Source of Primary Inoculum and Effects of Temperature and Moisture on Infection of Beans by Whetzelinia sclerotiorum

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ABSTRACT

White mold epidemics of beans in New York are initiated by ascospores produced by sclerotia of *Whetzelinia sclerotiorum*. Sclerotia are abundant in nonplowed bean fields in the spring; but after plowing, sclerotia are rare near the soil surface. Ascosporic inoculum late in the season originates mainly from sclerotia under duff in noncultivated wooded areas and fruit orchards adjacent to bean fields. In 1973, apothecia were produced abundantly from 23 April to 15 June but not during dry weather in June, July, and August. Potted bean plants exposed in commercial fields for 4 days when apothecia were abundant, became infected after incubation in a mist chamber. Also, there was a good correlation between presence of ascospores on bean tissue in the field, and subsequent development of white mold. An exogenous energy source such as bean blossoms, steamed celery stems, turnip extract, or sucrose solution was required for ascospores to infect healthy bean plants in the prebloom stage; however, ascospores readily infected injured plants at any stage of development, or noninjured plants in the blossom stage. Infection of beans by mycelium from sclerotia was not observed in the field. Presumably, this is because in the absence of an exogenous energy source sclerotia are incapable of infecting bean plants, even under ideal conditions in a mist chamber. Moisture is a limiting factor in the development of white mold on beans. Apothecia were produced only in saturated or near-saturated soils, and infection of beans occurred only if free moisture was maintained for a relatively long period at the interface of bean tissue and inoculum. Moisture was also essential for lesion expansion. Ascospore germination was not drastically affected by temperature, but 25 C was optimum for germ tube growth. Mycelial growth, sclerotial production, and lesion initiation and development were optimum at 20-25 C. The temperature range for apothecial production was 10 to 25 C, with an optimum of about 10 C. Temperature does not appear to be a limiting factor in the development of white mold under New York conditions.

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Additional key words: epidemiology, *Sclerotinia sclerotiorum*, ascospores, *Phaseolus vulgaris*.

Snap beans *(Phaseolus vulgaris* L.) are planted on about 55,000 acres in New York, representing 20% of the total acreage in the United States, and dry beans are grown on another 60,000 acres. Most of the snap beans grown in New York are for processing.

Severe epidemics of white mold of beans incited by *Whetzelinia (Sclerotinia) sclerotiorum* (Lib.) Korf and Dumont (10) sensu Purdy (15) have occurred repeatedly in recent years throughout the bean-growing areas of central and western New York (13). The disease causes considerable economic loss because, in addition to direct losses in the field, detection of >2% pod infection in a truckload at the processing plant results in rejection of the whole load.

Natti (13) reported that epidemics of white mold of beans in New York appeared some 8-14 days after full bloom, but were not correlated with planting date, cultivar, or selected environmental parameters. However, rainfall in July was reported to contribute to the incidence of disease, whereas the rainfall in August had no appreciable effect. Furthermore, he surmised that the primary inoculum causing white mold of beans is airborne, although ascospores of the fungus could not be found. He concluded that mycelium of the fungus is airborne in organic matter or in infected host tissues and that senescent host tissues are essential intermediaries for disease initiation.

An effective chemical control program was developed utilizing the systemic fungicide benomyl (13). However, a disease prediction method is needed to permit more efficient use of the chemical and restrict its usage to periods when white mold is forecast. To develop such a forecasting system, detailed information is needed concerning the effect of weather and other environmental factors on disease incidence and development, and the year-round activity of *W. sclerotiorum*. This paper reports on the primary source of inoculum responsible for initiating white mold of beans in New York, and the effects of temperature and moisture on production of inoculum, disease initiation, and development. A summary of a portion of this report was previously published (1).

MATERIALS AND METHODS.—The number of sclerotia in bean fields was determined either by counting sclerotia per 0.093 M^2 (1 ft^2) of soil surface, or number of sclerotia per 500 cc of soil in composite soil samples from 0 to 2.5, 2.5 to 10, and 10 to 17.5 cm deep. Soil samples were collected by soil augers or by digging with a shovel and trowel. Sclerotia were isolated by combination of flotation and sieving. Soil samples were first suspended in saturated NaCl solution and then passed through a 1.68-mm sieve (10-mesh screen).

Apothecial production was determined by incubating sclerotia in sterile distilled water (SDW) or in moist coarse sand at 15 C in a growth chamber with 4,304-5,380 lx (400-500 ft-c) fluorescent and incandescent lights, 14 hours per day. The effect of temperature (5, 10, 15, 20, 25, and 30 C) on apothecial production was tested similarly. Biological trapping and infection potential of ascospores produced under field conditions was determined by exposing greenhouse-grown bean plants of different ages (6 to 40 days old) in 10-cm diameter clay pots for 4 or 11 days in areas where apothecia were abundant. Plants were then placed in a mist chamber in the greenhouse for 1 week and the percent of white mold recorded.

Infectivity of ascospores of *W. sclerotiorum* obtained from field or growth chamber-produced apothecia was tested by atomizing ascospore suspensions on bean plants in the blossom stage, followed by incubation for 1 week in a mist chamber at temperatures which varied between 20 and 24 C. If plants were in prebloom stage, an exogenous energy source such as steamed celery segments, turnip extract, or sucrose solution was provided. In almost all the greenhouse experiments reported in this study, the bean cultivar 'Cascade' was used. The ability of sclerotia in soil to serve as a source of inoculum to infect beans was determined by placing sclerotia in close proximity to bean seeds, on the soil surface, or randomly mixing them with natural field soil or greenhouse-steamed soil. Usually, such experiments were conducted in 10-cm diameter clay pots with three seeds/pot. Also, the effect of an available energy source such as steamed celery stem segments, bean blossoms, or cotyledons on infection by mycelium produced from sclerotia was similarly evaluated.

Effect of temperature on ascospore germination and germ tube growth was tested by allowing ascospores to naturally eject onto PDA plates and incubating these at the desired temperature; the percentage germination and germ tube growth were recorded by microscopic examination. The effect of temperature on mycelial growth was tested by placing 4-mm mycelial disks of the fungus in the center of PDA plates and incubating these at the desired temperatures. At preselected intervals, colony diameter of advancing colonies was recorded, and the number and wet weight of sclerotia produced were determined 10 days after inoculation.

Effect of temperature on lesion initiation and expansion was determined by placing disks from the margins of advancing colonies of the fungus growing on PDA plates on detached bean leaves in plastic moist chamber boxes. Leaves were placed on a wire mesh about 2 cm above the bottom of the box, and high humidity was maintained by addition of water in the bottom, and by lining the sides of the box with filter paper that extended into the water. The amount and kind of food base required by ascospores and mycelium for infection of beans, was determined by growing the fungus either on agar media containing extracts of autoclaved natural energy sources, or on defined media with a few selective carbon sources. Natural media tested were celery, carrots, cabbage, and potato segments; autoclaved seeds of beet, lettuce, cabbage or sunflower; and celery, sunflower, beet seed ball, or turnip extracts. Turnip extract agar (TEA) at different concentrations was used most often. TEA was prepared by incorporating an extract from peeled turnip tissues boiled in distilled water (1:1, w/v) for 1 hour. The extract was filtered through four layers of cheesecloth, diluted as desired, and solidified by the addition of 2 % Oxoid agar. Pathogenicity to beans of the fungus grown on each medium was tested by placing discs from margins of colonies onto detached leaves maintained in plastic boxes as described above or onto leaves of intact plants kept under continuous mist in the greenhouse. Also, the effect of several of the natural and defined media on fungal growth was recorded by measuring colony diameters and number of sclerotia produced.

From 5 July until mid-September, bean plants were inoculated twice a week in the field with inoculum comprised mostly of colonized autoclaved celery segments; ascospore suspensions also were used during the blossom stage. Colonized celery segments were usually placed at the leaf axils near the stem.

A weather station consisting of a continuously recording rain gauge, leaf-wetness recorder, hydrothermograph and soil thermograph was located at the edge of the experimental plot. Also, complete meterorological data were collected in 1972 and 1973 through the Federal Northeast Cooperative Weather Station, Geneva, N.Y. which was located at a research farm where white mold epidemics of bean occurred in

1972, but not in 1973. Weather data were also collected from two additional locations in 1973.

Presence of ascospores of the fungus on bean blossoms and leaf tissues was checked periodically at six different farms. Blossoms, and occasionally bean leaf disks, were placed directly on acidified PDA plates and regrowth of *W. sclerotiorum* was recorded. Usually, however, each bean leaf collected was rubbed with a steamed celery segment which was then placed on acidified PDA. This method was quite satisfactory when checked by recovery

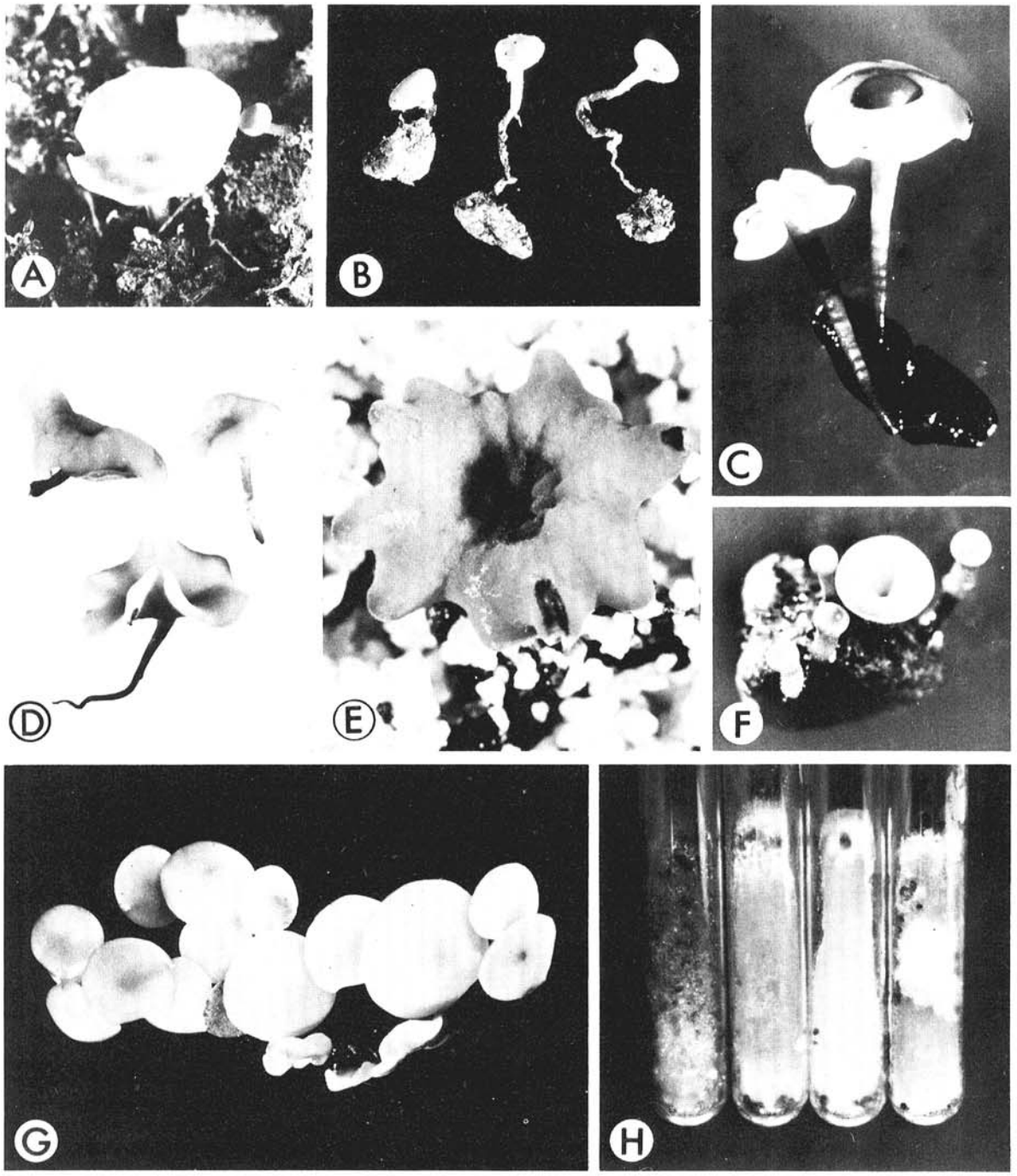

Fig. 1-(A to H). Gross morphological variation of *Whetzelinia sclerotiorum* in New York. **A,B,C)** Apothecia collected from bean fields. **D,E,F,G)** Apothecia produced in a growth chamber by field-collected sclerotia. **H)** Four single ascospore cultures representing the range of variation obtained on potato dextrose agar tubes.

of ascospores from artificially inoculated bean leaves under field and greenhouse conditions. Other methods and materials are described under appropriate sections in Results. Also, the number of replicates varied in different experiments and are indicated where data from each experiment are presented.

RESULTS.—*Production of apothecia; ascospores as primary inoculum.*—Several bean fields and adjacent areas where white mold epidemics occurred in 1972 were examined for apothecia at weekly intervals commencing in April and continuing throughout the 1973 growing season. Two of the fields were not plowed in the fall of 1972; thus, sclerotia produced on infected plants remained on the soil surface and were easy to locate. Apothecia of *W. sclerotiorum* were produced by sclerotia present in bean fields, but were also abundant in fruit orchards with sod culture. Apothecia were produced during early spring when soil moisture was maintained continuously at near saturation. Apothecia were found occurring naturally as early as 23 April and until about 15 June 1973. In the sod culture of apple orchards, sclerotia were often found around the base of dandelion plants and also near wild clover. Sclerotia collected during this same period that exhibited no signs of apothecial production, produced many apothecia in about 7-10 days when incubated in SDW at 15 C, indicating that they were ready to produce apothecia when given near optimum conditions. During April, May, and until 15 June 1973, intermittent showers kept the soil near saturation most of the time. However, after 15 June a prolonged drought occurred which continued throughout most of the growing season. No apothecia were found after 15 June 1973 in the same field locations where they had been found earlier. Furthermore, dried field-collected sclerotia from the same locations failed to produce apothecia when placed under near-ideal conditions in a growth chamber for as long as 3 months. Thus, it appears that drying of sclerotia greatly delayed, or possibly completely inhibited, apothecial production. However, sclerotia which failed to produce apothecia were viable as determined by growth of mycelium on acidified PDA plates.

Apothecia collected from the field differed considerably in size, shape, and color; as did those produced under growth chamber conditions by sclerotia collected from a single locality (Fig. 1-A to G). Similarly, single ascospore colonies grown on PDA slants varied considerably in appearance (Fig. 1-H). However, all were equally pathogenic on beans as tested by ascospore or mycelial disk inoculations.

Infection from ascospores under field conditions.—To demonstrate the infection potential of ascospores produced and released under field conditions, potted bean plants of different ages were exposed in the field for 4 or 11 days at two locations near Alton and Geneva, N.Y. where apothecia were abundant. Thirty-nine and 55% of the plants exposed for 4 and 11 days, respectively, to the natural inoculum (ascospores) at Geneva exhibited typical symptoms of white mold after 7 days of incubation in a mist chamber under greenhouse conditions. Also, 26% of the plants exposed to natural inoculum for 4 days at Alton developed white mold symptoms. There was a close positive correlation between plant age and percent of white mold infection. The average percent infection of 6, 12, 19, 22, 27 and 40-day-old plants was 4, 22, 34, 53, 68 and 100, respectively. Succulent greenhouse-grown plants suffered considerable injury from windburn during

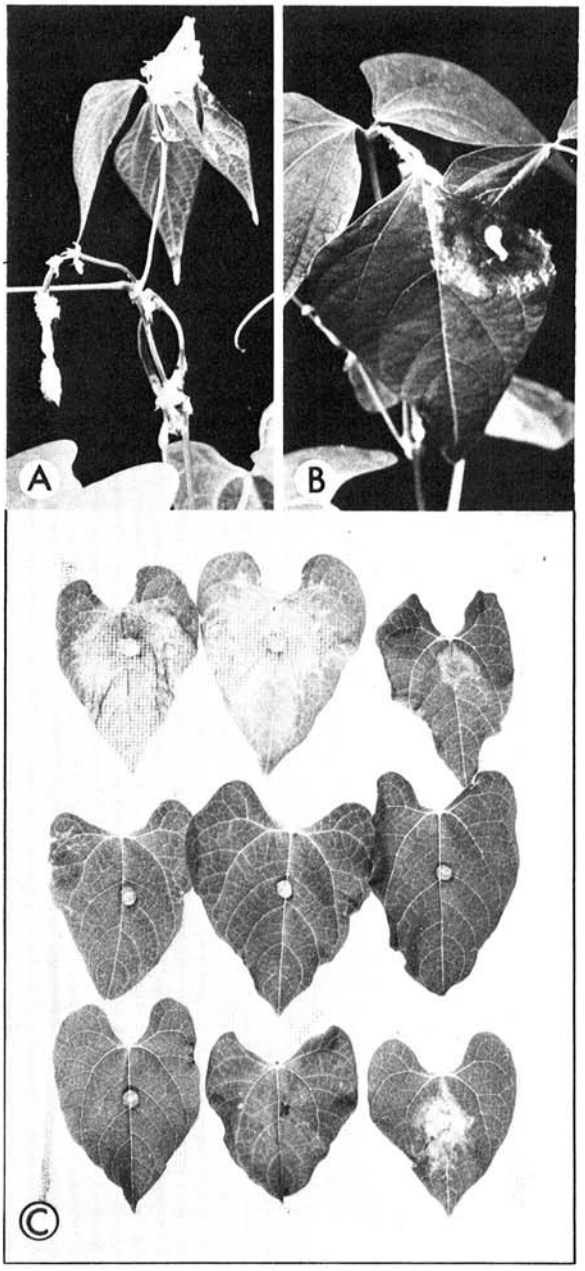

Fig. 2-(A to C). Relationship between nutrient availability and pathogenicity of ascospores of *Whetzelinia sclerotiorum* to beans. **A,B)** Symptoms and signs of infected blossoms and other plant parts in contact with infected blossoms. **C)** Lesion initiation and development in relation to level of nutrients in inoculum plugs. Dilutions of turnip extract agar were (top row L to R) 1:1 (w/v), 10^{-1}, and 10^{-2}; (middle row) 10^{-3}, 10^{-4}, and 10^{-5}; (bottom row) 10^{-6}, none (Oxoid agar), and 10^{-7} plus 1.0% each of glucose and asparagine, respectively.

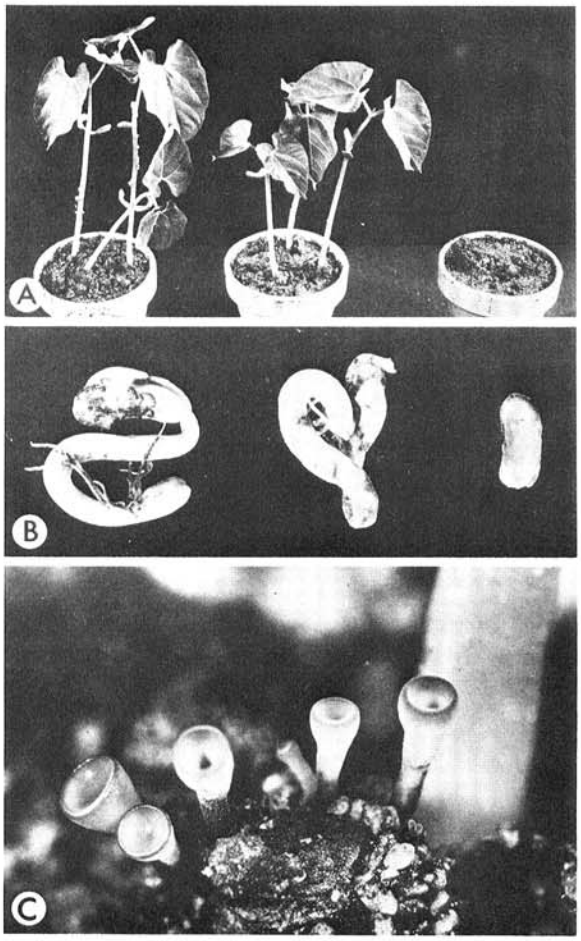

Fig. 3-(A to C). Role of sclerotia of *Whetzelinia sclerotiorum* in the initiation of disease under mist chamber conditions. **A)** L to R - Untreated check, sclerotia placed at seed level, and sclerotia placed in direct contact with steamed-celery segments which in turn were in contact with the seeds. **B)** Seed decay and pre-emergence damping-off symptoms which were induced only when sclerotia were placed in direct contact with steamed-celery segments. **C)** Apothecia produced by sclerotia placed in direct contact with bean seeds, but without an energy source. The bean stem on the right remained healthy.

field exposure which was more severe on the older, larger plants. This increased injury, plus the greater amount of tissue available to trap ascospores, probably accounts for the higher incidence of disease on the older plants. Although the 40-day-old plants had open blossoms, and the 27-day-old plants were in the green-bud blossom stage, most of the infections that occurred in this test were associated with injured areas, rather than with colonized blossoms.

Ascospores obtained from apothecia produced in the field or in growth chambers and atomized onto bean plants in the blossom stage, induced typical symptoms after the plants were incubated in a mist chamber for 7 days (Fig. 2-A, B). Signs of infection were usually evident in fully opened blossoms 2 to 3 days after inoculation. Also, cast blossoms lodged on leaves, petioles, axils of stems, or soil surface were colonized within the same period of time. Leaves injured mechanically or by heat prior to inoculation were also susceptible to infection by ascospores under mist chamber conditions.

Association between natural occurrence of ascospores on leaves and development of white mold.—An attempt was made to correlate the presence of primary inoculum of *W. sclerotiorum* on bean tissue during the growing season with subsequent development of white mold in six locations in central and western New York in 1973. One hundred or more bean blossoms and/or leaves were cultured as described previously to detect the fungus during prebloom and bloom stage at each location. The fungus was not recovered at five locations, but 12.7% recovery was obtained at one location. At harvest, several hundred plants at each location were examined closely for white mold symptoms. White mold developed (7.5% of the plants were infected) only at the location where ascospores had been recovered earlier.

Role of sclerotia in epidemiology of white mold.—The average number of sclerotia on the surface of nonplowed bean fields A and B, where severe epidemics occurred in 1972, was 16.1 and 5.4/30 cm^2, respectively; whereas after spring plowing only 0.2/30 cm^2 and none/30 cm^2 were found (10 replicates per nonplowed field, and 20 replicates/plowed field). The number of sclerotia/500 ml of soil (average of 10 replicates) in field B at depths of 0-2.5, 2.5-10, and 10-17.5 cm were 4.4, 1.2, and 0.3 (respectively) before plowing and 0.1, 0, and 0 after plowing, respectively. Sclerotia isolated from these fields, regardless of the depth at which they occurred, were viable as indicated by ability to produce apothecia when incubated in water under growth chamber conditions and also by production of mycelium when cultured on acidified PDA. The average number of apothecia produced by each sclerotium collected from the three depths was 3.3, 0.8, and 1.0, respectively. Similar results were obtained for field A.

Field- and laboratory-produced sclerotia repeatedly failed to infect bean plants grown in steam-treated or natural bean soil under greenhouse and mist chamber conditions, regardless of the number of sclerotia per 10-cm diameter pot (1 to 40) or the location of sclerotia; that is, next to bean seeds, on soil surface, or randomly mixed with the soil. Furthermore, bare sclerotia placed on the surface of intact or detached bean leaves, or in the axils of bean branches, failed to initiate infection after prolonged incubation in mist chambers. On the other hand, sclerotia placed next to bean seeds or seedlings, on or within the top 2.5-cm of the soil, produced abundant apothecia under continuous mist chamber conditions, Fig. 3-C. This did not occur under open greenhouse conditions where the plants were usually watered twice daily. In one experiment, sclerotia placed near bean seeds (2.0-2.5 cm below soil surface) produced an average of 3.2 apothecia per sclerotium; other sclerotia similarly placed near a piece of celery averaged 4.4 apothecia/sclerotium.

Infection of beans by sclerotial mycelium was successful only when a readily available energy source such as steamed celery stem segments, bean cotyledons, or other dead or senescent tissues were placed in direct contact between the sclerotium and a bean seed or bean stem (Fig. 3-A, B). Likewise, sclerotia placed in direct

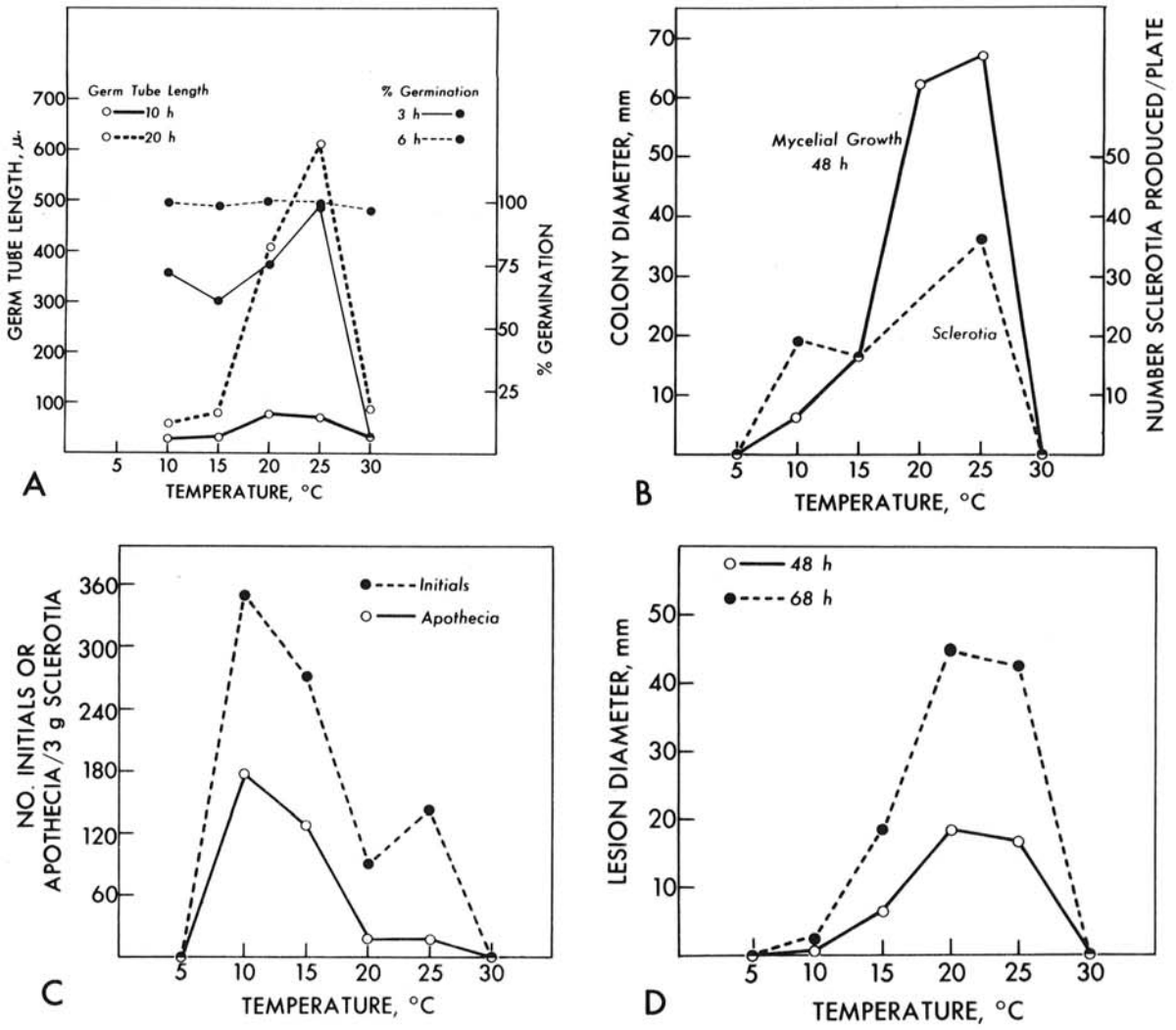

Fig 4-(A to D). Effects of temperature on the biology of *Whetzelinia sclerotiorum*. **A)** Germination and growth of germ tubes on potato-dextrose agar in relation to temperature and incubation period. **B)** Rate of mycelial growth and sclerotial production as influenced by temperature. **C)** Effect of temperature on apothecial production by sclerotia incubated in water. **D)** Relationship of temperature to lesion initiation and development on detached bean leaves in closed moist boxes.

TABLE 1. Effect of nutrient level of turnip extract agar (TEA), celery extract agar (CEA), and sunflower extract agar (SFEA) on initiation and development of lesions incited by *Whetzelinia sclerotiorum* on detached bean leaves[a]

	TEA		CEA		SFEA	
Conc.	No. infected/ no. inoculated	Avg. lesion diam after 48 hours (mm)	No. infected/ no. inoculated	Avg. lesion diam after 72 hours (mm)	No. infected/ no. inoculated	Avg. lesion diam after 72 hours (mm)
1×10^{-1}	68/68	20	5/10	40	9/10	49
8×10^{-2}	66/68	18	. . .	. . .	. . .	. . .
6×10^{-2}	64/68	17	10/10	40	8/10	29
4×10^{-2}	62/66	15	. . .	. . .	. . .	. . .
2×10^{-2}	35/61	7	9/10	43	9/10	24
1×10^{-2}	0/63	0	6/10	16	8/10	26
1×10^{-4}	0/66	0	0/10	0	0/10	0
1×10^{-6}	0/65	0	0/10	0	0/10	0

[a]Media were prepared as described for turnip extract agar in Materials and Methods.

contact with an energy source which was touching a bean stem at the soil line also caused lesions which rapidly progressed when continual misting was maintained. However, in many instances, sclerotia and the energy sources placed 1 cm away from the bean stem failed to cause infection.

Sclerotia collected from the field in early spring usually produced abundant apothecia when placed in water and incubated under growth chamber conditions favorable for apothecial production. The addition of an energy source such as steamed celery segments into the water surrounding the sclerotia did not inhibit or reduce apothecial production, but in contrast seemed to enhance the number and size of apothecia produced. Mycelial production by sclerotia in SDW was always very sparse and hardly evident except microscopically. Also, mycelial production from sclerotia plated on highly purified Oxoid water agar was comprised of only a few short strands and never advanced more than a few cm, even after prolonged incubation. In contrast, comparable sclerotia plated on nutritionally-rich agar media produced abundant mycelial growth and sclerotia. These results suggest that the food reserves in sclerotia are not available for mycelial growth, and that an exogenous source of energy must be provided for sclerotia to produce much mycelial growth. Furthermore, these results indicate that sclerotia, at least the large sclerotial type found exclusively on beans in New York, function primarily for production of apothecia, and that mycelium production is a secondary attribute requiring an exogenous energy source. Inasmuch as virtually all the infection of beans occurs on aboveground parts it seems highly unlikely that sclerotia are an important form of inoculum per se, but function essentially to produce airborne ascospores.

Nutrient levels required for infection.—In agreement with Natti's observations that infection in the field usually occurs through an intermediary of colonized bean blossoms (13), we observed that ascospores infected beans under mist chamber conditions through intact or detached bean blossoms. However, successful infections were readily obtained through tissues injured mechanically or by heat-scorching, or when other substrates such as autoclaved bits of celery, potato, carrot, or bean pods were substituted for bean blossoms. Also, infections were obtained when ascospore suspensions contained 1-2% sucrose or turnip extract. Likewise, ascospores ejected onto a water-agar medium readily colonized autoclaved beet seed balls or seeds of lettuce, sunflower, cabbage, or tomato. When such colonized substrates were placed on bean leaves under mist chamber conditions typical lesions developed within 2 or 3 days. Observations of the rate of expansion of lesions, indicated that colonized beet seeds were the best source of inoculum in comparison with the other types, but all consistently produced infections.

To determine more precisely the level of nutrients required for infection, various dilutions of turnip extract were tested. TEA plates were inoculated either by showering with ascospores, with disks of the fungus, or with sclerotia. After 2 or 3 days growth, disks from these plates were placed on plants maintained either under mist chamber conditions or on detached leaves enclosed in moistened plastic boxes. Lesions were consistently produced and the rate of lesion expansion on the 10^{-1} TEA was about the same as that induced by cornmeal agar disks, and only slightly less than those produced by disks from PDA cultures. The average lesion diameter produced by the fungus growing on PDA and CMA disks was 25 and 20 mm, respectively, after 48 hours of incubation. However, disks of the fungus growing on 10^{-2} TEA either failed to initiate lesions or those produced were quite small and remained small regardless of the time of incubation (Fig. 2-C). Additional experiments revealed that TEA containing a 2×10^{-2} dilution of turnip extract consistently produced small lesions while a similar medium containing a 10^{-2} extract often failed to induce

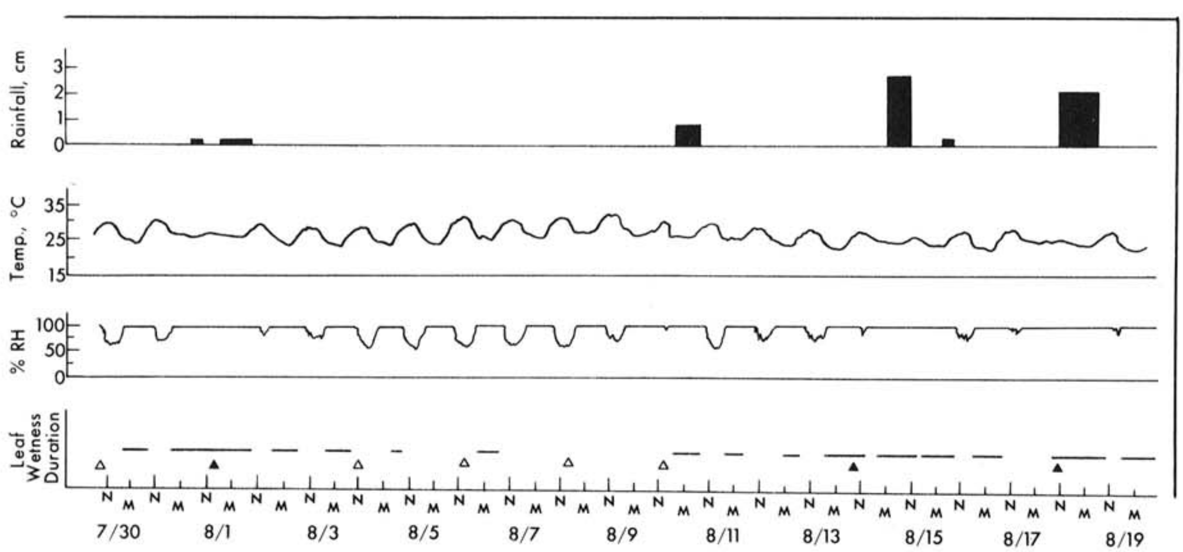

Fig. 5. Relationship of environmental conditions to successful (▲) and unsuccessful (△) artificial field inoculation of beans with *Whetzelinia sclerotiorum* during a 21-day inoculation period in the summer of 1973.

lesions despite prolonged incubation (Table 1). Similar results were obtained when extracts of celery, sunflower, or beet seeds were utilized, but the concentrations required for consistent infection varied with the different substrates from which the extracts were made. Oxoid agar is required in these tests because Difco water agar supported restricted growth due to impurities which allow the fungus to initiate lesions as quickly and as large as those produced by 2×10^{-2} TEA.

Microscopic examinations of ascospores ejected onto the surface of dialysis tube segments appressed to the surface of various dilutions of TEA revealed considerable differences in the amount and rate of growth. Appressoria-like structures were formed on the 10^{-3}, but not on the 10^{-4} TEA. Inasmuch as infection occurred only when more concentrated media were utilized, it appears that the appressoria apparently produced on the 10^{-3} TEA were not functional for infection of bean leaves. The rate of mycelial growth on the TEA media was closely correlated with the level of nutrients present, but sparse mycelial growth was produced even at the highest dilution (10^{-6}) of TEA.

Temperature and biology of W. sclerotiorum.—Ascospores ejected onto PDA plates germinated equally well (97% or more) at all temperatures tested when examined after 6 hours of incubation. However, after only 3 hours differential rate of germination of 98 and 6% occurred at 25 and 30 C, respectively (Fig. 4-A). After 10 hours, incubation germ tube growth was equally good at 20 and 25 C; however, after 20 hours of incubation, 25 C was best and growth was poor at 5, 10, and 30 C (Fig. 4-A). Mycelial growth, as indicated by colony diameter and number of sclerotia produced, was best at 20-25 C (Fig. 4-B). No measurable growth occurred at 30 C, even after 10 days incubation, but slight growth occurred at 5 C. More sclerotia were produced at 25 C than at 10 and 15 C. However, those produced at the lower temperatures were larger and heavier than those produced at 25 C. Average percent germination, germ-tube growth, and mycelial growth were calculated on more than 100, 20, and 10 measurements, respectively.

Lesions on detached bean leaves incubated in moist boxes developed about equally well when incubated at 20 C and 25 C (Fig. 4-D). No lesions developed at 30 C or 5 C, but very small (average diameter, 28 mm) expanding lesions did develop after 5 days of incubation at 10 C. Also, the minimum contact period between leaf surfaces and inoculum was 48, 24, 24, and 9 hours, respectively, at 10, 15, 20, and 25 C. At each temperature, eight inoculated and noninoculated leaves were used. This experiment was repeated and similar results were obtained.

Temperature and production of apothecia.—Field-produced sclerotia incubated in water at different constant temperatures produced the greatest number of initials and mature apothecia at 11 C; however, production at 15 C was quite good (Fig. 4-C). No apothecial initials were produced at either 30 or 5 C after 21 days of incubation. However, sclerotia that had incubated at 30 C initially produced a low number of apothecia (1.3 per sclerotium) after transfer to 15 C. Also, sclerotia initially incubated at 5 C produced spindly initials 2-3 months later, and normal apothecia were produced after transfer to 15 C. Data reported at each temperature are based on 3 g of sclerotia in which there were about 30 to 35 sclerotia.

Effect of moisture on initiation and development of infection.—Repeated inoculations of detached leaves or leaves, stems, and petioles of intact plants, with colonized tissues or agar disks, were not successful except when moisture was maintained continuously at the interface between the inoculum source and the surface of the inoculated tissues. Furthermore, even after lesions had formed, development was stopped abruptly if the surface of the inoculated tissue was allowed to become dry. RH, even near 100%, was not sufficient for lesion initiation and development. Likewise, successful inoculation with ascospores not only required free moisture, but for a much longer period of time (approx. 48-72 hours) before any visual signs of infection were evident. Similarly, dry colonized tissues or arrested lesions required 48 to 72 hours of continuous moisture before lesion initiation or expansion was evident.

In the summer of 1973, beginning on 5 July and thereafter, bean plants were inoculated twice a week in the field. The inoculum consisted of colonized celery stem bits and on a few occasions ascospore suspensions were atomized onto plants with blossoms in various stages of development. A total of 21 inoculations were made during July and August and the first two weeks of September. Lesions developed on only three inoculation dates in August; i.e., 1, 14, and 18 August. Examination of weather data collected from a weather station maintained in the same field revealed that the critical and only weather measurement correlated with infection was length of leaf wetness (Fig. 5). The duration of leaf wetness during the successful inoculation periods of Aug. 1, 14, and 18, was 16, 41 out of 48, and 25 hours, respectively. During each successful inoculation period, rain fell within the next 24 hours after inoculation, and temperatures fluctuated between 17-23 C (Fig. 5). The number of plants infected out of those inoculated on 1, 14, and 18 August were 12/12, 18/25, and 14/48, respectively. An inoculation with an ascospore suspension on 1 August resulted in only 3/51 infected plants. This result was not unexpected, however, because, in our experience, ascospores require longer periods of leaf wetness than other inoculum for infection, even under mist chamber conditions. An overall evaluation of data concerned with the effect of moisture on infection of beans in moist boxes, mist chambers, and under field conditions demonstrated that free water was required for initiation and development of white mold lesions on beans.

DISCUSSION.—Moisture is the most important climatic factor in the development of white mold epidemics under New York conditions. It is essential for production of ascospores, and for initiation and development of infection. Continuous moisture is required for apothecial formation, as indicated by the fact that even a slight osmotic water stress prevented apothecial formation (7), and that after desiccation sclerotia failed to produce apothecia even when incubated under near-optimum conditions for 2-3 months. Trevethick and Cooke (19) reported that the sclerotial rind does not restrict water loss and that sclerotial

survival during desiccation depends on resistance of individual cells rather than on the sclerotium as a whole. It is possible, therefore, that the sclerotium cells responsible for apothecial formation are not as resistant to desiccation as those responsible for mycelial regeneration. This may be due to physiological differences between these cells, or merely to their location or cell-wall composition.

Sclerotia were present both in old bean fields and in orchard sod culture, but more apothecia were produced in the latter situation. This was partly because most of the sclerotia in bean fields were turned under during land preparation for planting, and those that remained at or near the soil surface were more subject to fluctuations in moisture. Several other factors known to influence survival and germination of sclerotia of *W. sclerotiorum* were recently reviewed by Coley-Smith and Cooke (5).

Several workers (2, 12, 20) have reported that mycelium from sclerotia in soil infect beans, while others (6, 9, 14, 16) have reported direct infection of leaf tissues by ascospores. Our observations in New York indicate that sclerotia do not play an important role in initiating infections by the production of mycelium, because most infections occur aboveground. Furthermore, mycelial production by sclerotia was negligible except when an exogenous energy source was provided. Bare sclerotia failed to infect, even after prolonged incubation under near optimum conditions in a continuous mist chamber.

Natti (13) suggested that high amounts of rainfall in July were more important in influencing the amount of white mold than was rainfall in August. This suggestion was based on the fact that rainfall in July, 1966 and 1967 averaged about 9 cm each year, whereas August rainfall fluctuated between 4.3 and 13.5 cm. Despite these great differences in rainfall in August approximately 90% of the plants were infected each year by the end of August. A reasonable explanation for these results is that heavy rainfall in July results in apothecial production and in most years there are sufficient periods of dew formation in August to allow infection when blossoms and inoculum are present. It seems unlikely, however, that much infection would occur in the complete absence of rain in August because approximately 48-72, 16-24, and over 72 hours of continuous leaf wetness are required for the establishment of infection by ascospores, moist colonized tissues, and dry colonized tissues, respectively. Numerous other reports have stressed the importance of moisture in the development of white mold on beans (4, 7, 8, 11, 12, 13, 18, 21). In addition to weather parameters that affect the duration of leaf wetness, many other factors that also affect air movement, and thus duration of leaf wetness, appear to be involved. In New York State for example, white mold of beans is most prevalent in fields with heavy vegetative growth and in areas where air circulation is limited, such as low lying fields and particularly those surrounded by uncultivated wooded areas.

All of these factors considered together appear to explain why white mold of beans was epidemic in New York in 1972, but not 1973. During 1972, infection in some fields was as high as 68%. However, despite an extensive search in central and western New York during 1973, only a low incidence of disease (7.5%) was found in one late-planted field. Rainfall during June, July, and August was much more abundant in 1972 than in 1973, and no other significant differences in weather parameters between the 2 years was evident. For example, at one location near Geneva, total rainfall in May, June, July, and August was 9.6, 27.0, 9.6, and 9.7 cm (respectively) in 1972, and 9.4, 9.0, 4.1, and 6.3 cm (respectively) in 1973.

Our results suggest that abundant rainfall, particularly in June and July, is necessary for apothecial production because continuous water saturation of soil and sclerotia is required. Once ascospores are produced, they need not infect bean tissue immediately because both the germinated and ungerminated ascospores are capable of surviving desiccating conditions for considerable periods of time (7). This may explain why Natti concluded that airborne mycelium present in soil organic matter is a primary source of inoculum (13). This conclusion was based primarily on his failure to find apothecia in bean fields just prior to a sudden appearance of white mold. It seems likely that after ascospores have been produced and distributed and blossoms are available as an energy source, all that is needed to initiate an epidemic is sufficient duration of leaf wetness; thus, total rainfall in August may not be a limiting factor. Length of duration of leaf wetness when inoculum is present probably is more important than total rainfall.

Our results showing the dependence of ascospores and other types of inoculum of *W. sclerotiorum* on an exogenous energy source to initiate infection on beans is in agreement with reports of other workers (3, 12, 13, 14, 16, 17). Our demonstration that infection requires critical concentrations of the energy source, and that infection was precluded by one-fold dilution differences, suggests that critical adjustment of energy levels might facilitate detecting different levels of tolerance in germ plasm that is being screened for resistance.

Temperature exerted a significant effect on apothecial formation, ascospore germination and growth, and initiation of infection and expansion of lesions. It seems unlikely, however, that temperature per se would be a limiting factor under New York conditions: many years of weather records show that temperatures during the growing season are seldom too high or too low for the activities of *W. sclerotiorum*. Numerous reports in the literature concerning the effect of temperature on apothecial production by sclerotia of *W. sclerotiorum* are summarized by Coley-Smith and Cooke (5).

To be able to predict more precisely the incidence and severity of white mold, several gaps in our information need further study. While we are reasonably certain that primary inoculum is ascosporic, we need more information concerning the survival of ascospores under fluctuating field conditions. Also needed is additional information concerning the effect of drying on ability of sclerotia to form apothecia and the length of continuous wetting necessary for apothecial production after desiccation of sclerotia. Data obtained thus far suggest that a prolonged conditioning period may be required for sclerotia to produce apothecia after they have been subjected to drying. If so, epidemics of white mold would not likely occur in years when sclerotia have been severely desiccated during midsummer.

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