Changes in Carbon Dioxide Levels During Sclerotial Formation by Phymatotrichum omnivorum

Stuart D. Lyda and Earl Burnett

Associate Professor, Department of Plant Sciences, Texas A&M University, College Station, Texas 77843, and Research Soil Scientist, USDA, ARS, Blackland Conservation Research Center, Temple, Texas 76501, respectively.
The authors gratefully acknowledge the assistance of Barbara L. Holle, Technical Assistant I.

Supported in part by Contract Agreement 12-14-100-9339(41) from the USDA Soil and Water Conservation Research Division. A contribution of the Texas Agricultural Experiment Station (TA-8901) and the USDA Soil and Water Conservation Research Division.

Trade names are included for the benefit of the reader, and do not imply any endorsement or preferential treatment of the product listed by the USDA.

Accepted for publication 23 February 1971.

ABSTRACT

Carbon dioxide was monitored over a 12-week period from pint-sized culture chambers supporting sclerotial formation by *Phymatotrichum omnivorum* on a sterile soil-sorghum seed substrate. Carbon dioxide accumulated rapidly during the first 2 weeks after inoculation with the fungus, and declined rapidly during the period of maximum sclerotial development. Sclerotial initiation coincided with the elevated CO_2 concentration, whereas maximum sclerotial dry weight was obtained in 8 weeks. Apparently, CO_2 or the dissolved bicarbonate ion influences sclerotial formation in *P. omnivorum*. Soils with the capacity to absorb or otherwise accumulate high concentrations of CO_2 or bicarbonate might be suitable for sclerotial development of *P. omnivorum* and support the cotton disease caused by this fungus. There were no significant changes in O_2 and N_2 levels during the 12-week study period. Phytopathology 61:858-861.

Additional key words: fungus, sclerotia, soil microbiology.

Phymatotrichum omnivorum (Shear) Duggar is a soil-borne fungus that causes a severe root rot of over 2,000 species of dicotyledonous plants (1). The fungus is distributed widely throughout southwestern United States and northern Mexico, and is prevalent in the heavy, calcareous soils of central Texas. Comprehensive reviews on the disease, its distribution, and the fungus have been published by Rea (14) and Streets (18).

Sclerotia of *P. omnivorum* were first reported produced in soil-culture tubes by King & Loomis in 1929 (10). Subsequently, Neal (11) found them in naturally infested soils at Greenville, Texas. Although it has been 40 years since discovery of the sclerotial stage, the mechanisms that induce sclerotial formation and which cause the fungus to revert from a mycelial stage to production of sclerotia are unknown. Also, no one has determined why *Phymatotrichum* root rot occurs only in highly calcareous soils.

Numerous reports deal with the growth and distribution of *P. omnivorum* in soil and pure culture. The fungus may produce sclerotia at soil depths of at least 8 ft (15), and mycelial strands have been observed on the surface of roots at depths greater than 6 ft (9, 13, 18). This suggests that the fungus has the potential of inhabiting environments hostile to most soil microorganisms. Rogers (15) reported that most sclerotia were found at depths between 18 and 30 inches in the Blackland soils at Temple, Texas; however, he also found them in reduced numbers at depths to 96 inches. Soil at these depths is relatively uninhabited by other soil microorganisms that are concentrated in the upper 6 inches of soil.

Phymatotrichum omnivorum also has other unique survival powers. Taubenhaus et al. (20) observed that keeping naturally infested soil inundated for 120 days failed to eradicate the fungus. In laboratory studies, King (8) showed that 20% of the sclerotia survived immersion in water for 121 days at room temperature. Both of these procedures would be detrimental to most soil-borne fungi.

Neal & Wester (12) and Ezekiel et al. (5) noted the fungus grew as well in an atm of 25% CO_2 as in air, which is 833 times the natural level of CO_2 in air at standard conditions of temperature and pressure. When the CO_2 concentration was adjusted to 50%, there was ca. 40% reduction in growth rate.

The relationship between CO_2 tolerance and vertical distribution in soil of several fungi has been discussed by Burges & Fenton (2). They found that some fungi which normally occur in the upper 5 cm of soil were strongly inhibited by CO_2 concentrations greater than 5%, whereas those from lower depths were generally more tolerant of elevated CO_2 levels. Durbin (4) found a similar relationship between CO_2 tolerance and vertical distribution for different isolates of *Rhizoctonia solani* and a number of other plant-pathogenic fungi. Those isolates from ground level or aerial portions of plants were less tolerant of high CO_2 levels.

It has been reported many times that *Phymatotrichum* root rot is confined to the highly calcareous soils. Fraps & Fudge (6) found the soil-carbonate content closely correlated with the disease incidence. In the High Plains and east Texas where *Phymatotrichum* root rot does not occur to any extent, the soil carbonate content is 0-1%. In the Blacklands, Coastal Plain, and Lower Rio Grande regions of Texas where the disease occurs abundantly, the carbonate content is as high as 10%.

Apparently, no information is available on the gaseous content of the soil environment during sclerotial

formation by *P. omnivorum*. This paper presents information on changes in CO_2 during sclerotial formation in soil-culture chambers.

MATERIALS AND METHODS.—*Phymatotrichum* sclerotia were produced using a modification of the Dunlap method (3). Air-dried Houston black clay (HBC) was passed through 1-mm^2 wire screen. The screened soil was distributed into 39 wide-mouthed, glass culture chambers (Fig. 1) in 250-g quantities. Sorghum seed (25 g) was placed on the soil surface and moistened with 115 ml of tap water. All chambers were capped with a regular screw-type jar lid, which was ventilated by a 20-mm hole containing a cotton stopper. A gas-sampling tube constructed of 0.25-inch copper tubing was situated adjacent to the ventilation port. The gas-sampling tube was introduced through the lid into the center of the soil, and the external end of the tube was sealed to a brass male connector (¼ × ⅛ MPT) with epoxy resin. A silicone rubber septum was placed over the outside tube end, and the fitting was capped with a brass cap (Fig. 1).

The culture chambers were autoclaved for 2 hr at 20 psi steam and 131 C. After cooling, a 6-mm disc of *Phymatotrichum* growth on an agar base was introduced through the cotton-stoppered hole onto the sorghum seed. Three chambers were retained for the zero-time reading, and the remainder were placed in a constant-temperature incubator at 28 C.

The analytical procedure reported by Tackett (19) was used for all gas determinations. Concentrations of CO_2, O_2, and N_2 were determined in a Beckman Model GC-2A gas chromatograph with a thermal conductivity detector. Gas samples were injected into a serial column made of 100/120-mesh silica gel and 60/80 molecular sieve. The gas chromatograph was operated with an oven temperature of 70 C, current 150 ma, and helium pressure set at 40 psi. A Model SR Sargent recorder, equipped with a disc integrator, was used to record peak areas for the different gases. A series of standard gases were injected during each sampling period to establish known concentrations of gas. Quantitation also was made by area normalization, which agreed very closely with the data obtained from known standards.

Three cultural chambers were removed at weekly intervals for gas measurements. A 2-ml sample of gas was first removed from the sampling port to free the sampling tube of incumbent gases. A 1-ml sample was then withdrawn and injected into the gas chromatograph. After determining CO_2, O_2, and N_2 composition of the soil gases, the culture-chamber lids were removed and residual sorghum seed and surface mycelium were discarded. The soil was wet-sieved through a 16-mesh sieve (U.S. Standard Sieve Series No. 18) to recover newly-formed sclerotia. These were blotted, weighed on a Mettler balance for fresh wt, and oven-dried at 110 C for 24 hr to ascertain dry wt.

Sclerotial yields in sealed cultural chambers and in vented chambers with or without KOH.—To verify the influence of CO_2 on sclerotial yield, a test was conducted in which the sclerotial chambers were modified by three different procedures: (i) a nonvented lid covered the cultural chamber; (ii) a glass well containing 10 ml of 50% KOH was inserted in the center of the soil in chambers capped with a cotton-stoppered lid; and (iii) a glass well containing 10 ml of water was inserted in the center of the soil in chambers capped with a cotton-stoppered lid. The same cultural and analytical procedures were followed for this test as reported for the first 2 weeks. Nine chambers, three from each modification, were examined at weekly intervals for 12 weeks. Gas analyses and sclerotial yields were determined on each.

RESULTS.—Mycelium colonized the sorghum-seed substrate quickly and grew through the soil. The CO_2 level increased rapidly and reached its maximum concentration 2 weeks after inoculation (Fig. 2). At the end of the 1st week, the CO_2 concentration of the soil gas within the culture chambers was more than 30 times that of air. The test was conducted twice with similar results. Each time, maximum CO_2 concentrations were recorded at the end of 2 weeks of incubation.

A rapid accumulation of CO_2 in the soil appeared to shift the fungus into a sclerotial-forming stage. After 2 weeks, the fungus was actively producing sclerotia. Apparently little CO_2 was evolved during the remaining growth period, since the level of CO_2 dropped rapidly until the sclerotial growth curve peaked at about 8 weeks (Fig. 2). The levels of O_2 and N_2 did not change significantly during the sampling period. The O_2 concentrations never dropped below 19% in the cotton-stoppered chambers. A normal growth curve was obtained when the dry wt of sclerotia was plotted for each sampling period (Fig. 2).

Sclerotial yields in chambers with nonvented lids more than doubled those with vented lids (Fig. 4). The maximum accumulation of CO_2 was at 4 weeks (6.4%) in the sealed chambers; however, this level dropped rapidly as noted for the first 2 tests (Fig. 3).

Potassium hydroxide added to glass centerwells situated in the soil was completely neutralized within 1 week, and required changing. Even with weekly changes, sufficient CO_2 accumulated in the system to reach levels 10 times greater than found at standard conditions. Those chambers with water in the centerwell had CO_2 accumulating to 0.62%, which was lower than expected. It is possible that the glass well inserted in the center of the soil improved exchange of soil gases with the ambient environment, thus preventing a greater accumulation of CO_2.

DISCUSSION.—The rise in soil CO_2 that occurs during initial stages of sclerotial production by *P. omnivorum* suggests a relationship to the biology, habitat, and pathogenicity of the fungus. The CO_2 influence also might be related to distribution of the disease and to some of the inconsistencies noted in cultural practices for control, such as deep plowing.

It has been recognized for many years that *Phymatotrichum* root rot is confined to highly calcareous soils in southwestern United States and northern Mexico. These soils would be able to hold more CO_2 in the form

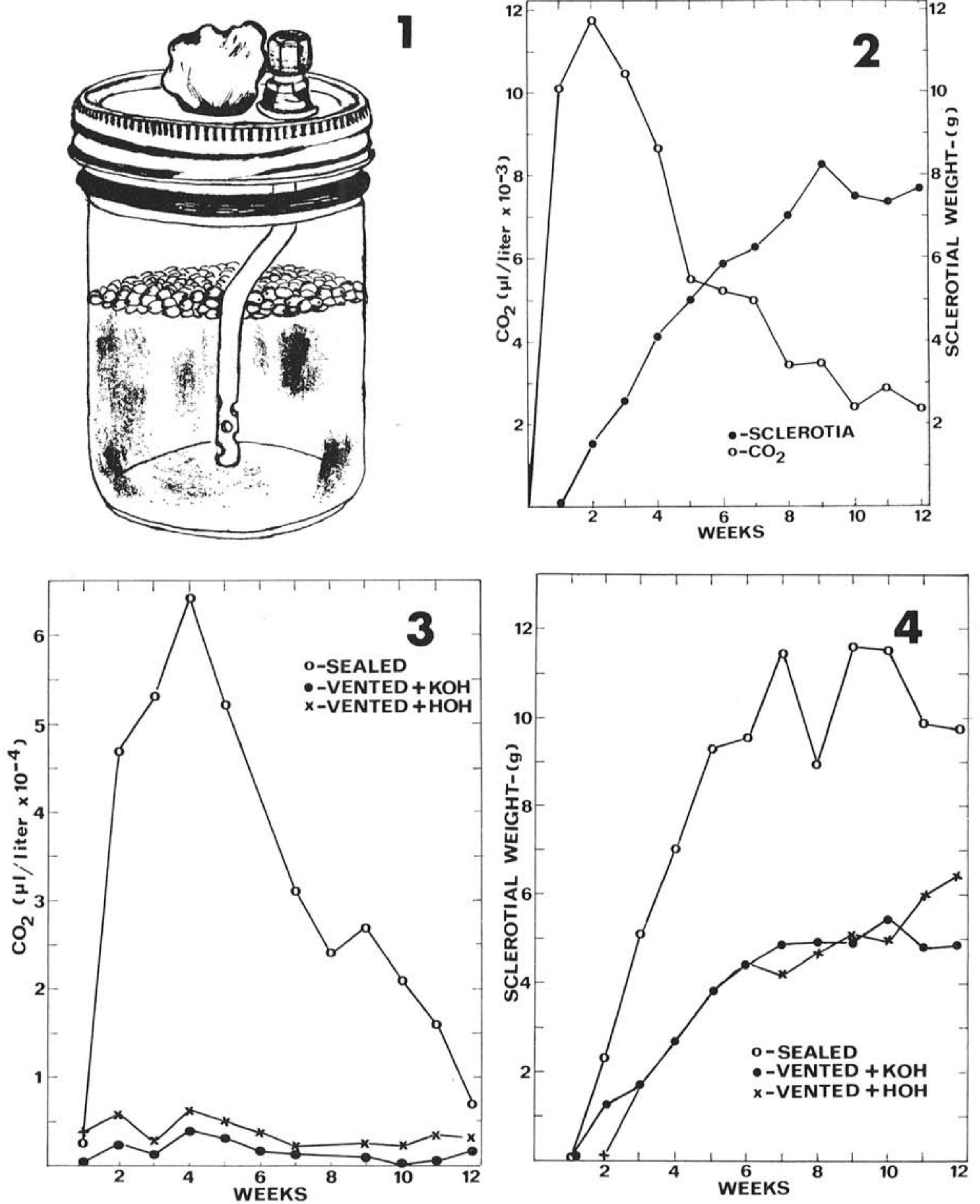

Fig. 1-4. **1)** A culture chamber used to monitor soil gases during sclerotial production by *Phymatotrichum omnivorum*. **2)** Changes in CO_2 concentration of soil gases during sclerotial formation by *P. omnivorum*. **3)** Carbon dioxide fluctuations in soil chambers supporting growth and sclerotial development of *P. omnivorum*. Chambers were (i) sealed to prevent loss of gases; (ii) vented with a cotton-stoppered hole and fitted with a centerwell containing 50% KOH; and (iii) vented and fitted with a centerwell containing water. **4)** Sclerotial yields of *P. omnivorum* growing in the soil chambers listed in Fig. 3.

of bicarbonate ions than noncalcareous soils. The heavier soils also would be less aerated and slow the loss of metabolic CO_2. An accumulation of high levels of CO_2 at the proper time might induce *P. omnivorum* to form sclerotia, serving to perpetuate the pathogen through adverse conditions. Sclerotia might not form as readily in well-aerated soils or those with an acid pH in which CO_2 would not accumulate as HCO_3^-.

Another hypothesis that could be advanced concerns the effect of deep plowing on *Phymatotrichum* root rot. In 1907 and 1908, Shear & Miles (16, 17) recommended deep fall plowing along with crop rotation for controlling *Phymatotrichum* root rot of cotton. Jordan et al. (7) found that the best control was achieved when the deep tillage was done early in the fall and when the soil was dry. King & Loomis (10) stated that deep spring plowing showed little or no benefit in controlling the disease. The inconsistencies noted with control by deep plowing could be explained by the timing of the operation. If the soils were deep-plowed before sclerotial formation, they might not accumulate sufficient CO_2 to induce the fungus to form sclerotia. If the operation were conducted late in the fall or in early spring, the sclerotia would already be formed and the plowing operation would appear to be fruitless.

LITERATURE CITED

1. BLANK, L. M. 1953. The rot that attacks 2,000 species, p. 298-301. *In* A. Stefferud [ed.]. Plant Diseases, the Yearbook of Agriculture. USDA, Washington, D.C.
2. BURGES, A., & E. FENTON. 1953. The effect of carbon dioxide on the growth of certain soil fungi. Brit. Mycol. Soc. Trans. 36:104-108.
3. DUNLAP, A. A. 1941. A convenient soil-culture method for obtaining sclerotia of the cotton root rot fungus. Amer. J. Bot. 28:945-947.
4. DURBIN, R. C. 1959. Factors affecting the vertical distribution of Rhizoctonia solani with special reference to CO_2 concentration. Amer. J. Bot. 46:22-25.
5. EZEKIEL, W. N., D. C. NEAL, P. R. DAWSON, & E. B. REYNOLDS. 1932. Report of the fifth annual cotton-root-rot conference. Phytopathology 22:983-993.
6. FRAPS, G. S., & J. F. FUDGE. 1939. Relation of the occurrence of cotton-root-rot to the chemical composition of soils. Texas Agr. Exp. Sta. Bull. 522. 21 p.
7. JORDAN, H. V., J. E. ADAMS, D. R. HOOTON, D. D. PORTER, L. M. BLANK, E. W. LYLE, & C. H. ROGERS. 1948. Cultural practices as related to incidence of cotton root rot in Texas. USDA Tech. Bull. 948. 42 p.
8. KING, C. J. 1937. A method for the control of cotton-root-rot in the irrigated southwest. USDA Circ. 425. 9 p.
9. KING, C. J., & C. HOPE. 1932. Distribution of the cotton-root-rot fungus in soil and in plant tissues in relation to control by disinfectants. J. Agr. Res. 45: 725-740.
10. KING, C. J., & H. F. LOOMIS. 1929. Further studies of cotton-root-rot in Arizona with a description of a sclerotium stage of the fungus. J. Agr. Res. 39:641-676.
11. NEAL, D. C. 1929. The occurrence of viable cotton-root-rot sclerotia in nature. Science 70:409-410.
12. NEAL, D. C., & R. E. WESTER. 1932. Effects of anaerobic conditions on the growth of the cotton-root-rot fungus, Phymatotrichum omnivorum. Phytopathology 22:917-920.
13. RATLIFFE, G. T. 1929. A prolonged saprophytic stage of the cotton-root-rot fungus. USDA Circ. 67. 8 p.
14. REA, H. E. 1939. The control of cotton-root-rot in the blackland region of Texas. Texas Agr. Exp. Sta. Bull. 573. 36 p.
15. ROGERS, C. H. 1942. Cotton-root-rot studies with special reference to sclerotia, cover crops, rotations, tillage, seeding rates, soil fungicides, and effects of seed quality. Texas Agr. Exp. Sta. Bull. 614. 45 p.
16. SHEAR, C. L., & G. F. MILES. 1907. The control of Texas root-rot of cotton. USDA Bur. Plant Indus. Bull. 102:39-42.
17. SHEAR, C. L., & G. F. MILES. 1908. Texas root-rot of cotton. USDA Bur. Plant Indus. Circ. 9. 7 p.
18. STREETS, R. B. 1937. Phymatotrichum (cotton or Texas) root-rot in Arizona. Arizona Agr. Exp. Sta. Tech. Bull. 71. p. 299-410.
19. TACKETT, J. L. 1968. Theory and application of gas chromatography in soil aeration research. Soil Sci. Soc. Amer. Proc. 32:346-350.
20. TAUBENHAUS, J. J., W. N. EZEKIEL, & J. P. LUSK. 1931. Preliminary studies on the effect of flooding on Phymatotrichum root rot. Amer. J. Bot. 18:95-101.