Sorghum Downy Mildew: Biology of Systemic Infection by Conidia and of a Resistant Response in Sorghum

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ABSTRACT

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Sorghum plants inoculated with conidia of *Peronosclerospora sorghi* were held under environmental conditions known to favor disease development. Hyphae from germinating conidia penetrated indirectly most successfully in shoot tissues. The fungus penetrated and established systemic infection only during early stages of seedling development. Resistance to sorghum downy mildew was manifested within 4 days after seeds of IS12661C and an IS12661 derivative imbibed water; whereas, the susceptible cultivar, TX412, remained susceptible for 7 days. A histological study revealed a hypersensitive-type reaction that restricted fungal development in resistant cultivars and was associated with the precocious formation of primary haustoria by the fungus in epidermal cells. Primary haustoria formed in mesophyll cells of susceptible cultivars and were associated both with unrestricted fungal development and, ultimately, either systemic symptoms or large local lesions. Histological studies provided evidence that resistance is manifested by different genotypes at different stages of the host-parasite interaction.

Sorghum downy mildew, which is caused by *Peronosclerospora sorghi* (Weston & Uppal) Shaw, is a destructive disease of sorghum and corn (5). *P. sorghi* perennially infects *Sorghum* spp. and *Zea mays* L. throughout the world and is characterized by distinct systemic and localized phases. Localized infections from conidia appear as stippled necrotic areas on leaves and may become systemic or remain localized (4). Systemic infection, the most destructive phase, may reduce sorghum yields as much as 50% (4). The disease is initiated by hyphae from germinating soil- or seedborne oospores or airborne conidia which infect seedlings. The first leaf to exhibit systemic symptoms may be chlorotic only on the basal half; whereas, later leaves have parallel stripes of chlorotic and white tissue which gives rise to the characteristic downy, white growth. The down which covers the undersides of the third or fourth leaves during cool and humid weather consists of fungal conidia and conidiophores.

The sites of foliar penetration by the conidial germ tubes which cause systemic infection have not been conclusively estabalished. Cosper (1) observed direct penetration by germ tubes with the production of appressoria at the junctions of epidermal cells. Jones (7) noted irregularly shaped swellings at or near epidermal cell junctions, but did not observe direct penetration in leaves of seedlings at the two- to three-leaf stage; however, he later reported that plants newly emerging from the soil were penetrated directly by conidial germ tubes at the soil surface (8). Only stomatal penetration was observed by Safeeulla (12). Although foliar infection by conidial germ tubes is known to cause systemic symptoms in sorghum, the process has not been sequentially described.

Sorghum downy mildew (SDM) is most effectively controlled in the United States by using the resistant commercial hybrids first made available in 1972 (3). Although some sorghums acquire resistance as they age (9,14), little is known of the nature or mechanism of resistance. Whether known sources of resistance restrict penetration and/or colonization and subsequent growth by the pathogen has not been established.

The purposes of this study were: to determine the site of foliar penetration which results in systemic infection following conidial germination; to follow postpenetration mycelial development in susceptible seedlings, and to compare and contrast host penetration and postpenetration fungal development in susceptible seedlings and in cultivars exhibiting field resistance.

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MATERIALS AND METHODS

Six grain sorghum cultivars, five resistant and one susceptible (TX412, a Feterita/Milo derivative), were used. The resistant cultivars were: four zerazera selections from the U. S. Department of Agriculture-Texas Agricultural Experiment Station Sorghum Conversion Program (an IS12661 derivative, IS12661C, IS2816C, and IS12610C) and a commercial hybrid (NK266). Seedlings were grown in a greenhouse maintained at 20–30 C before and after inoculation.

During the years 1971–1978, the six sorghum cultivars were observed under field conditions in disease nurseries in southern Texas in Bee, Nueces, and Jackson Counties. The cultivars were grown in replicated plots with a total of from 300 to 3,000 plants of each cultivar examined. The incidence of naturally occurring systemic SDM was noted.

Seedlings were inoculated with conidia as described by Craig (2) in a dark chamber at 20 C and 100% RH. Conidial inoculum was produced on 12-day-old, systemically infected TX412 plants and dispersed by a compressed air system onto test plants. Conidial release from infected leaves began 6–8 hr after the leaves were placed in the controlled environmental chamber and continued 2–4 hr.

To determine the primary site of infection and to compare the susceptible and resistant reactions, 3-day-old seedlings of the six cultivars were individually inoculated with conidia placed either on plumules, hypocotyls, or radicles by carefully covering all but the aforementioned parts with aluminum foil. Seeds were germinated in moist filter paper placed in a vertical position in order to correctly orient the plant parts. Selected seedling plumules were then threaded through small holes in the foil exposing the plumules. The hypocotyl (which is located between the primary root and the coleoptile) and radicles were similarly exposed to conidia by Craig's method (2). Fifteen replicates of each plant part were inoculated. After inoculation, seedlings were planted in sterile potting soil and grown for 3 wk in the greenhouse, at which time, the number of plants with systemic symptoms was recorded. For histological comparisons, representative resistant and susceptible leaf and stem samples were collected beginning 12 hr after commencement of the inoculation period and fixed in formalin-acetic acid-alcohol for 4 hr. Dehydration through a tertiary butyl alcohol series and embedding in paraffin was according to the method of Jensen (6). Sections 15 μm thick were affixed to glass slides, stained in a safranin-fast green series, mounted in Permount, and observed with a light microscope. A scanning electron microscopic technique was adapted for study of conidial germination and penetration. Representative leaf samples were collected 12 hr after inoculation and fixed in a 2.5% buffered glutaraldehyde solution for 1 hr. Dehydration through an ethanol series, drying in liquid CO_2, mounting, and gold-palladium coating was by the method of Royle and Thomas (11). Samples were examined with a JEOL JSM-U3 scanning electron microscope.

To follow mycelial development, 7-day-old seedlings of the susceptible cultivar were inoculated and grown for 4, 5, and 7 more days. At the end of each period, a representataive sample of three seedlings was prepared and observed with a light microscope as described above.

RESULTS

In four of the five resistant cultivars, systemic SDM infections initiated by hyphae from conidia were established in 3-day-old seedlings when only the plumules were inoculated (Fig. 1). Three of these resistant cultivars (IS12610C, IS2816C, and NK266) became systemically infected when only the hypocotyl was inoculated, but only IS12610C and the susceptible check, TX412, became systemically infected from inoculation of the radicle. These observations suggested that the young shoot was a primary site of infection and the likely site of the resistant response observed in the localized phase. Kenneth and Shahor (10), in similar inoculation experiments with maize seedlings, found that the pathogen primarily entered the aboveground parts of plants. Conidia, although primarily foliage-infecting propagules, were capable of direct penetration through the radicle in our tests.

The sequential processes of conidial germination, appressorial formation, stomatal penetration, and vesicle formation were similar in susceptible and resistant sorghum cultivars. Twelve hours after commencement of the inoculation period, conidia had germinated (Fig. 3 and 4) on the leaves of all cultivars. Germ tube swellings occurred at or near junctions of epidermal cells without evidence of penetration (Fig. 4). When germ tubes grew over stomata an appressorium was formed (Fig. 2), penetration followed, and a vesicle formed in the substomatal cavity (Fig. 6). Foliar penetration occurred only through stomata. No differences between susceptible and resistant cultivars were apparent during the first 48-hr period following inoculation. Haustoria-like structures were observed in the epidermal and mesophyll cells of both susceptible and resistant cultivars, but there were distinct differences in postpenetration mycelial development in resistant and susceptible cultivars. The susceptible cultivar had two fully expanded leaves and the third leaf growing out of the whorl at the time of inoculation. Three days after inoculation, a few local lesions developed on the first and second leaves. The number of local lesions increased on the 4th day, and small lesions developed on the third leaf. Examination of the cross-sectioned leaves at this stage revealed hyphae throughout the mesophyll tissue. As hyphae advanced, haustoria appeared in adjacent epidermal cells. In local lesion areas, host epidermal and mesophyll cells either darkened or shriveled and collapsed (Fig. 7). Few hyphae appeared to be restricted and the fungus continued to grow and spread into mesophyll cells. Aggregations of hyphae were observed in substomatal cavities prior to sporulation, but hyphae did not invade vascular bundles.

Five days after inoculation the basal half of the fourth leaf became chlorotic. Examination of longitudinally sectioned shoots between the leaf collar and crown showed hyphae growing intervenously downward in leaves to the basal end and through leaf sheaths. Hyphae were observed growing intercellularly both upward and downward at nodes. Growth progressed downward toward the base of the shoot and upward toward the shoot apex. Although hyphae grew near the base of the apical meristem (Fig. 9), colonization of meristematic tissue was not evident. Leaf initials were invaded, however, and new leaves emerged from the whorl with systemic symptoms. Infection hyphae in the vicinity of nodes and in developing new leaves produced haustoria in host cells. Hyphae terminated in conidiophores which emerged through stomata of the systemically infected plants incubated at high humidity (Fig. 5).

A restriction of hyphal growth in resistant cultivars became apparent 2 days after inoculation. Vesicles in substomatal cavities were smaller than those found in susceptible cultivars. Haustorium-like structures often formed immediately from the vesicles and expanded into epidermal cells (Fig. 8). Collapsed host cells appeared to restrict ingress (Fig. 6). Hyphal development stopped 3–4 days after inoculation. Host epidermal cells began to collapse at the site of infection and lysis of fungal vesicles and hyphae followed. In the moderately resistant cultivars, IS12610C

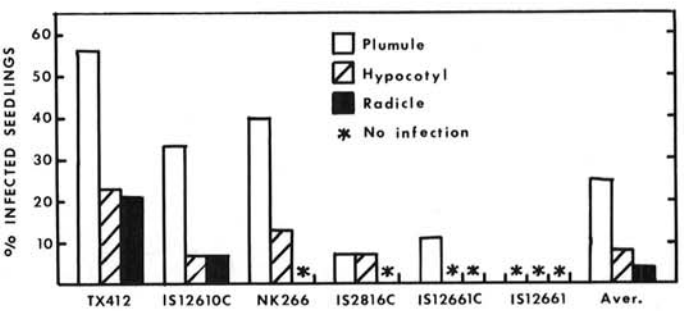

Fig. 1. Systemic infection in six grain sorghum cultivars inoculated with conidia of *Peronosclerospora sorghi* on plumules, hypocotyls, or radicles at 3 days of age. Asterisk (*) indicates no infection occurred.

and NK266, infection hyphae progressed to mesophyll cells where haustoria formed at the same rate as in the susceptible cultivar (Fig. 10). Shriveled and distorted mesophyll cells appeared to restrict fungal development in much the same manner as had the epidermal cells in the highly resistant IS12661C and the IS12661 derivative.

Lesion size in the host depended on the extent of mycelial invasion. Where vesicles and short hyphae invaded epidermal and mesophyll cells, relatively small lesions were observed. In contrast, larger lesions were associated with more extensive fungal invasions. Mycelium at these lesion sites appeared to be somehow restricted in resistant but not in susceptible cultivars.

We observed a good correlation with field data in the six cultivars for which rate of infection is shown in Fig. 11. The performance of IS12610C, for instance, suggests a possible vulnerability under field conditions since it remained susceptible (although at a low level) to artificial inoculation for at least 7 days.

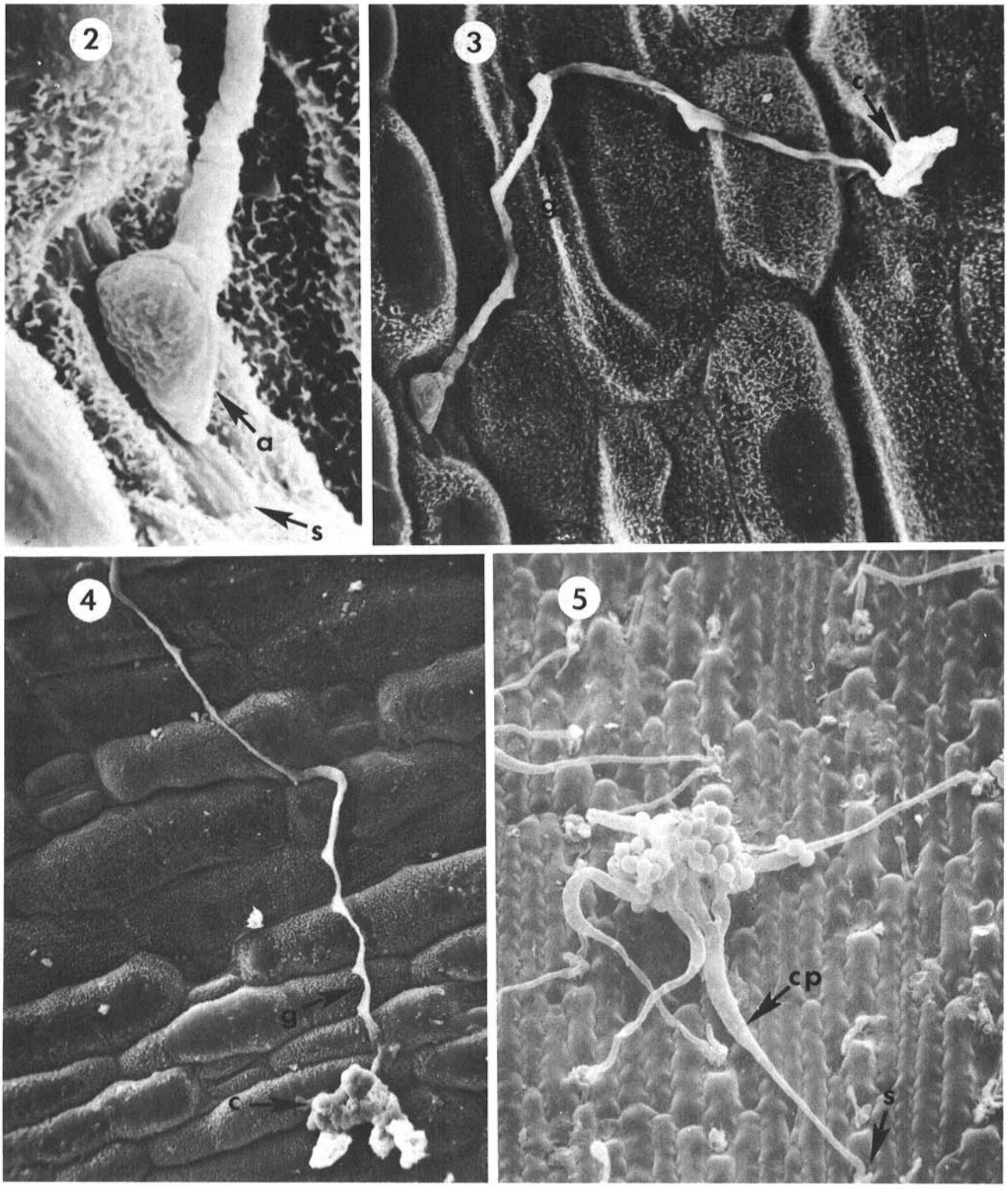

Fig. 2–5. *Peronosclerospora sorghi* on sorghum leaves. **2,** Enlargement of appressorium (a) over a in Fig. 3 (×2,500).**3,** Conidial germination and appressorium formed over stoma of IS2610C (×800). **4,** Conidial germination on TX412; note irregularly-shaped germ tube swellings over epidermal cell junctions (×600). **5,** Branched conidiophores emerging from stomata of systemically infected cultivar and terminating in conidia (Photo courtesy of A. Pedrosa). Abbreviations: a=appressorium, c=conidia, cp=conidiophore, g=germ tube, s=stoma.

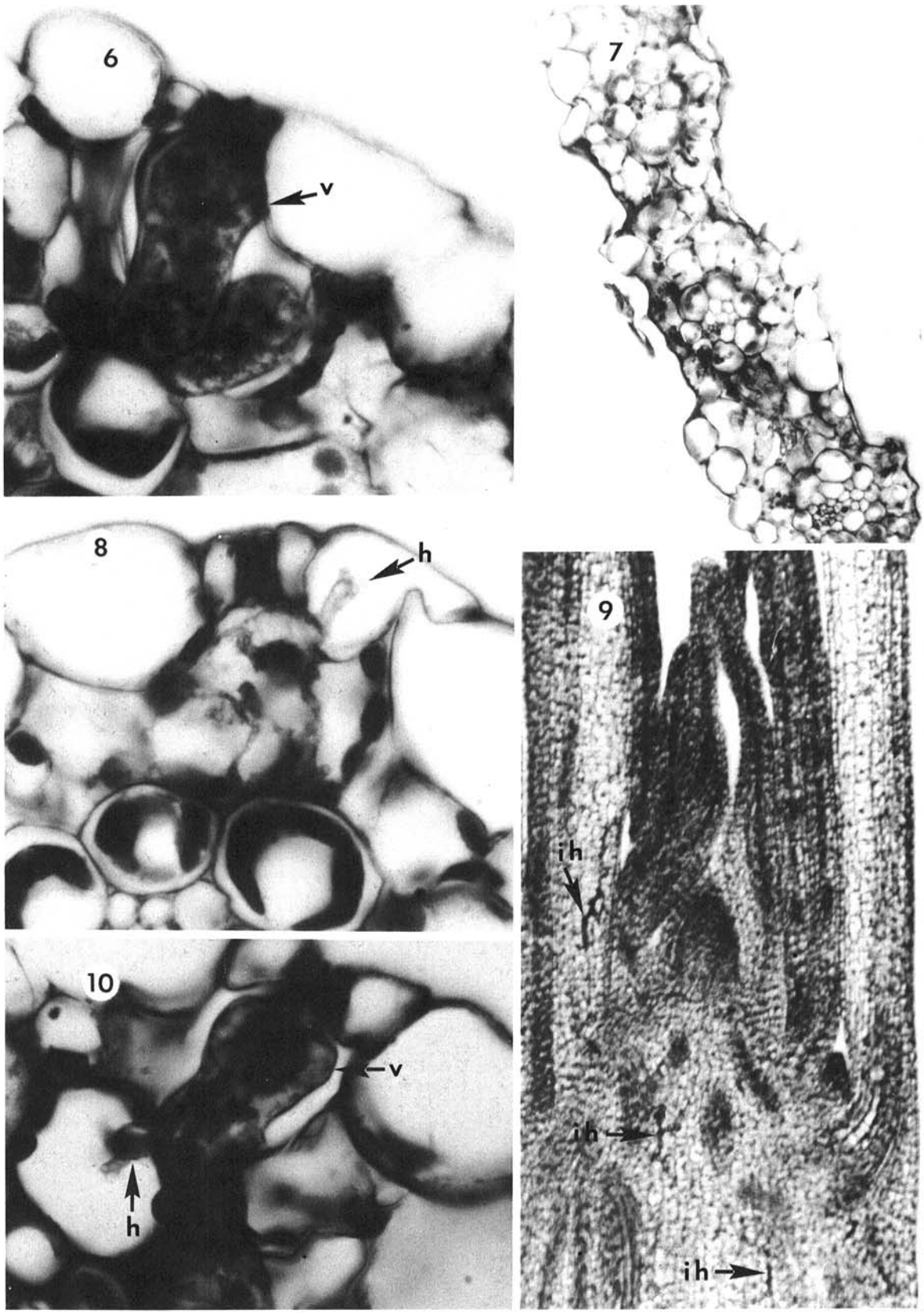

Fig. 6–10. Invasion of sorghum leaf cells by *Peronosclerospora sorghi,* and the resistant response. **6,** Vesicle (v) in substomatal cavity of IS12661 which appears to be restricted by collapsed host cells, 48 hr after inoculation (×900). **7,** Large local lesion in a leaf of IS12610C containing darkened and collapsed host cells and restricted hyphal growth 7 days after inoculation (×100). **8,** Haustorium-like projection (h) of substomatal vesicle into epidermal cells of IS12661C, 24 hr after inoculation (×900). **9,** Intercellular hyphae (ih) near the base of the apical meristem of TX412, 5 days after inoculation (×60). **10,** Haustoria-like projection of substomatal vesicle into mesophyll cells of NK266, 24 hr after inoculation (×900).

The more susceptible cultivars maintained their susceptibility for longer periods of time. Infection, although at less than 5%, was observed in IS12610C in 1974, in South Texas test plots and again in 1978. NK266 is also known to exhibit a similar incidence of infection in the field. Neither IS12661C nor the IS12661 derivative was seen to be infected in the years 1971–1978; trace infection was observed in IS2816C in 1978.

DISCUSSION

Although the conidial hyphae-induced local lesion phase of SDM can be extremely damaging in a single growing season, systemic infections caused by conidia are potentially more dangerous. Resistance to conidial-induced systemic infection is important, because systemic infections result in the formation of oosporic inoculum for primary infections in subsequent years.

Most of the penetration and infection occurred in plant shoots. Although crowns and roots were infected, the percent infection was lower than that of shoot-inoculated seedlings. Penetrations were generally indirect.

Resistance to SDM may be manifested in several ways. In a few cases infection fails entirely, either because the fragile conidia do not germinate or because their germ tubes cannot enter stomata and produce mycelium. Cosper (1) speculated that desiccation and/or unfavorable environmental conditions may render conidia noninfectious.

Sometimes the infecting fungus invades the host cells so rapidly that they are killed at once; the fungus then fails to advance possibly because dead host cells are unsuitable as a medium for its further growth. Ward (16), in 1902, first described this phenomenon for brome rust; it has since been referred to as hypersensitivity. The development of primary haustoria in the epidermal cells of resistant cultivars suggests a precocious infection which is characteristic of hypersensitivity. Salumu-Shabani (13) observed a similar penetration process in maize and noted that *P. sorghi* could invade resistant and susceptible maize cultivars with equal ease. As in sorghum, after the initial infection phase, progress of the fungus was restricted in resistant lines and rapid in susceptible ones.

Relationships between reactions to conidial inoculation and reactions to natural infection are complex and are not necesarily correlated since oospores constitute the primary inoculum in the soil. Also, artificial inoculation techniques may by-pass natural resistance mechanisms and induce infection in cultivars which are not normally susceptible in the field. Kenneth and Shahor (10) report that sorghum cultivars with field resistance were very susceptible to conidial inoculation in the greenhouse. Because of this they question the use of conidial inoculation tests to screen hybrids for resistance to systemic SDM. Craig (2), however, found that reactions to artificial inoculation agreed with field ratings for resistance. Whether field resistance was overcome by a copious amount of inoculum or by-passed because of optimum conditions for conidial infection during the inoculation period is not known.

Haustoria which predominated in mesophyll cells of susceptible and moderately resistant cultivars suggest a third type of resistance in which the host somehow restricts extensive fungal development. Slesinski and Ellingboe (15) observed, similarly, resistance expressed in different genotypes at different stages in the ontogeny of interactions between the wheat host and another obligate parasite, *Erysiphe graminis*. The factors which restrict hyphal growth of *P. sorghi* within its host tissues are presently unknown. The lessened susceptibility with plant age suggests a structural resistance in a developing seedling. Mature plant resistance and the specificity of this fungus for tissue other than meristem warrants further investigation into structural characters of the host which might prevent extensive fungal development.

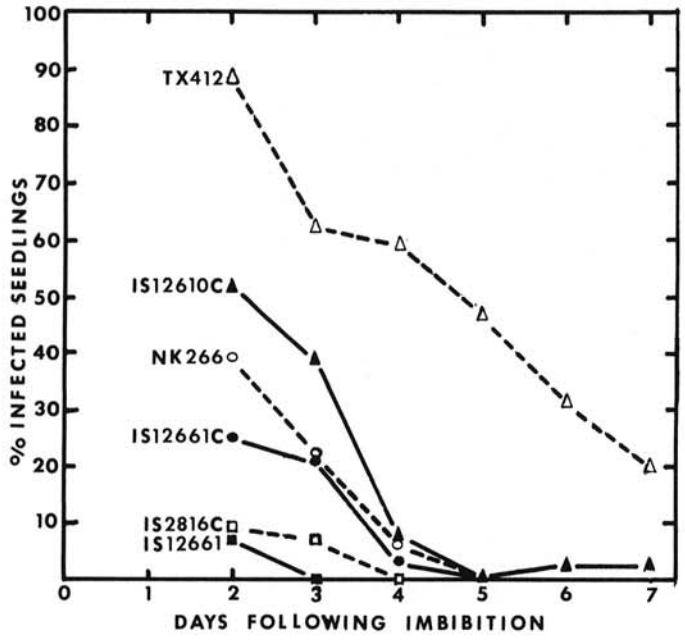

Fig. 11. Systemic infection in six grain sorghum cultivars inoculated with conidia of *Peronosclerospora sorghi* 2, 3, 4, 5, 6, and 7 days following imbibition of water by seeds.

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