A STUDY OF RUST RESISTANCE IN *PANICUM VIRGATUM* L.

by

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INTRODUCTION

Because of the fact that Panicum virgatum L. is an important grass in soil conservation work and in pastures on sandy loam soils, any disease such as rust, which hinders its growth, is worthy of investigation. The causal organism of the disease in this case is Uromyces graminicola Burr. which is a member of the order, Uridinales, a group of obligate parasites found only upon living plants that are members of the pteridophytes and spermatophytes. These fungi are very highly specialized in their parasitism; very closely related species of rusts usually have very closely related plants as hosts. However, with heteroecious rusts, the alternate hosts are members of very widely separated groups.

The factors causing high specialization have been approached through studies in morphology, cytology, anatomy, genetics, and physiology.

The plants themselves are classified as (I) immune, (II) resistant, and (III) susceptible.

HISTORY

The study of rusts is generally considered to have begun with the work of Anton de Bary (2). He was the first to discover that germ tubes from spores of rust fungi entered the leaves through their stomata and that after their entrance in some cases, further development ceased. In 1866, he (3) reported his observation of the germination of the urediniospores and the formation of the appresoria on the stomata. He was the first to recognize heteroecism.

Most of the early workers attempted to prove that immunity, resistance, and susceptibility resulted from morphological features which prevented the rust from developing within a plant. It was known that water was necessary for the germination of spores and any morphological feature such as bloom, which would prevent the collecting of water on the leaves would prevent the development of the rust.

There are some varieties of wheat upon which it seems exceedingly difficult for rust to gain a working hold, for example, Fulcaster, Egyptian, and Dietz Longberry. These are all hardy, stiff-strawed wheats, having smooth, fibrous leaves. In warm weather, any conditions of the soil which tend to keep the wheat leaves constantly wet are conducive to a rapid spread of the disease (Bolley 4).

Farrer (5), in his studies of wheat, emphasized the fact that wheats which successfully resist the summer-rust in one climate or even in one part of the country fail to do so in another. The cause of this latter circumstance is that the physical qualities which give resistance one place fail to protect in another; one variety may be resistant because its stomata are too small for the fungus to enter; another, because its leaves are covered by a glaucous film of a waxy substance, from which moisture runs quickly and are on that account not moist enough for the spores which lodge on them to germinate; another, because its cuticle is too thick for the enclosed fungus to break through; another, because its leaves are too erect and present too little of surface which is horizontal enough for spores to lodge on them; or because the narrowness of its leaves may to a certain extent protect a variety, or the habit of ripening early or quickly after flowering. Some of these qualities may be valuable for giving rust resistance in some localities or seasons. In a still atmosphere it may be erect and narrow leaves; in a moist, the smallness of its stomata; in a hot climate, a thick or tough cuticle; in a wet, a glaucous leaf-surface while all of these qualities may fail to protect if the climate be such as does not suit the constitution of the variety and give it abundant health and vigor without which it cannot resist the parasite.

Puttick (6), in his researches, explained that seedling plants were usually more easily infected than older plants because the older plants retained a film of moisture less easily than seedlings.

Freeman (7) found that as bloom on barley leaves increased the resistance to rust correspondingly increased. Plants growing in alkaline soils were more resistant to rust than others. The smaller percentage of pustules was undoubtedly due to the greater development of bloom on the barley foliage when grown in the strong alkali resulting in the rolling off of the water and hence in the loss of the inoculating material. The bluish color of the plants in the alkaline soils and the greater tendency of the water to run off were quite pronounced. Whether there was an actual difference in real rust resistance is a debatable question.

It has been pointed out by Cobb (8) that resistant wheats have narrow, still, upright foliage, while the reverse is true of susceptible varieties, especially if they are early, their foliage being broad, flabby, and pendant.

Considerable study has been made with regard to stomata in an effort to show a correlation or lack of correlation between the number and size of these structures to resistance.

In his work with asparagus, Norton (9) noticed that there is a definite relation between increase in size and rust resistance. Rust resistance in asparagus seems to be based upon structural differences. Vigor is not necessarily correlated with resistance. The evidence tends to show that resistant plants have smaller stomata than the nonresistant ones. The theory that vigor of growth is correlated with resistance cannot be accepted, for many resistant plants are quite small and never produce strong shoots.

According to Appel (10), the quick-drying varieties generally are less susceptible to the disease than the slow-drying varieties. Slow-drying is the result of the plant's habit of growth which hinders the evaporation of water. Such plants have flat leaves. Small, hairy leaves, as well as an airy, open growth of the whole plant facilitate drying. In the case of cereals immunity from rust is independent of the stomata.

Investigations of the rust of Antirrhinum led Doran (11) to conclude that resistant varieties have about one-half as many stomata as the susceptible varieties. The susceptible varieties showed approximately twice as many uredinia as the resistant varieties. This would indicate that in the snapdragon susceptibility is directly proportional to the number of stomata.

It is possible that hairiness is able to prevent a portion of the germ tubes from entering the stomata, but according to Cobb (8), resistance cannot be explained by this characteristic. It is probable for a leaf to have a tomentose covering sufficiently dense to prevent the germ tubes from reaching the stomata. However, this is not the case in the large group of economic cereals nor other grasses which are greatly infected by rust.

In his work on wheat, Cobb (8) found a correlation between the thickness of the epidermal cells and rust development. He found the epidermal cell walls twice as thick in the resistant wheats as those in the susceptible ones. Such a condition prevents the sori from breaking through the epidermis and restricts the dispersal of spores. The walls of the epidermal cells of the upper surfaces of the leaves were not as thick as those of the under surface. He believed this condition to be an explanation of the greater number of pustules on the upper surface. In order to prove this point, both surfaces of the leaf were exposed to the same conditions, more pustules forming on the upper surface, as would be expected. It was also found that the resistant variety of wheat has leaves with greater tensile strength than the susceptible variety. The result showed the resistant varieties with thick cuticle to be decidedly tougher than nonresistant varieties with thin cuticle. Also, a greater number of stomata per unit area were found on the resistant than on the susceptible variety.

Hursh (12), in his studies of the resistance of wheat to _Puccinia graminis tritici_ Erikss & Henn., concluded that in addition to fundamental protoplasmic resistance, wheat varieties possibly possessed other means of defense against the fungus. The number of leaf hairs and the size and number of the stomata were not considered important in restraining the germ tubes. The mycelium of _P. graminis_ within the host was limited almost entirely to chlorenchymatous tissue. The only important chlorenchymatous tissue of the stem in which the rust mycelium could grow is the collenchyma. Large amounts of sclerenchyma would break up the band of collenchyma into small bundles, thus reducing the possibility of the mycelium development. He found that the proportion of sclerenchyma to collenchyma in a given variety varied with the use of fertilizers. A greater amount of sclerenchyma was found in the mature plants than in the seedlings, thus explaining the greater resistance of the mature plants.

Allen (13) reported that the germination of spores and the formation of appressoria on the stomata take place in the same way in susceptible and immune hosts. In her work with Baart and Kanred Wheats infected by Puccinia graminis tritici Erikss & Henn., she found that in Baart the fungus entered freely and grew rapidly, while in Kanred only a few of the fungi passed through the stomata, the rest remaining outside, and shriveled. She found that in a congenial host, numerous haustoria were formed. A slender growing hypha came in contact with a host cell, whereupon it swelled at the tip, its paired nuclei divided, and a septum was formed, marking off a terminal cell. This haustorium mother cell was closely appressed to the host cell, formed a fine pore through its wall and the host wall. Its contents, including both the nuclei, which had decreased in size, and the cytoplasm, then passed in forming the haustorium. She noticed that the osmotic membrane of the host appeared to be invaginated by the haustorium but apparently was still intact. In Kanred, the process was similar until a small haustorium was formed which, either by its presence or by secreting some substance in the host cell, set up chemical reactions within the cell causing its collapse and death. The further diffusion of toxic substances into healthy host tissues is checked by the formation of thickened contact walls. One or more of the substances formed in the host cell diffused into the haustorium killing it and causing collapse of the mother cell and the death and plasmolysis of the hypha back of it for some distance. If this reaction is rapid, the haustorium is destroyed while still very small, if more sluggish, a full grown haustorium may be formed and some nourishment for further growth is extracted by the fungus. Kanred possesses three means of defense against this strain of stem rust: stomata which shut out the majority of the fungi, the heavy contact walls adjoining pathological cells, and a true immunity. She believed her observations to be in line with the theory that immunity is due to definite antagonistic chemical interactions between host and parasite.

The purpose of this study is to obtain facts which show whether or not rust resistance is due to morphological characteristics of the grass leaf, as the early investigators believed, or which substantiate the newer idea that rust resistance is due to the high specialization of the rust fungus. Morphological characters of the leaf which have been thought to have been responsible for preventing infection by rust fungi were: tensile strength of the leaves, amount of waxy bloom, erectness of the leaves, relative number, behavior, and size of stomata, thickness of cuticle, and pubescence.

MATERIALS

Panicum _virgatum_ L. (Switchgrass) is a grass upon which a study of specialization should be feasible as there are many strains of this species with varying degrees of rust resistance. The three following strains were used:

(1) G 203. The seed of this strain was collected in 1934 west of Muskogee, Oklahoma, in the Arkansas River bottoms. This strain has been found by the writer to be 100 per cent rust resistant (i.e., immune). It is of rank

growth usually attaining a height of six feet. The plants of this strain were of the second generation, that is, once removed from the plants growing in a native state in Oklahoma.

(2) G 122. Seeds of this strain were collected in October, 1935, near Colony, Anderson County, Kansas. It is of medium height reaching four feet. It is very susceptible to rust.

(3) G 200. This strain was collected in Holt County, Nebraska, in 1935 by the Soil Conservation Nursery of Lincoln, Nebraska, and is the first generation. This strain is very susceptible to rust and is small, comparatively, rarely reaching two and one-half feet.

Plants of these strains were removed in early spring while in a dormant state from the Federal Soil Conservation Nursery southwest of Manhattan, Kansas, to the Experiment Station Nursery, adjacent to the campus of Kansas State College where they were planted in a light sandy loam.

The uridine fungus which it was decided to use in the experiments was Puccinia panici Diet., the pycnia and aecia of which occur on Euphorbia marginata (Pursh) K&G., and the uredia and telia on Panicum virgatum L. Both hosts are endemic to Kansas. According to Arthur (14), the range for Panicum virgatum L. is as follows: Connecticut to South Dakota, southward to Alabama and Texas.

Seedlings of Euphorbia marginata (Pursh) K&G. were planted in rows adjacent to those of the three strains of Panicum virgatum L., so that infection by Puccinia panici Diet. would be aided by having the alternate hosts as near one another as possible. Both hosts became heavily rusted but not with Puccinia panici Diet. Rusted material of both plants was sent to Dr. H. A. Jackson of the University of Toronto, and to Dr. Frank D. Kern of the Pennsylvania State College for identification; both agreed that the Panicum virgatum L. was infected with Uromyces graminicola Burr. and that Euphorbia marginata (Pursh) K&G. with Uromyces proeminens (DC.) Pass, two different and unrelated species.

As a result of this, Uromyces graminicola Burr. was used in the experiments instead of Puccinia panici Diet.

Specimens of Panicum virgatum L. infected with Uromyces graminicola Burr. from five counties of the State of Kansas were found in the Pathological Herbarium at Kansas State College, but there were no specimens of the alternate host infected with the aecial stage of the rust. However, two of the species, Croton monanthogynus Michx.

and Stillingia sylvatica L., listed by Arthur (14) as alternate hosts, occur in this state.

METHODS

The following killing and fixing agent was used in preparing the rusted leaves for sectioning:

Ethyl alcohol (50 per cent)	100 cc.
Glacial acetic acid	2.5 cc.
Formalin	6.5 cc.

The material was dehydrated by the use of the ethyl-butyl series and changed twice in 100 per cent butyl alcohol; it was then infiltrated and imbedded in paraffin. Sections of 8u in thickness were made.

For staining sections which contained fungal filaments, Durand's (15) system was used. It consisted of first staining with Delafield's hematoxylin and counterstaining with one-half per cent solution of eosin (Grubler's alcohol soluble stain) in 95 per cent alcohol. Carbol-turpentine, three parts, melted carbolic crystals, two parts, was used as a clearing agent. Safranin and light green were used on sections not containing any of the fungus.

For clearing material for stomatal observations, Lloyd's (16) method of stripping the epidermis and immersing immediately in absolute alcohol was used. For the observation of stomata and hairs, leaves were placed in aceto-alcohol (glacial acetic acid, and 95 per cent alcohol, one part each). This is the method used by Hursh (12). All pigments were thoroughly removed and the leaves could be examined entire under the microscope using direct light. A saturated solution of chloral hydrate was also used for clearing leaves of all pigments.

ANATOMY OF THE LEAF OF PANICUM VIRGATUM L.

As is typical with grasses, the leaf of *Panicum virgatum* L. is composed of a blade and split sheath which encircles the stem. In this study, only the blade has been considered. The blades are linear and contain numerous parallel vascular bundles which vary in size and amount of mechanical and conducting tissue. Directly around the bundle is a bundle sheath of lignified cells which protects the bundle and especially the phloem. Directly outside the bundle sheath is found a layer of large chlorophyll-bearing cells, or parenchyma sheath. The secondary vascular bundles are completely surrounded by parenchyma sheaths which are in turn surrounded by girdles of chlorenchymatous tissue; however, in the primary bundles the parenchyma sheath is opened above and below, the sterome of both sides joining the bundle sheath, thus forming a continuous band of mechanical tissue between the upper and lower epidermis. All the bundles (except those in the apical region) are separated by strips of water-bearing tissue which connect the bulliform cells of the upper epidermis with those of the lower epidermis. The bulliform cells are found on the upper surface in the grooves between the undulations above the bundles.

In the region of the midrib are found several vascular bundles near the dorsal epidermis. Below the central vein of the midrib is found a large region of sclerenchymatous tissue. Above the bundles is a large region of non-chlorophyll-bearing parenchyma. The region of the leaf margin is composed entirely (i.e., within the epidermis) of sclerenchymatous tissue (Plate IV, Fig. 3).

In surface view, the epidermal cells are rectangular, varying in width and length, some of them being very much elongated and are in parallel rows lengthwise the leaf. The stomata are also in rows parallel to the epidermal rows and are found in the region between the bulliform cells and the fibrous tissue, thus permitting the substomatal chamber to be surrounded by chlorenchyma.

INVESTIGATIONS

Findings of the Susceptible and Resistant Strains

Comparative Anatomy. In comparing the anatomical structures of the resistant strain G 203 and the susceptible strain G 122, Table 1, it was found that one varied very little from the other. G 203 was found to have a pronounced bloom which is lacking in both of the susceptible strains, G 122 and G 200. As far as could be determined, the cuticle of both strains was identical in thickness, both being approximately 2.79μ in thickness for the lower epidermis and 1.89μ for the upper epidermis. There was no appreciable difference in the number of cells composing the sterome bundles between the two strains. As would be expected, due to the difference in size, the midrib of G 203 was 1.53 times as thick as that in G 122, and the average thickness in the regions of primary bundles varied, but to a much smaller extent. In comparing the number of stomata, Table 2, it was found that the immune strain G 203 had more stomata per unit area than either of the susceptible strains, G 200 and G 122.

Table 1. Comparative anatomical measurements of strains G 203 and G 122 (measurements in μ).

	Location on leaf	G 203: 25 mm. from base	G 203: 90 mm. from apex	G 203: 25 mm. from apex	G 122: 25 mm. from base	G 122: 90 mm. from apex	G 122: 25 mm. from apex
Thickness of leaf	Midrib region	415	320	210	270	320	175
	Region of primary bundle	176	184	163	166	174	166
Number of vascular bundles	Primary	18	16	13	8	8	5
	Secondary	34	37	25	21	19	15
	Total	52	53	38	29	27	20
Thickness of cuticle	Upper surface	1.86	1.86	1.86	1.86	1.86	1.86
	Lower surface	2.79	2.79	2.79	2.79	2.79	2.79

Table 2. Comparative number of stomata of three strains of <u>Panicum virgatum</u> L. (Total of 50 microscopic fields (.09179 sq. mm.), 10 fields on each surface of each leaf).

Strain	Distance from apex		Average for leaf	Average for both surfaces
	25 mm.	50 mm.		
G 203				
Upper surface	522.5	491.0	506.8	
Lower surface	396.5	403.0	399.8	453.3
G 122				
Upper surface	397.5	337.5	367.4	
Lower surface	319.0	283.0	301.0	334.2
G 200				
Upper surface	435.0	468.5	451.8	
Lower surface	322.0	343.5	332.8	392.3

<u>Observations</u> <u>of</u> <u>Infected</u> <u>Plants</u>. It was noticed at the Soil Conservation Nursery that the infection of G 122 was extremely heavy, being a 100 per cent infestation in most cases. It was growing in very dense stands, causing a higher humidity especially in the central part of the clumps; however, the stands were so heavy that a comparatively high humidity resulted throughout the entire plot. The same strain was also grown at the experiment station nursery in a single row, allowing sufficient air circulation and room for expanding. In the former case, where heavy infestation took place, the G 122 plants (susceptible) were growing directly next to a large plot of G 203 (resistant) plants, which were in dense stands but having a much more open form of growth than G 122. Thus, the source of urediospores was very near as well as abundant, yet practically no pustules were found in the entire plot and these were very close to the ground. The susceptible strain (G 122), when grown more openly at the experiment station nursery with no immediate source of urediospores, showed less infestation.

The mycelia of the fungus invades only the chlorophyll-bearing tissues (Plate IV, Fig. 4) and was thus restricted to the comparatively narrow region between the non-chlorophyll-bearing strip and the lignified strip. As a result of this restriction the pustules were forced to develop in a line. Many of them grew together forming very elongated pustules.

In order to determine the relative frequency of infection of the upper and lower surfaces of the susceptible strain, G 122, pustules intersecting a transverse line were counted (Table 3). In all cases there were more pustules in the upper surfaces than in the lower. An average of the ratios was taken and it was found that for every pustule in the lower surface there were 2.79 in the upper. This would be expected since there were more stomata per unit area on the upper surface (Table 2) and also a thinner cuticle (Table 1).

Table 3. The relative frequency of infection of the upper and lower surfaces of the leaves of Panicum virgatum L., strain G 122 (Frequency determined by the number of pustules intersecting a transverse line, samples being taken at random).

Samples 1 upper lower	2 upper lower	3 upper lower	4 upper lower
1.80:1.00	0.80:1.00	2.66:1.00	1.80:1.00
8.60:1.00	0.70:1.00	12.50:1.00	2.25:1.00
1.20:1.00	2.80:1.00	1.60:1.00	3.60:1.00
3.00:1.00	1.30:1.00	3.50:1.00	4.25:1.00
1.60:1.00	1.50:1.00	6.20:1.00	1.66:1.00
1.20:1.00	0.60:1.00	5.20:1.00	0.63:1.00
1.20:1.00	2.40;1.00	4.60:1.00	2.25:1.00
4.00:1.00	0.30:1.00	1.55:1.00	3.00:1.00
5.00:1.00	0.60:1.00	3.10:1.00	2.00:1.00
6.30:1.00	2.30:1.00	5.50:1.00	1.10:1.00
0.40:1.00	0.80:1.00	2.75:1.00	3.00:1.00
3.11:1.00*	1.28:1.00*	4.46:1.00*	2.32:1.00*

*Average

Average for all samples 2.79:1.00

<u>Relative Number of Stomata</u>. It has been thought that the size and relative number of stomata influenced the amount of infestation as it was believed that stomata might be small enough to prevent the germ tubes from entering and that the larger the number of stomata per unit area the larger would be the number of germ tubes entering.

Hursh (12) worked with wheat seedlings and found that the size of the stomatal apertures had little importance in influencing the entrance of the germ tubes. He found that, "the difference in the number of stomata seems unrelated to the degree of resistance to stem rust" because Khalpi, the most resistant wheat used for determining biologic forms of rust, has the largest number of stomata. It appears that what is more pertinent to the problem is the frequency and extent to which they open, but Miller (17) explained that the behavior of the stomata of the barley leaf, which is typical of the cereals, showed no opening during the night no matter how slight the day opening. Also, there are many closed stomata during the day occurring more or less in groups. They open and close comparatively rapidly, but even under favorable conditions they are fully open only for an hour or two daily.

According to Allen (18), only about 10 per cent of the germ tubes of a form from which Kanred is immune entered this variety, while approximately 30 per cent of the germ tubes of a form to which Kanred is susceptible penetrated. She explained that the presence of the appressorium might act as a stimulus by mere contact, by altering the gaseous exchange through the stoma or disturbing the moisture relations, by exerting a possible toxic influence upon the guard cells, or by its presence shutting off some of the light from the guard cells. It is at least conceivable that the guard cells might be sensitive to the appressorium and remain closed, thus excluding the fungus.

Whether the behavior of the stomata has any influence upon immunity still remains to be determined.

Duffas (19) made a detailed anatomical study of the wheat leaf and showed that there is no correlation between the number of stomata per unit area and rust resistance as there were more stomata in the resistant strain than in the susceptible. He believed that the germ tubes penetrated the epidermis directly as he found appressoria formed in regions which were void of stomata. He was unable to remove the appressoria with a fine needle which led him to be believe the germ tubes had penetrated the cuticle and epidermal cells. However, this is out of line with the researches of the mycologists who have proved that the germ tubes penetrate only through the stomatal apertures.

In all cases with Panicum virgatum L. the resistant strain had more stomata per unit area than the two susceptible strains. G 122 had only 73.7 per cent as many, and G 200 had 86.5 per cent as many stomata as G 203, the resistant strain. In that case, the number of stomata per unit area could not be considered of any importance in relation to rust resistance.

Leaf Hairs. Hursh (12), in working with wheat determined the number of leaf hairs per unit area in relation to resistance. He found that the germ tubes often came in contact with hairs and entwined about them. It is believable that this might reduce the number of germ tubes which are able to reach the stomata and form appressoria. It was found that on Kota, the variety with the largest number of leaf hairs, the actual number of appressoria observed on the average leaf is lower than that on varieties with fewer hairs, although enough appressoria were developed to permit infection. He believed the leaf hairs could not be considered as a serious obstacle to entrance.

The number of leaf hairs on Panicum virgatum L. is so small that they could not be considered as having any relation to resistance.

Waxy Bloom. One of the most outstanding differences between the resistant strain, G 203, and susceptible strains is the very conspicuous bloom. In the greenhouse the bloom did not develop to the extent that it did in the field. When grown in the field the waxy bloom becomes so thick that it gives to the leaves a light bluish-gray color.

In order to determine the efficiency of this characteristic, leaves were taken from all three strains and weighed; then they were immersed in water and allowed to drain only during the time it required to transfer them to the weighing pan, and then reweighed. The leaves were then blueprinted and areas determined with a planimeter. It was found that the resistant strain, G 203, retained only .000626 gram of water per square centimeter, while G 122 retained .002478 gram and G 200 retained .001591 gram. Thus, G 122 retained almost four times as much water as G 203, and G 200 over two and one-half times as much. There is no doubt that even a greater difference exists in the field as there the leaves of the two susceptible strains do not stand erect, as do those of G 203, but are pendant, and therefore expose much more horizontal surface for water to collect upon. After immersing the leaves in water, they were transferred in a vertical position, so that the two susceptible forms retained less water than they naturally would in the field.

DISCUSSION

The work of the early investigators was mainly along morphological lines. They believed that characteristics of the plant which prevented the fungus from entering to be solely responsible for resistance to rust. The trend today is to investigate the relations between host and parasite in regard to physiological incompatibility. A plant may be hypersensitive to invasion of the rust to the extent that the tissue dies rapidly, thus prohibiting the further development of the rust. In this case small necrotic flecks appear, but the host may be considered resistant (i.e., it is so susceptible that it is resistant).

Morphological characteristics which may be considered as producing resistance are: tensile strength of the leaves, amount of waxy bloom, erect leaves, relative number, behavior, and size of stomata, and pubescence. However, these are generally discredited as having any influence upon resistance, but rather the high specialization of the fungus is given credit for differences.

In studying the anatomy and growth habits of resistant and susceptible strains of *Panicum virgatum* L., the greatest differences were found in the amount of waxy bloom and the erectness of the leaves.

CONCLUSIONS

1. It is commonly known that the type of resistance varies with the species, and in the case of most of the economic cereals rust resistance is due in most cases to physiological-incompatibility, but in this case, evidence tends to point toward resistance due to morphological characteristics and growth habits.

2. The resistant and susceptible forms are essentially alike in gross anatomy.

3. There is no relation between the number of stomata per unit area and rust resistance.

4. As far as *Panicum virgatum* L. (Switchgrass) is concerned, leaf hairs have no connection with rust resistance.

5. The waxy bloom cuts down the amount of water retained by the leaf surfaces, lessening the chance of theurediospores lodging and germinating.

6. The vertical growing habit of the leaves lessens the chance for collecting of moisture and lodging of urediospores.

7. No difference could be found in the thickness of the cuticle between the susceptible and resistant strains.

8. In continuing this problem, it should be noted that experiments in removing the bloom from the leaves of the resistant strain would materially aid in solving the problem. The newer methods of inoculation should also be incorporated.

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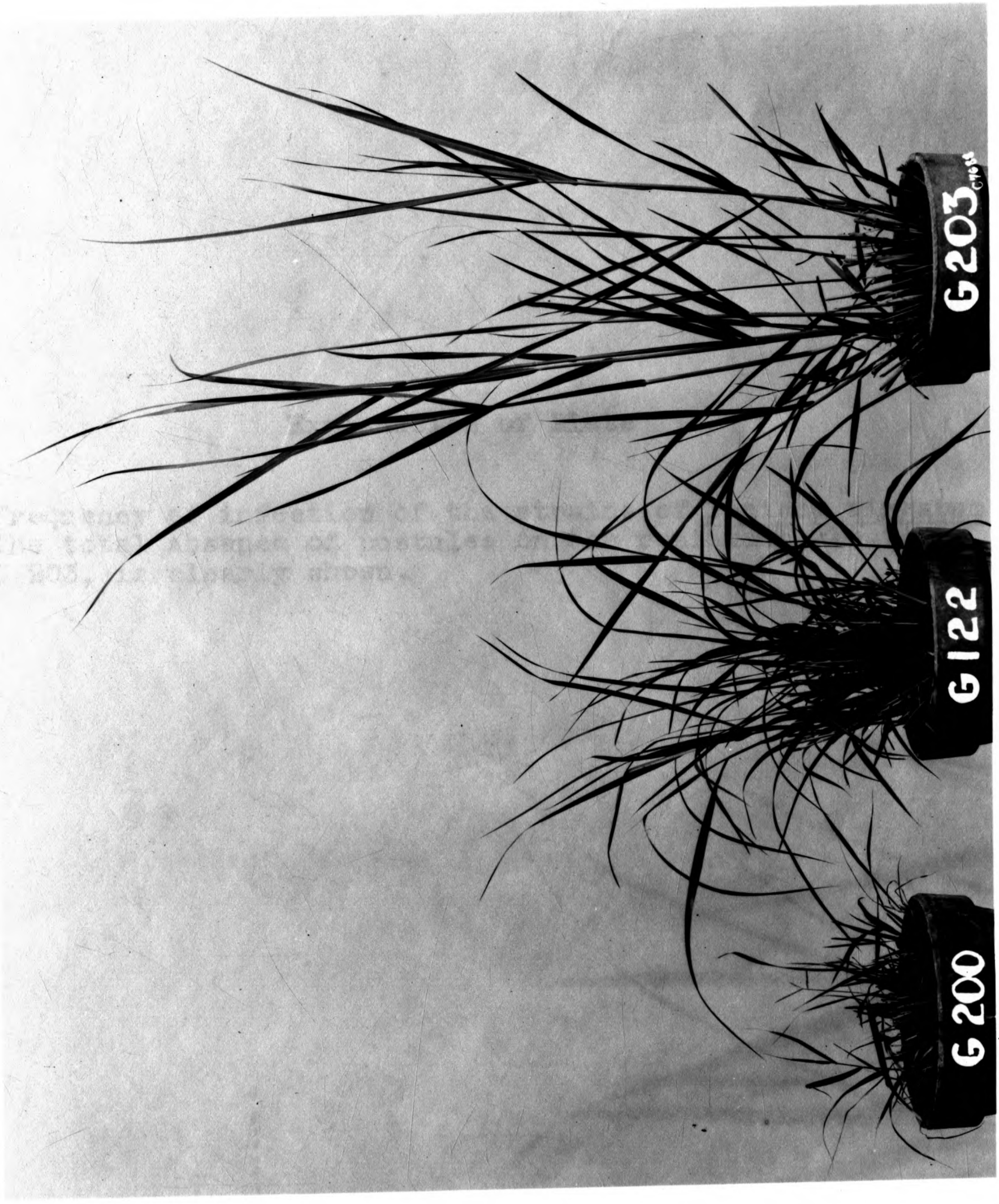
Explanation of Plate I

Growth habits of the susceptible and resistant strains of *Panicum virgatum* L. The erectness of the leaves of the resistant strain, G 203, is very pronounced.

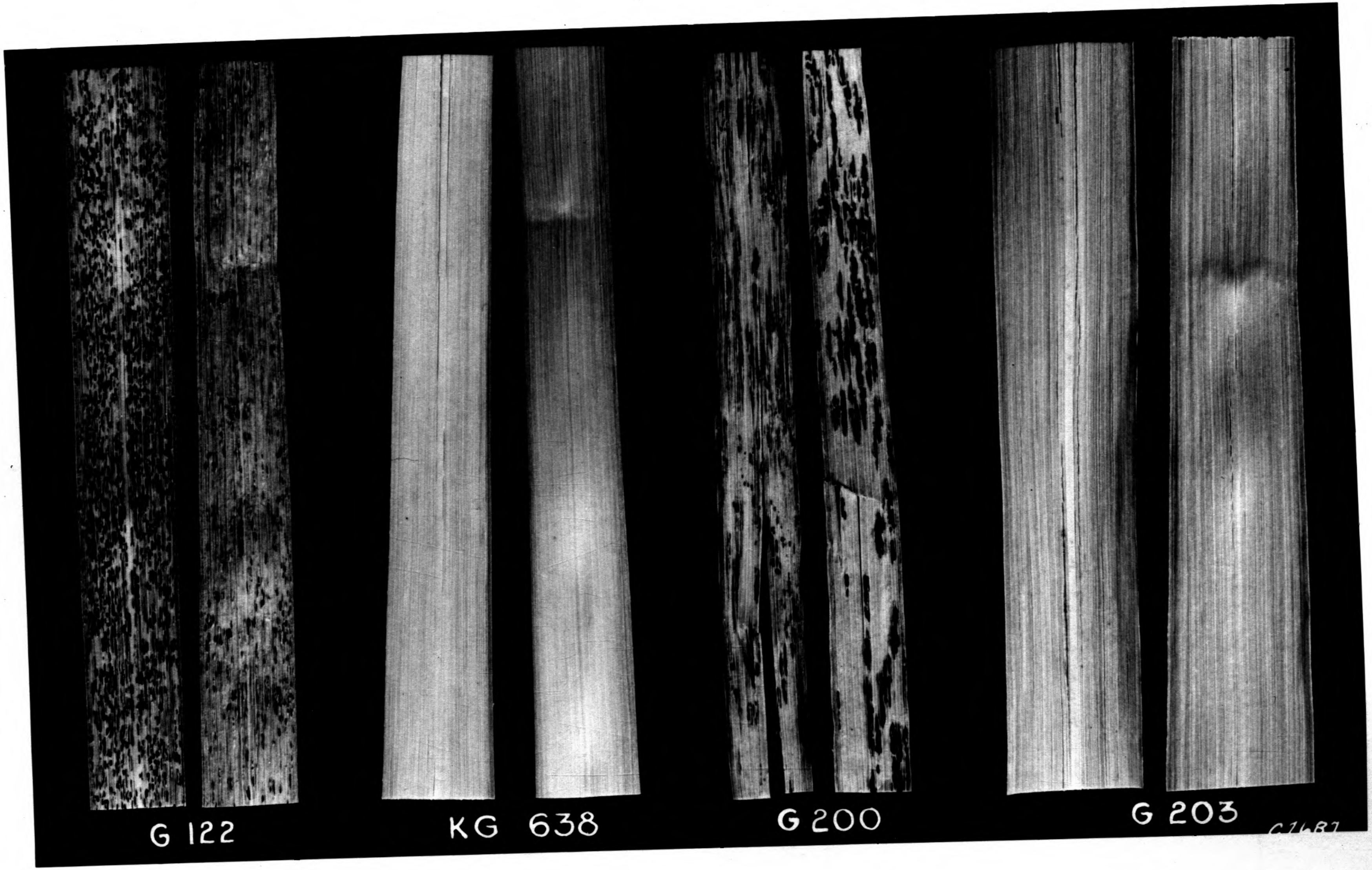
Plate I



Explanation of Plate II

Frequency of infection of the strains of *Panicum virgatum* L. The total absence of pustules on the resistant strain, G 203, is clearly shown.

Plate II



Explanation of Plate III

Fig. 1. A portion of the midrib region of strain G 203 showing the central bundle and the large amount of sclerenchymatous tissue directly below the bundle.

Fig. 2. A portion of a cross section of the leaf of strain G 203 showing a primary vascular bundle, the bundle sheath, parenchyma sheath, and the large bulliform cells.

Plate III

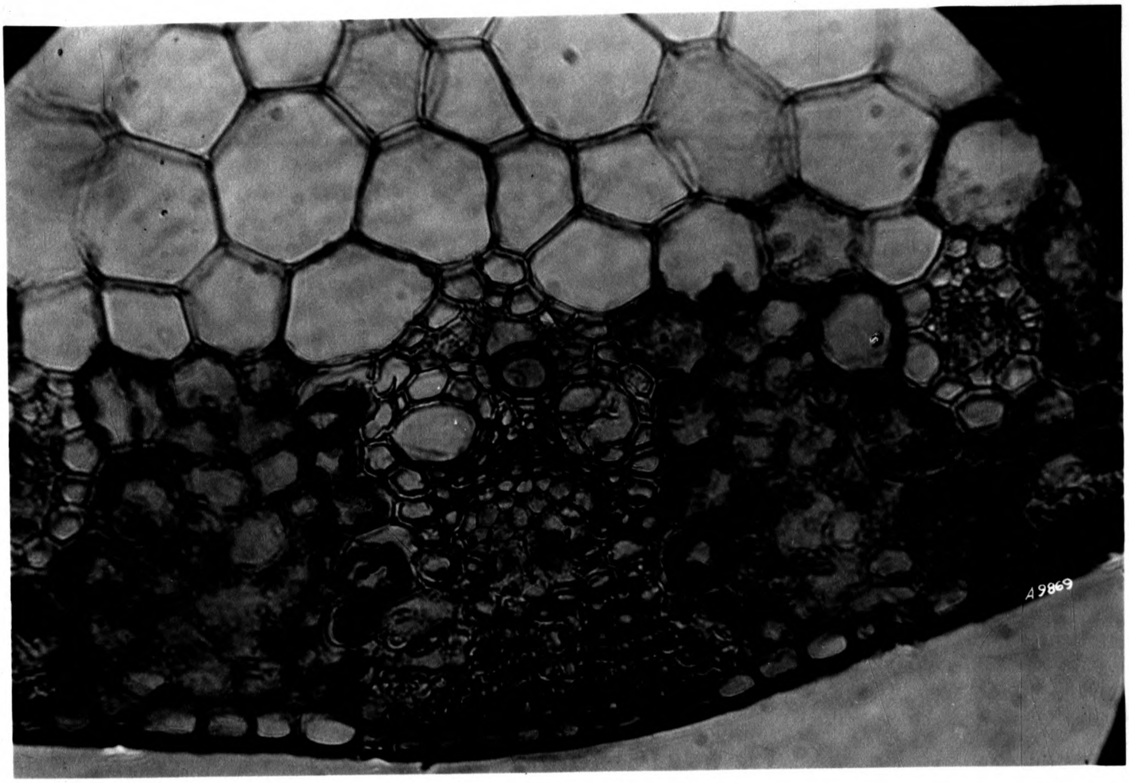


Fig. 1

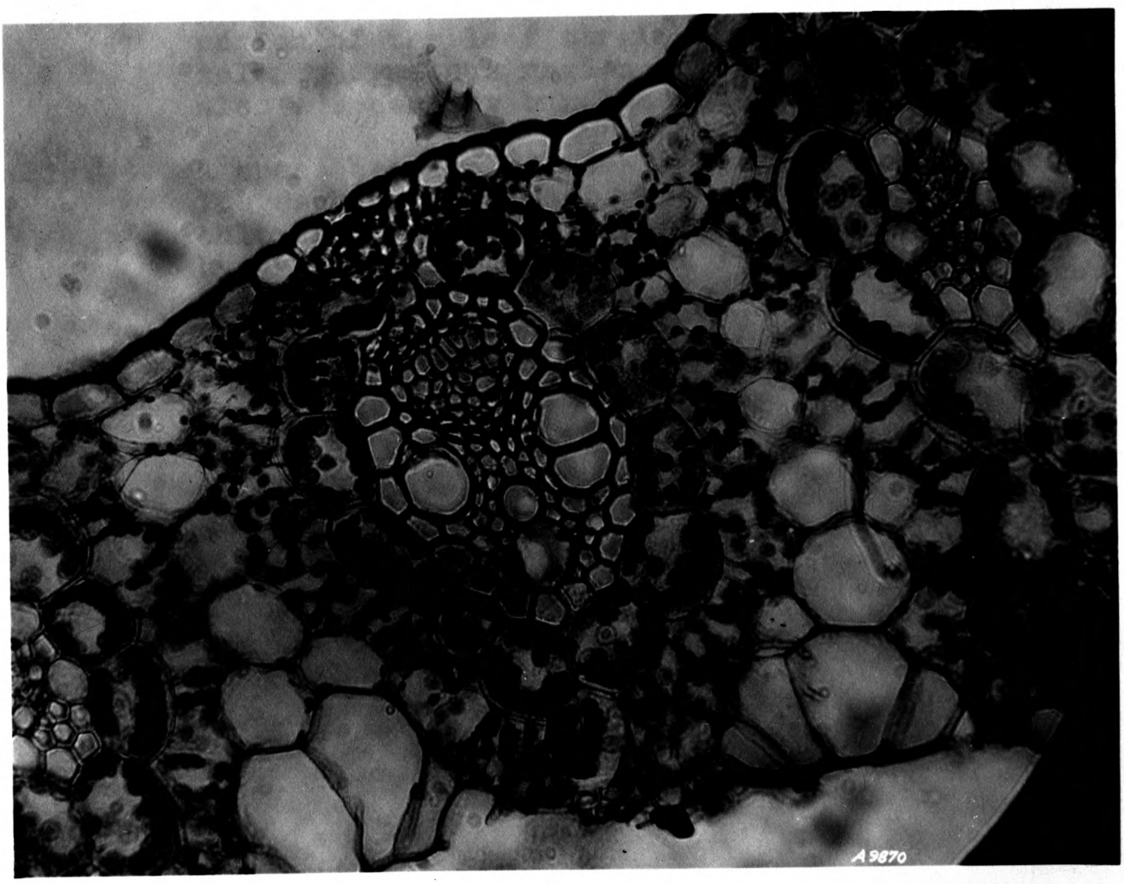


Fig. 2

Explanation of Plate IV

Fig. 3. A cross section of the leaf of strain G 203 in the region of the leaf margin showing the large sclerenchymatous region in the extreme margin and also a secondary bundle and a primary bundle.

Fig. 4. A cross section of the leaf through a pustule. The dark stained region is a portion of the chlorenchymatous girdle which is being parasitized by the mycelia of the rust fungus, Uromyces graminicola Burr.

Plate IV

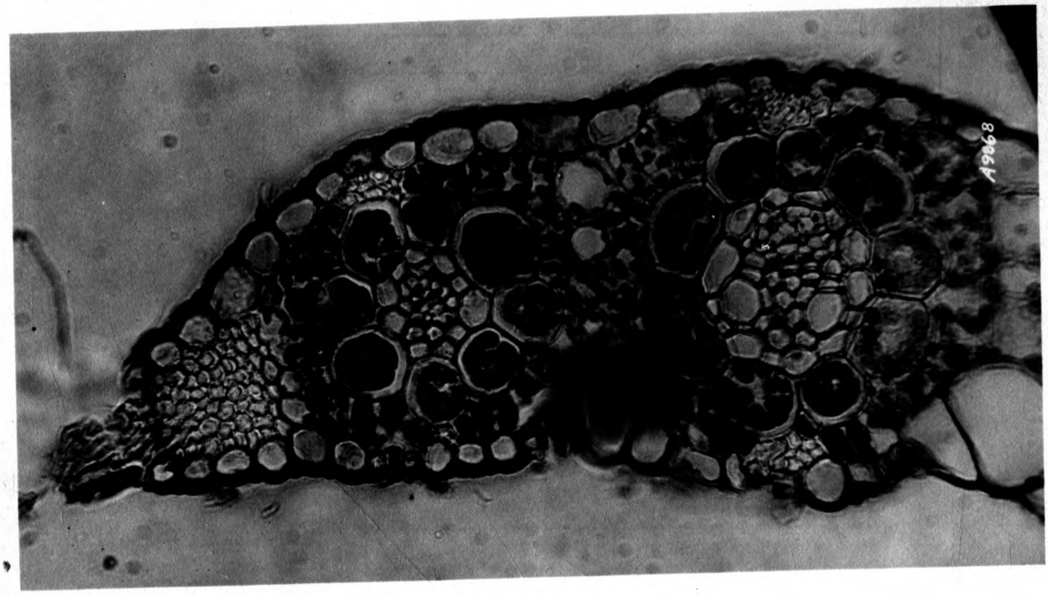


Fig. 3

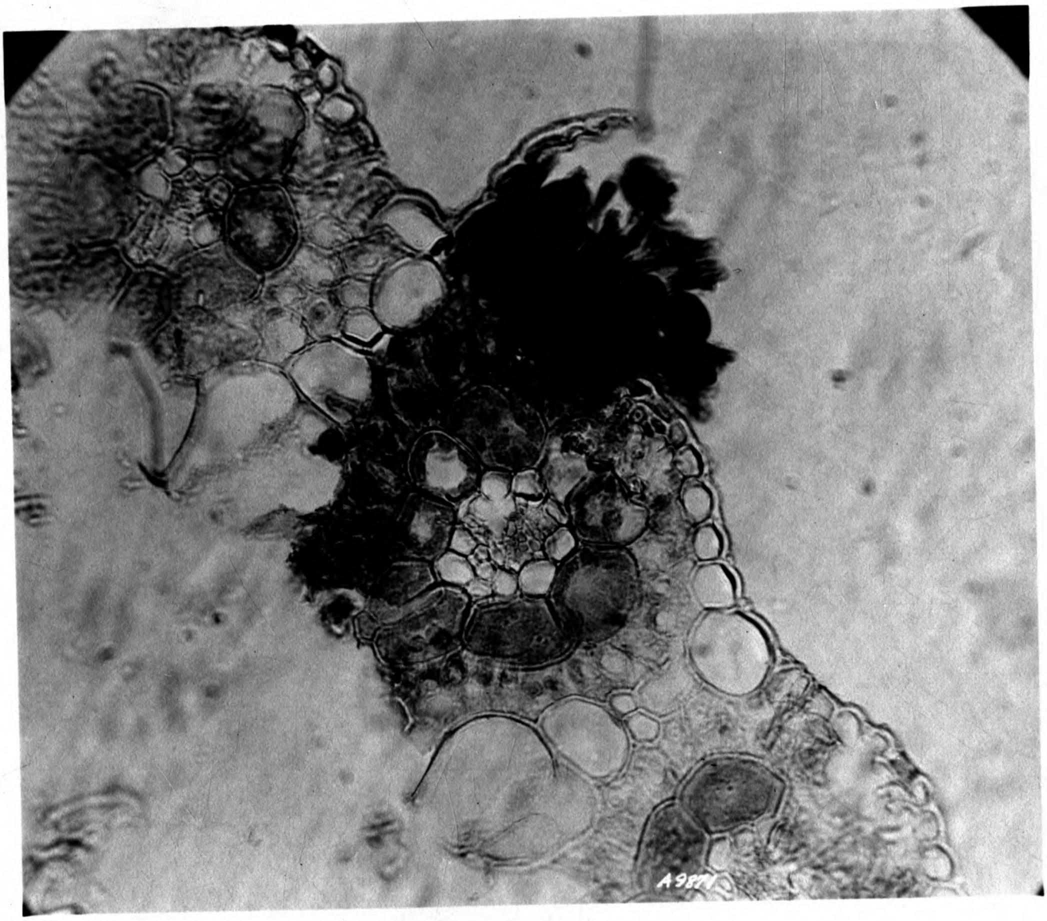


Fig. 4