

## Studies on the host range of *Septoria* species on cereals and some wild grasses in Iran

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**Summary.** In an attempt to determine the host range of *Septoria* species, 27 species/varieties of cereals and certain wild grasses were examined with inoculation experiments under controlled conditions. Most *Septoria* species were each pathogenic only on a particular host plant, and wild grasses played only a minor role as alternative hosts for these fungi. *Septoria tritici* isolates from *Triticum aestivum* infected *T. aestivum*, *T. durum*, *T. dicoccum* and *T. compactum*, species that may provide a primary inoculum source for *S. tritici*. *Septoria* isolates from *Aegilops tauschii*, *Lolium loliaceum*, *Lophochloa phleoides*, *Phalaris paradoxa* and *Hordeum glaucum* were pathogenic only on their original hosts. *S. passerinii* isolates from *Hordeum vulgare* and *H. distichon* were pathogenic on all *Hordeum* species/cultivars tested except *H. glaucum*. Thus various *Hordeum* species may play a role in the epidemiology of *Septoria* diseases on barley.

**Key words:** *Septoria* leaf spot diseases, alternative hosts, pathogenicity test.

### Introduction

Over 3000 published names of species and infra-specific taxa, many of them synonyms (<http://www.indexfungorum.org>), are listed under the genus *Septoria* Sacc.; however, estimates of the true diversity of this genus range from 1000 (Kirk *et al.*, 2001) to 2000 distinct species (Sutton, 1980). It is one of the largest genera of plant-pathogenic fungi, causing a range of disease symptoms including leaf and fruit spot in many agricultural crops (Holliday, 1989; Priest, 2006). Several *Septoria* species cause leaf spot diseases of cultivated cereals throughout the world, and some of these diseases are of considerable economic importance (Sprague, 1950a; Shipton *et al.*, 1971; King *et al.*, 1983; Eyal *et al.*, 1987).

*Septoria tritici* Roberge (teleomorph: *Mycosphaerella graminicola* [Fuckel] J. Schröt.) is a serious leaf-spot pathogen of wheat (*Triticum aestivum* L.) throughout the world (Eyal *et al.*, 1987). It is particularly important in some European countries and North America. This pathogen can cause losses of 30–40% (Eyal *et al.*, 1987); in 1998 in the UK alone, estimated losses were as much as £ 35.5 million (Hardwick *et al.*, 2001). Sprague (1950b) reported that *S. tritici* was a pathogen not only on wheat but on several grasses as well. Weber (1922) obtained isolates of *S. tritici* from *Triticum aestivum*, *Secale cereale* and *Poa pratensis*, each of which was pathogenic on its own host but not on the other cereals or wild grasses tested.

It has been suggested that *S. tritici* overwinters on alternative hosts, particularly on wild grasses (Hilu and Bever, 1957). Eyal (1999) suggested that *Bromus* spp., *Agrostis* spp., *Agropyron* spp., *Brachypodium* spp., *Dactylis* spp., *Festuca* spp., *Glyceria* spp., *Hordeum* spp., *Poa* spp., *Secale cereale* and *Triticum* spp. were alternative hosts for

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overwintering *S. tritici*. However, no concrete evidence has been provided to support this hypothesis.

Leaf blotch caused by *Septoria tritici* is a serious disease of wheat in Iran (Haghdel and Banihashemi, 2005) and under favourable environmental conditions causes considerable yield losses. From an epidemiological perspective, it is important to know whether *Septoria* species found on cereals are pathogenic on other wild grasses and *vice versa*. On the other hand, since the Fertile Crescent is the origin of domestication for many cereals and their associated pathogens (Stukenbrock *et al.*, 2007), a study of the host range of *Septoria* species is relevant to advancing our knowledge of septoria leaf spot diseases and their management.

## Materials and methods

During late May to early July 2004–06, samples of cereals and wild grasses with septorioses on the leaves or stems were collected from wheat fields of the Khuzestan, Ilam, Kermanshah, Fars and Golestan provinces of Iran. The leaf and stem samples were inspected under an Olympus dissecting microscope for any disease symptoms and for the occurrence of pycnidia. Microscopic slides from pycnidia and pycnidiospores were prepared and, based on morphological characteristics such as dimensions of pycnidia, pycnidiospores and number of conidial septa and type of conidiogenous cells, *Septoria* species were distinguished from other fungal species (Saccardo, 1883; Teterevnikova-Babayan, 1987; Priest, 2006).

The infected tissues (including leaves and stems) were surface-disinfected by dipping in a 0.5% sodium hypochlorite solution for 2 min, rinsed 3 times in sterile distilled water, transferred to Petri dishes containing 1.7% water agar (WA) and incubated at 21±2°C under continuous white fluorescent lighting. After 24 h a cirrus oozing out from a single pycnidium was transferred using a sterile needle to a Petri dish containing yeast malt dextrose agar (YMDA: yeast extract 4 g, malt extract [Merck, Darmstadt, Germany] 4 g, dextrose 4 g and agar 15 g, plus 200 ppm streptomycin in 1 L H<sub>2</sub>O). After three days, a subculture was taken from each Petri dish for further study. These *Septoria* cultures (Table 1) were used for host range studies carried out on various cereals and wild grasses (Table 2).

The seeds of the host plants originated from wheat fields in Khuzestan or Golestan, or were obtained from the seed bank of the Weed Research Department, Iranian Research Institute of Plant Protection (IRIPP), Tehran, or from the Genebank Research Department, Seed and Plant Improvement Research Institute (SPIRI), Karaj, Iran. Before the

infection experiments, seeds were grown in the greenhouse to ensure that they were not infected by any seed-borne disease and that their identity was correct. In this test none of the seeds showed any symptoms.

Seeds of the cereals and wild grasses were placed in Petri dishes containing filter paper moistened with 2 mL 10<sup>-4</sup> M KNO<sub>3</sub> in order to break seed dormancy, except for *Hordeum*, *Secale* and *Triticum* species which germinated rapidly in distilled water. The dishes were incubated in a growth chamber with a 16 h day with constant cool-white fluorescent lighting and day/night temperatures of 15 and 5°C. The Petri dishes were checked daily for seed germination. After germination, five seeds at the same stage of development were planted in a 15.5-cm diameter plastic pot (four pots per isolate) filled with a mixture of sterile loam soil, peat and perlite (3:2:1, v:v). The experimental design was completely randomized.

Inoculum of each isolate was prepared as follows. Pycnidiospores were inoculated in a 250 mL flask containing 125 ml yeast malt liquid medium (YM, 4 g yeast extract, 0.5 g malt extract, in 1 L distilled water, plus 0.15 mg kanamycin) and incubated in a shake incubator (SK-300, Lab Companion, Kimpo City, Korea) with 120 rpm at room temperature for 15 days. The conidial suspension was filtered through three layers of cheesecloth and the spore concentration was adjusted to 1×10<sup>7</sup> conidia mL<sup>-1</sup> using a hemocytometer; two drops of Tween 20 were added to 100 mL in each flask for subsequent application.

Seedlings of each pot were inoculated at the 3rd leaf stage with 20 ml of suspension with a hand sprayer; after spraying, the pots were covered with a clear polyethylene bag to increase humidity and prevent cross contamination. The pots were kept for 72 h on a greenhouse bench at 21±2/16±2°C (day/night) with a 16 h day. The bags were then removed and plants were kept in the greenhouse under the same conditions (Weber, 1922; Robert *et al.*, 2005). The plants were visually inspected every day; the appearance of disease symptoms, chlorosis and necrosis, and the latent period (the time between inoculation and the first appearance of a pycnidium producing a cirrus) were recorded. The *Septoria* species were re-isolated from leaf spots on inoculated seedlings, to satisfy Koch's postulates. The infected plant tissues were examined under the microscope and the pycnidia and pycnidiospores were measured to verify the fungal species inoculated.

## Results

Eleven cereal and grass species were found in wheat fields located in five provinces of Iran (Khuzestan, Ilam,

Table 1. *Septoria* species, host plants, locality and date of collection in four provinces of Iran.

Fungal species	Original host	Locality and date of collection
<i>Septoria tritici</i> Roberge ex Desm.	<i>Triticum aestivum</i>	Khuzestan (Dezful), Ilam (Dehloran), and Fars (Mamasani), 2006
<i>Septoria</i> sp. 1	<i>Aegilops tauschii</i>	Golestan (Gorgan, Kafshgiri), 2004
<i>S. passerinii</i> Sacc.	<i>Hordeum vulgare</i>	Kermanshah (Eslamabad, Sarpolezhehab), 2005
<i>S. passerinii</i>	<i>H. distichon</i>	Khuzestan (Dezful), 2005
<i>S. halophila</i> Speg.	<i>H. glaucum</i>	Khuzestan (Dezful), 2006
<i>S. calamagrostidis</i> (Lib.) Sacc.	<i>Avena ludoviciana</i>	Khuzestan (Dezful) and Golestan (Gorgan, Kafshgiri), 2004
<i>S. triseti</i> Speg.	<i>Phalaris minor</i>	Khuzestan (Dezful), 2004, and Ilam (Salehabad), 2006
<i>S. phalaridis</i> Cocc. & Morini	<i>P. paradoxa</i>	Golestan (Gorgan, Kafshgiri), 2004, and Ilam (Dehloran), 2006
<i>Septoria</i> sp. 2	<i>Lolium loliaceum</i>	Fars (Mamasani), 2006, and Khuzestan (Dezful), 2004
<i>Septoria</i> cf. <i>koeleriae</i>	<i>Lophochloa phleoides</i>	Fars (Mamasani) and Khuzestan (Dezful), 2006

Table 2. List of cereals and wild grasses used to determine the host range of *Septoria* species and incubation period in the solutions used for breaking seed dormancy.

Plant species	Locality	Incubation period (days)
<i>Aegilops cylindrica</i>	Gorgan, Kafshgiri	7
<i>A. triuncialis</i>	Karaj, SPIRI <sup>1</sup>	7
<i>A. tauschii</i>	Karaj, SPIRI	7
<i>Avena fatua</i>	Tehran, IRIPP <sup>2</sup>	10
<i>A. ludoviciana</i>	Tehran, IRIPP	10
<i>Bromus tectorum</i>	Tehran, IRIPP	15
<i>B. sterilis</i>	Tehran, IRIPP	15
<i>Echinochloa crus-galli</i>	Tehran, IRIPP	10
<i>Hordeum vulgare</i> cv. Reihan	Tehran, IRIPP	3
<i>H. vulgare</i> cv. Makoie	Tehran, IRIPP	3
<i>H. vulgare</i> cv. Karoon Dar Kavir	Tehran, IRIPP	3
<i>H. distichon</i>	Karaj, SPIRI	6
<i>H. glaucum</i>	Khuzestan, Dezful	6
<i>Lophochloa phleoides</i>	Khuzestan, Dezful	30
<i>Lolium loliaceum</i>	Khuzestan, Dezful	15
<i>Polypogon monspeliensis</i>	Khuzestan, Dezful	10
<i>Poa bulbosa</i>	Tehran, IRIPP	7
<i>Phalaris minor</i>	Tehran, IRIPP	7
<i>P. paradoxa</i>	Karaj, SPIRI	20
<i>Secale cereale</i>	Tehran, IRIPP	4
<i>Sorghum halepense</i>	Tehran, IRIPP	7
<i>Triticum aestivum</i>	Tehran, IRIPP	2
<i>T. durum</i>	Karaj, SPIRI	3
<i>T. dicoccum</i>	Karaj, SPIRI	3
<i>T. compactum</i>	Karaj, SPIRI	3

<sup>1</sup>SPIRI, Seed and Plant Improvement Research Institute.<sup>2</sup>IRIPP, Iranian Research Institute of Plant Protection.

Table 3. Host range, deposit number and morphological characteristics of *Septoria* spp.

Species	Host range	Isolate No.	Accession No.	Conidia		
				Length ( $\mu\text{m}$ )	Width ( $\mu\text{m}$ )	Septa
<i>Septoria calamagrostidis</i>	*	S1	IRAN 1484C	33–65	1–1.5	3–4
<i>S. triseti</i>	*	S2	IRAN 1486C	19–40	1–1.5	0–3
<i>Septoria</i> sp. 1	<i>Aegilops tauschii</i>	S3	IRAN 1489C	18–37	1–1.5	1–3
<i>Septoria</i> sp. 2	<i>Lolium loliaceum</i>	S4	IRAN 1487C	20–46	1–1.5	1–2
<i>S. halophila</i>	<i>Hordeum glaucum</i>	S5	IRAN 1483C	36–45	1.5–2	1 (2)
<i>Septoria</i> cf. <i>koeleriae</i>	<i>Lophochloa phleoides</i>	S6	IRAN 1488C	35–55	1–1.5	0–3
<i>S. tritici</i>	<i>Triticum aestivum</i> , <i>T. durum</i> , <i>T. dicoccum</i> and <i>T. compactum</i>	S7	–	47–80	1.5–2	1–4
<i>S. passerinii</i> <sup>a</sup>	<i>H. vulgare</i> <sup>b</sup> and <i>H. distichon</i>	S8	–	37–57	1.5–2	1–3
<i>S. passerinii</i> <sup>c</sup>	<i>H. vulgare</i> <sup>b</sup> and <i>H. distichon</i>	S9	–	36–55	1.5–2	1–3
<i>S. phalaridis</i>	<i>Phalaris paradoxa</i>	S10	IRAN 1485C	17–40	1.5–2	1–3

\*, The pathogenicity of these isolates was not proved in our experimental conditions.

<sup>a</sup> *S. passerinii* isolated from *H. vulgare*.

<sup>b</sup> *H. vulgare*, cv. Makoie, Karoon Dar Kavir and Reihan.

<sup>c</sup> *S. passerinii* isolated from *H. distichon*.

IRAN, Iranian Fungal Culture Collection (at RZ address).

Kermanshah, Golestan and Fars) with natural *Septoria* infection. Their morphological characteristics are presented in Table 3.

*Septoria tritici* was detected on hexaploid wheat (*Triticum aestivum* L.); it was common in most wheat growing areas of the country. Inoculation experiments of *S. tritici* on 25 cereal and grass species and varieties under controlled conditions showed that this fungus infected *T. aestivum*, *T. durum*, *T. dicoccum* and *T. compactum*, but it did not infect any other species and varieties of the cereals or grasses tested. The disease symptoms were observed on the leaves and leaf sheaths as mid-vein light green to yellow spots after 10 days. Pycnidia appeared after 12–15 days on brown, greyish-brown or yellowish-brown leaf spots; they were immersed, rarely erumpent, dark-brown to black (Figure 1 A–C, M, N, P).

*Septoria* sp. 1 was isolated from *Aegilops tauschii* Cosson. in Golestan; its pycnidia and pycnidiospores were similar to those of *S. tritici*. Its isolates inoculated on 27

cereals and grass taxa only infected its own host species. This *Septoria* sp. was not even pathogenic on other *Aegilops* species such as *A. triuncialis* and *A. cylindrica*. This indicated that the *Septoria* sp. 1 growing on *A. tauschii* was restricted to this host. Therefore, we designate it here as *Septoria* sp. 1.

The initial symptoms of the disease on *A. tauschii* under greenhouse conditions were pale-brown to necrotic lesions after about two months. The disease was more severe on older leaves. On infected leaves, pycnidia were dark-brown, usually solitary, sometimes aggregating (Figure 1J).

*Septoria passerinii* was isolated from *Hordeum vulgare* and *H. distichon* in Kermanshah and Khuzestan provinces respectively. Both isolates had the same host range and were pathogenic on all *Hordeum* species and cultivars tested ('Reihan', 'Makoie' and 'Karoon Dar Kavir'). Initial disease symptoms were pale brown, hologenous, elongate lesions on the upper and lower leaf surfaces and the leaf sheaths of *H. vulgare* and *H. distichon* after 20



days. Pycnidia appeared after one month and were dark-brown and solitary, turning black after another 5 days. This species characteristically causes compound lesions (Figure 1 D, E).

*Septoria halophila* was isolated from *H. glaucum* Steud. and *H. geniculatum* All., in Khuzestan and Ilam provinces respectively. Isolates were pathogenic on *H. glaucum* and *H. geniculatum*, but not on other plants tested. Initial disease symptoms on *H. glaucum* were dark-brown lesions after 12–14 days, which soon became pale buff in the center. After 20 days, the leaves were heavily mottled; pycnidia were mostly solitary, sometimes aggregated. The disease was more severe on the lower leaves (Figure 1 K, L).

*Septoria calamagrostidis* was isolated from *Avena ludoviciana* Durieu in Golestan and Khuzestan provinces. Naturally infected plants showed yellowish to pale-brown lesions with dark-brown to black pycnidia. Despite a number of attempts and four inoculation experiments, inoculated plants did not show any disease symptoms after 60 days. This *Septoria* species may require special environmental conditions to infect its host.

*Septoria triseti* Speg. was collected from *Phalaris minor* Retz. from Khuzestan and Ilam provinces. An inoculation experiment with this fungus was not successful and this will need more investigation.

Another species of *Septoria* was isolated from *Phalaris paradoxa* L. in Khuzestan and Golestan provinces with a morphology somewhat different from *S. triseti* (the conidia of *S. triseti* were 0–4-septate and longer than isolates obtained from *Phalaris paradoxa*, in which conidia were often 1-septate). Therefore, this isolate is identified here as *Septoria phalaridis*. The fungus infected only its own host species. Initial disease symptoms were pale-brown spots on the leaves three weeks after inoculation. Mature pycnidia were seen on infected leaves about one month later; pycnidia and pycnidiospores on tested plants were identical with those collected in the field (Figure 1G, H).

*Septoria* sp. 2 was isolated from *Lolium loliaceum* (Bory & Chaub.) Hand.-Mazz. in Fars and Khuzestan provinces. It infected only its own host plant. Initial symptoms of the disease were pale-brown lesions; brown to black pycnidia appeared 15 days after inoculation (Figure 1I).

*Septoria* cf. *koeleriae* was isolated from *Lophochloa phleoides* (Vill.) Reichenb. on the border of wheat fields in Khuzestan and Fars provinces. It again infected only its own host plant. The disease symptoms were ovate, buff leaf spots with off-white center; typical pycnidia began to appear three weeks after inoculation (Figure 1F).

## Discussion

In this study most *Septoria* species were pathogenic only on their own host plant, showing a very narrow host range. *S. tritici* isolates from bread wheat were pathogenic only on *T. aestivum*, *T. dicoccum*, *T. durum* and *T. compactum*, but not on other cereals and grasses. Weber (1922) examined the host range of *S. tritici* in numerous experiments under field and greenhouse conditions. He found *T. aestivum*, *T. dicoccum*, *T. compactum*, *T. monococcum*, *T. polonicum*, *T. spelta*, *T. turgidum*, *Secale cereale* and *Poa pratensis* to be susceptible to this species. However, when examining more than 4000 leaves of different oats and barley species and varieties under various moisture and temperature regimes at different times of year, he could not reproduce the disease on any of those plants. Pammel *et al.* (1901) and Cooke (1906) reported the disease in the United States on wheat and certain wild grasses. Sprague (1950a) reported natural infection of *S. tritici* on rye and Derevyankin (1969) observed a slight infection on rye and *Alopecurus pratensis* after artificial inoculation with *S. tritici*. This researcher concluded that *S. tritici* is a highly specialized pathogen of wheat, which infects other species only slightly and only under artificial conditions. Mäkelä (1975) studied the host range of *S. tritici* with the same inoculation method as was used in this study and found that isolates from winter wheat infected its own host and also spring wheat and rye.

Beach (1919) also reported *S. tritici* only on wheat species and certain grasses, but not on rye, oat or barley. However, Haghdel and Banihashemi (2005) succeeded in infecting *Lolium rigidum* and *Secale cereale* with *S. tritici* under greenhouse conditions. These conflicting findings on the infectivity of *S. tritici* on rye could be related to environmental conditions, which in our study may not have been suitable for the establishment of *S. tritici* on rye.

Isolates of *Septoria* sp. 1 from *Aegilops tauschii* only infected its own host species but not *A. triuncialis* or *A. cylindrica*. On the other hand, isolates of *S. tritici* from wheat did not infect *A. tauschii*.

*Septoria passerinii* was isolated from *Hordeum vulgare* and *H. distichon*. Isolates only infected species and varieties of the genus *Hordeum*, but no other cereal or grass species. This finding is in agreement with Weber (1923), who reported that *S. passerinii* isolates were pathogenic only on species and varieties of *Hordeum*. Shearer *et al.* (1977) similarly reported *S. passerinii* isolates infecting only cultivated and wild barleys. *S. passerinii* isolates from *H. vulgare* infected all cultivated *Hordeum* spp., but no other *hordeum* wild species except *H. brachyantherum*, and this only at a low level. Of the cultivated species, *H. distichon* and *H. vulgare* were the most susceptible and *H.*

*vulgare* var. *pallidum* was the most resistant to *S. passerinii* (Shearer et al., 1977).

Wild grasses that act as the source of primary inoculum of a crop pathogen should be widely distributed in the region and the host crop should be susceptible to the same pathogen (Shearer et al., 1977). In the present study, *S. passerinii* from *H. distichon* infected all *Hordeum* species. This finding suggests that *H. distichon* acts as an alternative host providing primary inoculum of *S. passerinii* for the infection of cultivated barley. Currently, *S. passerinii* is not regarded as a major pathogen of barley in Iran; however, if environmental conditions become suitable for development of the disease, *H. distichon* may become important in epidemics of *S. passerinii* on barley in the region. *Septoria halophila* from *H. glaucum* was pathogenic only on *H. glaucum*, not on the other *Hordeum* species and wild grasses tested. *H. glaucum* is an abundant weed in most cereal-growing areas of Iran (Minbashi Moeini, 2007); because of its high specialization *S. halophila* does not seem to play a role as a source of *Septoria* diseases in cultivated barley.

The *Septoria* species from *Lolium loliaceum*, *Lophochloa phleoides* and *Phalaris paradoxa* were also pathogenic only on their own host plants. They were thus highly specialized and did not play a role in septoria leaf spot on cereals.

Of the species studied, *S. tritici* was pathogenic on different species of *Triticum* and *S. passerinii* on species and varieties of *Hordeum* but not on other cereals and grasses. *Triticum* species are widely cultivated in Iran and are likely to provide primary inoculum of septoria leaf blotch of wheat; this possibility needs more investigation. Similarly, species and varieties of *Hordeum* provide sources of primary inoculum of septoria leaf blotch of barley, but they do not affect other cereals and wild grasses.

On certain wild grasses, several *Septoria* species may cause similar diseases, although these species are morphologically and physiologically distinct. But most *Septoria* species are pathogenic only on their own host. Thus wild grasses are unlikely to play a significant role as alternative hosts for leaf spot diseases of crops. However, related *Septoria* spp. may hybridize and expand their host ranges to cereal crops and thus potentially pose a new threat to crops in the future. This is evidenced in mycosphaerella leaf spot disease in banana, which is caused by a complex of at least three species. Close co-occurrence of multiple species of *Mycosphaerella* both in time and space on a single host can lead to close physical interactions and potential exchange of genetic material through inter-species mating, hybridization or anastomosis. This could ultimately result in development

of new species with altered virulence patterns or expand the host range (Conde-Ferraz et al., 2007).

In the present study, experiments were carried out under glasshouse conditions using a limited number of genotypes of wild grasses, which may not represent the real host range. In further studies different genotypes of grass species should be included for examination under both artificial and natural conditions.

Here the plants were kept in the glasshouse for about 4 months to allow *Septoria* species with a longer incubation period to develop pycnidia; however, *S. calamagrostidis* and *S. triseti* did not produce any disease symptoms on their original host plants *A. ludoviciana* and *P. minor* respectively. The conditions enabling infection seem quite complex for hemibiotrophic fungi such as *Septoria*. Shearer and Zadoks (1972) found that latent periods of *S. tritici* changed with temperature and moisture. Recently, Haghdel and Banihashemi (2005) showed that *S. tritici* could be pathogenic on *Secale cereale* and *Lolium rigidum* under certain artificial conditions, but they failed to establish the disease under natural field conditions. This emphasizes the importance of environmental conditions in disease establishment. The role of environmental parameters in the pathogenicity of *Septoria* species on wild grasses and cereal crops requires further study.

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