Evaluation of Organisms Antagonistic to the Sclerotoid Organs of *Drechslera teres*, the Causal Agent of Barley Net Blotch

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**ABSTRACT**


Twenty-seven microorganisms were evaluated for their ability to reduce sclerotoid organ formation and myceliogenesis in barley straw when applied prior to and following colonization by *Drechslera teres*. Ten of them were able to partly or totally inhibit the formation of sclerotoid organs on barley straw when applied as a precolonization treatment. All antagonists inhibited sclerotoid organ morphogenesis, whether through direct application or via the crude culture filtrates. Form *maculata* was more sensitive than form *teres* to culture filtrates of the antagonists. Treated barley straw did not display new sclerotoid organs in the postcolonization treatment, irrespective of the pathogen form. The postcolonization treatment of straw with certain antagonists also had a strong inhibitory effect on myceliogenesis of *D. teres* sclerotoid organs. The mycelium application also appeared more effectively inhibit myceliogenesis than did crude culture filtrates of the antagonists. All antagonists employed reduced the aggressiveness of the mycelium from germinating sclerotoid organs previously treated with antagonists in the *maculata* form.

Net blotch, caused by *Drechslera teres* (Sacc.) Shoemaker, teleomorphic stage: *Pyrenophora teres* Drechs.., is a major foliar fungal disease of barley (*Hordeum vulgare* L.). The two known forms of this pathogen, *D. teres* f. *teres* Smedeg. and *D. teres* f. *maculata* Smedeg., are present in France. The disease, which was previously latent, suddenly became epidemic about 15 yr ago (6). Studies initiated for

Accepted for publication 24 June 1993.

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**MATERIALS AND METHODS**

Pathogen isolation and storage. Barley leaves were harvested from plots on the Agricultural Experimental Station of Monlon near Toulouse, France. Two polyonidial strains of *D. teres* were isolated from surface-sterilized foliar tissues exhibiting typical symptoms and transferred onto 5% V8 medium (86% tomato, 6.4% carrot, 6.6% other vegetables, and 1% salt) containing 2% agar, until a pure culture was obtained. One strain belonged to the *maculata* or spot form of *D. teres*, and the other one to the *teres* or reticulate form. The pathogen was stored as sclerotia in the dark at 10°C using the method described by Barrault (6).

Production of sclerotoid organs. Strains of the *maculata* and the *teres* forms of *D. teres* were transferred onto a Czapek agar medium low in carbohydrates (1 g of K₂HPO₄, 0.5 g of MgSO₄, 0.5 g of KCl, 0.01 g of FeSO₄, 1251
7H2O, 3 g of NaNO3, and 1 g of sucrose per liter; pH 5.5), which favors formation of sclerotoid organs (30). Following incubation for 10 days in the dark at 23 C, barley straw fragments, previously autoclaved at 120 C for 20 min, were applied onto the surface of the cultures. Fructifications appeared after about 2 wk of incubation at 20 C in the dark.

Isolation of antagonists. Putatively antagonistic fungi and actinomycetes were isolated from barley straw sampled from heavily infected field plots of barley, as described above, and stored on agar slants of V8 juice medium. Putative fungal antagonists were transferred to a static liquid medium containing 10% V8 juice (pH 5.5) and incubated in the dark for 8 days at 23 C, and putative actinomycetous antagonists were transferred to a peptone yeast extract medium (5 g of peptone and 1 g of Difco yeast extract per liter; pH 5) for 13 days at 29 C. Each culture was then cool filtered on a 0.2-μm Gelman filter to separate mycelium from the secondary metabolites contained in the culture medium (crude filtrate). The mycelium was washed several times in sterile distilled water and ground with an Ultra-Turrax grinder.

Assessment of the effect of antagonists. 1. Sclerotoid organ morphogenesis. Both mycelial suspensions or crude culture filtrates of the putative antagonists were evaluated for their antagonistic effects on sclerotoid organ morphogenesis by D. teres. In the precolonization treatment, autoclaved barley straw fragments were immersed into a 20 ml solution of either the culture filtrate or the mycelial suspension of the various antagonistic fungal strains for 4 hr, then applied onto the D. teres cultures and incubated at 23 C in the dark. In the postcolonization treatment (i.e., after the appearance and counting of D. teres fructifications on straw), the straw fragments were dipped into the various solutions (mycelium or filtrates), then reapplied to the surface of the cultures and incubated. With each treatment, the controls consisted of sterile straw fragments dipped into sterile distilled water (mycelium control) or into sterile culture medium (filtrate control). Each trial included four straw fragments (3 cm × 0.5 cm) with three replications in a randomized-block design. Experimental units were examined at 2-day intervals, and the rate of existing sclerotoid organs or the occurrence of new sclerotoid organs was recorded. After incubation for 15 days, the visible (approximately 0.5 mm) sclerotoid organs forming on straw were counted using a dissecting microscope (magnification: 10 × 10) and expressed as numbers per unit surface area.

2. Sclerotoid organ myceliogenesis. After counting, sclerotoid organs were excised from the straw pieces under sterile conditions and transferred individually onto 5% V8 medium (2% agar). After incubation for 15 days at 23 C in the dark, the average diameter of each colony was measured.

3. Pathogen aggressiveness. Seeds of the susceptible barley cultivar Thibaut (4) were planted 1 cm deep in 60 × 40 × 8 cm tanks filled with vermiculite, with two seeds per furrow and six furrows per tank. Vermiculite was watered with Knop’s nutrient solution every week (30). The experiments were carried out in growth chambers with 12 hr of irradiance at 90 μmol·m⁻²·s⁻¹ at 23 C, and 12 hr of darkness at 18 C. The pure culture mycelium produced from treated sclerotoid organs was transferred onto a static liquid medium (10% V8) and incubated at 23 C in the dark. After 8 days, cultures were cool filtered (Durieux n°111 ash-free filter), and the mycelium (10 g/100 ml) were ground in sterile distilled water containing 0.25% gelatin and 0.01% polyoxyethylene ether (Triton CS 7, Rohm and Haas, France). Since inoculation with mycelium under controlled conditions had been shown to give the same infection types and levels on barley as inoculation with conidia (3), barley seedlings were inoculated at the two-leaf stage (27) by spraying a mycelium suspension (volume of 25 ml) over each tank. Six days after inoculation, disease intensity (measured as percentage of the foliar surface attacked) was estimated visually using the method described by Arabi (3). Infected leaves were surface sterilized, and the pathogen was isolated from necrotic foliar tissue and used for inoculation of new barley seedlings as described above. The percentage of the foliar surface attacked, obtained from the average of 10 observations per replication with three replications per treatment and per experiment, were then transformed into angular coordinates (back transformed means presented). Data were analyzed by ANOVA, and the means were separated by Newman and Keuls test (homogeneous groups) at the 0.05 level.

RESULTS

1. Morphogenesis of sclerotoid organs. Of the 27 microorganisms initially screened for antagonism to D. teres, 10 were able to partly or totally inhibit the formation of sclerotoid organs on barley straw when applied as a precolonization treatment (Fig. 1). These microorganisms included Trichoderma viride Pers.:Fr. (Tv); T. koningii Oudem. (Tk); T. pseudokoningii Rifai (Tp); one actinomycete (Micromonospora sp. a6 (Mic)); and five other unidentified fungi designated as 4a, 9a, 13b, 20a, and 20b. The most efficient antagonists of sclerotoid organ formation in order of decreasing efficiency were T. koningii; T. viride strain Tvo; the actinomycete Micromonospora sp. a6; T. viride strain Tv; T. pseudokoningii; and the unidentified fungi 4a, 13b, 20a, and 9a. Antagonists 4a and 20b were less effective against form teres than against form maculata, where sclerotoid organ morphogenesis was almost totally inhibited. Conversely, T. viride strain Tv was less effective against the form maculata than against the teres form (Fig. 1). All antagonists inhibited sclerotoid organ morphogenesis, whether through direct application or via the crude culture filtrates. The effects on morphogenesis of the sclerotoid organs varied with the antagonist. The antagonists also induced abnormalities in the morphology of sclerotoid organs. The majority of fructifications formed were less developed, displayed fewer setae, and were unable to develop myceliogenically. All mycelial treatments applied directly, inhibited completely the morphogenesis of sclerotoid organs of both D. teres forms;
whereas the inhibition was only partial with the filtrate of strain 4a, mostly with form teres, and ineffective with filtrate 9a. In contrast, the filtrate of strain 20b significantly stimulated sclerotoid ontogeny in form teres (Fig. 2). Form maculata was more sensitive than form teres to culture filtrates of the antagonists, with the exception of the T. viride filtrate, which only induced a slight inhibition of sclerotoid organ formation.

Treated barley straw did not display new sclerotoid organs in the postcolonization treatment, irrespective of the pathogen form, in contrast to nontreated straw, where a significant number of new sclerotoid organs appeared (Table 1).

2. Myceliogenesis of sclerotoid organs. Precolonization of barley straw with all the antagonists inhibited totally germination of the sclerotoid organs and therefore myceliogenesis (data not shown). The postcolonization treatment of straw with certain antagonists also had a strong inhibitory effect on myceliogenesis of D. teres sclerotoid organs (Fig. 3). Antagonists T. viride strains Tv and Tovo, and T. pseudokoningii completely inhibited germination of the sclerotoid organs. The other antagonists reduced myceliogenesis of these organs to a lesser extent. Myceliogenesis by form maculata was more sensitive than form teres to T. koningii and antagonists 4a and 20b, and to others to a lesser extent (Fig. 3).

The mycelium application also appeared to inhibit myceliogenesis more effectively than did crude culture filtrates of the antagonists. The inhibition of sclerotoid organ myceliogenesis was greater when the mycelium of antagonists 4a, 9a, 13b, 20a, and 20b was used against form teres (Fig. 3). Micromonospora sp. a6 was less effective in reducing myceliogenesis in both D. teres strains.

3. Aggressiveness of D. teres. Because the treated sclerotoid organs were unable to germinate, the effect of the precolonization treatment on D. teres aggressiveness could not be investigated. A high degree of variability occurred in the aggressiveness of both D. teres types of mycelium that developed from postcolonization-treated sclerotoid organs that had retained the ability to germinate on an agar medium (Fig. 4). All antagonists employed reduced the aggressiveness of the maculata form. In the case of the teres form and depending on the antagonist considered, an increase (Micromonospora sp. a6) or decrease (4a, 9a, 13b, 20a, and 20b) in level of aggressiveness by the pathogen was observed. Thus, only Micromonospora sp. a6 displayed a double effect: aggressiveness of the teres form was enhanced, whereas that of the maculata form was impaired. These effects were still reproduced after isolating the pathogen from surface-sterilized diseased tissue and inoculating new barley seedlings.

DISCUSSION
All antagonists selected for this study inhibited formation of sclerotoid organs and myceliogenesis in D. teres, particularly the microorganisms belonging to the genus Trichoderma, which were inhibitory when applied both as mycelium and as crude culture filtrates. This phenomenon was previously reported for D. teres in vitro (22) and was widely re-

![Fig. 2. Effect of a pretreatment of barley straw with mycelium or culture filtrates of antagonists on the morphogenesis of the sclerotoid organs of the (A) teres and (B) maculata forms of Drechslera teres on straw. UC = untreated control. See Table 1 for code identifications. Bars with the same letter are not significantly different according to Newman-Keuls test (P = 0.05).]

Table 1. Effect of different antagonist strains on morphogenesis of the sclerotoid organs of Drechslera teres f. teres and D. teres f. maculata on barley straw previously inoculated with either form of D. teres

<table>
<thead>
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<th>Treatments</th>
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<th>After treatment</th>
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<tr>
<td></td>
<td>teres form</td>
<td>maculata form</td>
</tr>
<tr>
<td>Untreated control</td>
<td>53.7 a¹</td>
<td>49.3 a</td>
</tr>
<tr>
<td>Trichoderma viride (Tovo)</td>
<td>55.1 a</td>
<td>50.8 a</td>
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<tr>
<td>T. viride (Tv)</td>
<td>52.9 a</td>
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<td>20b</td>
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¹ Means within columns followed by the same letter are not significantly different according to Newman-Keuls test (P = 0.05).
reported for other microorganisms in vitro and in vivo (11,19,20–23,26,32). These results indicate that potential antagonists of *D. teres* can be selected from the fungal populations naturally occurring on barley straw. The antagonists proved to be efficient inhibitors of *D. teres* development when applied prior or subsequent to *D. teres* colonization of the barley straw. Pfender (24) reported the inhibition of morphogenesis of *Pyrenophora tritici-repentis* (Died.) Drechs. sclerotia by a basidiomycete isolated from crop residues. Inhibition of perithecial morphogenesis and/or of in vivo sclerotia germination in *Rhizoctonia solani* Kühn by *Glisppadium virens* J.H. Miller, J.E. Giddens, & A.A. Foster (16), or in *Sclerotium rolfsii* Sacc. by actinomycetes (26), mycorrhizae (18), or various *Trichoderma* sp. (2,7,12,15,22) has been reported. These studies were carried out in vitro or in vivo under controlled conditions, and verification of the activity of antagonists under field conditions is necessary. Further studies are necessary to understand the mechanisms of sclerotia morphogenesis inhibition under natural conditions and the interaction between antagonists and *D. teres*, as well as among antagonists. It is possible that the suppression of sclerotia morphogenesis might be the result of the application of massive amounts of antagonist inocula, which induces an imbalance in the microbial population that is favorable to secondary colonizers of crop residues under natural conditions.

The antagonistic action of microorganisms investigated may proceed through mycoparasitism (contact, enzyme, or exocellular component production) via toxins or through a “toxin-mycoparasitism” synergistic effect (31–33), which could account for the striking differences observed between the effects of mycelia of the various antagonists and those of crude filtrates, particularly for *Trichoderma*, where the release of antibiotics has been demonstrated (7,22,32). A cytobiochemical investigation of the action of antagonists on *D. teres* mycelium that developed from treated sclerotoid organs should be considered, to provide information that would increase the efficiency of antagonists and to assess the possible ecological consequences of field applications. Decreases in disease severity through the application of various antagonists have often been reported (11–14,19,26,28–30). However, there are few published reports which have recorded inhibition or stimulation of the aggressiveness of *D. teres* or of any other pathogenic mycelium that developed from the germination of resting organs treated with an antagonist. Additional studies are required, involving more strains of the two pathogen forms, to account for the large spatiotemporal variability of *D. teres* aggressiveness (3,4,6). The forms *teres* and *maculata* reacted
differently to the antagonists; this is not surprising since both pathogen forms were already known to behave differently in intensive barley cropping systems (8, 30). These results would suggest that two different subspecies are actually involved, as already proposed by some authors (10, 25). The use of techniques in biochemistry and molecular biology (restriction fragment length polymorphism and RAPD) might contribute to advances in chemical and/or biological control through the development of markers for early detection of the infected seeds.

It would be interesting to evaluate the antagonists identified in this study on the other resting forms of D. teres such as chlamydospores, resting mycelium, or conidia, and on epidemiological parameters such as disease intensity and duration of lag phase and sporulation. An efficient biological control strategy will most likely be the result of a combination of different antagonists that will vary with the target species, strains, and even the nature of the substrate.

LITERATURE CITED


