A Histological Study of Host Parasite Relations of *Puccinia polysora* and *P. sorghi* on Different Genotypes of Maize[1]

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Introduction

Histological studies of plants resistant to rusts and other pathogens have led to two principle classifications of disease resistance, functional and protoplasmic. In functional resistance the host hampers penetration (1, 5, 6). In protoplasmic, the potential pathogen may enter, grow slightly and die, or may cause the collapse of host cells around the hyphae supposedly limiting nutrient supply and preventing sporulation. The immediate collapse of host cells in response to penetration has been termed hypersensitivity (13). At present, degrees of sporulation, which are often termed degrees of infectivity, are the means by which reaction types of the rust pathogens are distinguished, but these indicate little of what happens inside the host.

The amount of sclerenchyma tissue may limit the spread of the hyphae and thus the size of pustules (3, 6, 10, 12), but some workers have concluded that resistance was protoplasmic rather than structural (13), or suggested that protoplasmic resistance reduced the vigor of the parasite so that morphological factors might prevent sporulation (16).

Storey (15) described the resistant reactions to *P. polysora* as follows: "Ol-a," a slightly shrunken yellow fleck; "1++," a brown lesion with a tiny central pustule; "X," varying pustule sizes. Responses to *P. sorghi* have been noted as "1-" for chlorotic flecks (7) while Flangas et al. (4) mentioned only necrotic flecks and termed them "O" types. It is thus apparent that no uniform system of naming reaction types for the corn rusts exists.

This paper reports the histological examination of resistant and susceptible reactions of maize leaves to the two corn rusts, *Puccinia polysora* Underw. and *Puccinia sorghi* Schw.

Materials and Methods

Maize seedlings were inoculated by spraying a water suspension of the urediospores onto the leaves and placing the plants in a moist chamber in the dark for 16 hours. The plants were then kept in the greenhouse at 80°F. until symptoms developed.

The genotypes employed were selections from specific plant introductions and an inbred line derived from the variety Cuzco. Cuzco seed was originally obtained through the courtesy of P. M. le Roux, in 1954. The responses of the selections are shown in table I:

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TABLE I. Symptoms obtained on various hosts

hosts	*pathogens*	
	P. polysora	*P. sorghi*
P.I. 163558	minute pustule	necrotic fleck
P.I. 163597	chlorotic fleck	large pustule
P.I. 186208	chlorotic fleck	large pustule
P.I. 172332	necrotic fleck	large pustule
Cuzco	large pustule	chlorotic fleck

The above reactions represent the types observed except a water-soaked reaction. This appeared as a brownish orange, translucent fleck which was induced by high temperature and humidity acting on chlorotic flecks. "Watersoaking" often extended beyond the infected areas, notably on P.I. 186208 and P.I. 198902.

Leaf tissues were sampled at various intervals after inoculation. Frozen sections of 30 μ were prepared in water without fixation while other tissues were fixed in FAA or 1:1 acetic acid-ethanol. Before imbedding, tissues were dehydrated in tertiary butyl alcohol solutions and imbedded in "Histowax." Sections were stained with safranin in 70% ethanol followed by fast green in 95% ethanol. Geimsa's stain was used in water, and trypan blue in 95% ethanol containing 1% acetic acid by volume. The latter was used with whole mounts to show the infection structures, while the hyphae in whole mounts were demonstrated by boiling fresh tissues in 10% KOH until clear and soft, washing in hot 0.5% picric acid, staining in picro-aniline blue at 100°C. for 5-10 minutes, and washing out excess stain in 70% ethanol containing a trace of picric acid.

Results

Reactions to *Puccinia polysora* varied from necrotic flecks to large pustules and differed from those produced by *P. sorghi* on the same plants. Penetrations were similar on resistant and susceptible hosts, and were accompanied by large, thin-walled appressoria and vesicles (plate I) which for *P. sorghi* were unlike those formed on water agar (11). The chlorotic fleck type developed 3-5 days after inoculation and sections and whole mounts showed that the hyphae grew slowly between the cells without forming haustoria (plate II, fig. 2). Only two haustoria were found in chlorotic fleck inducing hosts; these were in an epidermal cell in a watersoaked area. A mass of compacted chlorenchyma cells surrounded by mycelium was found at 7-10 days. During this period the tissue collapsed slightly but no hypertrophy or hyperplasia was observed. At 7 days, frozen sections showed that the plastids were not destroyed but lacked chlorophyll. After 9-12 days the chloroplasts dissolved and the mesophyll collapsed, so that eventually only the vascular bundles and adjacent border parenchyma cells remained visible. In contrast, the necrotic fleck was accompanied by the complete and immediate collapse of the host cells at the penetration cite (plate III, fig. 4). At 80°F. the

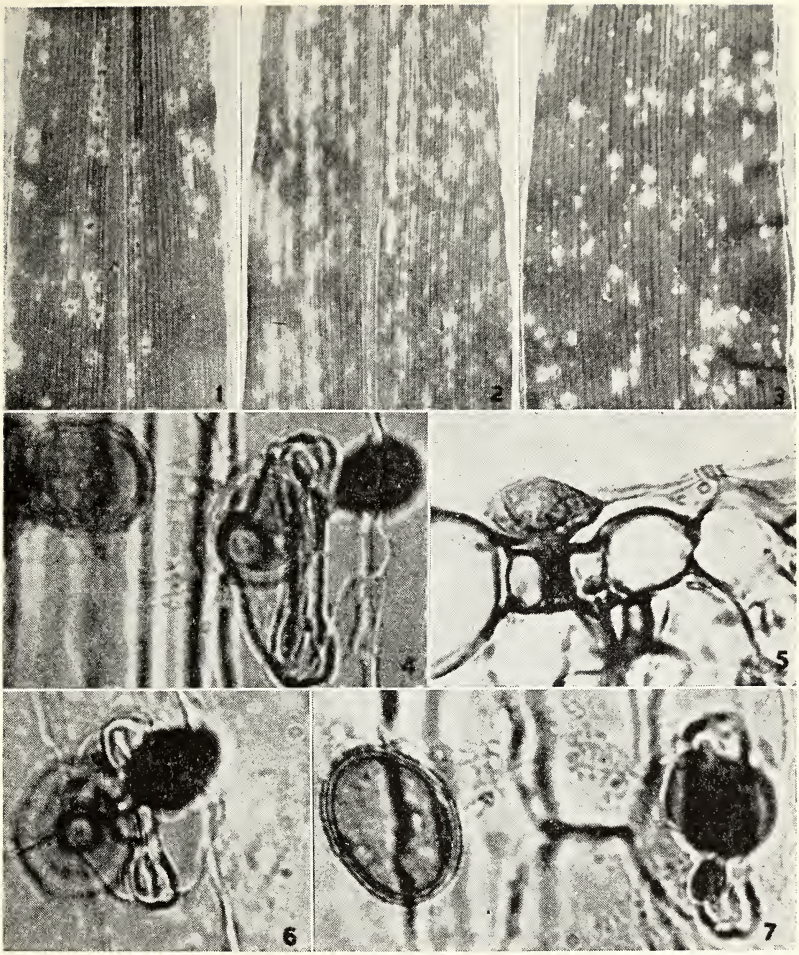

PLATE I

1. Minute pustules in necrotic flecks.
2. Diffuse chlorotic flecks, *P. sorghi* on Cuzco 8 days after inoculation.
3. Necrotic flecks, *P. polysora* on 172332 at 10 days.
4. Left to right: spore, appressorium, vesicle of *P. polysora* on susceptible leaf.
5. Cross esction of chlorotic fleck produced by *P. sorghi* on Cuzco, 6 days.
6. Appressorium and vesicle of *P. polysora* on P.I. 186208.
7. Spore, appressorium, and tiny vesicle of *P. sorghi* on Cuzco.

collapsed areas reached a maximum size 12 days after inoculation and appeared as white, parchment-like spots (pl. I, fig. 3) sharply delimited by healthy tissue. Border parenchyma cells remained turgid. At 12 days, only a thin line of broken and compacted cells remained so that both upper and lower epidermis were brought together. Cell penetration was not shown. Hyphae at the edges of the flecks suggested that growth of the parasite was essential for collapse. These hyphae appeared com-

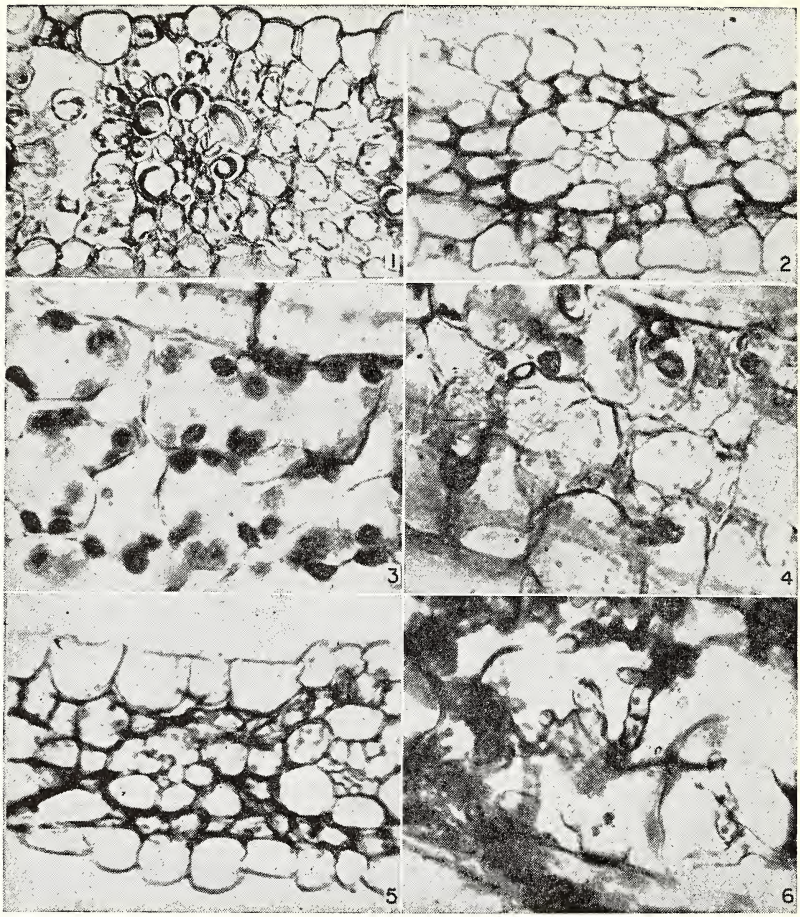

PLATE II

1. Healthy leaf on P.I. 186208, cross section.
2. Cross section showing intercellular hyphae of *P. polysora* at 8 days in a chlorotic fleck.
3. Mesophyll cells of healthy P.I. 186208.
4. Mesophyll of P.I. 186208 10 days after inoculation with *P. polysora* showing watery intercellular hyphae.
5. Cross section of chlorotic fleck produced by *P. sorghi* on Cuzco, 6 days.
6. Mass of hyphae of *P. sorghi* found in mesophyll of chlorotic fleck, 8 days.

pressed, forming ribbon-like bands above and below the border parenchyma cells. A third host response was a necrotic fleck with a minute central pustule. The pustules formed at 10 days at 80°F. Sections showed few haustoria were formed and sporulation was enhanced by placing the plants in a moist chamber 7-9 days after inoculation. Sporulation on susceptible plants required 8-10 days, and whole mounts showed the projection of haustoria (pl. III, fig. 2, 8) into cells from intercellular hyphae which were 2-4 μ in diameter. Often, the hyphae completely

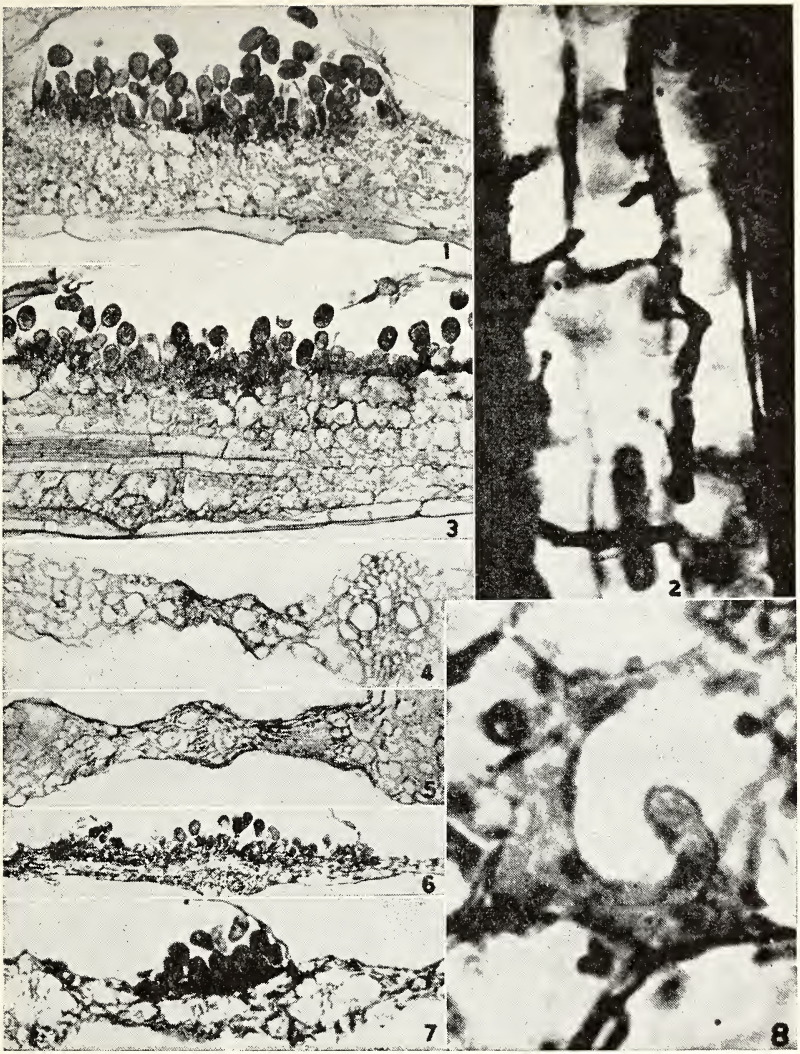

PLATE III

1. Susceptible reaction, *P. polysora.*
2. Hyphae and haustoria of *P. polysora* in susceptible leaf.
3. Susceptible reaction, *P. sorghi,* showing host cell distortion below pustule.
4. Cross section of necrotic fleck, 4 days, *P. polysora.*
5. Cross section of necrotic fleck, 5 days, *P. sorghi.*
6. Minute pustule reaction, *P. sorghi,* 7 days, note necrosis.
7. Minute pustule reaction, *P. polysora,* 9 days.
8. Haustorium of *P. sorghi* in susceptible leaf.

occupied the intercellular spaces at the junctures of 3 or 4 cells, forming large diamond and star-shaped structures. At places where pustules formed, the mass of hyphae might exceed the host tissue in volume and

considerable strain was evident by the distorted host cells (pl. III, fig. 1, 3). Hyphal growth appeared requisite for pustule formation as evidenced by the area of spread of the hyphae around pustules.

Host responses to *P. sorghi* were like those of *P. polysora* but developed 2-3 days faster than *P. polysora* at the same temperature. The chlorotic flecks were produced 2-4 days after inoculation and sections showed only intercellular hyphae. After 6-8 days there was a slight collapse of host cells. The hyphae grew abundantly between the cells but were compressed as if unable to move the host cells apart. Occasionally, at the centers of flecks a mass of hyphae was found (pl. II, fig. 6) but no true pustule initials. Similarly the hyphae were abundant in the necrotic fleck reactions but collapse was severe (pl. III, fig. 5) and sporulation was prevented. But where minute pustules were formed this collapse did not prevent spore formation and a few haustoria formed. Haustoria of *P. sorghi* always appeared as single hyphae and as many as three were found in one cell. No branching was observed. Numerous haustoria formed immediately below the pustules and were like those of *P. polysora.* The pustules of *P. polysora,* as with *P. sorghi* were accompanied by great host cell distortion to accommodate the pustule initials. Dicaryons were clearly seen in the hymenial-like layer below the spores.

Discussion

The results allow the comparison of similar resistant reactions on a single host species produced by different rust species. The immediate and complete collapse of host cells was associated with macroscopic white flecks but, where growth occurred between the cells without immediate collapse, a chlorotic fleck formed. There was no evidence of haustoria of either rust in the necrotic and chlorotic flecks. Since the collapse of host cells might have obscured any haustoria present, the statement "no haustoria" has some reservation. In all chlorotic and necrotic flecks the hyphal growth was restricted to the intercellular spaces more than in the susceptible leaves. This was particularly obvious in the chlorotic flecks and may have been the result of high cell wall strength. The rapidity of collapse in the necrotic flecks suggested an osmotic disturbance. If the differences between the chlorotic and the necrotic flecks are not basically morphological, then there may be two separate types of protoplasmic resistance based upon the collapse of host cells.

In the minute pustule reaction both rusts survived host cell destruction. Nutrients were probably transported from the healthy tissues at the periphery of the flecks to the cites of sporulation rather than the fungus gaining nourishment from the dead cells. Haustoria were associated with sporulation but were not necessary for hyphal growth, as evidenced by the chlorotic flecks.

On the basis of Stakman and Piemeisel's observations and an accepted classification of rust reaction types (14), both the chlorotic and necrotic flecks are classified as "O;" since no pustules form, yet there is a histological difference which has some genetic basis (16). It is suggested that a subdivision of the "O;" reaction be made for both maize rusts, perhaps "O;C" (chlorotic) and "O;N" (necrotic). These would serve as a better guide to studies of disease resistance.

Other studies of rust histology agree generally with the results presented here. Marryat (10) observed that, in wheat resistant to *Puccinia glumarum* (Schmidt) Erikss. & Henn., rust hyphae had fewer nuclei, appeared watery, and lacked haustoria. Stakman (13) found that the hyphae of stem rust in resistant wheat became closely appressed to the host cells and died to form a homogeneous mass. Allen (2) reported that the nuclei of the first cells attacked by *P. graminis* Pers. var. *tritici* Erikss. and Henn. in Mindum wheat were rapidly destroyed. This could not be shown for the necrotic fleck in maize leaves and was not true in the chlorotic fleck. Hursh (8) discovered that the mycelium of stem rust of wheat was limited to chlorenchyma which was also true for the maize rusts. Hart (6) reported the sclerenchyma cells in wheat as "unyielding" which was true of the border parenchyma and vascular elements in maize leaves. Regarding *P. sorghi*, Wellensiek (17) invoked the "starvation hypothesis" of resistance suggesting that necrosis was nutrient limiting. Results presented here are not strictly in accordance with this view since considerable mycelium was visible in the chlorotic flecks, and in the minute pustule reactions, necrosis did not prevent sporulation.

Regarding *P. sorghi*, the results agree with Mains (9) observations that, in the susceptible reactions, no haustoria were found in the border sheath and the vascular tissues.

Summary

The host-parasite interactions of corn plants resistant and susceptible to the two corn rusts, *Puccinia polysora* and *P. sorghi*, were studied. Resistance was defined as the prevention of sporulation and two symptoms accompanied lack of sporulation, a chlorotic fleck and a necrotic fleck. Both rusts behaved in the same manner. The chlorotic fleck was associated with hyphal growth without haustorial formation, and the host cells in this reaction remained turgid for 7-10 days and then collapsed slowly. The necrotic fleck resulted from the complete and immediate breakdown of host cells in the region of the hyphae. No haustoria were seen, but they may have been obscured by the collapse. These differences within a "O;" type response were given as a basis for subdividing this reaction in order to improve genetic and physiological studies of disease resistance. A third reaction type consisted of a minute pustule formed within a necrotic fleck. Haustoria were observed here and hyphae were more turgid. All reactions were compared to the susceptible example where hyphae grew freely through the tissues, separating cells, and forming numerous haustoria and spores.

Literature Cited

1. ALLEN, R. F. 1923. A cytological study of the infection of Baart and Kanred wheats by *Puccinia graminis tritici*. J. Agr. Res. **23**:131-151.
2. ALLEN, R. F. 1923. Cytological studies of infection of Baart, Kanred, and Mindum wheats by *Puccinia graminis* forms III and XIX. J. Agr. Res. **26**:571-604.
3. FARRAR, W. 1898. The making and improvement of wheats for Australian conditions. Agr. Gas. N. S. Wales **9(2)**:131-241.
4. FLANGAS, A. L. and J. G. DICKSON. 1961. The genetic control of pathogenicity, serotypes and variability in *Puccinia sorghi*. Am. J. Botany **48(4)**: 275-285.

5. HART, H. 1929. The relation of stomatal behavior to stem rust resistance in wheat. J. Agr. Res. **39**:929-948.

6. HART, H. 1931. Morphologic and physiologic studies on stem rust resistance in cereals. U.S.D.A. Bull. 266.

7. HOOKER, A. L. and W. A. RUSSELL. 1962. The inheritance of resistance to *Puccinia sorghi* Schw. in 6 corn inbred lines. Phytopathology **52(2)**:122-128.

8. HURSH, C. R. 1924. Morphological and physiological studies on the resistance of wheat to *Puccinia graminis tritici*. J. Agr. Research **27**:381-412.

9. MAINS, E. B. 1916. The relation of some rusts to the physiology of their hosts. Am. J. Botany. **4**:179-220.

10. MARRYAT, D.C.E. 1907. Notes on infections and histology of two wheats immune to attacks of *Puccinia glumarum*. J. Agr. Sci. **2**:129-138.

11. PAVGI, M. S. and J. G. DICKSON. 1961. The influence of environmental factors on the development of infection structures by *Puccinia sorghi*. Phytopathology **51(4)**:224-446.

12. EVANS, I. B. POLE. 1907. The cereal rusts I. The development of their Uredo mycelia. Ann. Botany **21**:441-446.

13. STAKMAN, E. C. 1915. Relation between *Puccinia graminis* and plants highly resistant to its attack. J. Agr. Res. **4**:193-200.

14. STAKMAN, E. C. and LEVINE, M. N. 1922. The determination of biologic forms of *Puccinia graminis* on *Triticum*. Minn. Agr. Exp. Sta. Tech. Bull. 8.

15. STOREY, H. H., and A. K. HOWLAND. 1957. Resistance in maize to the tropical American rust fungus, *Puccinia polysora* Underw. Heredity **11(3)**:289-301.

16. THATCHER, F. S. 1943. Cellular changes in relation to rust resistance. Can. J. Res. **21**:151-172.

17. WELLENSIEK, S. J. 1927. The nature of resistance to *Zea maize* to *Puccinia sorghi*. Phytopathology **17**:815-825.