Carbon Loss and Germinability, Viability, and Virulence of Chlamydospores of *Fusarium solani* f. sp. *phaseoli* After Exposure to Soil at Different pH Levels, Temperatures, and Matric Potentials

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Accepted for publication 13 November 1997.

ABSTRACT

Mondal, S. N., and Hyakumachi, M. 1998. Carbon loss and germinability, viability, and virulence of chlamydospores of *Fusarium solani* f. sp. *phaseoli* after exposure to soil at different pH levels, temperatures, and matric potentials. *Phytopathology* 88:148-155.

^{14}C -labeled chlamydospores of *Fusarium solani* f. sp. *phaseoli* were exposed to soil at 5, 15, 25, or 30°C at pH 5 or 8 and water potential of -1 kPa or to soil at 0, -1 , or -10 kPa at 25°C at pH 6.9. Total carbon loss was greatest at 25 or 30°C at pH 8 and -1 kPa. $^{14}\text{CO}_2$ from respiration of chlamydospores and from soil microbes utilizing chlamydospore exudates accounted for the largest share of total carbon loss under all con-

ditions. $^{14}\text{CO}_2$ from soil microbial metabolism of ^{14}C exudates of chlamydospores was greatest in soil at 15, 25, and 30°C, pH 8, and at either -1 or -10 kPa. Chlamydospore germinability in the absence of a C source (nutrient independence), viability in potato-dextrose broth, and virulence to kidney bean declined rapidly after exposure to soil at high temperatures (25 and 30°C), pH 8, and the higher matric potentials (0 to -1 kPa). By contrast, germinability remained high ($>50\%$), as did virulence, in soil at 5°C and -10 kPa even after 70 days of incubation. Carbon loss was inversely correlated with germinability, viability, and virulence after exposure to soil at different pH levels, temperatures, and matric potentials.

Fungistasis of nutrient-independent fungal propagules in non-sterile soil is related to the rapid loss of endogenous carbon through exudation (3,27) and respiration (15,22). In fungistatic soil, microorganisms increase exudation of nutrients from the fungal propagules, leading to their debilitation (19,20). Filonow and Arora (7) showed that the amount of exudation from fungal propagules was related to the numerical microbial population of the soil. Arora (1) showed increased carbon loss from *Bipolaris sorokiniana* conidia in the presence of microorganisms in vitro. Such results implicate soil microbes in the imposition of nutrient stress upon fungal propagules. Exudation from fungal propagules incubated on several nonsterile soils has been shown to be greater than that occurring on sterile sand saturated with dilute salts solution (8,27). Very recently, Mondal et al. (22) showed that chlamydospores lost more endogenous carbon through respiration in nonsterile than in sterile soil. These results further implicate microbial activity in enhanced propagule exudation and respiration.

Carbon loss from fungal propagules in soil, if excessive, can lead to decreased virulence (6,15,22), increased dependence on exogenous nutrients for germination (9), or death (5). Arora (1) and Arora et al. (2) showed that decreased virulence and loss of ^{14}C were imposed nonspecifically when different microorganisms were used to reinfest sterilized soil. Thus, soil environmental factors could affect the activity of soil microbes and could lead to biological control strategies against soilborne plant pathogens.

The influence of soil moisture, pH, and temperature on the expression of competitive stress deserves consideration, since numerous aspects of microbial life cycles, such as rate of exudation from fungal propagules, the exudate's movement toward sink microor-

ganisms (3), and the population density of microorganisms (28), are influenced by these factors. We demonstrated in previous work that chlamydospores of *Fusarium solani* (Mart.) Sacc. f. sp. *phaseoli* (Burkholder) W. C. Snyder & H. N. Hans. increasingly lost carbon after exposure to soil (22). However, the effect of soil environmental factors on carbon loss from chlamydospores is unknown. In this study, carbon loss from chlamydospores of *F. solani* f. sp. *phaseoli* was investigated in relation to germinability, viability, and virulence after exposure to soil at different pH levels, temperatures, and matric potentials.

MATERIALS AND METHODS

An isolate of *F. solani* f. sp. *phaseoli*, originally isolated from infected kidney bean (*Phaseolus vulgaris* L.), was used to produce chlamydospores in bean root extract (23). Root extract was supplemented with 2.5 μCi (1 Ci = 37 GBq) of [^{14}C]glucose (specific activity, 180 $\mu\text{Ci}/\mu\text{mol}$) to produce ^{14}C -labeled chlamydospores. The density was adjusted to 10^4 chlamydospores per 20 μl of spore suspension, and the suspension was kept on ice to prevent germination.

A sandy loam agricultural soil (pH 6.9 and 5% organic matter) from Gifu University Farm was used in all tests. The soil was collected from an area that had been fallow for several years and to which pesticides had not been applied. The relationship between soil matric potentials and gravimetric water content of the soil was determined by using tensiometers (11) consisting of 150-ml Büchner funnels with sintered glass plates of fine porosity (4.5 μm). Specific matric potentials (0, -1 , and -10 kPa) were achieved by adjusting the water content of the soil according to the relationship of matric potential to water content, determined by the tensiometers. For the sandy loam, 0, -1 , and -10 kPa corresponded to 38, 27, and 17% water (wt/wt), respectively. The pH of the soil was determined electrometrically in a mixture of soil and water (1/2, wt/vol). Soil was supplemented with aluminum sulfate, $\text{Al}_2(\text{SO}_4)_3$, or potassium carbonate, K_2CO_3 , to adjust the pH

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level to 5 or 8. The soils were thoroughly mixed and incubated in polyethylene bags in the laboratory at 25°C. Although there were initial changes in soil pH, the addition of more aluminum sulfate or potassium carbonate, thorough mixing, and further incubation stabilized the soil pH at the desired level.

Four circular polycarbonate membrane filters (1 cm in diameter, 0.2-µm pore size; Nuclepore, Pleasanton, CA), each containing approximately 10^4 ^{14}C -labeled chlamydospores, were placed between two larger circular Nuclepore polycarbonate filters (2.5 cm in diameter, 0.2-µm pore size). The edges of the filters were sealed with sterile (autoclaved) silicone vacuum grease to exclude microorganisms. These membrane filter envelopes were placed on the surface of approximately 5 g of nonsterile soil contained in plastic planchets (25 mm in diameter, 7 mm deep). The eight planchets were placed on 300 g of water-saturated sand in a glass container (9.5 cm in diameter, 7.5 cm high). The containers were tightly closed with rubber stoppers fitted with two glass tubes that allowed the passage of air for sampling but were connected by latex tubing and a plastic connector to maintain a closed system during incubation. The containers with soil samples of pH 5 or 8 and moisture content of -1 kPa were incubated separately in four incubation chambers (Sanyo, Japan), which were set at 5, 15, 25, and 30°C. Containers with soil moisture contents of 0, -1, and -10 kPa, pH 6.9, were incubated at 25°C. For each soil treatment, there were two replicates, each consisting of two containers. Each container contained eight planchets, all of one soil treatment. The initial radioactivity per container was 32×10^4 dpm.

Carbon loss was assessed by measuring two components of ^{14}C loss, evolved $^{14}\text{CO}_2$ and residual ^{14}C in the soil (10,17). Soil microbial respiration of ^{14}C exudates was estimated during 4 days after removal of the membrane from the soil (22).

The ability of chlamydospores to germinate in the absence of a C source (1% Pfeffer's salts solution) or in the presence of a C source (potato-dextrose broth [PDB]) was determined after incubation in soil. The filters bearing chlamydospores were placed in 5 ml of 1% Pfeffer's salts solution or in 3 ml of PDB in sterilized Petri dishes (90 mm) and incubated for 12 h at 25°C. Whenever propagules showed a decline in germinability, the incubation period was extended to 48 h. Chlamydospores were stained with 0.05% cotton blue, and 100 propagules were randomly counted for germination by using a light microscope (40×) with epi-illumination. Each circular filter bearing chlamydospores was considered one replicate. Three replicated filters per treatment were counted at each sampling time. Chlamydospore virulence to kidney bean cultivar Hatumidori Nigou after incubation on soil was tested in glass culture tubes (180 × 20 mm) filled with 5 g of autoclaved sandy loam soil (22).

Statistical analysis. The experiments were carried out in completely randomized design with two or three replicates per treatment. All the experiments were done twice. Since the outcomes of the experiments were similar, data from the first experiment are shown. Percentage data were transformed to angular scale to stabilize the variances (26). Statistical analyses were done with the software MSTAT (Michigan State University, East Lansing).

TABLE 1. Evolved $^{14}\text{CO}_2$, residual ^{14}C , and total ^{14}C loss from ^{14}C -labeled chlamydospores of *Fusarium solani* f. sp. *phaseoli* after 70 days of exposure to specific matric potentials

Matric potential (kPa)	^{14}C loss (% of total label) ^z		
	Evolved $^{14}\text{CO}_2$	Residual ^{14}C in soil	Total ^{14}C loss
0	22 c	13 a	35 b
-1	43 a	7 b	50 a
-10	31 b	5 b	36 b

^z Each value is the mean of two replicates. Values followed by the same letter do not differ significantly ($P = 0.05$) according to Duncan's multiple range test.

The treatment means were separated by Duncan's multiple range test ($P = 0.05$) after analysis of variance. Correlations on the means of first trial were performed where appropriate.

RESULTS

Carbon loss by chlamydospores. Chlamydospores of *F. solani* f. sp. *phaseoli* exposed to soil at different matric potentials evolved $^{14}\text{CO}_2$ at a low rate during the first 5 days. Thereafter, $^{14}\text{CO}_2$ evolution increased rapidly until about 30 days, and then rate of evolution became static (*data not shown*). After 70 days, total $^{14}\text{CO}_2$ evolution in soil at -1 kPa (43%) was significantly higher than at -10 (31%) or 0 (22%) kPa. These last two values also differed significantly (Table 1). $^{14}\text{CO}_2$ production increased as the soil temperature increased, and more $^{14}\text{CO}_2$ was evolved in soil at pH 8 than at pH 5 at all temperatures (Table 2). Residual ^{14}C in the soil increased as the moisture content of the soil increased (Table 1). After 70 days, residual ^{14}C in water-saturated soil (0 kPa) was 13% of total label, whereas the corresponding values in soils at -1 and -10 kPa were 7 and 5%, respectively. Residual ^{14}C in soil at different pH levels and temperatures differed little after 70 days of the experiment, ranging between 4.5 and 6.8% (Table 2).

Total carbon loss from chlamydospores was significantly higher in soil at -1 kPa (50%) than at -10 kPa (36%) or 0 kPa (35%)

TABLE 2. Evolved $^{14}\text{CO}_2$, residual ^{14}C , and total ^{14}C loss from ^{14}C -labeled chlamydospores of *Fusarium solani* f. sp. *phaseoli* after 70 days of exposure to soil at different temperatures and pH levels

Temperature (°C)	pH	^{14}C loss (% of total label) ^z		
		Evolved $^{14}\text{CO}_2$	Residual ^{14}C in soil	Total ^{14}C loss
5	5	10 a	6.3 a	16.3 a
	8	13 a	5.9 a	18.9 a
15	5	21 a	5.5 a	26.5 a
	8	25 a	4.9 a	29.9 a
25	5	26 b	6.8 a	32.8 b
	8	37 a	5.7 a	42.7 a
30	5	30 b	4.5 a	34.5 b
	8	45 a	4.9 a	49.9 a

^z Each value is the mean of two replicates. Values within a column followed by the same letter do not differ significantly ($P = 0.05$) according to Duncan's multiple range test.

Fig. 1. Microbial metabolism of ^{14}C in soil (as $^{14}\text{CO}_2$ collected) after the removal of ^{14}C -labeled chlamydospores of *Fusarium solani* f. sp. *phaseoli* from soil adjusted to varying matric potentials. Values are expressed as percentages of total residual ^{14}C in the soil at the time propagules were removed. Each point is the mean of two replicates. Values with the same letter do not differ significantly ($P = 0.05$).

(Table 1). Carbon loss was greater at pH 8 than at pH 5, regardless of temperature (Table 2). However, the differences were significant only at 25 and 30°C. Chlamydospores in soil at 30°C and pH 8 lost the most carbon (50%) followed by those in soil at 25°C at pH 8 (43%). Least carbon was lost at 5°C and pH 5 (16%) or 8 (19%).

Soil microbes respired most carbon in soil in the order $-1 \text{ kPa} > -10 \text{ kPa} > 0 \text{ kPa}$ (Fig. 1). After 4 days of incubation, microbes respired significantly more ^{14}C in soil at pH 8 than at pH 5 when incubated at 15, 25, or 30°C (Fig. 2). At 5°C, microbial metabolism was low and did not differ significantly between pH 5 and 8.

Fig. 2. Microbial metabolism of ^{14}C in soil (as $^{14}\text{CO}_2$ collected) after the removal of ^{14}C -labeled chlamydospores of *Fusarium solani* f. sp. *phaseoli* from soil adjusted to varying pH levels and temperatures. Values are expressed as percentages of total residual ^{14}C in the soil at the time propagules were removed. Each point is the mean of two replicates. Values with the same letter do not differ significantly ($P = 0.05$).

TABLE 3. Germinability and virulence of chlamydospores of *Fusarium solani* f. sp. *phaseoli* after exposure to soil with specific matric potentials

Germinability or virulence Matric potential (kPa)	Exposure period of chlamydospores to soil (days) ^x						
	10	20	30	40	50	60	70
Germinability in Pfeffer's salts solution ^y							
0	96 a, A	97 a, A	59 b, B	45 b, BC	39 b, C	0 c, D	0 b, D
-1	100 a, A	95 a, A	78 b, AB	42 b, B	32 b, BC	7 b, C	0 b, D
-10	100 a, A	100 a, A	90 a, A	58 a, B	47 a, B	32 a, B	18 a, C
Germinability in potato-dextrose broth ^y							
0	100 a, A	99 a, A	83 b, A	53 b, B	31 c, C	22 c, CD	0 c, D
-1	100 a, A	100 a, A	95 a, A	79 a, AB	45 b, BC	36 b, C	0 b, D
-10	100 a, A	100 a, A	100 a, A	79 a, AB	75 a, AB	67 a, B	57 a, B
Virulence to kidney bean ^z							
0	3.1 a, A	2.8 b, A	3.0 b, A	2.1 b, B	1.6 c, BC	1.2 c, C	0.6 c, D
-1	3.5 a, A	3.3 a, A	2.8 a, AB	3.0 a, A	2.1 b, B	1.9 b, B	1.2 b, C
-10	3.8 a, A	3.3 a, A	3.2 a, A	3.0 a, A	3.0 a, A	2.7 a, AB	2.0 a, B

^x Values within a column followed by the same lowercase letter and within a row followed by the same uppercase letter do not differ significantly ($P = 0.05$) according to Duncan's multiple range test.

^y Each value is the mean of three replicates.

^z Chlamydospore virulence was estimated on a scale of 0 to 4, in which 0 = healthy seedlings and 4 = dead seedlings.

Chlamydospore germinability and viability. Chlamydospores were highly germinable in the absence of a C source (i.e., in Pfeffer's salts solution before exposure to soil), but their germinability declined after 30 days' exposure to soil at 0 kPa and after 40 days at -1 and -10 kPa at 25°C (Table 3). On day 60, chlamydospores that had been incubated on soil at 0 kPa failed to germinate in the absence of a C source. At 70 days, chlamydospores in soil at -1 kPa had completely lost nutrient independence, and only 18% of chlamydospores in soil at -10 kPa remained germinable. The chlamydospores' loss of nutrient independence occurred more rapidly at pH 8 than at pH 5 when the soil was incubated at 15, 25, or 30°C; the rate of loss increased as temperature increased (Table 4). Chlamydospore germinability in Pfeffer's salts solution declined to zero in soil at 25 or 30°C and

pH 8. By contrast, germination levels at pH 5 at 25 and 30°C were 19 and 39%, respectively. Loss of nutrient independence was least in soil at 5°C and either pH 5 or 8.

Chlamydospore germinability in PDB (viability) declined after exposure to soil but at a lower rate than the loss of nutrient independence (Table 3). Chlamydospore viability remained greater than 80% for 40 days, after which viability declined sharply, except in soil at -10 kPa (Table 3) or 5°C and pH 5 or 8 (Table 5). Chlamydospores lost all germinability in PDB only when exposed to soil at 0 and -1 kPa or at 30°C and pH 8 for 70 days, whereas 57% of chlamydospores at -10 kPa and 25 to 58% at 15 or 25°C and pH 5 or 8 remained viable after 70 days (Tables 3 and 4). Carbon loss from chlamydospores during exposure to soil at different matric potentials or at different temperatures and pH levels

TABLE 4. Germination (%) of *Fusarium solani* f. sp. *phaseoli* chlamydospores in Pfeffer's salts solution after incubation on soil at different temperatures and pH levels

Temperature (°C)	pH	Exposure period of chlamydospores to soil (days) ^z						
		10	20	30	40	50	60	70
5	5	97 a, A	87 a, A	82 a, A	88 a, A	82 a, A	86 a, A	73 a, A
	8	97 a, A	95 a, A	84 a, A	78 a, A	81 a, A	76 a, A	63 b, A
15	5	100 a, A	100 a, A	100 a, A	88 a, A	57 a, B	50 a, B	35 a, C
	8	100 a, A	94 a, A	87 b, A	92 a, A	52 a, B	38 b, C	23 b, D
25	5	100 a, A	85 a, A	86 a, A	67 a, AB	77 a, A	55 a, B	39 a, C
	8	87 b, A	67 b, B	73 b, A	43 b, C	39 b, C	17 b, D	0 b, D
30	5	94 a, A	84 a, AB	81 a, AB	59 a, B	42 a, C	30 a, CD	19 a, D
	8	86 a, A	79 a, AB	77 a, AB	40 b, B	9 b, C	2 b, C	0 b, C

^z Each value is the mean of three replicates. Values within a column followed by the same lowercase letter and within a row followed by the same uppercase letter do not differ significantly ($P = 0.05$) according to Duncan's multiple range test.

TABLE 5. Germination (%) of *Fusarium solani* f. sp. *phaseoli* chlamydospores in potato-dextrose broth after incubation on soil at different temperatures and pH levels

Temperature (°C)	pH	Exposure period of chlamydospores to soil (days) ^z						
		10	20	30	40	50	60	70
5	5	100 a, A	100 a, A	100 a, A	100 a, A	100 a, A	94 a, A	85 a, A
	8	100 a, A	100 a, A	100 a, A	94 a, A	96 a, A	92 a, A	73 b, B
15	5	100 a, A	100 a, A	98 a, A	97 a, A	80 a, AB	72 a, B	58 a, C
	8	93 a, A	100 a, A	93 a, A	97 a, A	62 b, AB	58 b, B	35 b, C
25	5	100 a, A	100 a, A	100 a, A	83 a, AB	82 a, AB	77 a, B	50 a, C
	8	100 a, A	87 b, AB	79 b, AB	55 b, B	62 b, BC	59 b, C	25 b, D
30	5	100 a, A	89 a, A	78 a, B	76 a, B	48 a, C	37 a, CD	32 a, D
	8	100 a, A	85 a, AB	77 a, B	32 b, C	19 b, D	3 b, D	0 b, D

^z Each value is the mean of three replicates. Values within a column followed by the same lowercase letter and within a row followed by the same uppercase letter do not differ significantly ($P = 0.05$) according to Duncan's multiple range test.

Fig. 3. Relationship between ^{14}C loss from ^{14}C -labeled chlamydospores of *Fusarium solani* f. sp. *phaseoli* and their ability to germinate in potato-dextrose broth (PDB) and Pfeffer's salts solution. Chlamydospores of different ^{14}C content were prepared by incubating them on soil at different matric potentials. There was a negative relationship between ^{14}C loss from chlamydospores and germination in PDB or in salts solution (arcsine transformation).

was inversely correlated with germinability in Pfeffer's salts solution and PDB (Figs. 3, 4, and 5).

Chlamydospore virulence. Chlamydospore virulence to kidney bean was reduced significantly after 40 to 60 days of exposure to soil at 0 and -1 kPa and at 70 days at -10 kPa (Table 3) or 40 to 70 days at soil temperatures of 15, 25, or 30°C and pH 5 or 8 (Table 6). The decline of chlamydospore virulence was most rapid in the wet soils, at the higher pH levels, and as soil temperature increased. Virulence was not significantly decreased at 5°C at either soil pH level. Carbon loss from chlamydospores was negatively correlated with virulence to kidney bean (Figs. 6 and 7), except at 5°C.

DISCUSSION

Exposure of chlamydospores of *F. solani* f. sp. *phaseoli* to soil at 5, 15, 25, and 30°C, pH 5 and 8, or to soil at 0, -1, and -10 kPa at 25°C and pH 6.9 resulted in progressive loss of endogenous carbon. Carbon loss from chlamydospores was greatest at 25 or 30°C and pH 8 and at -1 kPa than under any other soil conditions. These soil conditions may have enhanced the efficiency of soil microbes in imposing nutrient stress upon chlamydospores. Arora et al. (2) observed that greater exudation from fungal propagules occurred in the presence of microorganisms than in their absence. Filonow and Arora (7) found the greatest carbon loss from conidia of *Cochliobolus victoriae* and sclerotia of *Macrophomina phaseolina* in soil in which the total microbial population was high. Filonow and Lockwood (8,10) also found a correlation between the loss of ^{14}C exudate from ^{14}C -labeled propagules of *C. victoriae* and the population density of microbes in soils. The soil

conditions imposing the greatest carbon loss in our experiments appear to be those of greatest microbial activity. This premise was supported by high microbial metabolism of chlamydospore exudates under these conditions (Figs. 1 and 2). Lower carbon loss rates by chlamydospores in soil at pH 5 than at pH 8 might cast a doubt about the adverse effect of Al in soil microbes at pH 5 (13,18). In one study, Mondal and Hyakumachi (*unpublished results*) estimated carbon loss by conidia of *C. sativus* after exposure to soil adjusted to pH 5 or 6 with HCl, H_2SO_4 , or $\text{Al}_2(\text{SO}_4)_3$ and to pH 7 or 8 with NaOH, KOH, or K_2CO_3 . However, carbon loss by the propagules did not differ significantly depending on chemicals but did differ significantly on the basis of pH. Hintikka (12) observed high Al tolerance among 48 saprophytic fungi in aluminum sulfate, potassium aluminum sulfate, and aluminum chloride.

High carbon loss rates were related to the rapid metabolism of endogenous reserves, which was favored by soil environments of 25 or 30°C, pH 8, and -1 kPa. Hyakumachi et al. (14) also noticed the greatest carbon loss from *C. sativus* conidia in soil at 25°C and pH 8 or 9.

Residual ^{14}C increased in soil as soil moisture content increased. Filonow and Arora (7) also observed enhanced ^{14}C exudation from propagules in water-saturated soil. In the present work, in wet soil (0 kPa), less ^{14}C was utilized by soil microbes, suggesting that ^{14}C remained either in soil solution or within the cells of soil microflora. However, Hyakumachi et al. (16) observed negligible ^{14}C in the soil solution. Thus, it is likely that much of the ^{14}C exudates remained within the cells of the soil microflora and were not metabolized by them, possibly because of a shortage of available O_2 in wet soil.

Fig. 4. Relationship between ^{14}C loss from ^{14}C -labeled chlamydospores of *Fusarium solani* f. sp. *phaseoli* and their ability to germinate in Pfeffer's salts solution. Chlamydospores of different ^{14}C content were prepared by incubating them on soil at different pH levels and temperatures. There was a negative relationship between ^{14}C loss from chlamydospores and germination in Pfeffer's salts solution (arcsine transformation).

Evolved $^{14}\text{CO}_2$ from respiration of chlamydospores and soil microbes utilizing chlamydospore exudates accounted for the largest share (>60%) of total carbon loss under all conditions. The share of microbial respiration of chlamydospore exudates was 3 to 5% of the total ^{14}C loss or 27 to 47% of the residual label in the soil under all conditions, except 5°C. Microbial respiration of $^{14}\text{CO}_2$ in soil at 5°C at either pH 5 or 8 was less than 1% of the total ^{14}C lost or less than 10% of the residual label. Thus, it appeared that chlamydospore respiration was the major route to the endogenous carbon (>55% of the total ^{14}C lost) when chlamydospores were exposed to soil at 5, 15, 25, or 30°C, pH 5 or 8, or to soil at water potentials of 0, -1, or -10 kPa at 25°C. In the previous studies, we demonstrated that chlamydospore respiration was the major sink for endogenous carbon (55 to 78% of the total ^{14}C lost) when chlamydospores were exposed to soil at -1 kPa (22). Similar results were also observed by Hyakumachi and Lockwood (15) in sclerotia of *Sclerotium rolfsii*.

Chlamydospore germinability, viability, and virulence were reduced to a greater extent during exposure at high matric potentials (0 to -1 kPa), at 25 and 30°C, and at pH 8 than under other conditions. This was attributed to the greater loss of endogenous carbon that occurred under these conditions. However, chlamydospore germinability, viability, and virulence declined more rapidly during exposure to water-saturated soil than at -1 or -10 kPa, although carbon loss from chlamydospores at 0 kPa was less than in the other soil treatments. This suggested that the low concentration of oxygen in water-saturated soil enhanced chlamydospore debilitation (4,11). Although we did not measure oxygen concentrations, the lower microbial metabolism of ^{14}C exudates in soil at 0 kPa than at -1 and -10 kPa supports this assumption. Further

experimentation is required to conclusively determine whether or not soil oxygen concentration has a direct effect on survival of this fungus. Other workers also noted a rapid decline in viability in conidia of *C. sativus* (24) and *Cephalosporium gramineum* (28), oospores of *Pythium aphanidermatum* (21), and chlamydospores of *F. oxysporum* f. *cubense* (29), *F. oxysporum* f. sp. *elaedis* (25), and *F. solani* f. sp. *phaseoli* (22) in water-saturated soil.

Fig. 6. Relationship between ^{14}C loss from ^{14}C -labeled chlamydospores and bean root rot indices caused by chlamydospores of *Fusarium solani* f. sp. *phaseoli* after exposure to soil. Chlamydospores of different ^{14}C content were prepared by incubating them on soil at different matric potentials. There was a negative relationship between ^{14}C loss from chlamydospores and bean root indices.

Fig. 5. Relationship between ^{14}C loss from ^{14}C -labeled chlamydospores of *Fusarium solani* f. sp. *phaseoli* and their ability to germinate in potato-dextrose broth (PDB). Chlamydospores of different ^{14}C content were prepared by incubating them on soil at different pH levels and temperatures. There was a negative relationship between ^{14}C loss from chlamydospores and germination in PDB (arcsine transformation).

TABLE 6. Virulence (%) of *Fusarium solani* f. sp. *phaseoli* chlamydospores to kidney bean after incubation on soil at different temperatures and pH levels

Temperature (°C)	pH	Exposure period of chlamydospores to soil (days) ²						
		10	20	30	40	50	60	70
5	5	3.8 a, A	3.4 a, A	3.5 a, A	3.8 a, A	3.8 a, A	3.4 a, A	3.2 a, A
	8	3.6 a, A	3.4 a, A	3.5 a, A	3.6 a, A	3.4 a, A	3.1 a, A	3.0 a, A
15	5	3.5 a, A	3.6 a, A	3.4 a, A	3.0 a, A	3.0 a, A	2.6 a, AB	2.1 a, B
	8	3.8 a, A	3.3 a, A	3.6 a, A	3.0 a, A	2.7 a, AB	2.3 a, B	2.0 a, B
25	5	3.5 a, A	3.6 a, A	3.3 a, A	3.7 a, A	3.5 a, A	2.6 a, B	1.7 a, C
	8	3.6 a, A	2.4 b, AB	3.2 a, A	2.9 b, A	2.3 b, AB	1.8 b, B	0.9 b, C
30	5	3.8 a, A	3.1 a, A	3.3 a, A	3.2 a, A	2.6 a, AB	1.4 a, BC	0.9 a, C
	8	3.2 a, A	2.7 b, AB	2.0 b, B	1.2 b, C	1.9 b, B	1.0 b, C	0.2 b, D

² Each value is the mean of three replicates. Values within a column followed by the same lowercase letter and within a row followed by the same uppercase letter do not differ significantly ($P = 0.05$) according to Duncan's multiple range test.

Fig. 7. Relationship between ^{14}C loss from ^{14}C -labeled chlamydospores and the reduction of bean root rot disease caused by chlamydospores of *Fusarium solani* f. sp. *phaseoli* after exposure to soil. Chlamydospores of different ^{14}C content were prepared by incubating them on soil at different pH levels and temperatures. There was a negative relationship between ^{14}C loss from chlamydospores and bean root rot indices.

The present study provided evidence, for the first time, that chlamydospores of *F. solani* f. sp. *phaseoli* lost carbon in soil at different rates depending on soil matric potential, temperature, and pH. Better understanding of the reduction of germinability, viability, and virulence of fungal propagules and their relationship to loss of endogenous carbon would seem to be of great importance in their ecology in soil and to the epidemiology of soilborne plant pathogens. Moreover, biological control of soilborne plant pathogens might be attained through maximizing stress on propagules.

ACKNOWLEDGMENTS

The authors gratefully acknowledge John L. Lockwood for helpful discussions and for a critical review of the manuscript and D. N. R. Paul for statistical help.

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