Toxin of *Pyrenophora tritici-repentis*: Host-Specificity, Significance in Disease, and Inheritance of Host Reaction

L. Lamari and C. C. Bernier

ABSTRACT

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Pyrenophora tritici-repentis differentially induces combinations of tan necrosis and extensive chlorosis in individual susceptible wheat cultivars. Crude and dialyzed culture filtrates from isolates of *P. tritici-repentis* contained a heat-labile (121 C for 20 min) toxin(s), which induced necrosis only on fungus-susceptible, tan necrosis-expressing cultivars within the genus *Triticum*. Cultivars resistant to the fungus as well as cultivars susceptible to extensive chlorosis-inducing isolates were insensitive. Segregation of F_2 populations from four different crosses between cultivars resistant and susceptible to tan necrosis indicated that susceptibility to the fungus and sensitivity to the toxin were controlled by the same dominant gene. Toxin production by the pathogen was associated with the ability of individual isolates to induce tan necrosis (nec+) in necrosis-expressing cultivars. Isolates that induced extensive chlorosis but not necrosis (nec− chl+) did not produce toxin in vitro. The toxin differentiated two near-isogenic lines from the cultivar Columbus. When inoculated with a nec+ isolate, only the toxin-sensitive line (Col+) developed tan necrosis. The toxin(s) of *P. tritici-repentis* is cultivar-specific, involved in the induction of necrosis in the host, and appears to be a pathogenicity factor. Its designation as Ptr necrosis toxin is proposed.

Pyrenophora tritici-repentis (Died.) Drechs., anamorph *Drechslera tritici-repentis* (Died.) Shoem. (syn. *Helminthosporium tritici-repentis* Died.), causes leaf spots on several gramineous hosts (7,9,17), including wheat, where the disease is known as tan spot. In recent years, tan spot has become a potentially destructive disease of wheat worldwide (8). In 1974, tan spot was observed to be the most important leaf spot disease in the Canadian Prairies (23), and in 1982, severe infections on seedlings were observed in Manitoba and Saskatchewan for the first time (24). The disease was subsequently found to be damaging to winter wheat (25).

The reaction of wheat germ plasm to *P. tritici-repentis* was evaluated with a rating scale based on lesion type, and resistant cultivars were identified (10). Susceptibility to *P. tritici-repentis* was expressed by the presence of either tan necrosis or chlorosis, and resistance was characterized by the absence of, or great reduction in, these symptoms. Some genotypes developed small brown to black spots and extensive chlorosis that covered most of the leaf.

Variation for virulence in *P. tritici-repentis* has been reported (3,5,16), and three pathotypes were identified in a population of the pathogen from western Canada (11). Pathotypes were classified on the basis of their ability to induce both tan necrosis and extensive chlorosis (nec+ chl+), extensive chlorosis only (nec− chl+), and tan necrosis only (nec+ chl−) on appropriate susceptible cultivars (11). The development of each reaction (tan necrosis or extensive chlorosis) depended on the individual wheat cultivar. Furthermore, the expression of tan necrosis as well as extensive chlorosis was found to be the result of specific interactions between individual host genotypes and pathotypes of the pathogen. Several lines of evidence suggest the involvement of toxin(s) in tan spot of wheat. These include: 1) the extensive chlorosis or necrosis observed in some wheat cultivars in response to infection by certain isolates (10,11), and 2) cytological evidence that hyphae of *P. tritici-repentis* grow intercellularly without penetrating the mesophyll cells (11-13).

The search for toxins in host-pathogen systems is stimulated not only by an interest in better understanding the pathogenic process but also by their potential use in identifying resistant cells or protoplasts in tissue cultures or in screening and breeding for disease resistance. There is presently one report of toxin production by isolates of *P. tritici-repentis* (26). The toxin(s) in culture filtrates of the fungus was reported to be cultivar-specific and to mimic the symptoms caused by the fungus. However, the potency of the toxin was very low compared with that of the host-specific toxins involved in other host-pathogen systems (1,14,20,21,27). The presence of toxic metabolites in fungal culture filtrates has been reported for many host-pathogen systems, but in only a few studies has their relevance to the disease been convincingly demonstrated (19). Toxins have been categorized as factors in virulence or pathogenicity (28). Criteria for establishing the significance of toxins in the disease syndrome have been reviewed (28). According to Yoder (28) and Scheffer (19), genetic analyses of the host and/or pathogen have provided the strongest evidence so far of toxin involvement in disease.

This report deals with the isolation and partial characterization of toxin(s) in the culture filtrates of certain isolates of *P. tritici-repentis*, the inheritance of host reaction to the toxin, and the significance of the toxin in the disease.

MATERIALS AND METHODS

Production of culture filtrates. One-milliliter aliquots of a conidial suspension (about 10^4 spores per milliliter) of the pathogen were transferred to 250-ml Erlenmeyer flasks containing 50 ml of Fries medium amended with 0.1% yeast extract (medium No. 66 in Dhingra and Sinclair [4]) and incubated at 20 C in the dark without agitation for 3 wk, or less if the mycelial mats began to lyse. The cultures were then filtered through Whatman No. 1 paper, and filtrates were passed through a 0.45-μm Millipore membrane, adjusted to pH 6.5 with 1 N NaOH, then stored at 4 C or kept in a freezer at −19 C until processed further.

Partial purification and stability. Initially, attempts were made to isolate and purify the toxin from culture filtrates of *P. tritici-repentis* by the methods of Smedegaard-Petersen (21); however, these were not successful. Subsequently, culture filtrates from 11 monoconidial isolates were dialyzed overnight against one change of distilled water (0.5 L) in 5-ml batches at 4 C (Spectrapore Spectrum Medical Industries Inc., Los Angeles, CA, with a cutoff

at 8,000 mol wt). The heat stability of toxin from *P. tritici-repentis* was assessed by subjecting crude and dialyzed filtrates (adjusted to pH 6.5) to autoclaving at 121 C for 20 min and filtering preparations through a 0.45-μm Millipore membrane to remove the precipitates and then testing filtrates for activity in a seedling bioassay. A nonautoclaved sample was retained as a control.

Bioassay. Seedlings were infiltrated with about 150 μl of a toxin dilution at the two- to-four-leaf stage; a Hagborg device (6) was used. Sterile distilled water and sterile Fries medium were used as controls throughout the study. Seedlings were examined daily for 3 days and classified as (+) if necrosis was present and (−) if no symptoms were evident.

Toxin specificity. Host range was assessed by infiltrating crude and dialyzed filtrates of isolates ASC1 and 86-124 of *P. tritici-repentis* into seedlings from 11 species outside the genus *Triticum* as well as from four wheat cultivars previously identified as susceptible to the fungus (10,11). Dilutions of 1:1, 1:10, 1:100, 1:500, and 1:1,000 were used. The test species and wheat cultivars were also inoculated with isolate ASC1 of the pathogen.

To avoid confusion in terminology, resistance and susceptibility are used hereafter to describe the reaction of wheat cultivars to the fungus, whereas sensitivity and insensitivity are used to describe the reaction to the toxin. The term necrosis refers to the tan area of dead tissue, usually visible 3–4 days after inoculation, surrounding a small, brown to black necrotic spot. Chlorosis refers to the zone surrounding the dark brown to black spot that consists of tissue exhibiting a gradual yellow discoloration. Chlorosis may be restricted, as a halo, or extensive, covering most of the leaf; in the latter case, it becomes necrotic within 7–8 days after inoculation. Although both symptoms express susceptibility to the pathogen, they result from two different interactions between individual isolates and specific wheat differential cultivars (11).

Twelve wheat cultivars, previously characterized for their reactions to the fungus (11), were used in a first test to assess cultivar specificity of the culture filtrates. These are listed in Table 1. To further evaluate the cultivar specificity and assess the relationship between the reaction to the toxin and the reaction to the fungus, a second test was conducted. Seedlings from 92 resistant and moderately resistant cultivars and from 69 susceptible lines and cultivars from a previous study (10) were

inoculated at the two-leaf stage with isolate ASC1 (nec+ chl+) of *P. tritici-repentis*. Dialyzed culture filtrates, at a dilution of 1:50, were infiltrated into the third leaf 6 days after inoculation, and reactions to the fungus and to the toxin were recorded 24–48 hr after toxin infiltration.

The relationship between virulence and toxin production by the pathogen was assessed with 11 isolates previously tested for virulence to a differential wheat set (11). Isolates ASC1, HY331-11, TK85-243, and TK85-245 were characterized by their ability to induce necrosis and chlorosis (nec+ chl+), and isolates 86-124 and HY331-3 induced necrosis only (nec+ chl−), whereas the remaining isolates (nec− chl+) induced chlorosis only (11) (Table 2). Dialyzed culture filtrates from each isolate were infiltrated into leaves of 30 wheat cultivars selected on the basis of their reaction to isolate ASC1 and its toxin.

Inheritance of wheat reaction to the toxin. One tetraploid and two hexaploid wheat cultivars previously identified as resistant to *P. tritici-repentis* (10) were crossed to the susceptible tetraploid cultivar Coulter and the hexaploid cultivars Celtic and Columbus (toxin-sensitive selection), respectively. F_1, F_2, and backcross progenies were tested for reaction to isolate ASC1 of *P. tritici-repentis* and its toxin (1:50 dilution).

Fungal inoculation. Inoculation was performed by procedures described previously (10). Seedlings at the two-leaf stage were sprayed until runoff with a conidial suspension, previously adjusted to 3,000 conidia per milliliter, with a DeVilbiss sprayer fitted to a compressed air outlet and operated at 10 psi. Ten drops of Tween 20 (polyoxyethylene sorbitan monolaurate) were added per liter of spore suspension before inoculation. The seedlings were then incubated under continuous leaf wetness for 24 hr at 20 C and with a 16-hr photoperiod. Seedling reactions were recorded at 6–8 days postinoculation, using a 1–5 scale, based on lesion type, where 1 denotes resistance and 5 susceptibility (10). When extensive chlorosis developed on seedlings from the "extensive chlorotic" lines, the symbol CH+ was given to the reaction. Absence of extensive chlorosis, on otherwise chlorotic lines, was denoted as CH−.

RESULTS

Toxin production. Crude and dialyzed filtrate from isolates ASC1 and 86-124 consistently contained a metabolite(s) that induced a severe necrosis within 24–36 hr on cultivars Glenlea, Celtic, Coulter, and BH1146 at dilutions of 1:100. Dilutions of 1:500 and occasionally of 1:1,000 also induced necrosis within 48–72 hr of infiltration. Non-*Triticum* species were not affected by the metabolites contained in the culture filtrates at any dilution and developed only minute necrotic or chlorotic flecks in response to inoculation with *P. tritici-repentis*. These were gramineous

TABLE 1. Reaction of 12 wheat cultivars to isolate ASC1 of *Pyrenophora tritici-repentis* and its culture filtrates[a]

Line or cultivar	Origin	Reaction[b]	
		Fungus	Toxin
Hexaploid			
BH1146	Brazil	3–4	+
Celtic	USA	4	+
Columbus	Canada	4	Seg (+,−)[c]
Erik	USA	1–2	−
Glenlea	Canada	5	+
Salamouni	Lebanon	1–2	−
T.p. × T.m.	Unknown	1	−
6B365	Lebanon	CH+	−
Tetraploid			
Coulter	Canada	5	+
4B242	USA	1–2	−
4B1149	Mexico	1	−
Diploid			
2B13	Germany	CH+	−

[a] Leaves were infiltrated with about 150 μl of a 1:100 dilution of dialyzed culture filtrate.
[b] Fungus reaction: rating scale of 1–5, where 1 = small brown to black spot without tan necrosis and chlorosis; 2 = small dark brown spot with minute amounts of tan necrosis or chlorosis; 3 = small brown to black spot surrounded by a distinct ring of tan necrosis or chlorosis and not coalescing; 4 = small brown to black spot surrounded by tan necrosis or chlorosis, sometimes coalescing; and 5 = most lesions consisting of coalescing tan necrotic or chlorotic tissues. CH+ = presence of extensive chlorosis. Toxin reaction: + = sensitive; and − = insensitive.
[c] Seg = Segregating for toxin reaction.

TABLE 2. Relationship between necrotic and chlorotic reaction patterns caused by 11 isolates of *Pyrenophora tritici-repentis* on two wheat cultivars and their ability to produce toxin in vitro

	Reaction		
	Extensive chlorosis[a]	Tan necrosis[a]	Toxin[b]
Isolate	on 6B365	on BH1146	production
86-124	−	+	+
HY331-3	−	+	+
ASC1	+	+	+
HY331-11	+	+	+
TK85-243	+	+	+
TK85-245	+	+	+
D308	+	−	−
HY331-1	+	−	−
HY331-2	+	−	−
HY331-6	+	−	−
HY331-7	+	−	−

[a] +,− = symptom expressed and not expressed, respectively.
[b] +,− = toxin produced and not produced in vitro, respectively, as revealed by the induction of visible symptoms on toxin-sensitive cultivars.

species: quackgrass (*Agropyron repens* L.), wild oat (*Avena fatua* L.), cultivated oat (*Avena sativa* L.), bromegrass (*Bromus inermis* Leyss.), barley (*Hordeum vulgare* L.), green foxtail (*Setaria viridis* (L.) Beauv.), corn (*Zea mays* L.), and broad leaf species: buckwheat (*Fagopyrum esculentum* Moench), sunflower (*Helianthus annuus* L.), tobacco (*Nicotiana tabacum* L.), and faba bean (*Vicia faba* L.). Neither water, uninoculated dialyzed Fries medium, nor undialyzed Fries medium, at the same dilutions as the culture filtrates, induced any symptom when infiltrated into the above test species.

Although a toxin(s) was present by the first week in the filtrates from cultures grown in the Fries medium, high concentrations of toxin, causing symptoms at 1:500–1:1,000 dilutions, were more consistently obtained when cultures were incubated 2–3 wk. Toxin levels from isolates ASC1 and 86-124 grown for 3 wk in the medium with low sugar content used by Tomás and Bockus (26) were only a tenth of those grown in the regular Fries medium, in spite of similar mycelial growth (unpublished). However, symptoms and host specificity of the toxin(s) produced in either medium were identical.

Cultivar specificity. In the first test involving 12 wheat lines and cultivars, all the lines resistant to the fungus and those that developed extensive chlorosis were insensitive to the toxin. In contrast, all lines that developed necrosis to the pathogen were toxin-sensitive (Table 1). The cultivar Columbus segregated for toxin reaction and 10–15% of the seedlings within the cultivar tested were found to be insensitive to the toxin. These are referred to as Col−, whereas their sensitive sister lines are referred to as Col+.

In the second test, all lines and cultivars resistant or moderately resistant to the fungus were insensitive to the toxin, whereas susceptible lines segregated into two groups. Of the 69 susceptible lines, 44 were sensitive to the toxin and 25 were insensitive. To confirm this finding, the 161 lines were inoculated once more with isolate ASC1 and checked daily to record their reaction type. The 92 resistant lines developed dark brown to black spots with very little or no chlorosis. All 44 lines previously found to be toxin-sensitive developed tan necrosis within 3–4 days of inoculation, whereas the 25 toxin-insensitive fungus-susceptible lines developed extensive chlorosis or chlorotic halos that became necrotic after 7–8 days.

Production of toxin by different isolates. All isolates previously known to induce necrosis on cultivar BH1146 produced toxin in vitro (Table 2). These included wild type (nec+ chl+) isolates, which also induced extensive chlorosis on line 6B365, as well as (nec+ chl−) isolates, which did not induce extensive chlorosis. All isolates lacking the ability to cause necrosis (nec− chl+) failed to produce detectable levels of toxin in the culture filtrates when bioassayed on the toxin-sensitive wheat line BH1146 (Table 2). The toxin had the same cultivar specificity when infiltrated in leaves of 30 cultivars, and it produced the identical symptoms, irrespective of the isolate from which it originated.

Effect of dialysis and autoclaving on toxin activity. The toxic metabolite(s) from the culture filtrates of all toxin-producing isolates listed in Table 2 were retained by the dialysis membrane, and no detectable loss of activity was observed in the bioassay. Also, infiltration of the dialysate failed to cause any symptoms on seedlings of toxin-sensitive cultivars Glenlea and BH1146, and on insensitive cultivars Erik, Salamouni, 4B1149, and 6B365. The crude and dialyzed culture filtrates, previously known to contain toxic metabolites, from all toxin-producing isolates lost their ability to induce symptoms (i.e., toxicity) after autoclaving.

Inheritance of toxin reaction. All F_1 progenies from each of four crosses between toxin-sensitive/fungus-susceptible and toxin-insensitive/fungus-resistant lines were sensitive to the toxin and developed necrosis when inoculated with the fungus, indicating that sensitivity and susceptibility were dominant. The F_2 and backcross progenies segregated into toxin-sensitive and toxin-insensitive plants (Table 3). The toxin-sensitive plants developed necrosis (to the fungus), whereas the toxin-insensitive plants were resistant to the fungus (no necrosis). The F_2 populations from all the crosses segregated in a ratio of one insensitive to three sensitive, indicating that sensitivity to the toxin (and susceptibility to the fungus) was dominant over insensitivity (resistance to the fungus). Progeny from the backcross to Erik (insensitive) segregated in a ratio of one insensitive to one sensitive, confirming that sensitivity was dominant over insensitivity. The segregation ratios of the backcross and the three F_2 populations are consistent with the hypothesis that a single gene is involved.

Comparison of Columbus selections for production of tan necrosis. The Columbus cultivar (pedigree RL4137 × Neepawa[6]) was found to be a mixture of two lines that were morphologically identical but had opposite reactions to the toxin. The toxin-sensitive (Col+) and the toxin-insensitive (Col−) lines were both susceptible to the fungus. When inoculated with a toxin-producing isolate, the Col+ line consistently produced tan necrosis 3–4 days after inoculation, whereas the Col− line developed chlorosis (Table 4, Fig. 1). Inoculation with a toxin-nonproducing isolate failed to induce the development of necrotic lesions in both lines (Table 4).

DISCUSSION

The toxin(s) present in the culture filtrates of *P. tritici-repentis* appears to be active only against wheat species. However, the cultivar specificity of the toxin corresponded to that of tan-necrosis-inducing isolates, which are characterized by their ability to induce tan necrosis on susceptible cultivars, such as BH1146 and Glenlea (11). Cultivars that develop chlorosis (line Col−) or extensive chlorosis (lines 6B365, 2B13) in response to fungal infection were insensitive to the toxin, suggesting that the induction of chlorosis and extensive chlorosis may involve a different toxin or mechanism(s) than the one operating in the necrotic reaction. This is in agreement with previous findings

TABLE 3. Segregation for reaction to isolate ASC1 of *Pyrenophora tritici-repentis* and its culture filtrates in three F_2 and two backcross wheat populations[a]

Type[b]	Cross	Ratio[c]		Chi square	Probability
		Observed R:S	Expected R:S		
BC$_1$	(Erik × Columbus) × Erik	34:42	1:1	0.6447	0.50–0.30
BC$_1$	(Erik × Celtic) × Erik	30:22	1:1	0.9423	0.50–0.30
F$_2$	Salamouni × Columbus	48:131	1:3	0.2253	0.70–0.50
F$_2$	Salamouni × Columbus	98:271	1:3	0.3983	0.50–0.30
F$_2$	4B242 × Coulter	68:218	1:3	0.1678	0.70–0.50

[a] Plants were inoculated with isolate ASC1 (which has the ability to induce both tan necrosis and chlorosis) at the two-leaf stage, and the third leaf was infiltrated on day 6 with about 150 µL of a 1:50 dilution of dialyzed culture filtrates of isolate ASC1. Reactions to both the fungus and the toxin were recorded 24–48 hr after toxin infiltration.

[b] All crosses are reciprocal, except 4B242 × Coulter. Erik, Salamouni, and 4B242 are used as toxin-insensitive/fungus-resistant cultivars, and Celtic, Columbus, and Coulter as toxin-sensitive/fungus-susceptible cultivars. BC$_1$ = F_1 plants were backcrossed once to the recessive parent. F_2 = F_1 plants were selfed to produce F_2 seeds.

[c] R = plants resistant to the fungus and insensitive to the toxin; S = plants susceptible to the fungus and sensitive to the toxin.

with the pathogen, in which the development of necrosis and extensive chlorosis resulted from interactions between individual isolates and specific wheat genotypes (11). Furthermore, the results of this study suggest that chlorosis and extensive chlorosis belong to the same system and are characterized by toxin-insensitivity and susceptibility to the chl− isolates. Until the mechanism(s) involved in the development of chlorosis and extensive chlorosis is understood, differentiation between susceptible chlorotic lines and resistant lines can only be achieved by testing with the appropriate isolates of the fungus. The association between toxin sensitivity and induction of tan necrosis by the fungus in the host is supported by several lines of evidence: 1) toxin sensitivity correlated with pathogen-induced necrosis in tests involving 161 cultivars; 2) sensitivity to the toxin and development of necrosis in response to fungal infection were controlled by a single dominant gene, in all crosses made (Table 3); 3) nec+ isolates, which induce necrosis in susceptible cultivars, also produced toxin in vitro (Table 2); 4) nec− isolates, which do not induce necrosis in susceptible cultivars, did not produce toxin in vitro (Table 2); and 5) Col+ lines developed necrosis in response to fungal infection (nec+) and were toxin-sensitive, whereas Col− lines did not develop necrosis (nec−) and were toxin-insensitive (Table 4, Fig. 1). The fact that sensitivity to the toxin is one of the few genetic differences between the two near-isogenic lines Col+ and Col− is significant in that the toxin can distinguish between tan necrosis- and chlorosis-expressing lines even when only slight amounts of chlorosis are induced.

The procedure for culturing the fungus and bioassaying the culture filtrates in the present study was not substantially different from that of Tomás and Bockus (26); however, the levels of toxin produced under our conditions seem to be considerably higher. Their use of a different medium does not in itself explain all the differences in activity observed, although trace elements as well as a considerably higher sugar content in the Fries medium may have enhanced toxin production under our conditions. Differences between the respective isolates of *P. tritici-repentis* used with regard to toxin production may also be a factor, as has been reported for this (26) and other pathogens (21,22). The process of concentrating the toxin under partial vacuum at 45 C used by Tomás and Bockus (26) may have partially inactivated the toxin. In the present study, the toxin produced by *P. tritici-repentis* was shown to be inactivated by autoclaving for 20 min at 121 C.

The ability of toxin-containing culture filtrates to reproduce all the symptoms of tan spot, as was reported by Tomás and Bockus (26), was not confirmed in this study. The symptoms that developed under our conditions consisted mainly of necrotic components. Occasionally, however, at very high dilutions of 1:1,000, symptoms of chlorosis did appear at 30–48 hr after infiltration, but by 4–6 days these subsequently became necrotic. The use, in this study, of cultivars such as 6B365 and 2B13, which support the development of extensive chlorosis, should have revealed the presence of any toxin(s) able to induce the chlorosis.

The toxin produced by *P. tritici-repentis* seems to have a much larger molecular weight than the toxins reported for *P. teres* (21) and other host-specific toxins (15). The dialysis process used in this study suggests a molecular weight larger than 8,000. The toxin produced by some isolates of *P. tritici-repentis* is not required for host penetration. This is supported by the fact that isolate HY331-6 (nec− chl+), avirulent on BH1146, has the ability to penetrate the epidermal cell and to invade the intercellular space in the mesophyll, although its growth is eventually restricted (11). Furthermore, the penetration of the epidermal cell, the formation of vesicles, and the initial invasion of the intercellular space of the mesophyll do not seem to be cultivar- nor isolate-specific processes in tan spot of wheat (11–13). Pathogenesis of *P. tritici-repentis*, which apparently does not penetrate living mesophyll cells (11–13), seems to differ from that of other necrotrophic pathogens. Resistance in these organisms was expressed at the penetration stage (18) or at the time that the fungus moves from the epidermis to the mesophyll (2), when the presence of a host-specific toxin is required for the continuation of the infection process. Additional studies are needed to determine toxin release in host tissues, as toxin production by *P. tritici-repentis* has been demonstrated in vitro only. The characterization of the toxin produced by *P. tritici-repentis* as a virulence or a pathogenicity factor depends on one's definition of *disease* for tan spot of wheat. If disease (or susceptibility) is associated with the presence of tan necrosis and resistance with its absence, then the toxin of *P. tritici-repentis* can be regarded as a pathogenicity factor, sensu Yoder (28). Because of the association of this toxin with tan necrosis, its designation as Ptr-necrosis toxin is proposed. The toxin produced by *P. tritici-repentis* has potential use in breeding and screening

Fig. 1. Reaction of two selections from wheat cultivar Colombus to toxin-producing isolate (ASC1) of *Pyrenophora tritici-repentis*. **A,** Symptoms of chlorosis developed by toxin-insensitive Col− selection. Note the chlorotic halos surrounding the small dark spots. **B,** Symptoms of tan necrosis developed by toxin-sensitive Col+ selection. Note the well-defined lesion borders.

TABLE 4. Reaction of two Columbus wheat selections to toxin-producer and -nonproducer isolates of *Pyrenophora tritici-repentis*[a]

Isolate	Host reaction	
	Col+[b]	Col−
ASC1 (Tox+)[c]	+[d]	−
HY331-6 (Tox−)	−	−

[a]Seedlings were inoculated at the two-leaf stage with a spore suspension adjusted to 3,000 conidia per milliliter and incubated for 24 hr under conditions of continuous leaf wetness.

[b]Col+ and Col− are, respectively, toxin-sensitive and -insensitive Columbus selections.

[c]Tox+ and Tox− are, respectively, toxin-producer and -nonproducer isolates.

[d]+ and − indicate tan necrosis induced and not induced by the fungus, respectively.

wheat for resistance to tan spot. However, its use would be limited to susceptibility expressed by necrosis. Caution would have to be exercised when using the toxin in screening germ plasm for resistance, because fungus-susceptible, chlorotic lines cannot be differentiated from resistant lines. The increasing use of tissue culture techniques in wheat may make the toxin of *P. tritici-repentis* useful in the early selection of resistant cells or protoplasts. Several questions regarding the mechanism(s) involved in the induction of chlorosis and extensive chlorosis in wheat cultivars remain to be resolved before a general model for pathogenesis in tan spot can be formulated.

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