Factors Affecting Sclerotium Populations of, and Apothecium Production by, Sclerotinia sclerotiorum

H. F. Schwartz and J. R. Steadman

Former Graduate Research Assistant and Associate Professor, respectively, Department of Plant Pathology, University of Nebraska, Lincoln, NE 68583. Present address of senior author is CIAT, Apartado Aéreo: 67-13, Cali, Colombia, South America.

Published with the approval of the Director as Journal Series Paper No. 5279 of the Nebraska Agricultural Experiment Station. The work reported was conducted under Nebraska Agricultural Experiment Station Project 21-015.

The authors gratefully acknowledge the use of research facilities provided by John L. Weihing, Director of the Panhandle Experiment Station, Scottsbluff, Nebraska. Appreciation also is extended to Dermot P. Coyne, Department of Horticulture, University of Nebraska, Lincoln, NE for the use of isogenic dry bean breeding lines which he developed.

Accepted for publication 4 August 1977.

ABSTRACT

SCHWARTZ, H. F., and J. R. STEADMAN. 1978. Factors affecting sclerotium populations of, and apothecium production by, Sclerotinia sclerotiorum. Phytopathology 68: 383-388.

Sclerotium populations of *Sclerotinia sclerotiorum* were variable during this 3-yr study; however, sclerotia did not accumulate in increasing numbers in fields planted to susceptible dry edible bean (*Phaseolus vulgaris*) cultivars despite annual white mold epidemics. Populations ranged between one and three sclerotia/kg air-dried soil in bean fields. A 3-yr crop rotation did not reduce sclerotium populations significantly. A low sclerotium population of 0.2/kg soil produced sufficient inoculum (ascospores) to infect 46% of the plant canopy during 1975. Sclerotia were redistributed within a field by irrigation water. During August, numerous sclerotia germinated to form 11-14 and 7-11 apothecia/m^2 in bean and sugar beet fields, respectively. An average of two apothecia were produced by each germinated sclerotium in both crops. An apothecium continued to produce ascospores in the field for about 7 days. Apothecium production was less beneath the open bush canopy of dark red kidney Charlevoix and the upright semi-vine canopy of small white Aurora when compared to production beneath the dense compact bush canopy of Great Northern (G.N.) Code P #92 and the dense viny canopies of G.N. UI #59 and G.N. Tara. Over 90% of the apothecia were located either adjacent to the plant or on the side of the irrigation furrow, regardless of plant growth habit. An irrigation application every 5 days increased apothecium production, especially beneath Tara, when compared to a 10-day irrigation frequency. Each apothecium produced about 2.3×10^6 ascospores under laboratory conditions.

Additional key words: *Whetzelinia sclerotiorum*, inoculum production, epidemiology.

Sclerotia are the primary survival structures of the white mold fungus, *Sclerotinia sclerotiorum* (Lib.) de Bary = *Whetzelinia sclerotiorum* (Lib.) Korf and Dumont (13). In western Nebraska 78% of the sclerotia buried at various depths survive for at least 3 yr under fallow conditions, and 72% of these sclerotia germinate and form stipes on water agar (4). In the field, sclerotia produce secondary sclerotia which can prolong *S. sclerotiorum* survival (1, 4, 25).

Sclerotia formed on or within host tissue may be dislodged onto the soil surface by the wind or during harvesting and threshing. Sclerotia are distributed within the vertical soil profile by land preparation (4) and between fields by irrigation runoff water (3, 22). The fungus also can be distributed throughout a bean-producing area in sclerotia- or mycelia-infested seed lots (2, 11, 21).

Suzui and Kobayashi (24) reported that 3.2 sclerotia/m^2 caused 60-95% infection in a kidney bean field. Additional data concerned with sclerotium populations and disease severity are lacking, especially for western Nebraska.

Apothecium production can be influenced by depth of sclerotium burial in the soil (14, 25), presence of a cover crop (25), chemicals (5, 10, 12, 17, 18), or periodic soil cultivation (18, 23). However, there are no reports on the influence of frequency of furrow-irrigation and bean plant-growth habit upon apothecium production.

Sclerotium populations contributing to local disease epidemics and cultural factors affecting apothecium production in western Nebraska are reported in this paper. A quantitative estimate of ascospore production also is reported.

MATERIALS AND METHODS

Sclerotium populations.—Soil samples were collected randomly along a zigzag path which traversed each selected field from end-to-end and side-to-side. Each soil sample was collected from a trench 10-cm wide by 20-cm long by 7.5- to 15-cm deep. In each field, 7 to 22 soil samples ranging from 1.34 to 3.67 kg dry weight were collected.

Soil samples were dried at 25-30 C for 2 to 5 days,

00032-949X/78/000 064$03.00/0

weighed, and passed through a 0.85-mm sieve (Tyler Screen, No. 20 mesh). Sclerotia then were removed from the residue and counted. Sclerotia were assayed for carpogenic germination by incubation on water agar (0.5-1.5%) at 18-20 C.

Fields were cultivated periodically for weed control. Alternate rows were ditched for furrow irrigations, which were applied at intervals of 7-10 days unless otherwise noted. Sugar beet fields were planted in early May and were irrigated at the same frequency. Fields designated B-1, B-2, B-3, F-3, Gdn, and Micro were planted annually to dry beans. A crop rotation of dry beans, sugar beets, field corn, and sugar beets was used in field G-3 in 1973-1976.

Apothecium production.—Apothecia were counted on a standard soil surface area of 0.42 m^2 beneath the bean and sugar beet canopy and of 0.68 m^2 beneath the corn canopy. Apothecium counts were converted to number/m^2. A randomized complete block design was used. In some experiments the standard surface area was subdivided into 0.07 m^2 samples as follows: (i) bottom of irrigated furrow; (ii) sides of irrigated furrow, two samples; (iii) adjacent to plant stems, two samples; and (iv) middle of cultivated row.

The number of carpogenically germinated sclerotia often was determined by excavating the sclerotia and attached apothecia. In some instances when estimates were made, however, sclerotia could not be excavated because of the effect inoculum destruction might have on disease severity.

Apothecium longevity was recorded in five randomly selected sample locations in a field of G.N. Tara which was irrigated every 5 days. Apothecia within a 0.42 m^2 area in the irrigated furrow were marked and observed daily from 19 to 30 August 1976. An apothecium was determined to have ceased functioning when it became shriveled and dried or detached from the buried sclerotium.

Ascospore production.—Field-produced sclerotia which had been buried in a greenhouse soil for 97 days (conditioned to produced apothecia) were recovered and placed on moist vermiculite. After 11-12 days individual sclerotia with a single apothecium (nine sclerotia in each of three replicates) were transferred to a trench formed in a 1.5% water-agar plate. Plates were inverted over a 25-mm diameter Millipore filter (type HA, 0.45 μm pore size) fastened to the lid, and incubated at 19-21 C (air temperature immediately above the apothecia) with constant fluorescent lighting (G.E. cool-white, 1,650 lux). Spore release began within 24-48 hr, and filters were replaced daily thereafter. The deposited ascospores were removed from the filter with a camel's-hair brush and suspended in 2 ml of sterile distilled water. The total number of ascospores produced daily by each apothecium was estimated by examining 10 1 m^2 fields in a Spencer bright-line hemacytometer.

RESULTS

Sclerotium populations.—In 1975, 70% of the sclerotia recovered from soil samples collected in the spring germinated carpogenically, whereas only 10-20% of those collected in the fall did so. Of those that did not germinate carpogenically, 30% or less were not viable, and the rest were newly-formed sclerotia that produced a few hyphae from the sclerotium surface when placed on agar.

Populations of sclerotia were variable. In most fields, fewer sclerotia were recovered in the spring of 1975, than in the spring of 1974 or 1976 (Table 1). In 1976, populations of sclerotia were similar in all fields even though the frequency of planting of a host crop in previous years varied. In one field (G-3) a severe white mold epidemic occurred on beans in 1972, and in subsequent years, sugar beets and field corn were planted. Although the populations decreased during this succession of nonhost crops, at least one sclerotium/kg soil was recovered. Populations of sclerotia ranging from the lowest (Micro Field in 1974) to the highest (B-1 Field in 1974) caused canopy infection of 28 and 48%, respectively. However, similar-sized populations of sclerotia resulted in large differences in disease severity, and there was no correlation between the size of populations and severity of white mold.

Soil samples were collected in the fall of 1975 to determine sclerotium populations immediately following bean harvest. Fewer sclerotia (average of $>$ 1 sclerotium/kg soil) were recovered from all fields at this time than in the spring, 1976, sampling (2 selerotia/kg soil).

Sclerotia were distributed evenly within the upper vertical soil profile. Populations of sclerotia at the depths of 0-7.5 and 7.6-15.2 cm averaged 2.7 ± 0.7 and 2.4 ± 0.5 sclerotia/kg soil, respectively, in the spring of 1975. However, sclerotia were distributed within a field by irrigation water. Plant debris was collected from the water surface of three irrigated furrows at the lower end of a field following the initial irrigation (3-4 hr) with well water on 28 June 1974. An average of eight sclerotia/10 g of dried plant debris was recovered.

Apothecium production in host and nonhost fields.—Apothecia were produced continuously throughout August and into September. Apothecia were initially detected about the first week of August during 1974-1976 in dry beans, field corn, sugar beets, or potato. No infection occurred in the corn or sugar beet fields. However, some leaf and vine infections were seen in the potato field. Numbers of apothecia and germinated sclerotia were similar whether beneath the plant canopies of the susceptible G.N. bean cultivars or nonsusceptible sugar beets (Table 2). Ranges of 7-11 and 10-14 apothecia/m^2 were observed in sugar beet and dry bean fields, respectively, during 1975 and 1976. An average of two apothecia was produced by each sclerotium, regardless of the crop. An apothecium was functional (not shriveled and dried or detached from the sclerotium) for about 7 days.

Effect of plant growth habit on apothecium production.—Higher numbers of apothecia were observed beneath the canopies of vines or short compact bush types than beneath the canopies of an upright semi-vine or a tall open bush, especially on 22 August (Fig. 1). Apothecia also were more numerous beneath a short compact bush than beneath a near-isogenic small vine. Similar differences were found between the bean breeding lines and cultivars when number of sclerotia which germinated carpogenically beneath the canopy were compared.

TABLE 1. Number of sclerotia of *Sclerotinia sclerotiorum* recovered from soil samples collected during May-June in 1974-1976 in western Nebraska

Year and field[a]	Sclerotia per kg soil	Disease severity (%)[b]	Great Northern cultivar	No. years planted to dry beans within last 9 yr
1974:				
B-1	6.2 ± 1.3	48	UI #59	4
B-2	0.2 ± 0.2	Data unavailable		1
B-3	3.3 ± 0.6	10	UI #59	>9
F-3	2.8 ± 0.5	25	Code P	3
Gdn	2.8 ± 1.2	41	Tara	1
Micro	0.1 ± 0.1	28	Tara	2
G-3	2.3 ± 0.9	Nonhost	(Sugar beet)	1
1975:				
B-1	2.5 ± 0.5	14	UI #59	5
B-2	0.2 ± 0.1	46	Tara	2
B-3	1.4 ± 0.2	31	UI #59	>9
F-3	1.8 ± 0.3	66	Code P	4
Gdn	0.5 ± 0.2	34	Tara	2
Micro	1.4 ± 0.4	39	Tara	3
G-3	0.9 ± 0.2	Nonhost	(Field corn)	1
1976:				
B-1	2.7 ± 0.4	50	Tara	6
B-2	1.6 ± 0.3	71	Tara	3
B-3	2.1 ± 0.3	43	Tara	>9
F-3	2.7 ± 0.7	90	Star	5
Gdn	1.4 ± 0.3	53	Tara	>4
Micro	2.3 ± 0.7	50	Tara	4
G-3	1.2 ± 0.3	Nonhost	(Sugar beet)	1

[a]All fields were located on the Panhandle Experiment Station, Mitchell, NE. Field G-3 was planted to dry beans, sugar beets, field corn, and sugar beets during 1973-1976, respectively, and had a severe white mold epidemic in 1973. All other fields were planted to dry beans each year and had annual white mold epidemics.

[b]Disease severity represents the average percentage of the above-ground plant showing signs of *S. sclerotiorum*; mean values of five to seven randomly selected replicates per field with 20-30 plants per replicate.

At least 90% of the apothecia observed were located adjacent to the plant stems near the irrigated furrow, regardless of plant growth habit (Fig. 1). Apothecia were observed in the nonirrigated furrow only beneath G.N. Tara.

Apothecium production was greatest on 22 August when most of the plants had attained maximum vegetative growth. By 31 August, 51% and 56% of the plant canopies of Code P #92 and Tara, respectively, were infected as compared to less than 1% of the canopies of Aurora or Charlevoix. At this time, 17 and 21% of the G.N. UI #59 and Code P #82 canopies, respectively, were infected.

Effect of irrigation frequency on apothecium production.—Apothecium production was monitored at different locations beneath the canopy of G.N. Tara and Aurora irrigated every 5 or 10 days. The majority of apothecia were produced adjacent to the plant stems near the irrigated furrow (Fig. 2). Greater numbers of apothecia were observed when Tara was irrigated either every 5 days or every 10 days than in either of the Aurora treatments. No significant differences were observed between the Tara treatments after 12 August 1976, or between the Aurora treatments. Similar results were found when comparisons were made on the number of sclerotia which germinated carpogenically beneath the canopy.

TABLE 2. Apothecium density of *Sclerotinia sclerotiorum* during August 1975 and 1976

Source of sample[a]	Year	Average inoculum density[b] Germinated sclerotia/m^2	Apothecia/m^2
Dry bean	1975	4.9 ± 0.4	10.5 ± 1.0
Dry bean	1976	6.0 ± 0.5	13.8 ± 1.6
Sugar beet	1975	5.3 ± 0.7	11.2 ± 1.7
Sugar beet	1976	2.9 ± 0.7	6.5 ± 2.0

[a]Data represents a composite of readings during August beneath the canopies of susceptible indeterminate growth, dry bean cultivars and nonsusceptible sugar beets grown only under standard cultural practices.

[b]A total of 224 and 121 or 80 and 30 samples were collected in 1975 and 1976 from dry bean or sugar beet fields, respectively. Each sample was from 0.42 m^2 area within the irrigated row.

Apothecium production reached a maximum around 23 August 1976 when only 1-3% of the plant canopy was infected in the 5-day Tara irrigation treatment. By 31 August, 30-40% of plant canopy was infected, whereas less than 3% canopy infection had occurred in any of the other treatments.

Ascospore production.—Sporulation was monitored daily, and apothecia released ascospores continuously for 2-17 days with an average of 9 days. Although many of the apothecia appeared shriveled or dried by the 11th day, detectable quantities of ascospores were still being discharged by a few. Average daily production by an actively discharging apothecium varied from 2.00×10^4 on day 17 to 4.62×10^5 on day 4, with an average of 2.58×10^5 per day. Maximum ascospore production occurred at 2- to 3-day intervals during days 4 to 9. Total ascospore production by an apothecium ranged from 1.50×10^5 to 7.27×10^6, with an average of 2.32×10^6.

DISCUSSION

The differential germination processes of conditioned (resulting in apothecia) and nonconditioned sclerotia of *S. sclerotiorum* provide a simple means to identify their particular stage of development. The majority (70%) of sclerotia collected in the spring-produced apothecia as compared with 10-20% of the fall-collected sclerotia. This decrease reflects: (i) removal of sclerotia that are capable of carpogenic germination from the upper soil profile by irrigation runoff water, (ii) loss of some conditioned sclerotia because their reserve of nutrients was consumed during apothecium production and/or degradation by microorganisms during the growing season, and (iii) increased number of sclerotia recently produced during the current season's white mold epidemic, but not capable of carpogenic germination.

Variability in populations of sclerotia was influenced by the extent of annual sclerotium replenishment which in turn was determined by the severity of white mold infection during the previous season. However, there was no correlation evident between populations of sclerotia and disease severity. Nevertheless, a minimum level evidently is necessary to incite a moderately severe disease epidemic; 0.2 sclerotia/kg soil caused 46% plant canopy infection.

There was no progressive accumulation of sclerotia in fields annually planted to susceptible bean cultivars. Conversely, there was no progressive decrease of sclerotia

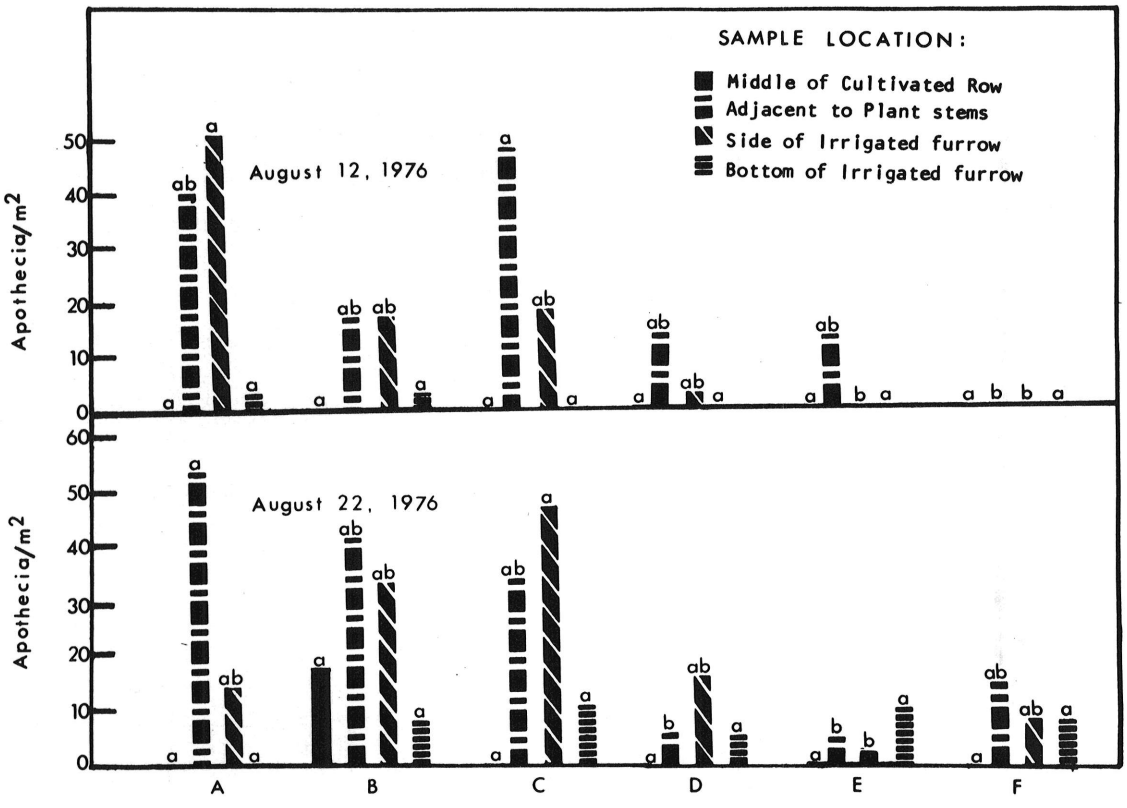

Fig. 1. Location of apothecium production by *Sclerotinia sclerotiorum* beneath the canopies of dry beans with different growth habits. A = G.N. UI #59; vine; B = G.N. Tara, vigorous vine; C = G.N. Code P #92, short compact bush; D = G.N. Code P #82, small vine; E = small white Aurora, upright semi-vine; and F = dark red kidney Charlevoix, tall open bush. Mean values of five replicates per sample location. Comparisons between breeding lines and cultivars (at each location for each date) are not significantly different unless graph values have different lower case letters; determined by Duncan's new multiple range test ($P = 0.05$).

in a field that was rotated with nonhosts during 1973-1976; about one sclerotium/kg soil survived and a moderately severe white mold epidemic developed when the field again was planted to dry beans. Haas and Bolwyn (9) also reported that the frequency of bean in the recent cropping history was not related to the amount of disease.

Populations of sclerotia were evenly distributed in the upper soil profile with numbers of sclerotia similar to that found in North Dakota (15). This distribution obviously is dependent upon tillage practices since lower populations of sclerotia and higher sampling error in fall soil samples compared to samples taken the following spring revealed that after harvesting, sclerotia are not evenly distributed on the soil surface and most are concentrated in small areas. Thus, a more representative sampling can be obtained after spring tillage. During the growing season, sclerotia can be moved within a field by irrigation water and can disseminate the fungus into low-lying areas of a field where microclimatic conditions may be more favorable for disease development. Sclerotia also have been reported to be spread from field to field by irrigation water (3, 22).

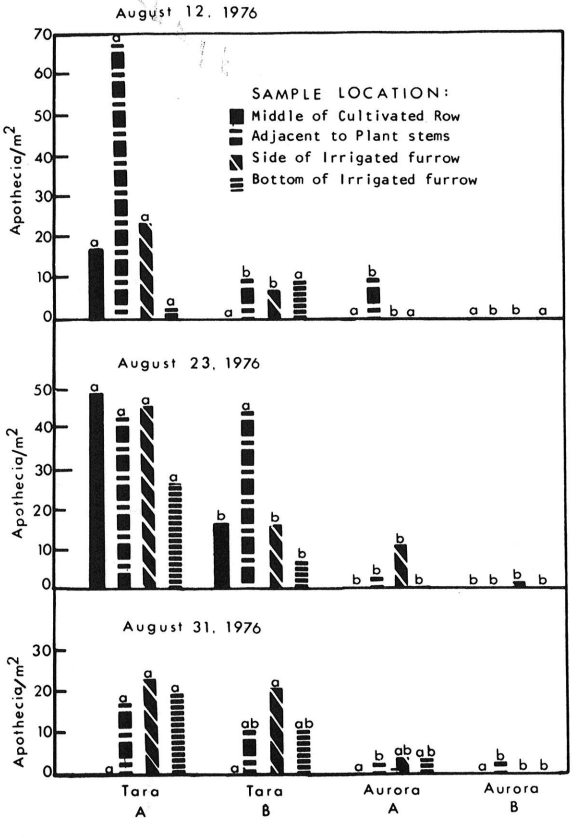

Fig. 2. Apothecium production by *Sclerotinia sclerotiorum* beneath the canopies of two dry bean cultivars grown with two furrow irrigation treatments. A and B = 5 cm irrigation water applied every 5 or 10 days, respectively. Mean values of seven replicates per sample location. Comparisons between cultivars-treatments (within each location on each date) are not significantly different unless graph values have different lower case letters; determined by Duncan's new multiple range test ($P = 0.05$).

Stevens and Hall (23) reported that a single apothecium contained 31×10^6 ascospores, but did not describe the method used to obtain this estimate. Our work revealed that a single apothecium could release an average of 2.32×10^6 ascospores during 9 days of spore release in the laboratory. Up to 35 (3, 23), 40 (Schwartz and Steadman, *unpublished*), and 100 (8) apothecia were produced by a single sclerotium under field conditions. Therefore, we estimate that the potential for production of ascospores by a single sclerotium to be as high as 2.3×10^8.

Most apothecia were produced adjacent to plant stems in or on the side of the irrigated furrow in bean fields. This location effect also was reported for lemon orchards (20). Production of apothecia is promoted by conditions which provide and maintain sufficient moisture. Thus, the irrigation furrow, especially the area near the plant stem where the canopy shades the soil surface, provides the most conducive environment for carpogenic germination. The association of apothecium production and amount of canopy covering the soil surface also was observed in the bottom of the irrigated furrow when abundant numbers of apothecia were not recorded until the plant canopy had covered the area over the furrow.

Plant growth habit and canopy architecture are known to influence white mold infection (6, 7, 19) and also can affect initial inoculum production (16). Therefore, a commercial cultivar which combines Great Northern seed type, high yield potential, and open plant architecture needs to be developed. A breeding program to achieve this objective is currently underway at the University of Nebraska.

LITERATURE CITED

1. ADAMS, P. B. 1975. Factors affecting survival of Sclerotinia sclerotiorum in soil. Plant Dis. Rep. 59:599-603.
2. BLODGETT, E. C. 1946. The Sclerotinia rot disease of beans in Idaho. Plant Dis. Rep. 30:137-144.
3. BROWN, J. G., and K. D. BUTLER. 1936. Sclerotiniose of lettuce in Arizona. Pages 475-506 *in* Agric. Exp. Stn. Bull. No. 63. 506 p.
4. COOK, G. E., J. R. STEADMAN, and M. G. BOOSALIS. 1975. Survival of Whetzelinia sclerotiorum and initial infection of dry edible beans. Phytopathology 65:250-255.
5. CORBIN, J. B., R. A. KITCHENER, and R. K. WARD. 1971. Benomyl foliar sprays for control of Sclerotinia and Cladosporium in tomatoes. Pages 205-209 *in* Proc. 24th N. Z. Weed and Pest Control Conf. 205-209. 226 p.
6. COYNE, D. P., J. R. STEADMAN, and F. N. ANDERSON. 1974. Effect of modified plant architecture of Great Northern dry bean varieties (Phaseolus vulgaris) on white mold severity, and components of yield. Plant Dis. Rep. 58:379-382.
7. COYNE, D. P., J. R. STEADMAN, and H. F. SCHWARTZ. 1977. Reaction of Phaseolus dry bean germplasm to Sclerotinia sclerotiorum. Plant Dis. Rep. 61:226-230.
8. DICKSON, F., and W. R. FISCHER. 1923. A method of photographing spore discharge from apothecia. Phytopathology 13:30-32.
9. HAAS, J. H., and B. BOLWYN. 1972. Ecology and

epidemiology of Sclerotinia wilt of white beans in Ontario. Can. J. Plant Sci. 52:525-533.
10. HAWTHORNE, B. T., and W. R. JARVIS. 1973. Differential activity of fungicides on various stages in the life cycle of Sclerotinia spp. N. Z. J. Agric. Res. 16:551-557.
11. HUNGERFORD, C. W., and R. PITTS. 1953. The Sclerotinia disease of beans in Idaho. Phytopathology 43:519-521.
12. JONES, D. 1974. Fungicidal effects of the fumigant dazomet on sclerotia of Sclerotinia sclerotiorum in soil. Trans. Br. Mycol. Soc. 63:249-254.
13. KORF, R. P., and K. P. DUMONT. 1972. Whetzelinia, a new generic name for Sclerotinia sclerotiorum and S. tuberosa. Mycologia 64:248-251.
14. KRUGER, W. 1975. The influence of some environmental factors on the development of apothecia and ascospores of Sclerotinia sclerotiorum (Lib.) de Bary. Z. Pflanzenkr. Pflanzenschutz 82:101-108. (In German, with English summary).
15. LLOYD, E. H. 1975. White mold of Pinto beans: Effects on yield and fungicidal control. N. D. Farm Res. 32:9-14.
16. NEWTON, H. C., and L. SEQUEIRA. 1972. Ascospores as the primary infective propagule of Sclerotinia sclerotiorum in Wisconsin. Plant Dis. Rep. 56:798-802.
17. PARTYKA, R. E., and W. F. MAI. 1958. Nematocides in relation to sclerotial germination in Sclerotinia sclerotiorum. Phytopathology 48:519-520.
18. 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.
19. SCHWARTZ, H. F., J. R. STEADMAN, and D. P. COYNE. 1978. Influence of Phaseolus vulgaris blossoming characteristics and canopy structure upon reactions to Sclerotinia sclerotiorum. Phytopathology 68:(In press).
20. SMITH, C. O. 1916. Cottony rot of lemons in California. Phytopathology 6:268-278.
21. STEADMAN, J. R. 1975. Nature and epidemiological significance of infection of bean seed by Whetzelinia sclerotiorum. Phytopathology 65:1323-1324.
22. STEADMAN, J. R., C. R. MAIER, H. F. SCHWARTZ, and E. D. KERR. 1975. Pollution of surface irrigation waters by plant pathogenic organisms. Water Resour. Bull. 11:796-804.
23. STEVENS, F. L., and J. G. HALL. 1911. A serious lettuce disease (Sclerotiniose) and a method of control. Pages 88-145 *in* N. C. Agric. Exp. Stn. Tech. Bull. 8. 145 p.
24. SUZUI, T., and T. KOBAYASHI. 1972. Dispersal of ascospores of Sclerotinia sclerotiorum (Lib.) de Bary on kidney bean plants. Part 2. Dispersal of ascospores in the Tokachi District, Hokkaido. Pages 61-68 *in* Hokkaido Nat. Agric. Exp. Stn. Res. Bull. 102. 82 p.
25. WILLIAMS, G. H., and J. H. WESTERN. 1965. The biology of Sclerotinia trifoliorum Erikss. and other species of sclerotium-forming fungi. II. The survival of sclerotia in soil. Ann. Appl. Biol. 56:261-268.