Relationship Between Production of a Self-Inhibitor and Inability of *Gaeumannomyces graminis* var. *tritici* to Cause Take-all

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ABSTRACT

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Cultures of *Gaeumannomyces graminis* var. *tritici* that were rated weakly pathogenic or nonpathogenic on wheat roots in a vermiculite rooting medium also showed a zone of inhibition when grown opposite to and in the same dish with a common (tester) culture of the same fungus on Difco potato-dextrose agar (PDA) at pH 4.0. Cultures rated as pathogenic showed little or no zone of inhibition when paired with the tester culture on the acidified PDA. Noninhibitory cultures grew 3.0 mm/day at 24 C on PDA at pH 4.0, whereas inhibitory cultures grew only 0.7 mm/day on the same medium, apparently because their own growth was inhibited (self-inhibition). Inhibitory and noninhibitory cultures both grew at 5.5–6.0 mm/day on PDA at pH 7.0, where little or no inhibition occurred. The correlation coefficient between amount of disease produced and size of zone of inhibition against the tester culture of *G. graminis* var. *tritici* at pH 4.0 was −0.75, −0.87, and −0.78, respectively, for three sets of 38 isolates after 9 mo of storage in culture (on unbuffered homemade PDA) and −0.68 for 118 monoascosporic cultures after 9 mo of storage on unbuffered PDA. Agar disks from the advancing margin of an inhibitory isolate grown on unbuffered homemade PDA at pH 5.5–6.0 (typical of disks used as a source of inoculum in pathogenicity tests) also were inhibitory. The tendency of cultures of *G. graminis* var. *tritici* to produce less disease after a period in culture on agar media may result, in part, from an increasing potential of the culture to produce inhibitor suppressive to its own growth. Ability to produce some inhibitor may be advantageous to the fungus in nature, but the tendency may be toward greater production (self-inhibition) after a period in culture.

Additional key words: hypovirulence, *Triticum aestivum*.

Cultures of *Gaeumannomyces graminis* (Sacc.) von Arx & Olivier var. *tritici* Walker that produced severe take-all on wheat when first isolated from Pacific Northwest wheat (*Triticum aestivum* L.) fields produced less disease and some were nonpathogenic after a period in culture (4). Others (1,3,5,8) have similarly observed a tendency in the take-all fungus to produce less disease after a period in culture on agar media.

Romanos et al (7) reported that certain cultures of *G. graminis* var. *tritici*, when grown on potato-dextrose agar (PDA) buffered at pH 3.5–4.5, produced inhibitors (termed Q factor) that were inhibitory to growth of *G. graminis* var. *tritici* as well as of other fungi. Producer isolates grew more slowly than nonproducer isolates on the acidified medium, possibly because of their own sensitivity to the inhibitors. The fact that producer cultures grow more slowly under conditions associated with production of inhibitors raised the possibility that inability to produce disease may be the result of a greater potential to produce inhibitor. This hypothesis was tested.

MATERIALS AND METHODS

A total of 424 cultures was tested for ability to cause take-all on wheat seedlings in a vermiculite rooting medium at pH 5.4–5.6 and also for ability to inhibit themselves or a common sensitive (tester) isolate on Difco potato-dextrose agar (PDA) buffered at pH 4.0. Of the 424 cultures, 267 were derived from 38 isolates obtained originally from diseased wheat plants from a field near Pasco, WA (PA isolates) (6). One set of the 38 PA isolates had been stored on dilute (one-fifth strength) unbuffered (pH 6.0 when fresh) PDA as mature cultures (enclosed in plastic bags to retard drying) at 24 C for 9 mo. Another set had been stored on the identical medium but at 12 C for 9 mo. A third set of the 38 isolates had been inoculated once onto wheat seedlings, reisolated from infected roots, and then stored for 9 mo as mature cultures on dilute unbuffered PDA at 24 C. In addition, 35 of the 38 isolates had been stored at 24 C for 10 mo at the time of this study, and 118 monoascosporic cultures representing the original PA isolates had been stored as mature cultures at 12 C for 9 mo. The other 157 cultures were derived from monoascospores from PAN parents (6) and had been transferred about every 10 days to fresh unbuffered homemade PDA for about 9 mo by the time tests were made of their ability to cause inhibition and to cause disease.

Pathogenicity of each culture was tested in a vermiculite rooting medium as previously described (4). The source of inoculum was two 6-mm-diameter disks of agar cut from the advancing margin of an actively growing culture on dilute unbuffered homemade PDA and placed 1 cm beneath two seeds of the wheat cultivar Fielder in a 2.5-cm-diameter Conetainer (Ray Leach Conetainer Co., Canby, OR 97013). To test for inhibitory activity, each culture was first grown on dilute unbuffered homemade PDA, then a 6-mm-diameter disk was taken from the advancing margin of the culture and placed on the edge of a dish of Difco PDA buffered at pH 4.0 (7). A 6-mm-diameter disk from a common sensitive (tester) isolate was placed immediately on the opposite side in each dish, 4 cm away from the disk of the potential producer. The tester isolate had also been maintained in culture for 9 mo but was still pathogenic and showed no sectoring or other signs of instability in culture. The two colonies were allowed to grow toward each other for 2 wk at 23–25 C, at which time the zone of inhibition, if any, was measured. The shortest distance between the longest hyphae of the respective colonies was taken as the zone of inhibition. Colony growth rate

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was determined for each culture grown on the Difco PDA at pH 4.0 by periodic measurements of the width of the colony (perpendicular to the direction of measurement of inhibition) during the 2-wk incubation period.

Experiments were also conducted to determine the influence of pH on the size of the zone of inhibition and on colony growth rates of selected isolates. Difco PDA was buffered at pH 4.0, 4.5, 5.3, 6.0, and 7.0 as described by Romanos et al (7). Three agar disks (6 mm in diameter, cut from the advancing margin) from each of four pathogenic (after 9 mo in culture) and four nonpathogenic (after 9 mo in culture) isolates growing on dilute homemade PDA were transferred to each buffered medium. The three disks were placed equidistantly around the periphery of the dish, then a 6-mm-diameter disk from a common tester (pathogenic) culture was placed in the center. Each of the eight cultures also was grown alone (as single colonies) in other dishes of buffered PDA at pH 4.0, 4.5. 5.3, 6.0, and 7.0, to measure their colony growth rates.

RESULTS

Of the 424 cultures tested, 41 were nonpathogenic (produced no disease); for 39 of these (95.1%), the zone of inhibition between the pathogenic (tester) and nonpathogenic colony was 8.3 mm wide (Table 1). By comparison, only 60 of 246 cultures that produced disease ratings of 3.5–5.0 (24.4%) showed zones of inhibition when paired with a tester colony, and the zones averaged only 2.0 mm wide (Table 1). These were possibly "barrages" caused by vegetative incompatibility (7). The remaining isolates produced only mild (rated 0.1–1.4) or moderate (rated 1.5–3.4) disease, and 89.6 and 70.8%, respectively, showed zones of inhibition averaging 7.0 and 5.3 mm, respectively, in width. The sizes of the zones of inhibition were progressively greater and were significantly different (P = 0.01) among the PA isolates rated, respectively, as nonpathogenic, weakly pathogenic, moderately pathogenic, and pathogenic (Table 1).

TABLE 1. Number of cultures of *Gaeumannomyces graminis* var. *tritici* that caused inhibition of a common sensitive (tester) isolate of *G. graminis* var. *tritici*, average size of zone of inhibition, and colony growth rate on potato-dextrose agar at pH 4.0 as related to pathogenicity of cultures

Pathogenicity[a] of culture	Cultures per pathogenicity category (no.)	Cultures producing zone of inhibition		Size of zone[b] (mm)
		No.	%	
0	41	39	95.1	8.3 ± 2.7 x
0.1–1.4	48	43	89.6	7.0 ± 2.5 x
1.5–3.4	89	63	70.8	5.3 ± 2.9 y
3.5–5.0	246	60	24.4	2.0 ± 1.4 z

[a] Based on a scale of 0–5, where 0 = no disease and 5 = seedling dead or nearly so.

[b] Measured after 2 wk of growth at 23–25 C by determining shortest distance between longest hyphae of two opposing colonies, one colony being the common sensitive (tester) isolate. Values not followed by the same letter are significantly different at P = 0.05 according to Duncan's multiple range test.

With each set of cultures, significant negative correlations occurred between the size of the zone of inhibition and both colony growth rate and ability to produce disease, and a significant positive correlation occurred between colony growth rate and ability .o produce disease (Table 2, Fig. 1).

A comparison was made of the range of variation in the ability of two groups of monoascosporic cultures to cause inhibition. One group of 40 was obtained from a pathogenic parent (PAN 4-27) and the other group of 38 was obtained from the same parent after it had been in culture for 9 mo and had become nonpathogenic. The 40 obtained from the parent while it was still pathogenic were stored at 12 C on dilute unbuffered homemade PDA for about 9 mo, then were tested simultaneously with the 38 fresh cultures from the same parent after it had become nonpathogenic. Of the 40 obtained from the pathogenic parent and stored for 9 mo, 37 showed no zone of inhibition when paired with the common tester isolate, and the other three showed zones less than 5.0 mm wide. Of the 38 obtained after the parent had become nonpathogenic, 12 showed zones between 5 and 10 mm wide when paired with the tester, 23 showed no zone of inhibition, and three showed zones less than 5 mm wide.

Colony growth rates and the sizes of the zones of inhibition were determined at pH 4.0, 4.5, 5.3, 6.0, and 7.0 for each of four pathogenic and four nonpathogenic cultures, all derived originally from PA isolates. Growth rates of the four pathogens averaged 3 mm per day at pH 4.0 and increased as pH was increased (Fig. 2). In contrast, growth rates of the four nonpathogenic cultures averaged only 0.7 mm/day at pH 4.0 but increased with increasing pH above 4.0 and were not significantly different from the growth rates of the pathogens at pH 7.0 (Fig. 2). The zones of inhibition between colonies of the four nonpathogens and the common tester culture were greatest at pH 4.0. No zones of inhibition occurred between colonies of the four pathogens and the common tester culture at any pH tested (Figs. 2 and 3).

When disks from a culture grown on dilute unbuffered homemade PDA (pH 6.0 when fresh) were transferred to Difco PDA buffered at pH 4.0, 4.5, and 5.3, a zone of inhibition occurred around the disks on the medium at pH 5.3 (Fig. 4). Close inspection of the zones revealed sparse hyphal growth of the fungus from the disk and across the zone, with normal growth beyond the zone of inhibition.

DISCUSSION

Cultures still able to produce severe take-all after 9–10 mo in storage on PDA produced small zones of inhibition when paired with a common tester isolate on PDA at pH 4.0, with 75% showing

TABLE 2. Correlation coefficients between rating as a pathogen and ability to produce a zone of inhibition, between growth rate and ability to produce a zone of inhibition, and between rating as a pathogen and colony growth rate for three groups of 38 isolates of *Gaeumannomyces graminis* var. *tritici* maintained in culture by various methods for 9 mo after isolation from the field and for a set of monoascosporic cultures obtained from the 38 isolates

Culture group	No. of cultures	Correlation coefficient per comparison		
		Disease rating[a] and size of zone of inhibition[b]	Colony growth[c] and size of zone of inhibition	Disease rating and colony growth
Stored on potato-dextrose agar (PDA) at 24 C for 9 mo	38	−0.75	−0.85	0.65
Inoculated onto and reisolated once from host, then stored on PDA at 24 C for 9 mo	38	−0.87	−0.86	0.70
Stored on PDA at 12 C for 9 mo	38	−0.78	−0.92	0.77
Ascosporic cultures stored on PDA at 12 C for 9 mo	118	−0.68	−0.79	0.59

[a] Based on a scale of 0–5 on seedlings exposed to agar disk inoculum of the culture in a vermiculite rooting medium.

[b] Based on zone of inhibition produced against a common sensitive (tester) isolate of *G. graminis* var. *tritici* after 2 wk on PDA at pH 4.0.

[c] Based on measurement of colony diameter for the culture grown by itself on PDA at pH 4.0.

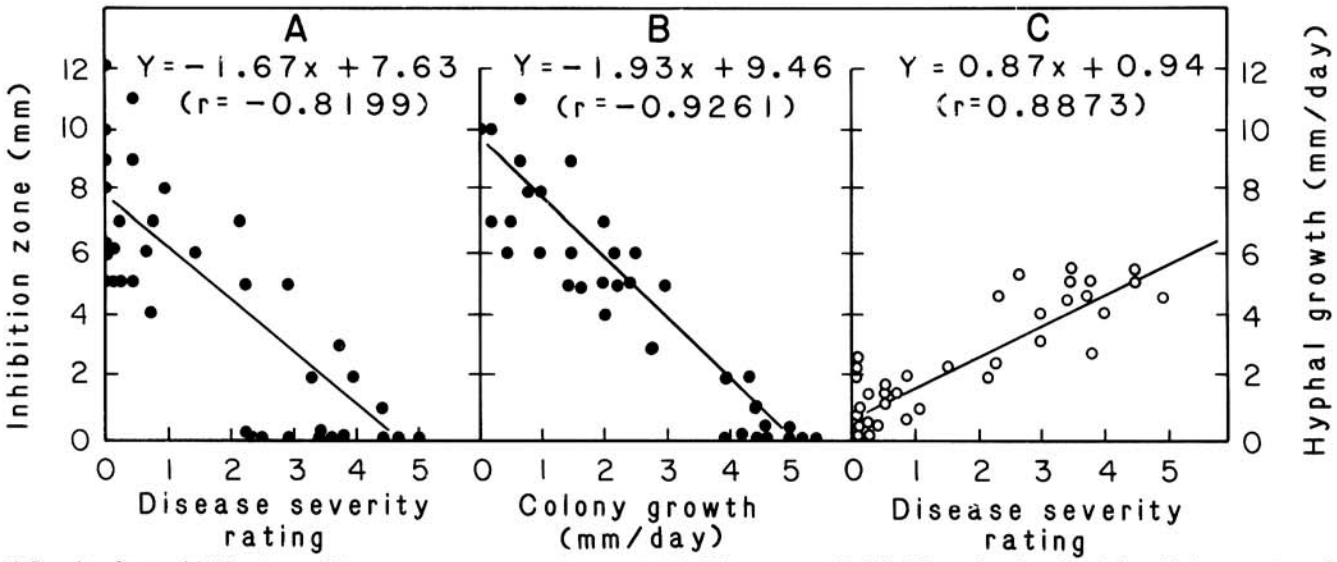

Fig. 1. Results of tests with 35 cultures of *Gaeumannomyces graminis* var. *tritici* held in storage at 24 C for 10 mo, showing the relationship between **A,** rating as a pathogen and the size of the zone of inhibition against a common sensitive (tester) culture on potato-dextrose agar (PDA) at pH 4.0, **B,** colony growth rate of the cultures on PDA at pH 4.0 and size of the zone of inhibition against the tester, and **C,** rating as a pathogen and colony growth rate on PDA at pH 4.0.

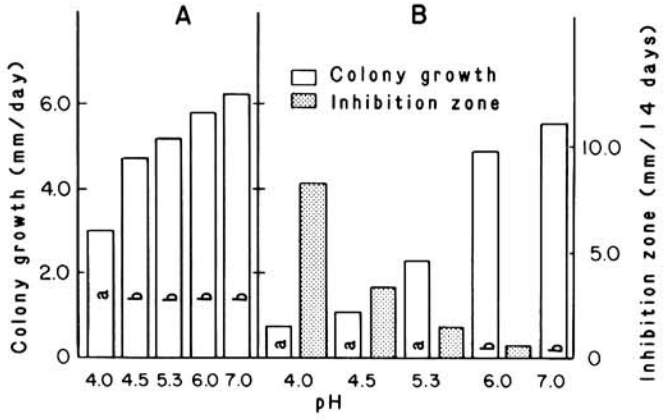

Fig. 2. Influence of pH of Difco potato-dextrose agar on colony growth rates and sizes of the zones of inhibition produced against a common sensitive (tester) culture for **A,** four pathogenic cultures of *Gaeumannomyces graminis* var. *tritici* and **B,** four cultures that had become weakly pathogenic after several months of maintenance in agar media. Bars having a different letter are significantly different at $P = 0.05$ according to Duncan's multiple range test.

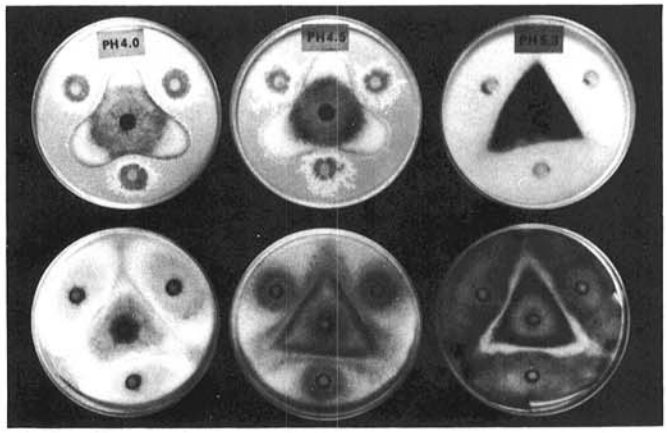

Fig. 3. Influence of the pH of Difco potato-dextrose agar medium on growth of a weakly pathogenic culture (three colonies near outer edge of each dish, top row) and a pathogenic culture (three colonies near outer edge of each dish, bottom row) of *Gaeumannomyces graminis* var. *tritici*. The colony in the center of each dish was started from the same pathogenic culture of *G. graminis* var. *tritici*.

no zones of inhibition. Our method did not distinguish between zones of inhibition caused by a diffusible inhibitor and barrages caused by vegetative incompatibility (7). Conceivably, the smaller zones evident with certain pairings of pathogenic (stable) cultures were barrages. In contrast, about 95% of the cultures unable to cause severe take-all showed a wide zone of inhibition when paired with the common tester (pathogenic and stable) culture. Highly significant negative correlations also occurred between colony growth rates on PDA and sizes of the zones of inhibition, evidence that inhibitory cultures inhibited themselves (7). Of particular significance is that zones of inhibition were sometimes observed on PDA buffered to pH 5.3 around agar disks taken from the advancing margin of an actively growing "normal" culture from unbuffered homemade PDA. This indicates that the inhibitory material may occur in agar disks such as commonly used (1,3,4,8) as a source of inoculum in tests for pathogenicity.

Perhaps the inability of a culture to cause disease under experimental conditions, where agar disks from culture dishes are the source of inoculum, is due not to loss of virulence per se but, rather, to an inability to express virulence because of the presence of inhibitory material in the disks. The inhibitory material is probably the Q factor described by Romanos et al (7). The Q factor

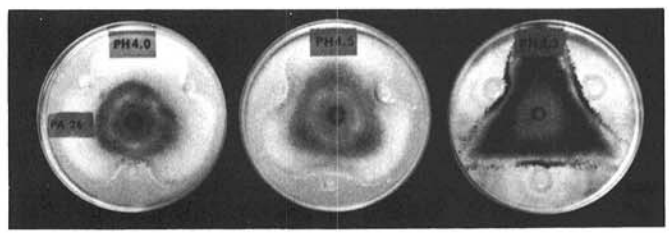

Fig. 4. Relative growth of a weak pathogen (three colonies near outer edge of each dish) compared with a pathogen (in the center of each dish) of *Gaeumannomyces graminis* var. *tritici* on Difco potato-dextrose agar buffered to three different pH values, showing the limited growth of the weak pathogen at pH 4.0 and 4.5 and a "clear" zone of inhibition around the agar disk inoculum at pH 5.3. Agar disks were from cultures grown on unbuffered (pH 6.0 when fresh) homemade PDA.

is most active or is produced in highest concentration in acidified PDA (7), an observation confirmed by our results. It is also apparent, as suspected by Romanos et al (7), that while inhibition is most evident at low pH, biologically active amounts of inhibitory material are produced and/or expressed in media at higher pH

values.

In our previous study (6), the frequency of nonpathogenic or weakly pathogenic monoascosporic cultures was much greater for those obtained after the parents had become nonpathogenic than for those obtained from the same parents before they became nonpathogenic. In the present study, the frequency of inhibitory monoascosporic cultures was much greater for those obtained from the parents after they had become nonpathogenic (12/38 showed zones 5–10 mm wide and 3/38 showed zones less than 5 mm wide) than for those obtained from the same parents before they became nonpathogenic (0/40 with large zones and 3/40 with small zones). Just as selection pressure on the fungus while in culture appears to favor a genetic change toward inability to produce disease, so it appears that selection pressure on nutrient agar media results in a greater ability of the fungus to produce inhibitor. Perhaps cells that produce the most inhibitor have an advantage on the agar plate over those that produce little or no inhibitor, resulting in a shift, when the fungus is in pure culture, in favor of cells (or hyphae) that produce inhibitor.

Romanos et al (7) found no relationship between the presence of mycovirus RNA and ability of a culture to produce the Q factor on PDA. We (6) have suggested that loss of ability to produce disease after prolonged vegetative culture is due either to a shift in nuclear makeup within heterokaryotic cells of the mycelium or to cytoplasmic (plasmid) determinants. Either of these two genetic mechanisms could also explain the increasing tendency of cultures to produce inhibitor. Nevertheless, the possibility of an involvement of other genetic mechanisms should not be ruled out.

The occurrence of genetic determinants for production of inhibitor within *G. graminis* var. *tritici* suggests some advantage of this trait to the fungus in nature. Perhaps ability to produce small amounts of inhibitor provides a means of defense for *G. graminis* var. *tritici* against competitors during saprophytic survival in wheat residue as shown by Bruehl et al (2) for antibiotic production by *Cephalosporium gramineum*. Selection pressure in nature would be against overproduction of inhibitor, since such strains would be eliminated because of their inability to grow in response to a host root. On the other hand, selection pressure would also be against underproduction of inhibitor, since strains unable to produce at least some inhibitor under the proper conditions might then be displaced during saprophytic survival by competitors for the crop residue.

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