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Greenhouse Studies on the Relationship of Age of Wheat Plants to Infection by Puccinia Graminis Tritici

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GREENHOUSE STUDIES ON
THE RELATIONSHIP OF AGE OF WHEAT PLANTS TO INFECTION
BY PUCCINIA GRAMINIS TRITICI

By

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INTRODUCTION

The resistance of wheat to the stem-rust organism (Puccinia graminis tritici) has been under investigation for a number of years. Although rust resistance has been investigated from various angles, there still remains a question as to the nature of resistance, the time when resistance first appears in plants and whether or not it is of a stable character. Peltier (11) found that environmental conditions could change the degree and type of stem rust infection on wheat plants; Miss Hart (5) proved that stomatal behavior was very important in rust resistance, and Harrington and Aamodt (4) studied the inheritance of rust resistance and found it to be of a fairly stable character.

Though heredity may play a large part in resistance, several investigators, Johnston and Melchers (7), Stakman and Peimeisel (14) have noted variations in reaction to rust even in the same variety of wheat and have found that marked differences occur coincident with differences in age of the wheat plant. Johnston and Melchers (7) in their work on leaf rust (puccinia triticina), stated that "certain varieties of wheat, very susceptible to leaf rust physiologic form 9 in the seedling stage, are highly resistant at heading time. Varieties resistant or rust free in the seedling stage are resistant at all stages of growth." Stakman and Peimeisel (14) found that seedlings of some grasses became infected with rust more readily than old plants of the same species. Melchers and Parker (10) concluded that "plants showing certain effects from rust when inoculated in the seedling stage may have an entirely different reaction to the same organism at time of heading." Puttick (12) likewise found that seedling plants were infected very easily while it was much more difficult for older plants to become infected. Finally Mains and Jackson (9), in their studies on physiological forms of Puccinia triticina, found that many varieties of wheat which were only moderately resistant, or even suscepti-

ble, in the greenhouse would show a very high degree of resistance in the field.

MATERIALS AND METHODS

This experiment was started in the winter of 1929-30, but due to the fact that the plants were raised in the laboratory where only the light from side windows was available, they were sickly and the resulting reactions were discarded. It may be noted, however, that a high degree of infection was obtained on Marquis wheat under these conditions. Work was started anew in the fall of 1930, using Marquis and Kota wheats and Physiologic form 56 of Puccinia graminis tritici. The latter was secured through the courtesy of the rust laboratories at the University of Minnesota.

Marquis and Kota wheats were selected because of their high susceptibility to rust, (including form 56), as found by Stakman and Levine (15). The plants were grown in six-inch pots, five plants to each pot. Fifty plants of each were grown, twenty-five used for the seedling tests and twenty-five permitted to reach maturity before they were inoculated.

A good stock culture of inoculum was secured by inoculating and re-inoculating small seedlings of Little Club wheat. Plants to be inoculated with the original culture were moistened with an atomizer and the uredospores were transferred to them by means of a fine brush. The plants were then placed in a moist chamber for 48 hours and at the end of 24 hours and 36 hours they were sprayed with distilled water. This afforded the most favorable conditions for the uredospores to germinate. After incubation the inoculated plants were placed on the greenhouse bench. Re-inoculation to secure a good stock culture was made by shaking a pot of heavily rusted plants over new seedlings. Transfers were frequently made to keep a good culture of viable spores on hand.

When the experimental plants had reached the desired stage of growth

they were placed in a moist chamber and the leaves and stems were moistened with a fine spray of water. Inoculations were made by shaking the infected plants of the stock culture above the experimental plants, which treatment afforded a uniform distribution of spores. The plants were then cared for in the same way as those of the original culture.

The plants used were inoculated at two different stages of growth, half at the seedling stage, which was taken two weeks after sowing, and half at the heading stage, taken as soon as the head emerged from the boot. Observations were made daily, after five days, to watch the development of infection.

In taking the final data, plants showing the average infection were considered and the class of rust reaction in each case was recorded as given by Stakman and Levine (13).

EXPERIMENTAL RESULTS

The results are summarized in Table I and substantiated by Figures 1, 2, 3, and 4, photographs of Marquis and Kota wheats showing the infection obtained at the two stages of growth.

Following the 5th day after inoculation, daily observations were made and the rust development was watched very closely. The seedlings of both Marquis and Kota showed necrotic areas at the end of the sixth day. Upon the development of the mycelium and production of pustules, this area became less pronounced and when the pustules were fully developed, the necrotic areas were practically gone. The pustules of the resulting infection were quite large and typical of those classed as 4 infection, Stakman and Levine (13). Photographs 1 and 3 were taken twelve days after inoculation and show the rust pustules fully developed. One set of seedling plants showed very little rust infection, which was somewhat puzzling until it was found that one of the members of the greenhouse

staff had done some sulphur dusting the day that the plants were placed on the main bench. The average seedlings of the other sets, which were inoculated a day later, reacted as shown in figures 1 and 3 and the rust development was quite high.

The mature plants after inoculation first showed minute hypersensitive flecks on the leaves, leaf sheath, and stem. As the mycelium developed, these flecks became slightly larger though after twelve days' incubation they were still quite small, as is shown in figures 2 and 4. Comparing the types of infection secured on the seedlings and mature plants, a marked difference in susceptibility is obvious; the infection on the seedlings gave rise to very pronounced pustules while the infection on the mature plants consisted of hypersensitive flecks.

The reactions obtained from the two stages of Marquis and Kota wheats show that there is a marked change in the degree of susceptibility to Puccinia graminis tritici physiologic form 36, ranging from very susceptible in the seedling stage to moderately resistant in the heading stage.

DISCUSSION

This experiment has shown that the varieties of wheat used (Marquis and Kota) may be highly susceptible to a definite physiologic form of the stem-rust fungus in the seedling stage and moderately resistant to the same form of rust at the heading stage. Whether or not the same reaction would take place in natural field conditions is impossible to determine without running a series of tests under these conditions for, as shown by Johnston and Melchers (7), varieties of wheat exhibiting a high degree of resistance to leaf rust under greenhouse conditions showed unmistakable signs of lowered resistance under field conditions. Just what causes this change in susceptibility is still questionable although several theories have been advanced to account for it.

Miss Helen Hart (5) states, "Such accurate observations and experiments as have been made indicate two types of resistance of wheat varieties to Puccinia graminis tritici, namely, physiological and morphological." Miss Hart has also shown that there may be a third factor, viz. in stomatal behavior, since the stomata offer the sole avenues of entrance for the rust fungus. The germ tubes of the rust fungus enter the plant only through the stomata, Hart (5), and Arthur (11) and since the stomata of seedlings and of rapidly growing plants open sooner in the day and remain open for a longer period of time than those on older plants they may have direct influence upon the degree of rust infection.

In my studies, Kota and Marquis wheats both show considerable flecking even when inoculated in the heading stage, which is evidence enough that the fungus germ tubes must have entered and given rise to growths of mycelium in the tissues of the hosts. From this it appears that the behavior of stomata has not been highly effective at any time in barring the entrance of the rust fungus on these wheat varieties. Miss Hart (5) found that the stomata of these varieties opened early in the morning and remained open until late in the afternoon and the time that the stomata remained open was greater in seedlings than in mature plants, therefore, the amount of infection would be greater in seedlings than in adults. Evidently, then, there is something else, either in the structure of the mature plant or the materials within the cells, that is detrimental to the development of the rust fungus after it has entered the tissues of the host.

Peltier (11) found that the development of rust was greatly influenced by environmental conditions such as soil temperature, soil moisture, air temperature and humidity. The effect of soil temperature and moisture would influence the rate and type of growth of the plants but had little, if any direct influence upon the degree of rust infection secured, while air temperatures vary-

ing between 69°F and 76°F seemed to be the optimum temperature for good rust infection. In general, he found that temperatures favorable for good plant growth were much the same as those at which the rust fungus would best develop. Environmental conditions could not have been responsible for differences which appeared in the young and mature wheat plants of my investigation, for these conditions were kept as near alike as possible for both sets of plants.

Hursh (6) has shown that the amount of sclerenchyma tissue is variable in different varieties of wheat, for example, Little Club wheat has a large amount of collenchyma tissue and very little of the sclerenchyma, while in Marquis and Kota wheats there is a large amount of sclerenchyma in comparison with the collenchyma, the latter being confined to small regions between the sclerenchymatous fibers. He has also shown that the lateral extent of mycelium development is checked by the sclerenchyma tissue. In Kota wheat where the sclerenchyma fibers are close together, the pustules, resulting from rust infection, are long and slender; while in Little Club, where sclerenchyma is scarce, the resulting pustules have greater width, which indicates that the mycelium has spread over a larger area. Now if sclerenchyma tissue in adult plants is more abundant and better differentiated than in seedlings, it follows that infection, even though equal in the two ages, would be the more conspicuous in the seedling, in which case the differences in infection between seedlings and adults would be more apparent than real. It should be added, however, that while infection in seedlings goes through to the production of pustules and viable spores, in the adult plants infection progresses only far enough in an equal period of time to produce flecking in the tissues.

Whatever causes the resistance, there is little doubt that a combination of morphological and physiological characters in varieties of plants, and at different ages of plants, has some direct effect upon it.

In the work carried out for this report, only greenhouse observations were made; no results were obtained under field conditions. Therefore the types of reaction of Marquis and Kota wheats to Puccinia graminis tritici, physiologic form 36, under natural field conditions are still a question. The mature plants may or may not show the same degree of resistance in field conditions that they showed in the greenhouse tests. Field tests would have to be carried out before any conclusions in regard to rust reaction under field conditions would be justifiable.

In connection with the failure of one set of inoculated seedlings to react to the rust inoculum after sulphur dust was used in the greenhouse, the works of Lambert and Stakman (8), Bailey and Greaney (2), and Broadfoot (3) may be mentioned. Bailey and Greaney (2), in their work on sulphur dusting for control of wheat rust, found that rust development and damage done to rust could be greatly reduced by applying sulphur dust to the plants. More bushels per acre were received from the dusted plots than from the undusted plots. Lambert and Stakman (8) concluded that stem rust of wheat could be controlled by dusting the plants with a suitable sulphur dust. Broadfoot (3) found that rust losses could be greatly reduced by using Kolo Dust. With three applications he received as high as 80.2 per cent reduction in the amount of stem rust and a 57.1 per cent increase in yield. From the work of these investigators, the failure of rust to develop on the one set of seedlings can possibly be accounted for. The plants were very moist at the time and a large amount of sulphur dust was applied to all plants in the greenhouse with the object of checking mildews and red spiders. That the sulphur dust must also have had a very definite effect upon the mycelium of the freshly germinating spores is obvious.

CONCLUSIONS

From the greenhouse studies carried on in this work it is evident that plants of Marquis and Kota wheat develop a very striking resistance to infection by Puccinia graminis tritici, physiologic form 36, as they grow from seedlings to mature plants. From the standpoint of grain growers, this fact is of importance because if seeds are planted early, the plants will be well along toward maturity before the rust organism is available in the uredospore condition, and when the uredospore stage is reached, the older plants will be more resistant to rust infection. However, it must be kept in mind that my investigation dealt with only one physiologic form of stem rust.

SUMMARY

1. Experiments were conducted in the greenhouse at South Dakota State College.

2. Two varieties of wheat were used: Marquis and Kota.

3. Uredospores of physiologic form 36 of Puccinia graminis tritici were used for inoculum.

4. Studies made proved that while the two varieties of wheat used possess low resistance in the seedling stage, their resistance increases noticeably as they approach maturity.

In conclusion, I wish to thank Prof. E. A. Walker for his assistance and suggestions throughout the investigation. I also wish to thank Dr. Ward L. Miller for his helpful criticism in the writing of this report and for his kind assistance throughout the investigation. The writer is also indebted to Dr. E. C. Stakman of the University of Minnesota for suggestions and for rust inoculum used in this investigation.

Table I

Wheat Varieties	Form of rust used	Type of infection obtained	Degree of infection obtained
Marquis, seedlings	Physiologic Form 38	4	(-)
" , adults	" " "	0-1	(;)
Kota, seedlings	" " "	4	(-)
" , adults	" " "	0-1	(;)

Explanation of symbols used follows in Table II.

Table II

(After Stakman & Levine[13])

Explanation of Symbols Used to Indicate Types and Degrees of Infection Produced by Biologic Forms of Puccinia Graminis on Varieties of Wheat

Types of Infection

0 - IMMUNE

No uredinia developed; hypersensitive flecks usually present, but sometimes there is apparent absolutely no trace of mycelial invasions in the host tissues.

1 - VERY RESISTANT

Uredinia minute and isolated; surrounded by sharp, continuous, hypersensitive, necrotic areas.

2 - MODERATELY RESISTANT

Uredinia isolated and small to medium in size; hypersensitive areas present in the form of necrotic halos or circles; pustules often in green, but slightly chlorotic, islands.

3 - MODERATELY SUSCEPTIBLE

Uredinia medium in size; coalescence infrequent; development of rust somewhat subnormal; true hypersensitiveness absent; chlorotic areas, however, may be present.

4 - VERY SUSCEPTIBLE

Uredinia large, numerous and confluent; true hypersensitiveness entirely absent, but chlorosis may be present when cultural conditions are unfavorable.

X - HETEROGENEOUS

Uredinia very variable, apparently including all types and

degrees of infection on the same blade; no mechanical separation possible; on reinoculation small uredinia may produce large ones, and vice versa. Infection ill defined.

Degrees of Infection

(=) - TRACE

Uredinia very few in number and covering a limited surface; development of rust generally poor and decidedly subnormal.

(-) - SLIGHT

Rust development below normal, but somewhat better than "trace".

(±) - MODERATE

Variation in rust development from "slight" to "considerable"; when infection is uniform but only medium in quantity the symbol is omitted.

(+) - CONSIDERABLE

Infection better than normal; uredinia fairly numerous and scattered.

(++) - ABUNDANT

Luxuriant development of rust; uredinia very many, covering large area of affected host.

MISCELLANEOUS SYMBOLS

(;) - Hypersensitive flecks

(.) - Necrotic lesions

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Explanation of Plates

Plate I

Fig. 1 Leaves of Marquis seedlings infected with Puccinia graminis tritici
(a) rust pustules

Fig. 2 Sections of mature stems of Marquis wheat infected with Puccinia graminis tritici
(b) flecking due to rust infection

Plate II

Fig. 3 Leaves of Kota seedlings infected with Puccinia graminis tritici
(c) rust pustules

Fig. 4 Sections of mature stems of Kato wheat infected with Puccinia graminis tritici
(d) flecking due to rust infection

Plate I

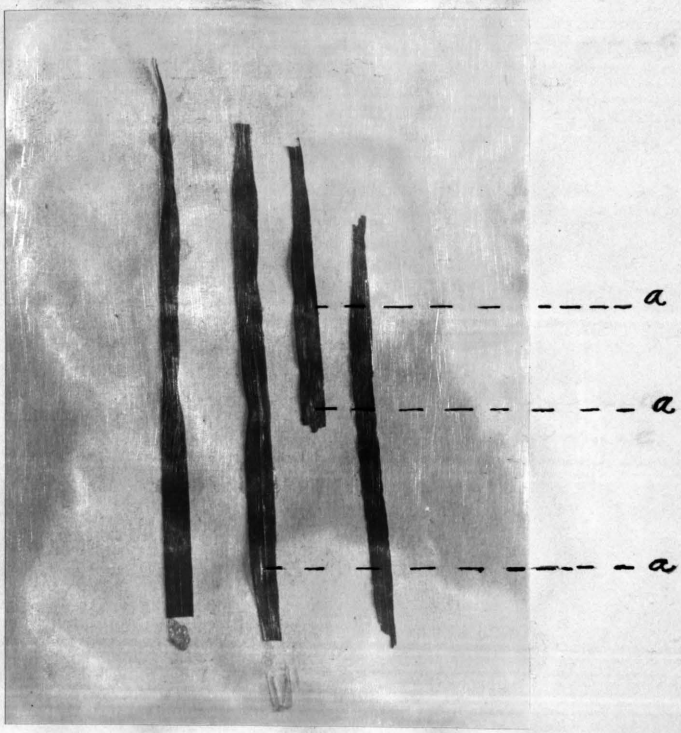

Fig. 1.

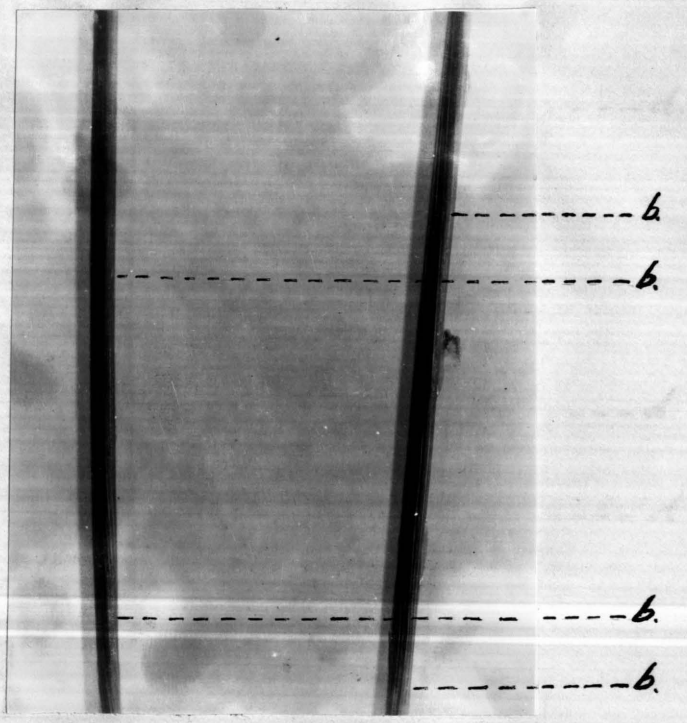

Fig. 2.

Plate II

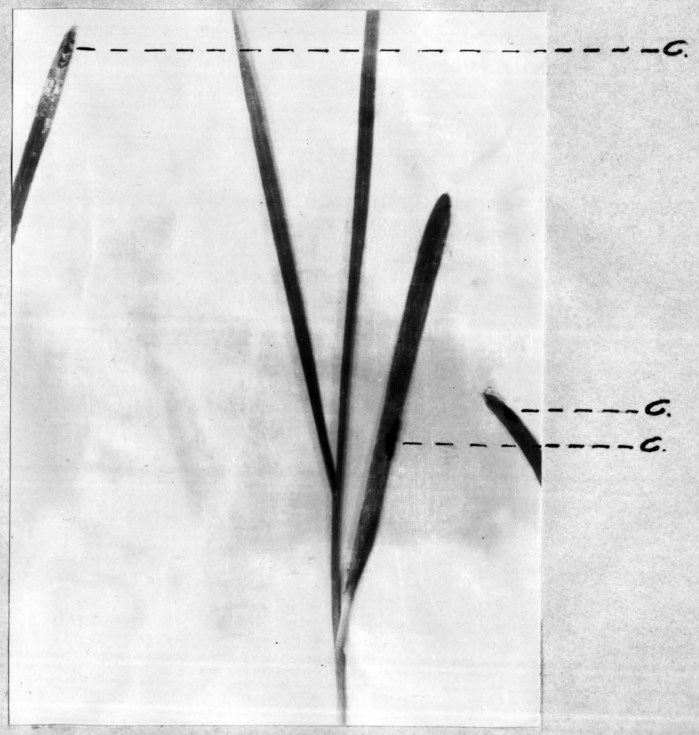

Fig. 3.

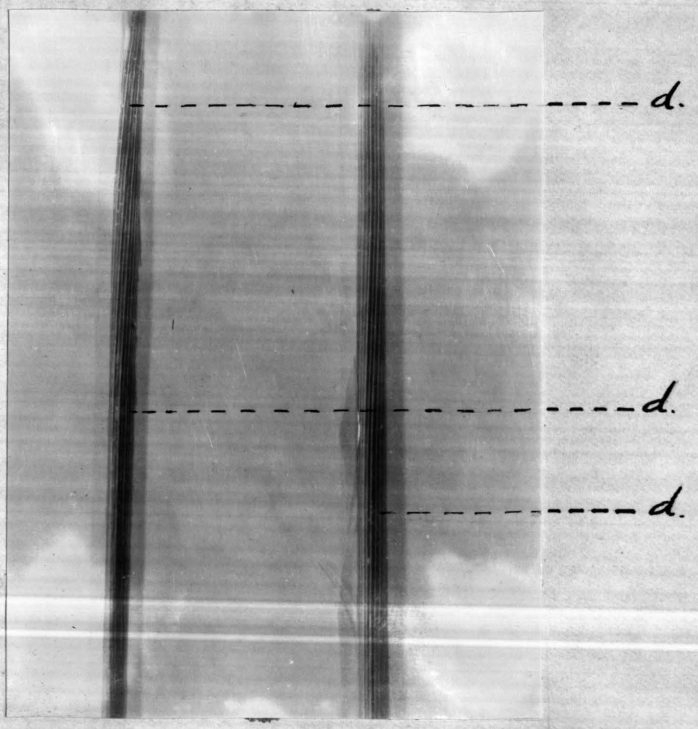

Fig. 4