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A MICROSCOPIC STUDY OF THE WHEAT-POWDERY MILDEW RELATIONSHIP AFTER APPLICATION OF THE SYSTEMIC COMPOUNDS PROCAINE, GRISEOFULVIN AND 6-AZAURACIL[1]

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On wheat seedlings systemically protected by root application of procaine-hydrochloride, griseofulvin or 6-azauracil, germination of oidia of *Erysiphe graminis* f. sp. *tritici* and penetration of this fungus into the epidermal cell wall was as high as on control plants. Inhibition of powdery mildew development became apparent only after the penetration process had started. About 85% of the infections were halted within 24 hours after the inoculation, and did not result in the formation of a haustorium. In the other cases usually not more than one haustorium per infection court was formed, which often showed anomalies, characteristic for each compound used. Development of mycelium was scanty or absent and no sporulation occurred. Similarly, on plants of a resistant wheat variety, powdery mildew inhibition became apparent only after penetration of the host had started. There was no development of mycelium or sporulation. A severe reaction of certain epidermal cells to penetration by powdery mildew was observed on resistant as well as on treated and untreated susceptible plants. However, in relation to the total number of infections, the percentage of this type of reaction was low.

INTRODUCTION

As early as the beginning of this century SALMON (1905) reported on a microscopical study of the infection process of a powdery mildew fungus on a non-congenial host plant, namely *Erysiphe graminis* DC. ex Mérat f. sp. *tritici* Em. Marchal on barley. He concluded that the resistance of barley to wheat powdery mildew was "not due to the failure of the germination tube of the oidia to penetrate the leaf cells of the plant, but to the inability of the fungus to develop further the incipient haustorium which is formed, or to the incapacity of the fully formed haustorium to adapt itself to the intracellular conditions". He called this phenomenon "subinfection". A rather similar behaviour of *E. graminis* f. sp. *tritici* on resistant wheat varieties was reported by CORNER (1935), who studied the infection process by microscopical examination of the epidermis, stripped from the underside of the leaf. On the highly resistant variety 'Norka', fungal growth stopped during the process of penetration or after formation of one or a few haustoria, and on the completely resistant variety 'Persian Black' no haustoria were formed at all.

LAST (BRIAN, 1952) found inhibition of barley powdery mildew after application of griseofulvin to the roots. Systemic activity against wheat powdery mildew has been reported for the compounds procaine-hydrochloride (DEKKER, 1961) and 6-azauracil (DEKKER, 1962; DEKKER & OORT, 1964). This paper

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reports a study of the infection process of wheat powdery mildew on plants treated with the three compounds mentioned. For comparison a resistant wheat variety was included in the experiments.

MATERIALS AND METHODS

The local anaesthetic procaine-hydrochloride was purchased from The British Drug Houses Ltd., England, and the antimetabolite 6-azauracil from Nutritional Biochemicals Corporation, Cleveland, Ohio, U.S.A. The antibiotic griseofulvin was kindly supplied by Dr. A. H. CAMPBELL, Glaxo Laboratories Ltd., England.

The wheat varieties used in our experiments were 'Leda' and 'Cleo', both susceptible to *E. graminis* f. sp. *tritici* and 'Riebesel', which shows natural resistance to this fungus. The seedlings were grown in flower pots containing steamed soil, in an isolated mildew-free part of the greenhouse. When the plants were large enough to be used for the experiments, they were transferred to the powdery mildew greenhouse. In this greenhouse a sunshade of cheesecloth was used during the summermonths to avoid too high temperatures. An average temperature of 19 $\pm$ 4 °C was maintained. From October to April additional light was given from 4 to 10 p.m.

The oidia to be used as inoculum were harvested from plants from which the old oidia had been removed 48 hours before by blowing. The plants to be inoculated were removed from the flower pots, and their roots rinsed with tap water. In order to distribute the oidia as equally as possible over the undersides of the leaves, the latter were stretched on hard board, ventral side up, and kept in place with cellophane during the inoculation. After dusting of the oidia on the leaf surface, the seedlings were placed with their roots in an aqueous solution of procaine-hydrochloride 550 ppm, griseofulvin 30 ppm, 6-azauracil 6 ppm or water. These concentrations were used in all experiments, since they were the lowest concentrations giving complete protection. At certain times after inoculation the epidermis was stripped from the underside of the leaves. The strips were stained with cotton blue in lactophenol, mounted in glycerin and examined microscopically.

GERMINATION AND PENETRATION

Susceptible untreated plants

Two weeks old seedlings of the varieties 'Leda' and 'Cleo' were inoculated and placed with their roots in water. Microscopical examination of the epidermis, stripped two and a half hours after inoculation, showed that some of the oidia had already started to germinate. After seven and a half hours the germ-tubes of these oidia had reached an average length equal to that of the oidia, and a secondary germ-tube could be observed. In the primary germ-tube or germination hypha a septum had formed between the oidium and the rest of the hypha, in which an appressorium-like thickening had formed.

In the following hours a small circular zone, stained by cotton blue, appeared around the tip of the germination hypha. In the centre of this a dark spot could be observed, which is the penetration hypha. The blue circular zone took the form of a halo, about 10–25 μ wide. The lignituber showed as a small refractive body in the centre of the halo, or, viewed from the side, as a conelike structure

(Fig. 1C). It was eventually pierced by the penetration hypha, after which formation of a haustorium started and the halo gradually disappeared. The formation of a germination hypha, appressorium and halo is illustrated in Figs. 1A and B, where the infection process has been arrested in an early stage by administration of procaine-hydrochloride to the plant.

Susceptible plants treated with procaine, griseofulvin and 6-azauracil

Two weeks old seedlings of the variety 'Cleo' were inoculated and placed with their roots in aqueous solutions of procaine-hydrochloride, griseofulvin or 6-azauracil. Twenty-four hours after inoculation the epidermis was stripped, stained and microscopically examined as indicated above. The numbers of ungerminated conidia, haloes without a haustorium and developing haustoria were counted on 20 epidermal strips per treatment, which is about 100 mm^2 surface area (Table 1).

TABLE 1. Development of powdery mildew on wheat seedlings treated with three systemic compounds. Epidermal strips (± 100 mm^2 per treatment) were made 24 hours after inoculation.

	Oidia not germinated	Haloes without a haustorium	Haustoria		Total number of infections
			Number	%	
Procaine-HCl. . .	131	2498	348	12.2	2846
Griseofulvin . . .	75	2461	344	12.3	2805
6-Azauracil. . . .	368	2678	467	14.9	3145
Water	118	1801	1514	45.7	3315

The table shows that the number of infections, as indicated by the formation of a halo or a young haustorium, was about equal on all treatments, the water-control included. The infection process, however, was more advanced on the control plants, as appears from the higher number of haustoria being formed. The number of ungerminated conidia was very small in comparison with the number of infection courts, indicating a high percentage of germination in all treatments, although some of the oidia may have been washed from the leaves during preparation of the epidermal strip. It may be concluded from these data that treatment of wheat seedlings with one of the systemic compounds mentioned did not inhibit the germination of the oidia or the initial stage of penetration.

Resistant plants

Since the natural resistance of the wheat variety 'Riebesel' was less pronounced on very young seedlings, also plants almost seven weeks old were used. As a control, plants of the same age of the susceptibele variety 'Leda' were used. The underepidermis was stripped 25 hours after inoculation and counts were made of the number of ungerminated oidia, haloes without a haustorium and haustoria. This experiment was carried out on a limited scale. There were no indications that the germination or the initial phase of penetration on the naturally resistant plants differed from those on the susceptible plants. The halo round the infection spot, however, seemed less pronounced on the 'Riebesel' plants.

Susceptible untreated plants

The haustorium, which is formed by the fungus after penetration of the lignituber, was at first more or less oval, without branches (Fig. 1C). Within 24 hours from the time of inoculation young haustoria in various stages of development were observed. After 48 hours full-grown haustoria (Fig. 2D) had formed. The germination hypha resumed its growth after completion of the primary haustorium and new penetrations occurred, followed by the formation of secondary haustoria. By the term "primary haustorium" is meant a haustorium, which is formed by the germination hypha of an oidium; the term "secondary haustoria" refers to haustoria formed later from the mycelium. From one oidium usually more than one germination hypha was formed. Three days after inoculation one primary haustorium and an average of 2.4 secondary haustoria were counted per infection court (Fig. 1F). Four days after inoculation the number of secondary haustoria per infection court had risen to 5.7 and the formation of sporophores was just starting. By that time almost all the haloes around the original penetration spots had disappeared, except in a few cases where penetration had not been followed by formation of a haustorium. The number of these unsuccessful infections on control plants was very low and estimated at only a few percent of all infections. On the fifth day after inoculation the first chains of oidia were observed.

Susceptible plants treated with procaine, griseofulvin and 6-azauracil

The observations were made on the same batch of plants as described under "Germination and penetration". The infection process was studied by counting daily the number of haloes without haustorium development, and the number of haustoria on about 100 mm² epidermal strip per treatment. Distinction was made between haustoria which had developed fingers, and haustoria which were still without branches. The latter might be called "abortive". Exact counts on epidermal strips of control plants could only be made at places where the superficial mycelium had been removed. On the basis of these counts an estimate was made of the total number of haustoria and haloes. On epidermal strips of the treated plants counts were easily made, since development of mycelium was scanty or absent. Table 2 gives the results of the observations on the fourth day after inoculation. The numbers of ungerminated conidia, being very small, have been omitted.

From Table 2 it appears that on the plants treated with one of the systemic

TABLE 2. The infection process of *E. graminis* f. sp. *tritici* on susceptible wheat seedlings treated with three systemic compounds. Epidermal strips (± 100 mm² per treatment) were made four days after inoculation.

	Haloes without a haustorium		Primary haustoria		Secondary haustoria	Total number of primary infections
			Unbranched	Branched		
Procaine-HCl	2075	81%	110	380	0	2565
Griseofulvin	3150	87%	245	233	0	3628
6-Azauracil	2206	84%	15	404	0	2625
Water	±150	±5%	0	±3000	±17000	3150

compounds a high percentage of penetrations did not result in the formation of a haustorium. Figs. 1A and B show this phenomenon on a procaine treated plant. It was noticeable that the haloes around penetration spots where no haustorium had been formed, persisted much longer than those around successful penetrations. Especially on griseofulvin-treated plants the abundant occurrence of haloes was very conspicuous, even seven days after inoculation, while on the control plants only a small number of persistent haloes were observed. On the control plants a number of secondary haustoria were formed (Fig. 1F), while none were visible on plants treated with procaine, griseofulvin or 6-azauracil (Fig. 1E). The primary haustoria that formed on the treated plants showed anomalies, which were often characteristic for the compound used. On the griseofulvin treated plants more than half of the haustoria remained abortive (Fig. 2A). The haustoria that did develop fingers were compact in appearance, the fingers being irregularly thickened and much shorter (Fig. 2B). On the 6-azauracil-treated plants most of the haustoria showed a reduction in size and number of fingers (Fig. 2C). In extreme cases only one finger was formed on each side of the haustorium. On the procaine-treated plants many haustoria showed anomalies. Severely affected haustoria were abortive, without branches or internal structure and with a rough surface (Fig. 3A). Less severe anomalies consisted of a reduction in size of the haustorium or the appearance of a balloon-like sheath (Fig. 3B), which is often barely visible round a haustorium on an untreated plant. From the more or less normal looking haustoria some mycelium developed, but subsequent penetrations were impeded (Fig. 1D). Sporulation was seldom observed.

Resistant plants

The observations were made on the same plants, as described under "Germination and penetration". Since it became increasingly difficult to strip the epidermis from resistant 'Riebesel' plants, inoculated more than three days previously, observations were made on whole leaves which had been left for some time in the fixation fluid (formaldehyde-propionic acid-alcohol 5:5:90v/v). On the seven weeks old plants almost no fully developed haustoria were formed. When a haustorium did form, it often appeared significantly different from that on susceptible 'Leda' plants. On 'Riebesel' plants a balloon-like swelling of the sheath around the haustorium was a common phenomenon (Fig. 3D). This occurred even in an early stage of haustorial formation, when fingers were not yet formed (Fig. 3C). In one experiment, with three weeks old seedlings, in which the leaves were examined seven days after inoculation, 136 out of 202 haustoria exhibited anomalies. Of these 136, 110 showed abnormal development of the sheath. Abortive haustoria, without branches, were also observed. No development of mycelium occurred.

REACTION OF THE HOST CELLS

As has been described earlier, the epidermal cell reacts to penetration by the powdery mildew fungus with formation of a lignituber and a halo. Around the lignituber and the haustorium the cell contents often showed a somewhat granular appearance. In some epidermal cells, however, a much stronger reaction to infection by the fungus occurred. The cell contents as well as the cell wall of such cells were intensely stained by cotton blue in lactophenol and very conspicuous between the other uncoloured epidermal cells. A few cells reacting in this way were observed within 24 hours of inoculation, but most of them became visible in the following days. This type of reaction started at the point of penetration. The granular appearance usually spread over the whole cell, but not to neighbouring epidermal cells (Fig. 4A). The cell wall, also, was heavily stained. Development of the fungus in such a heavily reacting cell seemed inhibited, formation of a haustorium being very rare. Usually, also, a reaction of the mesophyll cells immediately below the invaded epidermal cell was observed. After stripping the epidermis, prints of these mesophyll cells remained visible on the wall of the epidermal cell (Fig. 4B, C). The number of cells reacting to penetration by the fungus in the way described, was rather low. Counts were made on two weeks old 'Cleo' plants, four days after inoculation. The number of heavily reacting cells in an epidermal area of 100 mm^2 with about 3,000 infections was 61 on the control plants, 65 on procaine-, 159 on griseofulvin- and 90 on 6-azauracil-treated plants. It seems that griseofulvin enlarged the number of reacting epidermal cells somewhat. It is, however, obvious that even in this case the total number of reacting cells was low in comparison with the large number of infections. It may therefore be concluded that this heavy reaction type did not play a significant role in the inhibition of powdery mildew on plants treated with one of the three compounds mentioned. It is not clear why a small percentage of the epidermal cells reacted so heavily to attack by powdery mildew in contrast with the majority of the invaded cells.

For study of the host-cell reactions to powdery mildew on resistant plants, seven weeks old seedlings of the resistant 'Riebesel' and the susceptible 'Cleo' varieties were used. On both varieties reactions of the invaded epidermal cells and underlying mesophyll cells analogous to those described above, were observed (Fig. 4D, E, F). A difference between the resistant and the susceptible variety was the fact, mentioned earlier, that some time after inoculation it became increasingly difficult to strip the epidermis from the resistant plants. Apparently the cells invaded by powdery mildew stick more heavily to the underlying mesophyll cells, so that the strips become easily ruptured. Therefore, from the third day after inoculation, observations were made on whole leaves. The appearance of some granular material round the lignituber was more common on the resistant than on the susceptible plants. However, the percentage of heavy whole-cell reactions on resistant plants was small in relation to the number of infections, and not significantly different from that on susceptible plants. It appeared to be higher on older plants than on young seedlings. In some cases, also, a thickening of the epidermal cell wall was observed (Fig. 1F).

DISCUSSION

Mildew susceptible wheat plants treated with one of the systemic compounds procaine-hydrochloride, griseofulvin or 6-azauracil, showed a "highly resistant" reaction to powdery mildew. The majority of the infections were already halted within 24 hours of inoculation and before a haustorium had been formed. In the remaining cases only one, often abnormal, haustorium was formed almost without subsequent development of mycelium. The infection picture was similar in the case of seven weeks' old plants of the resistant wheat variety 'Riebesel'.

On younger plants of this variety the resistance to powdery mildew was less complete. The anomalies of the haustoria were characteristic for each compound used and for the naturally resistant plants. This might indicate that the modes of action on the powdery mildew are different in all four cases.

During the first days after inoculation some epidermal cells reacted much more severely than others to attack by powdery mildew. Growth of the fungus itself was inhibited in cells of this type, and usually no haustorium was formed. Since this phenomenon occurred only in a low percentage of the infected epidermal cells, but in all treatments, the control included, it offers no explanation for the inhibition of mildew development on plants treated with a systemic compound or on resistant plants. Apparently even on control plants a limited number of infections do not result in formation of a haustorium. In none of the cases studied was there any indication that powdery mildew inhibition was to be ascribed to necrosis or collapse of the epidermal host cell at the spot of penetration.

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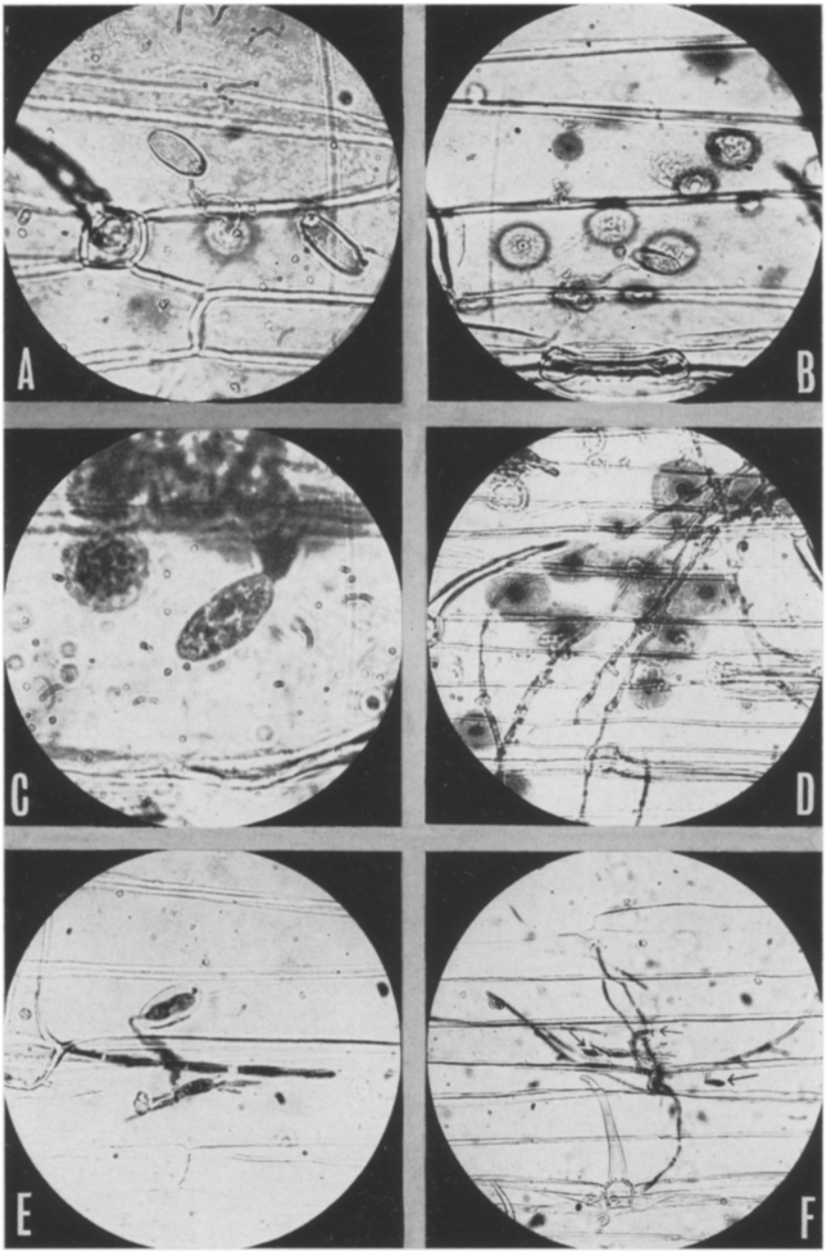

FIG. 1A, B. Unsuccessful infections by powdery mildew of wheat plants, var. 'Leda', treated with procaine-hydrochloride, × 450.

A. Germinated oidium with appressorium six days after inoculation; a halo but no haustorium has been formed.

B. Haloes without a haustorium, four days after inoculation; in centre of halo the lignituber with the penetration hypha (black dot).

FIG. 1C. Lignituber with young, still unbranched haustorium in epidermis of susceptible plant, var. 'Leda', 20 hours after inoculation, × 900.

FIG. 1D. Unsuccessful penetrations by powdery mildew on susceptible plants, var. 'Leda', treated with procaine-hydrochloride; numerous halo's without haustoria, 8 days after inoculation, × 340.

FIG. 1E, F. Development of powdery mildew on susceptible plants, var. 'Cleo', three days after inoculation. E. Only one haustorium and little mycelium has formed on a 6-azauracil-treated plant, × 675. F. One primary haustorium, three secondary haustoria (see arrows) and more mycelium have formed on control plant, × 340.

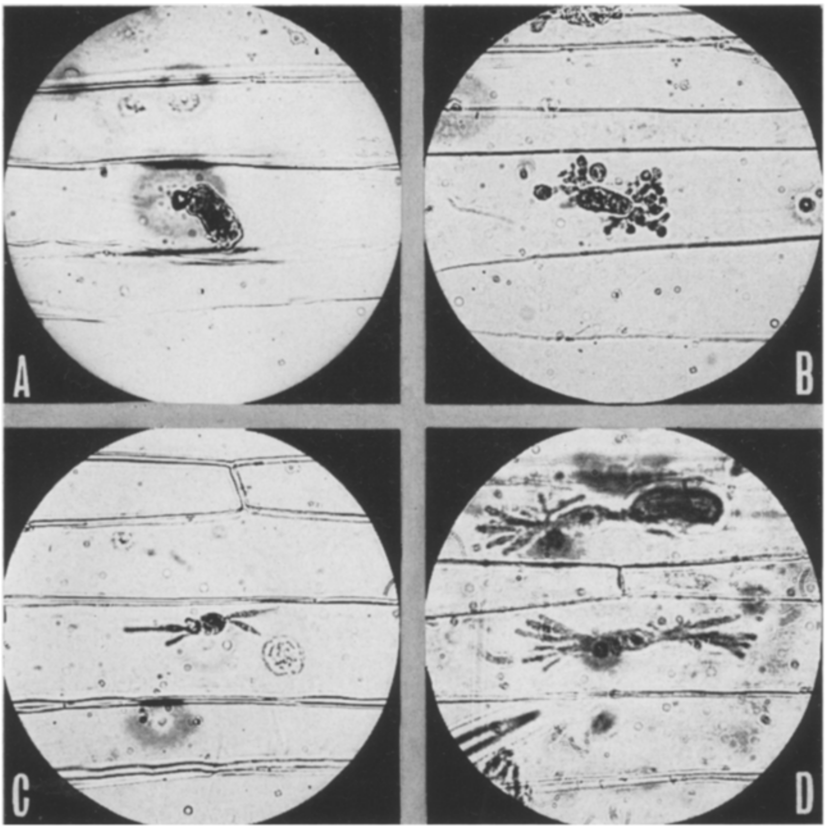

FIG. 2A, B. Formation of haustoria on griseofulvin treated susceptible wheat plants, var. 'Cleo', seven days after inoculation, × 675. A. Abortive haustorium without fingers. B. Abnormally formed haustorium with fingers.

FIG. 2C. Abnormal haustorium on a 6-azauracil treated susceptible plant, var. 'Cleo', seven days after inoculation, × 675.

FIG. 2D. Normal haustoria on untreated plant, var. 'Leda', 62 hours after inoculation, × 675.

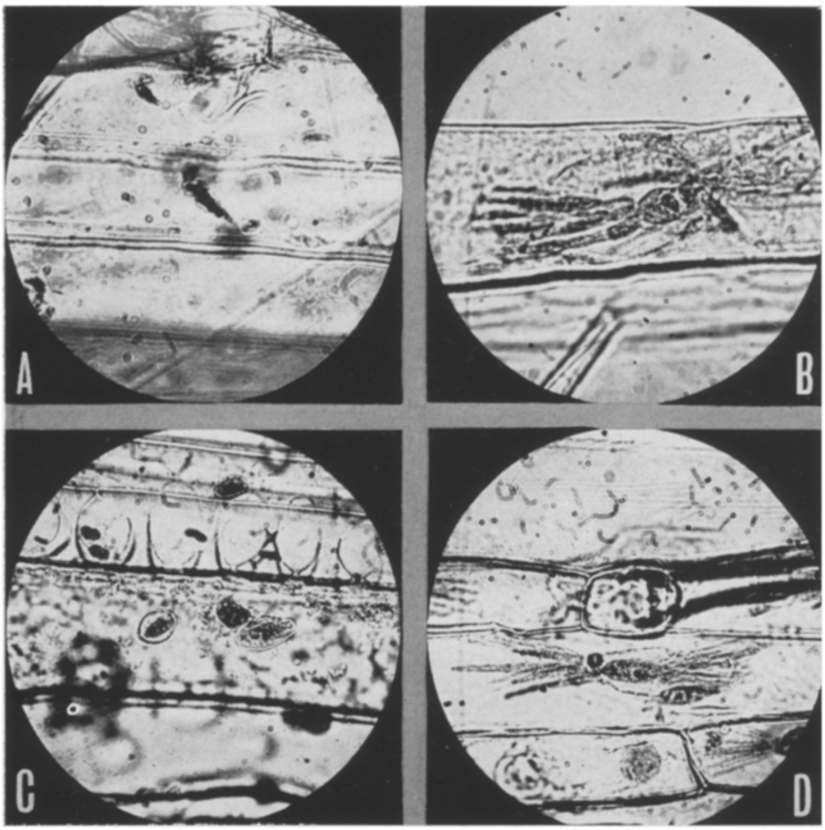

Fig. 3A, B. Formation of haustoria on wheat plants, var. 'Leda', treated with procaine-hydrochloride. A. Abortive haustorium, 62 hours after inoculation, × 675. B. Haustorium with a balloon like sheath nine days after inoculation, × 900.

Fig. 3C, D. Formation of haustoria on plants of the resistant wheat variety 'Riebesel'. C. Swollen sheaths around young haustoria without fingers, two days after inoculation, × 675. D. Swollen sheath around full-grown haustorium, seven days after inoculation of three weeks old seedlings, × 675.

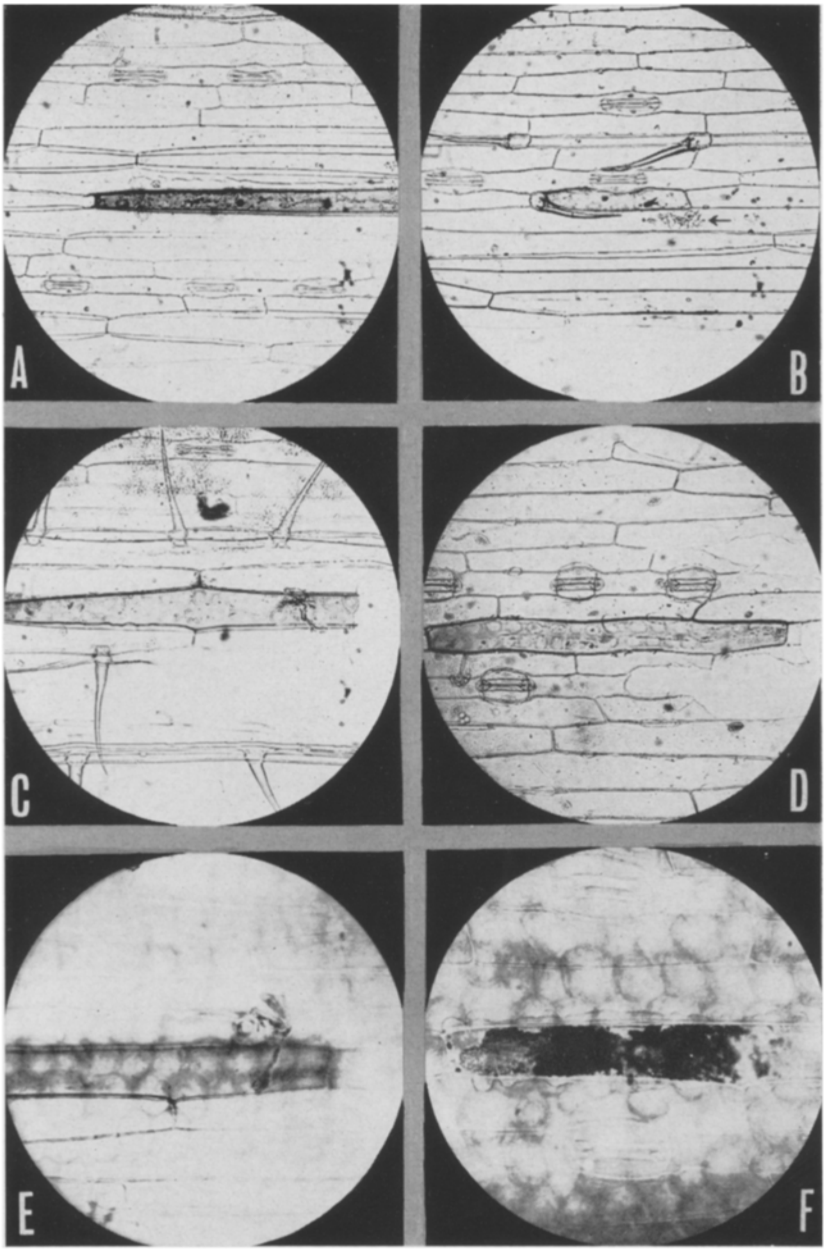

FIG. 4A, B, C. Reaction of epidermal cell to attack by powdery mildew on griseofulvin treated susceptible wheat plants, var. 'Leda', × 150. A. Invaded cell heavily stained by cotton blue, five days after inoculation. B. Branched haustorium in a non-reacting epidermal cell (see arrow) next to an abortive haustorium in a cell, which has reacted severely to invasion by the fungus. C. Prints of mesophyll cells visible on the underside of a stripped epidermal cell, which has reacted to invasion by the fungus.

FIG. 4D, E, F. Reaction of epidermal cell to attack by powdery mildew on plants of the resistant variety 'Riebesel'. D. Prints of mesophyll cells visible on underside of invaded cell, two days after inoculation, × 150. E. Cell contents stained, three days after inoculation; whole leaf cleared, × 340. F. Cell contents have become granular, cell wall has irregularly thickened, ten days after inoculation; whole leaf cleared, × 340.