Penetration of Maize Leaves by Helminthosporium turcicum

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ABSTRACT

Penetration of maize leaves by *Helminthosporium turcicum* was studied in trypan blue-stained whole leaf mounts. Penetration was direct and a fine infection hypha developed from the appressorium. The outer epidermal cell wall became thickened and the plasma membrane invaginated at the site of penetration. An intracellular vesicle was formed at the end of the infection hypha and stout colonization hyphae extended to adjacent cells. Initial subcuticular growth of the fungus and the development of more than one infection hypha from an appressorium were occasionally observed.

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Additional key words: Trichometasphaeria turcica, Zea mays.

Jennings and Ullstrup (5), and Hilu and Hooker (3, 4) studied the infection of maize leaves by *Helminthosporium turcicum* Pass. (*Trichometasphaeria turcica* Luttrell). Further details of maize leaf penetration were observed during a classroom study of the infection process. These are described.

Commercial yellow maize of unknown genetic origin was grown to the four-leaf stage in the greenhouse. Detached pieces of the three upper leaves were placed in petri dishes, with the cut ends placed between folds of moist cotton wool. Drops of spore suspension (with one drop of Tween 20 per 50 ml suspension) from a potato-dextrose agar culture of a South African maize isolate of *H. turcicum* were placed on the upper leaf surface. After 16-48 h in the dark at 26 C, leaf pieces were fixed and decolorized overnight in 3:1 (v/v) alcohol acetic acid, transferred through an alcohol series to water, and stained with trypan blue in lactophenol (9).

Acetic-alcohol fixation and trypan blue staining were suitable for demonstrating the fungus within host tissue. The ridges over the vascular bundles allowed oblique viewing of the leaf surface, therefore observation of certain stages normally seen only in microtome sections was possible.

Spore germination, germ tube growth, and appressorium formation were as previously described (3, 5, 7, 8). Stomata were occasionally penetrated; however, most penetration was directly through the cuticle and outer epidermal cell walls. It frequently occurred over the juncture of epidermal cell walls, but such development was not as common as reported by Jennings and Ullstrup (5). A blue-stained zone was observed around some infection sites (Fig. 11). It appeared to be located immediately beneath the cuticle or in the epidermal cell wall. Nearby lateral walls of the epidermal cells were also commonly stained (Fig. 11).

A fine infection hypha developed from the lower surface of the appressorium. It penetrated the cuticle and outer epidermal cell wall, which became thickened at the site of penetration (Fig. 1, 2, 5, 6). It invaginated the plasma membrane (Fig. 1, 2) and at this stage appeared to be surrounded by a sheath. It then penetrated the plasma membrane and formed a spherical intracytoplasmic vesicle. Hilu and Hooker (3) have also recorded vesicle formation during the early infection stages. There was some local granulation of cytoplasm (Fig. 3) but it was exceptional for sheaths to persist after vesicle formation (Fig. 4). Thick colonization hyphae then extended from the vesicle and granulation of the cytoplasm became more general (Fig. 5, 6, 7).

After developing in the primary infected cell, colonization hyphae penetrated adjacent cells. Narrow openings were formed in the intervening walls, with swelling of the hyphae both before and after penetration (Fig. 8). Further progress of the fungus in host tissue has been described (3, 4, 5), and it was not followed in this study.

There were occasional deviations from the above pattern of infection. After penetrating the cuticle, for instance, some infection hyphae developed subcuticularly as flat, branched, hand-shaped structures (Fig. 9) similar to those seen developing under cellophane in culture (6). The epidermal cell walls were eventually penetrated at one or more points and vesicles and colonization hyphae developed.

A further deviation was the formation of more than one infection hypha from an appressorium. Removal of germinated conidia from stained preparations left imprints of the appressorium with a central spot to mark the point of entry of the infection hypha (Fig. 10, 11). Occasionally two (Fig. 12) and in one case (Fig. 13) three points of entry from an appressorium were seen. An

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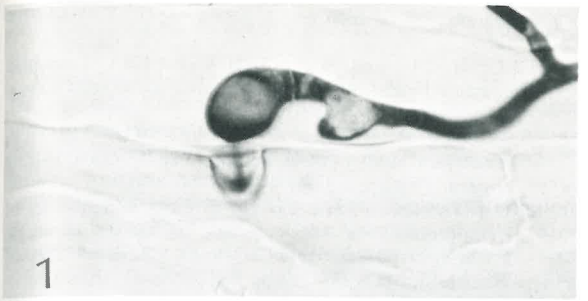
Fig. 1-13. Stages in the penetration of maize leaves by *Helminthosporium turcicum* (× 770, unless otherwise stated). **1)** Infection hypha from appressorium penetrating an epidermal cell, with thickening of the cell wall, invagination of the plasma membrane and sheath formation. **2)** Infection hypha from poorly-developed appressorium penetrating a subsidiary cell, also showing thickening of the cell wall, invagination of the plasma membrane and sheath formation (× 1250). **3)** Vesicle formed by infection hypha after penetrating the plasma membrane. Note granulation of cytoplasm around vesicle. **4)** Colonization hypha developing from the vesicle at the end of an infection hypha. **5)** Infected epidermal cell after vesicle formation and further colonization of the host, showing thickening of the wall at the infection site and granulation of the cytoplasm. **6)** Late infection stage showing thickening of the outer epidermal cell wall at the infection site, and granulation of the cytoplasm (× 1250). **7)** Infected epidermal cell with vesicle, colonization hyphae, and granulation of the cytoplasm. **8)** Extension of colonization hyphae to adjacent epidermal cells. **9)** Infection hypha developing subcuticularly before penetrating the epidermal cell wall. **10)** Impression left after removal of an appressorium, with central spot marking point of entry of infection hypha. **11)** Deeply-stained lateral epidermal cell walls, and lightly-stained area in vicinity of developing infection hypha. **12)** Impression of appressorium with central spots indicating formation of two infection hyphae. **13)** Impression of appressorium with central spots indicating formation of three infection hyphae.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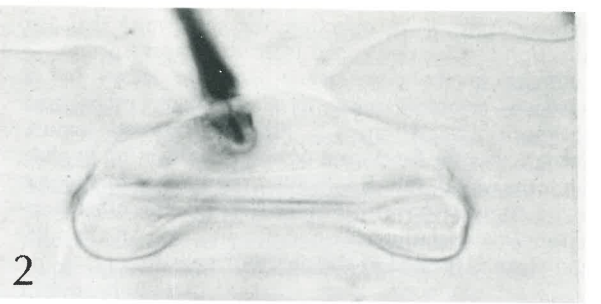
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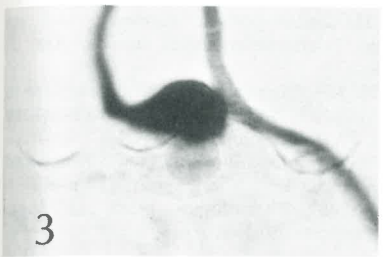
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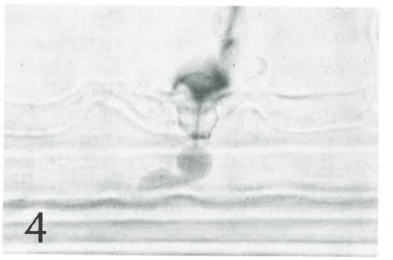
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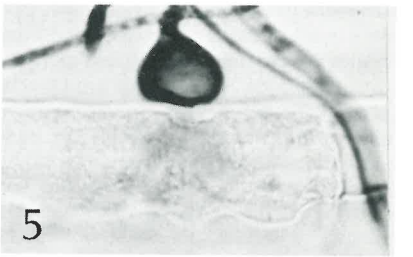
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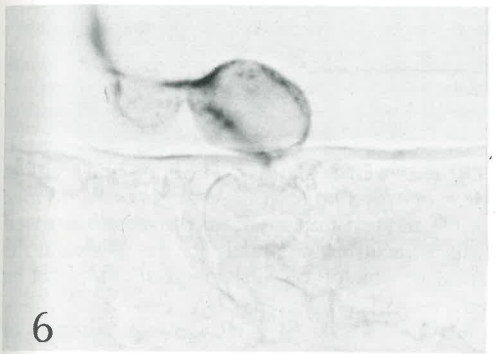
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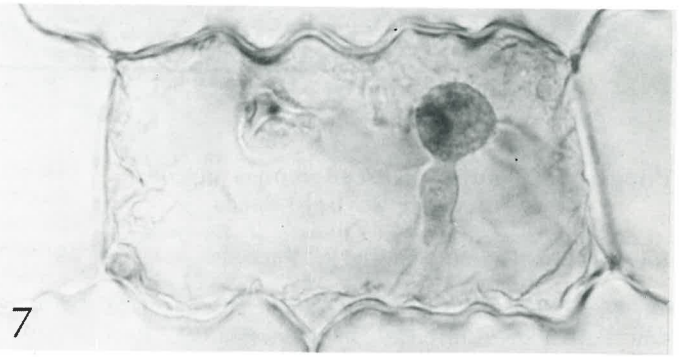
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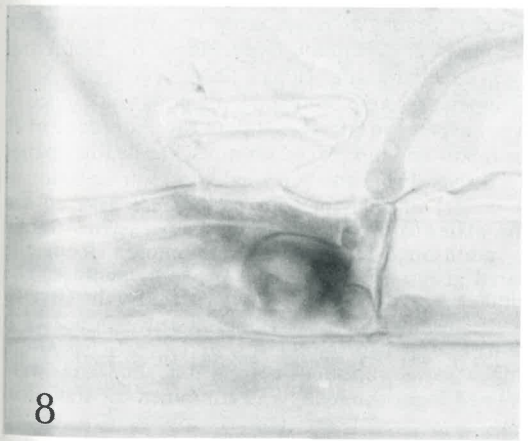
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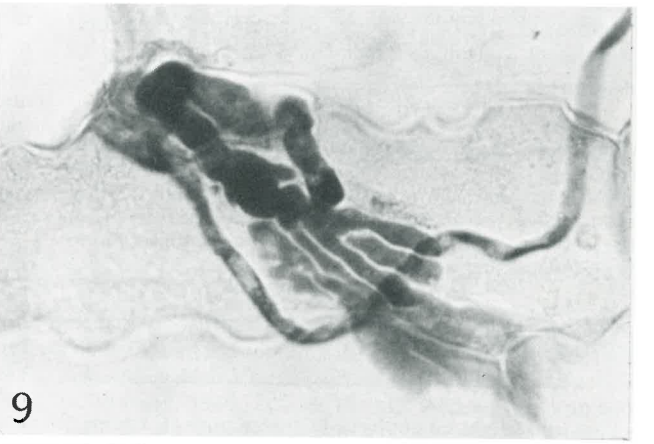
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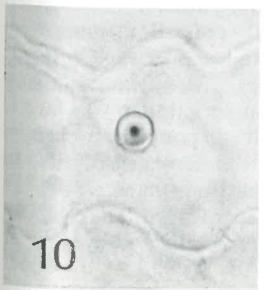
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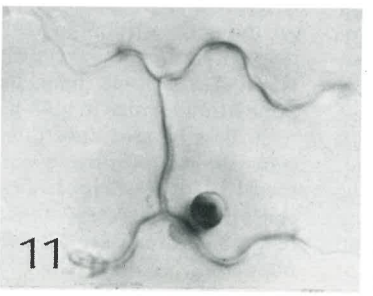
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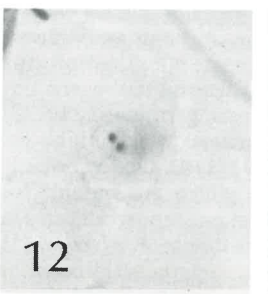
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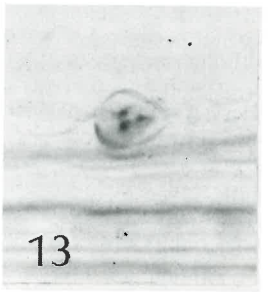
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oblique view of an infection site confirmed that two infection hyphae developed from one appressorium.

Stages in the penetration of maize leaves by *H. turcicum* resemble those in a variety of other host-pathogen associations (2, 10). An unusual feature, though, was the formation of multiple infection hyphae, while the well-developed sheaths around infection hyphae invite comparison with similar structures in other host-pathogen associations (2), and possibly also with papillae and sheaths formed at the necks of haustoria (1).

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