

## Influence of Water Potential on Growth and Survival of *Whetzelinia sclerotiorum*

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### ABSTRACT

Ascospore germination of *Whetzelinia sclerotiorum* was not adversely affected by osmotic potentials ( $\psi$ ) as low as -56 bars, but was reduced by 71% at -73 bars and completely inhibited at -91 bars. Mycelial growth was progressively stimulated as  $\psi$  was decreased from -1 to -14 bars, but growth was reduced at lower  $\psi$  values. Optimum growth was reduced 50% at  $\psi$  of -37 to -47 bars and was nil after 3 days at -91 bars. However, with longer incubation (2 to 3 weeks), measurable growth occurred even at -100 bars. Sclerotia were produced on agar media with  $\psi$  from -1 to -64 bars, but not at -73 bars. Apothecia were produced at 0, but not at -6 bars. Expansion of lesions on bean leaves was progressively increased as  $\psi$  in inoculum plug was decreased from -1 to -24

bars, and was not adversely affected until the  $\psi$  was reduced below -56 bars. However, lesion development was completely prevented at -91 bars. The different osmotica [KCl, sucrose, and salt mixture (KCl, NaCl, Na<sub>2</sub>SO<sub>4</sub>)] and different basal media (cornmeal agar or turnip extract agar) which were used produced similar effects at comparable  $\psi$ . Survival of ascospores ejected onto coverslips or dried bean blossoms was greater at lower relative humidities. Survival of germinated ascospores was closely correlated with length of incubation period and nutrient level of the incubation media, and was associated with the formation of appressoria and other unidentified resistant structures.

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*Whetzelinia (Sclerotinia) sclerotiorum* (Lib.) Korf and Dumont (12) is generally recognized to be most damaging under wet weather conditions (6, 7, 16, 17, 19, 27, 28). The fungus can function either as a soil-borne or as an air-borne pathogen, but on beans under New York conditions it operates almost entirely as an aboveground parasite that infects through senescent blossoms or wounds (19). Abawi and Grogan (1) reported that white mold epidemics in New York are initiated by ascospores produced mainly by sclerotia present under duff outside bean fields. Moore, et al. (18) suggested that ascospores of *W. sclerotiorum* can withstand drying for considerable time and initiate infection upon resumption of wet weather conditions. They presented no data to substantiate this, however. Partyka and Mai (20)

reported that ascospores of *W. sclerotiorum* produced by sclerotia collected from potato fields survived for 119, 94, and 64 hours when discharged onto sterile coverslips and stored at 60, 80, and 98% relative humidity (RH), respectively.

Numerous reports deal with the effect of water potential on growth, survival, and pathogenicity of various plant pathogens, particularly soil-borne pathogens (8, 9), but similar data on *W. sclerotiorum* are lacking. Preliminary inoculation tests conducted in closed moist chambers utilizing colonized agar disks or steamed celery pieces placed on detached bean leaves failed to infect when temperature fluctuation resulted in slight drying between the interface of inocula and leaf surfaces. Also, infection of intact plants was not possible

except under mist-chamber conditions. This led us to suspect that *W. sclerotiorum* is sensitive to water stress.

This paper deals with the influence of osmotic potential ( $\psi$ ) on the growth of *W. sclerotiorum* in vitro from ascospores, sclerotia, and mycelial disks; production of apothecia; initiation and expansion of lesions on bean leaves; and the effects of ambient relative humidity on survival of ungerminated and germinated ascospores after growth for various times on different concentrations of nutrient media. Results indicate that *W. sclerotiorum* is biologically quite active and even stimulated on artificial media of reduced  $\psi$ ; thus the apparent requirement for free water for successful infection seems anomalous.

**MATERIALS AND METHODS.**—A large sclerotial isolate of *W. sclerotiorum* (isolate WS-3) sensu Purdy (22) obtained from field-infected snap beans was used throughout this study. The fungus was maintained by periodic transfers on Difco potato-dextrose agar (PDA) at 20-22 C. Apothecia were produced from sclerotia grown at 15 C on autoclaved wheat, celery, carrots, or beans. To produce apothecia, sclerotia were placed either in sterile distilled water (SDW) or in moist coarse sand and held at 15 C in a growth chamber with 4,304-5,380 lx (400-500 ft-c) fluorescent and incandescent lights 14 hours/day; a minimum of 60 days was usually required for production of apothecia.

In most of the  $\psi$  experiments, the basal medium was Difco cornmeal agar containing 19 g of dehydrated material per liter which had a  $\psi$  of about -1 bar. However, turnip extract agar (TEA) also was used in a number of experiments. This medium 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% Difco or oxid agar.

The  $\psi$  of agar media were adjusted by adding required amounts of sucrose, KCl, or salt mixtures (NaCl, KCl, and  $\text{Na}_2\text{SO}_4$ ) as molal concentrations specified by Scott (26). The  $\psi$  of prepared media was calculated from the total relative water activities of these solutions ( $A_w$ ) as published by Robinson and Stokes (25) and Scott (26). About 20 ml of each medium were placed in plastic disposable petri dishes (15 × 100 mm). Inoculum was placed in the center of each dish and germination, colony diameter, and other measurements were made at preselected intervals. Plates were placed in plastic bags to reduce evaporation and, unless otherwise stated, were incubated at 25 C.

To determine the effect of  $\psi$  on ascospore germination and growth, sterilized coverslips showered with ascospores of *W. sclerotiorum* were placed so that ascospores were in contact with the medium. The effect of  $\psi$  on mycelial growth and sclerotial formation was determined by placing 4 mm disks of inoculum from the edge of young colonies on very low-nutrient media (Difco water agar, or  $10^{-4}$  TEA), in the middle of plates containing the osmotically modified media. The effect of different  $\psi$  on the germination and growth of laboratory-produced sclerotia was similarly determined.

The effect of  $\psi$  on apothecial formation by sclerotia was determined by placing overwintered field-collected

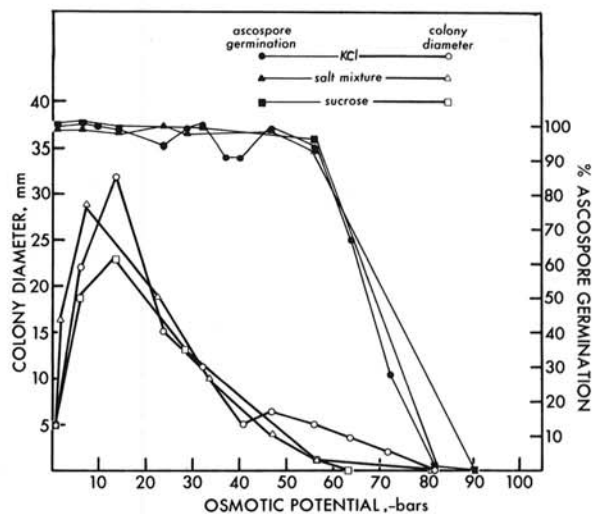


Fig. 1. Effect of osmotic potential ( $\psi$ ) on ascospore germination and radial growth of *Whetzelinia sclerotiorum* from ascospores on cornmeal agar amended with sucrose, KCl, or salt mixtures (NaCl, KCl, and  $\text{Na}_2\text{SO}_4$ ). Germination of ascospores and growth were determined after 9-12 and 59 hours of incubation, respectively.

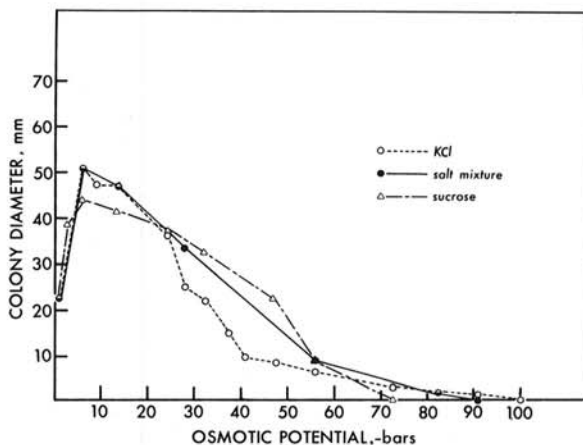


Fig. 2. Effect of osmotic potential ( $\psi$ ) on radial growth of *Whetzelinia sclerotiorum* from mycelial disks on cornmeal agar amended with three osmotica. Colony diameter was recorded after 25 hours of incubation.

sclerotia in SDW with different  $\psi$  in sterile glass petri dishes. Different  $\psi$  were obtained by addition of appropriate amounts of KCl, salt mixtures, or sucrose as previously described. Usually 10 overwintered sclerotia were placed in each dish, and the dishes were incubated in a 15-C growth chamber with lights. Controls consisted of comparable sclerotia incubated in SDW or water agar.

Survival of ascospores under different  $\psi$  was determined by ejecting ascospores onto coverslips or dried propylene oxide-sterilized bean blossoms. The  $\psi$  was adjusted by equilibrating the materials over salt solutions of various equilibrium humidities (RH). The different RH's tested (98.5, 85, 75.5, 64, 55, 46.5, 32.5,

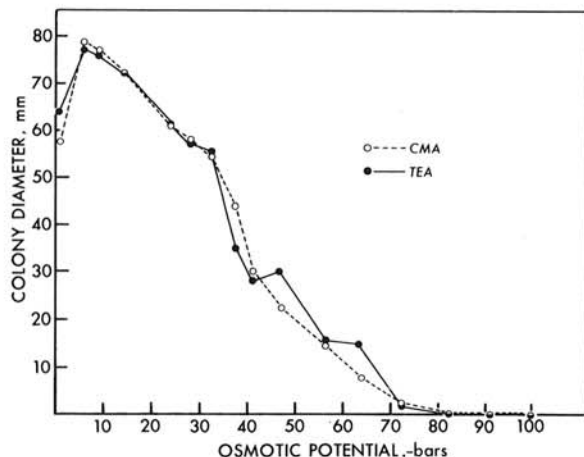


Fig. 3. Growth of *Whetzelinia sclerotiorum* on cornmeal agar (CMA) and turnip extract agar (TEA) adjusted to different osmotic potential ( $\psi$ ) with KCl. Colony diam was recorded after 72 hours incubation.

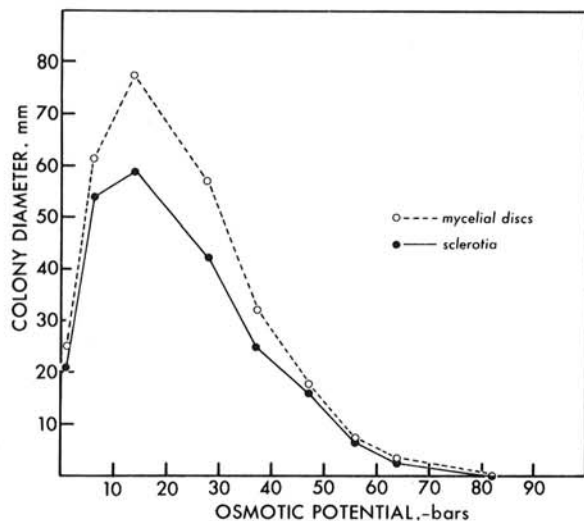


Fig. 4. Growth of *Whetzelinia sclerotiorum* from sclerotia or mycelial discs on turnip extract agar adjusted to different osmotic potential ( $\psi$ ) with KCl. Colony diam was recorded after 64 hours of incubation.

22.5, and 7%), were maintained in closed containers (Coplin jars) over pure water or saturated solutions of  $K_2SO_4$ , KCl, NaCl,  $NaNO_2$ , glucose, KCNS,  $MgCl_2$ , K-acetate, and NaOH, respectively, as reported by Winston and Bates (30). All tests were done at about 25 C. The relationship between RH and  $\psi$  values were calculated from the formula presented by Brown (6) in which each 1.0% decrease of RH equals approximately -14 bars. A long strip of modeling clay appressed to the inside of the jar supported the coverslips with ascospores above the surface of the saturated salt solutions. The jars were covered and sealed with Vaseline after insertion of the samples. At different intervals, the coverslips were removed and inverted onto the surface of acidified PDA

plates. Percentage spore germination usually was determined 9-12 hours later. Bean blossoms inoculated with ascospores were maintained above saturated salt solutions in deep storage dishes, or on top of large coverslips held in closed Coplin jars as described for the ascospore tests.

Survival of ascospores on bean blossoms was determined as ability to infect, by placing inoculated blossoms on detached bean leaves (cultivar, Cascade) in covered plastic dishes, or on leaves and petioles of potted plants maintained in a mist chamber under greenhouse conditions. Leaves in the plastic boxes 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 of the boxes. The sides of the boxes were lined with filter paper that extended into the water. The filter paper and inoculated leaves were kept wet continuously by misting with water as necessary with a fine atomizer.

The effect of length of incubation and nutrient level on the survival of germinated ascospores was studied on agar media. Surface-sterilized dialysis tube segments were placed on the surface of serial tenfold dilutions of TEA plates. The plates, showered with ascospores ejected from apothecia, were incubated at 25 C. After various periods of incubation, the dialysis tube segments were removed and placed right side up in empty petri dishes and maintained in a chamber at 98% RH. After various periods, the dry dialysis segments were inverted onto acidified PDA plates; germination and growth were recorded micro- and macroscopically.

The effect of  $\psi$  on pathogenicity was determined by growing the fungus on agar media with different  $\psi$ , and placing disks from the margin of colonies on detached bean leaves or on leaves of intact plants in greenhouse mist chambers. The detached leaves were kept moist by misting with SDW or with solutions of the same  $\psi$  as that in the inoculum disks. Further modification of this test was made by soaking the agar disks of *W. sclerotiorum* grown on the basal medium in salt solutions with different  $\psi$  for 2 hours before placing them on bean leaves. Other procedures used for specific experiments are described under the appropriate section under Results. All reported percentages are based on over 100 measurements per treatment and all other growth and pathogenicity measurements were usually based on six replicates per treatment in each experiment. However, replicate numbers varied between 3 and 10.

**RESULTS.—Osmotic potential ( $\psi$ ) and germination and growth of ascospores.**—Percentage and rate of germination of ascospores of *W. sclerotiorum* were not affected appreciably by lowering  $\psi$  from -1 to -56 bars. However, germination was appreciably reduced at -64 bars and lower  $\psi$  values, and was completely inhibited between -82 and -91 bars (Fig. 1). The observed effect was similar, with the different osmotica (KCl, salts mixture, and sucrose) and thus was apparently due to differences in  $\psi$  rather than to specific salt effects.

Growth of germ tubes was generally stimulated from -1 to -14 bars but decreased progressively with further decreases in  $\psi$ . Growth was reduced by about 50% of optimum at -28 bars and was essentially nil at  $\psi$  of -64 to -72 bars, as determined by measurements made after 59 hours of incubation (Fig. 1). After 10 days incubation, considerable growth had occurred at -82, but was nil at

-91 bars. As with the germination of ascospores, growth of germ tubes was affected similarly by the different osmotica of comparable  $\psi$ .

**Osmotic potential ( $\psi$ ) and mycelial growth.**—Mycelial growth was stimulated from -1 to -9 bars, and sometimes by -14 bars, but was decreased progressively with further lowering of  $\psi$  values (Fig. 2, 3, 4). Fifty percent reduction in optimum growth resulted between -37 and -47 bars, and growth was nil after 3 days incubation at -91 bars. However, considerable growth was evident 2-3 weeks after inoculation, even at -100 bars. Growth response was essentially the same on agar plates amended with the different osmotica with comparable  $\psi$ . Also, growth stimulation or reduction at different  $\psi$  was not affected by the different basal media used (CMA or  $10^{-1}$  TEA) (Fig. 3). KCl was used to adjust the  $\psi$  of both media. Growth experiments were repeated several times and similar results were obtained.

**Osmotic potential ( $\psi$ ) and sclerotial production and growth.**—Sclerotia were produced on media with  $\psi$  ranging from -1 to -64 bars. However, production was poor at -56 bars and lower  $\psi$ , as indicated by the small size and numbers of sclerotia formed. Sclerotia were not produced in agar media at -64 bars when ascospores were used as inoculum; however, a few small sclerotia were produced when mycelial disks were the inoculum. But no sclerotia were produced at -73 bars. Greater numbers of sclerotia were produced at -24 bars and lower  $\psi$  than at higher  $\psi$  values, regardless of the inoculum used. Sclerotial production on media amended with KCl or salt mixtures was similar, but greater numbers of sclerotia were produced on media of comparable  $\psi$  amended with sucrose. All sclerotia produced, regardless of  $\psi$  and solutes used, were viable as determined by mycelial regrowth on acidified PDA plates. Sclerotial germination and growth on  $10^{-1}$  TEA with different  $\psi$  obtained with KCl was essentially the same as that from mycelial disk inocula (Fig. 4). However, colony diameter originating from sclerotia was less at -1 to -37 bars than from mycelial disks. The best growth occurred at -14 bars, and ~50% reduction in optimum growth occurred at -37 bars. Growth was poor at -64 bars and was not measurable at -82 bars after 64 and 96 hours of incubation. Very poor growth occurred after 120 hours of incubation at -82 bars.

**Osmotic potential ( $\psi$ ) and apothecial formation.**—The effect of  $\psi$  on apothecial formation was tested by placing field-collected sclerotia in SDW adjusted to different  $\psi$  with KCl, salts mixture or sucrose. After 6 weeks incubation in a growth chamber, 70% of the sclerotia bathed in SDW alone had produced an average of 5.7 and 4.6 apothecial initials and mature apothecia/sclerotium, respectively. However, no apothecia were produced at  $\psi$  values of -6 bars and lower, and only an average of 0.5 apothecial initials per sclerotium was produced at -6 bars. All osmotica produced similar results by completely inhibiting apothecial formation at  $\psi$  values of -6 bars and lower. Unfortunately,  $\psi$  between 0 and -6 bars were not tested in a liquid medium. However, similar sclerotia placed on 2.0% water agar medium produced a few apothecia.

**Osmotic potential ( $\psi$ ) and lesion formation.**—Inoculation of bean leaves with agar disks of *W. sclerotiorum* grown on media with different  $\psi$

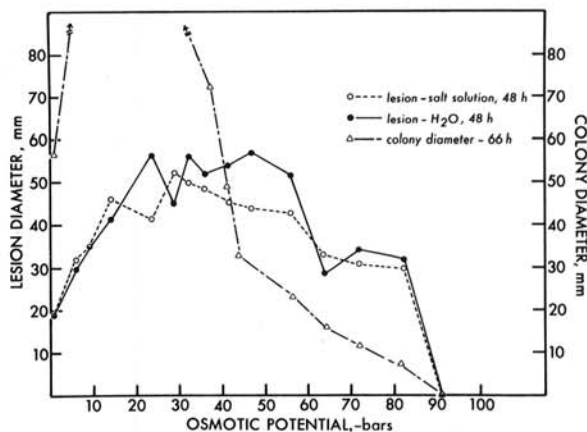


Fig. 5. Effect of osmotic potential ( $\psi$ ) of cornmeal agar adjusted with KCl on growth of *Whetzelinia sclerotiorum* in vitro and on lesion development when inoculum disks were moistened either with sterile water or KCl solutions of the same  $\psi$  as in the inoculum disks. Lesion diam recorded after 48 hours of incubation.

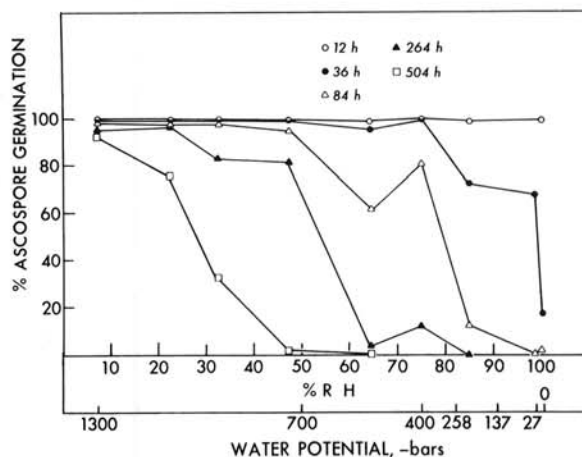


Fig. 6. Effect of relative humidity (RH) on survival of *Whetzelinia sclerotiorum* ascospores ejected onto coverslips and held in closed containers with RH adjusted with saturated salt solutions on different equilibrium humidities.

significantly affected lesion development (Fig. 5). Lesion diameter was progressively increased with decreasing  $\psi$  in the medium (CMA) from -1 to -24 bars. No significant decrease in lesion diameter resulted from inocula grown on media with  $\psi$  from -24 to -56 bars. Lesion diameter was progressively decreased as  $\psi$  was decreased below -56 bars, and was completely prevented at -91 bars. Similar results were obtained when TEA was used as the basal medium. Misting leaves with SDW or salt solutions with  $\psi$  equal to that of the agar medium used to produce the inoculum did not significantly change the diameter of the lesions produced (Fig. 5). Lesions produced on primary leaves were always smaller than lesions on young trifoliate leaves, irrespective of the  $\psi$  of the inocula or misting solutions utilized. Also, there was a close



correlation between mycelial growth of the fungus on the  $\psi$  media and the diameters of the lesions produced on plants (Fig. 5). However, near-optimal  $\psi$  for lesion formation covered a broader range than for mycelial growth in vitro.

Soaking mycelial disks of the fungus grown on basal medium for 2 hours in different concentrations of KCl also affected lesion development, but not to the same extent as when the fungus was grown on media with comparable  $\psi$ . However, significant differences in lesion diameter were produced when soaked disks were misted with SDW or solutions with similar  $\psi$  as that of the bathing solution. When SDW was used as a misting solution, diameter of lesions increased as the  $\psi$  of the bathing solution decreased from 0 to -56 bars, and lesion diameter was progressively decreased by lower  $\psi$  values of the bathing solution. In contrast, diameter of lesions developed from disks misted with KCl solutions with comparable  $\psi$  as that of bathing solutions increased as  $\psi$  of the bathing solution decreased from 0 to -24 bars, but progressively decreased with further decreases in  $\psi$  of the bathing solution. Lesion diameters produced by soaked disks was generally less than those produced by unsoaked disks irrespective of the  $\psi$ ; this result probably is due to the leakage of nutrients from soaked disks (Abawi and Grogan, unpublished).

Similar experiments to determine the effect of  $\psi$  on the size of lesions formed were performed on leaves of intact plants maintained under continuous mist conditions in the greenhouse. The results were in agreement with those obtained on detached leaves in closed moist chambers, although not as consistent. Generally, size of lesions increased as  $\psi$  of medium was decreased to -28 to -37 bars, but consistent reduction of lesion diameter occurred at  $\psi$  values of -56 and lower.

*Effect of RH on survival of ungerminated ascospores.*—1) On coverslips.—Ascospores ejected onto sterile coverslips survived for longer periods of time at the lower RH (Fig. 6). Percentage germination after desiccation for 21 days was 93, 77, 32, 2 and 0 at 7, 23, 33, 47, and 64% RH, respectively. Nearly all of the ascospores incubated for 60 hours at both 98.5 and 100% RH germinated on the coverslips. This was probably due to condensation water. The percent survival of these germinated ascospores as indicated by regrowth on PDA was 5 and 8% at 98.5 and 100% RH, respectively, after 60 hours of incubation. No germination of ascospores on coverslips was evident at any of the other RH's tested. At 85% RH, only 0.8% ascospores survived for as long as 6 days, but survival after 11 days of incubation at 75.5 and 64% RH was 13 and 4%, respectively. However, none of the spores had survived either RH when tested for regrowth after 14 days. In another test, the percentage of ascospore survival after 5 days was 0, 0, 0, and 86% at 100, 98.5, 75.5, and 32.5% RH, respectively. All tests were done at about 25 C; but in a separate experiment, ascospores suspended in SDW and stored at 5 C or in a frozen state survived for 10 and 4 weeks, respectively. Thus, the effect of RH at different temperatures needs further testing.

2) Survival on bean blossoms.—Bean blossoms were sterilized with propylene oxide and dried in an oven at moderate temperatures. Ascospores were ejected from apothecia onto the blossoms which were then subjected to

different RH's maintained over saturated salt solutions as previously described. Ascospore survival was tested at different time intervals, as in the survival studies on coverslips. Here again, the ascospores survived longer at the lower RH's, but none survived for 33 days at any RH tested. However, those stored at 7, 22.5, or 32.5% RH survived for 21 days and the highest survival was in those stored at 7.0% RH. Ascospores incubated at 64% RH survived for 7 days, but those stored at 75.5, 85, or 98.5% RH survived for only 65 hours.

3) Survival on bean leaves.—Bean plants maintained under laboratory conditions were showered with ascospores ejected from apothecia, while those under field conditions were sprayed with ascospore suspensions from a fine atomizer. After different time intervals, steamed celery stem segments were rolled over the leaves of inoculated and uninoculated plants (1 piece per leaf and 24 pieces per date) and placed on acidified PDA plates to determine percentage recovery of the fungus. Plants kept under laboratory conditions were placed under bell jars to maintain a high RH; condensed water was apparent on the inner walls of the jars. Under these conditions, ascospores survived for only 4 days. In contrast, ascospores atomized on bean leaves under field conditions where RH and temperature varied considerably survived for as long as 12 days. Percentage recovery of the fungus was 100, 25, 8, 0, and 4% at 0, 1, 5, 7, and 12 days after inoculation, respectively. In another field inoculation test, ascospore recovery was 60 and 50% after 4 and 9 days of incubation, respectively.

*Survival of germinated ascospores and resistant structures.*—Ascospores ejected onto the surface of sterilized dialysis tube segments were placed on different concentrations of TEA (diluted  $10^{-1}$ ,  $10^{-3}$ ,  $10^{-6}$ , or  $10^{-7}$  plus 1.0% (w/v) each of glucose and asparagine. After different incubation periods (15, 30, 62, and 86 hours at 20-22 C) the dialysis tube segments were removed, placed right side up in empty plastic petri dishes that were maintained in a desiccator at 98% RH. After 4, 11, 18, 26, and 32 days, the dialysis tube segments were placed with ascospores in contact with surface of acidified PDA plates and survival (as indicated by regrowth) was recorded. There was a close correlation between incubation time, level of nutrients in the agar medium, and duration of survival of germinated ascospores and other structures formed. After 62 and 86 hours of growth, the fungus survived for 32 days (longest time interval checked) irrespective of the nutrient levels in the media. Spores that had germinated and grown for 30 hours survived for 4 days irrespective of the media, and survived for 11 and 18 days on all media except the  $10^{-6}$  TEA. Germinated ascospores failed to survive for 26 and 32 days after 30 minutes incubation on  $10^{-6}$  and  $10^{-3}$  TEA. Also, they failed to survive for 4 days after 15 hours growth on  $10^{-6}$  and  $10^{-3}$  TEA. Likewise, incubation for 15 hours on  $10^{-7}$  TEA plus 1.0% each of asparagine and glucose did not allow survival of germinated spores for 26 days. Microscopic examination of germinated spores on the dialysis segments indicated that survival was closely correlated with the amount and kind of mycelial growth that had occurred during growth on the medium before drying. Regrowth of the fungus occurred from appressoria, hyphal cells with dense cytoplasm, and parenchymatous cells without obviously thickened walls.

The speed of development of appressoria and other structures from which regrowth occurred was clearly dependent on the level of the nutrients in the growth medium; appressoria formed much more quickly on the concentrated media. For example, well-formed appressoria were evident on dialysis tube segments on  $10^{-1}$  TEA after 24 hours incubation, while only early stages of appressoria had formed after 35 hours incubation on  $10^{-3}$  TEA.

*Survival of the fungus in infected host tissues.*—Greenhouse-grown bean blossoms were placed on actively growing cultures of the fungus for 72 hours, after which they were placed in empty petri dishes and maintained under laboratory conditions with a temperature that varied between 20–22 C and 50–70% RH. Survival of the fungus in these blossoms was tested by inoculating bean plants in a mist chamber in the greenhouse. Successful infection was accomplished with colonized blossom tissues that had been dried for 25 days after inoculation. Survival may have been longer, but it was not checked. Similarly, infected steamed celery segments were placed on bean plants and left on an open bench in the greenhouse with a fluctuating temperature of 20 to 27 C at 50 to 100% RH. The segments appeared to be thoroughly desiccated after 1 day. At different time intervals, bean plants with the dried inoculum on leaf surfaces, or on axils of branches, were replaced in mist chambers. Despite the desiccated appearance of the infected celery stem segments, infection resulted with segments dried for as long as 19 days, the longest period tested. Furthermore, colonized steamed celery segments placed on branch axils were still viable when tested after 16 days under field conditions as indicated by regrowth of the fungus on acidified PDA plates. Weather conditions in the field following inoculation with these segments was very dry and no lesions had developed on the inoculated plants.

**DISCUSSION.**—Failure of *W. sclerotiorum* to infect unless a film of water was maintained at the inoculated leaf surface suggested that the fungus is sensitive to low  $\psi$ . Preliminary evidence indicated that a minimal leaf wetness period of about 16 hours at 25 C is required for infection (Abawi and Grogan, unpublished). Results of this study showed, however, that growth of the fungus from ascospores, mycelial disks, or sclerotia is not adversely affected by  $\psi$  values of  $-40$  bars and higher. Furthermore, in common with several other fungi (8, 9), growth was stimulated by  $\psi$  of less than 0, i.e.  $-1$  to  $-14$  bars. Lesion expansion on bean leaves was also stimulated by lowering  $\psi$  from  $-1$  to  $-28$  bars and was not greatly reduced until about  $-64$  bars; yet, expansion of established lesions was stopped by removal from a moist chamber and allowing leaf surfaces to become dry (Abawi and Grogan, unpublished). Adebayo and Harris (2) reported that growth of *Phytophthora cinnamomi* and *Alternaria tenuis* is affected by both osmotically or matrix-induced reductions in  $\psi$  of agar media and soil. However, they found that both fungi were less tolerant of decreased matrix- than osmotic potentials. They suggested that lowered water potential cannot account for the total response, and that changes in other water-related soil properties (such as solute transport) may be involved.

*W. sclerotiorum* reportedly produces large amounts of

oxalic acid (16) and a variety of cell-wall-degrading enzymes (10, 11, 14, 15). The fungus also kills host cells in advance of actual hyphal invasion of healthy tissues (5, 16, 21, 24). Thus, we postulate that the free water required for lesion initiation and expansion may serve as a medium through which toxins and/or enzymes involved in pathogenicity move to infection sites and kill cells in advance of hyphal invasion; however, further research is needed to substantiate this hypothesis, and to evaluate further the role of moisture in initiation and development of infections.

Sclerotia of *W. sclerotiorum* subjected to slightly reduced  $\psi$  ( $-6$  bars) failed to produce apothecia, although apothecia were produced abundantly by other comparable sclerotia exposed to  $\psi$  of 0 bars. Several reports (3, 27) indicate that sclerotia of *W. sclerotiorum* require free water for apothecial production and that stipes do not form even near 100% RH. Furthermore, sclerotia exposed to desiccation in the field failed to produce apothecia when placed in free water in a growth chamber that provided near optimal conditions for apothecial production (Abawi and Grogan, unpublished). This indicates that exposure to extreme drying conditions has a lasting detrimental effect on apothecial production, although the sclerotia remained viable as indicated by consistent mycelial production on nutrient media. In a single test, several sclerotia soaked for 10 hours in saturated NaCl solution failed to produce apothecia within 6 weeks after transfer to SDW and incubation in the growth chamber. Several reports (3, 4, 23, 29) have dealt with factors influencing apothecial production, but the effects of low  $\psi$  have not been adequately explained. Possibly a reversible or nonreversible physiological change in the processes essential for apothecial production may be produced by low  $\psi$ . Whether such altered sclerotia eventually can regain the ability to form apothecia, has not been determined.

It was somewhat surprising to find that the ungerminated thin-walled ascospores of *W. sclerotiorum* are capable of withstanding continuous very low  $\psi$  for relatively long times (21 days). Also, ascospores atomized onto bean leaves under field conditions survived for as long as 12 days. These data indicate that ascospores need not immediately infect host tissues, but can survive dry conditions for a considerable time until wet conditions favorable for host infection prevail. However, the effect of fluctuating water potentials on survival of ascospores needs further evaluation. Survival of spores under dry conditions is not unique, however. Other fungi are known to survive better and for longer times at low RH. For example, the conidia of *Cochliobolus sativus* survived longer at approximately 10% RH than at 90 or 100% RH (13).

Survival of germinated ascospores was closely correlated with nutrient levels of the agar medium on which the spores had germinated and also with time of incubation. Microscopic examination revealed that survival was associated with amount of growth, and also with formation of appressoria and other unidentified resistant structures. Purdy (24) found that availability of a carbon source and mechanical stimulus were essential for appressorial formation by ascospores. He showed also that appressoria were involved in the direct penetration of

host tissues. Our results indicate that appressoria and other structures also may be involved in survival of the fungus during desiccation. These structures may play a major role in epidemiology of diseases incited by *W. sclerotiorum* under field conditions by functioning as secondary sources of inoculum.

## LITERATURE CITED

1. ABAWI, G. S., and R. G. GROGAN. 1974. Ascospores of *Whetzelinia sclerotiorum* as primary inoculum causing white mold of beans in New York. *Phytopathology* 64:578 (Abstr.)
2. ADEBAYO, A. A., and R. F. HARRIS. 1971. Fungal growth responses to osmotic as compared to matric water potential. *Soil Sci. Soc. Am. Proc.* 35:465-469.
3. BEDI, K. S. 1962. Light, air, and moisture in relation to the formation of apothecia of *Sclerotinia sclerotiorum* (Lib.) DeBary. *Proc. Ind. Acad. Sci., B* 55:213-223.
4. BEDI, K. S. 1963. The age and the size of sclerotia of *Sclerotinia sclerotiorum* (Lib.) DeBary in relation to the formation of apothecia. *J. Indian Bot. Soc.* 42:204-207.
5. BOYLE, C. 1921. Studies in the physiology of parasitism. VI. Infection by *Sclerotinia libertiana*. *Ann. Bot.* 35:337-347.
6. BROWN, R. W. 1970. Measurement of water potential with thermocouple psychrometers: construction and applications. U.S. Dep. Agric., For. Serv., Res. Paper INT-80. 27p.
7. CHUPP, C., and A. F. SHERF. 1960. Vegetable diseases and their control. Ronald Press, New York. 693 p.
8. COOK, R. J., and R. I. PAPENDICK. 1972. Influence of water potential of soils and plants on root disease. *Annu. Rev. Phytopathol.* 10:349-374.
9. GRIFFIN, D. M. 1969. Soil water in the ecology of fungi. *Annu. Rev. Phytopathol.* 7:289-310.
10. HANCOCK, J. G. 1966. Degradation of pectic substances associated with pathogenesis by *Sclerotinia sclerotiorum* in sunflower and tomato stems. *Phytopathology* 56:975-979.
11. HANCOCK, J. G. 1967. Hemicellulose degradation in sunflower hypocotyls infected with *Sclerotinia sclerotiorum*. *Phytopathology* 57:203-206.
12. KORF, R. P., and K. P. DUMONT. 1972. *Whetzelinia*, a new generic name for *Sclerotinia sclerotiorum* and *S. tuberosa*. *Mycologia* 64:248-251.
13. LEDINGHAM, R. J. 1970. Survival of *Cochliobolus sativus* in pure culture and in natural soil at different relative humidities. *Can. J. Bot.* 48:1893-1896.
14. LUMSDEN, R. D. 1969. *Sclerotinia sclerotiorum* infection of bean and the production of cellulase. *Phytopathology* 59:653-657.
15. LUMSDEN, R. D. 1970. Phosphatidase of *Sclerotinia sclerotiorum* produced in culture and in infected bean. *Phytopathology* 60:1106-1110.
16. MAXWELL, D. P., and R. D. LUMSDEN. 1970. Oxalic acid production by *Sclerotinia sclerotiorum* in infected bean and in culture. *Phytopathology* 60:1395-1398.
17. MOORE, W. D. 1955. Relation of rainfall and temperature to the incidence of *Sclerotinia sclerotiorum* in vegetables in South Florida during the years 1944 to 1954. *Plant Dis. Rep.* 39:470-472.
18. MOORE, W. D., R. A. CONOVER, and D. L. STODDARD. 1949. The sclerotinose disease of vegetables in Florida. *Fla. Agric. Exp. Stn. Bull.* 457. 20 p.
19. NATTI, J. J. 1971. Epidemiology and control of bean white mold. *Phytopathology* 61:669-674.
20. PARTYKA, R. E., and W. F. MAI. 1962. Effects of environment and some chemicals on *Sclerotinia sclerotiorum* in laboratory and potato field. *Phytopathology* 52:766-770.
21. PRIOR, G. D., and J. H. OWEN. 1964. Pathological anatomy of *Sclerotinia trifoliorum* on clover and alfalfa. *Phytopathology* 54:784-787.
22. PURDY, L. H. 1955. A broader concept of the species *Sclerotinia sclerotiorum* based on variability. *Phytopathology* 45:421-427.
23. PURDY, L. H. 1956. Factors affecting apothecial production by *Sclerotinia sclerotiorum*. *Phytopathology* 46:409-410.
24. PURDY, L. H. 1958. Some factors affecting penetration and infection by *Sclerotinia sclerotiorum*. *Phytopathology* 48:605-609.
25. ROBINSON, R. A., and R. H. STOKES. 1955. Electrolyte solutions. Academic Press, New York. 571 p.
26. SCOTT, W. J. 1953. Water relations of *Staphylococcus aureus* at 30°C. *Aust. J. Biol. Sci.* 6:549-564.
27. SPROSTON, T., and D. C. PEASE. 1957. Influence of thermoperiods on production of the sexual stage of the fungus *Sclerotinia trifoliorum* Erikss. *Trans. N. Y. Acad. Sci.* 20:199-204.
28. WALKER, J. C. 1952. Diseases of vegetable crops. McGraw-Hill, New York. 529 p.
29. WILLIAMS, G. H., and J. H. WESTERN. 1965. The biology of *Sclerotinia trifoliorum* Erikss. and other species of sclerotium-forming fungi. I. Apothecium formation from sclerotia. *Ann. Appl. Biol.* 56:253-260.
30. WINSTON, P. W., and D. H. BATES. 1960. Saturated solutions for the control of humidity in biological research. *Ecology* 41:232-247.