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

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ABSTRACT

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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 pycnidial 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 series. A sevenfold increase of conidia of the avirulent isolate (ISR398) resulted in a marked suppression of pycnidial 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(I)/ISR398(II)) on 'Seri 82' were ISR8036-like. The induced seedling resistance of 'Seri 82' to the virulent isolate may be associated with mechanism(s) triggering pycnidial production. The suppression of pycnidial 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. Schröt 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 quantitative assessment of pycnidial 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 pycnidial 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 pycnidial 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 pycnidial coverage was observed on seedlings of the winter wheat 'Kavkaz' 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 78.3% reduction in pycnidial 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 pycnidial 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.

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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 'Kavkaz' (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 (Kavkaz/BUHO"S"// KAL/BB, CM33027-F-5M-500Y-0M, VEERY#5) (CIMMYT, Mexico, D.F., Mexico) and Shafir (Sonora 64/Tezanos Pinto Precoz//Nainari 60/3/Florence Aurore) (Hazera Seed Co., Mivhor, M.P. Sde Gat, Israel) were inoculated following procedures used by Zelikovitch and Eyal (24).

Plants were inoculated with the first isolate (or treated with H_2O), moved into the humidity chamber for 48 h, dried, reinocu-

lated with the second isolate (or with H_2O), 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 daylength using cool white fluorescent light at 150 μE 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 in the first or second inoculation were replaced by H_2O (i.e., H_2O(I)/ISR398(II), ISR398(I)/H_2O(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 (7×10^7 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 H_2O(I)/ISR8036(II), ISR8036(I)/H_2O(II), and ISR8036(I)/ISR8036(II). Significance values between treatments were obtained using an analysis of variance (ANOVA).

Pathogenicity of subisolates. The identity of the resulting pycnidia in the challenge-inoculation treatments was verified by pathogenicity tests and DNA fingerprinting. Subisolates were from 31 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 subcultures 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], 25 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 *Pst*I and then separated on 0.8% agarose gels in Tris-acetate-EDTA (40 mM Tris-HCl, 10 mM Na_2OAc, 1 mM Na_2EDTA [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 Na_2HPO_4, 7% SDS, 1 mM EDTA, and 1% bovine serum albumin. Washes were performed at 65°C in 0.263 M Na_2HPO_4 and 1% SDS for 20 min, 2× SSC (1× SSC is 0.15 M NaCl, 0.015 M sodium citrate [pH 7]) and 0.1% SDS for 20 min, and twice with 1× 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)/H_2O(II) and H_2O(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), H_2O(I)/ISR8036(II), and ISR8036(I)/H_2O(II) were each used in an ANOVA.

Significant reductions in pycnidia 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 ISR398 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 seedlings of the wheat cultivars Seri 82 and Shafir by *Septoria tritici* isolates

Period (days)	Number of trials	ISR398(I)/ISR8036(II)[v]		ISR8036/ISR398(II)[w]	
		'Seri 82'	'Shafir'	'Seri 82'	'Shafir'
2	2	72.3**[x,y]	38.1*	43.8*	3.1
5	4	90.2**[z]	51.2*	41.6*	30.4
10	2	87.2**	17.0	–4.8	–27.8

[v] 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).

[w] Isolate ISR8036 was used in the first (I) inoculation and then isolate ISR398 was used in the challenge-inoculation (II).

[x] Percent reductions were calculated from the control treatments ISR8036(I)/H_2O(II) and H_2O(I)/ISR8036(II).

[y] * = Significant at $P < 0.05$, calculated from analysis of variance with treatments ISR8036(I)/ISR8036(II) and H_2O treatments of ISR8036.

[z] ** = Significant at $P < 0.01$, calculated from analysis of variance with treatments ISR8036(I)/ISR8036(II) and H_2O treatments of ISR8036.

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

Treatment	% suppression of pycnidial coverage	
	'Seri 82'	'Shafir'
ISR398(I)/ISR8036(II)[x]	63.6*[y]	62.9*
SupISR398(I)/ISR8036(II)[z]	–9.3	–10.8
ISR8036(I)/ISR398(II)	23.6	6.4
SupISR8036(I)/ISR398(II)	0.0	1.3

[x] Isolate ISR398 was used in the first (I) inoculation followed by the use of isolate ISR8036 in the second (II) inoculation.

[y] * = Significant at $P < 0.05$.

[z] 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 7×10^7 spores/ml followed 5 days later by isolate ISR8036 at a spore concentration of 1×10^7 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 ISR8036 at all three spore concentration ratios (1:1, 7:1, and 1:7). The reversal of the isolate order (ISR8036(I)/ISR398(II)) 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 subisolates. Subisolates 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)/ISR8036(II) and ISR8036(I)/ISR398(II) at periods of 2 and 5 days between the two inoculations. In the inoculation order ISR398(I)/ISR8036(II), 48% of the subisolates were ISR8036-like at the 2-day period, whereas 56% of the subisolates resembled the first isolate (ISR398) in the inoculation order at the 5-day period. The subisolates were categorized according to their distinct DNA hybridization pattern in Southern blots, after digestion of the total DNA with *Pst*I and probing with the minisatellite *S. tritici* probe ST398-3.7A. Subisolates of ISR398 could be identified by a typical band of 3.7 kb, whereas subisolates of ISR8036 were distinguished by a 4.8-kb band (Fig. 1). DNA fingerprints obtained from conidial cultures of subisolates from 'Seri 82' and 'Shafir' following inoculation with ISR398(I)/ISR8036(II) and ISR8036(I)/ISR398(II) combinations at 2- or 5-day periods clearly identified subisolate origin. In the inoculation order of ISR398(I)/ISR8036(II) on 'Seri 82', 23/23 (100%) subisolates were identified as ISR8036 (virulent on 'Seri 82') at the 2-day period and 21/28 (75%) at the 5-day period (Table 4). On the susceptible cultivar Shafir with the same inoculation order, 12/23 (52%) and 14/25 (56%) subisolates showed a DNA pattern of the first isolate in the order of inoculation (ISR398) at 2- and 5-day periods, respectively. In the reverse order (ISR8036(I)/ISR398(II)) on 'Seri 82', 17/20 (85%) subisolates were identified as ISR8036 at the 2-day period, and 23/24 (96%) subisolates had a DNA pattern of ISR8036 at the 5-day period. Of the subisolates secured from 'Shafir' in the same order of inoculation, 17/17 (100%) at the 2-day period and 25/31 (81%) at the 5-day period were similar to the first isolate used (ISR8036), despite the fact that this cultivar is highly susceptible to isolate ISR398.

Thirty-one subisolates secured from pycnidia on 'Seri 82' and 'Shafir' inoculated with two inoculation combinations were used to reinoculate seedling leaves of 'Seri 82', as well as for Southern analysis. The identity of the subisolates was categorized according to the resulting pycnidial coverage on 'Seri 82' (resistant to ISR398 and susceptible to ISR8036). Pycnidial coverage lower than 1% was categorized as isolate ISR398, whereas pycnidial coverage greater than 10% was indicative of isolate ISR8036. Subisolates secured from pycnidia on 'Seri 82' inoculated with ISR398(I)/ISR8036(II) at 5-day periods were similar to ISR8036 (8/9 = 88.8%) in both the pathogenicity test on 'Seri 82' and in the DNA pattern. The six subisolates secured from 'Shafir' all resembled ISR8036 in pathogenicity and DNA pattern. The majority (6/7 = 85.7%) of the subisolates secured from 'Seri 82' inoculated with ISR8036(I)/ISR398(II) were similar to ISR8036 in the pathogenicity and DNA tests. Whereas 4/9 (44%) subisolates secured from 'Shafir' resembled ISR8036 in the two tests. Identification of subisolates based on pathogenicity test using the pycnidial coverage classification and the DNA banding pattern agreed in all cases.

DISCUSSION

The two *S. tritici* isolates used in the present study (ISR398 and ISR8036) 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 ISR8036 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 Zelikovitch and Eyal (24) in which no significant suppression was recorded on seedlings of cultivars Ceeon and Shafir inoculated with a centrifuged culture filtrate of ISR398 mixed with conidia of ISR8036. However, 1:1, 8:2, and 2:8 conidial mixtures of ISR398 and ISR8036 in that study resulted in significant reductions in pycnidial coverage on cultivars Ceeon 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 acted as a growth inhibitor (25). Ride (18) suggested that the delay observed in lesion development in wheat inoculated with *Botrytis cinerea* (nonpathogen) and challenge-inoculated with *Stagonospora nodorum* (wheat pathogen) resulted from direct competition or inhibition between the fungal species.

It is possible that suppression processes were being 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

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*

Treatment			'Seri 82'		'Shafir'	
I	II	Ratio	Pycnidial coverage (%)[u]	% suppression ISR8036[v]	Pycnidial coverage (%)	% suppression ISR8036
ISR398/	ISR8036	1:1[w]	4.8 ab	68.8*[x]	10.1 a	62.2*
ISR398/	ISR8036	7:1[y]	1.1 a	92.9**[z]	14.0 a	47.6*
ISR398/	ISR8036	1:7	7.2 bc	56.8*	10.9 a	67.5*
ISR8036/	ISR398	1:1	6.9 bc	77.9**	11.1 a	63.8*
ISR8036/	ISR398	7:1	22.2 d	28.8	29.6 b	–1.4
ISR8036/	ISR398	1:7	12.5 c	19.3	28.4 b	7.5

[u] Within columns, means followed by the same letter are not significantly different at $P < 0.05$.
[v] Percent reductions were calculated from the control treatments ISR8036(I)/H_2O(II) and H_2O(I)/ISR8036(II).
[w] 1:1 = 30 ml of spore suspension/tray/inoculation at 1×10^7 spores/ml.
[x] * = Significant at $P < 0.05$.
[y] 7:1, 1:7 = first or second inoculation at 7×10^7 spores/ml; second or first at 1×10^7 spores/ml.
[z] ** = 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 subisolates would resemble the virulent isolate ISR8036. The findings confirmed that assumption, in which 100 and 84% of the subisolates 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 subisolates 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 the suppression of pycnidial production. Cohen and Eyal (3) have shown that in the resistant cultivar Kavkaz/K4500 L.6.A.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'.

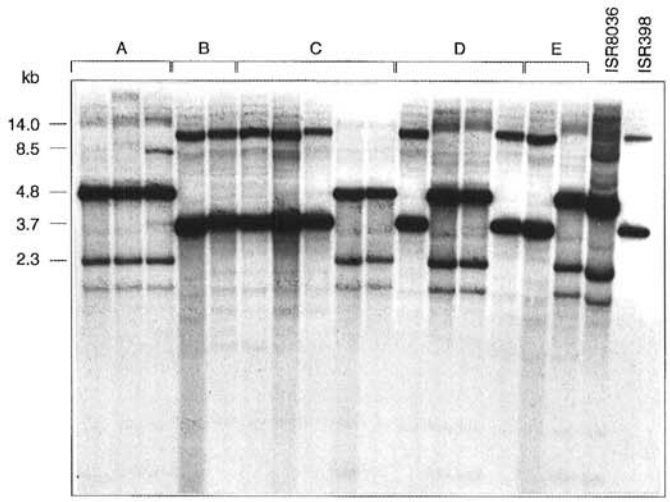

Fig. 1. Southern blot analysis of DNA from conidial preparations of subisolates of *Septoria tritici* isolated from pycnidia on seedlings of the wheat cultivar Shafir inoculated first with isolate ISR8036 and followed 5 days later with isolate ISR398. DNA was digested with *Pst*I and hybridized with the *S. tritici* minisatellite DNA probe ST398-3.7A. Lanes **A through E** correspond to single pycnidial subisolates 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.

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 mechanism(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.

TABLE 4. Identification of pycnidial subisolates by their DNA hybridization patterns with the ST398-3.7A probe following sequential inoculation at 2- or 5-day periods

Source of subisolate	Period (days)	ISR398(I)/ISR8036(II)[w]			ISR8036(I)/ISR398(II)[x]		
		ISR398	ISR8036[y]	Sum[z]	ISR8036	ISR398	Sum
'Seri 82'	2	0	23	23	17	3	20
'Seri 82'	5	7	21	28	23	1	24
'Shafir'	2	12	11	23	17	0	17
'Shafir'	5	14	11	25	25	6	31

[w] Isolate ISR398 was used in the first (I) inoculation followed 2 or 5 days later with isolate ISR8036 in the second (II) inoculation.
[x] Isolate ISR8036 was used in the first (I) inoculation followed 2 or 5 days later with isolate ISR398 in the second (II) inoculation.
[y] Values derived from the identification of subisolates according to specific bands by Southern blot of *Pst*I-digested DNA extracted from cultures obtained from pycnidia and hybridized with probe ST398-3.7A.
[z] Total number of subisolates.

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