Microscopic development of Puccinia Coronata CDA on resistant strains of oats

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MICROSCOPIC DEVELOPMENT OF PUCCINIA CORONATA CDA.

ON RESISTANT STRAINS OF OATS

by

Earnest LaGrande Hobbs

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INTRODUCTION

One of the problems concerning the cereal rusts has been determining the nature of resistance in different host varieties and species. Previous workers have made detailed drawings and accounts of infection showing the physical response of resistant varieties to invasion by a rust fungus. Of course, this approach cannot reveal the biochemical responses involved in resistance but information can be obtained on the intimate relationship of host and parasite. This information can be useful in future investigations of the physiology of host-parasite interactions. Any differences in reaction found in different varieties can be of use in a breeding program to more accurately classify types or sources of resistance.

The present study was undertaken to investigate the mechanism of resistance in two of the newer sources of resistance to crown rust of oats (caused by *Puccinia coronata* Cda. var. *avenae* Fraser & Ledingham). These strains show promise of being useful as sources of resistance to new races of the crown rust fungus which are capable of parasitizing all commercial oat varieties currently grown in the United States.

One strain, C.I. 7181, is susceptible in the seedling stage, but as the plant develops, older leaves become resistant. Such behavior suggested that some morphological character might be responsible for resistance in this case. C.I. 7181 is a good subject for a study of the nature of resistance, since it is possible to observe both resistant and susceptible reactions on the same plant. By inoculating at the proper time one leaf will show resistance and the next will be susceptible.
The other Strain, C.I. 7233, is a strain of *Avena abyssinica* Hochst., a tetraploid species. It is resistant in the seedling stage.

The specific purpose of the present study was to determine the mechanism of resistance in these particular lines. Another goal was to gain some understanding of the basic nature of resistance in oats to *P. coronata*.
REVIEW OF LITERATURE

The first theory concerning the development of cereal rust fungi within the host was advanced by Ericksson (1897). He hypothesized that the fungus was present in the plant cells in an intimate relationship with the host protoplasm. When conditions were right the fungus supposedly grew out of certain cells containing this "mycoplasm" and spread throughout the leaf in that area. Ward (1882) had already shown that the coffee rust fungus, *Hemileia vastatrix* Berk & Br., infected coffee by entrance of germ tubes through stomata. He showed that these germ tubes originated from germinated urediospores on the surface of the leaf. Ward (1902, 1905) refuted the "mycoplasm hypothesis" of Ericksson when he worked out the complete histology of *Puccinia dispersa* Erikss. on brome. He pointed out that the "corpuscles" that Eriksson said gave rise to the fungus mycelium were actually haustoria developed by the mycelium.

Pole Evans (1907) verified Ward's work when he traced the development of a number of cereal rusts including *P. coronata*. The spores germinated on the leaf surface producing germ tubes. When the germ tubes contacted a stomate a swelling, called the appressorium, was formed on the tip of the tube. From this swelling a hyphal peg grew through the stomate. Inside the leaf in the substomatal cavity a vesicle was formed which was characteristic in shape and form for the particular species of rust. Infection hyphae grew from this vesicle, and formed haustoria in host cells that were contacted. Ward also investigated the development of *P. dispersa* on resistant varieties of brome (1902).

Early theories had suggested that resistance was due to some
morphological character. Cobb (1892) advanced the theory that characters such as thickness of leaf cuticle, waxy coverings, leaf pubescence, size of stomata, or leaf angle might lend resistance to certain varieties. He noted some correlations of these characters with amount of infection which seemed to support his theory. Hitchcock and Carleton (1893) found that varieties with stiff, upright leaves were less likely to rust than those with flaccid leaves. They also found that a thick epidermis or a fine pubescence tended to inhibit entry of germ tubes into stomata. Ward, however, felt that these correlations were not valid. He performed a great number of inoculation experiments using P. dispersa on bromes. In addition, he made detailed histological investigations of infection. He concluded that there was no relationship between differences in morphology of brome varieties and their resistance to rust. Rather, resistance depended entirely on the physiological interactions of the protoplasm of the fungus with the cells of the host.

In further investigations of cereal rusts, Ward (1905) showed that spores of Puccinia glumarum (Schmidt) Erikss. & Henn. germinated equally well, or almost so, on resistant as on susceptible varieties of wheat. The germ tubes gained entry into both susceptible and resistant wheat varieties. However, on resistant varieties the development of the fungus was checked when the host cells contacted by the mycelium collapsed and the contents became disorganized into a shapeless, heavy-staining mass. Ward believed the fungus hyphae were "starving for want of food supplies or they are being poisoned." He felt that the death of the fungus was most likely due to starvation.
Gibson (1904) continued work concerning resistance to rusts in Ward's laboratory. She inoculated a wide range of non-host plants with *Puccinia chrysanthemi* Roze, and demonstrated that germination and stomatal penetration took place on all plants she used. The further development of the fungus depended on how closely the plant was taxonomically related to the normal host plant. In no case were haustoria formed in non-host plants. She felt that the failure of the fungus to develop was due to some toxic material secreted by the plant and not due to starvation. This conclusion was based on the observation that hyphae on the surface of the leaf appeared to remain alive when hyphae inside were shriveled.

When resistant varieties of chrysanthemum were inoculated, the hyphae entered and developed a mycelium with haustoria the same as in susceptible varieties. A few cells adjacent to the infected area died inhibiting the further spread of the mycelium. Resistance was based on this limitation of growth.

Gibson also noted that resistance was not transferred to susceptible shoots by grafting them onto resistant roots. From this she concluded that the factor responsible for resistance was not translocated.

Biffen (1907) stated that it was practicable to breed cereal varieties "- resistant to the attacks of certain parasitic fungi." He observed that resistance was independent of any discernible morphological characters and felt varieties could be bred which would be morphologically the same but would differ in their resistance to disease. This work along with that of Bolley (1908) initiated all subsequent work in breeding cereal varieties resistant to rust.
Marryat (1907), using varieties selected by Biffen, described in detail the infection of resistant and susceptible wheat plants inoculated with *P. glumarum*. The fungus entered and produced hyphae in both resistant and susceptible varieties. The hyphae grew vigorously in the susceptible host but in resistant plants the death and disintegration of host cells checked further development of the fungus. The hyphae became watery, nuclei were reduced in number and very few haustoria were formed. The cause of impoverishment of the fungus was thought to be starvation. However, Marryat also stated that the reaction was due to the "production of certain toxins and anti-toxins by host or parasite or both which are mutually destructive."

Stakman (1914) compared the course of infection on susceptible and resistant varieties of wheat by *Puccinia graminis* Pers. f. sp. *tritici* Erikss. & Henn. and found that in both cases the fungus entered the plant in much the same manner. After entrance, however, the behavior pattern was quite different. In the susceptible host the fungus grew vigorously and produced uredia. In the resistant varieties the reaction varied with the degree of resistance shown by the variety. In some cases the fungus failed to send out infection hyphae from the substomatal vesicle. When infection hyphae were produced they soon became vacuolated and never produced haustoria in the host cells. In all cases host cells in the vicinity of infection were killed. The more resistance exhibited by the host, the quicker a few host cells were killed and the progress of the fungus halted. The death of the host cells was the direct result of the presence of the hyphae, after which the hyphae themselves died. To
explain this phenomenon Stakman (1915) advanced the "hypersensitive" theory, i.e., immunity is due to some physiological incompatibility resulting in rapid death of the host cells when attacked by rust hyphae.

Newton (1922) investigated varieties she believed were more resistant to *P. graminis* than those Stakman (1914) used. However, the host reaction was much the same except that development of the fungus was checked earlier. On the variety Kanred only very few host cells were killed so that there was no macroscopic evidence of infection. In some cases development of the fungus was stopped after appressoria were formed. Despite the fact that infection was blocked at such an early stage Newton felt that the fungus died of starvation.

Allen (1921, 1923a, 1923b, 1926a, 1926b, 1927, 1928) made extensive investigations of the interaction of *P. graminis*, *Puccinia recondita* Rob. ex Desm. f. sp. *tritici* Erikss. (*P. triticina*) and *P. glumarum* on resistant, semi-resistant and susceptible varieties of wheat. She found essentially the same pattern of development as earlier workers had. Allen (1921, 1923a, 1923b) did note that on one variety, Kanred, only about 10 percent of the appressoria were able to send infection pegs through the stomata. This was true with races to which Kanred was susceptible as well as those to which it was resistant. The stomata on Kanred were narrower than those of other varieties so Allen thought they were excluding the fungus. Newton (1922) did not feel this was an important factor in the resistance of Kanred.

Allen (1923b) described a "secondary area" of damage to cells of Mindum wheat. Mindum was resistant to the race of rust used. Outside the area killed by contact with the mycelium, there was an area in which
the cells were plasmolysed and had swollen walls. She felt that these cells were possibly affected by diffusion of a toxic substance.

In the case of Khapli emmer, there was a partial adjustment to a more congenial relationship after the first cells were killed by *P. graminis* so that the fungus survived (Allen, 1926a). This was also true of Malakoff wheat when infected with *P. triticina* (Allen, 1927).

On Little Club, a variety susceptible to *P. recondita tritici*, Allen (1926b) noted that the cells in the infected area showed little impover­ishment or destruction. In fact, they were more turgid than those in other areas of the leaf.

Hart (1929,1931) correlated stomatal behavior of wheat varieties with resistance to *P. graminis tritici*. She noted that stomata of certain resistant varieties tended to open later in the morning than those of susceptible varieties. Peterson (1931) studied these same varieties and reached the conclusion that stomatal behavior was not an important factor in rust resistance. Caldwell and Stone (1932) found that closed stomata presented no barrier to penetration by *P. recondita tritici*. They stated "Often a small stomatal slit is evident between the appressorium and the substomatal vesicle, apparently resulting from the penetration of the tube pushing between the guard cells."

Ruttle and Fraser (1927) found that *P. coronata avenae* showed much the same relationship to a resistant and to a susceptible variety of oats as had been described for other rusts on their respective hosts. On the susceptible host the infected tissue appeared to be stimulated and the cells more turgid. On the resistant host there normally was rapid death of host cells and fungus due to some antagonistic interaction. This was
not always true though. In some cases the reaction was much less violent on the resistant variety and the fungus remained alive and produced small uredia. On the susceptible variety some signs of incompatibility were noted. Incompatibility was manifested by haustoria which appeared to be distorted and non-functional.

Hanes (1936) inoculated wheat, rye, barley, and Lolium sp. with P. coronata avenae and found penetration occurred as if on oats. Vesicles formed and infection hyphae were produced, but haustoria were never formed. Upon penetration of a stomate the guard cells were killed. Any mesophyll cells touched by the hyphae were either killed or the cell walls were thickened and further development of the fungus was stopped.

Rothman (1960) investigated the interaction between race 202 of P. coronata and eight varieties of oats showing varying degrees of resistance and susceptibility to race 202. He assigned the eight varieties to four groups according to their reaction to infection. Group I showed the normal susceptible pattern of development in which there was scarcely any effect on the host cells and the fungus developed freely. In varieties assigned to Group II development was slower and there was some cellular disorganization and impoverishment in areas surrounding the infected area. Cells of plants of Groups III and IV were killed and the further progress of the fungus checked. Groups III and IV were separated primarily by the time it took for the antagonistic reaction to start and the degree of penetration the fungus attained. In Group III host cells began to collapse after 72 hours and the fungus continued growth until 192 hours when cells in large areas of the mesophyll collapsed. In Group IV disorganization of host cells occurred 48 hours after inoculation and maximum development was attained at 144 hours.
MATERIALS

In this investigation the process of infection was studied using race 26U of *P. coronata avenae*. Race 26U was first described by Wahl and Schreiter (1953) in Israel and it was found in the United States the following year (Simons 1955). It attacks all commercial varieties being grown in the United States at this time. Resistance to race 26U has been found in some diploid and tetraploid species of oats. Some strains of hexaploid oats show field resistance to race 26U.

The development of the fungus and the host reactions were studied on a susceptible variety and two strains which are resistant, C.I. 7233 and C.I. 7181.

C.I. 7233 is a pure strain selected from P.I. 193958, which was introduced from Ethiopia (Simons 1959, Simons et al. 1959). It is a tetraploid oat of the species *A. abyssinica*, which usually shows heavy flecking when infected with race 26U in the seedling and later stages of growth. However, under the conditions of this investigation only light flecking occurred.

C.I. 7181 is a selection from an introduction from France designated P.I. 174544. It was shown to exhibit field resistance by Shands (1951).

The reaction of C.I. 7181 to infection by race 26U can be quite variable depending on the age of the plant part infected. In this study the older leaves "flecked" or small uredia were formed. Rather large susceptible type uredia were formed on the younger leaves.

The variety Clinton was used as the susceptible host. The development of the fungus on Clinton was considered to be the normal developmental
pattern and the reaction of Clinton to infection was used as the standard with which to compare the resistant reactions of C.I. 7233 and C.I. 7181.
METHODS

Plants of Clinton, C.I. 7233, and C.I. 7181 were grown in 6-inch pots in an air-conditioned chamber in the greenhouse. This chamber was maintained at a temperature of about 70°F.

When the fourth leaf was just beginning to emerge the plants were inoculated with race 264 of *P. coronata avenae*. Inoculation was accomplished by first spraying the plants with a 1% solution of Tween 20 (polyoxyethylene sorbitan monolaurate) in distilled water. The plants were then dusted with a mixture of urediospores and talc and placed in a moist chamber overnight. They were then placed back in the air-conditioned chamber.

Collections were taken from the second and third leaves at 1-day intervals for 10 days, beginning 24 hours after inoculation. The material was killed and fixed in Craf III (Sass, 1958). Dehydration of some of the material was carried out in an ethyl alcohol series and, the remainder was carried through a dioxan-n-butanol series (Sass, 1958) with the latter giving the best results. The material was infiltrated using commercial Parowax and embedded in Fisher Scientific Company's Tissuemat casting compound. Serial sections were cut 10 microns thick.

Safranin and fast green were used for preliminary staining. After determining that a particular batch of material had been satisfactorily fixed and embedded, hemalum was added to the safranin and fast green staining schedule to give better definition of the fungus hyphae.
RESULTS

Macroscopic Observations

Macroscopic symptoms of infection appeared first on the fully susceptible variety Clinton, occurring as small flecks the fifth day after inoculation. By the next day flecks were noticeable on the younger leaves of C.I. 7181. By the seventh day the leaves of C.I. 7233 and the older leaves of C.I. 7181 showed flecking.

Although the initial symptoms on all three strains were superficially similar flecks, the cause of the flecking was not the same on all three. Flecks on Clinton had developed into uredia by the eighth day after inoculation and on the upper leaves of C.I. 7181 by the tenth day. Flecks on C.I. 7233 and most of those on the older leaves of C.I. 7181 were necrotic spots by the ninth day after inoculation. Some type 2 or type 3 pustules were also found on the older leaves of C.I. 7181.

By the eleventh day after inoculation the pattern of development was clear. Upper and lower leaves of Clinton were highly susceptible, showing large type 4 uredia. The youngest, third leaves of C.I. 7181 were only a little less susceptible, the uredia being little smaller on C.I. 7181 than on Clinton. The second leaf of C.I. 7181 showed appreciable resistance. Some type 2 or 3 uredia developed, but most lesions found on the second leaf were necrotic flecks. C.I. 7233 was highly resistant showing scattered necrotic spots on both the second and third leaves. There were fewer necrotic areas on the second leaf than on the third.
Microscopic Observations

Development of the fungus on Clinton

Urediospores germinated on the surface of the leaf, producing germ tubes. When a germ tube contacted a stomate a swelling, which developed into the appressorium, formed over the stomatal opening. A small penetration peg then grew from the appressorium through the stomatal aperture (Figure 1). Inside the stomate another swelling, the substomatal vesicle, was formed. The vesicle was rather elongate, cylindrical, and had a single septum near the center. An infection hypha grew from each end of the vesicle into the mesophyll of the leaf (Figure 2).

All of the events up to this point occurred during the first 24 hours after inoculation, the time at which the first collections were taken.

When an infection hypha contacted a cell of the mesophyll of the host, a septum formed 10 to 15 microns back from the tip, cutting off a haustorium mother cell (Figure 3). A very fine thread entered the host cell by penetrating the cell wall (Figure 3). The distal end of this thread enlarged to form the haustorium. In the early stages of development the haustoria were small and globoid and stained deeply with safranin (Figure 4). This stage was reached 2 days after inoculation. Later the haustoria became more granular and one or two nuclei could be distinguished in each. The haustoria were usually rather long and more or less cylindrical with no branching or lobes (Figure 3). However, one haustorium was observed which was irregular in shape and appeared to branch and coalesce (Figure 5). The host nuclei were usually found tightly appressed
Figure 1. Top view of a stomate with a penetration peg (p) between the guard cells (Clinton, one day after inoculation, X 896)

Figure 2. Substomatal vesicle with an infection hypha growing from each end (Clinton, one day after inoculation, X 480)

Figure 3. Haustorium mother cell (hm) from which a haustorium (h) has developed (Clinton, six days after inoculation, X 896)

Figure 4. A small, developing haustorium (h) (Clinton, two days after inoculation, X 896)

Figure 5. An irregularly shaped haustorium (h) inside a host cell (Clinton, four days after inoculation, X 896)

Figure 6. Sheath cell invaded by three haustoria (h) (Clinton, six days after inoculation, X 480)
or close to the haustorium. As many as three haustoria were found in a single sheath cell (Figure 6).

During the time the haustorium was being formed from the terminal haustorium mother cell, a hypha branched off the cell just behind the haustorium mother cell. This branch grew until another mesophyll cell was contacted and the process of haustorium production was then repeated.

During the next few days the fungus grew rapidly, branching and spreading, until at 5 or 6 days after inoculation large areas of the leaf were filled with mycelium. These masses of mycelium are the cause of the flecking observed in susceptible varieties at this stage of development (Rothman, 1960).

There were numerous inclusion bodies in the cells of the infected area (Figure 7). They were round, homogeneous bodies which stained brightly with safranin. Inclusion bodies were found only on Clinton and susceptible leaves of C.I. 7181. None were found on resistant leaves of C.I. 7181 or on C.I. 7233 which was resistant. Inclusion bodies are thought by Humphrey and Dufrenoy (1944) to be made up of quinones enclosed in special vacuoles.

About 6 or 7 days after inoculation the growth of the hyphae became oriented toward either the upper or lower epidermis of the leaf, forming thick heavy-staining masses in intercellular spaces just below the epidermis (Figure 8). At about 7 days thickened knobs, which pushed up the epidermis, could be distinguished (Figure 9). These knobs soon became differentiated into urediospores. By 8 days the pressure from the urediospores was sufficient to rupture the epidermis of the host thus releasing the spores (Figure 10).
Figure 7. Inclusion bodies (i) in cells in an area with hyphae permeating the intercellular spaces (Clinton, six days after inoculation, X 480)

Figure 8. Massing of hyphae beneath the host epidermis prior to urediospore formation (Clinton, seven days after inoculation, X 480)
Figure 9. Early stage in the development of urediospores (Clinton, seven days after inoculation, X 480)

Figure 10. A uredium just beginning to release spores (Clinton, eight days after inoculation, X 344)
Even as late as the time of urediospore production the cells in the infected area were turgid. The cells not directly under the uredium appeared to be normal without any recognizable effects of infection. However, chloroplasts of cells just below the uredium were reduced in size or missing giving the cells a drained appearance.

Although most of the cells in an infected leaf were not affected severely by infection, some reacted violently. In a few cases there were small groups of three or four cells in which the protoplasm clumped and the cells died and formed heavy-staining disorganized masses (Figure 11). Such dead cells were observed as early as 3 days after inoculation.

**Development of the fungus on C.I. 7181**

**Development on upper leaves** The development of the fungus on the upper, susceptible leaves of C.I. 7181 was much the same as on Clinton. However, some slight disturbance of the normal condition of the cells was noticeable and the fungus developed more slowly.

Entrance into the stomatal cavity was the same as described for Clinton. The fungus developed as rapidly on C.I. 7181 as it did on Clinton during the first 24 hours. Infection hyphae were formed which contacted mesophyll cells. The host nucleus was found appressed to the cell wall at the point of contact by the hypha, and there appeared to be a slight clumping of the cytoplasm in that area (Figure 12).

Haustoria were initiated in the host cells by the second day. Some cells appeared to be unaffected (Figures 13 and 14) but in others the cell contents tended to gather around the haustorium (Figure 15).

By the seventh day following inoculation the difference in rate of
Figure 11. A hypersensitive area in the susceptible Clinton, containing dead cells (dc) (Clinton, three days after inoculation, X 896)

Figure 12. Infection hypha contacting a mesophyll cell and causing slight clumping of the protoplasm at the point of contact (third leaf of C.I. 7181, one day after inoculation, X 896)

Figure 13. Haustorium (h) in a mesophyll cell causing no recognizable effects on the cell (third leaf of C.I. 7181, three days after inoculation, X 896)

Figure 14. Haustorium (h) in a mesophyll cell causing no recognizable effects on the cell (third leaf of C.I. 7181, two days after inoculation, X 896)

Figure 15. Haustorium (h) in a mesophyll cell with the protoplasm of the cell clumped around the end of the haustorium (third leaf of C.I. 7181, four days after inoculation, X 896)

Figure 16. Hyphae in intercellular spaces showing some indication of growth to the epidermis prior to formation of a uredium (third leaf of C.I. 7181, eight days after inoculation, X 344)
development of the fungus on Clinton and C.I. 7181 was pronounced. On Clinton the beginnings of urediospore formation were taking place (Figure 9), but on C.I. 7181 the hyphae were just beginning to show oriented growth toward the surface of the leaf (Figure 16). The area of the leaf which was infected was smaller and the mycelium was not as thick as it was in Clinton when the hyphae began to grow toward the epidermis. Uredia did not develop on C.I. 7181 until 9 or 10 days after inoculation and were smaller when they did develop.

There were larger areas of dead, disorganized cells in C.I. 7181 than in Clinton and cells near the dead areas often showed some impoverishment (Figure 17). Dead cells were also found in areas where most of the cells were not damaged by the presence of haustoria. In one case observed, dead cells were found within 0.5 mm of a sporulating uredium.

**Development on lower leaves** As on Clinton and the upper leaves of C.I. 7181, the fungus gained entry into the stomatal cavity after 24 hours. The substomatal vesicle was formed and infection hyphae developed (Figure 18). From this point on development on the lower leaves of C.I. 7181 was slower than that on the upper leaves or on Clinton. No haustoria were noted until the seventh day after inoculation. Growth of the hyphae was very slow and very little branching was noted. By the sixth day the hyphae had only grown about 0.5 mm from the point of entry. In growing this distance a number of mesophyll cells were passed without any indication of haustorium formation or any effect on the cells (Figure 19).

On the seventh day there seemed to be a change in the action of the fungus. Haustoria were found in host cells (Figure 20). Most of these haustoria appeared to mature. They functioned only a short time, then
became irregular in shape and stained heavily (Figure 21). The mycelium at this stage was still scattered in the leaf and showed very little branching. The invaded cells of the host were affected in two ways. In some cases the cell contents clumped and stained deeply (Figure 22), while in others the chloroplasts were markedly reduced in size (Figure 21).

By 8 days the mycelium was branching more and spreading into rather large areas of the leaf several mm in diameter. The cells in heavily infected areas were almost completely devoid of chloroplasts (Figure 23). Small necrotic spots involving the death and disorganization of about six cells were also found at 8 days. These spots could be found in leaves in which the fungus was growing relatively well in other areas.

The first uredium formed was found in a leaf collected 9 days after inoculation. It was a small uredium only about 700 microns in diameter. There was an area around the uredium 3 or 4 mm in diameter in which the cells had collapsed. In contrast to other dead areas these cells did not stain heavily. These collapsed cells were found around the periphery of the uredium. The cells directly under the spore-bearing area appeared fully turgid but protoplasmic structure was absent giving the cells a drained appearance.

**Development of the fungus on C.I. 7233**

**Development on the upper leaves** Although C.I. 7233 is resistant to race 26½ in the seedling stage, there was a difference in the degree of resistance shown by the upper and lower leaves. The upper, younger leaves exhibited a range of reactions to the fungus. These varied from a reaction in which the fungus was able to feebly maintain itself without
Figure 17. Large area in the mesophyll in which cells are killed or impoverished by the parasite (third leaf of C.I. 7181, nine days after inoculation, X 344)

Figure 18. Substomatal vesicle and infection hyphae on the second leaf of C.I. 7181 (one day after inoculation, X 480)

Figure 19. Growth of hyphae in the mesophyll without formation of haustoria (second leaf of C.I. 7181, six days after inoculation, X 480)

Figure 20. Small developing haustorium (h) connected to a haustorium mother cell (hm) (second leaf of C.I. 7181, seven days after inoculation, X 896)

Figure 21. Irregular, heavy staining haustorium (h) in a host cell with chloroplasts reduced in size or missing (second leaf of C.I. 7181, ten days after inoculation X 896)

Figure 22. Sheath cell with protoplasm clumped in reaction to the fungus (second leaf of C.I. 7181, ten days after inoculation, X 480)
extensive damage to the host cells to one in which the fungus did not even gain entry into the leaf.

In one case the development of the fungus was stopped after appressorium formation. The guard cells of the stomate under the appressorium did not appear to be affected, but the cytoplasm of four or five cells just beneath the stomate was clumped (Figure 2k). Three examples were found in which some degree of development of the substomatal vesicle was attained before the fungus died. In one the guard cells appeared to be dead and stained heavily (Figure 25). In the other two sections, the guard cells were normal, but a number of cells in the immediate vicinity had clumped, heavy-staining cytoplasm (Figures 26 and 27).

In most cases the fungus successfully formed a substomatal vesicle and infection hyphae within 24 hours after inoculation. The first cells contacted were sometimes killed (Figure 28) but generally the fungus passed by one or more cells before affecting the host (Figures 29 and 30). A single haustorium mother cell was found by the second day (Figure 31). There was no evidence that a haustorium was formed in the host cell contacted. The cell collapsed and died and the haustorium mother cell was drained and the walls collapsed. A few haustoria were found in infections 5 or 6 days old. The cell contents of all cells invaded by haustoria were disorganized and aggregated around the haustoria. The haustoria attained near normal size but were irregular in shape and stained heavily (Figures 32 and 33).

For the first 7 or 8 days the fungus either caused death of the cells in its vicinity (Figure 34) or grew in the host tissue without forming
Figure 23. A heavily infected area of the second leaf of C.I. 7181 in which the cells have a drained appearance due to the dissolution of chloroplasts (ten days after inoculation, X 318h)

Figure 24. Development of the fungus stopped at the appressorium (a) stage after several cells are killed (third leaf of C.I. 7233, one day after inoculation, X 480)

Figure 25. Infection stopped during formation of the substomatal vesicle (s) and after death of the guard cells (third leaf of C.I. 7233, five days after inoculation, X 480)

Figure 26. Infection stopped during formation of the substomatal vesicle and after death of several mesophyll cells not yet contacted by the fungus (third leaf of C.I. 7233, one day after inoculation X 480)

Figure 27. Abnormal substomatal vesicle with infection stopped (third leaf of C.I. 7233, one day after inoculation, X 480)
Figure 28. Early stage of infection in which the first few cells contacted by infection hyphae have died (third leaf of C.I. 7233, one day after infection, X 480)

Figure 29. Early stage of infection in which an infection hypha has passed by one cell causing no effects but the cytoplasm of the next cell is clumped (third leaf of C.I. 7233, one day after inoculation, X 480)

Figure 30. An infection hypha growing in the mesophyll of the third leaf of C.I. 7233, five days after inoculation (X 480)
Figure 31. A collapsed haustorium mother cell (hm) near a dead, deformed cell with no evidence of a haustorium (third leaf of C.I. 7233, two days after inoculation, X 896)

Figure 32. A heavy staining, irregular haustorium (third leaf of C.I. 7233, five days after inoculation, X 896)

Figure 33. A heavy staining, irregular haustorium (third leaf of C.I. 7233, six days after inoculation, X 896)
haustoria or noticeably affecting the host cells (Figures 35 and 36). The particular pattern of infection appeared to be determined by the reaction of the host cells in a given area. In one case observed, the infection hypha from one end of the substomatal vesicle induced death of adjacent host cells (Figure 37), while the hypha from the other end had very little effect on the cells it encountered (Figure 38).

Ten days after inoculation most of the infected areas consisted of dead, collapsed, heavy-staining cells. However, there was still some sparse mycelium in some areas of the leaf at 10 days. In the vicinity of this mycelium host cells in a relatively large area of about 1 mm diameter were either dead or showed chloroplast disintegration giving a drained appearance (Figure 39). No haustoria were found in this area.

**Development on lower leaves** The same range of reactions occurred on the lower leaves of C.I. 7233 as on the upper leaves, but there was more tendency toward antagonistic relationships.

As on the upper leaves, there was one case in which development of the fungus was stopped at the appressorium stage. The appressorium formed over a stomate and the guard cells appeared to have been killed. The adjoining epidermal cells developed thickened walls and some of the cells inside the leaf were affected (Figure 40). Death of a few host cells and the fungus also occurred after formation of the substomatal vesicle as on the upper leaves.

In most instances the fungus entered and formed a vesicle and infection hyphae during the first 24 hours, as in susceptible strains. However, the first cells of the mesophyll to be contacted died and further development of the fungus was stopped (Figures 41 and 42). The small
Figure 34. An area of the leaf in which cells were killed and the fungus mycelium (m) has died (third leaf of C.I. 7233, 5 days X 896)

Figure 35. Mycelium growing in the third leaf of C.I. 7233 five days after inoculation (X 480)

Figure 36. Mycelium growing in the third leaf of C.I. 7233 at seven days after inoculation (X 480)
Figure 37. Mycelium (m) causing death of host cells. (This mycelium is from the same vesicle as that in Figure 38. Third leaf of C.I. 7233, eight days after inoculation, X 480)

Figure 38. Mycelium (m) causing little effect on host cells. (This mycelium is from the same vesicle as that in Figure 37. Third leaf of C.I. 7233, eight days after inoculation X 480)

Figure 39. A large area of impoverished cells in the vicinity of mycelium (m) still living ten days after inoculation (third leaf of C.I. 7233, X 480)
areas involved were not large enough to be macroscopically visible, hence the low incidence of flecking on the lower leaves of C.I. 7233. Flecks that were found were due to a few rather large areas of about one mm diameter in which the cells were either dead and disorganized or showed impoverishment without collapse of the cell walls. The very few bits of mycelium found in these areas appeared to be shrivelled and dead (Figure 13).

Only one haustorium was found in a host cell in all the sections examined. It was found in a 4 day old infection. The cytoplasm of the cell was disorganized and collected around the haustorium. The haustorium did not have the granular appearance of normal haustoria but appeared to be homogenous and stained deeply.

In only two of all the infections examined was the fungus able to survive up to 10 days in the host tissue, and then only as small isolated bits of mycelium (Figure 44). No haustoria were found in these areas and there was little or no damage to the host cells.
Figure 40. Infection stopped after formation of the appressorium and death of the guard cells (second leaf of C.I. 7233, one day after inoculation, X 896)

Figure 41. An area consisting of very few dead, disorganized cells (second leaf of C.I. 7233, five days after inoculation, X 480)

Figure 42. An area consisting of very few dead, disorganized cells (second leaf of C.I. 7233, two days after inoculation, X 480)

Figure 43. A large area containing impoverished cells and dead shrivelled mycelium (m) (second leaf of C.I. 7233, nine days after inoculation, X 896)

Figure 44. A small bit of mycelium (m) persisting in the host tissue without affecting host cells (second leaf of C.I. 7233, ten days after inoculation X 480)
DISCUSSION

The strains of oats and the different leaves of individual plants studied in this investigation can be classified as to the degree of susceptibility shown. Clinton was the most susceptible followed by the upper leaves of C.I. 7181, lower leaves of C.I. 7181, upper leaves of C.I. 7233, and lower leaves of C.I. 7233, in order of increasing resistance.

On Clinton haustoria were initiated by the second day after inoculation. The mycelium became well established and there was little recognizable effect on cells penetrated by haustoria until very late in the course of infection. The fungus grew rapidly and sporulated in about 8 days. There were a few very small areas of dead cells on Clinton indicating small areas where some maladjustment of fungus to host occurred.

On the upper leaves of C.I. 7181 the development of the fungus after the first 24 hours was 1 or 2 days slower than on Clinton. Spread of hyphae in the tissues was a little slower and uredia did not develop until about the tenth day after inoculation. There were some indications of a less-balanced interaction between haustoria and host cells in the form of slight clumping of cytoplasm around the haustoria. There were, also, larger, more numerous areas of dead cells where a completely antagonistic relationship was manifested between host and parasite.

A much different pattern of development was found on the lower leaves of C.I. 7181. As on Clinton and the upper leaves of C.I. 7181, entrance was gained and infection hyphae were produced by the end of the first 24
hours. No haustoria were initiated until 7 days after inoculation. The fungus grew very slowly in the tissues of the host without causing any recognizable damage to the host. It appeared as though some sort of inhibition was present. On the seventh day the fungus became more aggressive, initiating haustoria in the mesophyll cells as though the inhibitory effect had been overcome. The presence of haustoria in a host cell induced one of two effects on the cell. Either the chloroplasts were markedly reduced in size giving the cell an impoverished appearance, or the protoplasm massed around the haustorium in an irregular, heavy-staining mass. Despite the deleterious effects on the host cells the fungus evidently obtained enough nourishment to grow rapidly and spread through rather large areas of the leaf. In a few of these areas small uredia developed. They were surrounded by dead, collapsed cells. This arrangement of dead cells around a uredium produces the macroscopically visible type 2 reaction. In other areas host cells and mycelium died without sporulating, thus giving rise to necrotic flecks.

These inhibitory and antagonistic interactions in the lower, older leaves of C.I. 7181 presumably are the basis for "field resistance." In the upper Mississippi valley significant natural infection by urediospores carried by the wind from the south usually does not occur until after most of the leaves of C.I. 7181 are old enough to be resistant.

Previous investigations of resistance to *P. coronata* (Ruttle and Fraser, 1927; Rothman, 1960) had shown that the interactions responsible for resistance were apparently due to physiological incompatibility between host and parasite. In the present study, attempts to detect some
type of morphological basis for the resistance of C.I. 7181 were unsuccessful, indicating that the resistance of C.I. 7181 also is physiological rather than morphological in nature.

The range of types of host-parasite interactions on C.I. 7233 were the same on both the upper and lower leaves, but the lower leaves showed a higher proportion of highly antagonistic reactions. Only one haustorium was noted in all collections taken from lower leaves. Mesophyll cells died merely from the touch of the hyphae and sometimes even in advance of any mycelium. A number of haustoria were formed in cells of the upper leaves. All mesophyll cells invaded by haustoria died rapidly.

In contrast to the lower leaves of C.I. 7181, C.I. 7233 showed no extended period during which the mycelium grew slowly without forming haustoria or damaging host cells. Some mycelium persisted in the tissue of C.I. 7233 without causing any noticeable effect but in the great majority of infections observed host cells and hyphae died early on contact or with formation of haustoria by the fungus. This was especially true on the lower leaves. The fungus was never able to establish a heavy mycelial network within the leaf although some mycelium persisted up to 10 days.

Sometimes the antagonistic interaction was so rapid that the fungus did not successfully gain entrance into the leaf. A few cells and the appressorium or substomatal vesicle were killed and no further development followed.

No new knowledge was gained from this investigation concerning the physiology of the interaction between host and parasite. However, certain
points, apparently overlooked in previous studies, are worthy of consideration.

The fungus was able to live and grow slowly in the leaf tissue without formation of haustoria. It has generally been felt that haustoria are essential to obtaining nutrients needed for growth. However, Rice (1927) noted that the corn rust fungus was able to grow in the corn plant without forming haustoria. Other investigators (Rothman, 1960; Ruttle and Fraser, 1927; Allen, 1923b) have described runners, i.e.: long, unbranched hyphae, which grew considerable distances in the leaf without forming haustoria and without branching. They were thought to be drawing nourishment through haustoria in other parts of the mycelium. Such was not the case with certain hyphae noted in the present study. They were found in leaves of resistant varieties in which no successful haustoria occurred. Apparently the fungus is able to obtain nourishment from the host in some way other than through haustoria. Ward (1905) hypothesized that the mycelium in a resistant variety dies from starvation. However, the fact that the fungus can obtain nutrients and grow in resistant tissues without forming successful haustoria, suggests that death of the mycelium may be due to some toxic substance. Nevertheless, functional haustoria are necessary for the fungus to obtain sufficient nutrients to sporulate.

Litzenberger (1949) found a toxic substance in Victoria oats infected with P. coronata which killed mesophyll cells of oat leaves. He felt the fungus released this toxin at the time of sporulation. The death of a few host cells, the appressorium, and the substomatal vesicle on C.I. 7233
without formation of any infection hyphae strongly suggests that death of host cells and fungus may be due to a toxin. Cells could also be affected in differing degrees at some distance from areas where mycelium was present in the mesophyll. This further indicates the presence of diffusible toxic substances produced at the infection site.

Despite numerous instances of damage or death of cells of C.I. 7233 in advance of or on contact with mycelium, hyphae sometimes grew past mesophyll cells without any effect. The fungus passed one or two cells then killed the next one contacted. This same thing was noted by Stakman (1915) on wheat infected with *P. graminis tritici*. On the other hand, some cells in leaves of Clinton were killed and had the same appearance as cells killed in C.I. 7233. In Clinton these cells were surrounded by almost normal cells containing fully expanded haustoria. It, therefore, appears that resistance may be due to the reaction of individual cells or small groups of cells to infection. Some cells die rapidly when the fungus or a toxin secreted by it contacts them. Others are not affected. The difference in resistance in different varieties might then be due to the proportion of hypersensitive cells to cells that do not react.

A further indication of the cellular basis of resistance was noted in one particular infection observed. The hyphae originating from one end of the substomatal vesicle killed a number of cells and were dying themselves. Hyphae from the other end had grown through about the same amount of mesophyll tissue with little or no effect. The local nature of resistance was noted by Gibson (1904). She grafted susceptible shoots
of chrysanthemum in resistant plants without imparting resistance to the susceptible shoots. Roberts and Moore (1956) also demonstrated local effects on resistance. They used a variety of oats in which resistance to *P. graminis avenae* breaks down at high temperatures. They found that if different ends of resistant leaves were maintained at 75°F and 85°F, that resistance broke down on the parts maintained at 85°F with no effect on resistance in the parts held at 75°F.

The observation that older leaves of C.I. 7233 exhibited a higher degree of resistance than younger leaves was unexpected. Macroscopically visible differences in resistance were not readily discernible. Microscopically, death was more rapid on the older leaves and resulted in smaller numbers of cells being involved. This observation suggests the possibility that all oat varieties exhibit higher resistance on older leaves than on younger leaves. On strains such as C.I. 7181 this difference is sufficiently great to be readily visible. However, further observations on a wider range of varieties are necessary before any conclusion can be reached on this hypothesis.
An investigation was made of the microscopic development of race 2614 of the crown rust fungus on two strains of oats, C.I. 7181 and C.I. 7233, which show promise of being useful as sources of resistance in oat breeding programs. Clinton was used as a susceptible check variety. C.I. 7181 is a hexaploid strain which is susceptible in the seedling stage but becomes resistant as the leaves grow older. C.I. 7233 is a tetraploid strain which is resistant in both seedling and later stages of growth.

Clinton and the upper and lower leaves of C.I. 7181 and C.I. 7233 could be ranked in order of decreasing susceptibility. Clinton was the most susceptible followed by the upper leaves of C.I. 7181, lower leaves of C.I. 7181, upper leaves of C.I. 7233, and lower leaves of C.I. 7233. Clinton was highly susceptible; consequently the fungus generally caused little or no visible damage to host cells in the invaded area. There were, however, a few small areas in which host cells were killed, as they were on resistant plants.

Development on the upper leaves of C.I. 7181 was a little slower than on Clinton and there were more areas in which an antagonistic reaction occurred. Otherwise, the upper leaves of C.I. 7181 appeared to be completely susceptible.

On the lower leaves of C.I. 7181, growth of the fungus was very slow up to 7 days after inoculation, and there was no recognizable damage to the host. At 7 days the fungus began forming haustoria in host cells causing considerable damage to the cells invaded. Later, a few type 2
pustules, surrounded by necrotic areas, appeared.

On C.I. 7233 the types of interactions were the same on both upper and lower leaves, but the lower leaves showed a higher proportion of highly antagonistic reactions. The most common type of interaction was rapid death of a few mesophyll cells accompanied by death of the fungus. In some cases scattered hyphae persisted in host tissue up to 10 days after inoculation. This was more common on the upper than on the lower leaves.

Although no information was obtained on the intimate biochemical relationship between host and parasite, some observations were made which may help in our interpretation of host-parasite interactions.

1. Some diffusible toxic material was apparently produced by the fungus which caused death of mesophyll cells not in contact with the fungus.

2. Resistance appeared to be due to the reaction of localized cells or groups of cells.

3. There was a difference in degree of resistance exhibited by younger and older leaves. This difference was most pronounced on C.I. 7181.

4. Apparently the fungus was able to obtain sufficient nutrients from resistant strains of the host to maintain slow growth without the formation of haustoria. However, no sporulation was observed without haustoria being formed.
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