Control of *Drechslera teres* and Other Barley Pathogens by Preinoculation with *Bipolaris maydis* and *Septoria nodorum*

H. J. Lyngs Jørgensen, H. Andresen, and V. Smedegaard-Petersen

Plant Pathology Section, Department of Plant Biology, The Royal Veterinary and Agricultural University, Thorvaldsensvej 40, DK-1871 Frederiksberg C, Copenhagen, Denmark.

This work was financed by the European Union through the Competitiveness of Agriculture and Management of Agricultural Resources (CAMAR) project: Crop Protection Using Diversification and Induced Resistance in Low-Input Cereal/Legume Cropping Systems.

We thank H. Stryhn, Department of Mathematics and Physics, The Royal Veterinary and Agricultural University, Copenhagen, for statistical advice. We also thank S. Sangchote, Kasetsart University, Bangkok, Thailand; J. Ö. Jönsson, Svalöf-Weibull AB, Sweden; M. Houmøller, the Danish Research Service for Plant and Soil Science; and H. K. Manandhar, Central Division of Plant Pathology, Khumalthar, Lalitpur, Nepal, for providing some of the fungal isolates used in this study.

Accepted for publication 16 February 1996.

ABSTRACT

Jørgensen, H. J. L., Andresen, H., and Smedegaard-Petersen, V. 1996. Control of *Drechslera teres* and other barley pathogens by preinoculation with *Bipolaris maydis* and *Septoria nodorum*. Phytopathology 86: 602-607.

Preinoculation of barley leaves with either of two nonbarley pathogens, *Bipolaris maydis* from maize or *Septoria nodorum* from wheat, 24 h in advance of inoculation with a virulent isolate of *Drechslera teres* f. *maculata* resulted in significantly reduced infection by the latter organism. In a range of experiments, the reductions in disease severity measured 39 to 70% and 22 to 65% after preinoculation with *B. maydis* and *S. nodorum*, respectively. Furthermore, the disease-reducing capacity exerted by the two inducers is of a general nature since they were effective against *D. teres* in different barley cultivars and against other barley pathogens such as *B. sorokiniana*, *Erysiphe graminis* f. sp. *hordei*, and *Rhynchosporium secalis*. Light microscopy of infected leaves revealed that in noninduced control leaves, mycelial growth and sporulation of *D. teres* was abundant. In contrast, the reduced disease level of *D. teres* in barley leaves preinoculated with *B. maydis* or *S. nodorum* was associated with reduced lesion size, strongly restricted mycelial growth, and little or no sporulation, which are well-known factors of hypersensitive reactions. Hence, our results suggest that host resistance responses are activated and that induced resistance is involved in the suppression of *D. teres*.

Host plants can be protected against virulent pathogens by prior inoculation with virulent or avirulent isolates of pathogens, saprophytes, or other microorganisms. This is known as induced resistance, if the protection is mediated through activation of the host defense responses, and it has been observed in many plant species (10,17,27,35). For the last decade, induced resistance in the barley (*Hordeum vulgare* L.)-powdery mildew (*Erysiphe graminis* DC. f. sp. *hordei* Ém. Marchal) system has been examined at our department. Several aspects of induced resistance have been studied including measurements of disease reduction using different inducers (7,16,26,38), mechanisms possibly involved in suppression of the pathogen (8,39,43), and putative defense-response genes activated by resistance-inducing organisms and products of these genes (4,5,13,14,15,37,41,44).

Since previous studies at our department have almost exclusively been concerned with the induction of resistance against the biotrophic pathogen *E. graminis* f. sp. *hordei* in barley, we have now initiated studies on the induction of resistance against necrotrophic pathogens. For these investigations, we decided to examine the possibilities for controlling *Drechslera teres* (Sacc.) Shoemaker (teleomorph *Pyrenophora teres* Drechs., synanamorph *Helminthosporium teres* Sacc.), the causal agent of net blotch of barley, which is a serious disease worldwide.

The aim of the current work is to describe in detail the suppressive effect of two nonpathogens of barley, *Bipolaris maydis* (Nisikado & Miyake) Shoemaker from maize and *Septoria nodorum* (Berk.) Berk. from wheat, on subsequent infection by *D. teres* in barley. In addition, the effect of the two organisms was examined in less detail for a number of other pathogens of barley, i.e., *B. sorokiniana* (Sacc.) Shoemaker, the causal agent of barley spot blotch; *Rhynchosporium secalis* (Oudem.) J. J. Davis, the causal agent of leaf blotch or scald of barley; and *E. graminis* f. sp. *hordei*, the causal agent of barley powdery mildew.

The terminology of induced resistance is used in this report. Hence, the disease-reducing organisms are referred to as inducers, whereas the pathogens are denoted as challengers. This is done, although the mechanisms by which *B. maydis* and *S. nodorum* inhibit infection by *D. teres* is not yet investigated in detail. However, the current data and preliminary studies involving light microscopy and molecular techniques suggest that induced resistance is involved, although possibly not as the only mechanism. Results from the investigations on the mechanisms responsible for the disease reductions produced by *B. maydis* and *S. nodorum* will be the topic of a later report.

Corresponding author: H. J. L. Jørgensen; E-mail address: hjo@kvl.dk

Publication no. P-1996-0314-01R

MATERIALS AND METHODS

Plants and experimental design. Two spring barley cultivars, Canor Carlsberg and Lenka, were used in all investigations, except for one experiment testing two winter barley cultivars, Ermo and Frost. The plants were grown in a greenhouse (20 to 25°C) in plastic pots (12 by 13.5 cm) containing the soil mix 'Weibulls Enhetsjord' (K jord, Svalöf Weibull AB, Hammenhög, Sweden) (20). Twenty-four hours before inducer inoculation, the 14-day-old plants were transferred to a growth chamber as earlier described (18), except that the amount of light was increased to approximately 200 μE m^{-2} s^{-1} (16 h of light at 19°C with 50 to

60% relative humidity and 8 h of darkness at 16°C with 80 to 90% relative humidity). After the transfer to the growth chamber, the second developed leaf of each of 10 plants per pot was fixed in a horizontal position, adaxial side upwards, on bent plastic plates using unbleached cotton strings (18). At this stage, the plants had two fully emerged leaves and the third was expanding. Six different types of experiments were carried out (Tables 1 to 6). Two cultivars of barley were used in each type of experiment, and data for each cultivar were analyzed separately. Hence, each type of experiment consisted of two separate experiments designed as balanced, completely randomized block experiments with four blocks. Each block consisted of three pots, two of which received different inducer treatments before challenge inoculation, whereas the third pot represented the control, which was inoculated with the challenger only. To test whether the inducers alone caused symptoms on the test plants, three pots of each of the cultivars were treated in exactly the same way as the other test plants (i.e., with either of the inducers or left untreated), except for the challenge inoculation. The only exceptions to the general design were the type of experiment examining the effect of the different inducer concentrations (Table 4) and the type of experiment investigating the effect of the different inducer incubation lengths (Table 5) on subsequent infection by *D. teres*. In these types of experiments, only one of the inducers was tested at a time, on two cultivars of barley. In the former type of experiment (Table 4), each block in the small experiments consisted of four pots (three pots receiving different concentrations of either *B. maydis* or *S. nodorum* and one pot representing the control). In the latter type of experiment (Table 5), a block in each of the small experiments consisted of 10 pots (five pots receiving either *B. maydis* or *S. nodorum* as inducers at different times before the challenger and five pots representing the controls placed in plastic bags at the same times as the pots receiving inducers). Table 6 presents the results from the testing of three other challengers on barley. Each challenger was tested separately on two cultivars of barley.

Fungal isolates and inoculum. An isolate of each of the two nonbarley pathogens, *B. maydis* from maize and *S. nodorum* from wheat, were used as inducer organisms. Neither of the isolates is capable of causing disease on barley. Isolates of the barley pathogens *D. teres* f. *maculata* Smedeg., *R. secalis*, *B. sorokiniana*, and *E. graminis* f. sp. *hordei*, which all cause strong disease symptoms on barley, were used as challengers to measure the degree of protection incited by the inducer organisms.

Inoculum of *B. maydis* (isolate CP 2050) was produced on diluted potato-dextrose agar (PDA) (13.0 g/liter) plates (Difco Laboratories, Detroit). *D. teres* f. *maculata* (isolate CP 2051) was grown on grass agar (filtrate of 32.5 g/liter of boiled clover-rich grass fodder pills for cattle and 20 g/liter of agar) (26). *B. Sorokiniana* (isolate CP 1623) was grown on PDA plates (Difco Laboratories).

All organisms were incubated at 15 to 20°C under cycles of 16 h of near-UV light (Philips TLD 36W/08, Philips Lighting B.V., Roosendaal, Netherlands)/8 h of darkness. Inoculum of *S. nodorum* (isolate CP 2052) was produced on Czapek-Dox V8-juice agar (modified from Cooke and Jones [9]) under the same conditions as *B. maydis* but with an initial incubation period of 2 days in the dark. Inoculum of *E. graminis* f. sp. *hordei* (isolate 93-NÅ[Seg]) was produced on barley plants, isoline P-11 of the cultivar Pallas. After 7 days of incubation (16 h of light at 18 to 20°C and 8 h of darkness at 15 to 16°C), the powdery mildew fungus sporulated abundantly and was used for inoculation. *R. secalis* (isolate CP 1938-70) was grown as previously described (18). Inoculum of all organisms, except *E. graminis* f. sp. *hordei*, were harvested in glass-distilled water. For *B. maydis*, 14- to 18-day-old cultures were used; for *D. teres*, 7- to 10-day-old cultures were used; for *S. nodorum*, 14- to 16-day-old cultures were used; for *B. sorokiniana*, 8-day-old cultures were used; and for *R. secalis*, 9-day-old cultures were used. The inoculum concentrations of the inducer organisms *B. maydis* and *S. nodorum* were adjusted to 20,000 and 2,000,000 conidia/ml, respectively; exceptions were the type of experiment used to investigate the effect of lower inducer concentrations on the disease incidence of *D. teres* (Table 4) and the type of experiment that used *R. secalis* as the challenger (Table 6). The inoculum concentration of the challenge organisms *D. teres*, *B. sorokiniana*, and *R. secalis* were adjusted to 400 to 600, 4,000, and 10,000 conidia/ml, respectively, in the individual experiments. For *E. graminis* f. sp. *hordei*, 8 conidia/mm^2 were used. These concentrations were found to give satisfactory infection levels.

All types of inoculum, except that of *E. graminis* f. sp. *hordei*, were sprayed onto the fixed leaves until run-off. *E. graminis* f. sp. *hordei* was applied using the method of Cho and Smedegaard-Petersen (7). Following inducer inoculation, the test plants were incubated for 24 h in plastic bags to maintain a high relative humidity. Exceptions were the type of experiment used to investigate the effect of different inducer incubation periods on the suppressive effect on *D. teres* infection (Table 5) and the experiment that used *R. secalis* as the challenger (48 h of incubation, Table 6). After inducer incubation, the plastic bags were opened for about 10 to 30 min to let most of the inducer droplets dry before challenge inoculation. After application of necrotrophic challengers, the plants were sealed in plastic bags and incubated for 24 h in darkness (72 h for *R. secalis*). Subsequently, the bags were opened, and light at normal intensities was applied. When *E. graminis* f. sp. *hordei* was used as the challenger, plants were incubated in light and without plastic bags.

Assessment of disease. The percentage of necrotic symptoms caused by *D. teres* and *B. sorokiniana* was scored 6 and 9 days after inoculation, and symptoms caused by *R. secalis* were scored after 13 and 20 days. Leaf coverage by *E. graminis* f. sp. *hordei* was scored once after 6 days. A scale with 21 levels was used, each level consisting of a 5% interval: 0 = 0%, 1 = 0 to 5%, 2 = 5 to 10%, 3 = 10 to 15%, ..., 20 = 95 to 100%. A scale with this many levels was necessary to disclose differences between treatments resulting in only moderately different disease levels which, however, could be distinguished with the naked eye. Each pot was assigned one character for leaf coverage by necrotic symptoms (for *E. graminis* f. sp. *hordei*, coverage with colonies). Furthermore, differences in the size of lesions produced by *D. teres* were observed between inducer-treated and control plants. To examine

TABLE 1. Mean disease score, percent reduced infection, lesion length, and percent reduction in lesion size of *Drechslera teres* on the second developed leaf in the spring barley cultivars Canor Carlsberg and Lenka with and without inducer treatment with *Bipolaris maydis* or *Septoria nodorum*, respectively

	'Canor Carlsberg'				'Lenka'			
Inducer	Mean disease score	% reduction of disease score	Average lesion length (mm)	% reduction of lesion length	Mean disease score	% reduction of disease score	Average lesion length (mm)	% reduction of lesion length
Untreated	2.3	–	2.7	–	2.6	–	1.8	–
B. maydis	1.3	43.5	1.4	48.1	1.2	53.8	1.3	27.8
S. nodorum	1.7	26.1	2.4	11.1	1.3	50.0	1.3	27.8
$LSD_{0.95}$	0.5		0.9		0.5		0.2	
P value	0.0090		0.0281		0.0006		0.0019	

lesion size, the lengths of 10 individual lesions per pot were measured in one experiment at the last disease assessment.

The results of disease scorings and lesion sizes were analyzed using the general linear models procedure in PC-SAS (SAS Institute, Cary, NC). Only data for the last assessment are presented here. The disease-suppressive abilities of *B. maydis* and *S. nodorum* were each tested in more than 15 experiments, but only representative experiments are shown here.

Light microscopy of leaves. The development of *D. teres* in the leaf tissues was studied to determine the extent of hyphal growth within and outside the necrotic lesions. After the last disease assessment, segments were cut from control leaves as well as from leaves treated with either of the inducers. The segments were cleared in 96% ethanol, stained in toluidine blue O, and examined using light microscopy as previously described (18).

RESULTS

The two inducer organisms *B. maydis* and *S. nodorum* are both nonpathogenic on barley. However, occasionally they produced tiny spots barely visible to the naked eye. These spots did not develop further, and sporulation was never observed.

Effect of inducer inoculation on subsequent infection by *D. teres*. Table 1 shows that inducer inoculation of the second developed leaf of the barley cultivars Canor Carlsberg and Lenka with *B. maydis* or *S. nodorum* significantly reduced the average infection level of *D. teres* in both cultivars, reductions being 43 to 54% and 26 to 50% for *B. maydis* and *S. nodorum*, respectively.

The size of lesions caused by *D. teres* was also significantly reduced by inducer application (Table 1). Thus, *B. maydis* reduced the average lesion length by 48 and 28% in 'Canor Carlsberg' and 'Lenka', respectively. *S. nodorum* only significantly reduced the lesion length in 'Lenka' (28%).

Light microscopy studies showed that growth of *D. teres* was strongly inhibited in barley leaves preinoculated with either *B. maydis* or *S. nodorum*. Thus, lesions on the inducer-treated leaves were small and mycelial growth of *D. teres* was sparse compared with the controls. Only a very limited amount of hyphae was seen within the lesions, and almost no spreading took place into the neighboring tissue. Sporulation was not observed from such tissue. In contrast, mycelial growth of *D. teres* in the noninduced control leaves was abundant with hyphae proliferating from the large necrotic lesions through most of the surrounding leaf tissue. Sporulation was abundant on these leaves.

Table 2 shows that inducer inoculation of the fourth developed leaf of barley plants reduced the subsequent infection by *D. teres*. Disease reductions for *B. maydis* were 65 and 70% in 'Canor Carlsberg' and 'Lenka', respectively, whereas disease reductions for *S. nodorum* were 42 and 55% in 'Canor Carlsberg' and 'Lenka', respectively.

The two inducer organisms were also able to inhibit infection

TABLE 2. Mean disease score and percent reduced infection by *Drechslera teres* on the fourth developed leaf in the spring barley cultivars Canor Carlsberg and Lenka with and without inducer treatment with *Bipolaris maydis* or *Septoria nodorum*, respectively

	'Canor Carlsberg'		'Lenka'	
Inducer	Mean disease score	% reduction of disease score	Mean disease score	% reduction of disease score
Untreated	4.3	–	3.3	–
B. maydis	1.5	65.1	1.0	69.7
S. nodorum	2.5	41.9	1.5	54.5
$LSD_{0.95}$	1.1		1.0	
P value	0.0027		0.0043	

TABLE 3. Mean disease score and percent reduced infection by *Drechslera teres* on the second developed leaf in the winter barley cultivars Ermo and Frost with and without inducer treatment with *Bipolaris maydis* or *Septoria nodorum*, respectively

	'Ermo'		'Frost'	
Inducer	Mean disease score	% reduction of disease score	Mean disease score	% reduction of disease score
Untreated	4.0	–	2.3	–
B. maydis	2.0	50.0	1.0	56.5
S. nodorum	2.5	37.5	1.8	21.7
$LSD_{0.95}$	0.6		0.6	
P value	0.0004		0.0090	

TABLE 4. Mean disease score and percent reduced infection by *Drechslera teres* on the second developed leaf in the spring barley cultivars Canor Carlsberg and Lenka with and without inducer treatment with different concentrations of *Bipolaris maydis* or *Septoria nodorum*, respectively

	'Canor Carlsberg'		'Lenka'	
Inducer concentration (conidia/ml)	Mean disease score	% reduction of disease score	Mean disease score	% reduction of disease score
B. maydis				
0	4.9	–	7.1	–
1,000	4.9	0	6.5	8.5
7,500	3.6	26.5	2.9	59.2
15,000	3.0	38.8	2.3	67.6
$LSD_{0.95}$	1.3		2.6	
P value	0.0268		0.0041	
S. nodorum				
0	4.3	–	4.3	–
100,000	2.3	46.5	2.8	34.9
750,000	2.7	37.2	2.3	46.5
1,500,000	1.5	65.1	1.8	58.1
$LSD_{0.95}$	0.7		1.5	
P value	0.0003		0.0191	

TABLE 5. Mean disease score of *Drechslera teres* on the second developed leaf in the spring barley cultivars Canor Carlsberg and Lenka after inducer treatment with *Bipolaris maydis* or *Septoria nodorum*, respectively, and after different inducer incubation lengths before challenge inoculation

Duration of inducer incubation period (h)	Mean disease score	
	'Canor Carlsberg'	'Lenka'
B. maydis		
2	2.5	2.5
8	1.8	2.4
24	1.5	1.3
54	1.8	1.3
78	1.8	1.4
$LSD_{0.95}$	0.6	1.4
P value	0.041	0.150
Untreated[a]	3.6	4.7
P value[b]	<0.0001	<0.0001
S. nodorum		
2	3.5	2.5
8	5.0	4.5
24	3.0	2.8
54	2.5	2.3
78	2.5	1.8
$LSD_{0.95}$	1.1	2.9
P value	0.011	0.280
Untreated[a]	4.0	4.2
P value[b]	0.0270	0.0098

[a] Average of disease scores for all noninducer-treated plants.
[b] *P* value for comparison of disease scores for inducer-treated plants with the average for untreated plants (paired *t* test).

by *D. teres* in two winter barley cultivars (Table 3). *B. maydis* significantly reduced the average infection level of *D. teres* by 50 and 57% in 'Ermo' and 'Frost', respectively. *S. nodorum* was only able to significantly reduce infection by *D. teres* in 'Ermo' (38%).

Effect of low concentrations of the inducers on *D. teres* infection. Table 4 shows the effect of different inducer concentrations on the capacity of the inducer organisms to reduce subsequent infection by *D. teres*. A concentration of 15,000 conidia/ml of *B. maydis* significantly reduced infection by *D. teres* in 'Canor Carlsberg' by 39%. In 'Lenka', concentrations of 7,500 and 15,000 conidia/ml significantly reduced infection by the challenger by 59 and 68%, respectively.

All three concentrations of *S. nodorum* significantly reduced the infection by *D. teres* in both cultivars. Thus, for 100,000, 750,000, and 1,500,000 conidia/ml, the reductions were 47, 37, and 65%, respectively, in 'Canor Carlsberg' and 35, 47, and 58%, respectively, in 'Lenka'.

Effect of different inducer incubation periods on *D. teres* infection. For *B. maydis*, 2 h of inducer incubation in 'Canor Carlsberg' gave a higher infection level than the other incubation periods, which did not differ among themselves (Table 5). For *S. nodorum*, 8 h of inducer incubation before the challenger gave a higher infection level than the rest of the periods, which did not differ among themselves. For 'Lenka', there were no significant differences in disease severity for different inducer incubation periods for either *B. maydis* or *S. nodorum*. Table 5 shows that even though there were only a few inducer incubation periods in 'Canor Carlsberg' that supported significantly higher infection levels of *D. teres* than the rest, there were strongly significant differences in infection levels between inducer-treated and control plants for both cultivars and inducers.

Effect of the inducers on infection by other barley pathogens in 'Canor Carlsberg' and 'Lenka'. *B. sorokiniana* (Table 6) was significantly inhibited by preinoculation with both *B. Maydis* and *S. nodorum* in 'Lenka' (48 and 41%, respectively). In 'Canor Carlsberg', neither of the inducers had any significant disease-reducing effect.

In 'Canor Carlsberg', neither *B. maydis* nor *S. nodorum* significantly reduced infection by *E. graminis* f. sp. *hordei* (Table 6). On the other hand, significant reductions were found for both inducers in 'Lenka'; the reductions measuring 29 and 10% for *B. maydis* and *S. nodorum*, respectively.

R. secalis was not inhibited in experiments using the conditions employed in the other experiments (data not shown). However, in an experiment in which the concentration of *S. nodorum* was increased to 5,400,000 conidia/ml (the concentration of *B. maydis* unaltered at 20,000 conidia/ml) and the duration of the inducer incubation period was 48 h, significant reductions of infection by *R. secalis* were recorded (Table 6). Hence, *B. maydis* reduced infection by *R. secalis* by 69 and 48% in 'Canor Carlsberg' and 'Lenka', respectively, whereas the reductions by *S. nodorum* were 73 and 39% in 'Canor Carlsberg' and 'Lenka', respectively.

DISCUSSION

Preinoculation of four different barley cultivars with two nonbarley pathogens, *B. maydis* from maize and *S. nodorum* from wheat, as inducers resulted in significant and rather consistent reductions in the severity of disease incited by the barley pathogen *D. teres*. These reductions were quite substantial, measuring 39 to 70% and 22 to 65% for the inducers *B. maydis* and *S. nodorum*, respectively.

From our experiments, it is evident that the abilities of *B. Maydis* and *S. nodorum* to reduce disease severity are of a general nature. The inducers had an inhibiting effect on *D. teres* in different concentrations, after different inducer incubation periods, and in young seedlings as well as in older plants at the four-leaf stage. However, more important in this context is that the inducers were able to reduce infection by *D. teres* in a number of unrelated barley cultivars, representing two-rowed spring types ('Canor Carlsberg' and 'Lenka') as well as six-rowed winter types ('Ermo' and 'Frost'). In addition to these cultivars, significant reductions of disease severity were also observed in the six-rowed winter barley cultivar Jana and in the two-rowed spring barley cultivars Digger and Pallas (data not shown).

The results further show that both *B. maydis* and *S. nodorum* possess the capacity to induce protection against important barley pathogens other than *D. teres*, namely *B. sorokiniana*, *R. secalis*, and *E. graminis* f. sp. *hordei*. Although the protection incited by the two inducers varied among the different pathogens and with the different host cultivars, the results clearly demonstrate that the necrotrophic inducers have the capacity to induce protection against a range of barley pathogens representing both necrotrophic and biotrophic organisms.

Attempts to control *D. teres* by other microorganisms have previously been made. Thus, in vitro screening has revealed that certain fungal and bacterial species are able to reduce the growth of *D. teres* (1,2,24). In other investigations, the effect of various organisms on *D. teres* has been tested in vivo. Scharen and Bryan (30) observed that *Bacillus licheniformis* was able to reduce infection of barley by *D. teres* in the greenhouse as well as in the field. However, in their field trial, yield was not significantly in-

TABLE 6. Mean disease score and percent reduced infection by *Bipolaris sorokiniana*, *Erysiphe graminis* f. sp. *hordei*, and *Rhynchosporium secalis*, respectively, on the second developed leaf in the spring barley cultivars Canor Carlsberg and Lenka with and without inducer treatment with *Bipolaris maydis* or *Septoria nodorum*, respectively

	B. sorokiniana		*E. graminis* f. sp. *hordei*		*R. secalis*[a]	
Inducer	Mean disease score	% reduction of disease score	Mean disease score	% reduction of disease score	Mean disease score	% reduction of disease score
'Canor Carlsberg'						
Untreated	13.3	–	10.8	–	14.0	–
B. maydis	10.3	22.6	8.3	23.1	4.3	69.3
S. nodorum	10.5	21.1	10.5	2.8	3.8	72.9
$LSD_{0.95}$	4.8		3.2		5.4	
P value	0.3062		0.1938		0.0060	
'Lenka'						
Untreated	17.8	–	12.0	–	16.5	–
B. maydis	9.3	47.8	8.5	29.2	8.5	48.5
S. nodorum	10.5	41.0	10.8	10.0	10.0	39.4
$LSD_{0.95}$	3.5		1.0		5.8	
P value	0.0021		0.0005		0.0320	

[a] In this experiment, the concentrations of *B. maydis* and *S. nodorum* were 20,000 and 5,400,000 conidia/ml, respectively, and the incubation period was 48 h.

creased by the treatment. Blakeman et al. (3) reported that the nonpathogens of barley, *D. catenaria* (Drechs.) Ito and *D. siccans* (Drechs.) Shoemaker, could inhibit infection by *D. teres* in barley, but no further details were given. Substantial reductions of coleoptile and leaf infection by *D. teres* as well as of sporulation on naturally infected barley straws were observed by Mostafa (23), who tested five organisms previously selected for their inhibiting effect on *D. teres* (24). Mostafa et al. (25) also found that leaf infection by an aggressive isolate of *D. teres* could be inhibited by preinoculating with a mycelial suspension or a crude filtrate of a weakly aggressive isolate of *D. teres*.

Neither *B. maydis* nor *S. nodorum* apparently has been used before to control net blotch or other diseases in living cereal plants. However, Dickinson and Skidmore (11) reported a certain inhibiting effect of *S. nodorum* on the germination and germ tube growth of some common phylloplane organisms (e.g., the pathogen *B. sorokiniana*) on cellophane films and on detached barley leaves. Likewise, infection of wheat by *S. nodorum* was reported to reduce infection by other pathogens, e.g., *E. graminis* f. sp. *tritici* (42). Various *Bipolaris* species have been used to control certain cereal diseases. Hence, infection by *B. oryzae* in rice was suppressed in plants treated with conidial suspensions or extracts of conidia of the same pathogen (12,31,32,33,40). Also, infection of wheat by *B. sorokiniana* was reduced by preinoculation with a conidial suspension of *B. oryzae* or extracts of conidia of *B. Oryzae* or *B. sorokiniana* (6). *B. sorokiniana* infection of barley could also be inhibited by application of conidial suspensions of the same organism (28,29).

In our experiments, the disease severity of *D. teres* was measured as percentage of leaf coverage with necrotic symptoms alone, since chlorosis was only rarely observed. Normally, the symptoms produced by virulent isolates of *D. teres* are characterized by a combination of dark necrotic lesions and chlorosis, whereas avirulent isolates usually produce small necrotic lesions without chlorosis (34,36). A characteristic feature of the *D. teres* isolate we used in our experiments was, however, that it produced large necrotic lesions with little or no chlorosis at all. These distinct spot lesions without complicating chlorosis allowed us to use total necrotic area as one of the measures of reduced disease intensity of inducer-treated plants. Lesion size of *D. teres* was significantly reduced by inducer-inoculation with *B. maydis* and *S. nodorum*. In experiments on biological control of coleoptile infection in barley, Mostafa (23) also observed that the mean length of necrotic areas produced by *D. teres* on barley coleoptiles was considerably reduced after pretreatment with various fungal and bacterial species. Likewise, Keeling and Banttari (21), studying four cultivars of barley with varying degrees of resistance to *D. teres*, found that reduction of lesion size was an important factor in the expression of resistance of barley to this pathogen.

In noninduced control leaves, mycelial growth and sporulation was abundant. In contrast, the reduced disease severity of *D. teres* in barley leaves preinoculated with *B. maydis* or *S. nodorum* was associated with reduced lesion size, strongly restricted mycelial growth, and little or no sporulation, which are well-known factors of hypersensitive reactions. Our results therefore suggest that host resistance responses were activated and hence, that induced resistance is involved in the suppression of *D. teres*.

Disease reductions caused by various *Bipolaris* species have been claimed to be a result of 'acquired' or 'induced' resistance (6,12,28,29,31,32,33,40). This was stated even though detailed investigations were not made on the mode of action; it was not determined whether induced resistance, sensu Kloepper et al. (22), was actually responsible for the disease reductions observed.

Our investigations have clearly demonstrated that both *B. Maydis* and *S. nodorum* possess the ability to inhibit *D. teres* and other serious barley pathogens. *B. maydis* and *S. nodorum* could, therefore, be potential candidates for future work on the development of biological control agents to use commercially in barley. There are, however, many issues that need to be considered and the biological control organisms need to fulfill many requirements before a potential biological fungicide can be developed. One serious objection to the use of *B. maydis* and *S. nodorum* as biocontrol organisms is that they are pathogens of maize and wheat, respectively, and therefore could constitute a risk for maize and wheat crops. In this context, it should be emphasized that neither *B. maydis* nor *S. nodorum* from wheat is able to grow and sporulate on barley and, therefore, cannot serve as a source of inoculum for other crops. Furthermore, inoculum of either organism will not survive for a long time unless susceptible host plants are available. Hence, in our opinion, the only way nontarget crops could be infected is by direct and unintended inoculation which, of course, should be avoided. The risk of unintended inoculation of host plants depends on the extent maize and wheat are grown in an area. Maize is grown to a very limited extent in Scandinavia and hence, the risk in this geographic area is almost nonexistent. In contrast, wheat is a major crop and *S. nodorum* a major pathogen. Unintended inoculation of this crop during the use of *S. nodorum* for disease control in barley would, however, contribute only little to the existing population of *S. nodorum*. Other risks and problems in the development of a biological control agent based on induced resistance have been described elsewhere (19).

It is not possible to predict whether commercial inducers for barley based on *B. maydis* or *S. nodorum* can be developed. The current investigations may nevertheless prove to be valuable because they form the basis for studies of the resistance mechanisms activated in barley against infection attempts from *D. teres* and other important necrotrophic barley pathogens. Thus, we are currently performing detailed investigations on the mechanisms behind the observed suppression of *D. teres* infection. The current results and preliminary results based on further light microscopy and molecular studies suggest that induced resistance and possibly antibiosis are involved. Results from these studies on the mechanisms will be published later.

LITERATURE CITED

1. Al-Ali, B., Barrault, G., and Albertini, L. 1979. Action in vitro d'antagonistes fongiques et bactériens sur la croissance mycélienne de l'*Helminthosporium teres* Sacc. parasite de l'orge. Bull. Trimest. Soc. Mycol. Fr. 95:279-295.
2. Ali-Haimoud, D.-E., Mostafa, M., Barrault, G., and Albertini, L. 1993. Evaluation of organisms antagonistic to the sclerotioid organs of *Drechslera teres*, the causal agent of barley net blotch. Plant Dis. 77:1251-1255.
3. Blakeman, J. P., Mercer, P. C., O'Neill, R., and McGimpsey, H. C. 1986. Biological control of cereal diseases. Annu. Rep. Res. Tech. Work Dep. Agric. North. Irel. 1985, pp. 132-133.
4. Brandt, J., Thordal-Christensen, H., Vad, K., Gregersen, P. L., and Collinge, D. B. 1992. A pathogen-induced gene of barley encodes a protein showing high similarity to a protein kinase regulator. Plant J. 2:815-820.
5. Bryngelsson, T., Sommer-Knudsen, J., Gregersen, P. L., Collinge, D. B., Ek, B., and Thordal-Christensen, H. 1994. Purification, characterization, and molecular cloning of basic PR-1-type pathogenesis-related proteins from barley. Mol. Plant-Microbe Interact. 7:267-275.
6. Chakraborty, D., and Sinha, A. K. 1984. Similarity between the chemically and biologically induced resistance in wheat seedlings to *Drechslera sorokiniana*. Z. Pflanzenkr. Pflanzenschutz 91:59-64.
7. Cho, B. H., and Smedegaard-Petersen, V. 1986. Induction of resistance to *Erysiphe graminis* f. sp. *hordei* in near-isogenic barley lines. Phytopathology 76:301-305.
8. Christiansen, S. K., and Smedegaard, V. 1990. Microscopic studies of the interaction between barley and the saprophytic fungus, *Cladosporium macrocarpum*. J. Phytopathol. 128:209-219.
9. Cooke, B. M., and Jones, D. G. 1970. A field inoculation method for *Septoria tritici* and *Septoria nodorum*. Plant Pathol. 19:72-74.
10. Deverall, B. J., and Dann, E. K. 1995. Induced resistance in legumes. Pages 1-30 in: Induced Resistance to Disease in Plants. R. Hammerschmidt and J. Kuć, eds. Kluwer Academic Publishers, Dordrecht, Netherlands.
11. Dickinson, C. H., and Skidmore, A. M. 1976. Interactions between germinating spores of *Septoria nodorum* and phylloplane fungi. Trans. Br.

Mycol. Soc. 66:45-56.
12. Ganguly, D., and Padmanabhan, S. Y. 1962. *Helminthosporium* disease of rice–IV. Effect of cultural extract of the pathogen on the reaction of rice varieties to the disease. Indian Phytopathol. 15:133-140.
13. Gregersen, P. L., Brandt, J., Thordal-Christensen, H., and Collinge, D. B. 1993. cDNA cloning and characterization of mRNAs induced in barley by the fungal pathogen, *Erysiphe graminis*. Pages 304-307 in: Mechanisms of Plant Defense Responses. B. Fritig and M. Legrand, eds. Kluwer Academic Publishers, Dordrecht, Netherlands.
14. Gregersen, P. L., Christensen, A. B., Sommer-Knudsen, J., and Collinge, D. B. 1994. A putative *O*-methyltransferase from barley is induced by fungal pathogens and UV light. Plant Mol. Biol. 26:1797-1806.
15. Gregersen, P. L., Collinge, D. B., and Smedegaard-Petersen, V. 1990. Early induction of new mRNAs accompanies the resistance reaction of barley to the wheat pathogen, *Erysiphe graminis* f. sp. *tritici*. Physiol. Mol. Plant Pathol. 36:471-481.
16. Gregersen, P. L., and Smedegaard, V. 1989. Induction of resistance in barley against *Erysiphe graminis* f. sp. *hordei* after preinoculation with the saprophytic fungus, *Cladosporium macrocarpum*. J. Phytopathol. 124: 128-136.
17. Hammerschmidt, R., and Yang-Cashman, P. 1995. Induced resistance in cucurbits. Pages 63-85 in: Induced Resistance to Disease in Plants. R. Hammerschmidt and J. Kuć, eds. Kluwer Academic Publishers, Dordrecht, Netherlands.
18. Jørgensen, H. J. L., de Neergaard, E., and Smedegaard-Petersen, V. 1993. Histological examination of the interaction between *Rhynchosporium secalis* and susceptible and resistant cultivars of barley. Physiol. Mol. Plant Pathol. 42:345-358.
19. Jørgensen, H. J. L., and Smedegaard-Petersen, V. 1994. Constraints in the implementation of induced resistance against necrotrophic pathogens of cereals. Pages 77-85 in: Biological Control of Fruit and Foliar Disease. Proc. Workshop CEC Prog. "Competitiveness of Agriculture and Management of Agricultural Resources." (CAMAR), 1993. P. Lepoivre, ed. European Commission. Directorate-General for Agriculture. Working document for the European Commission reference F.II.3 - SJ/0010.
20. Jørgensen, H. J. L., and Smedegaard-Petersen, V. 1995. Pathogenic variation of *Rhynchosporium secalis* in Denmark and sources of resistance in barley. Plant Dis. 79:297-301.
21. Keeling, B. L., and Banttari, E. E. 1975. Factors associated with the resistance of barley to *Helminthosporium teres*. Phytopathology 65:464-467.
22. Kloepper, J. W., Tuzun, S., and Kuć, J. A. 1992. Proposed definitions related to induced disease resistance. Biocontrol Sci. Technol. 2:349-351.
23. Mostafa, M.-M. 1993. Biological control of *Drechslera teres*: Ability of antagonists to reduce conidia formation, coleoptile infection and leaf infection in barley (*Hordeum vulgare*). Cryptogam. Mycol. 14:287-295.
24. Mostafa, M., Barrault, G., and Albertini, L. 1992. Lutte biologique contre *Drechslera teres*: Action in vitro de microorganismes antagonistes sur la croissance mycélienne et la germination. Cryptogam. Mycol. 13:125-133.
25. Mostafa, M., Barrault, G., and Albertini, L. 1993. Essai de lutte biologique contre l'helminthosporiose de l'orge par l'utilisation d'une souche peu agressive de *Drechslera teres*. Cryptogam. Mycol. 14:229-238.
26. Møller, I. 1992. Resistance in Barley to the Net Blotch Fungus *Pyrenophora teres*. Ph.D. thesis. The Royal Veterinary and Agricultural University, Copenhagen.
27. Ozeretskovskaya, O. L. 1995. Induced resistance in the Solanaceae. Pages 31-62 in: Induced Resistance to Disease in Plants. R. Hammerschmidt and J. Kuć, eds. Kluwer Academic Publishers, Dordrecht, Netherlands.
28. Sarhan, A. R. T., and Kiraly, Z. 1986. Induction of systemic acquired resistance in barley infected with *Helminthosporium sativum*. Iraqi J. Agric. Sci. "Zanco" (Suppl.) 4:25-31.
29. Sarhan, A. R. T., Király, Z., Sziráki, I., and Smedegaard-Petersen, V. 1991. Increased levels of cytokinins in barley leaves having the systemic acquired resistance to *Bipolaris sorokiniana* (Sacc.) Shoemaker. J. Phytopathol. 131:101-108.
30. Scharen, A. L., and Bryan, M. D. 1981. A possible biological control agent for net blotch of barley. (Abstr.) Phytopathology 71:902-903.
31. Sinha, A. K., and Das, N. C. 1972. Induced resistance in rice plants to *Helminthosporium oryzae*. Physiol. Plant Pathol. 2:401-410.
32. Sinha, A. K., and Trivedi, N. 1969. Immunization of rice plants against *Helminthosporium* infection. Nature (Lond.) 223:963-964.
33. Sinha, A. K., and Trivedi, N. 1972. Resistance induced in rice plants against *Helminthosporium* infection. Phytopathol. Z. 74:182-191.
34. Smedegård-Petersen, V. 1971. *Pyrenophora teres* f. *maculata* f. nov. and *Pyrenophora teres* f. *teres* on barley in Denmark. Pages 124-144 in: Yearbook 1971. The Royal Veterinary and Agricultural University, Copenhagen.
35. Steiner, U., and Schönbeck, F. 1995. Induced disease resistance in monocots. Pages 86-110 in: Induced Resistance to Disease in Plants. R. Hammerschmidt and J. Kuć, eds. Kluwer Academic Publishers, Dordrecht, Netherlands.
36. Tekauz, A. 1985. A numerical scale to classify reactions of barley to *Pyrenophora teres*. Can. J. Plant Pathol. 7:181-183.
37. Thordal-Christensen, H., Brandt, J., Cho, B. H., Rasmussen, S. K., Gregersen, P. L., Smedegaard-Petersen, V., and Collinge, D. B. 1992. cDNA cloning and characterization of two barley peroxidase transcripts induced differentially by the powdery mildew fungus *Erysiphe graminis*. Physiol. Mol. Plant Pathol. 40:395-409.
38. Thordal-Christensen, H., and Smedegård-Petersen, V. 1988. Comparison of resistance-inducing abilities of virulent and avirulent races of *Erysiphe graminis* f. sp. *hordei* and a race of *Erysiphe graminis* f. sp. *tritici* in barley. Plant Pathol. 37:20-27.
39. Thordal-Christensen, H., and Smedegaard-Petersen, V. 1988. Correlation between induced resistance and host fluorescence in barley inoculated with *Erysiphe graminis*. J. Phytopathol. 123:34-46.
40. Trivedi, N., and Sinha, A. K. 1976. Resistance induced in rice plants against *Helminthosporium* infection by treatment with various fungal fluids. Phytopathol. Z. 86:335-344.
41. Walther-Larsen, H., Brandt, J., Collinge, D. B., and Thordal-Christensen, H. 1993. A pathogen-induced gene of barley encodes a HSP90 homologue showing striking similarity to vertebrate forms resident in the endoplasmic reticulum. Plant Mol. Biol. 21:1097-1108.
42. Weber, G. E., Gülec, S., and Kranz, J. 1994. Interactions between *Erysiphe graminis* and *Septoria nodorum* on wheat. Plant Pathol. 43:158-163.
43. Wei, Y. D., de Neergaard, E., Thordal-Christensen, H., Collinge, D. B., and Smedegaard-Petersen, V. 1994. Accumulation of a putative guanidine compound in relation to other early defense reactions in epidermal cells of barley and wheat exhibiting resistance to *Erysiphe graminis* f. sp. *hordei*. Physiol. Mol. Plant Pathol. 45:469-484.
44. Zhang, Z., Collinge, D. B., and Thordal-Christensen, H. 1995. Germin-like oxalate oxidase, a H_2O_2-producing enzyme, accumulates in barley attacked by the powdery mildew fungus. Plant J. 8:139-145.