Pathological Histology of Four Onion Cultivars Infected by Fusarium oxysporum f. sp. cepae

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ABSTRACT

Onion (*Allium cepa*) cultivars differentially tolerant or susceptible to basal rot caused by *Fusarium oxysporum* f. sp. *cepae* were equally susceptible to root and stem plate infection by the pathogen. Anatomical differences between the cultivars Treasure, Autumn Spice Improved, Grandee, and Elba Globe which could account for their different bulb decay reactions to the pathogen were not detected.

The pathogen invaded roots by both direct penetration and/or through wounds. Invasion of the stem plate was by growth of the pathogen from infected roots and/or through natural wounds in the lower portion of the stem plate area. Invasion of the fleshy leaf bases of the bulb in either tolerant or susceptible cultivars usually was from the stem plate, but occasionally it occurred through the lower portions of leaf bases below the soil surface when propagule levels in the soil were high.

The pathogen at first grew in the intercellular spaces of the root or the stem plate, but soon invaded cells. Tylose formation and occlusion of xylem vessels were observed equally in the stem plate tissues of the four cultivars. Chlamydospores formed in the cortex and vascular tissues of the root but not in the stem plate. Phytopathology 61:1164-1169.

Additional key words: Allium cepa, basal rot of onion.

Onion (*Allium cepa* L.) cultivars that are either tolerant or susceptible to *Fusarium oxysporum* Schlecht. emend. Snyd. & Hans. f. sp. *cepae* do not differ in time of initial root infection or percentage of root rot throughout the growing season under field conditions (2). Similarly, when tested under controlled environmental conditions in growth chambers at different levels of inoculum, differences in root rot reaction were not found between basal rot tolerant and susceptible cultivars. These same tolerant and susceptible cultivars differ considerably, however, in the percentage of bulb decay developed after storage. It has been suggested (2) that either physical or physiological factors or a combination of both function in tolerant cultivars to greatly reduce further penetration of the pathogen into the fleshy leaf bases after infecting the stem plate tissues.

The onion cultivars Treasure, Autumn Spice Improved, Grandee, and Elba Globe, susceptible, intermediate, intermediate, and tolerant to basal rot, respectively, (2, 10) were grown in organic soil artificially infested with a high population of *F. oxysporum* f. sp. *cepae* to initiate infection and provide material for the study of the pathological histology in diseased plants and bulbs. Two objectives were sought: (i) to determine if anatomical differences exist among these cultivars to account for the differences in reaction; and (ii) to observe the association of the pathogen with tissues of the root, stem plate, and fleshy leaf bases of the bulb of both susceptible and tolerant varieties.

MATERIALS AND METHODS.—*Onion cultivars and handling.*—Seeds of the cultivars Treasure, Elba Globe, Autumn Spice Improved, and Grandee obtained from Kenneth W. Stone, Regional Vegetable Specialist, Batavia, N.Y., were first wet for several seconds in 30% ethanol, then surface-sterilized for 5 min in 10% commercial "Sonny Sol" containing 5.25% by wt sodium hypochlorite. Both seedlings and small bulbs were grown from the same seed lot. Surface sterilized seeds were planted in 4-inch clay pots filled with steam-sterilized greenhouse soil (1 part peat moss:1 part sandy loam soil:1 part river sand). Plants were fertilized once a week with a dilute solution of a complete fertilizer. They were supplied with 16 hr of fluorescent light, and were grown under a temperature which varied between 20-25 C. Seedlings of desirable size were produced in 6 weeks, and small bulbs (0.5-1.5 inches in diam) in 4 to 5 months.

Inoculation techniques.—A sporodochial type isolate (No. 156) of *F. oxysporum* f. sp. *cepae* isolated from a naturally infected New York-grown onion and maintained by frequent monospore transfer on potato-dextrose agar (PDA) at 22-25 C and 12-hr fluorescent light day-lengths was used as the inoculum source. Spore suspensions were prepared by adding sterile distilled water to 3- to 5-week-old cultures, rubbing the culture surface with a sterile small spatula, shaking thoroughly, and filtering through four sterile layers of cheesecloth. The spore suspensions were adjusted to the desired concentration by means of a Spencer bright-line hemacytometer and added to soil. Six-week-old seedlings of the four cultivars were repotted in 6-inch clay pots filled with infested organic soil and maintained in a growth chamber with a day-night temperature of 27-21 C. Root and stem plate samples were taken at 28, 74, 122, 172, 246, and 372 hr after transplanting. Bulbs of the cultivars Treasure and Elba Globe were rooted in 4-inch clay pots filled with sterilized organic soil and maintained at a temperature which varied between 21-27 C. Three weeks later, these bulbs were inoculated by adding a spore suspension to the soil ball. Roots of half the plants used were wounded by making insertions of a sterile knife throughout the

soil-root ball. Stem plate tissues were dissected from the bulbs at 3 and 5 weeks after inoculation. Also, the stem plates of greenhouse grown bulbs of the cultivar Elite (intermediately susceptible to the fungus) were either dipped in a concentrated spore suspension or inoculated with hyphal and conidial masses of the fungus. Five bulbs/treatment were placed in a S/P seedpak growth pouch (Cat. No. B1220, Scientific Products, Evanston, Ill.), and received sterile water to maintain high humidity. They were incubated in the dark at 22-24 C. Samples of the root and stem plate tissues were dissected at 24, 48, 96, 144, 192, and 240 hr after inoculation. A series of additional experiments were conducted essentially in the same manner.

Histology.—Fixation, embedding, and staining of tissues sampled followed the method of Feder & O'Brien (5). Samples were fixed for 12-24 hr in 10% aqueous solution (v/v) of Acrolein, and then transferred to methyl cellosolve for dehydration. Two changes of this solvent were made in 4-24 hr. Dehydrated samples were placed sequentially for 4-24 hr in 100% ethanol, *n*-propanol, and finally in *n*-butanol. Exposure for 12 hr in each solution was the most satisfactory. All samples were then vacuum-extracted at room temperature to remove air from the tissues. Glycol methacrylate plastic was used as the embedding matrix. The fixed and dehydrated samples were transferred to the monomer mixture at room temperature. Three changes of this mixture were made at 6 to 24 hr/change; generally, 24 hr was used. The monomer mixture most often used in this study consisted of coconut charcoal-treated glycol methacrylate, 0.6% w/v polymerization initiator, and 5% v/v plasticizer. All treatments were conducted at about 0 C unless otherwise indicated. Infiltrated samples were placed in a No. 00 gelatin capsule, filled with the monomer mixture and capped firmly, and placed in an oven at 40 C for several days until the monomer mixture polymerized to form a hard block. The gelatin capsule was split open, the plastic block was fixed in a Collet-type holder and block, and the embedded samples were trimmed by the use of a fine file. Samples were sectioned by the use of a hand-made glass knife mounted on a Spencer "820" microtome. Generally, 3-μ-thick sections were cut and placed in distilled water (which had been passed through a Seitz filter) on microscope slides laid on a slide warmer overnight to allow the water to evaporate. Most sections were stained in 0.05% toluidine blue solution in benzoate buffer, pH 4.4, for 2 min. Stained sections were rinsed with running distilled water for 25 sec and left to dry at room temperature. Permount was applied, a cover slip added, and the slides were placed on a slide warmer for 12-18 hr. Several sections were stained with a combination of acid fuchsin and toluidine blue. With this procedure, the sections were stained first in 1% acid fuchsin in water and rinsed in water; then the toluidine blue schedule previously listed was followed. A few freezing microtome sections also were prepared. In this case, the tissues were fixed in Carnoy's solution (2 parts absolute alcohol and 1 part glacial acetic acid) and stained in 0.1% acid fuchsin in lactophenol or in 1% aqueous phyoxine and then sectioned.

RESULTS.—Anatomical differences in the four cultivars which might account for their differential susceptibility to Fusarium basal rot were not observed. The anatomies of healthy root, stem plate, and fleshy leaf base tissues examined for all four cultivars were identical to that described by Hoffman (6) for *Allium cepa*. There were no major differences in the relationship of the pathogen to the root and stem plate tissues of susceptible and tolerant varieties.

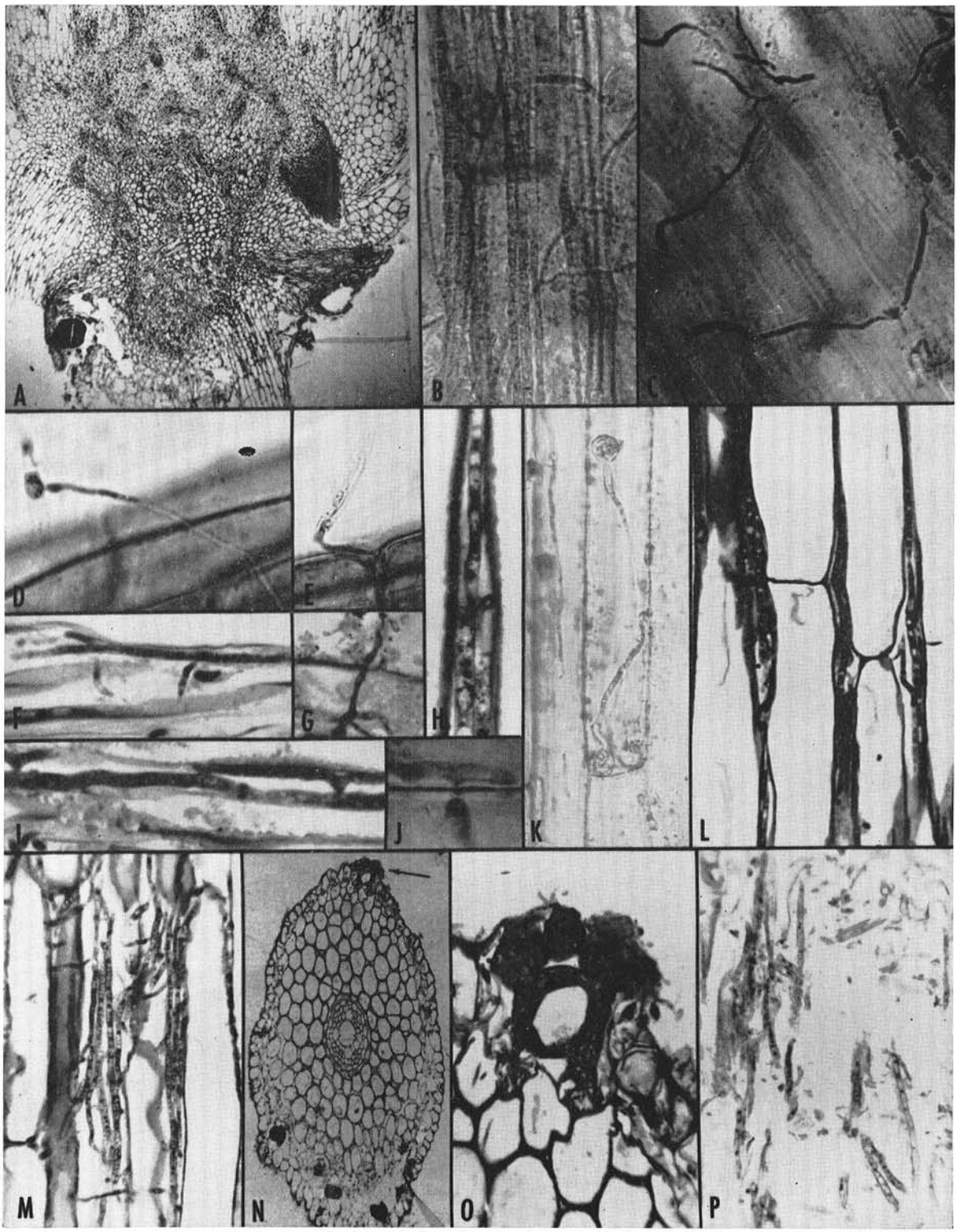
Fusarium oxysporum f. sp. *cepae* usually invades the roots, stem plate (modified stem), and the fleshy leaf bases of the onion plant, in that sequence. The modified onion stem connected to the fleshy leaves and the adventitious roots is shown in Fig. 1-A. These roots originate deep in the stem from the meristematic parenchyma of the inner cortical tissues close to the apical meristem of the stem. The roots elongate by pushing through the outer cortical cells and finally penetrate the bases of a few fleshy leaves (Fig. 1-A).

In soil, propagules of the fungus on or close to onion roots germinate usually by forming one or two germ tubes from one or both terminal cells, respectively (Fig. 1-C, E). A macroconidium containing a chlamydospore germinates only through the chlamydospore cell (Fig. 1-D). Germ tubes formed produce either a limited or extensive mycelial growth on the root surface. Occasionally, the end of the germ tube becomes swollen and divided by a septum which resembles an appressorium (Fig. 1-C). Hyphae on the root surface were often observed growing in groups parallel to each other along the longitudinal axis of the root (Fig. 1-B).

Penetration of the root was observed both through the uninjured root surface and wounds by direct penetration and without the formation of appressoria (Fig. 1-E, F, G). After ingress, hyphae of the pathogen grow mostly in the intercellular spaces of the parenchyma cells of the root cortex (Fig. 1-L). Later, these hyphae branch (Fig. 1-H), forming an extensive mycelium growing both intercellularly and intracellularly in tissues of the root cortex (Fig. 1-M). Peglike structures are formed by hyphae penetrating the cell walls of adjacent cells (Fig. 1-J). Hyphae often grow parallel to each other, and occasionally hyphal anastomosis was observed (Fig. 1-I). In the early stages of infection, a number of cells in the root cortex are completely filled with mycelium growth, whereas adjacent cells either lack or have only a few hyphae (Fig. 1-N, O).

At later stages of infection, the root cortex becomes completely disorganized and decomposed. Usually, hyphal fragments are the only structures observed in the region between the epidermis and the endodermis (Fig. 1-P, Fig. 2-A). The fungus invades the vascular system either through wounds or through the undifferentiated or partially differentiated tissues near the root tip. The latter was observed occurring with several points of ingress near the root tip in one experiment. Hyphae grew in and around the xylem vessels and in the xylem parenchyma of the root (Fig. 2-B, C).

Invasion of the stem plate tissues occurs either by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growth of the pathogen from infected roots or through natural wounds present at the lower portion of the stem plate, a result of the death of roots throughout the growing season (Fig. 2-E, H). The cortical cells of the roots are continuous with those of the outer cortex of the stem, as illustrated in Fig. 2-E which also shows hyphae (indicated by an arrow) in a xylem vessel of a root in the outer cortex of the stem. In the stem plate tissues, the fungus also grows in the intercellular spaces and intracellularly (Fig. 2-I). Occluded xylem vessels were occasionally observed in the roots, but more often in the stem plate (Fig. 2-E, I). Figure 2-G shows an early stage of the development of a tyloselike structure which eventually will occlude this xylem vessel completely. Occluded vessels were stained green with a shade of red color. The green color indicates the presence of lignin and polyphenollike compounds, whereas red indicates either (i) the presence of one; (ii) a combination of several; or (iii) all the following compounds: polyphosphates, polysulfates, and polycarboxylic acids, including alginic and pectic acids.

At later stages of disease development, the pathogen breaks through the stem area and grows quite rapidly and abundantly in the fleshy leaf base tissues and in the spaces between the leaf bases of the bulb (Fig. 2-F, J). Because of the heavy inoculum loads artificially added to the soil or in the inoculation procedures, masses of fungal hyphae sometimes were observed growing on the basal portion of the fleshy scales near the stem plate area. Direct penetration of the cells directly beneath this mycelial mass was observed (Fig. 2-I). Chlamydospores were formed in the root cortex (Fig. 1-K) and in and around the xylem vessels (Fig. 2-D). Microconidia were produced by the mycelium growing between the scale tissues in the bulb.

DISCUSSION.—Onion cultivars susceptible and tolerant to Fusarium basal rot were anatomically similar and equally susceptible to *F. oxysporum* f. sp. *cepae* in their root and stem plate tissue. It is suggested that the differential reaction of their leaf base tissue to infection by the fungus should be explained on another basis than structure. Penetration and subsequent growth of the pathogen in tissues of susceptible and tolerant cultivars was always similar under the conditions employed which were favorable for the development of the disease in the susceptible cultivars. These findings are in agreement with those of Beckman (4), who reported that wilt-producing fusaria grow equally well or better in resistant than in susceptible tissues. Also, results of this study showed that a number of xylem vessels of tolerant and susceptible cultivars were found occluded and clogged by tyloselike structures. Typical cork layer formation was not found although, on a few occasions, what resembled an early stage of cork layer formation was observed. Cells surrounding infected cells were found with few transverse cross walls. Tylose formation, occlusion of xylem vessels, and hypertrophy of infected onion tissues were reported earlier (14). Vascular occlusion is common, and has been considered by many workers a major factor in disease development and resistance to many wilt diseases incited by fusaria (3). Beckman (4) reported that resistant varieties exhibit vascular occlusion and foliar symptom development as great or greater than susceptible varieties. He obtained evidence that vascular occlusion occurred somewhat sooner and was more pronounced in resistant than in susceptible tissues. The latter has not been studied in this investigation.

Pierre (13), in his study on the nature of resistance of several bean lines differentially susceptible to *F. oxysporum* f. sp. *phaseoli* and *Thielaviopsis basicola*, reported that the formation of structural barriers such as wound peridium are of secondary importance in the resistance of bean to the abovementioned pathogens. He isolated two substances (phytoalexins) from infected bean tissues which were found to be inhibitory to spore germination and growth of several fungi. He clearly demonstrated that resistance in the bean lines tested provides a good example and is compatible with the phytoalexin concept. A preliminary study showed that an antifungal substance(s) is produced by onion tissues inoculated with pathogenic and nonpathogenic fungi (2). However, more work is needed to determine the role (if any) of phytoalexinlike compound(s) in the resistance of the onion plant.

Shalaby & Struckmeyer (14) reported that *Fusarium* infections of the onion hybrid Hickory were fewer and not as extensive as those of the onion inbred line W4. They found that the fleshy leaves of the bulbs of the hybrid Hickory differentiated at higher levels on the axis of the stem plate than did those in W4, and suggested this as an explanation for the difference in the extent of infection by *Fusarium*. They also reported that Hickory was more resistant to Fusarium bulb rot than W4. However, in their description of the disease they reported that the roots commonly turn pink and generally decay. In this and previous studies (1, 2), pink

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Fig. 1. Pre- and postpenetration growth of *Fusarium oxysporum* f. sp. *cepae* on and in onion tissues. **A)** Longitudinal section of the stem plate area attached to several fleshy leaves (top) and a few adventitious roots (bottom). Also shown is a young root originating deep in the stem plate area and its growth outward through the outer cortex of the stem and the base of two fleshy leaves. **B)** Groups of hyphae growing parallel to each other on the root surface. **C)** Macroconidial germ tubes with septa in the swollen ends. **D)** Germination of a chlamydospore in a macroconidium. **E)** Germination through a terminal cell of a macroconidium. The germ tube formed is penetrating the root surface through the juncture of two epidermal cells. **F)** Two peglike structures produced by hypha growing on the root surface. **G)** Hypha penetrating the intact root surface. **H)** Hyphal branch about to puncture the cell wall. **I)** Hyphal anastomosis in the cortical tissues of the root. **J)** Hyphal penetration from cell to cell through the formation of peglike structures by hyphal constriction. **K)** Terminal chlamydospores in a parenchyma cell in the root cortex. **L)** Hyphae growing in the intercellular spaces of the root cortex. **M)** Intracellular growth of hyphae in the root cortex. **N)** Cross section of a root showing several cells completely filled with mycelial growth. **O)** Close-up view of several of the cells seen in Fig. 1-N. **P)** Infected root cortex at a late stage of infection.

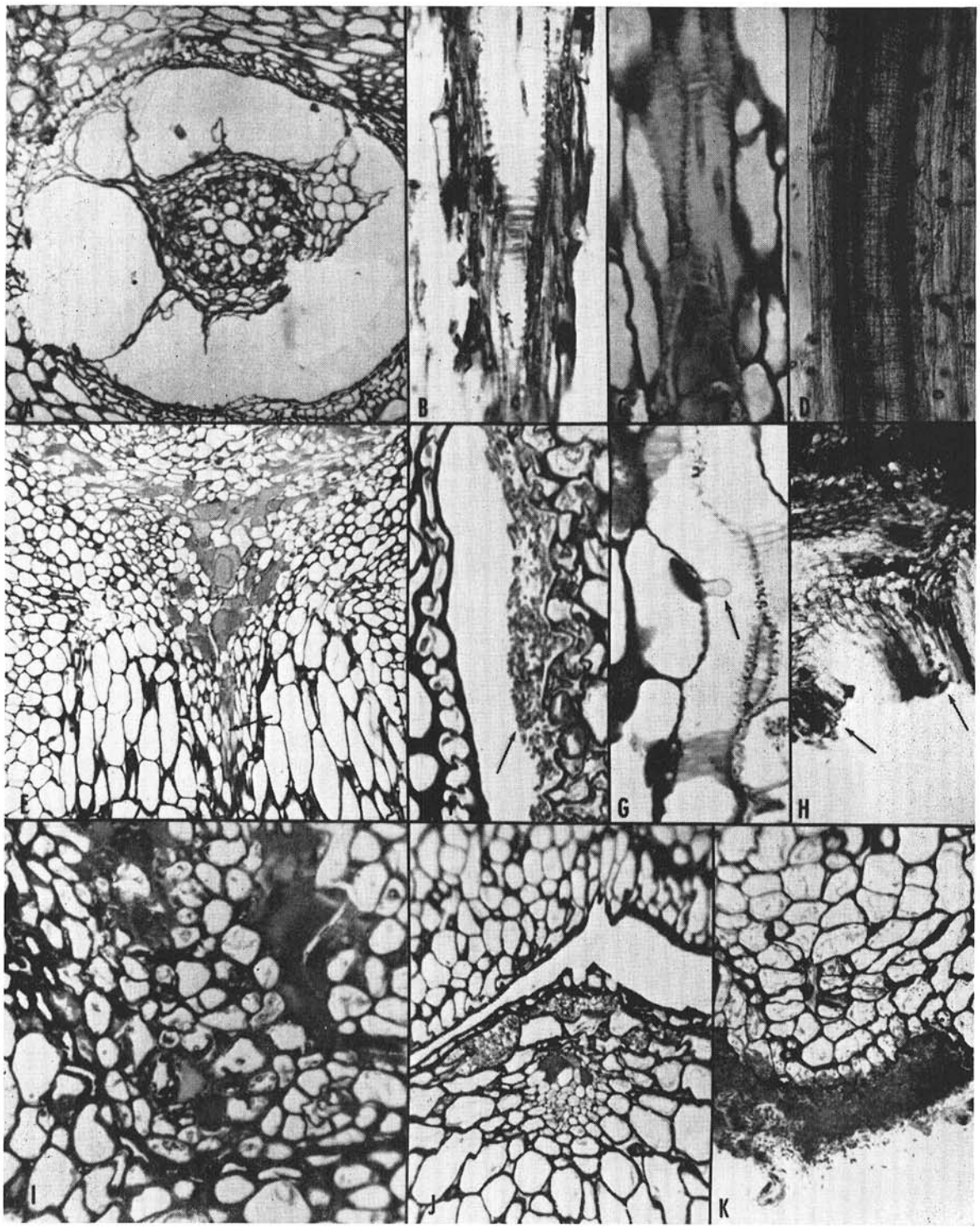

Fig. 2. Association of *Fusarium oxysporum* f. sp. *cepae* with onion tissues. **A)** A cross section of an infected root at a late stage of infection in the outer cortex of the stem plate. Most of the root cortex is completely digested. **B, C)** Hyphae growing in and around xylem vessels of the roots. **D)** Chlamydospores formed in and around a xylem vessel of a root. **E)** Infected root with hyphae (indicated by arrow) in contact with the stem plate tissues. The root cortex is continuous with the outer cortex of the stem. It also shows a number of occluded vessels. **F)** Mycelial growth (hyphae and microconidia) in the space between two fleshy leaves of the bulb. **G)** An early stage in the formation of a tyloselike structure in a xylem vessel. **H)** Natural wounds at the base of the stem plate area. **I)** Occluded vessels and an inter- and intracellular growth of hyphae in the stem plate tissues. **J)** Inter- and intracellular growth of hyphae in and around a vascular bundle of a fleshy leaf base which also exhibit occluded vessels. **K)** Mass of fungal growth on the outside of fleshy leaf base tissues near the stem plate area which resulted in a direct penetration of the host cells.

coloration of roots infected solely with *F. oxysporum* f. sp. *cepae* was never observed. However, this symptom was frequently present on roots of onions grown in naturally infested field soil cropped for several years to onion. The appearance of the pink root symptom was due to the presence of *Pyrenochaeta terrestris*. Kehr et al. (7) reported that *F. oxysporum* f. sp. *cepae* and *P. terrestris* independently infect the onion plant, and resistance to one of them is no measure of the protection against the other. Therefore, the conclusion of Shalaby & Struckmyer (14) that Hickory is more resistant than W4 on the basis of *F. oxysporum* f. sp. *cepae* acting as a primary pathogen is questionable.

Fusarium oxysporum f. sp. *cepae* was found to invade onion tissues by direct penetration through uninjured surfaces and/or through wounds. It is hoped that the results of the present study will lay to rest the controversy which has existed for some time in the literature concerning the mode of initial ingress of *F. oxysporum* f. sp. *cepae* into the onion root and stem plate. Several workers (7, 10, 12) reported penetration in the absence of any visible wounds; others (8, 9, 11, 15) suggested that penetration occurs only through wounds.

In this study, hyphal anastomosis was observed in infected root tissues. Abawi (2) reported that *F. oxysporum* f. sp. *cepae* exists in soil in one morphological (sporodochial) type. However, cultural variants were obtained from naturally infected onion tissues. Hyphal anastomosis may lead to morphological changes of the fungus, and thus may partially account for the presence and isolation of cultural variants from infected tissues and not from soil.

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