Effects of Oxygen, Carbon Dioxide, and Ethylene on Growth, Sclerotial Production, Germination, and Infection by *Sclerotinia minor*

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ABSTRACT

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At O_2 concentrations ranging from 4 to 21% (normal air) with CO_2 nearly constant at 0.03%, in vitro differences in radial growth or sclerotial production by *S. minor* were not significant, but at O_2 concentrations below 4% both parameters were greatly reduced. Sclerotial production was more sensitive to O_2 concentrations below 4% than was radial growth. At concentrations of $CO_2 \geq 8\%$ with O_2 kept at about 21%, both mycelial growth and sclerotial formation were reduced, but sclerotial formation was more sensitive to high levels of CO_2 than was radial growth. Germination of sclerotia increased with increasing O_2 concentrations between 1% and 8% with CO_2 kept nearly constant at 0.03%, but there were no significant differences in germination at O_2 contents ranging from 8 to 21%. With O_2 nearly constant at 21% there was a significant reduction in sclerotial germination at about 20% CO_2 but not at lower concentrations of CO_2. In the various O_2–CO_2 gas mixtures, sclerotial germination was similar to that obtained with similar levels of CO_2 when CO_2 levels alone were increased. No germination occurred in 19% CO_2 and 2% O_2. Results of in vitro tests with different combinations of concentrations of O_2 and CO_2 on infection of lettuce tissue by sclerotia indicated that O_2 concentrations of 7.8% and above were more favorable for infection than lower concentrations, and CO_2 concentrations $\geq$ 12.3% were less favorable than lower concentrations. Percent infection of lettuce tissues by sclerotia was less at the respective O_2 and CO_2 levels than was infection by mycelial disks. These results indicate that restriction of infection to lettuce tissues at or near the soil surface is not due to impaired aeration at the greater soil depths.

Additional key word: epidemiology.

Despite numerous reports on sclerotial formation, germination, growth, infection, and control of *Sclerotinia* spp. (1,3,6,7,12,13,16, 17,20,21,26,28), reports on effects of O_2, CO_2, and ethylene on the biology and epidemiology of these fungi are meager. Adams and Tate (2) reported that sclerotia in the upper 2-cm of the soil in the greenhouse were responsible for 90% of the infections of lettuce plants by *S. minor*, and Marcum et al (21) reported that about half of the infection of lettuce by *S. minor* in the field originated from sclerotia located within the upper 10 cm of soil. Stone and Smith (26) reported that covering the soil surface with a few additional inches of sterile soil resulted in control of lettuce drop. The effects of aeration were not discussed by the above authors (2,21,26), but Louvet and Bulit (20) reported that where two rows of lettuce were planted on the same side of ridges adjacent to the irrigation furrow, there was more infection by *S. minor* in the upper rows of lettuce which were in dryer and better aerated soil than the lower rows. Louvet and Bulit (20) also found that at low CO_2 levels in their experiments, infection occurred earlier and all plants became infected, whereas increase in CO_2 above 5.5% reduced the final percentages of infected plants and increased the time required for infection. Similarly, Adair (4) reported that a storage atmosphere with 1.4% O_2 controlled postharvest decay of cabbage by *S. sclerotiorum* and *Botrytis cinerea*.

The observed common occurrence of lettuce drop in the field at or close to the soil surface and the decrease in infection of lettuce plants with increase in depth of sclerotia in the soil (2,21,26) led to this investigation on the effect of O_2, CO_2, and ethylene levels on growth, sclerotial formation, germination, and infection by *S. minor*.

MATERIALS AND METHODS

Preparation of gas compositions. Required amounts of compressed air, CO_2, N_2, and ethylene were metered through capillary tubes of known resistance to gas flow and mixed to obtain various gas concentrations (19). The final gas compositions flowing at a constant rate of 6.25 L/hr were allowed to bubble through a 130-cm column of water to maintain a constant pressure before passage through 6.25 L, cylindrical glass jars containing the experimental material. The jars were equipped with air-tight lids containing inlet and outlet openings. The O_2, CO_2, and N_2 concentrations of introduced gas mixtures were determined with a Carle 8000 gas chromatograph (Carle, Inc., Fullerton, CA 92631) equipped with a thermal-conductivity detector and molecular sieve and silica-gel columns. The ethylene concentrations were determined with a Carle 211 gas chromatograph equipped with a flame-ionization detector and an alumina column. Sclerotia on moist quartz sand, inoculated lettuce leaf disks, or fungus cultures in 9.0-cm-diameter petri plates were exposed to the various gas mixtures in the jars. Sterilized paper clips were used to prop up the lid of petri plates to facilitate gas circulation. All the investigations were carried out in an incubator maintained at 20 C.

Effect of gas composition on growth, sclerotial production, and germination of *Sclerotinia minor*. Five-mm-diameter mycelial disks of *S. minor* (isolates SM-1, CSS-1, and SV-1) obtained from 3-to 5-day-old cultures on Bacto cornmeal agar (CMA) were used to inoculate the center of 9.0-cm-diameter plastic petri plates containing 20 ml of CMA. Four replicate plates were incubated in each gas composition. Radial growth was determined after 48 hr and sclerotia were counted on five 2.3-cm-diameter disks cut at random from each plate after 21 days. After 6 wk of incubation, sclerotia were harvested, washed, dried, and tested for eruptive germination on moist quartz sand (17). Three-month-old sclerotia produced on autoclaved oat seed were similarly tested for germination. Four plates each containing 25 sclerotia were held in the different gas mixtures for 5 days before germination percentages were determined.

Effect of gas composition on infection and sclerotial production on lettuce leaf disks. To determine the effect of gas compositions on infection of lettuce, leaf disks 5.0 cm in diameter and weighing approximately 1.25 g were cut from the outer leaves of nearly mature lettuce (*Lactuca sativa* L.) heads. Each disk was placed in a

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5.5-cm-diameter petri plate and inoculated with either a 5-mm mycelial disk of mycelium obtained from a 3- to 5-day-old culture or with one sclerotium of *S. minor* produced on autoclaved oat seed. Four such plates were exposed to each gas composition. Tissues were assessed for infection after 7 days and for sclerotial formation after 21 days. After 6 wk, sclerotia were harvested, washed, dried, and evaluated for ability to germinate eruptively on moist quartz sand.

RESULTS

Effect of O_2 on radial growth and sclerotial production. At 1% O_2, average radial growth of isolate CSS-I after 48 hr was 7.5 mm; growth was increased to 32.5 mm at 4.1% O_2, but further increases in O_2 above 4.1% did not significantly increase growth (Fig. 1). Results from tests of other isolates were similar to those presented in Fig. 1.

No sclerotia were produced at 1% O_2, but sclerotial production increased with increase in O_2 from 1 to 21% with CO_2 kept constant at about 0.03%. Sclerotial production at O_2 levels $\geq$ 8% did not differ significantly (Fig. 1). Sclerotial production was more sensitive to O_2 concentration below 4% than was radial growth.

Effect of CO_2 on radial growth and sclerotial production. Radial growth was decreased when CO_2 was increased from 0.03 to 20% while O_2 was maintained at about 21%. Growth after 48 hr of incubation in 0.03% CO_2 was 36 mm, and at 20.2% CO_2 growth was 25% of the maximum obtained in normal air (Fig. 2).

Production of sclerotia decreased with increasing CO_2 levels with O_2 kept constant at about 21%. Sclerotial production was more sensitive to higher CO_2 than radial growth and at 20.2% CO_2 no sclerotia were produced (Fig. 2).

Effect of O_2 and CO_2 mixtures on radial growth and sclerotial production. Radial growth increased as O_2 levels increased and CO_2 levels were correspondingly decreased in the gas mixtures. Sclerotial production also increased with increasing O_2 and decreasing CO_2 levels but was more sensitive to high CO_2 in the gas mixture than was radial growth (Fig. 3). Similar results were obtained when O_2 was kept constant and CO_2 levels were increased.

Effect of gas compositions on sclerotial germination. Six-week-old sclerotia produced on CMA or infected lettuce tissue in the different gas compositions were washed, dried, and tested for eruptive germination on moist quartz sand in normal air. Germination percentages ranged from 28 to 31% and differences were not significantly different. The effect of gas compositions on germination of 3-mo-old sclerotia produced on autoclaved oats in normal air also was tested. With increase in O_2 levels from 1 to 21% while CO_2 was kept at near constant level of 0.03%, germination increased to a maximum (85%) at 21% O_2 concentration (Fig. 1).

Sclerotial germination was high at low CO_2 levels, but increase from .03% CO_2 (normal air) to 20.2% CO_2 while O_2 was kept at near constant level of 21% resulted in a progressive decrease in germination. After 5 days of incubation at various CO_2 levels, germination at 20.2% CO_2 was about 50% of the maximum (Fig. 2).

The results of testing the effects of O_2 and CO_2 mixtures in which

TABLE 1. In vitro effects of O_2 concentrations on infection of and sclerotial formation on lettuce leaf tissue disks inoculated with *Sclerotinia minor*

	Infection and sclerotial formation			
	Inoculum: sclerotia		Inoculum: mycelial disks	
O_2[a] (%)	infection[b]	sclerotia[c]	infection[b]	sclerotia[c]
1.0	0 d	0 d	0 b	0 f
2.0	0 d	0 d	1 b	34 e
4.1	1 d	86 c	3 a	83 d
7.8	2 c	97 ab	4 a	123 c
12.2	3 b	91 bc	3 a	133 b
17.6	4 a	96 bc	4 a	144 a
21.0	4 a	109 a	4 a	136 a

[a] CO_2 concentration was maintained at about 0.03%.

[b] Average number of leaf tissue disks infected after 7 days from four replications in three trials. Values in the column with the different letters are significantly different according to Duncan's multiple range test (P = 0.05).

[c] Average number of sclerotia formed per tissue disk after 30 days. Values in the column with the different letters are significantly different according to Duncan's multiple range test (P = 0.05).

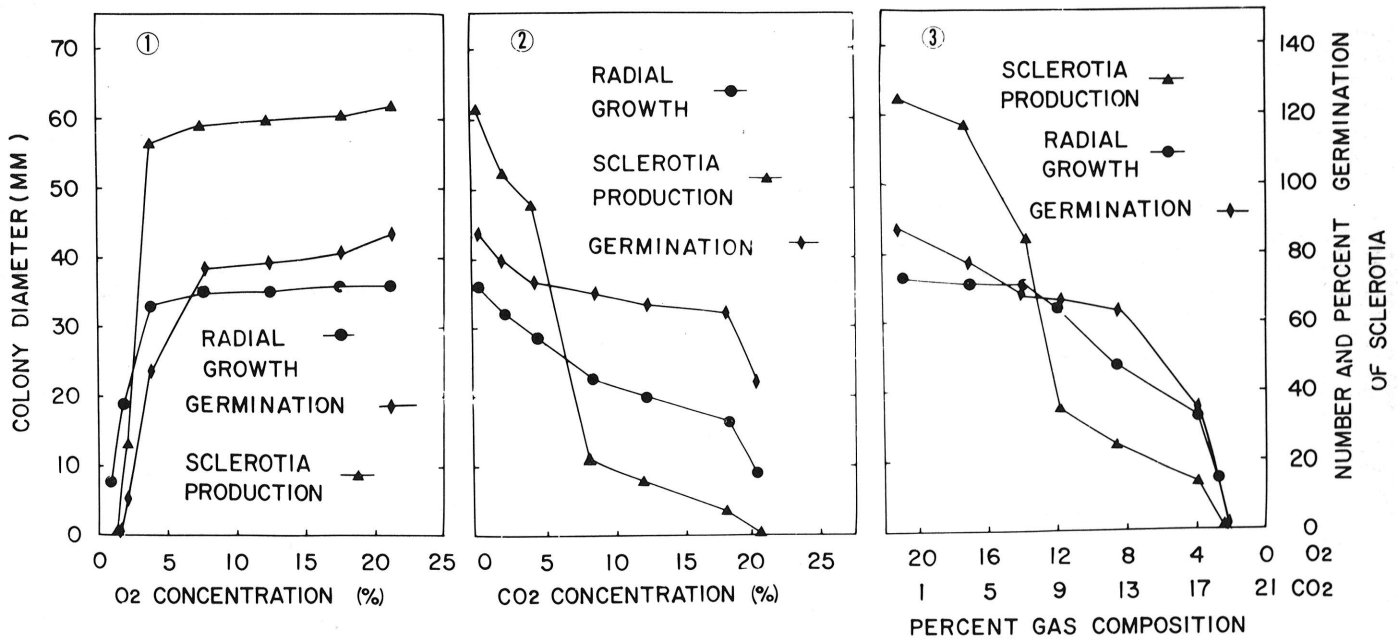

Fig. 1–3. In vitro effects of various concentrations of O_2 and CO_2 on radial growth, sclerotial production, and germination by *Sclerotinia minor*. Cultures of *S. minor* on CMA were introduced into jars with various combinations of concentrations of O_2 and CO_2. The rate of growth was assessed by measuring radial expansion after 48 hr. Sclerotial production was assessed after 21 days by counting sclerotia on five 2.3-cm-diameter disks cut at random from each plate. Sclerotia of *Sclerotinia minor* on moist quartz sand were introduced into jars with various combinations of concentrations of O_2 and CO_2 for 5 days after which their germination percentages were determined. **1,** Effect of O_2 concentrations on radial growth, sclerotial production, and germination by *Sclerotinia minor*. Carbon dioxide was held constant at 0.03% and O_2 was increased from 1.0 to 21%. **2,** Effect of CO_2 concentrations on radial growth, sclerotial production, and germination by *Sclerotinia minor*. Oxygen was held nearly constant at 21% and CO_2 was increased from 0.03 to 20%. **3,** Effect of varying inversely the concentration of O_2 and CO_2 on radial growth, sclerotial production, and germination by *Sclerotinia minor*.

O_2 and CO_2 were varied inversely (Fig. 3) were similar to results obtained by testing different concentrations of the individual gases (Figs. 1 and 2). At the lowest O_2 and highest CO_2 combination (2% O_2: 19% CO_2), no sclerotia germinated. Maximum sclerotial germination (85%) was obtained in normal air (0.03 CO_2 and 21% O_2).

Effect of O_2 and CO_2 concentrations on infection and sclerotia production on lettuce leaf disks. The infection of lettuce leaf disks by sclerotia of *S. minor* increased with increase in O_2 from 1 to 21%; differences at O_2 concentrations above 7.8% were not significant. Inoculation of tissues with mycelial disks resulted in more infections at the same O_2 levels than did inoculation with sclerotia. Numbers of sclerotia formed at the respective O_2 concentrations also increased with increase in levels of O_2 (Table 1), but the differences above 7.8% O_2 were not significant.

Sclerotia production on infected leaf disks was nil at 20.2% CO_2 and increased with decrease in CO_2. Infection of leaf disks by sclerotia of *S. minor* decreased with increase in CO_2; infection from mycelial disk inocula also decreased with increase in CO_2, but more infections were obtained with mycelial disks than with sclerotia (Table 2). More sclerotia were produced when the lettuce leaf disks were inoculated with mycelial disks than when sclerotia were used as inocula (Table 2).

When leaf disks inoculated with sclerotia were incubated in various concentrations of O_2–CO_2 mixtures, more infected leaf disks and increased sclerotial production occurred with increased O_2 and decreased CO_2 (Table 3). More infections and greater average numbers of sclerotia resulted from inoculations with mycelial disks rather than with sclerotia at the same gas levels.

Effects of ethylene on radial growth, sclerotial production, germination, and infection by *S. minor*. Radial growth, sclerotial production, and germination of sclerotia incubated in seven different levels of C_2H_4 ranging from 0–40 μl/L were not inhibited and were similar to controls incubated in normal air. Also, exposure of inoculated leaf disks to the same ethylene concentrations did not result in differences in infection and sclerotial formation on the infected leaf disks.

TABLE 2. In vitro effects of CO_2 concentrations on infection of and sclerotial formation on lettuce leaf tissue disks inoculated with *Sclerotinia minor*

CO_2[a] (%)	Infection and sclerotial formation			
	Inoculum: sclerotia		Inoculum: mycelial disks	
	infection[b]	sclerotia[c]	infection[b]	sclerotia[c]
0.03	4 a	109 a	4 a	146 a
2.1	2 b	104 ab	3 ab	153 a
4.4	2 bc	92 c	3 ab	149 a
8.5	2 bc	98 bc	4 a	135 b
12.3	1 cd	76 d	2 b	127 c
18.0	1 d	0 e	3 ab	89 d
20.2	0 d	0 e	0 c	0 e

[a] O_2 was maintained at ~21%.
[b] Average number of leaf tissue disks infected after 7 days based on four replications in three trials. Values in the column with different letters are significantly different according to Duncan's multiple range test ($P = 0.05$).
[c] Average number of sclerotia formed per tissue disk after 30 days. Values in the column with different letters are significantly different according to Duncan's multiple range test ($P = 0.05$).

TABLE 3. In vitro effects of inversely varying the concentrations of O_2 and CO_2 on infection of and sclerotial formation on lettuce leaf disks inoculated with *Sclerotinia minor*

O_2:CO_2[a] (%)	Infection and sclerotial formation			
	Inoculum: sclerotia		Inoculum: mycelial disks	
	infection[b]	sclerotia[c]	infection[b]	sclerotia[c]
2.5 : 18.4	0 c	0 d	0 c	0 c
3.7 : 17.3	0 c	0 d	2 b	95 b
8.7 : 12.3	1 c	41 c	2 b	101 b
11.4 : 9.6	1 c	56 b	3 a	112 b
14.1 : 6.9	2 b	61 b	4 a	146 a
17.6 : 3.4	4 a	94 a	4 a	148 a
21.0 : .03	4 a	98 a	4 a	146 a

[a] Carbon dioxide was increased from 0.03 to 18.4% whereas O_2 was decreased from 21 to 2.5% in the gas mixtures.
[b] Average number of leaf tissue disks infected after 7 days, based on four replications in three trials. Values in the column followed by different letters are significantly different according to Duncan's multiple range test ($P = 0.05$).
[c] Average number of sclerotia formed per tissue disk after 30 days. Values in the column followed by different letters are significantly different according to Duncan's multiple range test ($P = 0.05$).

DISCUSSION

Infection of lettuce by *S. minor* from eruptive germination of sclerotia can occur either at the soil surface, where old leaves or the crown may be infected or belowground where roots may be infected. However, most infections occur at or very close to the soil surface (2,21,26). It appears that germination of sclerotia and infection of lettuce may be reduced or prevented at soil depths greater than about 10 cm. However, the levels of O_2 and CO_2 present in most arable soils probably cannot account for the reduced germination and infection below the soil surface, because in most soils the concentration of O_2 rarely is less than 10% and CO_2 is usually 0.2–2% and rarely exceeds 10% (11,14). As with *S. minor*, there is a consensus that infections caused by *Sclerotium rolfsii* generally occur very close to the soil surface (5), even though this fungus also germinates and grows at O_2 and CO_2 levels existent in most soils (15).

Although Smith (23) and Smith and Cook (24) reported that ethylene is commonly produced in soil and that a concentration of about 1 μl/L is fungistatic to germination of sclerotia of *S. rolfsii*, seven levels of ethylene ranging from 0 to 40 μl/L did not affect sclerotia germination and infection by *S. minor*. The possibility of ethylene causing an indirect effect on germination by influencing other soil microorganisms (23,24) is not ruled out, however.

The preponderance of lettuce drop resulting from infection at or near the soil surface may be due to the requirement for drying of sclerotia to induce eruptive germination rather than to impaired aeration at the greater soil depths. This possibility is supported by the report by Smith (25) and Beach (6) that incidence of drop caused by *S. minor* was increased greatly in crops grown in infested soil that had been allowed to dry prior to being planted to lettuce. Another reason for greater damage from infections near the soil surface may be that infection on the lower portion of a root may not move upward rapidly enough to girdle the root-crown in the vital zone prior to harvest. Therefore, plants may not develop drop even though they have been infected.

Besides environmental factors affecting germination and infection, numbers of germinable sclerotia also influcence the incidence of drop. For example, we found that sclerotia on the soil surface survived longer than those at lower depths (18); thus, more sclerotia of *S. minor* would be expected to occur near the soil surface.

Our results indicate that sclerotia production by *S. minor* was more sensitive to low levels of O_2 and high levels of CO_2 than was radial growth (Figs. 1–3). Similar findings have been reported for other fungi wherein high CO_2 inhibited the formation of resistant resting structures, but stimulated mycelial growth (8,9–11,15,19, 22,27). Pilkington (22) suggested that this difference is due to the increased energy requirements for the formation of melanized resting structures. However, the levels of O_2 and CO_2 usually present in soil probably would not limit sclerotial production and germination. Therefore, it seems unlikely that lack of infection by *S. minor* at depths greater than 10 cm is attributable to the influence of soil gas composition.

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