Estimated Distances for Infection of Wheat Roots by *Gaeumannomyces graminis* var. *tritici* in Soils Suppressive and Conducive to Take-All

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ABSTRACT

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A Shano silt loam (SSL) cropped consecutively to irrigated wheat for 22 yr (suppressive to take-all of wheat caused by *Gaeumannomyces graminis* var. *tritici*) and a Ritzville silt loam (RSL) never cropped to wheat (conducive to take-all) were compared to determine if suppressiveness is expressed as a stricture on the distance an inoculum source can be from the root and still cause infection. Data for the number of infections per plant produced by three sizes of inoculum particles (0.25–5.0, 5.0–1.0, and 1.0–2.0 mm in diameter), each at nine particle densities (0.1 to 10.0 mg/g soil), were used in a statistical model to obtain estimated mean distance for infection (EDI). For any given particle size, the mean EDI value was about the same in SSL and RSL even though these soils are suppressive and conducive, respectively, to take-all. The EDI values were about 1.7 mm in both soils with inoculum particles 0.25–0.50 mm and 2.8 and 3.9 mm in diameter in SSL and RSL, respectively, with particles 0.50–1.0 mm. Following an adjustment for multiple infections per particle, the EDI values for particles 1.0–2.0 mm were estimated at 11.3 and 10.8 mm in SSL and RSL, respectively. Fumigation (methyl bromide) and treatment with moist heat (60 C for 30 min) resulted in significantly greater EDI values for the two smaller particle sizes in both soils, but not for the larger (1.0–2.0 mm) particles. The distance the fungus can grow from an inoculum source to a host root is thus affected by the associated microbiota, to which the fungus is most vulnerable when its food base is near the minimum threshold size. However, this form of antagonism was independent of the antagonism responsible for take-all decline.

Additional key words: antagonism, biological control, *Triticum aestivum*.

Gaeumannomyces graminis var. *tritici*, cause of take-all of wheat and barley, lives saprophytically in soil as mycelium within the decaying fragments of parasitically colonized plants. The pathogen grows from these fragments (remnant stems and roots of the host) onto the roots of the next wheat or barley crop and the cycle is repeated. Gilligan (4,5) proposed a model to estimate the mean distance between the inoculum and the root over which infection can occur. By using colonized millet seeds as the inoculum particles, and by placing the colonized seeds in a two-dimensional, horizontal plane through which the wheat roots passed vertically, he estimated that *G. graminis* var. *tritici* can grow about 11.6 mm from an inoculum particle to reach a wheat root. The millet seeds were about 2.5 mm in diameter and the rooting medium was an artificial, sterile, sand-soil mixture. No estimates have been made of distances the fungus can grow from inoculum particles to reach and infect roots in natural soil where competition is more intense and nutrients available for prepentration growth are more limiting than in artificial rooting media.

Previously (13), we showed that the threshold particle size of infested plant fragments required for infection is 0.25–0.5 mm for fragments of wheat crowns or axenically colonized oat grains and probably larger for infested root fragments. These estimates are similar to the figure of 0.42 mm determined by Hornby (6–8). The number of lesions per milligram of inoculum (infection efficiency) was greater in fumigated or pasteurized soil than in nontreated soil, which suggests that antagonism from the associated microbiota limits the growth of the fungus from an inoculum particle to the root.

The objectives of this investigation were to develop a model from our previous data (13) for estimating the mean distance for infection (EDI) by *G. graminis* var. *tritici* and to determine whether the distance is affected by suppressiveness of the soil associated with take-all decline. A preliminary report has been published (12).

MATERIALS AND METHODS

Estimation of EDI. The EDIs were estimated by using a statistical model developed from data for the number of lesions produced by three inoculum particle size categories (0.25–0.5, 0.5–1.0, and 1.0–2.0 mm in diameter) tested in all combinations at nine different inoculum particle concentrations (0.1–10.0 mg/g soil) and in either a Shano silt loam (SSL) suppressive to take-all (from a field cropped 22 consecutive years and where take-all decline had occurred) or a Ritzville silt loam (RSL) conducive to take-all. The original data (13) were for the number of lesions per particle weight of axenically colonized oat grains. The study was limited to these data because actual numbers of viable inoculum particles (determined by plating on potato-dextrose agar) could be determined only for oats axenically colonized by *G. graminis* var. *tritici*. The viability of the pathogen could not be estimated in fragments of infested wheat crown tissue and infested wheat roots because of the other microorganisms also present in those fragments. The numbers of infections per root caused by the pathogen in inoculum particles of the three size-categories were tested in nontreated (natural), fumigated (methyl bromide at 0.34 kg/90 kg), and pasteurized (60 C, 30 min.) SSL and RSL soils in all possible combinations with the 10 concentrations of inoculum. The plants from which the data were collected were grown singly in plastic tubes (Ray Leach Containers, Canby, OR) containing 5 g of infested soil sandwiched between layers of vermiculite.

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To simplify our work, we used only those data for inoculum particles that produced an average of about one lesion per particle (0.25–0.50 and 0.50–1.0 mm). By selecting data produced with the smaller inoculum particle sizes, we were able to estimate the distances based on the assumption that each inoculum particle could infect only one root via the shortest distance to the root. The roots in our experimental systems (average of 4.2 per plant) grew in a nonparallel nonlinear manner. Therefore, we treated the total number of lesions per plant as if they occurred on one root(and not on 4.2 roots), with inoculum particles distributed randomly around the one root. In addition, we assumed the three-dimensional experimental systems could be reduced to two dimensions, since each inoculum particle was likely to interact with only one root and to do so in a plane perpendicular (the shortest distance) to the long axis of the root.

Once we could estimate the distance of infection for these particles, the distances for infection by particles causing more than one lesion per particle were then estimated by assuming that the mean for multiple infections was the same as the overall mean. The concentration of inoculum particles in the soil can be described as the number of particles in an area around the root and their arrangement can be modeled by a Poisson distribution. Since inoculum particles greater than 1.0 mm in diameter can produce more than one lesion, and since hyphae of *G. graminis* var *tritici* apparently are chemotactically attracted to wheat roots (9), it will be necessary in the future to incorporate into our method parameters that account for infectious hyphae not following the shortest path to the root.

Our method allowed estimation of the mean distance from the root to the *n*th particle, assuming the particles were randomly distributed around the root and there were lesions on the root. We used the distribution of the distance to the *n*th nearest neighbor to determine the mean distance to the *n*th nearest neighbor as given by Thompson (10),

$$E(r_n) = (1/m^{0.5}) \cdot [(2n)!n/(2_n \cdot n!)^2].$$

Note that in this equation $E(r_n)$ is the mean distance to the *n*th nearest neighbor (ie, *r* is the radius of a circle that contains the *n*th nearest neighbor on its circumference) and that *m* is the density of individuals per unit area. By rearranging the equation and letting $E(r_n)$ equal EDI for inoculum particles randomly distributed around a root, we obtain

$$EDI = \left\{(2n-1)!/2^{2n-1}[(n-1)!]^2\right\} \cdot m^{-0.5}. \quad (1)$$

By using our method, EDI values for particles 1.0–2.0 mm also were calculated by assuming the number of particles, *n*, would equal the number of lesions per plant divided by the number of infections a particle could produce. In this case, *n* is often not an integer so a more general method is required than that used to obtain equation 1. The squared distance to the *n*th inoculum particle from the root has a gamma distribution. This distribution may be used to obtain an estimate of the distance to the *n*th inoculum particle by using results for the gamma integral. The result is

$$EDI = \left\{\Gamma(2n)/2^{2n-1}[\Gamma(n)]^2\right\} \cdot m^{-0.5} \quad (2)$$

in which Γ is the gamma function. When *n* is an integer, equation 2 reduces to Equation 1.

Estimating the number of viable inoculum particles. It was necessary to know not only the number of particles per unit weight of soil, but also the number of particles with viable *G. graminis* var. *tritici*. This was determined by distributing four sample weights (0.1, 0.5, 1.0, and 5.0 mg) of each size unit onto the surface of 1/5 potato-dextrose agar in a petri dish and then recording the total number of particles per plate and the total number of particles from which at least one hypha of *G. graminis* var. *tritici* emerged and grew onto the medium. The percentages of viable particles were 99.8, 89.0, and 70.0 for the three particle size categories (1.0–2.0, 0.5–1.0, and 0.25–0.50 mm), respectively. The number of viable inoculum units per milligram was therefore 1.6, 12.5, and 134.4, respectively, for particles 1.0–2.0, 0.5–1.0, and 0.25–0.50 mm in diameter.

RESULTS

Multiple infections from infested oat particles. Infested oat particles 1.0–2.0 mm in diameter produced an average of 1.0 and 1.4 lesions per particle in nontreated SSL and RSL, respectively. All smaller particles produced an average of about 1.0 lesion per particle in the two nontreated soils that were used. Multiple infections per particle were more frequent in soils fumigated with methyl bromide or pasteurized, but were still limited to particles 1.0–2.0 mm. The number of infections per particle 1.0–2.0 mm was 6.0 and 2.0 in fumigated and pasteurized (respectively) soil and 1.8 and 4.3 in fumigated and pasteurized (respectively) RSL.The soil treatments had no apparent effect on the number of lesions per particle 1.0 mm in diameter compared with their activity in nontreated soils.

EDIs in naturally suppressive SSL and naturally conducive RSL. The EDI of inoculum particles 0.25–0.50 mm was 1.7 mm in nontreated SSL and RSL (Table 1). Fumigation and pasteurization of the two soils resulted in greater EDI values than those obtained for nontreated soils, but the increases were not significant for this particle size category (Table 1).

The EDI values for inoculum particles 0.5–1.0 mm were 2.8 mm and 3.9 mm in nontreated SSL and RSL, respectively. Fumigation and pasteurization of SSL increased the values to 7.4 mm and 6.0 mm, respectively (significant at $P = 0.05$), and the same treatments of RSL increased the EDI values to 4.6 and 5.0 (difference not significant), respectively (Table 1).

Estimated distances for infection of inoculum particles 1.0–2.0 mm in diameter were made following an adjustment for multiple infections. The EDI values for these particles in nontreated SSL and RSL soils were 11.3 mm and 10.8 mm, respectively, and were not significantly greater in fumigated and pasteurized soils (Table 1). These EDI values approached the physical limits of the tube radius (15 mm).

TABLE 1. Estimated distances for infection of wheat roots by *Gaeumannomyces graminis* var. *tritici* in fumigated (Fum) and pasteurized (Past) Shano silt loam (SSL) and Ritzville silt loam (RSL) soils

Particle size category[y] (mm)	Distance from root surface (mm)[z]					
	SSL			RSL		
	Untreated	Fum	Past	Untreated	Fum	Past
1.0–2.0	11.3 a	11.3 a	10.9 a	10.8 a	11.6 a	11.0 a
0.5–1.0	2.8 a	7.4 b	6.0 b	3.9 a	4.6 a	5.0 a
0.25–0.5	1.7 a	3.2 a	2.8 a	1.7 a	2.2 a	3.1 a

[y] Oat grains axenically infested with *G. graminis* var. *tritici* were fragmented and separated into three sizes.

[z] The estimated distances were based on a random distribution of particles tested at nine different particle densities (0.1–10.0 mg/g of soil) for each particle size. SSL and RSL soils were sieved through 2.0-mm screens and either untreated, fumigated with methyl bromide, or pasteurized at 60 C for 30 min. Means followed by the same letter within each particle size by soil combination are not significantly different according to Duncan's multiple range test ($P = 0.05$).

DISCUSSION

Thompson's (10) model was used with data for inoculum particles that are randomly distributed around the root and that produce only one lesion each to develop a method for estimating distances for infection. Gilligan's (5) model for infection of wheat by *G. graminis* var. *tritici* is also based on inoculum particles randomly arranged around wheat roots. Gilligan used millet seeds (about 2.5 mm in diameter) axenically colonized by *G. graminis* var. *tritici* as a source of inoculum, and probably each seed supported multiple infections. He placed the inoculum particles in a horizontal plane to reduce the probability of multiple infections

and recorded only the number of roots infected and not the number of infections per plant. Despite these and other differences between our respective experimental approaches and assumptions, when our data are analyzed with his model, the calculated mean widths (w) from his equation 3 for each of our three particle sizes differed from the three mean EDI values calculated with our model by only about 1.0 mm.

In fact, the estimates of mean distance by Gilligan's model were consistently greater than the EDI values developed from our method. One reason for this is that Gilligan's w is based on the square root of the expected squared distance to the rth inoculum particle whereas our EDI is correctly determined as the expected distance to the rth inoculum particle. In other words, $E(r_n)$, which we estimate and call EDI is not the same as the square root of $E(r_n^2)$ which Gilligan estimates and calls w.

The EDI was a function mainly of the size of the oat fragment (particle) containing viable mycelium of *G. graminis* var. *tritici*. This is not surprising, considering that the larger the food base occupied by the mycelium of this fungus, the greater the inoculum potential (3). Conceivably, the pathogen may respond as hyphal growth from smaller particles at estimated mean distances greater than the values provided by our model, but either the energy available in these smaller particles is insufficient for the hyphae to reach the root, or the hyphae reach the root but have insufficient energy by that time to initiate infection. We can also assume that more than one hypha grows from any given particle toward the root, even when each infested particle produced only one lesion. The multiple infections produced by the larger particles should be viewed as part of a continuum between, at the one extreme, only one or a few hyphae growing from the smallest particles and producing only one infection (or no infection) per particle, to the other extreme, where the number of hyphae growing from the larger particles is so large that not all from a given particle make contact in the same area on a given root or even contact the same root.

The energy available for infection of wheat roots by *G. graminis* var. *tritici* will be a function not only of the size of the food base, but also the quantity of available organic carbon, nitrogen, and other nutrients in the rhizosphere. Fumigation and pasteurization of the soil both tended to increase the EDI values for the two smaller particle sizes. Assuming that root exudation is no different in the treated and nontreated soils, then the greater distances in the treated soils can be attributed to less antagonism, probably to less competition from the associated microbiota in these soils. The lack of any real change in EDI values between treated and nontreated soils for particles 1.0–2.0 mm in size could indicate that any difference in nutrient availability in treated and nontreated soil was insignificant compared with the nutrients available to the fungus in these larger particles. It is also possible that the physical limit of the tubes (15-mm radius) precluded detection of EDI values much beyond the 11–12 mm determined for these larger particles in the natural soils. Nevertheless, the values of 11–12 mm for the EDI from mycelium in oat particles 1.0–2.0 mm in diameter agrees with Gilligan's (5) estimate of 11.6 mm maximum distance for infection from mycelium in millet seeds 2.5 mm in diameter. The fact that our smaller oat particles were as effective as the larger millet seeds might reflect a superior nutrient status per unit-size of oat than millet grains.

Both our estimates and Gilligan's (5) for distance the fungus can grow from a food base and infect a root probably are greater than actually occurs from particles of equivalent size in nature. In both cases, the estimates are for growth from particles containing the fungus in pure culture. Moreover, our respective tests were conducted with the inoculum particles added fresh and the wheat seeds sown immediately. In nature, secondary colonists cohabit the food base with *G. graminis var. tritici* and probably compete with the pathogen for nutrients otherwise used for prepenetration growth. In addition, the inoculum particles are rarely if ever fresh in soil at the time of sowing, but rather, are exposed to microbial degradation for weeks or even months between the harvest of one crop and the sowing of the next. Obviously, a given size particle of natural inoculum will need to be in closer proximity to the root than axenically colonized particles; nevertheless, our estimates provide a useful point of reference.One significant finding by our method is that antagonists(s) responsible for take-all decline have no effect on the EDI values from oat particles. Results published in a previous paper (13) likewise showed no effect of the take-all decline factor(s) either on the minimum particle size required for infection, or on infection efficiency for different particle sizes at the different population densities. Suppressiveness of the SSL to take-all has been confirmed many times by a pot bioassay method with oat inoculum (2), and this soil was the source of *Pseudomonas fluorescens* strains 2-79 and 13-79 shown by Weller and Cook (11) to give biocontrol of take-all when applied as a seed treatment. Our evidence continues to indicate that the suppressiveness responsible for take-all decline limits lesion development after infection (1) but has little effect on the prepenetration growth phase leading to infection. It must be remembered, however, that these tests and interpretations are based on particles of axenically colonized oat grains. Inhibitory strains of root-colonizing pseudomonads gave the best protection when infiltrated into the inoculum particles (14). In nature, the antagonist(s) responsible for take-all decline may serve initially to suppress lesion development, but once established as a cohabitant with the pathogen in the host, they could then persist during saprophytic survival and subsequently interfere with ability of the fungus to respond to new host roots.

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