Factors in Loss of Pathogenicity in *Gaeumannomyces graminis* var. *tritici*

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ABSTRACT

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Fewer than half of 111 pathogenic cultures of *Gaeumannomyces graminis* var. *tritici* were still pathogenic after maintenance for 9 mo on dilute (one-fifth strength) homemade potato-dextrose agar. Cultures lost ability to cause disease at about the same rate whether kept continuously growing (transferred every 10 days) at 24 C or stored (without transfer) at 12 or 24 C. Cultures were more likely to remain pathogenic if passed through the host (reisolated from infected roots of inoculated seedlings) at approximately 1-mo intervals beginning as soon as possible after the fungus was isolated from nature. Monoascosporic cultures also lost ability to produce disease during maintenance in culture, but less often than did their parents. The proportion of monoascosporic cultures rated as pathogenic was 36/38 for those obtained from three pathogenic parents but only 106/198 for those obtained from the same three parents after the parents had lost ability to produce disease. Results were similar for cultures started from single cells from fragmented hyphae; 35/50 of single-celled cultures from pathogenic parents, but only 2/150 of those from nonpathogenic parents, were rated pathogenic. The results are evidence against mycovirus infections being responsible for loss of pathogenicity and suggest that the fungus either is commonly heterokaryotic, with a low frequency of nuclei lacking genes for virulence and/or carrying genes that can suppress expression of virulence, or has a cytoplasmic determinant (plasmid) that affects expression of virulence. Possibly, selection pressure during maintenance in agar culture favors a shift in genetic determinants toward inability to cause disease.

Additional key words: cytoplasmic factors, hypovirulence, *Triticum aestivum*, wheat.

Like many phytopathogenic fungi, isolates of *Gaeumannomyces graminis* (Sacc.) von Arx & Olivier var. *tritici* Walker maintained in culture for several months on agar media tend to lose their ability to cause disease, and they commonly change in cultural appearance (1,5,8,9,14). Several explanations have been or can be offered to account for loss of pathogenicity in this fungus during prolonged vegetative culture (4), including expression of a latent mycovirus (9), "vegetative death" (11) associated with a cytoplasmic genetic element (plasmid), and a shift in ratios of genetically different nuclei if the fungus is heterokaryotic (1). Mature hyphae of this fungus are commonly multinucleate (6), and heterokaryons have been synthesized in the laboratory (7,12). This study was undertaken to determine if any of these factors might be responsible for the changes in cultural appearance and inability of *G. graminis* var. *tritici* to cause disease after being maintained in culture.

MATERIALS AND METHODS

Cultures selected for the study. Two culture collections (PA and PAN) representing, respectively, isolates from two irrigated fields near Pasco, WA, (8) were selected. The study began with 40 PA isolates and 71 PAN isolates, but as the study progressed, some cultures were lost owing to contamination and mites. When first tested (during the first month after isolation), all isolates showed strong virulence and most formed perithecia on roots and stem bases of seedlings. The study included 182 monoascosporic cultures representing 18 of the PA parents and 100 monoascosporic cultures representing five of the PAN parents.

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Methods of handling the cultures in the laboratory and periodic testing for virulence. Two complete sets of the original PA and PAN cultures (parents) and one set of all the monoascosporic cultures, all on dilute homemade potato-dextrose agar (PDA) (one-fifth strength potato extract and dextrose, 2% agar), were held as mature cultures in storage at 24 and 12 C, respectively, for the 9-mo duration of the study. Another complete set of monoascosporic cultures representing the five PAN isolates was stored at 3 C. In addition, each of the original 40 PA and 71 PAN cultures was maintained at 24 C in a state of continuous growth by transfer to the edge of a petri dish of fresh dilute PDA about every 10 days (as the advancing mycelium reached the opposite edge of the dish). A 6-mm-diameter disk of agar containing mycelium from the advancing edge of a growing colony was used for each transfer. All cultures, whether stored or kept continuously growing, were enclosed in plastic bags (to retard drying) when not being handled.

The virulence of each culture was tested approximately every month. For cultures in storage, a 6-mm-diameter disk was transferred to fresh dilute PDA and the original culture returned to storage. Disks cut from the advancing margin of the colony produced were then used as a source of inoculum in the test. For isolates kept continuously growing, two 6-mm-diameter disks were cut from the advancing margin of each culture and used as sources of inoculum. Storage for 1 mo, 2 mo, 3 mo, etc., corresponded to three transfers, six transfers, nine transfers, etc., respectively. The two agar disks were placed in a plastic tube (15 × 2.5 cm with drain holes; Ray Leach Conetainer Co., Canby, OR 97013), with vermiculite as the rooting medium, and sown with two seeds of the wheat (*Triticum aestivum* L.) cultivar Fielder (8). A 1-cm-thick layer of vermiculite separated the seeds from the agar disks containing the pathogen. Each culture was tested in duplicate. Seedlings were rated 6 wk after planting on a scale of 0–5, where 0 = no disease and 5 = the most severe disease (8).

Each of the 40 PA and 71 PAN isolates was also passed through the host approximately every month. The isolates were recovered by isolation from a root lesion on an inoculated seedling, using

dilute PDA amended with 100 ppm each of rifampin (Calbiochem-Behring Corp., La Jolla, CA 92037) and streptomycin (Sigma Chemical Co., St. Louis, MO 63178). Each isolate was then transferred to dilute PDA without antibiotics and the actively growing cultures used immediately as a source of inoculum (in agar disks) to repeat the cycle.

Perithecia (selfed cultures) were obtained either from infected roots of seedlings inoculated with the test isolates or by growing the fungus on sterilized stems of wheat seedlings on moist filter paper. Monoascosporic cultures were obtained as described previously (8). Single cells were obtained by growing the cultures on sterile cellophane on dilute PDA, then submerging the cellophane in 20 ml of sterile water in a petri dish to remove the mycelium. The water with floating mycelium was transferred to a 50-ml container and homogenized at low intensity for 20 sec with a Virtis homogenizer. The fragmented hyphae were dispersed on the surface of dilute PDA with antibiotics and allowed to settle for 3–5 min on the agar surface, then the water was decanted. The dishes were incubated at an angle and examined periodically over the next 72 hr. Single cells with one or more emerging hyphae (germ tubes) were transferred with a small piece of adhering agar to dilute PDA as described for making monoascosporic cultures (7).

RESULTS

Influence of method of handling the cultures on their ability to cause disease. Cultures lost ability to cause disease whether held in storage or kept continuously growing (Table 1). The second test for virulence of the 40 PA isolates was not begun until after the isolates had been in culture for 4 mo, at which time only about 73% caused severe disease (rated 3.5 or greater); all 40 caused severe disease when fresh from nature. The percentage of PA isolates rated 3.5 or greater continued to decrease with each month in culture, to only 15.8% after 9 mo. A similar pattern was observed for the PA cultures kept continuously growing during the 4–9 mo after their isolation from diseased plants (Table 1). Tests with the 71 PAN

TABLE 1. Pathogenicity ratings for cultures of *Gaeumannomyces graminis* var. *tritici* maintained on dilute potato-dextrose agar without transfer (stored at 24 C as mature cultures) or transferred approximately every 10 days for up to 9 mo after isolation from diseased wheat from the field

Treatment	Isolates tested (no.)	Mean pathogenicity rating[a] ± SE	Isolates (no.) per pathogenicity rating[a]				Pathogenic[b] (%)
			0	0.1–1.4	1.5–3.4	3.5–5.0	
PA isolates							
Fresh	40	4.9 ± 0.2	0	0	0	40	100.0
Stored 4 mo	40	4.0 ± 1.3	0	1	9	30	79.0
Stored 5 mo	39	3.2 ± 1.9	2	9	8	20	51.3
Stored 6 mo	38	3.2 ± 1.8	2	6	11	19	50.0
Stored 7 mo	38	2.1 ± 2.1	9	11	5	13	34.2
Stored 8 mo	39	2.5 ± 1.9	4	10	10	15	38.5
Stored 9 mo	38	1.6 ± 1.6	7	15	10	6	15.8
Transferred 3 times	39	3.4 ± 1.8	2	6	6	25	64.1
Transferred 6 times	38	2.0 ± 2.0	4	10	7	17	44.7
Transferred 9 times	38	3.0 ± 2.1	4	9	5	20	52.6
Transferred 12 times	38	3.0 ± 1.9	2	7	11	18	47.6
Transferred 15 times	36	2.1 ± 1.8	4	13	8	11	30.6
PAN isolates							
Fresh	71	4.7 ± 0.4	0	0	1	70	98.6
Stored 1 mo	71	4.5 ± 1.1	1	4	4	62	87.3
Stored 2 mo	71	4.0 ± 1.2	0	3	15	53	74.7
Stored 3 mo	71	3.6 ± 1.5	1	5	22	43	60.6
Stored 4 mo	70	4.1 ± 1.2	2	0	13	55	78.6
Stored 9 mo	59	3.1 ± 1.7	8	4	17	30	50.9
Transferred 3 times	65	4.4 ± 1.0	1	1	6	57	87.7
Transferred 6 times	64	4.1 ± 1.1	0	2	12	50	78.1
Transferred 9 times	64	4.0 ± 1.2	0	4	10	50	78.1
Transferred 12 times	64	4.5 ± 1.3	2	2	2	58	90.6
Transferred 15 times	59	3.2 ± 1.8	5	7	18	29	49.2

[a] On a scale of 0–5, where 0 = no disease, 1 = one or two seminal roots (less than half) with one or more lesions each, 2 = three, four, or all (more than half) of roots with one or more lesions each, 3 = all roots infected and lesions also on the coleoptile, 4 = lesions uniformly distributed 1–2 cm up the coleoptile and most extending into the stem, and 5 = seedling dead or nearly so.
[b] Rated 3.5 or greater.

TABLE 2. Pathogenicity ratings for monoascosporic cultures of *Gaeumannomyces graminis* var. *tritici* maintained on dilute potato-dextrose agar without transfer (stored at 24 C as mature cultures) or transferred approximately every 10 days after derivation from a virulent parent

Treatment	Mean pathogenicity rating[a] ± SE	Isolates (no.) per pathogenicity rating[a]				Pathogenic[b] (%)
		0	0.1–1.4	1.5–3.4	3.5–5.0	
Representing 18 PA isolates						
Stored 2 mo	4.5 ± 0.9	1	3	11	160	91.4
Stored 5 mo	3.3 ± 1.8	17	24	30	102	59.0
Stored 9 mo	3.1 ± 1.8	16	20	42	89	53.6
Representing 5 PAN isolates						
Fresh	4.4 ± 0.6	0	0	6	94	94.0
Stored 1 mo	4.7 ± 0.7	0	2	0	97	98.0
Stored 9 mo	4.3 ± 0.2	0	3	14	81	82.7
Transferred 3 times	4.6 ± 1.1	3	1	0	81	95.3
Transferred 12 times	4.6 ± 0.7	0	0	8	69	89.4

[a] On a scale of 0–5, where 0 = no disease, 1 = one or two seminal roots (less than half) with one or more lesions each, 2 = three, four, or all (more than half) of the roots with one or more lesions each, 3 = all roots infected and lesions also on the coleoptile, 4 = lesions uniformly distributed 1–2 cm up the coleoptile and most extending into the stem, and 5 = seedling dead or nearly so.
[b] Rated 3.5 or greater.

isolates were begun almost immediately after they were obtained from diseased plants, and after only 1 mo, several caused less disease than in their first test. Only about half of the PAN isolates still rated 3.5 or greater after 9 mo of storage or after 15 transfers. The original PA and PAN isolates lost ability to cause disease at about the same rate whether stored at 24 or 12 C.

Of the 71 PAN cultures passed through the host about once a month, 69 were still included in the experiment after 5 mo and five passages; these were still very pathogenic, with an average disease severity rating of 4.3.

Ninety-four of the 100 monoascosporic cultures representing the five PAN parents and 166 of the 182 monoascosporic cultures representing the 18 PA parents were pathogenic when tested immediately after isolation (Table 2). Slightly more than half of the monoascosporic cultures representing the PA parents still produced severe disease (rated 3.5 or greater) after 9 mo in culture (compared with only about 15% of those from PA parents; Table 1). Most (83–90%) of the monoascosporic cultures from PAN parents still produced severe disease after 9 mo in culture (compared with only about half of the PAN parents; Table 1). Thus, monoascosporic cultures also lost ability to produce disease during prolonged vegetative culture, but the proportion still able to cause severe disease after 9 mo in culture was consistently higher than for their parents.

Virulence of monoascosporic and single-celled cultures from nonpathogenic and pathogenic parents. Virulence tests were made with monoascosporic cultures immediately after isolation and again after 9 mo in storage. The ascosporic cultures were from two types of parents: those no longer able to cause severe disease and those still able to cause disease after 9 mo in culture. The loss among ascosporic cultures of ability to cause severe disease after 9 mo in culture was no different for those derived from parents that remained pathogenic during 9 mo in culture than for those derived from parents that lost ability to cause severe disease (Table 3). Thus, the tendency of a parent culture to become nonpathogenic gave no indication of the likelihood of progeny becoming nonpathogenic.

Three parents—PA-26, PA-30, and PAN 4-27—produced selfed perithecia both before and after losing ability to cause disease. Fifty monoascosporic cultures from each parent after it had lost ability to cause disease were compared with the monoascosporic cultures obtained from the same parent when it was still pathogenic. Only 11, 7, and 20 monoascosporic cultures were obtained from PA-26, PA-30, and PAN 4-27, respectively, while they were still pathogenic, but most (85–100%) of these caused severe disease (average rating, 3.5) and the remainder caused moderate disease (1.5–3.4 rating); none produced mild disease (0.1–1.4 rating) or were rated as nonpathogenic (Table 4). By comparison, as few as 12/50 and as many as 39/50 of the monoascosporic cultures obtained from the three parents after they had lost ability to cause disease were highly pathogenic (Table 4). Most of the remainder caused moderate (1.5–3.4) or mild (0.1–1.4) disease, but a few were nonpathogenic.

Fifty single-celled cultures were made from PA-26, PA-30, and PAN 4-27 and also from PA-12, which still caused severe disease after 9 mo in culture. Thirty-five of the 50 from PA-12 caused severe disease (rated as pathogenic as the parent) and the other 15

TABLE 3. Pathogenicity ratings for monoascosporic cultures of *Gaeumannomyces graminis* var. *tritici* when originally isolated and after 9 mo of storage (without transfer) as mature cultures of dilute potato-dextrose agar and representing parents that either lost or did not lose ability to cause disease after 9 mo of storage on dilute potato-dextrose agar

Isolate	Months from original	No. of ascospore progenies used	Variation in pathogenicity rating[a] among ascospore progenies: 0	0.1–1.4	1.5–3.4	3.5–5.0
Parent caused only mild or no disease after 9 mo in culture						
PA-3	0	9	0	0	2	7
	9	9	0	1	1	7
PA-16	0	12	0	0	5	7
	9	12	1	1	1	9
PA-20	0	11	0	0	2	9
	9	11	2	2	4	3
PA-25	0	11	0	0	2	9
	9	10	2	1	4	3
PA-26	0	11	0	0	1	10
	9	9	2	3	2	2
PA-28	0	12	0	0	0	12
	9	12	2	0	0	10
PA-30	0	7	0	0	1	6
	9	7	0	0	3	4
PA-32	0	11	0	0	2	9
	9	8	1	2	3	2
PA-37	0	11	0	0	5	6
	9	11	1	1	3	6
PA-39	0	12	0	0	1	11
	9	10	0	2	3	5
PA-40	0	6	0	0	0	6
	9	3	3	0	0	0
PAN 4-13	0	20	0	0	0	20
	9	19	0	0	2	17
PAN 4-27	0	20	0	0	0	20
	9	20	0	0	3	17
PAN 4-38	0	20	0	0	0	20
	9	20	0	0	4	16
	Total	173	0	0	21	152 (88%)
		161	14	13	33	101 (63%)
Parent still able to cause severe disease after 9 mo in culture						
PA-12	0	11	0	0	3	8
	9	12	0	0	2	10
PA-29	0	11	0	0	5	6
	9	11	0	1	2	8
PA-41	0	12	0	0	0	12
	9	12	1	1	2	8
PA-42	0	11	0	1	5	5
	9	12	0	2	5	5
PA-43	0	11	0	0	0	11
	9	9	0	1	3	5
PA-44	0	9	0	0	0	9
	9	8	1	2	3	2
PAN 4-4	0	20	0	0	6	14
	9	20	0	1	3	16
PAN 4-9	0	20	0	0	0	20
	9	19	0	2	2	15
	Total	105	0	1	19	85 (81%)
		103	2	10	22	69 (67%)

[a] On a scale of 0–5, where 0 = no disease, 1 = one or two seminal roots (less than half) with one or more lesions each, 2 = three, four, or all (more than half) of the roots with one or more lesions each, 3 = all roots infected and lesions also on the coleoptile, 4 = lesions uniformly distributed 1–2 cm up the coleoptile and most extending into the stem, and 5 = seedling dead or nearly so.

TABLE 4. Pathogenicity ratings for monoascosporic cultures of *Gaeumannomyces graminis* var. *tritici* obtained from three pathogenic parents and for other monoascosporic cultures obtained from the same parents after they had lost ability to cause severe disease

Isolate	Pathogenicity rating[a] of parent isolate	Ascospore cultures (no.)	Cultures (no.) per pathogenicity rating[a]: 0	0.1–1.4	1.5–3.4	3.5–5.0	Pathogenic[b] (%)
PA-26	5.0	11	0	0	1	10	90.9
	0.8	50	0	4	29	17	34.0
PA-30	5.0	7	0	0	1	6	85.7
	0.5	50	9	9	20	12	24.0
PAN 4-27	5.0	20	0	0	0	20	100.0
	1.7	98	1	4	16	77	78.6

[a] On a scale of 0–5, where 0 = no disease, 1 = one or two seminal roots (less than half) with one or more lesions each, 2 = three, four, or all (more than half) of the roots with one or more lesions each, 3 = all roots infected and lesions also on the coleoptile, 4 = lesions uniformly distributed 1–2 cm up the coleoptile and most extending into the stem, and 5 = seedling dead or nearly so.

[b] Rated 3.5 or greater.

caused moderate disease (1.5–3.4 rating) (Table 5). Of the 150 single-celled cultures from PA-26, PA-30, and PAN 4-27, 126 produced either no disease or mild disease (as their parents did) but the remaining 24 produced severer disease than their parents did, with two having disease ratings greater than 4.0.

Cultural variation among monoascosporic and single-celled cultures from pathogenic and nonpathogenic parents. Cultures started from single cells from nonpathogenic parents showed greater variation in appearance, including sectoring, than those from pathogenic parents. Monoascosporic cultures from nonpathogenic parents also showed greater variation in appearance than those from pathogenic parents. Some single-celled cultures from the nonpathogenic PA-30 were hardly recognizable as *G. graminis* var. *tritici*.

DISCUSSION

All of the original 111 field isolates obtained from diseased plants produced severe disease when tested initially, yet about 10% of the monoascosporic cultures derived from them produced only moderate or mild disease (rated less than 3.5) when tested initially. Our earlier study (8) likewise indicated that about 10% of monoascosporic cultures produce disease milder than that produced by their parents freshly isolated from diseased plants. Monoascosporic cultures are probably homokaryotic, except for rare mutations. Virulence is controlled by many genes (2), which can account for part of the variation in virulence ratings among the ascospore cultures. However, the frequency of monoascosporic and single-celled cultures that produced disease ratings less than 3.5 was markedly greater for those from parents no longer able to cause disease than for those from the same parents before they lost ability to cause severe disease. This result clearly indicates a shift within the respective parents toward inability to cause disease.

If one or more mycoviruses were responsible for loss of pathogenicity, one would expect few selfed ascosporic cultures to be less pathogenic than their parents, since the mycoviruses in *G. graminis* var. *tritici* are transmitted inefficiently into the ascospore (13). Indeed, the frequency of pathogenic ascosporic cultures from nonpathogenic parents should have been higher than recorded in our experiments. It might be argued that regardless of the low frequency of transmission of mycovirus through the ascospores, the frequency of transmission should be proportional to the degree of infection, ie, greater from the severely infected than from the mildly infected or noninfected parents. This cannot explain, however, why about 10% of the selfed progeny from highly pathogenic cultures produced less disease than their parents did, nor is this argument consistent with the observation that groups of monoascosporic cultures (all obtained from parents that were pathogenic at the time) lost ability to cause disease at about the same rate whether or not their parents eventually became nonpathogenic during maintenance in culture. Chambers (5) similarly observed that monoascosporic cultures become nonpathogenic during maintenance in culture. In our study, the tendency of the parent to become nonpathogenic had no value for predicting whether the ascosporic progeny would become nonpathogenic. It therefore seems highly improbable that mycovirus infections can explain the loss of pathogenicity in either the parent or the ascospore cultures.

The recovery by single-cell isolation of the occasional pathogenic culture from a parent that had become nonpathogenic (after prolonged vegetative culture) could be interpreted to indicate a variable nuclear condition from cell to cell, ie, heterokaryotic hyphae (1). Conceivably, selection pressure during maintenance on nutrient agar media could favor a greater diversity of nuclear makeup from cell to cell within the mycelium, with only the occasional cell having a nuclear condition similar to that of the original pathogenic parent. On the other hand, the results of this aspect of our study are similar to reports of dsRNA agents being absent from some conidia of hypovirulent strains of *Endothia parasitica* (10) and of strains of *Aspergillus glaucus* carrying cytoplasmic determinants for "vegetative death" (11).

The consistent recovery of a low frequency (about 10%) of weakly pathogenic monascosporic cultures from highly pathogenic parents might also be interpreted to indicate that the parent cultures were heterokaryotic. With ascosporic cultures, the weakly pathogenic homokaryons would be separated from the pathogenic homokaryons by "single sporing," thereby resulting in a frequency of weakly pathogenic cultures roughly equal to the frequency of nuclei in the mycelium that lacked genes for virulence or carried genes for suppression of virulence. The frequency of weakly pathogenic and nonpathogenic monoascosporic cultures was considerably higher from selfed parents after the parents had lost ability to produce disease than from the same selfed parents while still pathogenic, which would be expected if loss of pathogenicity results from a shift in nuclear condition in the heterokaryotic mycelium toward a higher frequency of nuclei associated with inability to cause disease. Chambers and Flentje (7) were unsuccessful in getting their weakly pathogenic cultures to produce perithecia but observed, nevertheless, that monoascosporic cultures from perithecia on a living host were all strongly pathogenic, whereas only half of those from a perithecium produced in culture were strongly pathogenic. Our results indicate that the genetic changes associated with inability to produce disease begin soon after the fungus is in pure culture, and presumably some genetic change occurs even before expression as a nonpathogenic phenotype.

Monoascosporic cultures, on the other hand, also lost ability to cause disease during maintenance in culture. The rate at which monoascosporic cultures lost ability to produce disease was slower than the rate for their parents but was nevertheless much higher than would be expected if the loss was due solely to mutations. Nearly half of those derived from PA isolates and about 18% of those derived from PAN isolates lost ability to cause disease during 9 mo in culture. Chambers (5) reported that of 12 monoascosporic cultures strongly virulent with the first subculture, eight had become either weakly pathogenic or nonpathogenic after subculturing for 11 mo. A rare mutation, toward avirulence, if that genetic change also conferred an advantage to the fungus on agar media, could possibly account for the apparent high rate at which ascospore cultures become nonpathogenic. These results, however, can also be explained by a cytoplasmic genetic determinant (plasmid) transmitted from parent to progeny that eventually (after prolonged vegetative culture) is lethal or suppressive to the carrier (4). This might also explain why monoascosporic cultures lost ability to cause disease at the same rate during maintenance in culture, whether or not their respective parents eventually became nonpathogenic. Burnett (3) has suggested that variation in some Ascomycetes and Deuteromycetes attributed in the past to reassortments of nuclei in heterokaryotic mycelia, and possibly also some examples of "saltation," may be reassortments of extrachromosomal elements.

The cultures recovered directly from nature were remarkably uniform in phenotype. This can be attributed to the selection pressure in nature, which would be against the nonpathogenic types

TABLE 5. Pathogenicity ratings for single-celled (from fragmented hyphae) cultures of *Gaeumannomyces graminis* var. *tritici* obtained from four parent cultures ranging between pathogenic and weakly pathogenic

Isolate	Pathogenicity rating[a] of parent isolate	Cultures (no.) per pathogenicity rating[a] 0	0.1–1.4	1.5–3.4	3.5–5.0	Pathogenic[b] (%)
PA-12	4.0	0	0	15	35	70.0
PAN 4-27	1.7	11	31	6	2	4.0
PA-26	0.8	17	18	15	0	0
PA-30	0.5	4	45	1	0	0

[a] On a scale of 0–5, where 0 = no disease, 1 = one or two seminal roots (less than half) with one or more lesions each, 2 = three, four, or all (more than half) of the roots with one or more lesions each, 3 = all roots infected and lesions also on the coleoptile, 4 = lesions uniformly distributed 1–2 cm up the coleoptile and most extending into the stem, and 5 = seedling dead or nearly so.

[b] Rated 3.5 or greater.

(4). Of special significance is that, although our study lasted only 9 mo, most or all cultures, given enough time, apparently would eventually become nonpathogenic. Evidently, most or all cultures from nature carry factors that can result in suppression of virulence. It may be that the fungus in nature has the correct balance of the different nuclear and cytoplasmic genes necessary for its success in both parasitism and saprophytism.

LITERATURE CITED

1. Asher, M. J. C. 1981. Pathogenic variation. Pages 199-218 in: Biology and Control of Take-all. M. J. C. Asher and P. J. Shipton, eds. Academic Press, London. 538 pp.
2. Blanch, P. A., Asher, M. J. C., and Burnett, J. H. 1981. Inheritance of pathogenicity and cultural characters in *Gaeumannomyces graminis* var. *tritici*. Trans. Br. Mycol. Soc. 77:391-399.
3. Burnett, J. H. 1975. Mycogenetics. John Wiley & Sons, London. 375 pp.
4. Caten, C. E. 1972. Vegetative incompatability and cytoplasmic infection in fungi. J. Gen. Microbiol. 72:221-229.
5. Chambers, S. C. 1970. Pathogenic variation in *Ophiobolus graminis*. Aust. J. Biol. Sci. 23:1099-1103.
6. Chambers, S. C., and Flentje, N. T. 1967. Studies on oat-attacking and wheat-attacking isolates of *Ophiobolus graminis* in Australia. Aust. J. Biol. Sci. 20:927-940.
7. Chambers, S. C., and Flentje, N. T. 1967. Studies on variation with *Ophiobolus graminis*. Aust. J. Biol. Sci. 20:941-951.
8. Cook, R. J., and Naiki, T. 1982. Virulence of *Gaeumannomyces graminis* var. *tritici* from fields under short-term and long-term wheat cultivation in the Pacific Northwest, USA. Plant Pathol. 31:201-207.
9. Férault, A. C., Tivoli, B., LeMaire, J. M., and Spire, D. 1979. Etude de l'evolution comparee du niveau d'agressivité et du contenu en particules de type viral d'une souche de *Gaeumannomyces graminis* (Sacc.) Arx et Olivier (*Ophiobolus graminis* Sacc.). Ann. Phytopathol. 11:185-191.
10. Grente, J., and Sauret, S. 1969. L'hypovirulence exclusive est-elle contrôlee par des determinants cytoplasmiques? C. R. Acad. Sci. Paris. 268:3173-3176.
11. Jinks, J. L. 1959. Lethal suppressive cytoplasms in aged clones of *Aspergillus glaucus*. J. Gen. Microbiol. 21:397-409.
12. Lemaire, J. M., Jouan, B., and Tivoli, B. 1975. Un exemple de prémunition chez le blé contre l'agent du Piétin-échaudage: *Ophiobolus graminis*. Z. Pflanzenkr. Pflanzenpathol. Pflanzenschutz 82:50-51.
13. Rawlinson, C. J., and Buck, K. W. 1981. Viruses in *Gaeumannomyces* and *Phialophora* spp. Pages 151-174 in: Biology and Control of Take-all. M. J. C. Asher and P. J. Shipton, eds. Academic Press, London. 538 pp.
14. Russell, R. C. 1939. Pathogenicity tests with cultures of *Ophiobolus graminis* Sacc. Sci. Agric. 19:662-669.