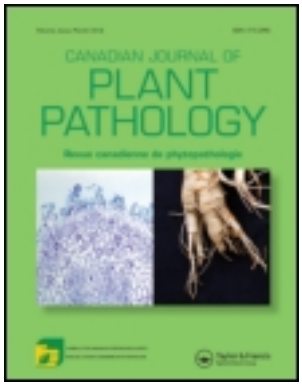

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Virulence of Pyrenophora tritici-repentis in the Southern Cone Region of South America

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Genetics and resistance/Génétique et résistance

Virulence of *Pyrenophora tritici-repentis* in the Southern Cone Region of South America

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Abstract: *Pyrenophora tritici-repentis* induces tan spot, one of the most important fungal diseases of wheat. At least eight races of the pathogen are known to occur based on their virulence on a wheat differential set. In 2009 and 2010, 87 commercial wheat fields were sampled for tan spot in the Southern Cone region of South America, namely Argentina, Brazil and Uruguay. An average of three isolates from each field were tested for their virulence on the differential wheat hosts 'Glenlea', 6B365, 6B662, 'Salamouni', 'Coulter' and 4B1149. Of 273 isolates tested, 52.4% were classified as race 1 and 47.6% were classified as race 2, with no other races identified. This low level of pathogenic diversity was somewhat expected, since the bread wheat grown in this region has a narrow genetic diversity with respect to tan spot resistance. To fully assess virulence patterns in the Southern Cone, use of this differential set is recommended. To our knowledge, this is the first report of the race structure of *P. tritici-repentis* in South America.

Keywords: *Pyrenophora tritici-repentis*, races, South America, tan spot, virulence, wheat

Résumé: *Pyrenophora tritici-repentis* cause la tache helminthosporienne, une des plus importantes maladies fongiques du blé. D'après leur virulence à l'égard d'un groupe de lignées différentielles de blé, au moins huit races de l'agent pathogène sont actuellement connues. En 2009 et 2010, 87 champs de blé ont été échantillonnés pour la tache helminthosporienne dans le cône sud de l'Amérique du Sud, notamment en Argentine, au Brésil et en Uruguay. Trois isolats en moyenne pour chaque champ ont été testés pour leur virulence à l'égard des hôtes différentiels 'Glenlea', '6B365', '6B662', 'Salamouni', 'Coulter' et '4B1149'. Des 273 isolats testés, 52.4 % ont été classifiés dans la race 1 et 47.6 %, dans la race 2, sans que d'autres races soient identifiées. On s'attendait quelque peu à ce faible degré de diversité pathogénique étant donné que le blé panifiable cultivé dans cette région possède une faible diversité génétique quant à la résistance à la tache helminthosporienne. Afin d'évaluer systématiquement les patrons de virulence propres au cône sud, il est recommandé d'utiliser ce groupe différentiel. À notre connaissance, il s'agit de la première mention de la structure de la race de *P. tritici-repentis* en Amérique du Sud.

Mots clés: Amérique du Sud, blé, *Pyrenophora tritici-repentis*, races, tache helminthosporienne, virulence

Introduction

Tan spot, caused by the ascomycete *Pyrenophora tritici-repentis* (Died) (anamorph *Drechslera tritici-repentis*, Died), is one of the most important leaf diseases of wheat worldwide (Rees *et al.*, 1988; Perello *et al.*, 2003; Tekauz *et al.*, 2004; Duveiller *et al.*, 2005). Yield losses up to 50%, reduced kernel weight and a high degree of kernel shrivelling have been reported in severe epidemics (Rees *et al.*, 1988; Cheong *et al.*, 2004). Previous researchers (Kholi *et al.*, 1982; Dubin, 1983; Annone, 1998) indicated that this disease started to become important in the Southern Cone region of South America, Colombia,

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Peru and Ecuador in the early 1980s, causing yield losses of between 20 and 70% in Paraguay and Argentina. In Uruguay, tan spot was first detected in 1982 and identified by Luzzardi *et al*. in 1985. Díaz de Ackermann & Kholi (2003) reported that high levels of the disease have occurred in the northern provinces of Uruguay since 1990. Yield losses of 32% have been estimated, ranging from 3 to 84% between 1998 and 2009, in fields where wheat was grown after wheat (Díaz de Ackermann, 2011).

The increase in tan spot prevalence has been primarily associated with conservation tillage practices, shorter crop rotation, continuous wheat cultivation and the planting of susceptible wheat cultivars (Rees & Platz, 1992; Bailey, 1996; Lamari *et al*., 2005*a*). Changes in cultivar genotypes have also been reported to play an important role in the increasing importance of this disease (Cantrell, 1982). Much of the high susceptibility observed in cultivars released after 1960 is associated with the deployment of semi-dwarf wheat germplasm (Rees & Platz, 1982). Although the genetic basis for resistance to tan spot in the predominant wheat cultivars grown in the Southern Cone has not been fully documented, it appears to have a narrow basis, at least in Uruguay (Díaz de Ackermann, 2011).

Studies on the variation in virulence (da Luz & Hosford, 1980; Schilder & Bergstrom, 1990) based on quantitative approaches were of little contribution in understanding the basis of the *P. tritici-repentis*–wheat interaction. Shah & Fehrmann (1992) did not find physiological races, although significant interactions could be detected between genotypes and isolates from four geographic locations. Lamari & Bernier (1989*b*) grouped isolates of *P. tritici-repentis* into pathotypes based on their ability to induce tan necrosis and extensive chlorosis (nec+, chl+), tan necrosis only (nec+, chl−), extensive chlorosis only (nec−, chl+), or neither symptom (nec−, chl−) on susceptible host genotypes. In 1995, Lamari *et al*. reported a new virulence type in the nec−, chl+ pathotype of *P. tritici-repentis* and proposed the adoption of a race classification system similar to the one used in cereal rusts (Lamari & Sayoud, 1997). This race classification system is based on the virulence of isolates on specific differential wheat genotypes and allows the identification of races based on lesion type (Lamari *et al*., 1995).

To date, eight races of *P. tritici-repentis* have been identified and characterized based on their ability to induce necrosis and/or chlorosis on a differential set composed of four hexaploid wheats, 'Glenlea', 6B365, 6B662 and 'Salamouni', and two tetraploid wheats, 'Coulter' and 4B1149 (Lamari *et al*., 1995, 1998, 2003; Strelkov *et al*., 2002; Strelkov & Lamari, 2003). Symptom development is mediated by the differential production of host-specific toxins by isolates of the fungus, which serve as pathogenicity factors (Strelkov & Lamari, 2003) or virulence factors (Friesen *et al*., 2003) for *P. tritici-repentis*. Three host-specific toxins are known (Lamari & Bernier, 1989*c*; Orolaza *et al*., 1995; Effertz *et al*., 2002). Ptr ToxA is a small, necrosis-inducing protein (Ballance *et al*., 1989; Tomás *et al*., 1990; Tuori *et al*., 1995; Zhang *et al*., 1997) encoded by the *ToxA* gene, which is conserved among all *P. tritici-repentis* isolates characterized to date (Ballance *et al*., 1996; Ciufetti *et al*., 1997; Friesen *et al*., 2006). Ptr ToxB is also a small protein (Strelkov *et al*., 1999), encoded by the *ToxB* gene, which causes chlorosis on sensitive wheat genotypes. Unlike *ToxA*, the *ToxB* gene has been found to be variable among isolates of *P. tritici-repentis* (Strelkov & Lamari, 2003; Martinez *et al*., 2004; Strelkov *et al*., 2006). Another chlorosis-inducing host-specific toxin, termed Ptr ToxC, is also produced by *P. tritici-repentis*. While it remains to be fully characterized, genetic studies suggest that it has an important role as a pathogenicity factor for the tan spot fungus (Lamari & Bernier, 1991; Gamba & Lamari, 1998; Gamba *et al*., 1998). Races of *P. tritici-repentis* are defined by their ability or inability to produce these toxins, with 'basic' races producing only a single toxin (race 2, ToxA; race 3, ToxC; race 5, ToxB) and 'composite' races producing multiple toxins (race 1, ToxA/ToxC; race 6, ToxB/ToxC; race 7, ToxA/ToxB; race 8, ToxA/ToxB/ToxC) (Lamari & Strelkov, 2010). Race 4 isolates produce no active toxins and are avirulent.

Genetic studies demonstrated that only genotypes susceptible to the necrosis and/or chlorosis-inducing isolates of *P. tritici-repentis* are also sensitive to the respective toxins (Ptr ToxA, Ptr ToxB and Ptr ToxC), and that both reactions are controlled by three dominant and independent genes (Lamari & Bernier, 1989*c*; Gamba & Lamari 1998; Gamba *et al*., 1998; Friesen & Faris, 2004). Most of these studies have also shown that sensitivity to the toxins and susceptibility to the producing fungal isolates co-segregate and appear to be controlled by the same genes (Lamari & Bernier, 1991; Gamba & Lamari, 1998). These studies, along with the demonstration that the acquisition of Ptr ToxA-producing ability is a sufficient condition for virulence in *P. tritici-repentis* (Ciufetti *et al*., 1997), suggest that the eight races of the fungus currently described account for all of the virulence phenotypes expected from three toxins matching three respective loci in the host (Lamari *et al*., 2003). Nonetheless, this does not preclude the possibility of the differential production of non-specific toxins by the pathogen and/or uncharacterized susceptibility factors in the host (Friesen *et al*., 2003; Strelkov & Lamari, 2003; Bouras & Strelkov, 2008).

In order to achieve complete and more effective resistance to tan spot, the genetic basis of both components

of the disease syndrome and their relationships need to be understood. The monogenic and recessive nature of the inheritance of resistance to tan spot and the absence of epistatic effects in tetraploid and hexaploid wheats, irrespective of the isolate involved, suggest that this pathosystem follows the toxin or inverse gene-for-gene model of host–parasite interactions, where the specificity of the host–pathogen system is determined by the compatible interaction (*i.e.* susceptibility) (Lamari & Bernier 1991; Orolaza *et al.*, 1995; Gamba & Lamari, 1998; Gamba *et al.*, 1998; Lamari *et al.*, 2003). Moreover, the development of effective and stable genetic resistance requires a good understanding of the nature and extent of variation for virulence in pathogen populations.

The race structure of *P. tritici-repentis* has been studied in several regions worldwide. In North American collections of the pathogen, Lamari *et al.* (1995) first identified races 1 to 4, with races 1 and 2 being prevalent (Lamari *et al.*, 1998). These races were also later identified from the wheat centre of diversity (Lamari *et al.*, 2005*b*). In addition, Lamari *et al.* (1995) first reported race 5 in Algeria, and this race was also later reported in Canada (Strelkov *et al.*, 2002), the United States (Ali *et al.*, 1999), Syria and Azerbaijan (Lamari *et al.*, 2005*b*). In contrast, race 6 has been only reported in North Africa (Strelkov *et al.*, 2002), while races 7 and 8 have been only found in the Middle East and the Caucasus (Lamari *et al.*, 2003, 2005*b*). To our knowledge, however, the race structure of *P. tritici-repentis* in South America has not been examined. Therefore, the goal of this study was to characterize the virulence profiles from an extensive collection of isolates from the Southern Cone region of South America, in order to assess the race structure in this important wheat-growing area.

Materials and methods

Fungal isolates

In 2009, 20 wheat crops were surveyed and sampled for tan spot in Uruguay. A larger survey was conducted in 2010, with 14 wheat crops sampled in Uruguay, 31 crops sampled in Entre Ríos (Argentina), and 22 crops sampled in Rio Grande do Sul (Brazil). Wheat growth stages at the time of surveying ranged from the boot (ZGS 41) to the milk stage (ZGS 71-73) (Zadoks *et al.*, 1974).

In each field, 20–30 leaves were collected, placed in paper envelopes and allowed to air dry at room temperature for approximately 24 h. Leaves with visible lesions were cut into 2–3 cm segments and placed in Petri dishes containing lightly moistened filter paper. In order to induce fungal sporulation, the leaf segments were incubated at 20 °C for 16–20 h under fluorescent light, followed by 16–18 h in the dark at 15 °C as per the protocol of Lamari *et al.* (1995).

From a total of 457 single-spore isolates obtained (representing three to four single-spore isolates per field), 273 were studied for virulence phenotype on the wheat host differential set; the latter included 97 isolates from Argentina, 74 from Brazil and 102 from Uruguay.

Plant material

The differential set consisted of the six wheat genotypes ('Glenlea', 6B365, 6B662, 'Salamouni', 'Coulter' and 4B1149) used by Lamari *et al.* (2003) to previously characterize the eight known races of *P. tritici-repentis*. Five to seven seeds of each genotype were sown in 15 cm-diameter pots filled with 50% peat moss and 50% soil mix and kept in a growth chamber at 21 °C (day) and 18 °C (night) with a 16-h photoperiod.

Inoculation and disease rating

Inoculum was produced on V8–potato dextrose agar following the procedure described by Lamari & Bernier (1989*b*). The conidial suspension was adjusted to 3000 conidia mL^{-1}, with Tween® 20 (polyoxyethylene sorbitol) added to reduce surface tension (1 drop 100 mL^{-1}). Seedlings were inoculated at the two-leaf stage with a sprayer at a constant pressure of approximately 67 kPa. After inoculation, the seedlings were moved to a room with more than 90% relative humidity for 24 h at 21 °C (day) and 18 °C (night) with a 16-h photoperiod, following which they were returned to the previous conditions for 6 to 8 days. The tan spot disease severity was recorded at this time, based on a 1 to 5 scale developed by Lamari & Bernier (1989*a*), where: 1 = small, dark-brown to black spots, without any surrounding chlorosis or tan necrosis (resistant); 2 = small dark-brown to black spots, with very little chlorosis or tan necrosis (moderately resistant); 3 = small, dark-brown to black spots, completely surrounded by a distinct chlorotic or tan necrotic ring, with lesions generally not coalescing (moderately resistant to moderately susceptible); 4 = small, dark-brown to black spots, completely surrounded with chlorotic or tan necrotic zones; with some of the lesions coalescing (moderately susceptible); 5 = most lesions consisting of coalescing chlorotic or tan necrotic tissue (susceptible). A water control treatment was included in every run. A randomized complete block design was used, with two to three replicates (pots) per treatment.

Results and discussion

Reaction types on the six wheat differentials proposed by Lamari *et al.* (2003) corresponded to those exhibited by races 1 and 2 of *P. tritici-repentis* (Table 1). No other races were found. Race 1 isolates caused severe necrosis on the differential 'Glenlea' and extensive chlorosis on 6B365, but were avirulent on 6B662, 'Salamouni', 'Coulter' and 4B1149. Similarly, race 2 isolates also caused severe necrosis on 'Glenlea', but were avirulent on 6B365 as well as on 6B662, 'Salamouni', 'Coulter' and 4B1149.

The proportion of fungal isolates classified as races 1 or 2 in the various countries surveyed is summarized in Table 2. Of the 97 isolates characterized from Argentina, 45 (46.4%) were classified as race 1, while the other 52 (53.6%) were classified as race 2. Among the 74 Brazilian isolates, 39 (52.7%) were classified as race 1 and 35 (47.3%) were classified as race 2. Among the 102 Uruguayan isolates, 59 (57.8%) were classified as race 1 and 43 (42.2%) were classified as race 2. In total among the three countries, 143 (52.4%) of the isolates were classified as race 1, while 130 (47.6%) were classified as race 2 (Table 2). Isolates belonging to each of the two races were always found within the same field.

Table 1. Reaction of six differential wheat genotypes to 273 isolates of *Pyrenophora tritici-repentis* collected in Argentina, Brazil and Uruguay.

	Reaction type [a]	
Host genotype	Race 1	Race 2
'Glenlea'	4-5 (N) [b]	4-5 (N)
6B365	4-5 (C)	1-2
6B662	1	1-2
'Salamouni'	1	1
Coulter	1	1
4B1149	1	1

[a] Plants were rated on a scale of 1–5 based on disease severity, where 1 and 2 represent resistant reactions, and 3–5 represent susceptible reactions (Lamari & Bernier, 1989*a*).
[b] (N) = necrosis; (C) = chlorosis.

Table 2. Race structure of 273 isolates of *Pyrenophora tritici-repentis* collected in Argentina, Brazil and Uruguay.

		Race	
Country	Total number of isolates	1 No. (%)	2 No. (%)
Argentina	97	45 (46.4)	52 (53.6)
Brazil	74	39 (52.7)	35 (47.3)
Uruguay	102	59 (57.8)	43 (42.2)
Total	273	143 (52.4)	130 (47.6)

The narrow virulence spectrum found in *P. tritici-repentis* populations from the Southern Cone region of South America was somewhat expected, since wheat production in Argentina, Brazil and Uruguay relies almost exclusively on bread wheat cultivars of which the genetic resistance is poorly diversified or of unknown origin. This is in contrast to the high amount of diversity in race composition observed in isolates of *P. tritici-repentis* collected from the wheat centre of diversity (Lamari *et al.*, 2005*b*). The races found to be prevalent in South America, races 1 and 2, also represent more than 90% of fungal isolates characterized from North America (Lamari & Strelkov, 2010). Races 1 and 2 are known to produce Ptr ToxA, the necrosis-inducing toxin (Strelkov & Lamari, 2003), sensitivity to which is conferred by the *Tsn1* gene in the wheat host (Friesen *et al*., 2003). The prevalence of races 1 and 2 in South America suggests that the wheat cultivars grown in this region are sensitive to Ptr ToxA, conferring a selective advantage to isolates of *P. tritici-repentis* that can produce this toxin. Furthermore, sensitivity to Ptr ToxA was found to be common in Canadian bread wheat cultivars (Lamari *et al.*, 2005*a*), and may have contributed to the increased prevalence of races 1 and 2 in that country.

The six wheat genotypes ('Glenlea', 6B365, 6B662, 'Salamouni', 'Coulter' and 4B1149) used by Lamari *et al*. (2003) as a differential set in earlier studies are proposed here to evaluate and monitor the race composition of *P. tritici-repentis* in the Southern Cone Region of South America. As noted by Lamari *et al.* (2003), a subset of differentials consisting only of 'Glenlea', 6B365 and 6B662 is in most cases likely to be sufficient to effectively differentiate and characterize all races identified so far. These differentials have been proven to be effective in regions where diversity is higher, and they have been well characterized both genetically and for sensitivity to the toxins. Individual races can now be used to identify gene(s) for resistance in wheat and wheat differential genotypes can be used to identify new races, as reported by Lamari *et al*. (1995b). The use of a common set of differentials, as well as of a universally accepted rating system for the tan spot reaction, also allows for meaningful comparisons among datasets collected by different groups of workers. The ability to easily and objectively characterize races on a qualitative system should be of direct benefit to breeding programmes wanting to develop wheat cultivars resistant to *P. tritici-repentis*.

To achieve complete and effective resistance to *P. tritici-repentis*, resistance to both tan necrosis and chlorosis must be incorporated into newly developed cultivars. From a breeding perspective, the resistance gene(s) identified must be tested against representative isolates of

the tan spot population commonly found in those regions intended for cultivar deployment. Screening procedures should be based on the ability to easily and objectively characterize isolates of *P. tritici-repentis* on a qualitative basis. Failure to correctly identify pathogen races will affect selection efficiency during resistance screening and, eventually, the development of tan spot-resistant cultivars. To our knowledge, this is the first report on the virulence patterns of *P. tritici-repentis* populations from the Southern Cone Region of South America.

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