Decreased Virulence of *Cochliobolus victoriae* Conidia After Incubation on Soils or on Leached Sand

A. B. Filonow, C. O. Akueshi, and J. L. Lockwood

Former research associates and professor, respectively, Department of Botany and Plant Pathology, Michigan State University, East Lansing 48824-1312. Present address of first and second authors, respectively: Department of Plant Pathology, Oklahoma State University, Stillwater 74078; and Department of Botany, University of Jos, P.M.B. 2084, Jos, Nigeria.

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ABSTRACT

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Conidia of *Cochliobolus victoriae* on membrane filters were aseptically incubated for 15–30 days on leached sand or on five soils. Virulence of conidia was assessed using susceptible, aseptic oat seedlings in glass vials inoculated with 10–10^4 incubated or nonincubated conidia per vial. Seedlings in vials inoculated with incubated conidia usually had significantly (P = 0.05) reduced disease indices and longer roots and coleoptiles than seedlings inoculated with nonincubated conidia. Similar results were obtained with seedlings in soils. Incubated conidia also showed an increased dependence on exogenous nutrients for germination, retarded rates of germination, and reduced viability. Incubated conidia at inoculum densities two or more times those of nonincubated conidia produced less disease than nonincubated conidia, suggesting that decreased viability after incubation was not solely responsible for decreased virulence. The results indicate that prolonged exposure to soils can reduce the virulence of conidia of *C. victoriae*.

Research on the status of energy-yielding nutrients in soils (2,9,15,18) provides a solid basis for the concept that fungi and other microorganisms in soil exist in an environment frequently deprived of suitable carbon needed for sustained growth. Thus, competition for energy-yielding nutrients in soil is often very great, and results in the rapid utilization by the soil microflora of readily available nutrients that contact soil. Since propagules of most plant pathogenic fungi contact soil at some point in their life cycles, they are also subjected to competitive nutrient stress. One consequence of such exposure, is fungistasis, in which fungal germination and growth are repressed (4,6,7,12,19). Enhanced exudation by fungal propagules incubated on soils or on a model apparatus that simulates soil-imposed nutrient stress has been associated with repressed germination of propagules (4,6,7,19). Moreover, prolonged exposure of propagules to nutrient stress can elevate their nutrient requirements for germination and result in a progressive loss of viability (4,8). Other consequences of competitive nutrient stress on fungal propagules in soil include mycolysis (10,13) and the formation of persistent structures (5,11).

Virulence is the relative capacity to cause disease, and it is well established that the prior nutritional history of fungal plant pathogens has an influence on their potential virulence (17,21,22). Moreover, addition of nutrients to inoculum has been shown to increase the disease produced by several plant pathogens (3,14,20).

Considering these results, we tested whether fungal propagules debilitated by competitive nutrient stress imposed over time by soil or by a model system simulating soil-imposed stress would show reduced virulence. A brief report of this research has been given (1).

MATERIALS AND METHODS

Fungus. *Cochliobolus victoriae* Nelson (= *Helminthosporium victoriae* Meehan and Murphy) (isolate 3, obtained from R. P. Scheffer, Michigan State University) was maintained on carrot agar containing, per liter: 250 ml of a carrot decoction (30 g of diced carrots in 250 ml of distilled water, autoclaved 10 min, and filtered through cheesecloth) plus 20 g of agar. Plate cultures were incubated at 24 C under the diffuse light of the laboratory for 1–3 mo prior to use. Conidial suspensions were prepared from agar cultures flooded with 15 ml of dilute (10^{-2}, v/v) Pfeffer's salts solution (4). The conidial suspension was passed through a 80-μm mesh sieve into a 50-ml centrifuge tube. Conidia were washed twice with salts solution by resuspension and centrifugation at 10^4 g for 5 min at 5 C. The density of the final suspension (kept in ice to prevent germination) was determined with a hemacytometer and various dilutions were made as needed.

Incubation of conidia on soils. The following soils were used: Boyer sandy loam, Spinks sandy loam, Dryden loam, Parkhill clay loam, and Brookston clay loam. Characteristics of these soils are reported elsewhere (7). Soils were sieved (<2 mm) and stored at 4 C until use. Twenty grams of soil in 90 × 15-mm plastic petri dishes was adjusted to about −0.05 bar matric potential by adding distilled water. The petri dishes containing moistened soil were kept in polyethylene bags at 24 ± 2 C for 4 days prior to use.

Conidia were incubated inside a sterile incubation chamber (8), which consisted of an autoclavable plastic bottle with the bottom replaced by a Nuclepore membrane filter (0.4-μm pore size) sealed to the bottle with silicone cement. A foam plug was in the top of the bottle. The chamber was placed on a sintered glass suction head and conidia (10^6 to 10^7) were deposited by gentle suction onto the interior surface (52-mm diameter) of the membrane filter. Each chamber was then placed on a dish of moist soil, thus permitting conidia to contact the soil solution. There were six dishes (with chambers) for each of the five soils and each was placed in a small polyethylene bag.The bags were placed inside a 52 × 42 × 15-cm tray, which was then enclosed in a large plastic bag containing several pinholes. To maintain contact between conidia inside the chambers and soil solution, 0.5–1.0 ml of distilled water was added to the soils every 5–10 days. Incubation was for 15, 30, or 60 days.

After incubation, the chambers were removed and the conidia were suspended in dilute, sterile, Pfeffer's salts solution. Conidia were washed twice with salts solution by centrifugation (10^4 g for 5 min at 5 C) and appropriate dilutions were made with salts solution for use in the virulence assays. In addition, conidia were deposited on 15 × 15-mm Nuclepore membrane filters (about 10^3 conidia per membrane) to test for nutrient-independence, germination response to seedling exudate, and viability. As a measure of nutrient-independence, one filter was floated for 24 hr on 1 ml of sterile, dilute salts solution (24 C) in each of three stainless steel

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planchets (24-mm diameter × 6 mm). In addition, two 2-day-old oat seedlings (*Avena sativa* 'Park'), surface disinfested as described below, were added to each planchet and filters were floated over them for 24 hr. To test viability, two filters were incubated on PDA for 12–18 hr. After incubation, the conidia on the filters were stained in phenolic rose bengal and destained on moist filter paper. Germination was determined by using incident light microscopy; at least 100 conidia per filter were counted.

Incubation of conidia on leached sand. Conidia on membrane filters were incubated in an aseptic leached sand apparatus (4). Three to four large filters (0.4-μm pore; 47-mm diameter; $\sim 10^6$ conidia per filter) and two to three smaller ones (0.4-μm pore; 15 × 15 mm; 10^3 conidia per filter) were placed on sand in 13.5-cm-diameter glass leaching dishes and the sand was percolated with sterile, dilute salts solution at 85–90 ml/hr. At this flow rate, germination of the conidia on the sand was <5%. In each experiment there were three to four dishes.

After 15 or 30 days, filters were removed from the dishes and placed in 50-ml sterile centrifuge tubes with 10 ml of salts solution. The capped tubes were mixed for 1 min at high speed on a vortex mixer to remove conidia from the filter. The filters were removed, the conidia were washed twice with salts solution, and the density of the final suspension was determined by using a hemacytometer. Appropriate dilutions were made for use in virulence assays. In addition, conidia on the smaller filters were tested for germination on salts solution, in seedling extract, and on PDA as described above.

Assays of inoculum virulence. Two assay techniques were used to determine the virulence of conidia incubated on soils or on leached sand. Oat seeds (*Avena sativa* 'Park') susceptible to *C. victoriae* were used. The glumes were removed by using fine forceps. Seeds were surface-disinfested in 0.2% sodium hypochlorite for 4 min, then in ethanol for 2 min, followed by five washes with sterile distilled water. Seeds were placed on sterile moist paper towels and aseptically incubated for 2 days.

In the "vial" assay, three seedlings were placed in a sterile 22-ml glass scintillation vial and 0.3 ml of diluted conidial suspension was added. Inoculum densities were 10, 10^2, 10^3, and 10^4 conidia per milliliter. Controls were uninoculated seedlings and seedlings that were inoculated with nonincubated conidia from cultures prepared at the same time as those used for incubated conidia. There were four replicates in each treatment. Foam plugs were kept in the vials for the first 2 days, then were removed to avoid restricting coleoptile growth. Vials were kept in a large tray on wet paper towels, and the tray was enclosed in a plastic bag to minimize evaporation from the vials. Seedlings were incubated for 7 days and then rated for disease severity. Measurements of root and coleoptile lengths were also made.

In the "cup" assay, 30 g of each of five soils was placed in 142-ml (5-oz) wax-coated cups. Soils were adjusted to about −0.05 bar matric potential and equilibrated in a large plastic bag for 2 days prior to use. Two-day-old seedlings were placed in holes (1.0-cm deep) in the soil, measured volumes of conidial suspensions (adjusted to inoculum densities of 10, 10^2 or 10^3 conidia per seedling) were added, and soil was added to cover the roots. For each inoculum density, there were three seeds per cup and five cups per soil. Controls were uninoculated seedlings and seedlings inoculated with nonincubated conidia. The cups were placed in a large tray with moist paper towels and enclosed in a plastic bag. After 10 days of incubation, seedlings were removed from the soils, gently washed with tap water, assessed for root and coleoptile length, and rated for disease.

Disease severity index. The disease index was rated on a scale of 1–7 in which: 1 = healthy, no lesions on roots; 2 = lesions present as small patchy brown areas on roots; 3 = brown lesions 1–5 mm long, girdling the root, slight external colonization of the roots; 4 = lesions 6–20 mm long, moderate colonization by mycelium up to coleoptile; 5 = dark brown lesions, 21–40 mm long, extensive colonization of roots and coleoptiles, coleoptiles were chlorotic; 6 = roots black and necrotic, nearly complete colonization of seedlings by mycelium, chlorotic coleoptiles were flaccid; and 7 = roots completely decayed, complete colonization of seedlings, seedlings damped-off. In the "cup" assay, less emphasis was placed on the extent of mycelial colonization of the plant surface.

All experiments were repeated at least once. Results were subjected to analysis of variance and differences between means were tested for significance by using Duncan's multiple range test or Student's paired *t* test, both at $P = 0.05$.

RESULTS

Increasing inoculum densities of conidia resulted in increasing disease severity (Fig. 1). Ten nonincubated conidia per vial produced noticeable disease, increasing the disease index from 1.0 (healthy) to 2.9, and reducing root and coleoptile lengths of seedlings by 60 and 32%, respectively, compared to uninoculated controls. Very severe disease occurred at 10^3 nonincubated conidia per vial and only slightly greater disease occurred at 10^4 conidia per vial. Age of the conidia on agar culture (1–3 mo) did not appear to affect the virulence of conidia, although this point was not specifically addressed.

Seedlings inoculated with conidia incubated on soils or leached sand for 15 days showed a tendency for decreased disease severity as compared with those inoculated with nonincubated conidia. At 10^3 incubated conidia per vial, all disease indices of seedlings inoculated with incubated conidia differed significantly from those of seedlings inoculated with nonincubated conidia (P = 0.05) (Table 1). Disease indices of seedlings inoculated with incubated conidia at 10 or 10^2 conidia per vial were in all cases lower than those of seedlings inoculated with uninoculated conidia; however, differences were statistically significant only with conidia taken from Dryden loam (10^2 conidia per vial) or from Dryden loam or leached sand (10 conidia per vial) (P = 0.05). Coleoptiles of seedlings inoculated with incubated conidia were longer than those of seedlings inoculated with nonincubated conidia, and most values differed significantly at 10 and 10^3 conidia per vial (P=0.05). Roots of seedlings inoculated with conidia pretreated on soils for 15 days were typically longer than those of seedlings inoculated with nonincubated conidia. These differences, however, were not always significant (P = 0.05).

Germination on salts solution of conidia taken from leached sand or soils after 15 days of incubation was 16–25% compared with 29% for nonincubated conidia (Table 2). Germination of incubated conidia in seedling exudate was 29–38% compared with 35% for nonincubated conidia. On PDA, incubated conidia germinated 39–59%, whereas nonincubated conidia germinated 79%.

Seedlings inoculated with conidia incubated for 30 days on soils or leached sand showed significantly less disease and longer coleoptiles and roots, in almost every case (P= 0.05), than seedlings inoculated with nonincubated conidia (Table 3). Conidia taken

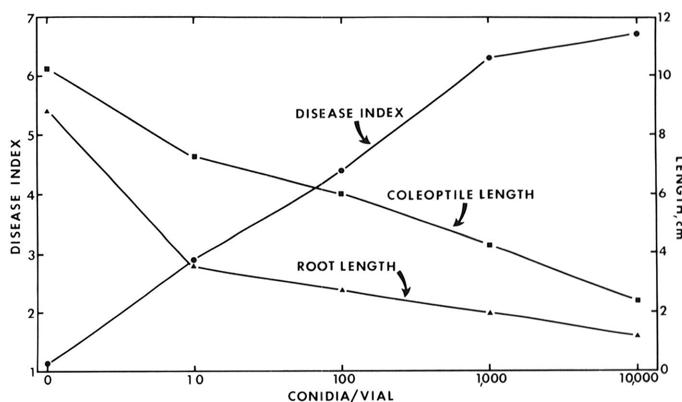

Fig. 1. Disease severity indices and root and coleoptile lengths of oat seedlings in vials containing nonincubated conidia of *Cochliobolus victoriae*. There were three seedlings per vial and five vials for each inoculum concentration of 0–10^4 conidia per milliliter. Data points are the means of five vials. Disease index was on a scale of 1–7 in which 1 = healthy and 7 = complete colonization and decay of seedlings.

from leached sand or from soils after 30 days of incubation germinated 15–21% on salts solution, compared to 43% germination for nonincubated conidia (Table 2). Germination of incubated conidia on seedling exudate was 23–34% compared to 56% for nonincubated conidia. On PDA, incubated conidia germinated 34–40%, whereas nonincubated conidia germinated 75%. Incubated conidia typically required an additional 6–10 hr of incubation on salts solution or on PDA than did nonincubated conidia to achieve maximum germination.

In general, seedlings inoculated with conidia that had been incubated for 30 days developed less disease than those inoculated with conidia that had been incubated for 15 days. For instance, at 10 conidia per vial, seedlings inoculated with conidia taken from three of the soils after 30 days of incubation had significantly lower disease indices (1.7–2.2) than seedlings inoculated with conidia after 15 days on these soils (3.2–3.6) ($P=0.05$). A similar trend was also seen at 10^2 conidia per vial, although differences were significant only at $P=0.10$. At 10^3 conidia per vial, disease indices of seedlings inoculated with conidia incubated on four of the soils or from leached sand (3.8–5.2) for 30 days were also significantly lower than those for seedlings inoculated with conidia incubated for 15 days (4.7–6.0) ($P=0.05$). Coleoptile and root lengths tended to be longer in seedlings inoculated with conidia incubated on soils or on leached sand for 30 days. However, statistically significant differences were fewer than for disease indices because of greater variability. Similarly, the depression in germination of conidia was greater after 30 than after 15 days of incubation on soils or leached sand (Table 2). For example, mean germination of conidia incubated on the five soils for 15 days was 76, 96, and 53% of that of nonincubated conidia in salts solution, seedling exudate, and on PDA, respectively, as compared with 43, 50, and 49% for conidia incubated for 30 days.

In the "cup assays," seedlings in soil infested with conidia previously incubated on the same soil for 30 days typically showed less disease than did seedlings inoculated with nonincubated conidia (Table 4). For instance, disease indices of oats in soils

TABLE 1. Disease indices and lengths of coleoptiles and roots of oat seedlings in vials inoculated with nonincubated conidia of *Cochliobolus victoriae* or with conidia incubated on leached sand or on soils for 15 days

Incubation on	Disease index[y]			Coleoptile length (cm)			Root length (cm)		
	10[z]	10^2	10^3	10	10^2	10^3	10	10^2	10^3
Leached sand	2.8 ab	4.2 b	6.0 d	8.5 c	5.6 ab	4.3 bc	4.9 b	3.9 c	2.5 c
Dryden loam	2.4 a	3.3 a	4.7 a	7.2 bc	6.2 b	4.4 bcd	5.2 b	3.2 abc	1.4 ab
Spinks sandy loam	3.2 bc	3.6 ab	4.0 a	7.7 c	7.3 c	5.6 d	4.0 a	4.1 c	1.9 bc
Boyer sandy loam	3.6 c	3.9 ab	5.1 bc	7.3 bc	5.9 ab	4.1 b	4.7 b	3.6 bc	2.4 c
Parkhill clay loam	3.2 bc	4.3 b	5.7 d	7.3 bc	5.0 ab	5.5 cd	4.2 ab	2.7 ab	1.6 ab
Brookston clay loam	3.2 bc	3.6 ab	5.2 c	6.0 ab	5.8 ab	5.2 bcd	4.1 a	2.5 a	2.2 c
Nonincubated	3.7 c	4.4 b	6.8 e	5.1 a	4.8 a	2.3 a	3.1 a	2.6 a	1.2 a

[y] Scale of 1–7 in which 1 = healthy and 7 = complete colonization and decay of seedling.
[z] Two-day-old seedlings (cultivar Park) in glass vials (three seedlings per vial, five vials per treatment) were inoculated with conidial suspensions (10, 10^2, and 10^3 conidia per vial) and incubated for 7 days prior to disease rating. Means in a column followed by the same letter are not significantly different ($P=0.05$) according to Duncan's multiple range test.

TABLE 2. Germination of conidia of *Cochliobolus victoriae* on salts solution, seedling exudate, and PDA after prior incubation of conidia on leached sand or on soils for 15 and 30 days

Incubation on	Germination (%)[a]					
	Salts solution		Seedling exudate		PDA	
	15 days	30 days	15 days	30 days	15 days	30 days
Leached sand	16 ± 2	15 ± 3	34 ± 5	25 ± 3	59 ± 6	40 ± 8
Dryden loam	20 ± 5	16 ± 2	33 ± 2	23 ± 6	40 ± 7	35 ± 5
Spinks sandy loam	23 ± 6	19 ± 3	29 ± 7	25 ± 7	42 ± 9	38 ± 5
Boyer sandy loam	20 ± 6	17 ± 4	33 ± 5	27 ± 6	39 ± 11	34 ± 11
Parkhill clay loam	22 ± 5	19 ± 4	35 ± 10	32 ± 9	40 ± 8	38 ± 5
Brookston clay loam	25 ± 8	21 ± 1	38 ± 6	34 ± 3	49 ± 5	39 ± 7
Nonincubated	29 ± 6	43 ± 7	35 ± 8	56 ± 3	79 ± 8	75 ± 2

[a] Mean germination ± one standard deviation. One membrane filter bearing conidia was floated for 24 hr on 1 ml of sterile, dilute (10^{-2}) Pfeffer's salts solution alone or Pfeffer's solution containing two surface-disinfested, 2-day-old oat seedlings, in each of three planchets. Two filters bearing conidia were incubated on PDA for 12–18 hr. Conidia were stained with phenolic rose bengal and the germination of at least 100 conidia per filter was determined.

TABLE 3. Disease indices and lengths of coleoptiles and roots of oat seedlings in vials inoculated with nonincubated conidia of *Cochliobolus victoriae* or with conidia incubated on leached sand or on soils for 30 days

Incubation on	Disease index[y]			Coleoptile length (cm)			Root length (cm)		
	10[z]	10^2	10^3	10	10^2	10^3	10	10^2	10^3
Leached sand	2.4 b	3.8 bc	5.2 c	8.7 c	6.5 bc	4.4 b	4.9 b	3.1 ab	3.1 b
Dryden loam	2.3 b	3.4 ab	3.8 a	8.7 c	6.9 bc	5.7 c	6.7 d	4.2 c	3.1 b
Spinks sandy loam	2.7 b	4.2 bc	4.7 bc	9.4 c	7.4 cd	5.9 c	6.2 bcd	4.2 c	3.3 b
Boyer sandy loam	1.7 a	3.2 ab	4.3 ab	9.1 c	7.0 bcd	6.1 c	6.3 cd	3.5 bc	3.1 b
Parkhill clay loam	2.1 ab	3.1 a	3.9 a	7.2 b	6.1 b	5.3 bc	5.0 bc	4.1 c	3.6 b
Brookston clay loam	2.2 ab	3.2 ab	3.9 a	8.9 c	8.3 d	5.3 bc	5.5 bcd	4.1 c	1.9 a
Nonincubated	3.7 c	4.4 c	6.8 d	5.1 a	4.8 a	2.3 a	3.1 a	2.6 a	1.2 a

[y] Scale of 1–7 in which 1 = healthy and 7 = complete colonization and decay of seedling.
[z] Two-day-old oat seedling (cultivar Park) in glass vials (three seedlings per vial, five vials per treatment) were inoculated with conidial suspensions and incubated for 7 days prior to disease rating. Means in a column followed by the same letter are not significantly different ($P=0.05$) according to Duncan's multiple range test.

infested with incubated conidia were reduced by 43–63% at 10^2 conidia per seedling and by 35–39% at 10^3 conidia per seedling. Disease indices of seedlings in soils infested with conidia incubated for 15 days were similarly reduced (by 37–72%) at 10^2 conidia per seedling, but there was only a slight reduction (0–11%) in disease at 10^3 conidia per seedling, except for conidia taken from Dryden loam soil (26% reduction).

DISCUSSION

Results of this study show that incubation of conidia of *C. victoriae* on soils or on leached sand for several weeks resulted in decreased inoculum virulence. In general, conidia incubated for 30 days on soils or leached sand were less virulent than those incubated for 15 days. Decreased virulence of incubated conidia was accompanied by an increased dependence on exogenous nutrients for germination and retarded germination rates. Germination of conidia on seedling exudate was also reduced by incubation for 30 days on soils or leached sand, but not for 15 days. Viability of the conidia was also reduced somewhat, but this did not appear to account for the decreased virulence of conidia. This is evident from a graphical presentation of the disease indices from Table 3 (Fig. 2). As determined by germination on PDA, viability of incubated conidia was 45–53% that of nonincubated controls. Thus, to attain an inoculum density equivalent to nonincubated controls, twice as many (using 50% viability) incubated conidia would be needed. From Fig. 2 it can be seen that twice as many incubated conidia would produce less disease than would nonincubated control conidia. For example, at 20 incubated conidia per vial, predicted disease indices were 2.2–3.2, whereas 10 nonincubated conidia produced a disease index of 3.7. Even at 100 incubated conidia per vial, only conidia incubated on Spinks sandy loam and on leached sand would produce more disease than 10 nonincubated conidia per vial. Similar comparisons can also be made at other inoculum densities, eg, 200 incubated conidia per vial compared with 100 nonincubated conidia, with the same conclusion. Therefore, an equal, and in many cases, a much greater density of incubated inoculum produced less disease than nonincubated inoculum. This was also true when root and coleoptile lengths were compared, and it was generally true for conidia after 15 days of incubation, as well.

The association of increased dependence on nutrients for germination with decreased virulence suggests that a nutrient stress was imposed on conidia incubated on leached sand or on soils. Although nutrient exudation by conidia of *C. victoriae* was not measured in this study, previous work has shown that ^{14}C-exudation from this isolate of *C. victoriae* (isolate 3) was increased by incubation on leached sand and that exudation was still detectable after 8 days of incubation (8). In this regard, conidia of *C. victoriae* (isolate 3), which were made nutrient-dependent after incubation for 9–12 days on leached sand, contained only 20% as much endogenous carbohydrate as nonincubated conidia (A. B. Filonow and J. L. Lockwood, *unpublished*). Previous work has also shown that ^{14}C-exudation from *C. victoriae* (isolate 3) occurred on each of five different soils (7), and that a prolonged incubation of 30 days on these soils resulted in an increased dependency on exogenous nutrients for germination and some loss in viability of conidia (8).

Soils differ in the intensity of the nutrient stress they impose on propagules (6,7), and it is reasonable to assume that differences in the virulence of fungal propagules due to nutrient stress may also occur in different soils. Previous work has shown that loam or sandy loam soils generally induced a greater exudation by *C. victoriae* conidia than did clay loam soils (6,7). In the present study, conidia incubated on Dryden loam, Spinks sandy loam, or Boyer sandy loam soils did not appear to produce less disease than conidia incubated on the two clay loams. However, virulence assays with increased precision might detect such differences.

The cellular mechanism by which nutrient stress reduces virulence of conidia is not known. Although the nature and quantity of nutrients lost from conidia under stress have not been precisely determined, several monosaccharides, sugar alcohols, amino-sugars, and amino acids have been identified in conidial exudate (A. B. Filonow and J. L. Lockwood, *unpublished*). The importance of these nutrients to cellular processes controlling virulence has yet to be assessed. However, these types of

TABLE 4. Disease indices of oats sown in soil and treated with 10^2 or 10^3 nonincubated conidia of *Cochliobolus victoriae* per seedling or with conidia previously incubated on the same soils for 30 days

Soils	Treatment of conidia[a]	Disease index[b]	
		10^2 conidia	10^3 conidia
Dryden loam	Nonincubated	4.2	4.6
	Incubated	2.1*	2.9*
Spinks sandy loam	Nonincubated	4.3	4.7
	Incubated	1.6*	3.0*
Boyer sandy loam	Nonincubated	4.3	5.2
	Incubated	2.4*	3.2*
Parkhill clay loam	Nonincubated	4.4	5.7
	Incubated	2.5*	3.6*
Brookston clay loam	Nonincubated	4.6	5.5
	Incubated	2.6*	3.6

[a] Two-day-old oat seedlings (cultivar Park) were placed in 1-cm-deep holes in 50 g of soil (three seedlings per cup, five cups per treatment). Conidia were pipetted directly on the seedlings and their roots were then covered with additional soil. Plants were harvested after 10 days.

[b] Scale of 1–7 in which 1 = healthy and 7 = complete colonization and decay of seedling. Mean disease indices followed by an asterisk are significantly different ($P = 0.05$) from its corresponding control (nonincubated conidia) as determined by a paired t test.

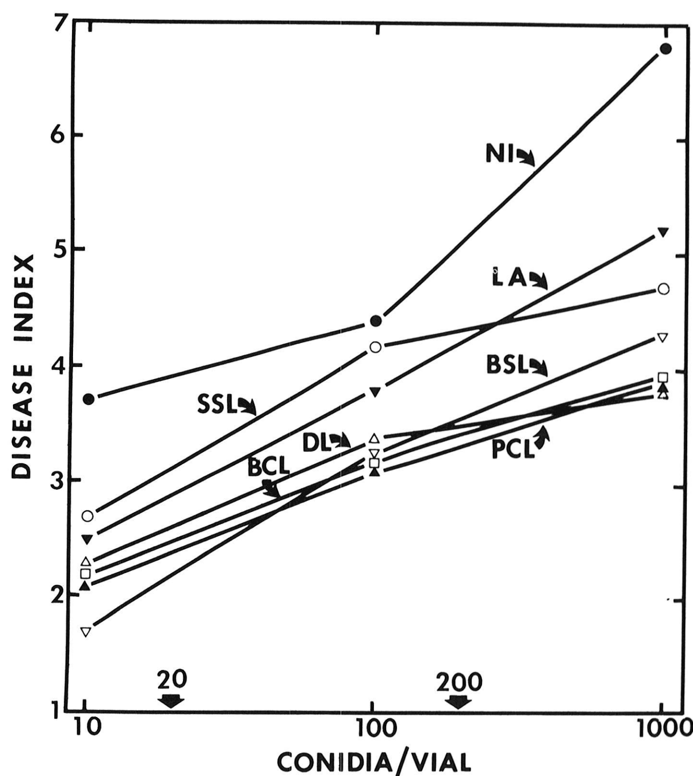

Fig. 2. Disease severity indices of oat seedlings in vials containing conidia of *Cochliobolus victoriae* that had been incubated on five soils or the leached sand model system for 30 days. Conidia were subjected to the following treatments: NI = nonincubated; LA = leaching apparatus; SSL = Spinks sandy loam; BSL = Boyer sandy loam; DL = Dryden loam; BCL = Brookston clay loam; PCL = Parkhill clay loam. At 10 and 10^2 nonincubated conidia per vial, 20 and 200 incubated conidia per vial would give equivalent inoculum densities, assuming 50% viability of incubated conidia. There were three seedlings per vial and five vials for each inoculum concentration of 10, 10^2, or 10^3 conidia per vial. Data points are means for five vials. Disease index was on a scale of 1–7 in which 1 = healthy and 7 = complete colonization and decay of seedlings.

compounds have been shown to affect the virulence of inoculum grown on media of different nutrient contents (17,21,22) and to increase disease when applied exogenously to inoculum (3,14,20). Therefore, the depletion of such nutrients from spores via exudation may also decrease virulence. Since pathogenicity in *C. victoriae* has been directly correlated with the presence of toxin in nongerminated conidia (16), it is possible that stored toxin in the conidia and/or toxin production after germination was also decreased by exposure to leached sand or soils. The isolate of *C. victoriae* (isolate 3) used in the present study was a toxin producer (R. P. Scheffer, *personal communication*). However, no determination of toxin presence in conidial exudate was made in this study.

To our knowledge, this study is the first to demonstrate that the virulence of fungal inoculum can be decreased by exposure to soil. Since this study was done, conidia of *Cochliobolus sativus* were also found to be less virulent to wheat seedlings after 5–15 days of incubation on leached sand or soil (D. K. Arora, A. B. Filonow, and J. L. Lockwood, *unpublished*). The extent to which the virulence of propagules of other soilborne fungal plant pathogens would be reduced by exposure to soil is not yet known, but if shown to occur widely, could be a potentially significant component of the epidemiology of fungal plant pathogens in soil.

LITERATURE CITED

1. Akueshi, C., Filonow, A. B., and Lockwood, J. L. 1981. Attenuation of virulence of *Cochliobolus victoriae* after incubation on soils or leached sand. (Abstr.) Phytopathology 71:1115.
2. Babiuk, L. A., and Paul, E. A. 1970. The use of fluorescein isothiocyanate in the determination of the bacterial biomass of grassland soil. Can. J. Bot. 16:57-62.
3. Banttari, E. E., and Wilcoxson, R. D. 1964. Relation of nutrients to severity of spring black stem of alfalfa. Phytopathology 54:1048-1052.
4. Bristow, P. R., and Lockwood, J. L. 1975. Soil fungistasis: Role of spore exudates in the inhibition of nutrient-independent propagules. J. Gen. Microbiol. 90:140-146.
5. Christias, C., and Lockwood, J. L. 1973. Conservation of mycelial constituents in four sclerotium-forming fungi in nutrient-deprived conditions. Phytopathology 63:602-605.
6. Filonow, A. B., and Lockwood, J. L. 1979. Conidia exudation by *Cochliobolus victoriae* on soils in relation to soil mycostasis. Pages 107-119 in: Soil-borne Plant Pathogens. B. Schippers and W. Gams, eds. Academic Press, London.
7. Filonow, A. B., and Lockwood, J. L. 1983. Mycostasis in relation to the microbial nutrient sinks of five soils. Soil Biol. Biochem. 15: (In press).
8. Filonow, A. B., and Lockwood, J. L. 1983. Loss of nutrient-independence for germination by fungal propagules incubated on soils or on a model system imposing diffusive stress. Soil Biol. Biochem. 15:(In press).
9. Gray, T. R. G., and Williams, S. T. 1971. Microbial productivity in soil. Pages 225-286 in: Microbes and Biological Productivity. D. E. Hughes and A. H. Rose, eds. Cambridge University Press, London.
10. Hsu, S. C., and Lockwood, J. L. 1971. Responses of fungal hyphae to soil fungistasis. Phytopathology 61:1355-1362.
11. Hsu, S. C., and Lockwood, J. L. 1973. Chlamydospore formation in *Fusarium* in sterile salt solutions. Phytopathology 63:597-602.
12. Ko, W. H., and Lockwood, J. L. 1967. Soil fungistasis: Relation to fungal spore nutrition. Phytopathology 57:894-901.
13. Ko, W. H., and Lockwood, J. L. 1970. Mechanism of lysis of fungal mycelia in soil. Phytopathology 60:148-154.
14. Kraft, J. M., and Erwin, D. C. 1968. Effects of inoculum substrate and density on the virulence of *Pythium aphanidermatum* to mung bean seedlings. Phytopathology 58:1427-1428.
15. Lynch, J. M., and Panting, L. M. 1980. Cultivation and the soil biomass. Soil Biol. Biochem. 12:29-33.
16. Nishimura, S., and Scheffer, R. P. 1965. Interactions between *Helminthosporium victoriae* spores and oat tissue. Phytopathology 55:629-634.
17. Phillips, D. J. 1965. Ecology of plant pathogens in soil: IV. Pathogenicity of macroconidia of *Fusarium roseum* f. sp. *cerealis* produced on media of high or low nutrient content. Phytopathology 55:328-329.
18. Shields, J. A., Paul, E. A., Lowe, W. E., and Parkinson, D. 1973. Turnover of microbial tissue in soil under field conditions. Soil Biol. Biochem. 5:753-764.
19. Sneh, B., and Lockwood, J. L. 1975. Quantitative evaluation of the microbial nutrient sink in soil in relation to a model system for soil fungistasis. Soil Biol. Biochem. 8:65-69.
20. Toussoun, T. A., Nash, S. M., and Snyder, W. C. 1960. The effect of nitrogen sources and glucose on the pathogenesis of *Fusarium solani* f. *phaseoli*. Phytopathology 50:137-140.
21. Trainor, M. J., and Martinson, C. A. 1978. Nutrition during spore production and the inoculum potential of *Helminthosporium maydis* race T. Phytopathology 68:1049-1053.
22. Weinhold, A. R., Bowman, T., and Dodman, R. L. 1969. Virulence of *Rhizoctonia solani* as affected by nutrition of the pathogen. Phytopathology 59:1601-1605.