Relation of Inoculum Size and Concentration to Infection of Wheat Roots by *Gaeumannomyces graminis* var. *tritici*

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ABSTRACT

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In this work we examined the relationship between different sizes and concentrations of plant fragments infested with *Gaeumannomyces graminis* var. *tritici* and their efficiency in producing lesions on wheat roots. Colonized wheat crowns from the field, infected roots produced in the greenhouse, and oat grains colonized under axenic conditions each were fragmented and then separated by sieving into particles ranging from 2.0 to 0.10 mm. Each of two soils, a Ritzville silt loam (RSL) and a Shano silt loam (SSL) were fumigated with methyl bromide and then amended with 10 concentrations (between 10 and 0.01 mg/g soil) of each type of infested plant fragment in all combinations. The number of root lesions produced per wheat seedling in each treatment was assessed after 28 days at 15 C. Fragments of colonized oat grains were the most infectious treatment at a given size and concentration but fragments of colonized crowns from the field were only slightly less infectious; both were significantly more effective as sources of inoculum than equivalent sizes and concentrations of colonized root fragments. In RSL, the threshold size was 0.25–0.50 mm, below which few lesions developed regardless of concentration, and above which the number of lesions increased proportionally with concentration for both crown and oat fragments. In SSL, the threshold size for crown tissue was 0.15–0.25 mm, and for oat grain fragments, 0.10–0.15 mm. For root particles in SSL, the threshold size was 0.5–1.0 mm; in RSL it was >1.0 mm (the largest particle size tested). An analysis of plant fragments from field soils (mainly crowns) showed that only inoculum in particles with diameters ≥ 0.5–1.0 mm produced lesions.

Gaeumannomyces graminis (Sacc.) Muller and Von Arx var. *tritici*, Walker, causal agent of take-all of wheat (*Triticum aestivum* L.) and barley (*Hordeum vulgare* L.), survives saprophytically in soil as mycelium in host plant debris (4,5). The debris includes crowns (including stem bases) and roots colonized by the pathogen while the tissues were still part of a living plant. The infested residue becomes fragmented into smaller pieces with tillage and because of decomposition by *G. graminis* var. *tritici* and other soil microorganisms.

Most research on the quantification of inoculum of soilborne plant pathogens has been with chlamydospores, oospores, and sclerotia. These are distinct survival structures formed by the pathogen and can be quantified by direct observation or by counting colonies produced on selective media. Crop residue infested with *G. graminis* var. *tritici* may range from a large crown fragment with attached roots and stem bases on the one extreme, to very small crown fragments containing only a few hyphal strands at the other extreme (15). Large particles represent a large food reserve for the pathogen and can be expected to house more mycelium of the fungus able to initiate subsequent infection. Hornby (7,8) found that the infective inoculum was predominately associated with colonized residue particles 0.42 mm and greater in diameter. However, he later reported that colonized particles <0.42 mm may be ineffective as a source of inoculum in one soil, but could produce lesions in another soil if conditions were more conducive for pathogenesis (9,10). Microorganisms living in the fragments as cohabitants with *G. graminis* var. *tritici* affect pathogenesis (1,3,14,15). Whether a few large colonized particles have the same infectivity as many smaller particles of the same mass and whether colonized crowns, roots, or axenically colonized oat grains (commonly used as an inoculum source in experiments) influence infectivity are generally unknown.

This research was undertaken to study the relationship of kind and size of colonized residue and size and concentration of colonized particles to infection of wheat roots by *G. graminis* var. *tritici* in soil.

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MATERIALS AND METHODS

Sources and preparation of inoculum. Three sources of inoculum were used: crowns from naturally colonized plants taken from a field, roots from plants colonized in the greenhouse, and oat grains colonized by the pathogen in flasks under axenic conditions in the laboratory. The naturally colonized crowns were recovered at harvest from a field near Pasco, WA, where cultivar Daws winter wheat had been grown under pivot irrigation the previous 3 yr. About 75% of the mature plants were infected with *G. graminis* var. *tritici*. The root inoculum was produced by growing cultivar Fielder spring wheat in plastic pans (15 × 25 × 30 cm) with perforated bottoms and filled (10-cm deep) with sterile vermiculite as a root medium. A 1-cm layer of vermiculite containing 1% (w/v) ground oat inoculum was added, wheat seeds were planted (2 cm apart), and the seeds were covered with a 2-cm layer of sterile vermiculite. The pans were maintained at 15 C with light for 16 hr each day and moistened daily. After 21–26 days, the seedlings were washed free of vermiculite and the colonized roots were excised and air-dried. To produce oat inoculum, 200 ml of untreated whole oat grains plus 250 ml of water were combined in a 1-L flask and autoclaved for 90 min on each of two successive days. The sterile oats were seeded with agar disks of a virulent isolate of *G. graminis* var. *tritici* and the fungus allowed to grow for 19–21 days. The contents of each jar were air-dried and tested for contaminants by

placing a few infested grains on potato-dextrose agar (PDA). Contaminated batches were discarded.

Each kind of colonized residue was fragmented in a mill and then separated by screening into size classes: 1.0–2.0 mm, 0.5–1.0 mm, 0.25–0.5 mm, 0.15–0.25 mm, 0.10–0.15 mm, and <0.10 mm. Inoculum particles were used within a 4-mo period.

The assay system for infection efficiency. Each test for infection efficiency of different inoculum particle sizes and concentrations in soil involved a single wheat seedling with roots allowed to grow through 5.0 g of soil containing a known mass and size of inoculum particles. The 5.0 g of soil formed a 2-cm-thick layer between sterile vermiculite contained in plastic tubes ([Fig. 1]; Ray Leach Conetainer Co., Canby, OR) 3 cm in diameter at the open end, 15-cm deep, and perforated at the bottom to permit drainage. One wheat seed (cultivar Fielder) (surface-sterilized [0.5% sodium hypochlorite for 45 sec] and axenically washed) was placed directly on the infested soil in each tube. A layer of vermiculite 1.5 cm thick covered the seed. Each tube was watered with 10 ml of sterile distilled water every other day for 28 days. All assays were conducted at 15 C with a 16-hr photoperiod. Soil and vermiculite were removed from the seedlings and the total number of lesions caused by *G. graminis* var. *tritici* were visually counted on each seedling without the aid of magnification. Isolations from test seedlings were made occasionally to confirm the presence of *G. graminis* in a lesion.

Soils and methods of infestation. Two soils were used (Table 1): a Shano silt loam (SSL) from a field near Moses Lake, WA, where wheat had been grown continuously for the previous 22 yr; and a virgin Ritzville silt loam (RSL) from the Washington State University Dryland Research Station, Lind, WA. Soils were collected from the upper 25 cm of the profile in October 1979, air-dried, and sieved through 2.0-mm screens. In their natural state, SSL and RSL were conducive and suppressive to take-all, respectively, but this difference was eliminated by fumigation (0.34 kg of methyl bromide per 90 kg of soil) for purposes of this study.

Each type and size of inoculum particle was tested at 10 concentrations in each 5-g aliquot of soil (ie, 10.0, 5.0, 2.5, 1.5, 1.0, 0.5, 0.25, 0.15, 0.10, and 0.01 mg/g). Inoculum particles were thoroughly mixed into test soil by using a small-volume soil blender. Each treatment (type, size, and concentration of inoculum particle) was replicated 10 times (10 tubes, each with one seedling), and each assay was repeated at least once.

The percentage of oat inoculum particles with viable *G. graminis* was determined for each size-class by counting the number of inoculum particles from which the fungus grew into dilute (1/5-strength) PDA. Similar determinations for the crown and root inoculum particles were not possible because of contaminants.

Occurrence of infective plant debris from field soil. Wheat residue remaining after harvest was screened from the soil of three different irrigated fields to determine the occurrence of infective plant fragments. Field 1 had been cropped to wheat for 1 yr after a nonsusceptible break crop and had mild take-all. Field 2 had been cropped for 2 yr after a nonsusceptible break crop and had moderate take-all. Field 3 was cropped for 3 yr to wheat and take-all was severe. Soil samples (4.2 kg, air-dried) were prepared by bulking 10 subsamples taken at random from the top 25 cm of the soil profile. Organic debris was separated from this soil on 1.25-, 0.64-, 0.40-, 0.20-, 0.10-, and 0.05-cm (mesh) screens and the total weight (W) of each fraction was recorded. The four largest sizes were then fragmented in a mill to particles <1.0 mm. Twenty 0.5-g samples of each fraction were mixed with 4.5 g of methyl bromide-fumigated soil (collected from the same field yielding the residue) giving a total test sample of 5.0 g. This 5.0-g infested sample was sandwiched between layers of vermiculite in tubes as described above. An inoculum unit was defined as having a weight (W[grams]) of 0.5 g. Infectivities (I) of the 20 samples for each fraction were averaged and the total infectivity for each fraction was calculated:

$$\text{Total infectivity} = (W/0.5) \times I. \quad (1)$$

Statistical analysis of infection efficiencies. Data for each of the three inoculum sources were analyzed by the General Linear model (GLM) procedure (Statistical Analysis System, Cary, NC). Each model was similar and related the lesions per plant (Y), soil type (S), inoculum particle size (I), soil × inoculum particle size interaction (S^*I), and a regression coefficient (β) times the logarithm of the inoculum particle concentration (C), ie,

$$Y = \mu + S + I + S^*I + \beta C, \quad (2)$$

in which μ represents a general mean, which is a convenient reference point for examining treatment effects. The logarithm of inoculum particle concentrations was used to linearize the infection efficiencies (ie, the relationship between lesion number and inoculum concentration). The response of the root system to inoculum particle type and size, concentration, and soil type were predicted well by the model, giving R values of 0.92, 0.91, and 0.94, respectively, as computed from the linear model. An F test for interactions between the logarithm of the inoculum particle concentration (C) and the soil type-inoculum particle size interaction (S^*I) was significant at $P = 0.05$. It was meaningful, therefore, to calculate and compare separate slope coefficients for the logarithm of the inoculum particle concentration (C) considering each soil-inoculum particle size combination. Tests of hypotheses for common slopes among soil-inoculum particle size combinations were conducted and followed by pairwise comparisons to identify which slopes from the model were different by using likelihood ratio F-tests (6) (Tables 2 and 3). Particle size was treated as a discrete independent variable for ease of interpretation and since each level represented an interval of sizes. The slope values represent the relationship between the number of lesions and the logarithm of the concentration; therefore, the interpretation of these values must be done on a relative basis, ie, relating the slope for one soil by inoculum size combination and those of another soil by inoculum size combination.

RESULTS

Naturally colonized crown particles. The threshold size of naturally colonized crown particles, below which few lesions developed, was 0.15–0.25 mm in SSL and 0.25–0.50 mm in RSL, even with inoculum concentrations of 10 mg/g soil (Fig. 2a and b, Table 2). For particles at or above the threshold size, the number of lesions increased in proportion to the concentration of crown particles to a maximum of 31.1 lesions per plant. Infection efficiency of colonized crowns, ie, lesions produced per milligram of inoculum particle, was consistently greater in the SSL compared with the RSL (Table 2). Overall trends and the threshold particle size required to produce disease were similar in the two soils (Fig. 2a and b).

Colonized wheat root particles. The threshold size of greenhouse-produced, colonized wheat roots was larger than that for colonized crowns, and also differed according to soil type. The

TABLE 1. Analysis of Shano silt loam (SSL) and Ritzville silt loam (RSL) soils[a]

Character[b]	SSL	RSL
pH	6.0	7.5
% Organic Matter	3.8	1.7
P ppm	74.4	3.7
K ppm	1,070.0	795.0
Zn ppm	23.0	5.0
Cu ppm	4.9	1.0
Fe ppm	72.6	7.4
Cl ppm	100.0	100.0
Ca (me/100 g of soil)	16.0	5.0
Mg (me/100 g of soil)	4.0	2.0
Salts (mmhos/cm^3)	1.4	1.4

[a] SL soil is located near Moses Lake, WA; SL soil is located near Lind, WA. Samples were sieved through 2.0-mm screens.
[b] Soil analysis was performed by the Washington State University Department of Agronomy and Soils, Soil Testing Laboratory.

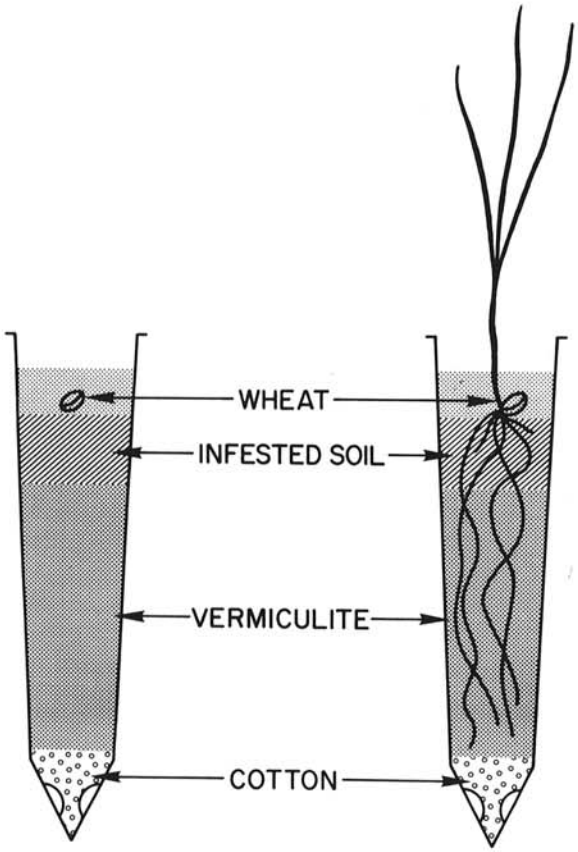

Fig. 1. Diagram of tube assay system. The polyvinylchloride tube is slightly tapered, perforated at the bottom, 15 cm tall, and about 3.0 cm in diameter at the top and about 2.5 cm near the bottom.

TABLE 2. Lesions per plant produced by three sources of *Gaeumannomyces graminis* var. *tritici* in two soils fumigated with methyl bromide[a]

Inoculum particle size (mm)	Maximum infection efficiency (lesions/mg inoculum particle)[b]			
	Crowns	Roots	Oats[c]	
			Unadjusted	Adjusted
Shano silt loam (fumigated)				
<0.10	0.4 ± 0.5	0.0 ± 0	7.6 ± 3.0	31.7 ±12.3
0.10–0.15	1.8 ± 0.9	0.0 ± 0	23.4 ± 3.0	47.8 ± 6.1
0.15–0.25	4.4 ± 3.3	0.0 ± 0	18.0 ± 7.9	31.8 ±13.8
0.25–0.50	7.0 ± 6.7	0.1 ± 0	50.6 ± 6.4	72.4 ± 9.2
0.50–1.00	31.1 ±10.0	12.9 ± 0.7	77.0 ± 8.9	86.6 ±10.0
1.00–2.00			79.0 ±17.9	79.0 ±17.9
Ritzville silt loam (fumigated)				
<0.1	0.0 ± 0	0.0 ± 0	0.1 ± 0.1	0.3 ± 0.3
0.10–0.15	0.3 ± 0.1	0.0 ± 0	0.3 ± 0.3	0.6 ± 0.6
0.15–0.25	0.9 ± 0.5	0.0 ± 0	2.7 ± 2.0	4.7 ± 3.5
0.25–0.50	5.0 ± 0.6	0.6 ± 0.1	27.2 ± 4.9	38.9 ± 7.0
0.50–1.00	20.0 ± 0.8	0.9 ± 1.2	30.0 ± 7.0	33.7 ±19.1
1.00–2.00			22.0 ± 5.1	22.0 ± 5.1

[a] Means ± standard error of mean for 20 plants..

[b] Maximum infection efficiency was determined at that concentration of inoculum in soil (0.01–1.0 mg/g soil) that resulted in the greatest number of lesions per wheat seedling per milligram of inoculum. Efficiencies were determined for each size of particle in two soils for three sources of inoculum: fragments of naturally colonized wheat crowns, colonized wheat roots (greenhouse), and axenically colonized oat grains. Ritzville silt loam from near Lind, WA, and Shano silt loam from near Moses Lake, WA, were fumigated with methyl bromide (0.34 kg/90 kg soil).

[c] Infection efficiency of axenically colonized oat grain particles were calculated before and after adjustments were made for the number of fragments containing viable fungus. Starting with the smallest particle, the percentages of particles with viable propagules were: 24, 49, 57, 70, 89, and 99:8, respectively.

threshold size was estimated at 0.5–1.0 mm in SSL and greater than the largest particle tested (0.5–1.0 mm) in RSL (Fig. 2c and d). Infection efficiency was again consistently greater in SSL than in RSL (Table 2); in SSL, only 1.5 mg of root inoculum per gram of soil produced 12.9 lesions per plant compared with RSL in which 2.5 mg/g produced an average of only 0.9 lesions per plant.

Axenically colonized oat particles. Colonized oat grains are commonly used as a source of inoculum in studies of take-all in field plots or under controlled conditions. The threshold particle size of oats was therefore studied to determine how this artificial source of inoculum compared with natural sources. In SSL, the threshold size was smaller than in RSL and particles smaller than 0.10–0.15 mm produced an average of 7.6 lesions per plant at 10 mg/g soil (Fig. 3a). In RSL, the threshold size was 0.25–0.50 mm (the same as for naturally infested crowns), below which an average of only 2.7 lesions per plant developed, even with 10 mg/g soil (Fig. 3b). In both soils, lesions produced by particles at or above the threshold size increased in proportion to the concentration of colonized oat particles up to a maximum of 20–22 lesions per plant, but infection efficiency was again consistently greater in SSL than in RSL (Table 2). In SSL, 0.5 mg of particles 0.10–0.15 mm in size produced an average of 23.4 lesions per plant compared with RSL in which 2.5 mg of particles of 0.10–0.15 mm produced an average of only 0.3 lesions per plant.

Axenically colonized oat grain inoculum adjusted for viability. Nearly 100% (99.8%) of 1.0–2.0 mm particles contained *G. graminis* var. *tritici*. The percentages for smaller particles were: 89% (0.5–1.0 mm), 70% (0.25–0.5 mm), 57% (0.15–0.25 mm), 49% (0.10–0.15 mm), and 24% (0.10 mm). This viability factor was used to determine more critically the threshold size and infection efficiencies for colonized oat grain fragments (Table 2). On this basis, in SSL, 0.1-mm particles produced 31.7 lesions per milligram and the next larger two particle sizes produced 47.8 and 31.6 lesions per milligram, respectively.

Comparison of infection efficiencies of three inoculum sources in SSL and RSL. The model (equation 2) relating lesions per plant to soil type, inoculum particle size, concentration, and source, statistically predicted the same threshold size of particles as did the analysis of infection efficiencies given in Table 2. For example, the threshold size of colonized crown particles in both soils (0.25–0.5 mm) had slope values (lesions per plant per log of the number of milligrams of inoculum particles) of 7.92 and 7.75 SSL and RSL, respectively, below which the slopes were significantly lower and decreased with particle size (Table 3).

The estimated slopes (lesions per plant per log milligram of particles) from the linear models provided a means of comparing colonized oat particles as a substitute for naturally colonized crown particles in experimental systems. The linear model showed that the slope for threshold size (0.10–0.15 mm) of colonized oat particles in

TABLE 3. Lesions per plant per log inoculum particle concentration estimated from linear models for three sources of *Gaeumannomyces graminis* var. *tritici* in two soils[a]

Inoculum particle size (mm)	Lesions/log (mg particles)					
	Crowns		Roots		Oats	
	SSL	RSL	SSL	RSL	SSL	RSL
<0.10	0.49 a	0.00 a	0.00 a	0.00 a	3.32 a	0.17 a
0.10–0.15	1.19 a	0.05 a	0.00 a	0.00 a	8.11 b	0.74 a
0.15–0.25	1.98 a	1.67 a	0.00 a	0.00 a	7.71 b	9.85 b
0.25–0.50	7.92 b	7.75 b	0.24 a	1.16 a	8.97 b	9.91 b
0.50–1.0	8.42 b	8.16 b	9.05 b	0.98 a	8.47 b	7.90 b
1.00–2.0	···	···	···	···	9.55 b	9.45 b

[a] Each size of inoculum particle was tested at ten concentrations (0.01–1.0 mg/g soil) in a tube assay (15). Ritzville silt loam (RSL) from near Lind, WA, and Shano silt loam (SSL) from near Moses Lake, WA, were fumigated with methyl bromide (0.34 kg/90 kg soil). A linear model was designed to describe the data for each inoculum source. For each size of inoculum particle, the lesions per plant per log inoculum particle concentration was estimated by linear model slope values. Slope values followed by the same letter, within each source, were not significantly different according to the likelihood ratio F-tests ($P = 0.05$).

SSL was less than the slope for threshold size (0.25–0.5 mm) of particles in RSL. Particles 0.25–0.5 mm had similar slope values for colonized oat grains in SSL and RSL soils, and also similar slope values for colonized crown particles in SSL and RSL (Table 3). In each soil, and for each source of inoculum, the lesions per plant/log particle concentration decreased with decreasing sizes of inoculum particles tested. For example, data for colonized crown particles of 0.15–0.25, 0.10–0.15, and 0.10-mm sizes tested in SSL resulted in slope values of 1.98, 1.19, and 0.49, respectively; for the same size particles tested in RSL, the slopes were 1.67, 0.05, and 0.0, respectively.

Infection efficiency of field-colonized wheat tissue. The minimum size of infectious particles was largest (12.5 mm) in soil from a field cropped to wheat for only 1 yr after a nonsusceptible crop, smaller (6.4–12.5 mm) in field soil cropped two consecutive years to wheat, and smallest (0.5–1.0 mm) in field soil cropped three

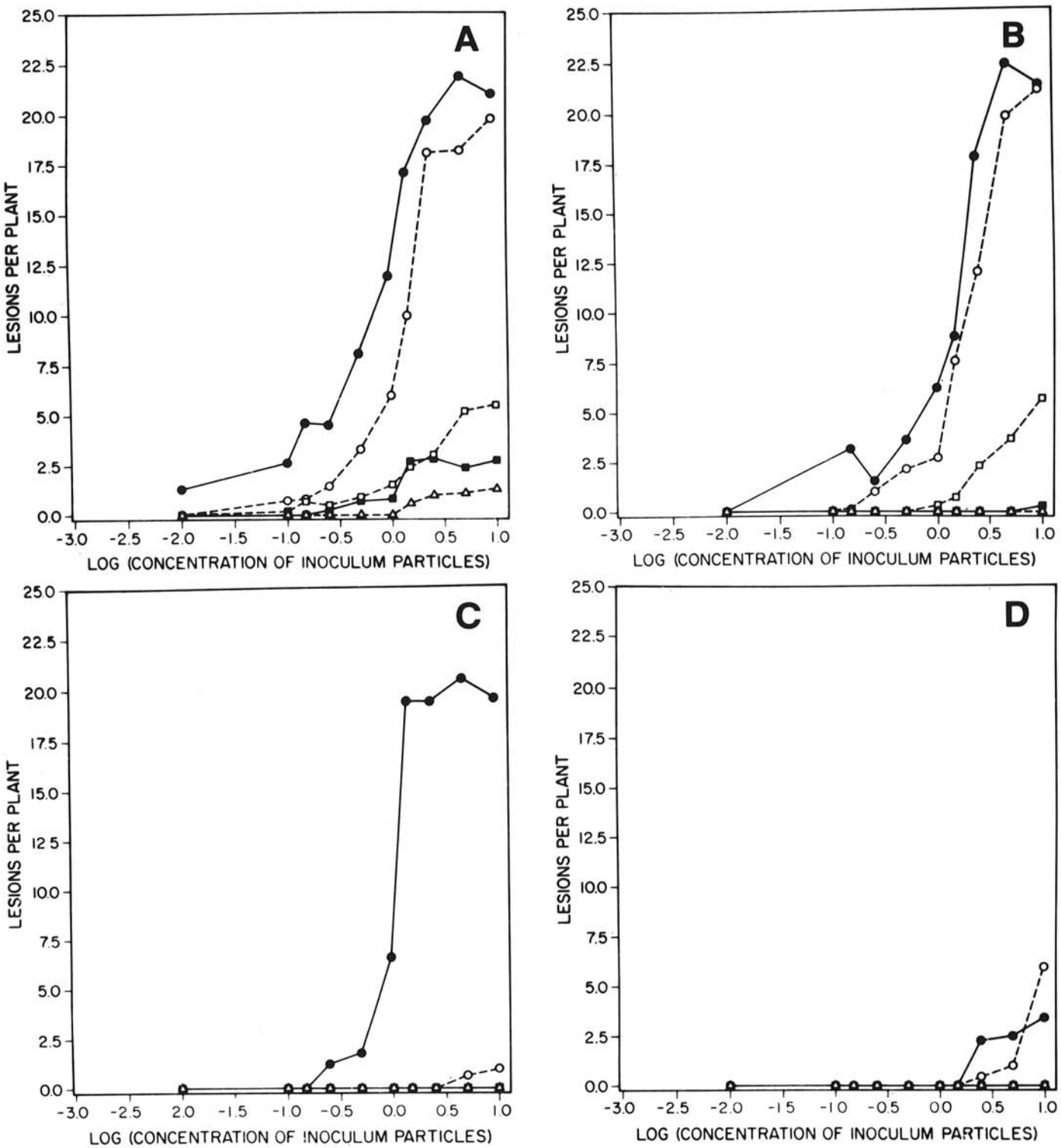

Fig. 2. Mean number of lesions per plant as a function of the logarithm of the weight of inoculum particles (mg) added to the soil. Ritzville silt loam (RSL) from near Lind, WA, and Shano silt loam (SSL) from near Moses Lake, WA, were fumigated with methyl bromide (0.34 kg/90 kg). The data represent the following treatments: **A,** SSL soil + colonized wheat crown particles, **B,** RSL soil + colonized wheat crown particles, **C,** SSL soil + colonized wheat root particles, and **D,** RSL soil + colonized wheat root particles. Median inoculum unit sizes: 0.75 mm (•), 0.375 mm (o), 0.20 mm (□), 0.128 mm (■), and 0.053 mm (Δ).

consecutive years to wheat (Table 4). The number of lesions produced per plant seeded into 5 g of each naturally infested soil was 1.0, 4.7, and 5.7 for fields cropped to wheat for 1, 2, and 3 yr, respectively. Total infectivity (a measure of the infectivity of all particle sizes combined, equation 1) was least in the field cropped to wheat only 1 yr following a nonsusceptible crop and proportionally greater in fields cropped to wheat for 2 and 3 yr (Table 4).

DISCUSSION

There was a threshold particle size of colonized crowns (0.25–0.50 mm) in RSL, below which few lesions developed at any of the inoculum particle concentrations tested, and above which the number of lesions increased in proportion to the weight of particles added. Large particles produced no more infections than the same weight of smaller particles, provided all were of the threshold size or larger. The concept of threshold particle size for infection is supported by field data in which the smallest infectious particles sieved from soil of the three fields were fragments 0.5–1.0 mm in size. Hornby (8,9) reported that infectious particles sieved from field soil in England were of similar size (≥0.42 mm).

TABLE 4. Infectivity of *Gaeumannomyces graminis* var. *tritici* in plant debris recovered from three fields of Shano silt loam

Plant debris diameter (mm)	Lesions per field[a]		
	Field 1	Field 2	Field 3
<0.25	0	0	0
0.25–0.50	0	0	0
0.5–1.0	0	0	20 ± 3
1.0–2.0	0	0	61 ± 9.8
2.0–6.4	0	0	75.3 ± 7.4
6.4–12.5	0	105 ± 9	146.3 ±14.5
>12.5	192 ±14	2,359 ±54	1,872 ±61.8

[a] The total lesions produced by inoculum found in plant debris from 4.2 kg of field soil. Field 1, first year of wheat under irrigation; Field 2, second year of wheat under irrigation; Field 3, third year of wheat under irrigation. Take-all was very mild in Field 1, moderate in Field 2, and severe in Field 3. Means ± standard error of mean for 20 replicates.

The minimum size of colonized particles recovered from fields decreased with increasing number of years wheat was grown in a field. Only particles ≥12.5 mm housed inoculum in the field cropped for the first year to wheat after a rotation crop. Smaller colonized particles were recovered from the field where wheat was grown for 2 yr and the smallest (0.5–1.0 mm) were recovered where wheat was grown three consecutive years. Probably after the first year of wheat, the inoculum is still contained in fresh (undecomposed) host fragments (eg, crowns), which become further fragmented and decomposed into smaller sizes with time and cultivation until finally they are below the threshold size. Cultivation practices or environmental conditions that lead to breakdown of the debris should accelerate the reduction of inoculum *G. graminis* var. *tritici* in soil. Moore and Cook (12) showed that take-all is less severe with conventional tillage than with no-till practices because tillage reduced the amount of inoculum available in the upper few centimeters of soil to infect crowns of the next crop.

The threshold size for colonized root particles varied with the soil type, but was larger than that for colonized crowns in both soils. The threshold size in SSL soil was 0.5–1.0 mm for root fragments compared to 0.15–0.25 mm for crown fragments. In RSL, the threshold size for colonized root particles could not be determined since even the largest particle tested (0.5–1.0 mm) produced less than one lesion per milligram of particles. Brown and Hornby (2) similarly found that roots were less effective than crowns as an inoculum source of this fungus. Our estimates for threshold size of infected roots (produced in the greenhouse) may be conservative since naturally infected roots support a greater variety of microorganisms potentially suppressive to *G. graminis* var. *tritici*. The ratio of surface area to volume is greater for roots than crowns and would result in roots decomposing faster than crowns in natural soil. Thus, the use of practices that help confine take-all to root infection, ie, reduce or eliminate colonization of the crown (13), not only benefits the current wheat crop but should result in inoculum with a lower potential to damage subsequent crops.

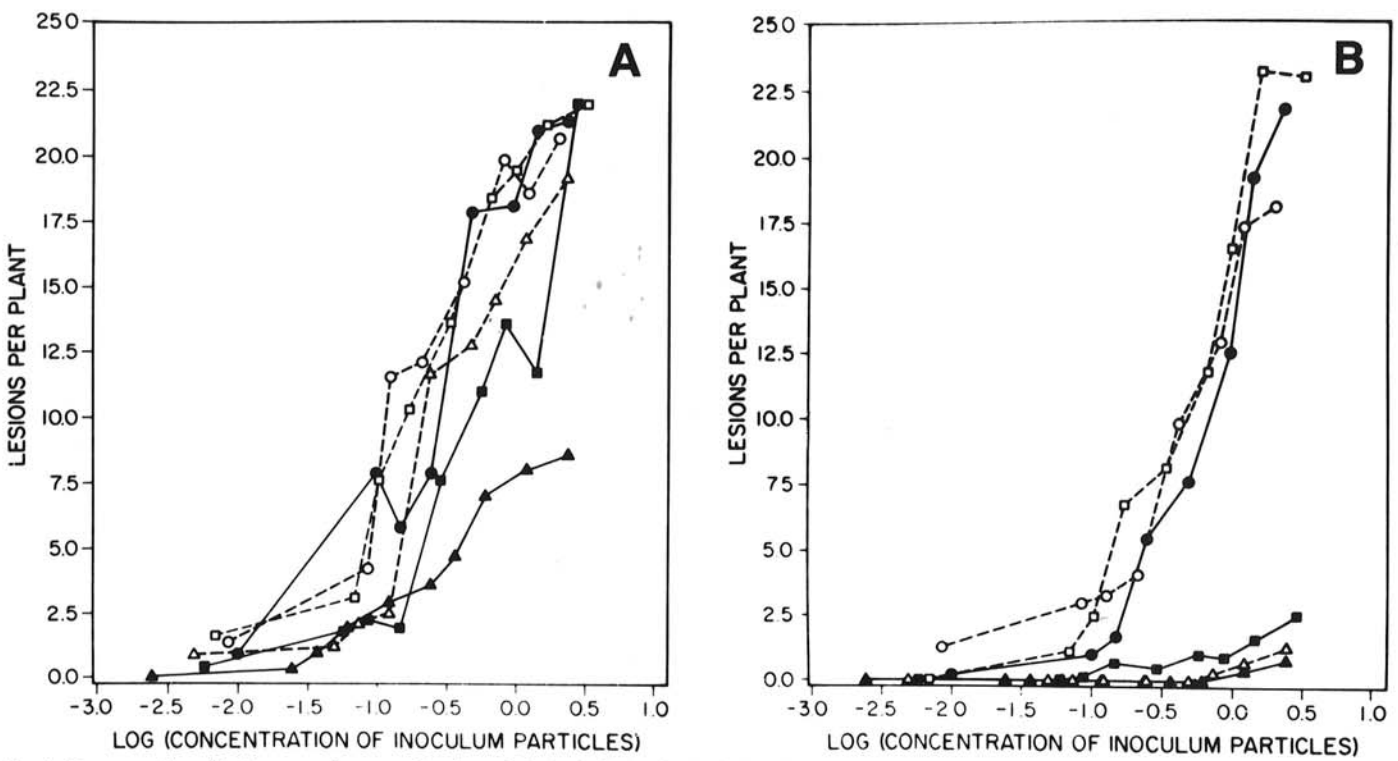

Fig. 3. Mean number of lesions per plant as a function of the logarithm of the weight of inoculum particles (mg) added to the soil. Ritzville silt loam (RSL) from near Lind, WA, and Shano silt loam (SSL) from near Moses Lake, WA, were fumigated with methyl bromide (0.34 kg/90 kg). The data represent the following treatments: **A,** SSL soil + artificially colonized oat particles; **B,** RSL soil + artificially colonized oat particles. Median inoculum unit sizes: 1.0 mm (o), 0.75 mm (□), 0.375 mm (•), 0.20 mm (■), 0.128 mm (Δ), and 0.053 mm (▲).

The threshold size (0.10–0.15 mm) for particles of axenically colonized oat grains was smaller than that for naturally colonized crown particles in SSL and similar in size for the crown particles in RSL. On the other hand, colonized oat grain fragments had a greater infection efficiency than did equivalent sizes of naturally colonized crown particles in both soils. Adjustments to consider only oat particles having viable *G. graminis* var. *tritici* resulted in greater apparent infection efficiency of colonized oat grains and allowed for experimental inoculum to be standardized.

A given size, mass, or source of inoculum was consistently more efficient in causing a lesion in SSL soil compared with RSL soil. This difference probably relates to a greater availability of nutrients to *G. graminis* var. *tritici* in SSL soil. Both soils were air-dried and fumigated with methyl bromide, which eliminates organisms that could interfere with pathogenesis (1). These treatments also cause a flush of readily available carbon and other nutrients (11). However, the SSL soil, having a higher content of organic matter and probably a much higher total microbial biomass, would release more nutrient after fumigation than would the RSL. This availability of nutrients in the soil solution would be highly significant to *G. graminis* var. *tritici* (2), which must grow as mycelium from the plant debris through the rhizosphere and ectotrophically over the root before infection occurs. The pathogen also uses nutrients from the plant residue occupied, which may explain the greater infection efficiency of colonized oats compared with colonized crowns or roots; axenically colonized oat grains contain more available nutrients than either naturally colonized crowns or roots. This may explain why the pathogen in inoculum particles with limited nutrient supply, could still infect wheat roots in SSL soil. In RSL, the nutrients in the soil combined with those in the largest colonized root particles (0.5–1.0 mm) probably were still limiting to the pathogen, and hence no infection occurred. In contrast, in SSL colonized oat particles 0.10–0.15 mm produced 23.4 infections at 0.5 mg/g soil. This is evidence that the infection of wheat by *G. graminis* var. *tritici* is influenced by the total nutrients available from the residue as well as from the soil external to the residue.

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