The Suppression of Pycnidal Production on Wheat Seedlings Following Sequential Inoculation by Isolates of Septoria tritici

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ABSTRACT


Cross-inoculation of two wheat cultivars with two isolates of Septoria tritici was studied. Inoculation of seedlings of the wheat cultivar Seri 82 with the avirulent S. tritici isolate ISR398 followed at 2, 5, or 10 days later by inoculation with the virulent isolate ISR8036 resulted in marked reductions in pycnidal coverage. Significant reductions were also recorded on 'Shafir', which is susceptible to both isolates. Inconsistent reductions resulted from inoculating 'Shafir' with the virulent isolates first, followed by the avirulent isolate. No reductions were observed when the culture filtrates were used instead of conidia of the first isolate in the inoculation sequence. A sevenfold increase of conidia of the avirulent isolate (ISR398) resulted in a marked suppression of pycnidal coverage compared with a 1:1 ratio between the isolates. Subisolates produced by reisolation from pycnidia of 'Seri 82', which was inoculated first with ISR398 and then with ISR8036 (ISR398(I)/ISR8036(II)), were ISR8036-like as verified by virulence on 'Seri 82' and by probing with the S. tritici minisatellite DNA probe ST398-3.7A. The majority of the subisolates resulting from the reversed order of inoculation (ISR8036(II)/ISR398(I)) on 'Seri 82' were ISR8036-like. The induced seedling resistance of 'Seri 82' to the virulent isolate may be associated with mechanism(s) triggering pycnidal production. The suppression of pycnidal production in the susceptible cultivar Shafir can be explained in part by endogenous competition between the two isolates during colonization of wheat leaf tissue.

Additional keywords: induced resistance, Mycosphaerella graminicola, Triticum aestivum.

Septoria tritici blotch of wheat incited by Mycosphaerella graminicola (Fuckel) J. Schrötl in Cohn (anamorph Septoria tritici Roberge in Desmaz.) can attain epidemic proportions and cause significant reductions in yield (11,17). Breeding for host resistance is considered the main defense against this disease and several sources of resistance have yielded adequate protection (5). Resistant germ plasm is not abundant and is often associated with undesirable late maturity and excessive plant height (1,11). Differentiation of host response to the pathogen is based on quantative assessment of pycnidal coverage, separately or in combination with percent necrosis (7,15).

Several reports have indicated that physiologic specialization exists in S. tritici (9,10,22,23). Deviations from the expected host response, following inoculation with specific isolates, were reported upon inoculation with a mixture of isolates (6,24) and in challenge-inoculation trials (24). In the challenge-inoculation trials, the first isolate (avirulent or virulent) was followed several days later with another isolate (virulent or avirulent). Inoculation of wheat seedlings and adult plants in the field with isolate mixtures resulted in significant suppression of pycnidal coverage as compared with inoculation results with the most virulent single isolate in the mixture. Symptom suppression on seedlings of the susceptible cultivars used in Zelikovitch and Eyal's study (24) was neither isolate- nor cultivar-specific. The suppression of pycnidal coverage from the expected results was attributed to the production of substance(s) by S. tritici that can regulate fungal development in the host tissue. Eyal (6) suggested that the suppression of symptoms under field conditions may be indicative of differential aggressiveness of isolates, regardless of their virulence. A 74.6% suppression of pycnidal coverage was observed on seedlings of the winter wheat 'Kvakaz' inoculated first with the avirulent isolate ISR398 and followed 7 days later with the virulent isolate ISR8036 (24). Reversing the order of inoculation, i.e., the virulent isolate followed by the avirulent isolate, resulted in a 78.5% reduction in pycnidal coverage when compared with coverage of plants inoculated separately with isolate ISR8036. The authors attributed the suppression of symptoms to an antagonistic product of S. tritici and to a differential production/sensitivity/competition mechanism, but they did not rule out an isolate-cultivar interaction response that is triggered by the first inoculation.

The objective of this study was to test the hypothesis that the reduction in pycnidal coverage following challenge-inoculation using isolates of S. tritici differing in their specific virulences can be attributed to the induction of resistance or to competition or both.

MATERIALS AND METHODS

Cultures. Culturing of isolates ISR398 (ATCC 48507) and ISR8036 followed that of Zelikovitch and Eyal (24). Both isolates are virulent on 'Shafir'; whereas ISR398 is avirulent and ISR8036 is virulent on 'Kvakaz' (Lutescens 314 H 147/Bezostaya 1) and 'Seri 82'.

Wheat cultivars and inoculation. Ten-day-old seedlings (two-leaf stage) of the wheat cultivars Seri 82 (Kvakaz/BUHO'S//KAL/BB, CM33027-F-5M-500Y-0M, VERRY45) (CIMMYT, Mexico, D.F., Mexico) and Shafir (Sonora 64/Tezanos Pinto Precizo/Naínari 603/Florencio Aurore) (Hazaer Seed Co., Mivhor, M.P. Sde Gai, Israel) were inoculated following procedures used by Zelikovitch and Eyal (24).

Plants were inoculated with the first isolate (or treated with H2O), moved into the humidity chamber for 48 h, dried, reinocu-
lated with the second isolate (or with H₂O), and moved again to the humidity chamber for 48 h. Plants were removed to a bench in the same growth chamber and illuminated for a 12-h light period using cool white fluorescent light at 150 μmol·m⁻²·s⁻¹ with a constant temperature of 18°C. Severity (estimation of percentage of pycnidial coverage) was visually assessed 21 days after inoculation with the aid of standard drawings (8).

Sequential inoculation. The time periods between the first and second inoculations were 2, 5, or 10 days. In the control treatment, conidia for the first or second inoculation were replaced by H₂O (i.e., H₂O/I/ISR398 [II], ISR398/I/H₂O [II], in which I denotes the first and II the second inoculation). A second set of control treatments within a trial consisted of wheat seedlings inoculated with the same isolate in the first and second inoculations.

In one trial, the conidial preparation of the first inoculation was replaced by a culture filtrate of either ISR398 or ISR8036 and followed 5 days later with conidia of the other isolate. The effect of spore concentration on the suppression of symptoms was measured in another trial by increasing sevenfold (7 × 10⁷ spores/ml) the concentration of either the first or the second isolate in the inoculation order.

The suppression of pycnidial production was calculated from the treatments inoculated with viable spores or culture filtrate from only ISR8036, namely H₂O/I/ISR8036 [II], ISR8036/I/H₂O [II], and ISR8036/I/ISR8036 [II]. Significance values between treatments were obtained using an analysis of variance (ANOVA).

Pathogenicity of subsolates. The identity of the resulting pycnidia in the challenge-inoculation treatments was verified by pathogenicity tests and DNA fingerprinting. Subisolates were from 51 pycnidia on infected leaves of 'Shafir' and 'Seri 82'. Subcultures were used to reinoculate seedlings of 'Seri 82' to differentiate between the avirulent isolate (ISR398) and the virulent isolate (ISR8036).

Fungal DNA analysis. Total DNA was extracted from the same subsolates using the method described by Linde et al. (14) with modifications. Conidia of S. tritici were grown in liquid sucrose + yeast extract medium for 10 days at 18°C. The conidia were separated by centrifugation at 1,200 × g for 10 min. The conidia were then ground in liquid nitrogen and resuspended in 5 ml of extraction buffer (200 mM Tris·HCl [pH 8.5], 5 mM EDTA, 250 mM NaCl, 0.5% sodium dodecyl sulfate [SDS]). The suspension was deproteinized by two phenol extractions. The DNA was precipitated by adding an equal volume of isopropanol, stored overnight at 4°C, and then centrifuged at 12,000 × g for 20 min. The pellet was washed in 70% EtOH, dried, and resuspended in Tris·EDTA (10 mM Tris·HCl, 1 mM EDTA [pH 8]). The preparation yield was 20 to 50 mg of DNA/100 ml of spore culture. The DNA was further purified by Rnase A treatment (100 mg/ml) at 37°C for 2 h, followed by phenol extraction and ethanol precipitation. The DNA was digested with PstI and then separated on 0.8% agarose gels in Tris-acetate-EDTA (40 mM Tris·HCl, 10 mM Na₂Ac, 1 mM Na₂EDTA [pH 8]) or Tris-borate-EDTA (90 mM Tris·HCl, 90 mM boric acid, 2 mM EDTA [pH 8]) buffers and transferred to Hybond-N° membranes (Amersham International, Amersham, United Kingdom) following alkaline denaturation (19).

Hybridization with the ST398-3.7A probe. The ST398-3.7A genomic fragment (3.7 kb) that was isolated from ISR398 of S. tritici (16) was used as a probe. This fragment contained a 0.4-kb repetitive sequence with minisatellite characteristics (12,16,26). The ST398-3.7A fragment was labeled by random priming (19). Hybridization was performed overnight at 65°C in 0.263 M Na₂HPO₄, 7% SDS, 1 mM EDTA, and 1% bovine serum albumin. Washes were performed at 65°C in 0.263 M Na₂HPO₄ and 1% SDS for 20 min, 2x SSC (1x SSC is 0.15 M NaCl, 0.015 M sodium citrate [pH 7]) and 0.1% SDS for 20 min, and twice with 1x SSC and 0.1% SDS for 20 min. The membranes were then autoradiographed using Agfa RP2 film (Agfa-Gevaert, Mortsel, Belgium).

RESULTS

Time periods in sequential inoculations. The effect of inoculation combinations and time periods between the first and second inoculation on pycnidial coverage is presented in Table 1. The results are expressed in percent reduction in pycnidial coverage from the control treatments ISR8036/I/H₂O [II] and H₂O/I/ISR8036 [II]. The actual pycnidial coverages recorded on seedlings of 'Seri 82' and 'Shafir' inoculated with the combinations ISR398/I/ISR8036 [II] and ISR8036/I/ISR398 [II], and compared with the control treatments ISR8036/I/ISR8036 [II], H₂O/I/ISR8036 [II], and ISR8036/I/H₂O [II], were each used in an ANOVA.

Significant reductions in pycnidial coverage (P < 0.05) ranging from 72.3 (2-day periods), 87.2 (10-day periods), and 90.2% (5-day periods) were recorded on 'Seri 82' inoculated first with ISR8036 followed with ISR8036. Significant reductions were also recorded on the susceptible cultivar Shafir inoculated with the same isolate combination at 2- and 5- but not at 10-day periods.

Reductions in pycnidial coverage of 42 and 44% were recorded on 'Seri 82' inoculated first with the virulent isolate ISR8036 and followed 2 or 5 days later with the avirulent isolate ISR398. Inconsistent, statistically nonsignificant reductions or enhancement in pycnidial coverage were recorded on 'Shafir' inoculated with the same order of isolates.

The replacement of the conidial preparations of the first isolate with culture supernatant did not result in any significant reduction in pycnidial coverage on either cultivar when sequentially inoculated 5 days later with conidia of the second isolate (Table 2).

**Table 1.** The suppression of pycnidial coverage following sequential inoculation of seedings of the wheat cultivars 'Seri 82' and Shafir by Septoria tritici isolates.

<table>
<thead>
<tr>
<th>Period (days)</th>
<th>Number of trials</th>
<th>ISR398/I/ISR8036 [II]</th>
<th>ISR398/I/ISR8036 [II]</th>
<th>ISR8036/I/ISR8036 [II]</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>2</td>
<td>72.3**</td>
<td>38.1*</td>
<td>43.8*</td>
</tr>
<tr>
<td>5</td>
<td>4</td>
<td>90.2**</td>
<td>51.2*</td>
<td>41.6*</td>
</tr>
<tr>
<td>10</td>
<td>2</td>
<td>87.2**</td>
<td>17.0</td>
<td>-8.4</td>
</tr>
</tbody>
</table>

* Isolate ISR398 (avirulent on 'Seri 82' and virulent on 'Shafir') was used in the first (I) inoculation and then isolate ISR8036 (virulent on 'Seri 82' and 'Shafir') was used in the challenge-inoculation (II).

**Table 2.** The effect of culture supernatant of the first isolate on pycnidial coverage in sequential inoculation.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>'Seri 82'</th>
<th>'Shafir'</th>
</tr>
</thead>
<tbody>
<tr>
<td>ISR8036/I/ISR8036 [II]</td>
<td>63.6**</td>
<td>62.9*</td>
</tr>
<tr>
<td>Sup ISR398/I/ISR8036 [II]</td>
<td>-9.3</td>
<td>-10.8</td>
</tr>
<tr>
<td>ISR8036/I/ISR398 [II]</td>
<td>23.6</td>
<td>6.4</td>
</tr>
<tr>
<td>Sup ISR8036/I/ISR398 [II]</td>
<td>0.0</td>
<td>1.3</td>
</tr>
</tbody>
</table>

* Isolate ISR398 was used in the first (I) inoculation followed by the use of isolate ISR8036 in the second (II) inoculation.
** Significant at P < 0.05.

* Culture supernatant (Sup) replaced conidia of isolate ISR398 in the first (I) inoculation followed by conidia of isolate ISR8036 in the second (II) inoculation.
Spore concentration. The suppression of pycnidial coverage on 'Seri 82' was increased to over 90% when spore concentration of ISR398, used as the first isolate, was elevated to 7 x 10^{10} spores/ml followed 5 days later by isolate ISR306 at a spore concentration of 1 x 10^{10} spores/ml (7:1) (Table 3). Significant reductions in pycnidial coverage were recorded on 'Shafir' inoculated first with ISR398 and followed 5 days later with ISR306 at all three spore concentration ratios (1:1, 7:1, and 1:7). The reversal of the isolate order (ISR306/ISR398 or ISR398/ISR306) in a 7:1 spore concentration ratio did not result in a reduction in pycnidial coverage as compared with the significant reduction of 53 to 78% in a 1:1 spore concentration.

Identity of subsolates. Subsolates were isolated from pycnidia that developed on seedling leaves of 'Seri 82' and 'Shafir' following inoculations with the two wild-type isolates in the combinations ISR398 (I) / ISR306 (II) and ISR306 (I) / ISR398 (II) at periods of 2 and 5 days between the two inoculations. In the inoculation order ISR398 (I) / ISR306 (II), 48% of the subsolates were ISR306-like at the 2-day period, whereas 56% of the subsolates resembled the first isolate (ISR398) in the inoculation order at the 5-day period. The subsolates were categorized according to their distinct DNA hybridization pattern in Southern blots, after digestion of the total DNA with PsfI and probing with the minisatellite S. tritici probe ST398-3.7A. Subsolates of ISR398 could be identified by a typical band of 3.7 kb, whereas subsolates of ISR306 were distinguished by a 4.8-kb band (Fig. 1). DNA fingerprints obtained from conidial cultures of subsolates from 'Seri 82' and 'Shafir' following inoculation with ISR306 (I) / ISR398 (II) and ISR306 (II) / ISR398 (I) combinations at 2- or 5-day periods clearly identified subsolate origin. In the inoculation order ISR398 (I) / ISR306 (II) on 'Seri 82', 23/23 (100%) subsolates were identified as ISR306 (virulent on 'Seri 82') at the 2-day period and 21/28 (75%) at the 5-day period (Table 4). On the susceptible cultivar 'Seri 82' and the susceptible cultivar 'Shafir' to the two isolates was consistent throughout this study whenever a single isolate was used in the inoculation. The results when the supernatant replaced spores of the first isolate were consistent with the findings of Zeleniovich and Eyal (24) in which no significant suppression was recorded on seedlings of cultivars Cencon and Shafir inoculated with a centrifuged culture filtrate of ISR398 mixed with conidia of ISR306. However, 1:1, 8:2, and 2:8 conidial mixtures of ISR398 and ISR306 in that study resulted in significant reductions in pycnidial coverage on cultivars Cencon and Shafir. Mycelial growth of these two isolates was partly reduced when grown in strips adjacent to each other on wheat leaf agar. The authors attributed the suppression in growth in vitro to production in the culture medium of indole-3-carboxylic acid (ICA,Me), which acts as a growth inhibitor (25). Ride (18) suggested that the delay observed in lesion development in wheat inoculated with Botrytis cinerea (nonpathogen) and challenged-inoculated with Stagonospora nodorum (wheat pathogen) resulted from direct competition or inhibition between the fungal species.

It is possible that suppression processes were initiated in the leaves of inoculated 'Seri 82' and 'Shafir' in response to signals associated with pycnidia formation (3,13,20). This hypothesis was supported by the findings that suppression of pycnidial production on 'Seri 82', and partly on 'Shafir', was enhanced by increasing conidial concentration (×7) and to a lesser degree by

**DISCUSSION**

The two S. tritici isolates used in the present study (ISR398 and ISR306) were selected on the basis of differential interaction on 'Kavkaz' and 'Seri 82'. The specific interaction recorded in the present study was consistent with that reported in previous studies (5,24) in which isolate ISR398 produced low pycnidial coverage (<5%) on 'Seri 82' or on its parent 'Kavkaz', isolate ISR306 produced pycnidial coverage (>20%) on both cultivars, and both isolates produced dense pycnidial coverage on 'Shafir'. The response of the resistant cultivar 'Seri 82' and the susceptible cultivar 'Shafir' to the two isolates was consistent throughout this study whenever a single isolate was used in the inoculation. The results when the supernatant replaced spores of the first isolate were consistent with the findings of Zeleniovich and Eyal (24) in which no significant suppression was recorded on seedlings of cultivars Cencon and Shafir inoculated with a centrifuged culture filtrate of ISR398 mixed with conidia of ISR306. However, 1:1, 8:2, and 2:8 conidial mixtures of ISR398 and ISR306 in that study resulted in significant reductions in pycnidial coverage on cultivars Cencon and Shafir. Mycelial growth of these two isolates was partly reduced when grown in strips adjacent to each other on wheat leaf agar. The authors attributed the suppression in growth in vitro to production in the culture medium of indole-3-carboxylic acid (ICA,Me), which acts as a growth inhibitor (25). Ride (18) suggested that the delay observed in lesion development in wheat inoculated with Botrytis cinerea (nonpathogen) and challenged-inoculated with Stagonospora nodorum (wheat pathogen) resulted from direct competition or inhibition between the fungal species.

**TABLE 3. The effect of pycnidiospore concentration on percent pycnidial coverage on wheat seedlings sequentially inoculated at 5-days periods with alternating isolates of Septoria tritici**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>'Seri 82'</th>
<th>'Shafir'</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Ratio</td>
<td>Pycnidial coverage (%)</td>
</tr>
<tr>
<td>ISR398 / ISR306</td>
<td>1:1*</td>
<td>4.8 ab</td>
</tr>
<tr>
<td>ISR398 / ISR306</td>
<td>7:1*</td>
<td>1.1 a</td>
</tr>
<tr>
<td>ISR306 / ISR398</td>
<td>1:7</td>
<td>7.2 bc</td>
</tr>
<tr>
<td>ISR306 / ISR398</td>
<td>1:1</td>
<td>6.9 bc</td>
</tr>
<tr>
<td>ISR306 / ISR398</td>
<td>7:1</td>
<td>22.2 d</td>
</tr>
<tr>
<td>ISR306 / ISR398</td>
<td>1:7</td>
<td>12.5 c</td>
</tr>
</tbody>
</table>

* Within columns, means followed by the same letter are not significantly different at P < 0.05.
* Percent reductions were calculated from the control treatments ISR306(I)/H_{2}O(I) and H_{2}O(I)/ISR306(II).
* 1:1 = 30 ml of spore suspension/entry inoculation at 1 x 10^{10} spores/ml.
* 7:1: 7:1 = first or second inoculation at 7 x 10^{10} spores/ml; second or first at 1 x 10^{10} spores/ml.
* ** Significant at P < 0.05.
* 7:1:1:7 = first or second inoculation at 7 x 10^{10} spores/ml; second or first at 1 x 10^{10} spores/ml.
* *** Significant at P < 0.01.
decreasing conidial concentration of ISR398 when used first or second in the inoculation order. Suppression of pycnidial coverage, especially on 'Seri 82' inoculated first with the avirulent isolate ISR398 and then with the virulent isolate ISR8036, can be explained by resistance induced in wheat seedlings by the avirulent isolate. Induction of resistance in barley against powdery mildew (Erysiphe graminis f. sp. hordei) was reported to be promoted both by virulent and avirulent races (2). Thordal-Christensen and Smedegard-Petersen (21) reported that the ability to induce resistance in barley was similar for both virulent and avirulent races of powdery mildew for up to 10 to 12 h of induction, and was increased significantly thereafter for the avirulent race. The suppression of pycnidial production on 'Seri 82' by the reverse order of inoculation (virulent/avirulent) may be explained in part by the same cross-protection mechanism in which a dosage effect is operative, or in combination with a suggested competition between the two isolates within the wheat leaf tissue (24). Suppression of pycnidial coverage in the susceptible cultivar may be governed by competition between the two colonizing isolates in the intercellular spaces prior to pycnidial production.

The distinction between the two suggested mechanisms can be indirectly assessed by the identification of the resulting pycnidia based on distinct markers. Pathogenicity on 'Seri 82' and typical DNA fingerprinting can readily identify the two isolates. It was expected that in the combination ISR398(I)/ISR8036(II) on 'Seri 82' the majority of the subs isolates would resemble the virulent isolate ISR8036. The findings confirmed that assumption, in which 100 and 84% of the subs isolates were ISR8036-like at 2- and 5-days periods, respectively. Whereas on the susceptible cultivar Shafir, the ratio of pycnidia produced by the two isolates would be close to 1:1 or skewed towards isolates in the first inoculation, especially in longer inoculation periods. It was expected that the majority of the subs isolates resulting from the reversal of the inoculation order (ISR8036(I)/ISR398(II)) on 'Seri 82' should be ISR8036-like either because of a defense mechanism induced by the avirulent challenger that is incapable of producing pycnidia or because of competition. DNA fingerprinting of the resulting pycnidia on 'Seri 82' and 'Shafir' revealed dominance of the virulent isolate ISR8036 in the population that may be indicative of rapid colonization (fitness) by this isolate. There was a strong indication that cross-protection and competition were operating singly, and in certain cases in combination, that resulted in suppression of pycnidial production. Cohen and Eyal (3) have shown that in the resistant cultivar Kavakaz/K4500, L6A.4 inoculated with the avirulent isolate ISR398 an autofluorescence developed at the penetration site within 24 h after inoculation. Moreover, the authors reported that mycelium could be detected in this cultivar in the intercellular spaces between mesophyll cells; necrosis developed but no pycnidia were produced. They suggested that the high level of resistance in this cultivar is because of a mechanism suppressing the formation and maturation of pycnidia. The results obtained in the present work were supportive of this hypothesis. Methods are being developed to distinguish between the two isolates within the leaf tissue and quantitatively assess the amount of colonized mycelium of each isolate in the inoculation combination. It is likely that in certain pathogen × host interactions colonization is halted past penetration (4), but such a mechanism would not exclude the development of pycnidia by the challenger isolate, and it would not explain suppression in which the challenged isolate is virulent on 'Seri 82'.

The composition of a population of S. tritici isolates on a wheat leaf is strongly affected by virulence, their fitness, the interaction between cultivar and isolates, and interaction between isolates (13,20). It was shown that under environmental conditions conducive for a Septoria tritici blotch epidemic, the suppressive effect of isolate mixtures was almost negligible (6). The conditions under which cross-protection is still effective needs further elucidation in terms of isolate range, systemic protection beyond seedling, effect of plant growth stage and age, environmental conditions, and so on. It is not clear whether the suppression of pycnidial coverage exhibited upon inoculation with a mixture of S. tritici isolates or upon sequential inoculations with different isolates are governed by the same or different mechanisms(s). Since the two phenomena differ from one another in the length of time elapsed between tandem inoculations, with a minimum effective interval of 48 h separating the first and the second inoculation, it is possible that the suppression manifested by inoculation with isolate mixture and sequential inoculation are governed as suggested by cross-protection and competition.

![Fig. 1. Southern blot analysis of DNA from conidial preparations of subs isolates of Septoria tritici isolated from pycnidia on seedlings of the wheat cultivar Shafir inoculated first with isolate ISR8036 and followed 5 days later with isolate ISR398. DNA was digested with PstI and hybridized with the S. tritici minisatellite DNA probe ST398-3.7A. Lanes A through E correspond to single pycnidial subs isolates from leaves inoculated with ISR398 and ISR8036. A 3.7-kb band is typical of S. tritici isolate ISR398, and a 4.8-kb band is characteristic of isolate ISR8036.](image)

<table>
<thead>
<tr>
<th>Source of subs isolate</th>
<th>Period (days)</th>
<th>ISR398</th>
<th>ISR8036</th>
<th>Sum</th>
<th>ISR8036(I)/ISR398(II)</th>
<th>ISR8036(II)/ISR398(I)</th>
</tr>
</thead>
<tbody>
<tr>
<td>'Seri 82'</td>
<td>2</td>
<td>0</td>
<td>23</td>
<td>23</td>
<td>17</td>
<td>3</td>
</tr>
<tr>
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<td>5</td>
<td>7</td>
<td>21</td>
<td>28</td>
<td>23</td>
<td>1</td>
</tr>
<tr>
<td>'Shafir'</td>
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<td>11</td>
<td>23</td>
<td>17</td>
<td>0</td>
</tr>
<tr>
<td>'Shafir'</td>
<td>5</td>
<td>14</td>
<td>11</td>
<td>25</td>
<td>25</td>
<td>6</td>
</tr>
</tbody>
</table>

a Isolate ISR398 was used in the first (I) inoculation followed 2 or 5 days later with isolate ISR8036 in the second (II) inoculation.

b Isolate ISR8036 was used in the first (I) inoculation followed 2 or 5 days later with isolate ISR398 in the second (II) inoculation.

c Values derived from the identification of subs isolates according to specific bands by Southern blot of PstI-digested DNA extracted from cultures obtained from pycnidia and hybridized with probe ST398-3.7A.

d Total number of subs isolates.

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LITERATURE CITED


