Infection of Bean by Ascospores of Whetzelinia sclerotiorum

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ABSTRACT

Bean blossoms serve as an energy source to support infection of beans by *Whetzelinia sclerotiorum;* hence, under New York conditions, an epidemic of white mold occurs only after flowering. On bean petals, ascospores germinated by forming one or two germ tubes within 6 hours after inoculation. Simple, club-shaped appressoria were formed, and direct penetration of the host cuticle by infection pegs occurred within 24 hours. A vesicle was produced under the cuticle layer which eventually produced hyphae of different diameters that grew intercellularly and intracellularly in blossom tissues. By 72 hours the infected blossoms were completely invaded and disintegrated by the fungus. Hyphal strands protruding from infected blossoms produced multicellular, cushion-shaped appressoria when contact was made with other host tissues. A depression in the host tissue was first observed beneath the appressorium. Infection pegs were produced by the flattened ends of the appressoria, and penetrated the cuticle directly. Once beneath the cuticle, the fungus grew rapidly throughout the leaf tissue. Appressorial development of *W. sclerotiorum* on dialysis tubing segments was positively correlated with the nutrient level in the agar medium used. These results further substantiate the role of blossoms as an energy source in the epidemiology of white mold of beans in New York State.

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In recent years, white mold of bean (*Phaseolus vulgaris* L.) incited by *Whetzelinia sclerotiorum* (Lib.) Korf and Dumont (5) sensu Purdy (10), has repeatedly occurred in epidemic proportions in central and western New York (2, 8) causing significant economic losses.

Abawi and Grogan (1, 2) concluded that white mold epidemics of bean in New York are initiated by ascospores of *W. sclerotiorum* produced mainly outside bean fields by sclerotia of the fungus. They also reported that infection of beans by mycelium from sclerotia was not observed under field conditions. Natti (8) observed that symptoms of white mold of beans in New York appeared 8-14 days after full bloom and that senescent blossoms were an essential intermediary for disease development. Uninjured, healthy bean plants in a prebloom stage were not infected by ascospores. However, successful infections by ascospores were readily obtained in tissues injured mechanically or by heat-scorching, or when other energy sources were substituted for bean blossoms (2).

Histological investigations on the infection of leaf and stem tissues of beans and other hosts by *W. sclerotiorum* have been reported by several workers (3, 7, 9, 11). This paper reports the infection of bean blossoms by ascospores of *W. sclerotiorum,* subsequent infection of healthy green tissues of bean in contact with infected blossoms, and some observations of appressorium formation on dialysis tubing segments and host tissues.

MATERIALS AND METHODS.—A large-sclerotium isolate of *W. sclerotiorum* (WS-3) obtained from field-infected snap beans was maintained on Difco potato-dextrose agar (PDA) and used throughout this study. Sclerotia were produced by growing the fungus on autoclaved celery stem segments. After 4 weeks, sclerotia were removed and placed in sterile distilled water and held at 15 C in a growth chamber with 4,304-5,380 lx fluorescent and incandescent lights for 14 hours/day. About 60 days were usually required for growth of the fungus and production of apothecia. Ascospore suspensions were prepared by attaching apothecia onto the inner side of a petri dish cover with the disk opening facing down. Ascospores were subsequently ejected into sterile distilled water placed in the petri dish bottom.

Usually four seeds of the snap bean cultivar Cascade were planted in 10-cm diameter clay pots filled with a pasteurized soil mix. The plants were grown in a greenhouse at 20-24 C and fertilized weekly with a dilute solution of a complete fertilizer. Generally, the plants reached the white-bud stage approximately 5 weeks after seeding.

Ascospore inoculum was adjusted to 5- to 6×10^4 spores/ml suspension with a Spencer bright-line hemacytometer and atomized onto bean plants. Plants were also inoculated by placing bean blossoms sprayed with ascospores 72 hours earlier in the center of bean leaves. Immediately after inoculation, plants were placed in a mist chamber in a greenhouse at 20-24 C.

Blossom and leaf tissues were removed from the mist chamber at intervals of 6, 12, 18, 24, 48, 72, 96, and 120 hours. Tissues were fixed in formalin-acetic acid-alcohol, dehydrated in an alcohol series, cleared in xylene, and embedded in Paraplast-Plus as described in Sass (13). Sections ranging from 6-10 μm thick were cut on an American Optical, Model 820 rotary microtome, stained in 0.5% safranin, and counter stained with 0.5% fast green. Whole mounts were prepared by fixing and staining in 0.1% acid fuchsin in lactophenol. Tissues were mounted on slides for observation.

Germination of ascospores and formation of appressoria were studied on bean tissues, and on dialysis tubing segments placed on different concentrations (10-fold series) of turnip extract agar (TEA). This extract was prepared by boiling peeled turnips in distilled water (1:1, w/v for 1 hour. The extract was filtered through four layers of cheesecloth, diluted with water, and solidified by adding 2% Oxoid agar. Dialysis tubing was cut into approximately 0.5 × 1-cm segments, surface-sterlized for 30 seconds in 95% ethanol, and rinsed several times in sterile distilled water. The sterilized segments were then placed on the surface of the TEA medium. The plates were placed on a turntable and were showered with ascospores from apothecia attached to the inner side of a bell jar inverted over the turntable. Plates were incubated at 20-24 C. At preselected intervals, dialysis tubing segments were removed, stained in 0.1% acid fuchsin in lactophenol, and examined.

RESULTS.—*Appressorium formation on dialysis tubing segments.*—Ascospores on dialysis tubing segments incubated on 10^{-1} TEA germinated within 3 hours by either one or two terminal germ tubes (Fig. 1-a, b). Appressorial initials were first observed after 10 to 12 hours of incubation as an enlargement of the ends of germ tubes which then branched dichotomously (Fig. 1-c, d, e). Appressorial branches were usually club-shaped; within 24 hours they divided again to form multicellular, compound appressoria. After 48 hours of incubation, many ascospore colonies had produced several compound appressoria (Fig. 1-f, g). Further development of these appressoria resulted in the formation of finger-shaped structures (Fig. 1-h, i). The finger-shaped appressoria proceeded to form a cushion-shaped structure (Fig. 1-j). Ends of the fingers of appressoria germinated either by producing thin, long hyphae which subsequently produced appressoria, or by forming short branches which terminated with an appressorium (Fig. 1-h, i, j).

Appressorium formation was closely correlated with the nutrient level of the incubating medium. Fig. 1-l through 1-q illustrate the different stages of appressorium formation produced after 40 hours of incubation on 10^{-6}, 10^{-5}, 10^{-4}, 10^{-3}, 10^{-2}, and 10^{-1} TEA, respectively, in comparison to growth on Oxoid agar alone (Fig. 1-k). Definite appressorium formation occurred at concentrations of 10^{-3} and higher, with most advanced development occurring at 10^{-1}.

Appressoria formed on dialysis tubing segments, but not on the surface of TEA. Appressorium formation on bean tissues was essentially similar to that produced on dialysis tubing segments and is described later.

Infection of bean blossoms by ascospores.—Ascospores enlarged prior to germination on petals of bean blossoms. Germination occurred within 6 hours after inoculation and extensive germ tube branching occurred within 24 hours (Fig. 2-j, k). Ends of germ tubes doubled in width and resembled appressorial initials prior to penetration of bean petal tissues (Fig. 2-l).

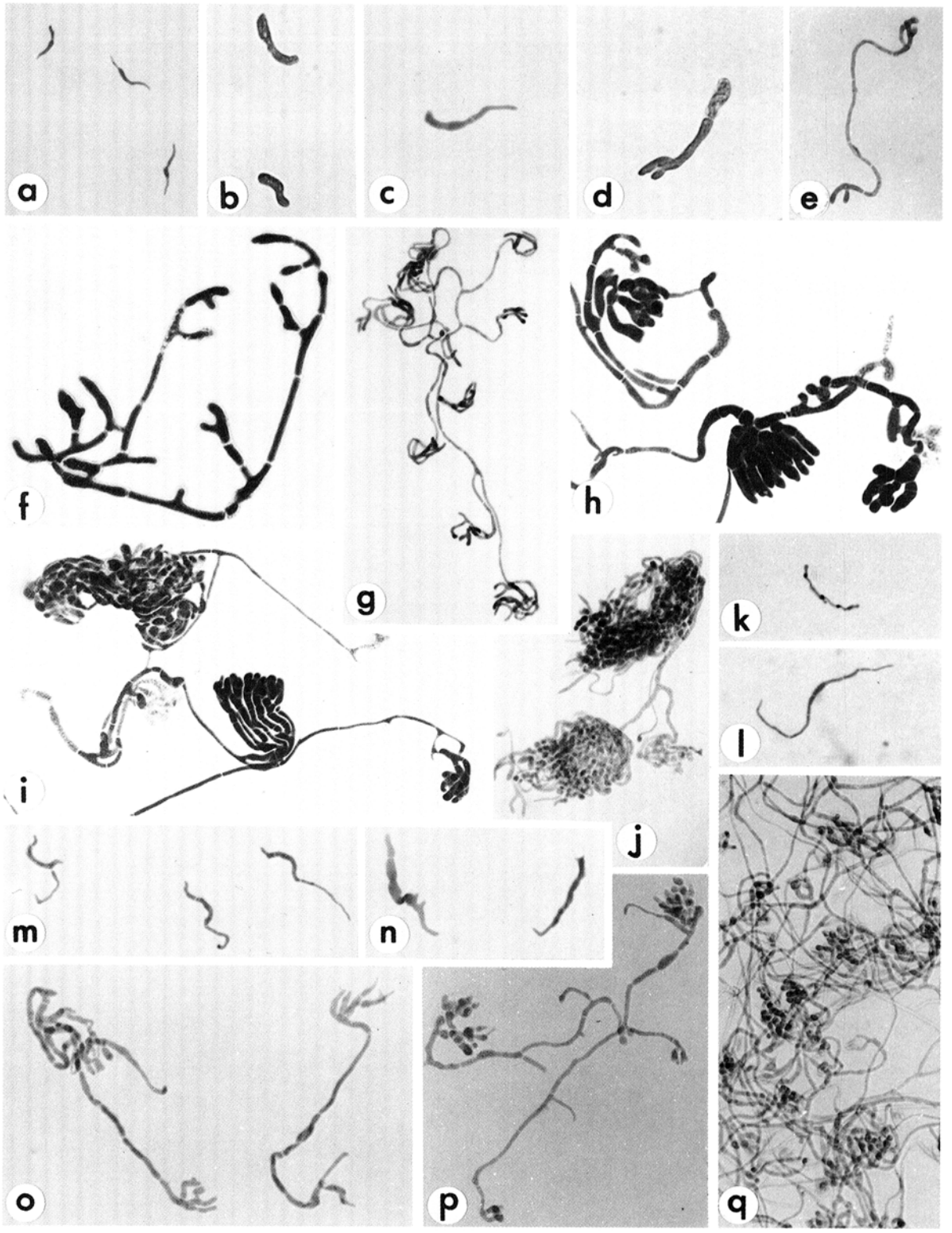

Fig. 1-(a to q). Germination of ascospores and production of appressoria of *Whetzelinia sclerotiorum* on dialysis tubing segments. **a to j)** Sequential development of appressoria on 10^{-1} turnip extract agar. **k to q)** Appressorial development on Oxoid agar and concentrations of turnip extract agar from 10^{-6} to 10^{-1} after 40 hours incubation. Magnification of a, e, g, j, and q = × 162; b, d, h = × 404; c i, l, m, n, and o = × 253; f = × 323; and k and p = × 202.

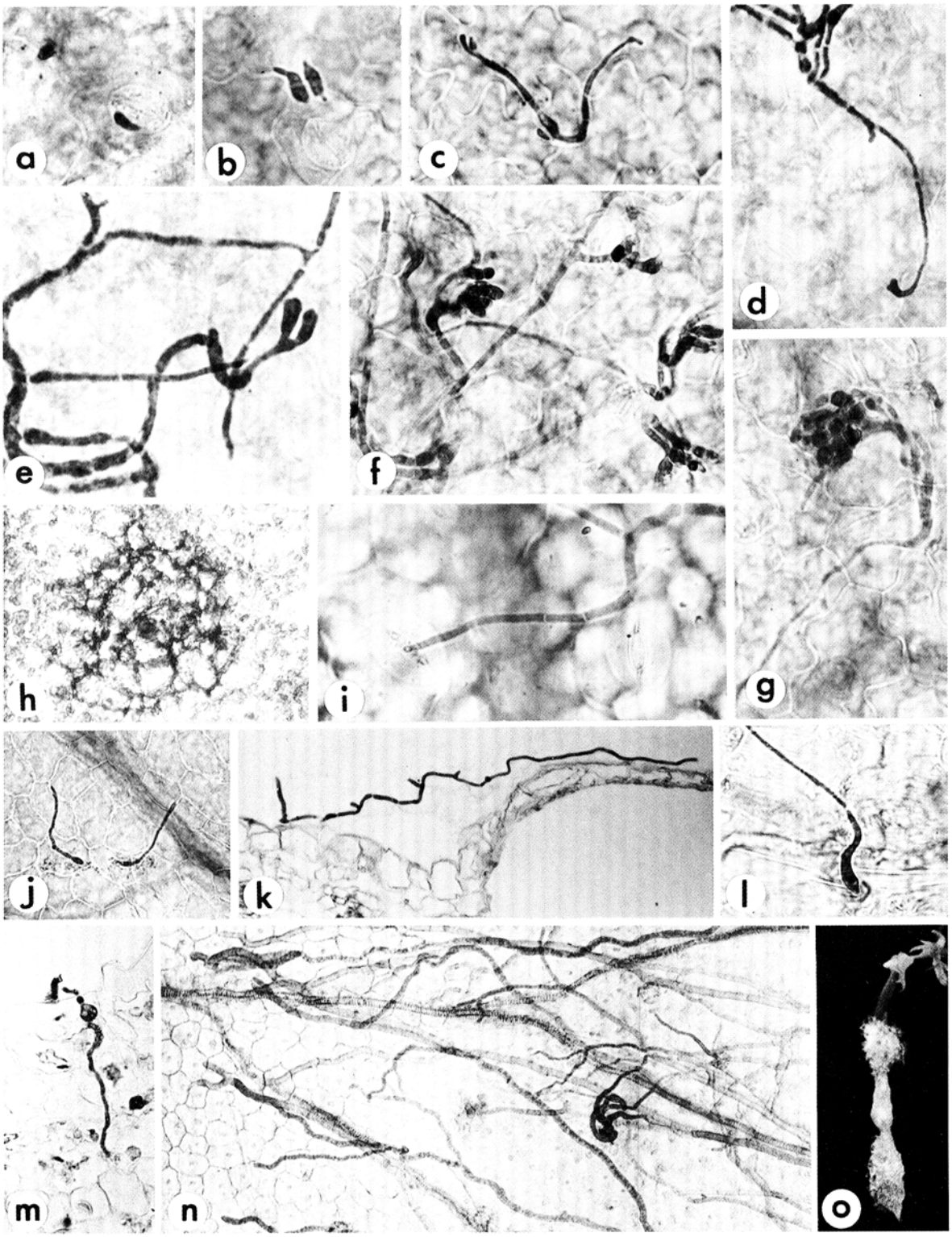

Fig. 2-(a to o). Infection of bean blossoms and leaves by ascospores of *Whetzelinia sclerotiorum*. **a to g)** Ascospore germination, appressorial formation and infection of bean leaf tissues in the presence of 10^{-1} turnip extract. **j to o)** Stages of infection of bean blossom and pod tissues by *W. sclerotiorum*. Magnification of a, b, e, g, i, and m = × 400; c and d = × 320; f = × 250; h = × 100; j and l = × 640; k = × 510; n = × 175 and o = × 3/4.

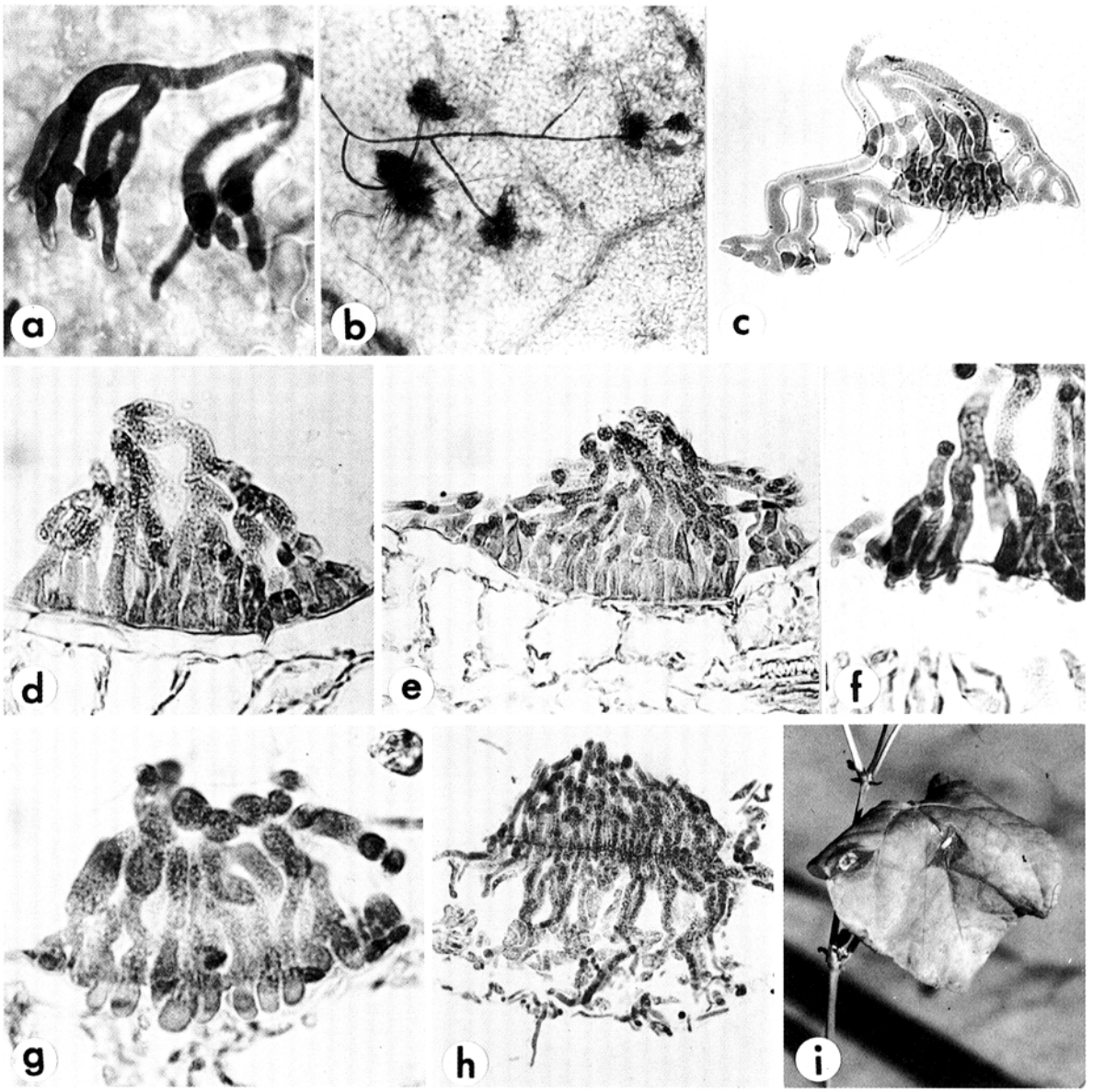

Fig. 3-(a to i). Appressorial formation and infection of bean leaf tissue by mycelial strands of *Whetzelinia sclerotiorum* originating from infected blossoms (× 560, 63, 225, 385, 385, 575, 770, 320, and 1.0 magnifications, respectively).

The appressoria formed infection pegs which penetrated the epidermal layer and formed vesicles therein (Fig. 2-m). Vesicles gave rise to secondary hyphae that were approximately twice the width of external hyphae and grew intercellularly and intracellularly throughout the petal tissues. The centers of established lesions were void of cellular organization; however, the cell walls at the margin of the lesions remained intact in the presence of invading hyphae (Fig. 2-n). Within 48-72 hours all flower parts were infected with many mycelial strands protruding from the floral parts. After 72 hours of incubation, the blossoms were completely covered with a dense, cottony mycelial growth (Fig. 2-o). Hyphal strands extruding from the blossoms produced multicellular, cushion-shaped appressoria when they came in contact with other tissues (Fig. 3-b).

Infection of leaves by mycelium from infected blossoms.—Hyphal strands growing out from infected blossoms branched dichotomously after making contact with leaves (Fig. 3-a). These structures continued to branch, forming multicellular, compact, cushion-shaped appressoria on leaf surfaces (Fig. 3-b, c, d). Figures 3-(d to h) illustrate the different stages of epidermal penetration and advanced leaf tissue invasion. A slight depression in the leaf surface directly beneath the appressorium was evident (Fig. 3-d). The depression became more pronounced with disorganization of the epidermal layer (Fig. 3-e). Epidermal penetration was accomplished by

infection pegs produced by several flattened branches of the appressorial cushion (Fig. 3-f, g). The peg-like structures enlarged in diameter, and the mycelium grew intercellularly and intracellularly, completely disrupting the cellular organization of the leaf (Fig. 3-h). Macroscopic symptoms on the upper leaf surface and protruding hyphal strands on the lower surface were evident about 48 hours after inoculation of leaves with infected blossoms (Fig. 3-h, i). Well-developed sclerotia were observed on infected tissues 5-7 days after inoculation.

Infection of leaf tissues by ascospores with the addition of an energy source.—Ascospores of *W. sclerotiorum* ejected onto leaf surfaces germinated and infected bean leaves only when an energy source (10^{-1} TE) was added to the spore suspension. Ascospores on leaf tissues without TE had germ tubes that were shorter than the spore length when observed 72 hours after inoculation (Fig. 2-a). With the addition of TE, ascospores germinated by forming one or two short germ tubes within 12 hours of inoculation (Fig. 2-b). Germ tubes were about 10 times the spore length with the first signs of branching apparent at about 24 hours (Fig. 2-c). After an incubation period of 24-48 hours, simple and multicellular compound appressoria were observed (Fig. 2-d, e, g). Extensive mycelial growth with large numbers of multicellular, cushion-shaped appressoria were apparent 48-72 hours after inoculation (Fig. 2-f). Macroscopic lesions were evident at 72 hours (Fig. 2-h). In only two instances were hyphae observed entering open stomata (Fig. 2-i).

DISCUSSION.—Results of these histological investigations further substantiate earlier reports (2, 8) that bean blossoms are essential intermediaries for the development of white mold epidemics of beans under New York conditions. Infected bean blossoms are an efficient source of inoculum for infecting leaf, stem, or pod tissues of beans. Ascospores of *W. sclerotiorum* completely colonized mature and senescent bean blossoms in 2 to 3 days, but colonized healthy bean leaf tissues only when an exogenous energy source was provided. These results explain why control of white mold was possible when only the bean blossoms were sprayed with benomyl [methyl 1-(butyl carbamoyl)-2-benzimidazole carbamate], even though the whole plant was inoculated with ascospores of *W. sclerotiorum* under mistchamber conditions (Abawi and Hunter, *unpublished*). This study also supports the earlier hypothesis that bean blossoms function mainly as an energy source (2, 8). The requirement of an exogenous energy source for ascospores and hyphae to infect host tissues is well documented (3, 6, 11, 12).

The type and developmental stages of appressoria of *W. sclerotiorum* produced by germ tubes or hyphae observed in this study agree with those reported by other workers (3, 7, 9, 11). As Boyle (3) and Purdy (11) reported, appressoria were produced in contact with host tissues and other stimuli, or, as found here, on dialysis tubing segments placed on agar. Appressoria were not produced directly on agar media regardless of the nutrient level of the medium. Only simple, club-shaped appressoria were produced by ascospores on bean blossoms. On leaf tissue, however, ascospores produced more advanced appressorial structures in the presence of an energy source (TE). Hyphae protruding from infected blossoms or other plant materials (beet seeds, celery stem segments, grain seeds; Abawi and Grogan, *unpublished*) always produced the multicellular, cushion-shaped appressoria. The different types of appressoria observed are possibly due to differences in the nourishment of the fungal mycelium producing the appressoria. On dialysis tubing segments on a dilution series of TEA, appressorium formation was positively correlated with the nutrient level of the medium [Fig. 1-(k to g)]. These results are in agreement with those reported earlier by Purdy (11). In addition to their importance in host penetration, appressoria may also function as survival structures as suggested earlier (4).

Infection of bean leaves by mycelia of *W. sclerotiorum* growing out from infected blossoms was essentially the same as that reported by Lumsden and Dow (7) and Prior and Owen (9). However, a true vesicle was not formed under the cuticle layer. Differences in diameters of hyphae were observed in infected blossom and leaf tissues; however, their distribution appeared to be more random than that suggested by Lumsden and Dow (7) who identified three distinct modes of hyphal diameters in infected hypocotyl tissues. Furthermore, hyphae of the fungus were found beyond the margins of actively expanding lesions, while Boyle (3) and Lumsden and Dow (7) reported that hyphal growth was always within the lesion area.

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