leaf base. Thus it is impossible for germ tubes of rust to enter the leaf base directly. The only opportunity for infection would be by means of the rust mycelium that developed in the susceptible tissue above or below the leaf base. This part of the culm generally is free from rust, for even in cases of very severe infection when the culm appears to be a solid mass of red-brown or black spores, the pustules stop abruptly above and below the leaf base. In only two cases have pustules been noticed on the leaf base, once on quack grass and once on Ceres wheat that had been heavily inoculated with a virulent from of rust. The infected Ceres was sectioned and studied. Evidently the rust had entered the leaf sheath above its swollen base and the hyphae had grown in all directions. The mycelium was found throughout the nonlignified tissues, although its progress in the leaf base must have been hindered by the thick cell walls of the ground tissue. Spores had been formed beneath the outer epidermis, and several pustules appeared on the leaf base. Spores also had formed within the tissues; thus there were numerous internal pustules with spores deeply embedded in the host tissues. (Fig. 14.)

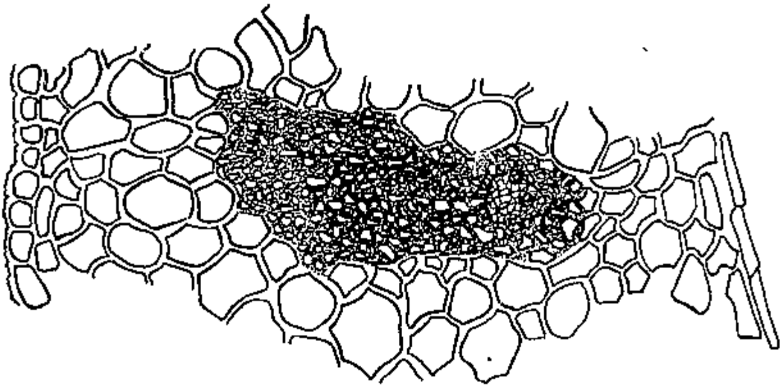


FIGURE 13.—Cross section of the swollen leaf base, showing the thick-walled ground tissue in which is embedded the vascular bundle with its semielliptical sheath of sclerenchyma. × 100

The leaf blade and sheath, compared with the peduncle, contain relatively large areas of rust-susceptible tissues. In all the wheat varieties studied, the areas of susceptible tissues were extensive enough to permit the formation of moderately large pustules. Nevertheless, there are definite structural limitations to the spread of rust in the blades and sheaths, and the possible size of a rust pustule is greater in some varieties than in others. In all varieties the swollen leaf base has a definite morphological resistance to rust. The slight morphological restrictions in the other parts of the leaf probably are never so effective a means of limiting rust development as is the structure of the peduncle. They are present, however, and may have some effect in cases of severe infection.

A structural peculiarity that may be more important in rust resistance than the extent of rust-susceptible tissue is to be found in the leaf sheath of einkorn. In einkorn the outer epidermis is often reinforced by a group of sclerenchyma cells placed between the epidermis and the chlorophyllous tissue. Such a reenforcing strand has not been noticed in any other cereal or grass yet examined.

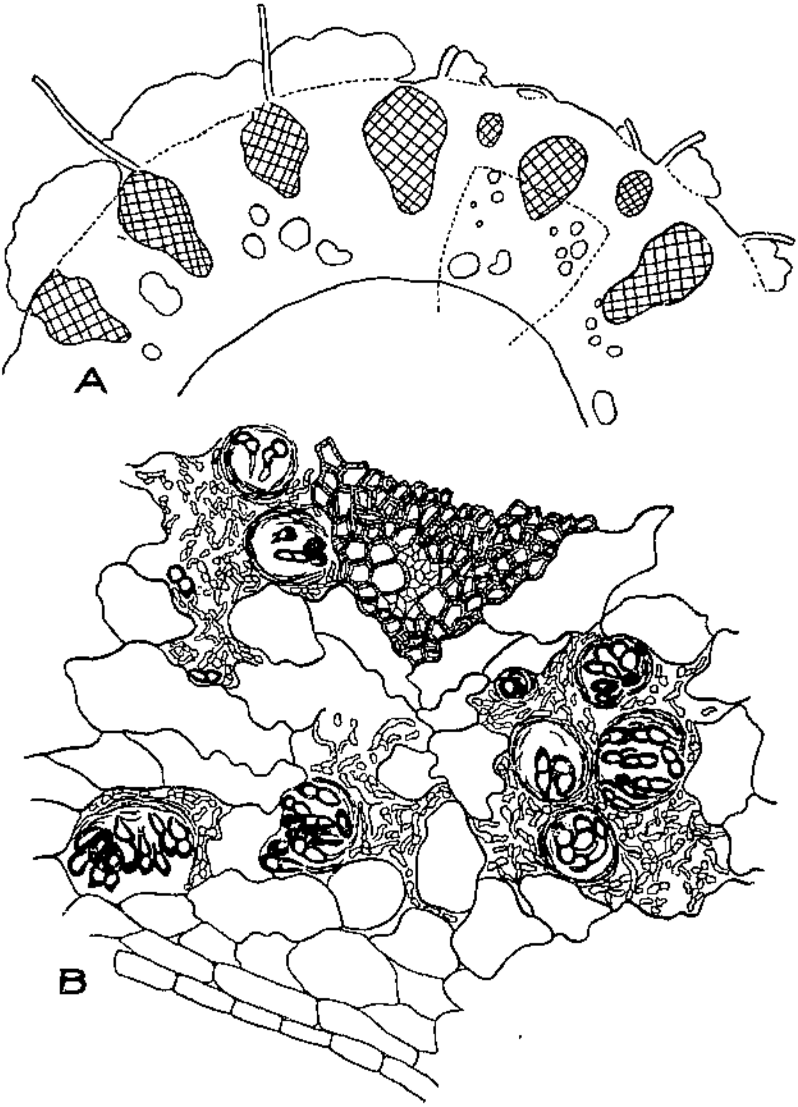


FIGURE 14.—A, Diagram of a cross section of the rusted leaf base of Ceres wheat. × 25. B, Camera-lucida tracing of part of the infected leaf-base tissue with several internal pustules. × 250

It undoubtedly would be a factor in morphological resistance in the leaf sheath, probably by increasing the resistance to rupture of the epidermis. This question will be discussed later.

THE RACHIS

Very often the heads of wheat, like the peduncles and sheaths, are rusted. The lower part of the rachis frequently is rusted on the convex side of the individual joints, but the flattened side of a joint never is rusted. At first one might think that this flattened side escaped infection because it was pressed against the inflorescence, but, on examining the structure of the rachis, no rust-susceptible tissue is found in that part. (Fig. 15.) The convex side of a rachis joint is structurally similar to that of the peduncle. Strands of chlorophyllous collenchyma are set in the sclerenchyma sheath and border on the epidermis. The vascular bundles form a broken cylinder within the sclerenchyma sheath and the inner part is made up of parenchyma.

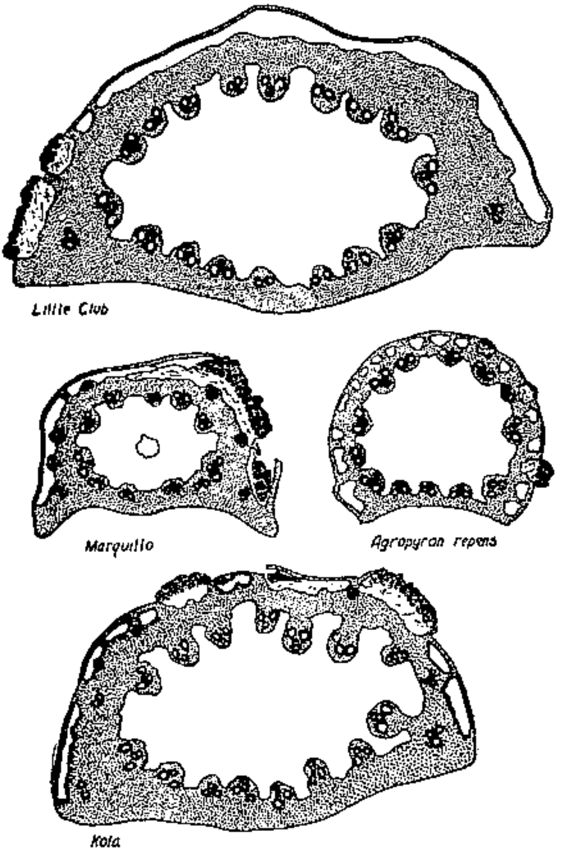


FIGURE 15.—Cross sections of the rachises of three varieties of wheat and of *Agropyron repens*, showing the variation in size of rachis and in the distribution of the collenchyma. × 25

The flattened side has no collenchyma, but just beneath the epidermis lies a wide and fairly deep band of sclerenchyma. Although the rachillar structures have not been compared in many different varieties, in the sections examined there have been indications of structural differences in the rachis as well as in the peduncle and the leaf of different varieties. The collenchyma areas in the rachis, as well as in the peduncle, are more extensive in the upper region of each rachis joint than in the middle and lower sections of a joint, so that it is necessary that corresponding parts be studied when varieties are being compared.

The rachis of Little Club (fig. 15) contains extensive collenchyma areas, its structure approaching that of the peduncle at a point very close to the head. One broad, confluent collenchyma area extends about three-quarters of the distance around the convex side of the rachis. In Marquillo the rachis is smaller and more slender than in Little Club. In Figure 15 the collenchyma of the Marquillo rachis is divided into three broad areas, the largest of which occupies about half of the convex side of the rachis joint. The other two areas are smaller, but are nevertheless quite broad. Infection evidently occurred at one side of the large collenchyma area, the mycelium has spread across two-thirds of the area, and spores are being produced in the earliest-infected part of the collenchyma.

In the rachis of Kota the collenchyma is broken up into many relatively narrow strands. Thus, in the joint sectioned (fig. 15) there are 10 distinct collenchyma areas on the convex side of the rachis, while in the rachis of Marquillo there are but 3. Each of the 10 strands must be infected separately, for the rust can not spread from one to another. As far as concerns the rachis, therefore, Kota has more structural protection against rust than either Marquillo or Little Club.

In this connection it is of interest to note the extreme division of the rust-susceptible tissue in the rachis of *Agropyron repens*. A section is shown in Figure 15 in which the collenchyma is divided into 20 narrow strands, 4 of which are infected with stem rust. It is obvious that each infecting germ tube is capable of doing far less damage in the rachis of quack grass than in that of Little Club wheat.

THE GLUME

The glumes also contain rust-susceptible tissues and often are rusted in the field. There are longitudinal strands of chlorophyllous tissue between the colorless bands of sclerenchyma. In cross section at about the middle of the glume (fig. 16), the largest areas of collenchyma are on either side of the midrib. Smaller strands are located near the margins of the glume. There is a great deal of sclerenchyma around the midrib of the glume, but on the inside and near its margins the cells have thick, nonlignified walls. The thick-walled cells seem impervious to rust hyphae, however, and the pustules are confined to the collenchyma strands. Pustules are often formed on the inner face of the glume as well as on the outer.

In some varieties, Khapli for example, there is no prominent midrib in the lower third of the glume. When the material is sectioned near the base, the chlorophyllous tissue is found distributed in more or less regular strands, one approximately as extensive as the next one, and all of them bordering on the outer epidermis. (Fig. 17.) In Marquis, Kota, and Ceres, however, the ridge of the midrib extends the entire length of the glume and the two largest areas of chlorophyllous tissue always lie on either side of it. There are smaller strands of the rust-susceptible tissue near the other vascular bundles of the glume. The entire width of the large strand may border on the epidermis, or only part of it may do so, the rest of the strand being embedded beneath the thick-walled cells which make up most of the ground tissue of the glume.

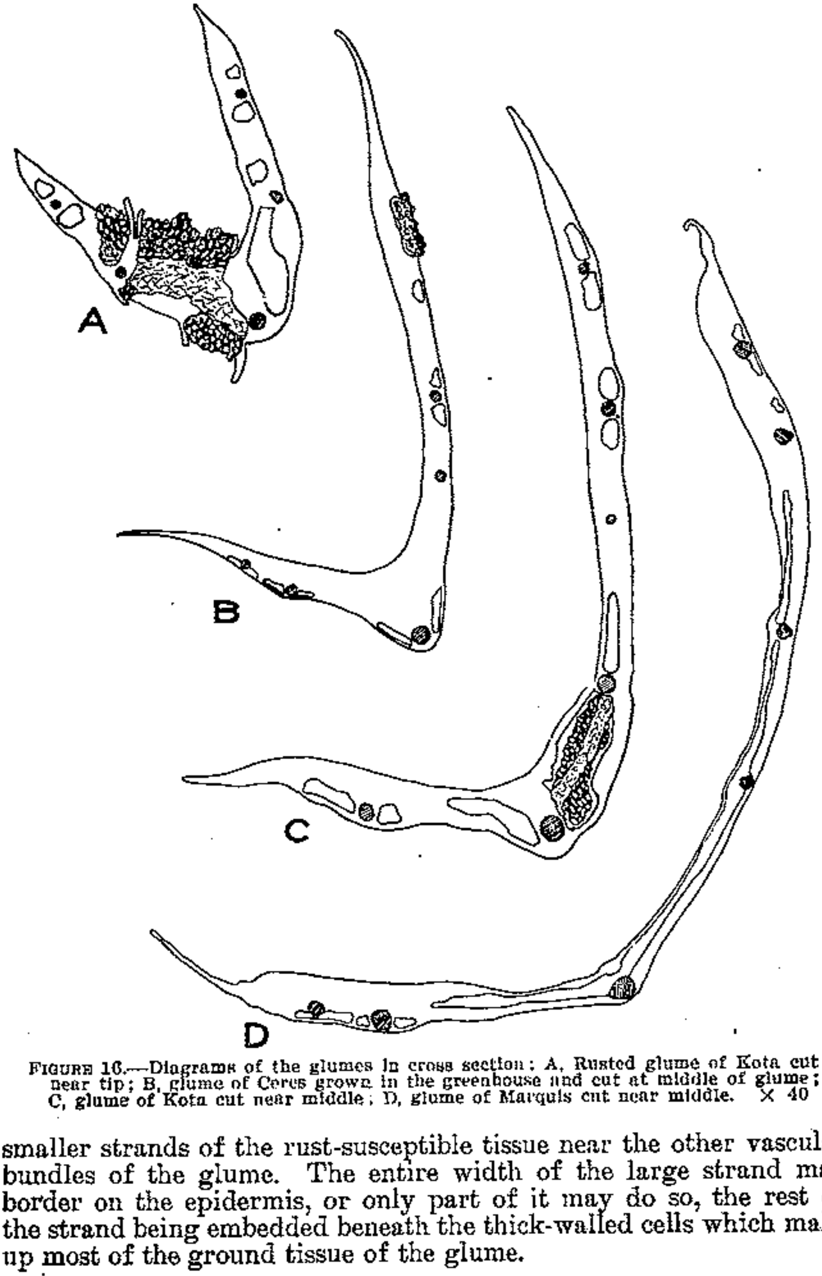


FIGURE 16.—Diagrams of the glumes in cross section: A, Rusted glume of Kota cut near tip; B, glume of Ceres grown in the greenhouse and cut at middle of glume; C, glume of Kota cut near middle; D, glume of Marquis cut near middle. × 40

The two larger chlorophyllous areas more often are infected with stem rust than the smaller strands, and if infection be severe there usually are more spores on the inner face of the glume than on the outer. (Fig. 16, A.)

The structure of the glumes has not been studied in sufficient detail to determine whether varietal differences in structure might influence rust infection. From Figure 16 it is evident, however, that the chlorophyllous areas beside the midrib in Ceres are very small and shallow when compared with the corresponding areas in Marquis. Ceres resembles Kota more closely. The glumes of Kota often are rusted, although they do not have so much rust-susceptible tissue as the glumes of some other varieties.

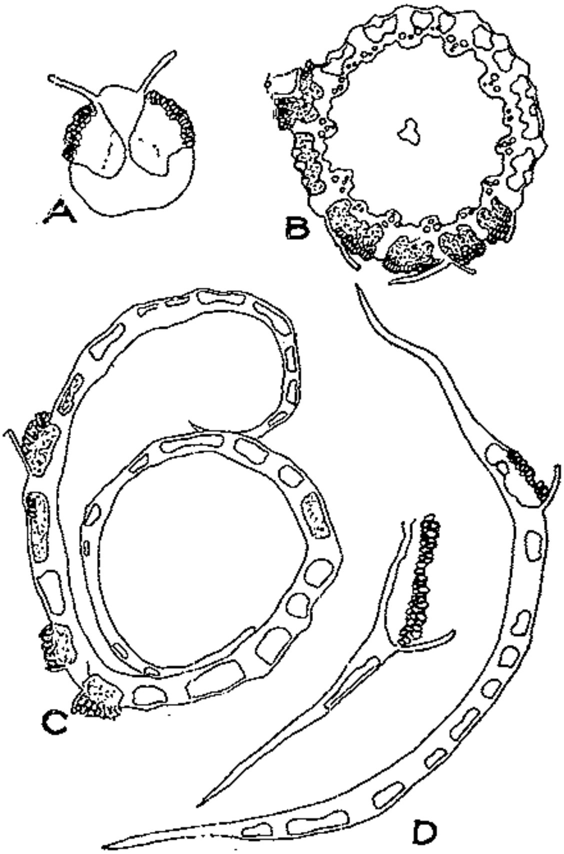


FIGURE 17.—*Diagrams of cross sections of Khapli emmer infected with Puccinia graminis tritici form 41*: A, Awn; B, peduncle; C, leaf sheath; D, glume near the base, and a small part of the glume nearer the middle. × 30

The glume becomes quite narrow at its tip, just before it tapers into the beak or the awn. At this level the two large chlorophyllous areas become even larger and more conspicuous in relation to the rest of the tissue, and the small insignificant, lateral collenchyma areas dwindle into minute patches of tissue and finally disappear altogether. Figure 16 shows the structure of Kota near the tip of the glume.

THE AWN

The awns, when present, are merely extensions of the midribs of the glumes. The median vascular bundle runs through the awn, and usually there also are two smaller bundles. In cross section the awn is triangular in shape and there are two collenchyma strands, one on each side of the large vascular bundle. (Fig. 18.) Sclerenchyma surrounds the collenchyma strands and the vascular bundles, but the center of the awn is filled with cells whose walls are thick but not lignified. The rust pustules are confined to the collenchyma strands. Even in the awns the collenchyma strands differ in different varieties of wheat. The collenchyma strands of Iumillo are more nearly surrounded by sclerenchyma and less of the strand borders on the epidermis than is the case in Ceres or Kota. In the awns of Khapli there also are relatively narrow collenchyma strands that limit the size of the pustules.

Each part of the head contains rust-susceptible tissue, although the proportion of susceptible to resistant tissue is less than in the peduncle or leaves. The most extensive areas of chlorophyllous tissue are those which occur near the top of each rachis joint, and sometimes large pustules are found on this part of the head. Stem rust on the lower joints of the rachis probably is fully as injurious as that on the upper part of the peduncle.

If the glumes are infected with stem rust, the pustules develop near the base of the glume, where the chlorophyllous tissue is most abundant, or along either side of the midrib, where the two largest strands of susceptible tissue are located. Pustules on the glume usually are very narrow, for they are greatly restricted by host structure, but there is no restriction to their longitudinal development.

Pustules on the awn likewise are narrowly linear, for only two strands of susceptible tissue run the length of the awn. If spores are produced in abundance, the pustule may spread out and cover an entire side of the long triangular awn.

Thus nearly all parts of the wheat plant contain tissues that are morphologically susceptible to stem rust. Infection occurs if the virulent rust forms gain access to those particular tissues which have protoplasmic as well as morphologic susceptibility. Pustules are formed only after the infecting fungus begins to produce spores and develops enough pressure to rupture the epidermis.

PUSTULE FORMATION

Different stages in the process of pustule formation are shown in Figures 19 and 20. The drawings were made from an infected leaf sheath, but the process is the same for pustules on all parts of the plant. The pathogene produces a considerable mass of mycelium throughout the susceptible chlorophyllous tissue and then begins to form spores. Thus considerable pressure is exerted within the affected tissue. All surrounding sclerenchyma is rigid and unyielding, but the epidermis is stretched and lifted by the underlying masses of spores and hyphae. (Fig. 19.) Finally, the epidermis breaks and is forced back. (Figs. 19 and 20.)

The number and size of pustules indicate the degree of infection of any wheat variety. In the field, unless a rust form is capable of producing abundant mycelium and spores, it can not be determined whether the rust may have entered the plant. Nevertheless, there are many cases of abnormal pustules and incomplete development of the infecting rust.

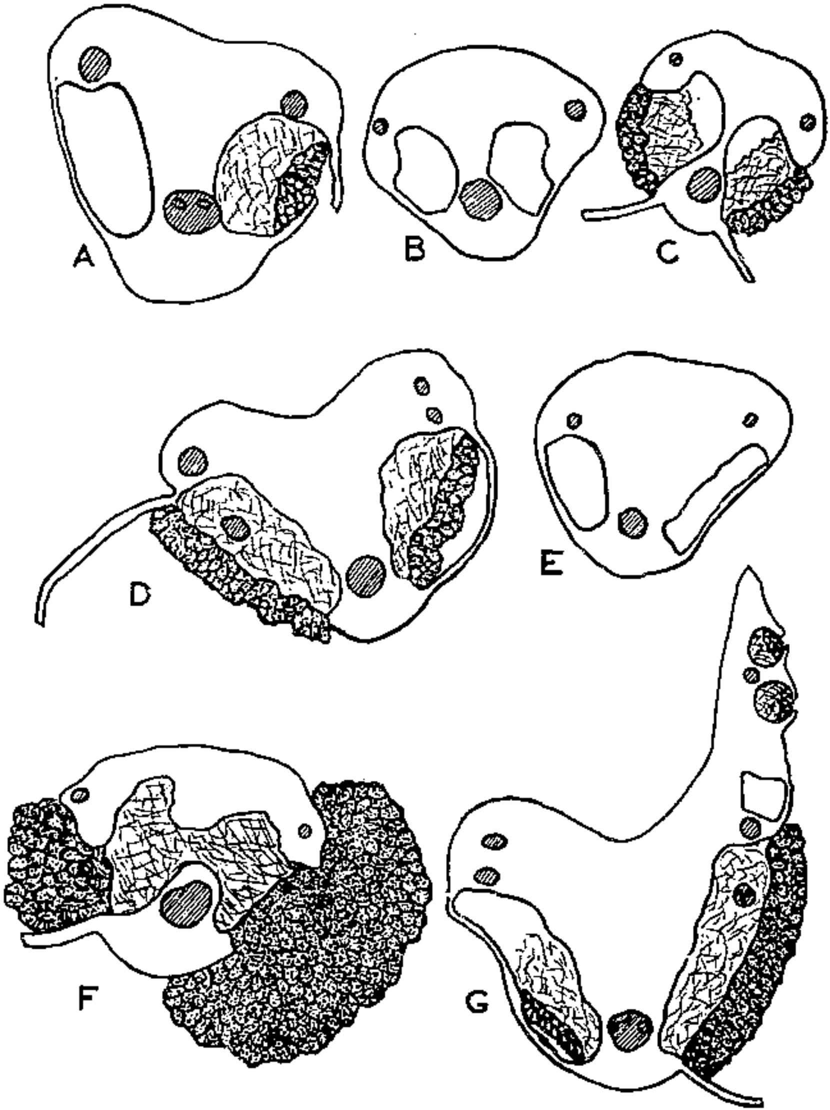


FIGURE 18.—Diagrams of cross sections of the awns of several wheat varieties; A, Kota; B, Iumillo; C, Khapli; D, Ceres; E, H—44; F, Ceres, near tip of the awn; G, Ceres, in the transition area between the glume and near the base of the awn. × 50

Sometimes the germ tubes enter the stomata, and the collenchyma becomes so severely infected that a great mass of mycelium is produced without forming spores. In this case infection is discernible only by means of a microscope. In some cases the mycelial development is so great that the epidermis is slightly distended. and protrusions are easily felt if the culm is drawn between the fingers. In some seasons Khapli emmer, growing on University Farm, St. Paul, may have many of these obscure infections. In other varieties numerous normal pustules may appear on the culms along with a few or many mycelial infections that are only microscopically evident. This often has been the case in Kota, Webster, Marquillo, Ceres, Khapli, Mindum, and Kubanka (C. I. 2094).

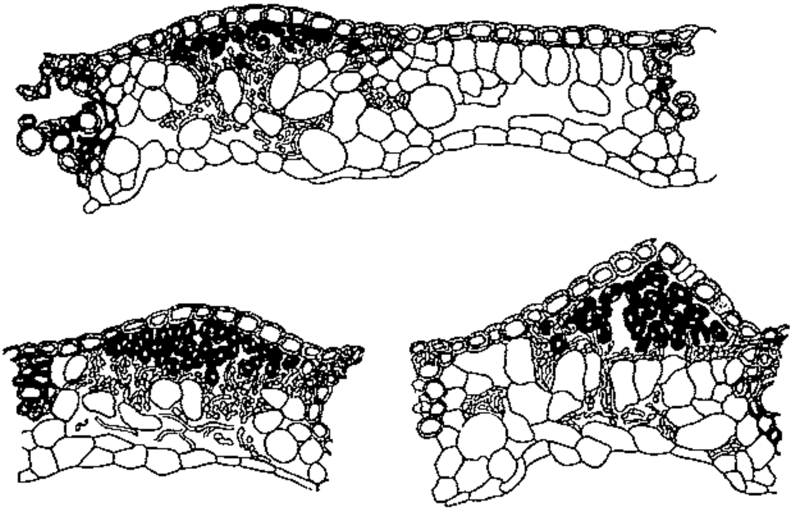


FIGURE 10.—Cross sections of the leaf sheath, showing different stages in pustule formation. The epidermis is distended and finally ruptured by the mass of mycelium and spores beneath it. × 60

At other times the infection begins normally. Spores are formed just beneath the epidermis, but the rust is unable to exert sufficient pressure to rupture the epidermis. In these cases the infection is apparent, for the dark spore masses can be seen beneath the semitransparent epidermis. Such imperfectly developed pustules have been found in great abundance on Webster and in fewer numbers on Kota, Ceres, and Marquillo, and on some of the durums. It is probable that the protoplasmic resistance of a variety hinders the development of the rust so much that its growth pressure is insufficient to rupture the epidermis. In addition, however, there is a possibility that such morphologic factors as size of collenchyma strand and structure of the epidermal membrane affect the rupturing of the epidermis and influence the formation of pustules quite as much as the physiologic reaction of the wheat and the mass development of the fungus.

RELATION OF SIZE OF COLLENCHYMA STRANDS TO FORMATION OF PUSTULES

It is impossible to determine precisely the force required to rupture the epidermis, but some general conclusions may be drawn regarding the relative amounts required in different types of collenchyma strands. In the early stages of pustule formation the distended epidermis behaves more or less like a spherical membrane and the pressure per unit area is dependent on the tensile strength of the membrane and the radius of the sphere of which it is a part. Thus, $p=\frac{2t}{r}$ where p=pressure per unit area of the membrane, t=tension per unit length of the membrane, and r=the radius of the sphere of which the membrane is a part. In a wheat variety containing several different types of collenchyma strands the distended epidermal membrane over a narrow, isolated strand infected with rust would have a greater curvature than that over a broad, confluent strand. (Fig. 21.) The greater the curvature, the lower will be the value of r; and, if it is assumed that the tensile strength (t) of the two membranes is the same, the value of p, or the force per unit area required to rupture the epidermis, is greater in the case of the single narrow strand than the broad, confluent one. Likewise, the force per unit area required to rupture the epidermis of the peduncle is greater than that required to rupture the epidermis of the leaf sheath, for the angle of curvature of the membrane over the infected leaf sheath would be less and the value of r would be relatively high.

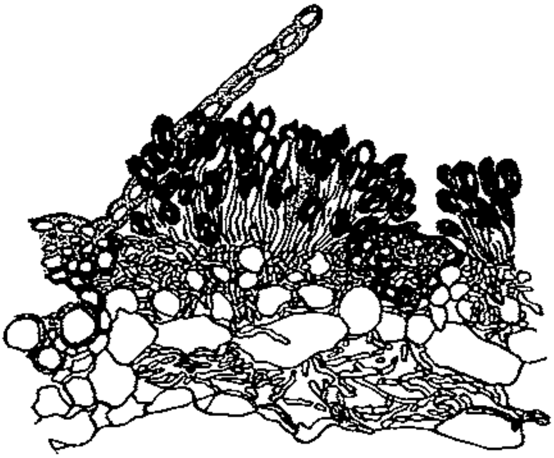


FIGURE 20.—Cross section of a mature and normal pustule on the leaf sheath of Ceres wheat. The epidermis is broken and forced out, and the mass of spores has pushed out beyond the periphery of the culm. × 80

Other factors being equal, it is plausible to assume that the epidermis of the peduncle is more easily ruptured in Marquis, Quality, and other varieties having broad confluent collenchyma strands than in Kota or Webster or other varieties with narrow, isolated strands.

The mycelial masses and the imperfectly developed pustules were found in none of the common wheats that have extensive areas of collenchyma. In the studies here reported they were observed only in varieties of *Triticum vulgare* characterized by narrow collenchyma strands. Similar infections have been found on the durums and emmers, but in these cases the collenchyma strands are only partially fused into broad, confluent collenchyma areas. The groups of sclerenchyma cells beneath the epidermis and between the component parts of the rust-susceptible area are so arranged that only small parts of the area actually border on the epidermis. Usually a pustule is confined to one or two of the individual parts of the area, and it is only in cases of very severe infection that the entire area is converted into one large, broad pustule. The epidermis over one individual part of the collenchyma area may act as a spherical membrane distinct from the other membranes over other parts of the area, so that pustule formation may be almost as difficult as in a single narrow strand of a common wheat.

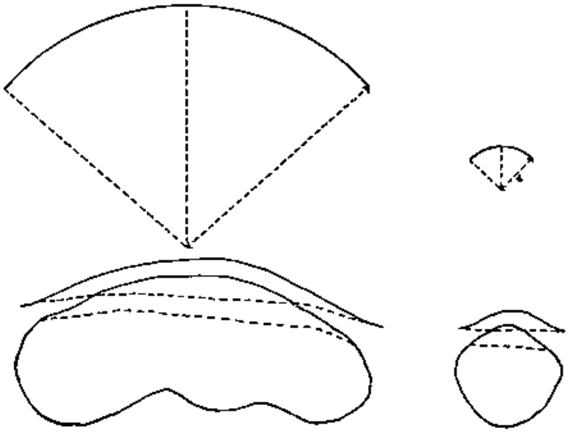


FIGURE 21.—Diagrams illustrating the distention of the epidermis over broad and narrow collenchyma strands. The broken lines in the lower figures indicate the position of the epidermal membrane before the collenchyma is infected by rust, while the solid lines indicate its position after distention by the fungus mass. The arcs and the corresponding radii of their circles show how the value of r varies in the two cases. × 115

If the tensile strength of the membranes of all varieties were the same, it might be concluded that pustule formation always would be more difficult in narrow collenchyma strands than in broad ones. It seems reasonable to assume, however, that there is considerable variation in tensile strength of the epidermis in different wheat varieties, just as there is in size of collenchyma areas or any other measurable character. If it were possible to determine tensile strength of the epidermal membrane, accurate data could be obtained on the forces expended in pustule formation in different varieties. At present, however, efforts are confined to a study of different factors that may affect the tensile strength of the membrane. The epidermis varies in structure in different parts of the plant, and it is probable that slight structural differences in the epidermal membranes of certain varieties may have a profound effect on the tensile strength and the resistance to rupture of these membranes.

STRUCTURE OF THE EPIDERMIS AND ITS RESISTANCE TO RUPTURE

Percival has fully described the epidermis of different parts of the wheat plant (*31, p. 52, 53–56, 92, 93, 107–109*). The epidermal cells in seedlings are very similar to ordinary parenchyma cells. In surface view some of the cells are long and rectangular and arranged end to end along the blade; the rows of these are interrupted occasionally by smaller cells, square in outline. All these cells have thin, straight, cellulose walls, thus constituting a delicate, flexible epidermis over the mesophyll of the seedling blade. The bulliform cells of the upper epidermis are shorter but deeper than the rest of the epidermal cells; and their walls are thin and elastic, providing for the rolling and well-known adjustment of the leaf blades of cereals to unfavorable humidity. On either side of the rows of bulliform cells, stomata are arranged in one or two parallel rows. The entire membrane is thin and delicate, and, if it contained chlorophyll, probably would be as often infected and would furnish as much nutriment for the rust fungus as do the mesophyll cells. In fact, haustoria often are formed in the epidermal cells when the mycelium is abundant in the intercellular spaces bordering the epidermis. When pustules are formed the spores are aggregated just beneath the epidermis, and small portions of the membrane are distended and broken, without any indication of a definite resistance to rupture. Sometimes, when rust development is exceedingly rapid and vigorous, spores push out between the epidermal cells without lifting any part of the membrane. In such cases, since the epidermal cells are too weakly cemented to act as a restraining membrane, they are pried apart by the young spores. Apparently there is only slight and ineffective resistance to rupture in the epidermis of the seedling.

The epidermis over the collenchyma in the peduncle consists of a single layer of more or less elongated cells with sinuous, lignified walls. A few small, isodiametric cells occur at intervals. This membrane, interrupted only by the stomata, extends as a protective covering over the collenchyma of the stem. The epidermis over the sclerenchyma of the peduncle usually is made up of narrower cells, and it has a tendency to merge with the other small-cell lignified tissue and become part of the sclerenchyma band rather than to remain as a distinct covering layer. It is the epidermal membrane over the collenchyma strand that is important in the process of pustule formation, and, when compared with the delicate membrane of the seedling, it seems to be a hard, tough layer with its sinuous, heavily lignified cell walls so firmly cemented together that a great deal of force is required to break it.

The epidermis of the leaf sheath is similar to that of the peduncle. Most of the cells are elongated, and the walls are sinuous and greatly thickened. Not all of the cells, however, have lignified walls. The walls of the epidermal cells covering the chlorophyllous cells often are composed of cellulose, with no indications of lignification. The degree of lignification of the epidermis of the leaf sheath and the leaf blade varies greatly, but no reliable data have yet been secured to determine whether it is constant in a given variety. Sometimes lignification is complete, and all cells covering both the chlorophyllous and strengthening tissues take the lignin stain. At other times only the epidermal cells over the strengthening sclerenchyma and the three or four rows of epidermal cells adjacent to them are lignified. The walls of the epidermal cells over the central part of the chlorophyllous area are thickened but not lignified. In other cases lignification is closely restricted to those epidermal cells that border the sclerenchyma, and the entire strand of collenchyma is covered by epidermis made up of cells with cellulose walls. Whether the degree of lignification affects the resistance to rupture in the epidermis has not been determined, but it is reasonable to suppose that the quality of the membrane may be of very great importance. It seems possible that a membrane composed of cells with thickened cellulose walls would be more pliant and have a greater elasticity than one made up of cells with thickened lignified walls, very effective as supporting tissue, but easily broken when subjected to stretching and distention caused by the fungus mass. The quality of the epidermal membrane, as well as certain other characters, should be studied in relation to pustule formation.

THICKNESS OF THE EPIDERMIS

The thickness of the epidermal membrane probably is almost as important as the quality in determining its resistance to rupture. Given two membranes of the same quality and the same extent, but differing in thickness, it is certain that the thicker membrane would break less easily than the thinner one.

Measurements were made of the epidermal membranes of the peduncles of several wheat varieties, and in a few membranes the outer cell walls with their layers of cuticle were measured separately. The results are given in Tables 3 and 4. In Little Club, Ruby, and an unnamed variety (C. I. 3465), the epidermal membrane is relatively thin. (Table 3.) These three varieties have protoplasmic susceptibility to several rust forms and they usually rust copiously in the field. The pustules on these varieties are nearly always normal and fully developed, and there is no indication of subepidermal spore masses. Apparently the epidermis is broken rather easily. In other varieties, for example, Marquis, the epidermis is only slightly thicker than that of Little Club. Marquis also has a protoplasmic susceptibility to a large number of rust forms and often is heavily rusted in the field. Most of the rust forms appear sufficiently virulent and powerful to break the epidermis without difficulty. In only a few cases have subepidermal pustules been noted on Marquis, and in these cases the few abnormal pustules were accompanied by numerous fully developed and normal ones.

TABLE 3.—*Measurements of the thickness of the epidermal membrane over the collenchyma of the peduncle in different wheat varieties grown in the field at Coon Creek or at St. Paul, Minn., in 1927*

Variety	Thickness of epidermis	Variety	Thickness of epidermis
	Microns		*Microns*
Marquis	31.5	Webster	38.2
Ruby	29.2	Hope	33.6
Little Club	30.6	Marquillo	34.5
No. 3465 [1]	29.9	Velvet Don	35.0
Kota	35.0	Acme	35.7
Ceres	31.1	Khapli	40.0

[1] Selection from an introduction from Egypt, C. I. 7261, and grown at University Farm in 1927. It is very susceptible to stem rust.

Some varieties have considerably thicker epidermal membranes. The epidermis of Khapli seems to be extremely thick, and that of Webster, Acme, Kota, and Velvet Don also is very thick, as compared with the epidermis of other varieties.

If the epidermis of the peduncle is examined in detail it is found that the outer walls of the epidermal cells are thickened much more than the lateral and inner walls. The exterior cell wall, with its accompanying layer of cuticle, probably plays an important part in determining the rigidity and the strength of the epidermis. Cobb (*13*) studied the structure of the epidermis in the leaves of several wheat varieties in Australia and found that the cell walls in the rust-resistant Ward's Prolific were twice as thick as those in the rust-susceptible Zimmerman. Twelve varieties were examined by Cobb. With one exception, the cell walls were thicker in the resistant wheats than in the susceptible ones. Cobb was of the opinion that the structure of the epidermis was a very important factor in rust resistance, a thick tough epidermis preventing the fungus from ripening and disseminating its spores.

In addition to the measurement of epidermal membranes, a few measurements were made of the outer cell walls and the cuticle layer of the epidermis. (Table 4.) The peduncles of only a few varieties were used, but, from the measurements obtained, it is evident that there are distinct varietal differences in this character as well as in the thickness of the epidermal membrane. The outer cell wall of the epidermis of Webster is extremely thick, while that of C. I. 3465 is relatively thin. The cell walls in Hope are thicker than the walls of Kota and Marquillo in spite of the fact that the thickness of the epidermal membrane of Hope is less than that of the membranes of Kota and Marquillo. In Quality and C. I. 3465, two highly susceptible varieties, the outer cell walls are thin and, since the entire epidermis is thin, there is less resistance to rupture.

TABLE 4.—*Measurements of the thickness of the outer wall and cuticle of the cells in the epidermis over the collenchyma of the peduncle in several wheat varieties and the rust reaction of those varieties when grown in the field at Coon Creek or at University Farm, St. Paul, Minn.*

Variety	Source	Rust reaction[1]	Thickness of cell wall
			Microns
Marquillo	Coon Creek	30 SR	6.33
Kota	do	30 SR	6.87
Webster	do	60 R	7.66
Hope	University Farm	Tr.	7.17
Quality	do	45 S	5.62
No. 3465	do	100 S	5.63

[1] Numbers indicate percentage of infection; S=susceptible; R=resistant; Tr.=trace.

The effect of thick epidermal membranes on pustule formation often is evident in Kota and Webster, two varieties which have been studied particularly in the field. These two varieties have a moderate protoplasmic resistance to a large number of rust forms. Webster has been moderately resistant to all forms with which it has been inoculated (*42*), but Kota is resistant to some and susceptible to others (*41*). Kota and Webster also have a morphologic resistance to stem rust because of the distribution of rust-susceptible tissues of the peduncle in narrow isolated strands that are sunken in the furrowed stems. Moreover, their relatively thick epidermal membranes often prevent the development of normal rust pustules. Figure 22, A, represents a section through an infected collenchyma strand in the peduncle of Kota. The epidermis has just been broken, and it is evident that the membrane is so thick and tough that before the epidermis was ruptured the sclerenchyma bands were cracked and split by the force exerted by the fungus. (Fig. 22, A, *a*.) The force has been great enough even to deform the spores slightly. The spore walls assume the contours of the epidermis against which they have been pressing. (Fig. 22, A, *b*.)

A more extreme case illustrating the effect of thickness of epidermal membrane is shown in Figure 22, B. This shows a cross section through an infected collenchyma strand in the peduncle of the variety Webster. The epidermis apparently is so thick and tough that it does not yield to the pressure of the fungus, and as an increasing number of spores are produced the more delicate collenchyma is crushed when the rigid epidermis does not yield.

In both these cases, and especially in the infected Webster, there is a possibility that protoplasmic resistance is an important contributing factor. A rust form grows less rapidly and vigorously in a moderately resistant host than in a susceptible one, and it is reasonable to assume that in the former the fungus can not exert so much pressure as in the latter. Unless the fungus is very weakly parasitic it is probable that a number of small but normal pustules will be formed. If, on the other hand, the moderate protoplasmic resistance of the host is enhanced by a very limited space for development and by a particularly thick and tough epidermal membrane, it often happens that the pustules are formed with great difficulty or remain buried in the host tissues. Subepidermal pustules are very common in Webster, and occur in the leaf sheath as well as in the peduncle. (Fig. 23.) Very often at least 60 per cent of the pustules on the culm of Webster never break the epidermis. Spores may be formed in abundance, but they remain within the host.

A similar phenomenon might readily occur in Khapli, although it is not so often apparent. Khapli has a protoplasmic resistance to virtually all of the rust forms occurring in the United States, and when stem rust gains entrance to the culm of Khapli it seldom produces an abundance of spores. Mycelium sometimes is produced so abundantly as to distend the epidermis, but no dark mass of spores is evident beneath the membrane to show that infection has taken place. In the greenhouse, *Puccinia graminis tritici* form 41 is very virulent on Khapli and attacks plants in all stages of development.

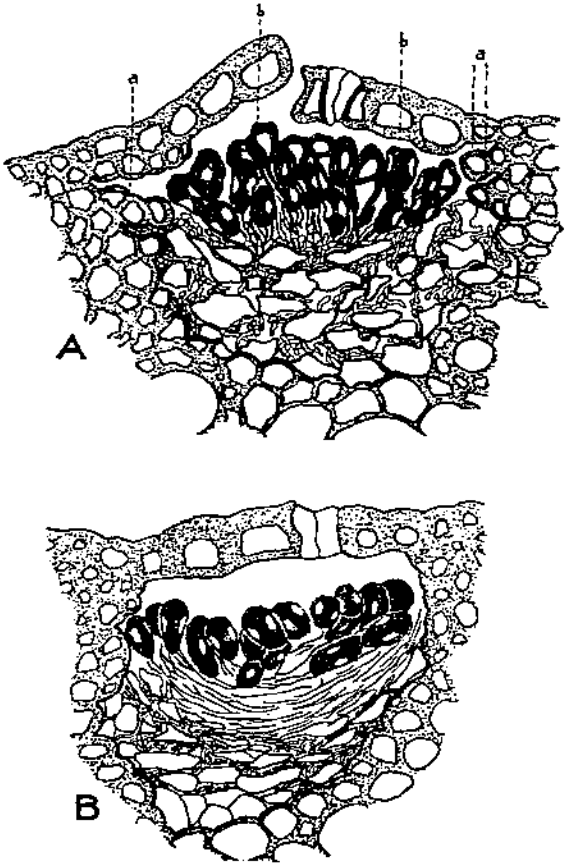


FIGURE 22.—Diagrams of cross sections of rusted collenchyma strands in the peduncles of two varieties of wheat. A, Kota. The epidermal membrane is thick and tough and before it is broken the sclerenchyma bands are cracked (*a*). The resistance of the membrane is so great that some of the spores are slightly deformed (*b*). B, Webster. The fungus is unable to break the epidermis and the collenchyma is crushed by the mass of urediniospores. × 250

Khapli is so favorable a host for this rust form that the latter grows rapidly and luxuriantly. Most of the pustules formed are normal in spite of the thick epidermis, but there is evidence of difficulty in the breaking of the membrane, and occasionally pustules are not formed on the infected areas.

Sometimes the epidermis is reenforced by sclerenchyma cells, constituting a more complex and irregular membrane than the single-cell layer that usually is considered.

REENFORCEMENT OF THE EPIDERMIS

Reenforcing strands of sclerenchyma cells beneath the epidermis are very common in the durums and emmers, in which the individual collenchyma strands are only partially fused into broad and extensive collenchyma areas and are separated by the strands of sclerenchyma which lie just beneath the epidermis but do not form a complete band in the general sclerenchyma sheath of the peduncle. (Fig. 24.) In a cross section of the stem the reenforcing strands always form the prominent ridges, for the sclerenchyma is in a compact mass approximately as deep as it is wide. These sclerenchymatous masses tend to make the epidermis even more heterogeneous than it usually is and they divide the entire covering layer of the broad collenchyma area into smaller units. These often behave as narrow individual membranes rather than as part of one wide membrane. Thus it sometimes happens that a number of distinct pustules form when one large collenchyma area is infected, and only in exceptional cases is the entire area converted into one large pustule. Figure 24 shows that two separate pustules have been formed in one of the double collenchyma strands in the peduncle of Khapli emmer. In the neighboring double strand of collenchyma one large pustule is forming and the entire membrane, with its central reenforcement of sclerenchyma, is being lifted and pushed out as one unit. Although *P. graminis tritici* form 41 is extremely virulent on Khapli, the sclerenchyma mass reenforcing the epidermis sometimes is effective in limiting the size of the pustule. The same is true also of the other emmers and durums.

FIGURE 23.—Cross section of an infected leaf sheath of Webster wheat. Spores are produced in abundance but the epidermis is not broken and the pustules remain submerged in the host. × 100

Reenforcing strands of sclerenchyma are more unusual in the common wheats. They are not found in such varieties as Quality and Little Club, which have broad, confluent strands of collenchyma, but they may occur in other varieties, such as Kota, Ceres, Marquillo, Webster, and Progress. All of these wheats have few or several double collenchyma strands that appear as though two narrow strands were fused. When the sclerenchymatous reenforcements occur they are just beneath the epidermis and between the two parts of the double strand. The reenforcing strands are never so strongly developed as in the durums and emmers. Often they are merely three or four longitudinal rows of cells aggregated in a mass that does not greatly affect the epidermal membrane nor divide it into distinct units. Occasionally they are sufficiently developed to limit the size of rust pustules as effectively as the reenforcing sclerenchyma sometimes does in the durums.

Another effective reenforcement of sclerenchyma has been noted in the leaf sheath of einkorn. (Fig. 25.) In this section the sclerenchyma is in a broad strand and not crowded into a deep mass. The membrane over the infected tissue is thus strengthened along a large part of its width and still acts as a single membrane rather than as two units of membrane separated by sclerenchyma. No such reenforcements have been found in the leaf sheaths of any other varieties.

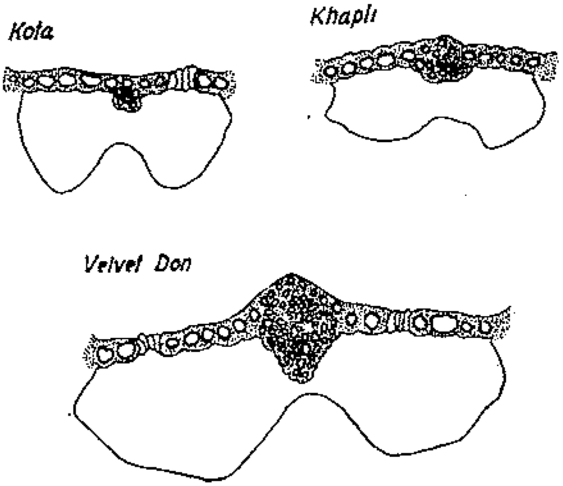


FIGURE 24.—Diagrams from peduncles of common wheat (Kota), durum (Velvet Don), and emmer (Khapli), showing reenforcing sclerenchyma beneath the epidermis and between the parts of compound collenchyma strands. × 115

According to general observations and detailed examinations it seems certain that the structure of the epidermis is an important factor in the formation of pustules. It is generally recognized that the rust fungus often has difficulty in gaining entrance to its host and establishing itself within the tissues, but it is not so generally realized that there may be great difficulties involved in breaking through the epidermis of the host after extensive mycelial growth and abundant spore production have occurred. The structure of the infected area and the nature of the protective membrane are factors that greatly influence the liberation of the rust spores from the infected host after the fungus has completed its development in the area in question.

The development of the fungus in a given area may be slow or rapid, depending on a number of factors, such as compatibility of the host and parasite, light, temperature, and other external factors. In the peduncle the rate of development of rust may be profoundly affected by the structure of the infected tissue, and the extension of the mycelium may be hindered by morphological peculiarities of the collenchyma, as well as by the sheath of sclerenchyma surrounding the susceptible collenchyma.

STRUCTURE OF THE COLLENCHYMA

If the cell walls of the chlorophyllous tissues in the peduncle are irregularly thickened, as are the walls of other collenchyma, it seems probable that the fungus would have more difficulty in sending haustoria through them than through the thin walls of ordinary chlorophyllous parenchyma cells. The rust mycelium might ramify the tissue at a slower rate, and a longer time would be required for the development of a mass of mycelium sufficient to produce an abundance of spores and form conspicuous rust pustules. This might then be one reason for the differences in length of incubation period in the seedlings and in the peduncles of mature plants. In a physiologically susceptible seedling the fungus advances rapidly in the mesophyll tissue, sending numerous haustoria quickly and easily through the thin cellulose walls of the cells. The development is so rapid that the first pustules usually appear on the seventh day after inoculation, and by the twelfth or fourteenth day pustules may be fully formed and accurate rust notes taken. When, on the other hand, the peduncles of mature plants are inoculated with virulent forms, the rust mycelium progresses more slowly and the first pustules generally appear from 12 to 15 days after inoculation. Very often rust notes are not taken until about the twenty-first day and sometimes even later.

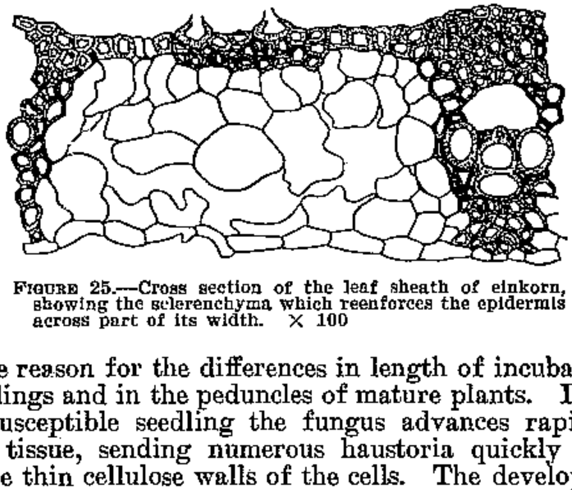


FIGURE 25.—Cross section of the leaf sheath of einkorn, showing the sclerenchyma which reenforces the epidermis across part of its width. × 100

Whether the haustoria penetrate equally well the thick and the thin walls of the collenchyma cells has not been determined experimentally, but observational evidence indicates that they are formed more slowly in cells with thick walls than in those with thin, delicate ones. In the case of the infected leaf base of Ceres, the general appearance of the sections indicated that the rust had difficulty in advancing through the ground tissue, made up of compact cells with very thick cellulose walls. The absence of large intercellular spaces and the heavy, regular thickening of the cell walls retarded the fungus considerably. It is possible also that the collenchyma cell walls of some wheat varieties are thicker than those of other varieties and that the fungus develops even more slowly in peduncles of the former.

EFFECT OF FERTILIZERS ON STRUCTURE

For many years there has been a general impression that heavy applications of nitrogenous fertilizers render plants susceptible to rusts. According to Little (*27*), Bolley (*12*), Voelcker (*46*), Sorauer (*36*), Remer (*33*), Armstrong (*10*), Spinks (*37*), Freeman and Johnson (*17*), Johnson (*24*). and others, the severity of rust attack is likely to be greatly increased on low, rich soils, and especially on soils heavily manured with nitrogenous material. If there is any excess of potassium or phosphorus, however, the plants are not likely to be so heavily rusted. The literature on the subject has been fully reviewed by Eriksson and Henning (*15*), and Stakman and Aamodt (*40*).

Whether the fertilizers exert a direct effect on the physiologic reaction of a plant to rust is not yet definitely determined. It has been suggested by Armstrong (*10*), Freeman and Johnson (*17*), and by Vavilov, according to Stakman and Aamodt (*40*), that the effect is indirect. The opportunities for infection are greater since nitrogenous fertilizers often delay maturity and increase the plant surface and density of stand. Ward (*50*) found that nutrient solutions alone did not alter the susceptibility or resistance of bromegrass to brown rust. Stakman (*38*) found in 1914 that fertilizers had no direct effect on the immunity or susceptibility of wheats to stem rust. Again, in 1924, Stakman and Aamodt (*40*) concluded that natural and artificial fertilizers did not change the degree of physiologic susceptibility of wheat to stem rust. They suggested, however, that the morphologic resistance of a variety might be changed somewhat by fertilizers.

Several authors have suggested that fertilizers may affect the internal structure of the cereals and protect or predispose them to disease. According to Hursh (*23*) and Stakman and Aamodt (*40*), Petermann believed that the strength of the cell walls was affected by fertilizers and that differences in resistance were due to differences in the resistance of the walls to puncture. According to Hursh (*23*) and Stakman and Aamodt (*40*), Russell also states that fertilizers affect the structure of the cell walls, the nitrogenous manures causing the plants to produce thin cell walls easily penetrated by the rust hyphae. According to Stakman and Aamodt (*40*), Miyake and Adachi are of the same opinion. The latter also report that the increased resistance of plants fertilized with potassium is due to the fact that potassium fertilizers strengthen the cell walls.

Hursh (*23*) studied the effect of nutrient salts on the morphology of leaves of Haynes Bluestem wheat. In cultures deficient in nitrogen the leaves had relatively large amounts of sclerenchyma; the girders surrounding the vascular bundles extended from one epidermis to the other and separated completely the areas of susceptible chlorenchyma. The epidermis was composed of small, heavily cutinized cells with thick walls, and the entire leaf was firm and compact. In cultures supplied with excess nitrogen the leaves were more porous and succulent. Very little sclerenchyma accompanied the vascular bundles, and each area of chlorenchyma was continuous with the next. The epidermal cells were large, thin-walled, and slightly cutinized. Hursh concludes that the effect of excess nitrogenous fertilizers is to increase the area in which the fungus can grow without altering the fundamental protoplasmic resistance of the plant.

In the present study it was thought advisable to determine the effect of fertilizers on the structure of the peduncle. If the relative proportions of sclerenchyma and collenchyma are greatly altered, or if the division of collenchyma into strands is changed by different fertilizers, it would be impossible to rely on morphologic resistance in any variety of wheat. If nitrogenous fertilizers prevent a normal development of sclerenchyma, which results in very little division of the collenchyma areas, it is obvious that the rust may develop extensively after it enters the peduncle of a physiologically susceptible host. And if the epidermal membrane is greatly modified by the application of fertilizers, the process of pustule formation in certain varieties may be affected.

Experiments were made at University Farm, St. Paul, Minn., in 1925 and 1926, and at Coon Creek, Minn., in 1927 and 1928. Triplicate rows of each variety were grown in square-rod plots, and border rows of Marquis or Mindum were grown around each plot. The fertilizers were applied in moderate and in excessive quantities just before seeding, and, in the case of nitrates, about three or four weeks after seeding. In 1925 and 1926 six varieties of wheat were grown: (1) Marquis, a common wheat with a relatively large proportion of collenchyma in the peduncle, usually susceptible to stem rust; (2) Kota, with a smaller proportion of collenchyma and often resistant in the field; (3) Ceres, a hybrid between Marquis and Kota, intermediate with respect to structure of its peduncle, sometimes resistant in the field; (4) Pentad, a durum wheat, apparently highly resistant to or almost immune from stem rust; (5) Mindum, a durum, physiologically susceptible to a number of forms of stem rust, often rusted in the field if virulent forms are present; and (6) Acme, also a durum, with protoplasmic susceptibility to most rust forms but very seldom rusted in the field. Acme is said to have an effective morphologic resistance by reason of the occurrence of sclerenchyma between the epidermis and the collenchyma strands in the peduncle (*23*).

A different set of varieties was grown at Coon Creek, Minn., in 1927, namely, the durum wheats, Acme and Mindum; Khapli emmer; the morphologically susceptible Marquis; Little Club, highly susceptible physiologically and morphologically; the rust-resistant hybrid Marquillo; and the resistant common wheat, Webster. In 1928 Marquis, Marquillo, Kota, Ceres, Hope, Acme, and Mindum were grown.

Fertilizers were applied both in moderate quantities and in excessive quantities. At University Farm the following fertilizers were used:

	Pounds per acre
Treble superphosphate	125
Potassium chloride	240
Ammo-phos (13–48)	80
Sodium nitrate	240

Two check plots were left without fertilizers, and one plot was treated with a combination of superphosphate, potassium chloride, and sodium nitrate. A second series of plots was treated with excessive quantities of fertilizers and received double the quantity applied to the first series. The same fertilizers were used at Coon Creek, except that either ammonium sulphate or urea was used in place of the sodium nitrate. The Ammo-phos series was omitted. In addition to the single fertilizer plots, there were plots treated with combinations of two of the fertilizers, and one plot treated with all three compounds. Plots of a second series were treated with excessive quantities of fertilizers.

The peduncles were clipped about two weeks after the plants headed and were preserved in acetoalcohol. Later they were sectioned on a sliding microtome, stained, and examined. All peduncles were sectioned about 1½ inches below the rachis, and the results are based on the averages of 10 to 15 peduncles in each sample.

EFFECT OF FERTILIZERS ON THE RELATIVE PROPORTIONS OF SCLERENCHYMA AND COLLENCHYMA IN THE PEDUNCLE

In 1925 samples of each variety from the different plots were measured to determine the proportion of collenchyma adjacent to the periphery of the peduncle. It is possible that the prevalence of large and numerous pustules on plants heavily fertilized with nitrogen is due to an increase in amount of collenchyma, which is a direct result of nitrogenous fertilization. The proportion of collenchyma in the peduncles was expressed in percentages, and the measurements for the entire series, with stem rust notes in addition, are shown in Table 5.

TABLE 5.—*Rust notes and percentages of collenchyma in the peduncles of six varieties of wheat grown on different fertilizer plots at University Farm, St. Paul, Minn., 1925*

Variety and fertilizer	Collenchyma	Rust infection [1]	Variety and fertilizer	Collenchyma	Rust infection [1]
	Per cent			*Per cent*	
Marquis:			Acme:		
Check	72.3	25–30 S.	Check	72	Tr.–50 R.
Superphosphate [2]	69.3	25 S.	Superphosphate [2]	77.9	Tr.–50 R.
KCl	70.1	35 S.	KCl	73.5	5 R.
Ammo-phos	71.5	35 S.	Ammo-phos	77.4	5 R.
$NaNO_3$	71.5	25 S.	$NaNO_3$	74.5	Tr. R.
Combination [3]	71.1	35 S.	Combination [3]	73.6	Tr. R.
Kota:			Mindum:		
Check	58	10 R.	Check	75.6	20 S.
Superphosphate [2]	54.9	5 R.	Superphosphate [2]	77.4	25 S.
KCl	57.8	15 R–SR.	KCl	78.8	15 S.
Ammo-phos	63.1	20 R–SR.	Ammo-phos	78.2	10 S.
$NaNO_3$	59.3	10 R.	$NaNO_3$	78.9	15 S.
Combination [3]	61	20 R–SR.	Combination [3]	75.0	15 S.
Ceres:			Pentad:		
Check	63.2	10 R.	Check	74.8	Tr. R.
Superphosphate [2]	62.9	5 R.	Superphosphate [2]	81.2	0.
KCl	61.9	5 R.	KCl	78.4	0.
Ammo-phos	65.3	Tr.–5 R.	Ammo-phos	76	0.
$NaNO_3$	66.5	5 R.	$NaNO_3$	75.8	0.
Combination [3]	63.6	5 R.	Combination [3]	73.3	0.

[1] Numbers indicate percentage of infection; S=susceptible; R=resistant; Tr.=trace.
[2] Treble superphosphate.
[3] A combination of superphosphate, KCl, and $NaNO_3$.

It is obvious that the variation in rust infection is not extreme within a variety, and that the heaviest infection is not always on the plants that receive the nitrogenous fertilizers. In the case of Marquis, the percentages of rust infection were almost the same on all plots, the highest estimates (35 S)[5] being those for plants supplied with potassium chloride, Ammo-phos, or a combination of potassium, phosphorus, and nitrogen. When the structure of the peduncle was studied, there were only very slight differences in percentages of collenchyma, the range being between 69.3 and 72.2 per cent.

The results obtained with Ceres were somewhat similar to those obtained with Marquis, there being no great differences in rust infection nor in the structure of the peduncle. In the case of Ceres, however, the severest rust infection (10 R) was on plants in the check plot, and the mildest (trace to 5 R) was on plants fertilized with Ammo-phos. There was not the slightest indication of change in proportion of collenchyma in peduncles of plants in different plots. In fact, the percentage range was from 62.9 to 66.5, and the percentage of collenchyma in the least heavily rusted peduncles from the Ammophos plots was 65.2, as compared with 63.1 per cent in those from the more heavily rusted check plots.

The only indications of a relationship between the severity of rust and the proportion of collenchyma, as a result of the application of fertilizers, was found in Kota wheat. The plants on the plot treated with treble superphosphate had the least collenchyma and the rust infection was lower than that in any of the other plots. The pustules were small and well isolated, for the reaction was recorded as R. Conversely, the plants on the two plots treated with Ammo-phos and with a combination of potassium, phosphorus, and nitrogen contained larger percentages of collenchyma, and the rust infection was relatively high (20 per cent) and reaction varied from R to SR.

In the case of durum wheats, also, there was no evidence that fertilizers have any profound effect on the proportion of susceptible tissue in the peduncle. The Mindum plants which received the treble superphosphate were more severely rusted than other plants of the same variety, but they did not contain the largest percentage of collenchyma. On the other hand, plants of the Pentad variety, which received treble superphosphate, contained slightly more sclerenchyma than similar plants of other plots. Pentad has a marked protoplasmic resistance to stem rust, and only a trace of rust developed in the check plot on a few plants of this variety that had been injured. The Acme plants that received treble superphosphate also contained the highest percentages of collenchyma, but the differences in measurements were so slight that they were not considered significant. In this variety also the plants injured by foot rot, by bunt, or by clipping seemed to be more liable to rust infection. For this reason, the rust infection in the check plot and the phosphate plot was recorded as trace to 50 R.

Neither the results of this experiment nor those of the 1926 experiment support the supposition that nitrogenous fertilizers alter

---

[5] For explanation of symbols for rust reactions see footnote 1 to Table 5, p. 45.

the structure of plants by increasing the proportion of rust-susceptible tissue. Measurements made in 1926 were as inconsistent as those made in 1925. There was not the slightest correlation between fertilizers used and percentage of collenchyma in Kota wheat, even when the fertilizers were applied in excessive quantities. The range of variability in percentage of collenchyma was not great in any variety, and the very slight differences that occurred were not consistent with the differences which occurred in 1925.

In the next two years, 1927 and 1928, tests were made at Coon Creek, Minn., to determine whether there would be greater response to fertilizers on the sand than on the loam at University Farm. Although stands were poor because of unfavorable growing conditions, some varieties were studied in detail. The results were about the same as those previously obtained. The differences in percentage of collenchyma were so slight as to be of no significance, and the highest percentages of collenchyma were not always in the peduncles of plants fertilized with nitrogenous materials. The conclusion was that fertilizers applied in the quantities used in these experiments did not appreciably alter the proportion of collenchyma in the peduncle of any variety studied. The next problem was to ascertain the possibility of fertilizers affecting the distribution of collenchyma in the peduncle.

EFFECT OF FERTILIZERS ON THE DISTRIBUTION OF COLLENCHYMA IN THE PEDUNCLE

Although the application of nitrogenous fertilizers did not cause the formation of a larger proportion of collenchyma in the peduncle, it is possible that the collenchyma present in such a peduncle might comprise fewer and larger areas and might not be divided into many smaller and narrow ones, as would be the case in an unfertilized plant. In order to obtain definite information on this question the numbers of different types of collenchyma areas in each peduncle were tabulated, and averages were secured for 10 samples from each fertilizer plot. In this way it is easy to tell at once how many single collenchyma strands occur in the average stem of a plant from a certain fertilizer plot, how many double strands, and how many collenchyma areas consisting of three or more coalesced strands. Of course, there is no comparison of size of single collenchyma strands, but the degree of division of the rust-susceptible tissue is determined.

The results of two of these tabulations are presented in Table 6. In Marquis wheat the great majority of the collenchyma areas consisted of single strands and a moderate number of them were double strands. Larger areas of collenchyma, three or four coalesced strands, were more unusual, but occurred in one or two peduncles in a group of 10 examined culms. In the durum wheat, Acme, the average number of single collenchyma strands in each peduncle was considerably lower, and relatively more of the collenchyma areas consisted of several coalesced strands.

TABLE 6.—*Distribution of the collenchyma of the peduncle into single, double, and coalesced strands in Marquis and Acme wheats grown on different fertilizer plots at University Farm, St. Paul, Minn., 1926*

Variety and fertilizer	Average number of collenchyma strands of indicated type					
	Single	Double	3-coalesced	4-coalesced	5-coalesced	Over 5-coalesced
Marquis:						
Check	21.8	5.2	0.2	0.1	0	0
KCl	21.9	7.8	.2	0	0	0
Superphosphate	20	8.8	.1	.1	0	0
Ammo-phos	22.3	5.9	.3	0	0	0
$NaNO_3$	20.7	7.7	0	0	0	0
Combination[2]	21	7.1	.1	.1	0	0
Check	19.3	7.5	.2	0	0	0
Excess KCl	21.3	6.7	.1	.1	0	0
Excess superphosphate	21.3	7.4	0	0	0	0
Excess Ammo-phos	22.9	6.4	.1	.1	0	0
Excess $NaNO_3$	21.8	8	.1	.1	0	0
Excess combination	24.8	7.7	.1	0	0	0
Acme:						
Check	7.4	4.7	.8	1.8	.4	.3
KCl	10.3	4.1	1.1	1.9	0	.6
Superphosphate[1]	8.8	4.6	1.4	1	.1	.7
Ammo-phos	7.7	5.2	2	.7	.4	.3
$NaNO_3$	6.2	5.1	1.7	1.9	.3	.6
Combination[2]	8.2	5	1.4	2	.3	.5
Check	8.3	6.3	1.2	1.8	.1	.2
Excess KCl	11.4	6.1	1.2	1.3	.4	.3
Excess superphosphate	12.3	4.3	1.8	1	.2	.4
Excess Ammo-phos	10.2	4.3	1.4	1.2	.1	.5
Excess $NaNO_3$	7.9	4.6	1.2	2.1	.1	.6
Excess combination	8	6.4	2	1.1	.1	.8

[1] Treble superphosphate.
[2] A combination of superphosphate, KCl and $NaNO_3$.

If the averages obtained for each fertilizer plot be compared, it is evident that in Marquis the fertilizers appeared to have had no effect on the division of the collenchyma. The proportions of single, double, and coalesced strands were approximately the same in all plants from the different plots; nitrogen did not tend to produce fewer single strands and more double or coalesced strands. In the case of Acme there were greater differences when the averages for the single collenchyma strands were compared. The plants supplied with excessive quantities of potassium chloride or treble superphosphate contained more single collenchyma strands than plants on the other plots, thus indicating the possibility of a greater division of collenchyma. In spite of the fact that there were more single strands in these plants, the number of coalesced collenchyma strands remained about the same as in plants from other fertilizer plots. It also is true that the average number of single collenchyma strands was high in the case of plants furnished with Ammo-phos in excess.

The peduncles of Kota, Ceres, Pentad, and Mindum were similarly analyzed but gave no evidence that fertilizers exerted any appreciable or consistent effect on the division of collenchyma in the peduncle.

EFFECT OF FERTILIZERS ON THE STRUCTURE OF THE EPIDERMAL MEMBRANE

If fertilizers affect the strength and thickness of cell walls in cereals and grasses, as Petermann and Remer have suggested (*23, 40*), it is possible that they may affect the thickness of the epidermal membrane and the strength and thickness of its cell walls. Such modifications in the epidermis would alter the tensile strength of the membrane and change its resistance to rupture. If the modifications were great enough, it is conceivable that the formation of pustules would be greatly affected. Thus, in a variety like Kota, in which evident difficulty in pustule formation is sometimes found when only moderately virulent rust forms attack the peduncle, it is probable that a thickening and strengthening of the membrane or its cell walls, as a result of fertilization with potassium or phosphorus, would prevent entirely the formation of normal pustules. On the other hand, if the epidermis were thinner than normal and had weaker and thinner cell walls as a result of nitrogenous fertilization, the tensile strength of the membrane might be decreased sufficiently to permit the formation of normal pustules without any difficulty whatever. As only a few actual measurements have been made on the thickness of the epidermal membrane and of the cell walls, it is impossible to say now whether the slight differences in measurement are consistent and can be correlated with the different types of fertilizers used. More data must be obtained before the problem is solved.

EFFECT OF FERTILIZERS ON THE DIAMETER OF THE PEDUNCLE

During the examination of plants on the fertilizer plots it was noticed that the peduncles of some plants were thick and succulent, while those of others were more slender. Hursh (*23*) suggested that the diameter of the peduncle might be an important factor in the morphologic resistance to rust, by determining the angle of curvature of the epidermis and thus affecting the resistance to rupture of the membrane. After studying the deeply furrowed peduncle of some varieties, as contrasted with the smooth, even peduncle of others, the writer decided that the width and curvature of the epidermal membrane over each collenchyma area were far more important than the diameter of the peduncle. Nevertheless, the diameters of peduncles of Marquis and Kota, grown on different fertilizer plots at Coon Creek, Minn., were determined. There seemed to be more variation in diameter of peduncles of Kota than in those of Marquis. (Fig. 26, A, B.) Variation in Marquis seemed too slight to be of any importance, but in Kota the nitrogenous fertilizers seemed to have a decided effect in increasing the diameter of the peduncle. Whether these differences are great enough to alter the degree of curvature in the usually somewhat furrowed peduncle of Kota is uncertain. The rust infection on the plants at Coon Creek was not severe enough to indicate the effect of fertilizers. If diameter were an important factor in this case, however, it would be such as to decrease the resistance to rupture in the epidermis of plants treated with nitrogen. It seems probable that the diameter of the peduncle, therefore, would be a more important factor in rust resistance in the case of a smooth-stem wheat than when the peduncle is furrowed, as it is in Kota.

RELATION OF STOMATAL BEHAVIOR TO STEM-RUST RESISTANCE IN WHEAT

Since the germ tubes of *Puccinia graminis* enter the wheat plant only through the stomata, it seems likely that stomatal behavior may have an influence on rust infection. While there is not necessarily any correlation between number, distribution, and size of the stomata and the behavior of wheat varieties with respect to rust, stomatal movements may be important, as Hursh (*23*) and Allen (*7*) have suggested. Pool and McKay (*32*) found that germ tubes of *Cercospora beticola* were excluded from the beet leaf when the stomata were closed, so it seems reasonable to presume that similar conditions hold for *P. graminis* on cereals. The writer (*20*) found that the germ tubes of stem rust enter the cereals only through open stomata, for, apparently, they are unable to force their way between the guard cells when the stoma is closed. Consequently, infection occurs only during certain favorable periods—that is, while there is sufficient moisture for the germination of spores and the survival of germ tubes—while temperature is favorable for spore germination and growth of germ tubes, and while the stomata are open so as to permit the entry of germ tubes.

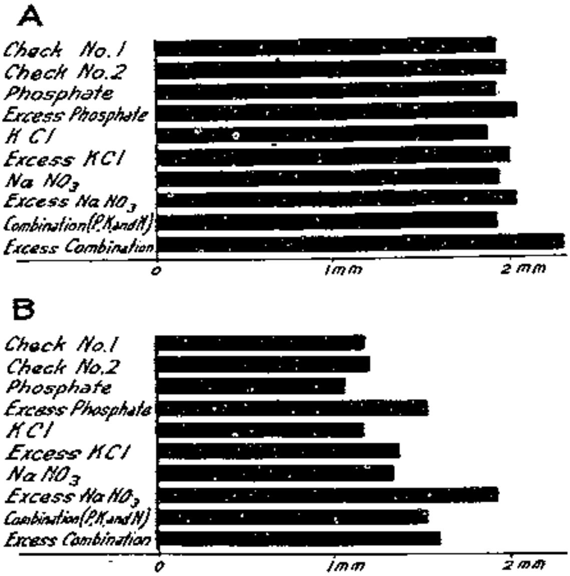


FIGURE 26.—Graphs showing relative diameters of the peduncles of wheat grown on different fertilizer plots in 1926: A, Marquis; B, Kota

According to Loftfield (*28*), the stomatal movements in cereals follow a definite daily rhythm. He found that under favorable conditions the stomata open at sunrise or soon after, remain open during the morning, close gradually during the late afternoon, and then remain closed all night. If conditions are unfavorable, most of the stomata remain closed during the day or are only partially open for an hour or so after sunrise. Night opening does not occur under ordinary conditions, favorable or unfavorable.

Allen (*7*), while studying the early stages of infection of *Puccinia triticina* on Little Club wheat, found that in material fixed in the morning many of the germ tubes had just entered the host and formed the substomatal vesicles. In the material fixed in the afternoon she found that infection was always more advanced, and most of the substomatal vesicles had sent out infecting hyphae. She suggested that this daily rhythm might be due to the fact that the time of entrance is conditioned by the daily stomatal movements of the host and that entrance waits upon the natural opening of a stoma rather than upon mechanical force or chemical action.

Allen (*6, p. 720*) also has reported that only a certain percentage of the germ tubes that form appressoria over the stomata actually gain entrance to the host. In studying the behavior of three physiologic forms of *Puccinia graminis tritici* on Khapli emmer she found that—

> The appressoria of all three forms of the fungus secrete some substance, which, if present in sufficient amount, penetrates and kills the guard cells and alters their walls. So far as can be judged by appearances, it is the same substance in all three, but varies in quantity * * *. On comparing the percentage of entries with the degree of stomatal injury, it is to be noted that where injuries are least serious the entries are greatest in number.

In these cases it is probable that the stomata closed as soon as the substances penetrated and killed the guard cells. Any outside influence which affects the guard cells and decreases their turgor tends to close the opening between them.

The writer (*20*) has found that the rhythm of stomatal movements fluctuates somewhat in different wheat varieties, although it follows the general trend of the rhythm as determined for the cereals as a class. The stomata of cereals are always closed at night. Light is the most important stimulus for opening, but the stomata of some varieties respond to the stimulus very quickly, while those of other varieties respond slowly. A study of stomatal behavior in the upper Mississippi Valley during the early morning hours, immediately after sunrise, indicates that a large proportion of germinating rust spores will gain entrance to some varieties and prove very effective inoculum, while on other varieties the majority of germ tubes will be excluded and only relatively few will enter and infect the host. The spores may germinate at any time during the night, if temperatures are favorable, because the necessary moisture usually is supplied by heavy dews during the growing period in the upper Mississippi Valley. The dew remains on the plants for a considerable time after sunrise, so that conditions often are ideal for spore germination and survival of germ tubes. Although many spores may germinate during the night, none of the germ tubes can enter the host until the stomata open the following morning. Thus the critical period for infection is the time between sunrise and the disappearance of the heavy dew, after which the delicate fungus germ tubes on the plant surfaces perish. If the stomata respond quickly to the stimulus of light and open soon after sunrise, there is a longer period during which infection may take place than if the stomata respond to sunlight slowly. Figure 27 shows the behavior of stomata in five wheat varieties during the early morning of a typical July day.

The stomata of Little Club opened almost instantly at sunrise. For 20 minutes after sunrise, when the first observations were made, the stomata were about half open. It was possible for the germ tubes of the rust to enter Little Club easily between 5 a. m. and 6.20 a. m., the time when the heavy dew ended. Of course, the dew disappeared gradually and the plants were not thoroughly dry until about 8.20 a. m., so that the period for infection probably extended beyond the disappearance of the heavy dew. It is impossible to say whether the period was one and one-half hours or three hours, but it was long enough for a large number of germ tubes to effect entry. The stomatal behavior in Quality, Reward, and Early Baart is similar to that in Little Club. The stomata open very quickly in the morning and there is a relatively long and favorable infection period.

The stomata of Marquis open a little less rapidly than those of Little Club, and at 20 minutes after sunrise the openings appear as narrow slits, which probably are too narrow for the germ tubes to penetrate. At the next observation period, 40 minutes after sunrise, the stomata were about half open, in condition for the admittance of germ tubes. Thus in Marquis the period favorable for infection was only slightly shorter than in Little Club. Other varieties with stomatal behavior similar to that of Marquis are Haynes Bluestem, Ruby, Ceres, Arnautka (C. I. 1493), and Mindum.

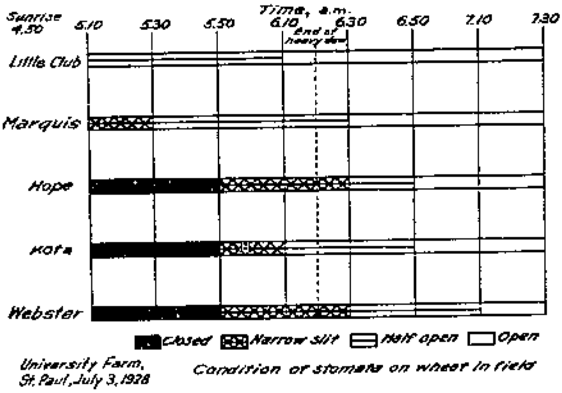


FIGURE 27.—Stomatal behavior in the field in five varieties of wheat—Little Club, Marquis, Hope, Kota, and Webster—during the period immediately after sunrise

The stomata of the other varieties observed responded to light more slowly. The stomata of Kota, Webster, and Hope were closed when the first observations were made and they did not begin to open until some time after 5.30 a. m. At 5.50 a. m. most of the openings in these three varieties were only narrow slits, but by 6.10 a. m. the stomata in Kota were about half open. From Figure 27 it is evident that the stomata of Kota were suitable for germ tube entry just a short time before the end of the heavy dew. The period for infection may have been about 15 minutes, or it may have been between one and one-half and two hours. In any case it was a much shorter period than that of either Little Club or Marquis.

The stomata of Webster and Hope opened even more slowly than those of Kota. At 6.10 a. m. these stomata were only narrow slits, and they did not open far enough to permit infection until near the disappearance of the heavy dew. If most of the germ tubes perished when the dew began to disappear, there was almost no time during which the stomata were sufficiently open for the passage of germ tubes. Even though the germ tubes remained viable after the end of the heavy dew, the period during which infection was possible is very short when compared with the same period for Little Club.

It is seen, therefore, that certain wheat varieties may escape stem-rust infection because of their characteristic stomatal behavior. Such varieties are said to possess a "functional resistance" to stem rust. The functional resistance may vary in different seasons and in different localities, but in some varieties it is remarkably constant and probably accounts for the marked field resistance of those wheats.

Velvet Don (C. I. 1445) has been resistant to stem rust at University Farm every season since 1909. Not more than a trace, or occasionally 5 to 10 per cent, of rust develops on it in the field, in spite of the fact that it is highly susceptible to some of the rust forms actually present in abundance in the field. The stomatal behavior in this variety is similar to that in Webster and Hope, so that Velvet Don probably possesses a functional resistance and stem rust seldom gets inside this variety when it is growing in the field. Greenhouse experiments give further evidence that the resistance of Velvet Don is functional. When seedlings of this variety are inoculated in the greenhouse with *Puccinia graminis tritici* form 21, a type-4 infection results, indicating great susceptibility to that form. When inoculations are made later on plants in the 4-leaf stage, in the boot or after heading, excellent infection results, an abundance of rust pustules develop, and there is no indication of resistance. (Fig. 28.) In the greenhouse the older plants are kept in the moist chamber for three days after inoculation, and during that period there probably is considerable time in which the stomata are open and germ tubes are entering the plants. In the field form 21 is very often present, but the periods of stomatal opening probably do not coincide with the periods favorable for spore germination and growth of germ tubes. The functional resistance of Velvet Don has been very effective at University Farm. Whether it would be effective under different conditions is uncertain, as this type of resistance in other varieties sometimes is more effective in one locality than in another.

There is great variation in the percentage of stem rust on Webster wheat. In the uniform rust nursery at University Farm only 5 to 10 per cent of stem rust developed on Webster in 1927. On the sand experimental plots at Coon Creek, Minn., there was 60 to 90 per cent of infection in 1927, but the pustules were all relatively small and a great majority of them never ruptured the epidermis, because Webster possesses a morphologic and a protoplasmic resistance to rust as well as a functional one. Stomatal behavior was not studied at Coon Creek, but it is certain that a great many more entries were effected there than at University Farm.

Likewise, the percentage of stem rust on Kota varies according to locality. In some of the uniform rust nurseries the percentage of infection on Kota is very high, while in other nurseries only a small percentage of rust develops on this variety. In 1927 there was 28 to 35 per cent of stem rust on Kota at Madison, Wis., and 38 to 75 per cent at Morris, Minn. In the same year there was only 1 to 2 per cent of stem rust on Kota at Manhattan, Kans., and 4 to 10 per cent at Fargo, N. Dak. Rust forms to which Kota is highly susceptible were present at all places, for forms 18 and 21 were identified. Nevertheless, the percentage of infection varied greatly and stomatal behavior may have been responsible for some of the differences.

Infection on Kota varies also from season to season. At University Farm infection on Kota ranges from a trace to about 33 per cent. In 1919, 1921, and 1922 only a trace of rust was reported on Kota; in 1925 and 1927 there was between 12 and 20 per cent, and in 1920 there was about 33 per cent. Some of this variation may have been due to the absence of virulent physiologic forms of rust, but it is possible that stomatal behavior also was important. In many seasons Kota is almost free from stem rust, there is very little evidence of mycelial infection, and the functional resistance of Kota seems to be operative. In other seasons there are numerous infections on Kota, and stomatal behavior seems to be ineffective in excluding the rust.

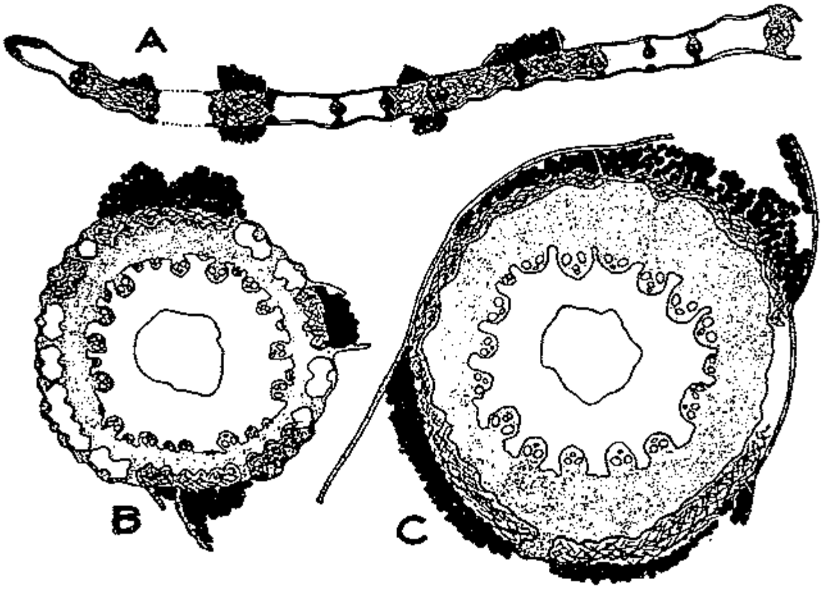


FIGURE 28.—Diagrams of cross sections of Velvet Don infected with *Puccinia graminis tritici* form 21: A, Leaf blade; B, peduncle 2 inches below the head; C, peduncle just below head. × 22

The same variability in number of infections is evident in Khapli emmer. Khapli has a marked protoplasmic resistance to all forms of stem rust yet found in the United States, but if the stomata are open it is possible for *Puccinia graminis tritici* to enter a resistant host as easily as it enters a highly susceptible one. The stomatal behavior of Khapli in some seasons is such that the rust is excluded entirely. In others a large number of germ tubes enter and the mycelial invasions are numerous. As fruiting pustules usually are not formed, a cursory field examination of Khapli reveals no evidence of the infection. The mycelium develops enough so that the epidermis is distended, and if the culm is drawn between the thumb and forefinger the surface feels uneven and bumpy. If a microscopic examination be made the mycelium may be seen in the chlorophyllous tissues. In some years this type of infection is very usual on Khapli. In other years the germ tubes are unable to enter and the emmer is free from rust.

Stomatal behavior has been studied in some of the wheat hybrids that have been grown and tested for their resistance to stem rust at University Farm. A number of crosses have been made in which one parent possesses a marked functional resistance to stem rust, while the other parent has none. If the functional resistance is transmitted to the new individuals, then, under field conditions, the hybrids usually will be resistant to all forms of *Puccinia graminis*. A few observations were made to ascertain if there was any correlation between stomatal behavior and the rust resistance of the selected hybrid lines.

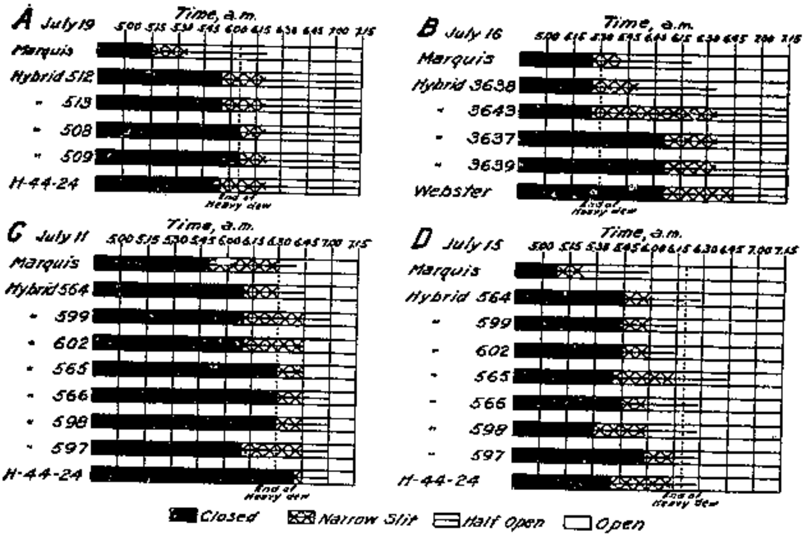


FIGURE 29.—Stomatal behavior in the field of several hybrid wheats and their parents

Goulden (*19*) discusses the inheritance of "mature plant resistance" in hybrid wheats and suggests that it offers much greater possibilities in the production of resistant varieties than the building up of protoplasmic resistance by a complicated series of crosses.

The stomatal behavior of four F_5 hybrid lines of a Marquis × H-44-24 cross was compared with that of the two parents. Marquis generally is susceptible to rust in the field, while H-44-24 has a decided functional resistance, being similar in this respect to its sister selection, Hope. As may be seen in the graph of Figure 29, A, the stomatal movements in the four hybrids resembled stomatal movements of the H-44-24 parent rather closely; not one hybrid had a tendency to behave like Marquis in this respect. Evidently when the selections were made all of the hybrid lines chosen for propagation possessed a functional resistance. The stomatal behavior did not seem related to the morphologic type of the hybrids, for hybrids 509 and 512 seemed to be Marquis types, while hybrids 508 and 513 resembled H-44-24.

Seven hybrid lines of an H-44-24 × Marquis cross also were studied. They were in the F_4 generation, four of them closely resembling the H-44-24 parent morphologically, while two of them were distinct Marquis types, and one line apparently was still segregating for awns and other head characters. Figure 29, C and D, illustrates stomatal behavior in these hybrid lines and in the parent lines on two days in July, 1929. The two days differed in several respects, and particularly in sunlight and relative humidity. The cloudiness and lower relative humidity of July 11 delayed 25 to 60 minutes the opening of stomata in nearly all hybrids and their parents, Marquis, and H-44-24. Thus, on that date, Marquis and hybrid 564 were the only lines in which the stomata may have been open early enough to permit even a few germ tubes to enter before 6.30 a. m., at which time the plants were almost dry. (Fig. 29, C.) Stomata of the H-44-24 parent and six hybrids were closed or but slightly open until after the moisture had disappeared. On July 15, a day of sunshine and fairly high relative humidity, the stomata opened earlier and in all cases may have permitted germ-tube entry. The stomata of Marquis were open for the longest period before the disappearance of the dew, but none of the hybrids behaved like Marquis. Hybrids 564, 599, 602, 566, and 598 seemed to take an intermediate position between the parents, Marquis and H-44-24, with regard to functional resistance, because their stomata were half open about 40 minutes later than those of Marquis and about 15 minutes earlier than those of H-44-24. The stomata of hybrids 565 and 597 and the parent H-44-24 were open almost an hour later than those of Marquis, so that these wheats were exposed to rust infection for a relatively short period. Here again there seemed to be no correlation between stomatal behavior and morphologic type.

When, however, a few of the F_3 hybrids of Marquis × Webster were studied, there was one line (hybrid 3638) whose stomata behaved very much like those of Marquis. (Fig. 29, B.) It happened that this hybrid also resembled Marquis morphologically, while the other hybrids appeared to be like Webster or were still segregating for head characters. From the observations of July 16 it seems that hybrids 3637, 3639, and 3643, and the parent Webster have a functional resistance. Their stomata were in the narrow-slit stage at the end of the heavy dew; and those of Webster opened later than all the rest. Unfortunately, none of the Marquis × Webster hybrids were promising enough to be selected for further propagation.

A functional resistance may explain some of the "mature plant resistance" in certain wheat varieties. Most of the evidence of a mature plant resistance has been based on the field reactions of mature plants, but it is necessary that the phenomenon be studied under more controlled conditions. Stomatal behavior undoubtedly is the cause of the mature plant resistance in Velvet Don, as has been concluded from the greenhouse studies. Such is the case with Kota and Ceres, also, for the mature plants of these varieties are easily infected in the greenhouse and seem to acquire no resistance to the rust forms to which they were susceptible as seedlings. Khapli also has been grown in the greenhouse and inoculated at different stages of growth to determine whether the older plants developed a resistance to *Puccinia graminis tritici* form 41, an

Egyptian form to which Khapli seedlings are highly susceptible. The seedlings gave a type-4 reaction, and the plants were again inoculated when they were in the boot, and once more immediately after heading. The inoculum was applied with flattened steel needles to certain definite areas rather than being scattered promiscuously by dusting the mature plants with other rusted plants.

Inoculated seedlings are usually left in the moist chamber for about 48 hours after inoculation, but the older plants were left for 72 hours in order to be reasonably sure that a large portion of the germ tubes entered the stomata. Rust notes were read three weeks after inoculation, because the pustules develop more slowly on the older plants than on the delicate, succulent seedlings. Figure 17 illustrates the results of the inoculations on the mature plants. Pustules developed on all inoculated parts, peduncle, leaf, leaf sheath, glumes, and awns. In all sectioned material, morphologic resistance to stem rust was evident, for the pustules were well separated by the sclerenchymatous tissues. In some cases it was easy to see that the rupture of the epidermis was a difficult process. Form 41 is a virulent rust form, however, and it could be seen that infection occurred in every collenchyma strand facing on the side of the culm that was covered by the inoculating needle. There was no evidence of the so-called mature plant resistance.

Stomatal behavior in wheats may not explain all cases of mature plant resistance, however, because Johnston and Melchers (*35*) have recently studied the relation of age of wheat plants to infection by *Puccinia triticina* and found that certain varieties were definitely more resistant in the heading than in the seedling stage. Their work was done in the greenhouse, and, although their plants were left in moist chambers for only 48 hours, the fungus appeared to have entered the plants without difficulty. The notes were taken 14 to 16 days after inoculation, but pustules seemed to be fully developed on those plant parts which they considered susceptible. The resistant mature plants often were only flecked and in many cases when pustules developed there was a good deal of necrosis. They also found that the younger leaves of a mature plant usually were more resistant to leaf rust than the older leaves of that plant. They believed that the change from susceptibility in the seedling stage to resistance at heading time was due to differences in the chemistry or physiology of the young, active leaves.

In the case of stem-rust resistance at University Farm, there seems to be a very high correlation between functional resistance due to stomatal behavior and the general field reaction of a variety. (Table 7.) Almost all of the wheat varieties possess protoplasmic resistance to one or more rust forms. Certain varieties also possess morphologic resistance, and this, whether the host has protoplasmic susceptibility or resistance, accounts for the limitation of the fungus after it enters the plant. A few varieties are generally resistant and often free from rust in the field, in spite of the fact that they may have no protoplasmic resistance and no marked morphologic resistance to the form of rust present. Stomatal behavior has been studied in some of these peculiar varieties and it has been concluded that their general freedom from rust in the field is due to functional resistance based on stomatal behavior. The most generally resistant wheats, Webster, Hope, Acme, and Velvet Don, and Khapli emmer probably have all three types of resistance, so that, even if stomatal behavior becomes ineffective as a means of resistance in some seasons or in some localities, the protoplasmic reactions and the structures of the host may protect it from severe attack. A functional resistance is not a selective one but operates against all forms of rust. It probably is a more effective type of resistance than a morphologic or a moderate protoplasmic resistance.

TABLE 7.—*Summary of the different types of rust resistance possessed by some wheat varieties and the correlation between their stomatal behavior and their general host reaction to stem rust when grown in the field at University Farm, St. Paul, Minn., in 1928*

Stomatal opening and variety	Types of resistance			Host reaction in the field [1]
	Functional	Morphologic	Protoplasmic	
Rapid:				
Little Club	−	−	+	S.
Quality	−	−	+	S.
Early Baart	−	−	+	S.
Reward	−	−	+	S. or R.
Moderately rapid:				
Marquis	−	−	+	S. or R.
Haynes Bluestem	−	−	+	S. or R.
Ruby	−	−	+	S. or R.
Ceres	−	+	+	MS. or R.
Arnautka	−	+	+	S. or R.
Mindum	−	+	+	S. or R.
Moderately slow:				
Kota	+	+	+	MS. or R.
Marquillo	+	+	+	MS. or R.
Kubanka	+	+	+	S. or R.
Khapli	+	+	+	R.
Slow:				
Webster	+	+	+	R.
Hope	+	+	+	R.
Velvet Don	+	+	+	R.
Acme	+	+	+	R.

[1] S.=Susceptible; MS.=moderately susceptible; R.=resistant.

Abbott (*1*, *2*) has reported that Webster, Hope, and Khapli were rusted severely in Peru in 1928 and 1929, Hope being so severely injured that the grain was badly shriveled. Apparently morphologic resistance did not afford complete protection against rust, for in Khapli, although each pustule was relatively small, the pustules were so numerous on the culms that they coalesced and completely girdled the stem. The evidence obtained in Peru indicates that extremely virulent forms of *Puccinia graminis tritici* are present and that in all the wheats and emmers tested functional resistance due to stomatal behavior was not effective. Abbott pointed out, however, that environmental conditions in Peru are especially favorable for the development of stem rust. Under such extreme conditions of high humidity and favorable temperature it therefore is probable that morphologic and functional resistance do not protect plants so completely as under ordinary conditions.

UNEVEN OR ONE-SIDED INFECTION OF PEDUNCLES IN THE FIELD

During the past few years it often has been observed that one side of a wheat culm may be severely rusted and covered with numerous stem-rust pustules, while the other side is almost free from rust, with only a few scattered pustules. A large number of these unevenly rusted peduncles were sectioned with the thought in mind that the morphology might vary sufficiently in the two halves of some stems to account for the restriction of the rust. The collenchyma areas varied somewhat, and occasionally those of one-half of the peduncle were broad and extensive while those of the other half were broken up into several smaller strands. But that half of the stem with broad collenchyma strands was not always the severely rusted half. Often the rusted half had fewer and smaller collenchyma strands than the nonrusted half. In the great majority of these unevenly rusted peduncles the structure of the one half was approximately the same as that of the other half and there was no indication that the morphology of the host was responsible for the one-sided rust infection.

Later, Stakman and Aamodt[6] noticed that the north side of the culm usually was severely infected by rust, while the south side sometimes remained relatively free from rust. The cases of uneven infection often were numerous in the awnless wheats, but they were very infrequent in the awned varieties. These field observations suggested that intensity of sunlight might be concerned in the problem. The south side of the peduncle is exposed to the sunlight all day long, while the north side usually is protected and in the shade most of the time. The awns of certain varieties might have a shading effect on the culms so as to permit an even rust infection all around the stem.

There was a possibility that the south side of the stem was scalded during periods of intense sunlight and heat, for the uneven infections generally were noticed after hot, sunshiny weather. In this case part of the tissues might be destroyed, so that rust could not develop, or the guard cells of the stomata might cease to function and thereby exclude the germ tubes of the rust. The north side of an unevenly rusted culm often remains green longer than the south side, which loses its chlorophyll and turns yellow. The stomata remain closed on the yellowed half of the culm, but whether they cease to function before the tissues yellow has not been determined.

It seemed possible that the north side of the culms might furnish a food supply more favorable to the production of rust pustules than the south side. The sugar contents of the north and south halves of stems were determined to see whether there were differences in this respect. The samples were collected at 2 p. m. from Marquis and Marquillo checkrows scattered throughout the uniform rust nursery. The peduncles were split lengthwise as they were collected. Samples were taken of green nonrusted and green unevenly rusted material and of scalded stems that had turned yellow on the south side. Each sample was analyzed for reducing sugars and for nonreducing sugars, but the results were not entirely satisfactory. The wheat peduncles contained very little sugar and there were no appreciable or significant differences in the percentages of

---

[6] Unpublished data.

reducing or total sugars in the samples used. It is possible that the sugars are not so important in fungus nutrition as some of the other materials such as proteins or pentosans.

EFFECT OF OTHER DISEASES ON RESISTANCE OF WHEAT TO STEM RUST

There is a possibility that the rust resistance of cereals breaks down when the normal metabolism of a host is interfered with, especially if that host is attacked by other pathogenes. Stakman, Piemeisel, and Levine (*45*) have reported that the physiologic forms of *Puccinia graminis* are remarkably constant and do not gradually adapt themselves to resistant or semicongenial hosts. They state that there is no evidence that the pathogenicity of rust forms is easily changed by host influence. Hayes and Stakman (*21*), in reporting on the constancy of stem-rust forms from the standpoint of plant breeding, stated that hybrid wheats did not increase the infection capabilities of rust. The F_3 families of a Kanred × Marquis cross continued to be immune from rust after they had been proved homozygous for rust immunity in greenhouse tests. The majority of workers now are probably agreed that the protoplasmic rust resistance of a wheat variety can not be broken down by the application of fertilizers, and that any increased rust infection in the field attendant on nitrogenous fertilizers probably is produced indirectly. The important factors seem to be increased density of stand and lodging and delayed maturity rather than a change in the protoplasmic reaction to rust.

On the other hand, there have been a few reports of the apparent loss of resistance to rust. Lang (*26*) believed that bunt infection usually renders wheat plants more susceptible to yellow rust, and Armstrong (*10*) reported a disturbance in the resistance to yellow stripe rust in hybrids derived from a cross between Wilhelmina and American Club. Seventeen of the F_2 plants were free from rust in the field in 1918 and were considered "genetically immune." The F_3 plants from these 17 were slightly to moderately rusted in 1919, a season very favorable for stripe rust, and a few of the plants were badly rusted. Later it was found that some of the badly rusted individuals also were infected with bunt, and it was thought that the bunt had broken down the rust resistance. Armstrong, however, was of the opinion that the resistance of a plant varied somewhat with its physiologic condition.

Later, Weston (*52*) also reported an apparent loss of resistance to yellow stripe rust in some bunt-infected Little Joss wheat. Little Joss is a hybrid endowed with the stripe-rust resistance of its Ghirka parent, which is only slightly susceptible to *Puccinia glumarum* in its later stages of growth. In years when yellow rust is severe in England the young leaves of Little Joss plants sometimes are infected early in the season. Later, as the plants develop, they "grow away" from the disease and only occasional, subepidermal pustules are found on the mature plants. Whether this resistance is truly protoplasmic is not certainly known. It may be a field resistance of mature plants similar to some cases that have been discussed in this paper. In 1925–26 at Cambridge, England, all bunted plants of this variety also were severely rusted. There seemed to be a definite correlation between the two diseases; when bunt infection was high the yellow stripe rust was severe, and if copper-carbonate treatments reduced the amount of bunt the percentage of stripe rust was lower. *Tilletia tritici* seemed to be capable of breaking down the stripe-rust resistance of Little Joss in its later stages.

An apparent breakdown of stem-rust resistance also has been noted at University Farm. Very often a large number of pustules will appear on individual plants of a rust-resistant variety severely infected with bunt or with loose smut. Likewise, in 1926, when foot rots of wheat were very prevalent in Minnesota, the affected plants of certain varieties seemed rather severely rusted. For example, Acme usually is almost free from rust at University Farm, but in 1926 it was possible to find in the plots a certain small percentage of severely rusted plants. At first glance it appeared as if the seed had been impure and that a plant of another variety, susceptible to stem rust, was growing in the plot. The plants were typical of Acme, however, and in every case these plants also were severely attacked by root rots. There was a definite correlation between the two diseases. Similar observations have been made on Mindum, Kota, and Marquis wheats.

The phenomenon was not studied in detail because the observations were made rather late in the season, when the final rust notes were being taken. It is impossible to say whether or not the protoplasmic resistance of Acme was broken down. It seems more probable that the rust infection was due to the ineffectiveness of the functional resistance usually characteristic of Acme in the field. Apparently, the breakdown in resistance was not because of any specific fungus in the plants, for the same effect was produced when the heads were removed about flowering time. A great many samples of different wheat varieties were taken in that year and the heads and part of the peduncle were clipped for morphologic studies. The clipping process was just as effective in breaking down resistance as was foot rot or bunt infection. The same effect was noticed in the oat plots of the uniform rust nurseries, for rust was exceptionally severe on plants from which the heads had been clipped for a study of blast. The physiologic processes were undoubtedly disturbed by clipping, and it is conceivable that the stomatal behavior might have been altered to such an extent as to have facilitated entry of the organism.

One controlled experiment was made in the greenhouse, designed to break down the resistance of Khapli emmer to *Puccinia graminis tritici* form 17. The Khapli seedlings give a type-1 reaction with form 17, so it may be safely assumed that one is working with the stable protoplasmic resistance and not confusing it with the morphologic or functional resistance of Khapli. Some of the heads were clipped just as they were emerging from the boot, and the boots, leaves, and culms of clipped and nonclipped plants were inoculated with form 17. The plants were kept in moist chambers for three days to allow the germ tubes plenty of time to enter. The leaves and boots of all plants were flecked slightly and there was a good deal of local yellowing. Whether this was due to rust infection or to early ripening is uncertain. No rust pustules appeared, however, so that apparently the protoplasmic resistance of Khapli was not broken down by clipping the heads.

Field experiments were made in 1929 to test further the possibility of breaking down rust resistance by subjecting plants to the attacks of other virulent pathogenes. Seed of Anthony oats, a variety usually resistant to rust in the field, in the United States, was dusted so heavily with spores of *Ustilago avenae* and *U. levis* that 35 to 40 per cent of the resulting plants became smutted. The plants were subjected to natural rust infection only, with the hope that there would be plenty of inoculum. The rust infection was unsatisfactory, however, and the plants apparently maintained their resistance, as there was only a trace of stem rust in the entire plot. On August 5, after most of the oats in the field were thoroughly ripe, the plants were cut and the smutted plants sorted from the nonsmutted. Eighty-five per cent of the nonsmutted plants had ripened and were yellow and dry. On the other hand, almost 100 per cent of the smutted plants were still green and fairly succulent. Thus the smutted plants might have been in condition for rust infection over a longer period than the healthy plants. In case of an epidemic of the oat-rust forms it is probable that smutted plants might be more severely rusted than the nonsmutted, but, under the field conditions at University Farm in 1929, there was no indication that the rust resistance of Anthony oats was altered by the smut infection.

Adjacent to the plot of Anthony oats there was a plot of Kota wheat in which 75 per cent of the plants were infected with *Tilletia tritici* and 4 per cent with *Ustilago tritici*, while 21 per cent remained healthy. Kota wheat often is rust resistant in the field, and it was thought possible that its resistance might be broken down in plants which were smutted. On August 5 the smutted, bunted, and clean plants were sorted and examined carefully. All the bunted plants and about 95 per cent of the clean plants were yellowed and ripe, but 50 per cent of those attacked by Ustilago were still green and somewhat succulent. Thus Ustilago infection in wheat as well as in oats seemed to delay ripening and prolonged the period during which plants were in condition for rust infection. Tilletia infection in wheat did not have this effect. The stem-rust infection varied in the three lots, there being only a trace in both the clean and bunted plants, but from 5 to 8 per cent in those attacked by *Ustilago tritici*. Unfortunately, the rust forms that infected the smutted plants were not determined, but it is believed they were forms that might attack clean Kota plants in the field. Thus the explanation would not be a breakdown in rust resistance, but increased opportunity for stem-rust infection due to the delayed ripening of the wheat or an alteration in its functional resistance.

More complete experiments and more detailed observations are necessary before the question can be settled. There also must be certainty that one is dealing with a protoplasmic resistance rather than with a morphologic or functional type. At present the last two types of resistance are easier to alter or overcome experimentally than is the protoplasmic resistance.

DISCUSSION

The problem of rust resistance evidently is extremely complex. At various times certain ideas have dominated the concept of the nature of resistance, but all of these ideas seldom have been harmonized with all of the known facts. Cobb many years ago concluded that resistance was due to morphologic characters of host plants. He thought there was a correlation among resistance and the tensile strength of host tissues, the number and size of stomata, and the presence of waxy bloom on the leaves. This idea had considerable vogue. Then Ward concluded from his investigations of the brown rust of bromes that no observable morphologic characters were responsible for resistance. He suggested, on the other hand, that there was some sort of protoplasmic antagonism between resistant host plants and the pathogene. The work of Marryat, Gibson, and Stakman supported this view. For some time after these investigations resistance was attributed solely to physiologic incompatibility between host and pathogene. Stakman and Piemeisel, however, suggested in 1917 that resistance sometimes might be due to structural characters. Hursh investigated this possibility quite thoroughly and came to the conclusion that morphologic characters sometimes were responsible for resistance. The investigations of Allen called attention again to the fact that the size and number of the stomata may have had some influence on resistance. The writer has investigated the problem to discover whether these various ideas can be reconciled. The results presented in this bulletin show clearly that resistance of gramineous hosts to *Puccinia graminis* does not depend on any one factor. It has been shown that resistance may be due to protoplasmic, morphologic, or functional peculiarities of the host. Any one of these types of resistance, or any combination of the three, may be present in a single variety. One should not generalize too much. Even the morphologic resistance may differ in different parts of plants of the same variety.

The percentage and distribution of rust-susceptible tissue probably is of primary importance in determining the degree of morphologic resistance to rust in different parts of plants of a single variety and in comparable parts of plants of different varieties. In the seedlings of all wheat varieties examined, only the vascular bundles and a few slender, isolated strands of sclerenchyma cells offer resistance to rust. In mature wheat plants there is less susceptible tissue than in the seedlings, and in some parts there is none at all. The leaf blade probably has more susceptible tissue than any other part of the mature plant, for the susceptible mesophyll areas, distributed in large parallel strands, are separated from each other only by the vascular bundles and the girders of sclerenchyma. There is slightly less susceptible tissue in the leaf sheath than in the leaf blade, for the sclerenchyma about the vascular bundles usually is more strongly developed. The susceptible tissue is distributed in parallel strands, as in the blade, but near the base of the leaf sheath one strand may be continuous with the next at the periphery of the culm, because the vascular bundles with their sheaths of sclerenchyma are embedded in the leaf sheath and do not extend to the surface.

The peduncle of a plant contains much less rust-susceptible tissue than either leaf sheath or blade, but the proportion and distribution of that tissue differ in different parts of the peduncle. The greater proportion of susceptible tissue occurs just beneath the head, and it is distributed in one to four or five broad strands encircling the peduncle. Farther below the head the quantity of susceptible tissue is reduced and distributed in numerous strands that are extremely narrow in some varieties and broad and coalesced in others. Just above and within the leaf sheath there is much less susceptible tissue in the peduncle. The collenchyma areas are very small and there are very broad bands of sclerenchyma between them. The uppermost portion of the peduncle always is susceptible morphologically to rust, the middle portion may be either susceptible or resistant, and the lower portion usually is resistant morphologically.

The rachis is somewhat similar to the peduncle, and its rust-susceptible tissue is distributed in strands around the convex side of the joint. There is, however, no susceptible tissue on the flattened side of each joint of the rachis, so this part of the rachis is immune morphologically from rust.

There is some susceptible tissue in the glume and in the awn, but the quantities are small when compared with those in the leaf blade and the leaf sheath. The two largest strands of susceptible tissue are on either side of the midrib in the glume and are continuous with the two strands that run the length of the awn. In the glumes other smaller strands run parallel with the two principal strands, but they are not so often rusted as the central strands.

After surveying the rust-susceptible tissue in different parts of the grass plant, it is found that some parts have more mechanical restrictions to rust development, and hence more morphologic resistance to rust, than other parts. The seedlings seem to have no appreciable morphologic resistance, and in the mature plants the base of the leaf sheath, the upper extremity of the peduncle, and the leaf blade have but little. The lower portion of the peduncle has a very decided morphologic resistance, and the flattened side of each joint of the rachis has a morphologic immunity from rust. There may be morphologic resistance in the middle part of the peduncle also, in the leaf sheath, and in various parts of the head, but the effectiveness of such resistance differs greatly in different varieties of wheat.

There may be structural differences affecting relative susceptibility even in susceptible tissues. When the tissue is made up of very thin-walled chlorophyllous cells, as it is in all seedlings, there is actually very little to prevent the penetration by haustoria; and when there are many large intercellular spaces, as is the case in seedlings, there are no mechanical obstacles to the rapid and abundant development of fungus mycelium. However, if the rust-susceptible tissue consists of compact collenchyma cells with cellulose walls of varying thickness, as is the case in the peduncle, it is probable that the mycelium will progress more slowly than it does in the tissues of the seedling. An extreme effect of tissue structure on rust development is evident in the swollen leaf base. This part of the plant is seldom infected, but on rare occasions the rust mycelium may pass from the base of the leaf sheath or from the upper extremity of the lower internode into the ground tissue of the swollen leaf base. The ground tissue can be infected by the pathogene, but the cells are so compactly arranged and their cellulose walls so greatly thickened that the ramification of hyphae must require considerable energy.

During the later stages in the development of rust infection, other morphologic characters are apt to be more important than the quantity and distribution or the structure of rust-susceptible tissue. When the pathogene produces spores and forms pustules the structure of the epidermal membrane is a factor to be considered. The epidermis of the seedling is a delicate membrane composed of thin-walled, only slightly cutinized cells. It is easily ruptured by the expanding pustules and the spores even may be pushed out between the cells without lifting a layer of them. The epidermis in the peduncle, on the other hand, usually is a very thick, heavily cutinized membrane. The cell walls are extremely thick and seem to be as heavily lignified as the walls of the sclerenchyma. Obviously, the epidermis of the peduncle is much more resistant to rupture than that of the seedling. The thickness and the extent of the epidermis of the peduncle differ greatly in different wheat varieties, and in some varieties the resistance to rupture is so great that pustules are formed with difficulty or not at all. The epidermis in the rachis, the glume, and the awn is similar to that in the peduncle. In the leaf blade and sheath, however, it seems weaker and less tough. It is far thicker than in the seedling, and the cell walls are thick, but the degree of lignification varies considerably. Occasionally the entire epidermis is composed of cells with lignified walls, but more often this is not the case. Sometimes only those cells over the sclerenchyma are lignified, while those over the rust-susceptible tissue have thickened cellulose walls.

The epidermis sometimes may be reenforced by strands of sclerenchyma cells, resulting in increased resistance to rupture because of thickening of the membrane or because of the separation of the wide membrane into several narrower ones which act independently of each other. Such reenforcements never occur in seedlings, but are extremely common in the peduncles of some varieties of wheat. They undoubtedly contribute to a morphologic resistance in the durum wheats and in the emmers, but are of little importance in the common wheats. Reenforcing sclerenchyma strands have not been found in the leaf blades or leaf sheaths of any of the wheat varieties, but in einkorn there is a broad band of sclerenchyma just below the epidermis of the leaf sheath; hence the membrane over the rust-susceptible tissue is about twice as thick as it would otherwise be. The nature and structure of the epidermal membrane, therefore, probably are responsible for considerable morphologic resistance to stem rust, because of the fact that pustules can not be formed readily.

In the peduncle of the plant, other factors probably influence somewhat the severity of rust infection. The shape and position of the rust-susceptible areas may affect the size of rust pustules. In culms of the durums and emmers the width of the collenchyma bundles a short distance beneath the surface is greater than that of the periphery. Thus the individual pustules are slightly smaller than they would be if the radial borders of the infected areas were regular and approximately perpendicular to the surface of the peduncle, as they are in the great majority of common wheats. It is probable also that it is harder for the pathogene to burst the collenchyma strand of a durum than that of a common wheat, because in the durum a larger proportion of the growth pressure exerted by the mycelial mass would be expended on the rigid unyielding sclerenchyma, and the narrower epidermal membrane would be more resistant to rupture than the wide membrane covering the entire width of the collenchyma strand in the common wheat. The position of the collenchyma strands also must be taken into consideration. In the durums and emmers they are situated in longitudinal furrows in the peduncle. There is a tendency toward this condition in some of the common wheats, but the furrowing of the stem is never so marked as in the durums and emmers in which the pustules are likely to be narrow because they are flanked by prominent ridges of sclerenchyma on both sides. It is possible that these morphologic characters are often of minor importance, but if the host plant has only moderate protoplasmic resistance to a certain rust form, the shape and disposition of the infected areas may be of major importance and may serve to emphasize the moderate protoplasmic resistance.

The pathogene does not easily enter to many resistant varieties of wheat. In a few cases the exclusion is due to morphologic causes. For example, the epidermis of the swollen leaf base of a wheat culm has no stomata; consequently the fungus can not invade directly this part of the plant. The leaf base is almost never rusted in the field, but it is possible for rust mycelium to penetrate the tissue of the leaf base from the adjacent infected leaf sheath or stem. Occasionally in the peduncle a strand of collenchyma is completely buried in the sclerenchyma, no part of the strand abuts on the epidermis, and there is no way for the fungus to pass through the epidermis and the sclerenchyma to reach the susceptible strand of collenchyma. In most cases, however, the exclusion of the rust fungus is due to peculiarities in stomatal behavior. In certain wheat varieties the stomata usually are closed during the periods when the germ tubes of the fungus are ready to initiate infection. A certain degree of moisture and favorable temperatures are necessary for spore germination, and moisture probably is the prime requisite for the survival of the germ tubes. In the upper Mississippi Valley there generally is sufficient dew to enable the spores to germinate during the night or the early morning hours. But when the dew disappears in the morning the germ tubes shrivel and perish if they are exposed. During the night the stomata of the wheat plant are closed and they do not open until affected by the stimulus of sunlight in the morning. The response to sunlight is rapid in some varieties and slow in others. The first stages of rust infection depend upon the rate of opening of the stomata, for the germ tubes do not force their way through closed stomata. In some varieties the stomata open so late and so slowly that the dew disappears and the germ tubes dry up before they can enter the host. Such varieties are functionally resistant to stem rust, and this type of resistance is about equally effective against all physiologic forms.

Clearly, then, rust resistance may be due to several different factors operating independently or in conjunction. This holds true for different varieties and species of Gramineae and for different parts of individual plants. For example, Kota wheat has considerable morphologic resistance because of the amount and distribution of sclerenchyma in the peduncle, but this is not true of Quality wheat. On the other hand, although there are some varietal differences there is relatively little morphologic resistance in the leaf blade of Kota or of most other varieties, because the susceptible areas are relatively large in all of them. For this reason morphology probably is relatively unimportant in resistance to *Puccinia triticina*. In studying resistance to this rust one probably would decide that there was no effective morphologic resistance in any variety. It is always necessary to take into account the structural characters in the different parts of the host attacked by a particular rust fungus. The leaf sheath and the peduncle are the principal organs severely infected with *P. graminis*, but it is the leaf blades that are principally infected with *P. triticina*, *P. dispersa*, and *P. glumarum*. The problem of resistance to *P. graminis* has been dealt with in this bulletin, and a good deal of the detailed work has been done on the structural characters of the peduncle of the plant, the region most severely damaged by rust when the attack is heavy. Had the leaf rusts been investigated the conclusions might have been quite different

It may be well to summarize the factors influencing resistance in several of the outstanding wheat varieties to illustrate the importance of considering more than one factor when such a problem is being studied.

Kota, a variety of *Triticum vulgare*, has protoplasmic resistance to a number of physiologic forms of *Puccinia graminis tritici*. Besides its protoplasmic resistance, Kota has a very definite morphologic resistance to stem rust. Hursh showed that this resistance was due to the occurrence of the susceptible tissue of the peduncle in narrow, isolated strands. The size of the pustules is limited by the width of the strands and if many pustules are produced it is necessary that each strand be infected separately, for the mycelium does not spread from one strand to the next. There are other factors, however, that probably contribute to the morphologic resistance of Kota. There is a tendency for the peduncle to be furrowed, and the rust pustules produced in these furrows must contain great masses of spores if they are to expand beyond the sclerenchyma borders of the furrows. The epidermis of Kota is very thick in comparison with that of other varieties of common wheat. The cell walls are thick and the membrane appears to be extremely tough. In some cases, on the peduncle especially, the rust pustules remain subepidermal, as they seem unable to break the epidermis. In many cases the force exerted by the fungus cracks the sclerenchyma bands and separates the lignified cells before the epidermal membrane gives way. Thus Kota has several structures contributing to its morphologic resistance to stem rust. Moreover, Kota probably has some functional resistance to stem rust. Its stomatal behavior indicates that under some conditions a large proportion of germ tubes are excluded. The effectiveness of this moderate functional resistance may vary in different seasons and localities, but it sometimes assumes major importance. Kota thus actually has the three types of resistance to rust, namely, protoplasmic, functional, and morphologic; and at least three different structural characters are responsible for the morphologic resistance.

Marquis is a direct antithesis to Kota. Marquis has a protoplasmic resistance to some physiologic forms of *Puccinia graminis tritici*, but it seems to have neither morphologic nor functional resistance to stem rust. The rust-susceptible tissue of the peduncle is distributed in broad, double strands, and the stem of the plant usually is smooth and not furrowed as in Kota. The epidermal membrane is not so thick as in Kota and there is less resistance to rupture. Imperfect pustules are seldom found in Marquis wheat. The stomatal behavior of Marquis is such that the stomata open rather rapidly and there is ample opportunity for the germ tubes of the rust to enter. It seems that Marquis must rely solely on its protoplasmic resistance to stem rust. Therefore, in comparison with Kota, it is much handicapped.

Webster, like Kota, possesses the three types of resistance to stem rust. In its protoplasmic reactions it has been either highly or moderately resistant to all the physiologic forms of *Puccinia graminis tritici* with which it has been inoculated at University Farm, St. Paul, Minn. There also is a very definite morphologic resistance in Webster. The peduncle is inclined to be furrowed, and the collenchyma is distributed in narrow, isolated strands beneath the trough of each furrow. The epidermis is extremely thick and very resistant to rupture. Subepidermal pustules are common on the peduncles and the leaf sheaths of Webster. It may be that normal pustules would be formed more often if Webster had less protoplasmic resistance, but it is evident that most physiologic forms of the fungus have great difficulty in breaking the epidermis. Webster also has very marked functional resistance, for its stomata respond to light so slowly that many germ tubes of rust probably never have a chance to enter. The functional resistance of Webster is even more marked than that of Kota.

Hope, a common wheat, is very resistant to stem rust in the field, in the United States, and it, too, possesses the three types of resistance. Hope is not so uniformly resistant in a protoplasmic way as Webster seems to be, but it is resistant to a large number of forms of stem rust. The morphologic resistance of Hope is due to the fact that there are only narrow, isolated strands of suspectible tissue in the peduncle, the stem is slightly furrowed, and the moderately thick epidermis has exceptionally thick outer cell walls. However, the very high degree of rust resistance of Hope in the field may be due principally to its very pronounced functional resistance.

The two hybrid wheats, Ceres and Marquillo, are protoplasmically highly resistant to a number of rust forms. There is less evidence of morphologic resistance, however, for both varieties have numerous moderately broad, coalesced collenchyma strands in the peduncle. The only factor that might account for a morphologic resistance is the strength of the epidermis. In Marquillo it is thick and tough, and in sectioned material there is evidence that the fungus has difficulty in forming pustules. The importance of the epidermis in Ceres is less obvious, and it is not certain that Ceres has any effective morphologic resistance. When the stomatal behavior is studied, Ceres seems to have no functional resistance, but Marquillo has a moderate functional resistance like that of Kota.

The durum wheats have protoplasmic resistance to certain forms of stem rust, but if they have morphologic resistance it is due to structures other than those responsible for resistance in the common wheats. It is doubtful whether such morphologic resistance as the durums have is so effective as their protoplasmic and functional resistance. All of the durums have broad, coalesced collenchyma strands in the peduncles; therefore, the amount and distribution of susceptible tissue are not factors in their resistance. The shape and the position of the collenchyma in the furrowed peduncle may be responsible for some resistance to rust but probably they are not such effective factors as size of collenchyma strand. They do not give any morphologic resistance to Mindum wheat, for example, for Mindum may be highly susceptible in the field. The most effective morphologic resistance in the durums seems due to thickness and the reenforcements of the epidermal membrane and its increased resistance to rupture.

Some of the durums have decided functional resistance to stem rust. Acme and Velvet Don may be classed with Webster and Hope with regard to stomatal behavior and functional resistance. This type of resistance seems to account for the relative freedom of Acme and Velvet Don from stem rust in the field. Mindum and Arnautka, on the other hand, seem to have no more functional resistance than Marquis, while Kubanka has a moderate functional resistance similar to that possessed by Kota.

In the emmers there also are the three types of rust resistance. Khapli, for example, has protoplasmic resistance to most of the physiologic forms of *Puccinia graminis tritici.* It also has morphologic resistance because of the following structural characters, namely, the relatively small amount of susceptible tissue and its distribution in narrow, isolated strands in the peduncle, the irregular shape of collenchyma strands and their position in the furrowed culm, the extreme thickness of the epidermis and its sclerenchymatous reenforcements which increase its resistance to rupture. Khapli also has a moderate functional resistance to stem rust because of stomatal behavior.

From the facts available, it is clear that there are several causes for stem-rust resistance of varieties of wheat. These can be grouped into three general categories—protoplasmic, morphologic, and functional. In the case of protoplasmic resistance, there is some sort of antagonism between the pathogene and the host plant. The pathogene may enter in a normal manner but is unable to grow extensively within the tissues, because it usually kills some of them and then itself dies. While it is known what happens in this type of resistance, it is not known why it happens because of the meager knowledge of the physico-chemical process involved. This protoplasmic resistance usually is effective only against certain physiologic forms. No varieties of wheat yet known have protoplasmic resistance to all known forms of *Puccinia graminis tritici.* Morphologic resistance may protect plants against the attack of all but the most virulent physiologic forms, but it is itself due to many factors and is somewhat variable. Functional resistance, due largely to the failure of stomata to open promptly in the morning, thus excluding the germ tubes of the pathogene, is likely to be more universally effective than either of the other two. Some varieties have only one type of resistance, while others have two, and still others have all types. It is likely that both the morphologic resistance and the functional resistance are particularly important because varieties with these means of resisting rust attack probably can resist invasion by all physiologic forms. Therefore, they are likely to be very useful in the development of rust-resistant varieties. It is obvious that varieties possessing all known types of resistance may be most useful. Fortunately, some varieties, such as Hope, seem to possess in some degree, at least, all of the three general types of resistance. The implications of these facts in the development of rust-resistant varieties are, of course, self-evident.

SUMMARY

There are three general types of resistance to stem rust (*Puccinia graminis*) in wheat—protoplasmic, morphologic, and functional. A number of different structures that are responsible for morphologic resistance have been investigated; and stomatal behavior has been studied in wheat as the principal cause of a functional resistance.

All of the aboveground parts of a wheat plant contain rust-susceptible tissue—leaf blade, leaf sheath, peduncle, rachis, glume, and awn. The quantity and distribution of the tissue vary in different parts of the plant. The wheat seedling is composed almost entirely of susceptible tissue, and a large proportion of the mature leaf blade is susceptible, but in the peduncle and rachis there are smaller amounts of susceptible tissue distributed in longitudinal strands beneath the epidermis.

Wheat seedlings offer virtually no morphologic restrictions to the development of rust. Most of the tissue is thin-walled chlorenchyma, with numerous intercellular spaces. The epidermis is a thin, delicate, easily ruptured, cellulose-walled membrane.

The relative proportions of collenchyma and sclerenchyma vary in different parts of the peduncle and in comparable parts of the peduncles of different wheats. Just beneath the head about 90 to 98 per cent of the periphery of the peduncle is bordered by collenchyma, distributed in from one to four or five broad coalesced strands. In the middle of the peduncle from 50 to 80 per cent of the periphery is bordered by collenchyma, distributed in numerous strands that are separated by bands of sclerenchyma. Just above and within the leaf sheath the peduncle has only 25 to 35 per cent of the periphery bordered by collenchyma, the strands of which are extremely narrow and shallow. The relative proportions of collenchyma in the middle portion of the peduncle differ somewhat in different wheats, being about 60 to 70 per cent in the common wheats, about 72 to 80 per cent in the durums, and 50 to 65 per cent in the emmers.

The size, shape, and disposition of collenchyma strands in the peduncle are important factors in determining the spread of the mycelium and the size of rust pustules. In the common wheats some varieties are characterized by very broad and coalesced strands of collenchyma, while others usually have very narrow single strands of collenchyma with strong wide bands of sclerenchyma between them. Each strand is regular in shape with borders almost perpendicular to the surface of the culm. Most of the common wheats have smooth culms, but a few varieties have stems that are slightly furrowed. All varieties of durum wheat have broad extensive areas of collenchyma formed by three to seven or more confluent strands. The shape of the strand is irregular and the portion adjacent to the epidermis is less than its total width. The stem of durum wheats usually is deeply furrowed, with the result that the rust pustules lie at the bottom of the furrow. The shape and position of the collenchyma strands in emmers are very much like those in the durums, but the collenchyma strands of the emmer are likely to be narrow and isolated rather than coalesced into large extensive areas.

The rust-susceptible tissue in the leaf blade occupies the area between the parallel vascular bundles and extends from the lower to the upper epidermis. The girders of sclerenchyma that accompany some of the vascular bundles separate one collenchyma area from its neighboring area. The width of the area varies in different varieties of wheat and in different species of Triticum, but the differences are seldom sufficient to affect greatly the size of the rust pustules. An increase in the number of sclerenchyma girders and a decrease in the distance between them make necessary a greater number of infections if the rust is to cover much of the surface of the leaf blade.

The leaf sheath contains broad strands of rust-susceptible tissue continuous with those of the leaf blade and separated by the vascular bundles with their sheaths of sclerenchyma. The rust-susceptible chlorenchyma lies just beneath the outer epidermis and below it is a large lysigenous cavity in the parenchyma that forms the inner part of the leaf sheath. The general structure of the leaf sheath does not differ greatly in different varieties of wheat.

The base of the leaf sheath is relatively thick, the lysigenous cavities tend to disappear, and the vascular bundles with their accompanying sclerenchyma are embedded in the leaf sheath and do not extend to the outer epidermis. The rust-susceptible areas thus merge with neighboring rust-susceptible areas at the periphery of the leaf sheath, and there is excellent opportunity for the formation of large rust pustules.

The rust-susceptible tissue of the rachis is distributed in longitudinal strands as in the peduncle, but the flattened side of each joint of the rachis is composed of sclerenchyma and is never infected by rust. There are indications that the amount and distribution of collenchyma in the rachis differ in different varieties of wheat just as they do in the peduncles of different wheats.

There is relatively little rust-susceptible tissue in the glumes and awns.

Certain varieties of wheat may be resistant because of structural peculiarities that make it difficult for pustules to rupture the epidermis. The size and shape of the collenchyma bundles, the thickness and toughness of the epidermis, and sclerenchymatous reenforcements of the epidermis appear to be most important in this connection.

Not all of the tissues in which the rust can grow are equally susceptible. The degree of compactness and the thickness of the cell walls may determine the rapidity with which the pathogene can grow.

Some varieties of wheat are very resistant to all physiologic forms of stem rust under natural conditions because their stomata open so slowly in the morning that the germ tubes of the pathogene usually dry up before they can enter.

Results of experiments to determine the effect of fertilizers on the structure of wheat varieties and their susceptibility to rust attack were somewhat inconsistent. There were no consistent and significant differences in the proportion or distribution of collenchyma in the peduncles of wheats grown in different fertilizer plots, although in a few cases there was an effect which indicated a possible correlation with the degree of rust infection. A study of the effect of fertilizers on the structure of the epidermal membrane was not conclusive. The application of nitrogenous fertilizers did not increase the diameter of the peduncles of Marquis wheat. In the case of Kota the diameter was increased, but it is not clear whether rust susceptibility was increased thereby. No one fertilizer or combination of fertilizers, in the experiments so far made, seemed consistently to alter the structure of plants sufficiently to affect their susceptibility or resistance to rust.

In the field, stem rust often develops almost exclusively on the north side of the plants. The exact reason for this phenomenon is not yet known, but it does not seem to be due to differences in structure on the two sides of the stem nor to differences in total sugars nor reducing sugars.

Wheat plants affected with smuts or foot rot are likely to become more heavily rusted than healthy plants; clipping the heads has the same effect.

It is evident that there are several types of resistance to stem rust. A variety of wheat may have one or more of these types of resistance. Some have only protoplasmic resistance to certain physiologic forms, and none, so far as known, is resistant to all forms. Such varieties are likely to be severely rusted in those years and localities in which the physiologic forms to which they are susceptible happen to occur. While morphologic and functional resistance may be somewhat variable, varieties with these types of resistance are likely to be more nearly universally free from rust than those with protoplasmic resistance only. And, obviously, varieties like Hope and Webster, which have at least in some degree all known types of resistance, are likely to be almost universally resistant.

LITERATURE CITED

(1) ABBOTT, E. V.
1929. STEM RUST OF WHEAT IN PERU. Phytopathology 19: 1041–1043.

(2) ———
1930. STEM RUST INFECTION OF KHAPLI EMMER AND HOPE WHEAT IN PERU. (Abstract) Phytopathology 20: 143.

(3) ALLEN, R. F.
1921. RESISTANCE TO STEM RUST IN KANRED WHEAT. Science (n. s.) 53: 575–576.

(4) ———
1923. A CYTOLOGICAL STUDY OF INFECTION OF BAART AND KANRED WHEATS BY PUCCINIA GRAMINIS TRITICI. Jour. Agr. Research 23: 131–152, illus.

(5) ———
1924. CYTOLOGICAL STUDIES OF INFECTION OF BAART, KANRED, AND MINDUM WHEATS BY PUCCINIA GRAMINIS TRITICI FORMS III AND XIX. Jour. Agr. Research (1923) 26: 571–604, illus.

(6) ———
1926. CYTOLOGICAL STUDIES ON FORMS 9, 21, AND 27 OF PUCCINIA GRAMINIS TRITICI ON KHAPLI EMMER. Jour. Agr. Research 32: 701–725, illus.

(7) ———
1926. A CYTOLOGICAL STUDY OF PUCCINIA TRITICINA PHYSIOLOGIC FORM 11 ON LITTLE CLUB WHEAT. Jour. Agr. Research 33: 201–222, illus.

(8) Allen, R. F.
1927. A CYTOLOGICAL STUDY OF ORANGE LEAF RUST, PUCCINIA TRITICINA PHYSIOLOGIC FORM 11, ON MALAKOFF WHEAT. Jour. Agr. Research 34: 697–714, illus.

(9) ———
1928. A CYTOLOGICAL STUDY OF PUCCINIA GLUMARUM ON BROMUS MARGINATUS AND TRITICUM VULGARE. Jour. Agr. Research 36: 487–513, illus.

(10) Armstrong, S. F.
1922. THE MENDELIAN INHERITANCE OF SUSCEPTIBILITY AND RESISTANCE TO YELLOW RUST (PUCCINIA GLUMARUM, ERIKSS. ET HENN.) IN WHEAT. Jour. Agr. Sci. [England] 12: 57–96.

(11) Biffen, R. H.
1907–1912. STUDIES IN THE INHERITANCE OF DISEASE RESISTANCE. I–II. Jour. Agr. Sci. [England] 2: [109]–128, 1907; 4: [421]–429, 1912.

(12) Bolley, H. L.
1889. WHEAT RUST. Ind. Agr. Expt. Sta. Bul. 26, 19 p., illus.

(13) Cobb, N. A.
1890–92. CONTRIBUTIONS TO AN ECONOMIC KNOWLEDGE OF AUSTRALIAN RUSTS (UREDINEÆ). Agr. Gaz. N. S. Wales 1: [185]–214, illus., 1890; 3: 44–68, 181–212, illus. 1892.

(14) Eames, A. J., and MacDaniels, L. H.
1925. AN INTRODUCTION TO PLANT ANATOMY. 364 p., illus. New York.

(15) Eriksson, J., and Henning, E.
1896. DIE GETREIDEROSTE. 463 p., illus. Stockholm.

(16) Farrer, W.
1898. THE MAKING AND IMPROVEMENT OF WHEATS FOR AUSTRALIAN CONDITIONS. Agr. Gaz. N. S. Wales 9: [131]–168, [241]–260, illus.

(17) Freeman, E. M., and Johnson, E. C.
1911. THE RUSTS OF GRAINS IN THE UNITED STATES. U. S. Dept. Agr., Bur. Plant Indus. Bul. 216, 87 p., illus.

(18) Gibson, C. M.
1904. NOTES ON INFECTION EXPERIMENTS WITH VARIOUS UREDINEAE. New Phytol. 3: 184–191, illus.

(19) Goulden, C. H.
1929. BREEDING RUST RESISTANT VARIETIES OF WHEAT—FUNDAMENTAL ASPECTS OF THE PROBLEM. Sci. Agr. 10: 258–267, illus.

(20) Hart, H.
1929. RELATION OF STOMATAL BEHAVIOR TO STEM-RUST RESISTANCE IN WHEAT. Jour. Agr. Research 39: 929–948, illus.

(21) Hayes, H. K., and Stakman, E. C.
1922. WHEAT STEM RUST FROM THE STANDPOINT OF PLANT BREEDING. Western Canad. Soc. Agron. Proc. 2: 22–35, illus.

(22) Hitchcock, A. S., and Carleton, M. A.
1893. PRELIMINARY REPORT ON RUSTS OF GRAIN. Kans. Agr. Expt. Sta. Bul. 38, 14 p., illus.

(23) Hursh, C. R.
1924. MORPHOLOGICAL AND PHYSIOLOGICAL STUDIES ON THE RESISTANCE OF WHEAT TO PUCCINIA GRAMINIS TRITICI ERIKSS. AND HENN. Jour. Agr. Research 27: 381–412, illus.

(24) Johnson, A. G.
1911. FURTHER NOTES ON TIMOTHY RUST. Ind. Acad. Sci. Proc. 1910: 203–204.

(25) Johnston, C. O., and Melchers, L. E.
1929. GREENHOUSE STUDIES ON THE RELATION OF AGE OF WHEAT PLANTS TO INFECTION BY PUCCINIA TRITICINA. Jour. Agr. Research 38: 147–157, illus.

(26) Lang, W.
1917. ÜBER DIE BEEINFLUSSUNG DER WIRTPFLANZE DURCH TILLETIA TRITICI. Ztschr. Pflanzenkrank. 27: 80–99.

(27) Little, W. C.
1883. REPORT ON WHEAT-MILDEW. Jour. Roy. Agr. Soc. England (2) 19: 634–693.

(28) Loftfield, J. V. G.
1921. THE BEHAVIOR OF STOMATA. 104 p., illus. Washington, D. C. (Carnegie Inst. Wash. Pub. 314.)

(29) Marryat, D. C. E.
1907. NOTES ON THE INFECTION AND HISTOLOGY OF TWO WHEATS IMMUNE TO THE ATTACKS OF PUCCINIA GLUMARUM, YELLOW RUST. Jour. Agr. Sci. [England] 2: [129]–138, illus.

(30) Newton, M.
1922. STUDIES IN WHEAT STEM RUST (PUCCINIA GRAMINIS TRITICI). Roy. Soc. Canada Proc. and Trans. (3) 16 (sect. 5): 153–210, illus.

(31) Percival, J.
1921. THE WHEAT PLANT: A MONOGRAPH. 463 p., illus. London.

(32) Pool, V. W., and McKay, M. B.
1916. RELATION OF STOMATAL MOVEMENT TO INFECTION BY CERCOSPORA BETICOLA. Jour. Agr. Research 5: 1011–1038, illus.

(33) Remer, W.
1904. DER ROST DES GETREIDES IN SCHLESIEN IM SOMMER 1903. Ztschr. Pflanzenkrank. 14: [65]–70.

(34) Ruttle, M. L., and Fraser, W. P.
1927. A CYTOLOGICAL STUDY OF PUCCINIA CORONATA CDA. ON BANNER AND COWRA 35 OATS. Calif. Univ. Pubs. Bot. 14: [21]–72, illus.

(35) Sappin-Trouffy, P.
1896. RECHERCHES HISTOLOGIQUES SUR LA FAMILLE DES UREDINÉES. Botaniste 5: 59–244, illus.

(36) Sorauer, P.
1897. DER EINFLUSS EINSEITIGER STICKSTOFFDÜNGUNG. Ztschr. Pflanzenkrank. 7: 287–290.

(37) Spinks, G. T.
1913. FACTORS AFFECTING SUSCEPTIBILITY TO DISEASE IN PLANTS. Jour. Agr. Sci. [England] 5: [231]–247, illus.

(38) Stakman, E. C.
1914. A STUDY IN CEREAL RUSTS: PHYSIOLOGICAL RACES. Minn. Agr. Expt. Sta. Bul. 138, 56 p., illus.

(39) ———
1915. RELATION BETWEEN PUCCINIA GRAMINIS AND PLANTS HIGHLY RESISTANT TO ITS ATTACK. Jour. Agr. Research 4: 193–200, illus.

(40) ——— and Aamodt, O. S.
1924. THE EFFECT OF FERTILIZERS ON THE DEVELOPMENT OF STEM RUST OF WHEAT. Jour. Agr. Research 27: 341–380, illus.

(41) ——— and Levine, M. N.
1922. THE DETERMINATION OF BIOLOGIC FORMS OF PUCCINIA GRAMINIS ON TRITICUM SPP. Minn. Agr. Expt. Sta. Tech. Bul. 8, 10 p., illus.

(42) ——— Levine, M. N., and Griffee, F.
1925. WEBSTER, A COMMON WHEAT RESISTANT TO BLACK STEM RUST. Phytopathology 15: [691]–698, illus.

(43) ——— Parker, J. H., and Piemeisel, F. J.
1918. CAN BIOLOGIC FORMS OF STEMRUST ON WHEAT CHANGE RAPIDLY ENOUGH TO INTERFERE WITH BREEDING FOR RUST RESISTANCE? Jour. Agr. Research 14: 111–124, illus.

(44) ——— and Piemeisel, F. J.
1917. BIOLOGIC FORMS OF PUCCINIA GRAMINIS ON CEREALS AND GRASSES. Jour. Agr. Research 10: 429–496, illus.

(45) ——— Piemeisel, F. J., and Levine, M. N.
1918. PLASTICITY OF BIOLOGIC FORMS OF PUCCINIA GRAMINIS. Jour. Agr. Research 15: 221–250, illus.

(46) Voelcker, J. A.
1912. [THE WOBURN] POT-CULTURE EXPERIMENTS, 1910–11–12. Jour. Roy. Agr. Soc. England 73: 314–338, illus.

(47) Ward, H. M.
1901. THE BROMES AND THEIR RUST-FUNGUS (PUCCINIA DISPERSA). Ann. Bot. [London] 15: 560–562.

(48) ———
1902. ON THE RELATION BETWEEN HOST AND PARASITE IN THE BROMES AND THEIR BROWN RUST, PUCCINIA DISPERSA (ERIKSS.). Ann. Bot. [London] 16: [233]–315, illus.

(49) ———
1902. ON THE QUESTION OF "PREDISPOSITION" AND "IMMUNITY" IN PLANTS. Cambridge Phil. Soc. Proc. (1900–1902) 11: [307]–328, illus.

(50) **Ward, H. M.**
1902. EXPERIMENTS ON THE EFFECT OF MINERAL STARVATION ON THE PARASITISM OF THE UREDINE FUNGUS, PUCCINIA DISPERSA, ON SPECIES OF BROMUS. Roy. Soc. [London] Proc. 71: 138–151, illus.

(51) Wellensiek, S. J.
1927. THE NATURE OF RESISTANCE IN ZEA MAYS L. TO PUCCINIA SORGHI SCHW. Phytopathology 17: 815–825, illus.

(52) Weston, W. A. R. Dillon.
1927. THE INCIDENCE AND INTENSITY OF PUCCINIA GLUMARUM ERIKSS. AND HENN., ON WHEAT INFECTED AND NONINFECTED WITH TILLETIA TRITICI WINTER, SHOWING AN APPARENT RELATIONSHIP BETWEEN THE SUSCEPTIBILITY OF WHEAT PLANTS TO YELLOW RUST AND TO BUNT. Ann. Appl. Biol. 14: [105]–112, illus.

ORGANIZATION OF THE UNITED STATES DEPARTMENT OF AGRICULTURE WHEN THIS PUBLICATION WAS LAST PRINTED

Secretary of Agriculture	Arthur M. Hyde.
Assistant Secretary	R. W. Dunlap.
Director of Scientific Work	A. F. Woods.
Director of Regulatory Work	Walter G. Campbell.
Director of Extension Work	C. W. Warburton.
Director of Personnel and Business Administration.	W. W. Stockberger.
Director of Information	M. S. Eisenhower.
Solicitor	E. L. Marshall.
Weather Bureau	Charles F. Marvin, *Chief.*
Bureau of Animal Industry	John R. Mohler, *Chief.*
Bureau of Dairy Industry	O. E. Reed, *Chief.*
Bureau of Plant Industry	William A. Taylor, *Chief.*
Forest Service	R. Y. Stuart, *Chief.*
Bureau of Chemistry and Soils	H. G. Knight, *Chief.*
Bureau of Entomology	C. L. Marlatt, *Chief.*
Bureau of Biological Survey	Paul G. Redington, *Chief.*
Bureau of Public Roads	Thomas H. MacDonald, *Chief.*
Bureau of Agricultural Engineering	S. H. McCrory, *Chief.*
Bureau of Agricultural Economics	Nils A. Olsen, *Chief.*
Bureau of Home Economics	Louise Stanley, *Chief.*
Plant Quarantine and Control Administration	Lee A. Strong, *Chief.*
Grain Futures Administration	J. W. T. Duvel, *Chief.*
Food and Drug Administration	Walter G. Campbell, *Director of Regulatory Work, in Charge.*
Office of Experiment Stations	James T. Jardine, *Chief.*
Office of Cooperative Extension Work	C. B. Smith, *Chief.*
Library	Claribel R. Barnett, *Librarian.*

This bulletin is a contribution from

Bureau of Plant Industry	William A. Taylor, *Chief.*
Division of Cereal Crops and Diseases	M. A. McCall, *Principal Agronomist, in Charge.*

U. S. GOVERNMENT PRINTING OFFICE: 1931

END