Epidemiology and Control of Seed-borne *Drechslera teres* on Barley

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Net blotch caused by *Drechslera teres* is an important disease in most barley-growing areas. To prevent the introduction of this pathogen into the field, seed treatment is recommended. The objectives of this research were to evaluate different fungicides for eradicating *D. teres* from the seed and the role of both infected and treated seeds in the epidemiology of the disease under field conditions. The three fungicides tested in vitro (iminoctadine, guazatine, and thiram + iprodione) were able to eliminate *D. teres* at the highest dose used in this study. Under field conditions, eradication of the pathogen was not achieved, but net blotch was significantly reduced.

*Keywords: Pyrenophora teres, fungicides, Hordeum distichum*

Introduction

Two-row barley (*Hordeum distichum*) is one of the most important cereals grown in Argentina, with a cultivated area of 250,000 ha in the 2002–2003 growing season. In recent years, with the increasing use of no-tillage agriculture and the popularity of susceptible cultivars in combination with monoculture, net blotch of barley caused by *Drechslera teres* (Sacc.) Shoem has become an increasing problem. There was a severe net blotch epidemic in the Pampean Region in the 1990–1991 crop season (Carmona 1994). Disease surveys from 1990 to 2000 showed that net blotch was the most important barley disease in Argentina, with average yield losses of 20% (Carmona et al. 1999). Besides, *D. teres* was the

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most frequent seed-borne pathogen in 1992 (Barreto and Carmona 1993). The main sources of *D. teres* inoculum are infected seeds and infected crop residues. Several reports have indicated that the infected seed is an important means by which *D. teres* survives, spreads, and initiates primary foci in net blotch epidemics (Piening 1968; Shipton et al. 1973; Hampton 1980; Jordan 1981). To prevent re-introduction of the pathogen, mainly in areas with crop rotation, fungicide seed treatment is recommended. Data of seed treatment studies in vitro indicate that the fungicides iminoctadine, guazatine and iprodione are efficient against *D. teres* with a control of up to 99% (Carmona et al. 1999). However, for crop protection actions, epidemiology studies are needed to understand the disease development and to assess yield losses under field conditions caused by *D. teres* seed-borne disease. The objectives of this study were to evaluate the efficacy of different fungicides for the eradication of *D. teres* from barley seeds and to study the role of both infected and chemically-treated seeds on net blotch epidemiology under field conditions.

**Materials and methods**

**Eradication study**

Seeds collected from severely infected barley fields planted with the susceptible cultivar Quilmes Pampa in 1999 were tested on a selective agar medium developed for *Cochliobolus sativus* (Reis 1983). Three fungicides were applied as slurries: iminoctadine (experimental, 25% EC), guazatine (as Kenopel 40% LS) and thiram + iprodione (as Rovrin 60 + 20% WP) at the following doses: 150, 200, 250 and 300 g or ml per 100 kg of seed. Each chemical treatment was applied to 300 g of seed in a 500-ml Erlenmeyer flask, plus 2% water. The flasks were shaken to ensure a uniform distribution and then air-dried at room temperature (24 °C). Untreated seeds (controls) were similarly processed using sterile distilled water. The effectiveness of the fungicides was evaluated in vitro using 400 seeds plated onto selective agar medium plates, as previously mentioned, with four replicates of 100 seeds per fungicide and dose. Plates were incubated for 10 to 12 days in a growth chamber at 25 ± 2 °C, exposed to 12 h alternating periods of near-ultraviolet light (NUV, TL 40W/08) and darkness. Seeds were examined for conidiophores and conidia of *D. teres*. The effects of fungicides and doses and their interactions on *D. teres* seed infection and seed germination were first analyzed by the GLM procedure (SAS 1988). Later, the significance of the differences between fungicides and doses were evaluated by the Tukey’s test.
**Epidemiology study**

Field trials were conducted at our experimental field at Castelar (INTA, Buenos Aires province) in 2000. To avoid inoculum from other sources, the field was never sown with barley and there were no barley fields in the vicinity. Two 80 m² plots (2 × 40 m), spaced 20 m apart were sown on July 19th. One plot was planted with treated barley seeds (iprodione + thiram at a dose of 300 g/100 kg of seed) and the other plot was planted with untreated infected barley seeds. Seeds were planted at a density of 110 kg/ha in rows spaced 15 cm apart. Incidence of net blotch in plumules was registered from August 15, in five subplots of 2 m² per plot (Wester 1992) at 5–7-day intervals starting 19 days after emergence. The symptomatic transmission efficiency of the pathogen (STE%) was calculated with the following formula: STE (%) = PS (%) / S (%) × 100; where PS = Incidence of *D. teres* in plumules and S = Incidence of *D. teres* in seeds. Intensity of leaf disease was recorded from five plants sampled from each subplot. In the laboratory, a leaf was considered infected when it had at least one spot of 2 mm. Each infected leaf was examined after passing through the humid chamber to detect *D. teres* sporulation. Leaf disease incidence and severity between the untreated and fungicide-treated barley plots were registered at each observation date and the data were analyzed by paired comparison *t*-tests. Differences in disease intensity (plumule disease incidence, leaf disease incidence and leaf disease severity) and trapped airborne spores between the untreated and fungicide-treated barley plots were calculated at each observation date and analyzed by paired comparison *t*-tests (SAS 1988).

**Airborne spores**

To quantify airborne conidia, spore traps (Reis and Santos 1985) were placed at the centre of each plot, 5 cm above the canopy from 14 days after sowing. A vaseline-coated microscope slide was mounted inside the trap orifice and changed weekly. Spore number of *D. teres* conidia was recorded on the slide using a microscope.

**Comparison of yield and seed infection**

At maturity, yield of each plot (non-treated and treated) was hand-harvested. Total seed weight of 375 heads per plot (75 spikes taken at random in each of the five subplots) and the weight of 1000-grain were determined. Seed infection of *D. teres* of each plot was evaluated using four replicates of 100 seeds plated on the...
agar selective medium (Reis 1983) and incubated, as described above. The means of both 1000-grain weight and seed re-infection of *D. teres* for untreated and fungicide-treated barley plots were analyzed by the *t*-test (SAS 1988).

**Results**

**Eradication study**

The seed lot used in this study showed 68.75% of *D. teres* seed infection and 84% of germination. The analysis of variance of the effects of fungicide seed treatments and their interactions on seed infection resulted significant. According to this last result, Tukey’s test, using previously transformed data by the arcsine of the square root, was utilized to evaluate the significance of the differences between the three fungicides per dose and the four doses per fungicide. The three fungicides gave a total control of the pathogen at a dose of 300 g or ml. Besides, iminoctadine reached the same control at a lower dosage of 250 ml (Table 1). In contrast, the fungicides did not affect significantly seed germination (range from 81.3 to 87%).

**Table 1. Effect of fungicide seed treatments at different doses on seed infection (%) of *Drechslera teres***

<table>
<thead>
<tr>
<th>Fungicide</th>
<th>Dose (g or ml/100 kg seed formulated product)</th>
<th>150</th>
<th>200</th>
<th>250</th>
<th>300</th>
</tr>
</thead>
<tbody>
<tr>
<td>Iminoctadine</td>
<td></td>
<td>1.25</td>
<td>0.25</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>Iprodione + thiram</td>
<td></td>
<td>6.0</td>
<td>2.25</td>
<td>1.5</td>
<td>0.0</td>
</tr>
<tr>
<td>Guazatine</td>
<td></td>
<td>9.5</td>
<td>4.0</td>
<td>2.25</td>
<td>0.0</td>
</tr>
</tbody>
</table>

Seed lot used with 68.75% of natural *D. teres* infection. Tuckey’s tests were performed using the arcsine of the square root transformation of the original seed infection values (in percentage). A,B,C = Values in files (differences between doses per fungicides) followed by the same capital letter are not significantly different based on Tukey’s test (*P* < 0.05). a,b,c = Values in columns (differences between fungicides per dose) followed by the same lower-case letter are not significantly different based on Tukey’s test (*P* < 0.05).

**Epidemiology study**

Net blotch was the only disease observed in the barley field plots. Although symptoms on plumules were observed in both plots 19 days after emergence, the incidence of *D. teres* in plumules was 6.5% and 0.30% in the non-treated and fungicide-treated plots respectively. The maximum *D. teres* symptomatic transmis-
sion efficiency (MSTE) values registered from seed to plumule was 18.8% and 0.75%, with mean values of 14.5% and 0.59%, for untreated and treated plots respectively. Disease intensity on leaves was also significantly different in both plots. Incidence increased progressively from the primary foci of inoculum reaching the maximum level 37 days after sowing (12.97 and 0.5%, Table 2). During the spring, the disease increased dramatically in the untreated plot and it was significantly higher than that observed in the treated barley plot. This situation remained during the entire growing season (Table 2). From October to November, the disease progressed faster in the untreated plot than in the treated plot, reaching the maximum incidence and severity on November 12th (100% and 38%, respectively). However, in the treated plot, disease measurements presented values of 49% and 3.9%, respectively (Table 3).

Table 2. Plumule net blotch incidence in non-treated and treated seed fungicide iprodione + thiram in barley field plots in 2000

<table>
<thead>
<tr>
<th>Observation Date</th>
<th>Plumule net blotch incidence (%)*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Non-treated plot</td>
</tr>
<tr>
<td>August 15th</td>
<td>6.46</td>
</tr>
<tr>
<td>August 20th</td>
<td>8.60</td>
</tr>
<tr>
<td>August 27th</td>
<td>11.88</td>
</tr>
<tr>
<td>September 3rd</td>
<td>12.97</td>
</tr>
</tbody>
</table>

\[ t\text{-test value:}**\ 6.59 \]
\[ P > t: 0.0071 \]

* Data of incidence taken from five subplots of 2 m2 of each non-treated and treated plots at each observation date.

** Paired comparison t-test: differences in plumule net blotch incidence between non-treated and treated plots were calculated. The average of these differences was significantly different from zero.

Table 3. Net blotch incidence and severity in non-treated and treated seed with iprodione + thiram in barley field plots during 2000

<table>
<thead>
<tr>
<th>Observation date</th>
<th>Leaf disease incidence (%)</th>
<th>Leaf disease severity (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Non-treated</td>
<td>Treated</td>
</tr>
<tr>
<td>October 1st</td>
<td>43.0</td>
<td>17.8</td>
</tr>
<tr>
<td>October 8th</td>
<td>55.3</td>
<td>20.3</td>
</tr>
<tr>
<td>October 15th</td>
<td>70.5</td>
<td>28.6</td>
</tr>
<tr>
<td>October 22th</td>
<td>72.0</td>
<td>30.0</td>
</tr>
<tr>
<td>October 29th</td>
<td>75.0</td>
<td>31.7</td>
</tr>
<tr>
<td>November 5th</td>
<td>89.6</td>
<td>33.3</td>
</tr>
<tr>
<td>November 12th</td>
<td>100.0</td>
<td>49.1</td>
</tr>
</tbody>
</table>

\[ t\text{-test value:}* 10.86 \]
\[ P > t: 0.0001 \]

*Paired comparison t-test: differences in leaf disease incidence and severity between non-treated and treated plots were calculated. The average of both disease incidence and disease severity differences were significantly different from zero.
Airborne spores

No *D. teres* conidia were trapped before sowing, thus indicating the absence of additional source of inoculum in the area. Later, significant differences between treatments in the amount of airborne inoculum were detected. The first conidia were captured on August 27\(^{th}\) (1 month after sowing) only in the control plot. A large number of conidia occurred on November 12\(^{th}\) in the untreated control plot (total 46). On the other hand, the number of conidia registered in the treated plot from October 8\(^{th}\) to November 12\(^{th}\) was between 0 and 7.

Comparison of yield and seed infection

The weight of 1000 grains was significantly affected by the seed fungicide treatment. This yield component corresponding to the treated plot resulted in 11.0% higher than the observed in the non-treated plot. Regarding to re-infection of the pathogen in the harvested seeds, there were significant differences between the non-treated (26% of seed infection) and treated (3.5%) plots (Table 4).

**Table 4.** Mean values of 1000-grains weight and seed re-infection (%) of *Drechslera teres* calculated for both non-treated and fungicide-treated (iprodione + thiram) seeds in barley field plots in 2000

<table>
<thead>
<tr>
<th>Variable*</th>
<th>Mean value</th>
<th>Non-treated</th>
<th>Fungicide-treated</th>
<th>t-test value</th>
<th>P &gt; t**</th>
</tr>
</thead>
<tbody>
<tr>
<td>1000-grain weight (g)</td>
<td></td>
<td>45.49</td>
<td>50.51</td>
<td>5.85</td>
<td>0.001</td>
</tr>
<tr>
<td>Seed re-infection (%)</td>
<td></td>
<td>26.0</td>
<td>3.5</td>
<td>-9.52</td>
<td>0.001</td>
</tr>
</tbody>
</table>

* Data are means of 4 samples of 100 seeds (400 seed/plot).

** For each variable analyzed, values from non-treated and treated seeds were significantly different using mean difference t-test (P < 0.001)

Discussion

The three fungicides tested in vitro as seed treatments of barley were completely effective on both inhibition of mycelium growth and sporulation of *D. teres* at the highest concentration used in this study (300 g or ml per 100 kg of seed). Similar effects were obtained with iminoctadine at a lower dose (250 gr/100 kg seed) in agreement with previous reports (Reis et al. 1995). However, *D. teres* was not completely inhibited at lower concentrations of the fungicides. In practice, seed treatment is often limited to a reduction of inoculum of both infection percentage and amount of inoculum in individual seeds (Neergaard 1977). Since a 100% of control can be rarely be obtained, the fungicide treatments should
be judged by their capacity of reducing the inoculum level under field conditions (Maude 1983). Despite the eradication obtained in vitro with iprodione + thiram, net blotch developed in plants grown from both non-treated and treated seeds under field conditions. Due to the absence of other sources of inoculum at our study, plumule infection observed after seedling emergence indicated that the infected seed is an important source of inoculum in the field. However, the number of primary foci of infection was larger in seedlings in the control plot when compared to the treated plot. Taking into account the amount of infection in seeds and the incidence of the disease in the plumule, seed transmission efficiency reported in this study was 18.8% and 0.75%, respectively. About one month after sowing, the disease incidence in plumule increased in the control plot from 6.46 in mid-August to 12.97 at the beginning of September, values much higher than in the treated plot for the same period (0.3 to 0.5). During the spring, *D. teres* conidia in the air indicated the secondary spread of the pathogen in the field. This fact was in agreement with the symptoms observed in the leaves. Although the disease was detected in both plots, the rate of increase was reduced significantly in the treated plot. According to our results and taking into account that the alarm threshold for net blotch disease control has been established in 60% for leaf incidence and 5% for severity (Carmona and Schmidt, unpublished), the development of the disease did not reach this threshold in the treated plot. However, disease score was higher than the alarm threshold during October for both leaf incidence (70.5%) and severity (6.4%) in the plot planted with naturally infected seeds. Although seed transmission of *D. teres* has been reported previously, our results confirm the importance of its contribution to net blotch development under field conditions. Seed transmission of *D. teres* from seed to plumule has been shown to be 9% in greenhouse (Carmona et al. 1999). In this study, we established a maximum rate of seed transmission efficacy of 18.8% under field conditions. Both similar and higher rates of seed transmission have been reported for other leaf spots of cereals such as *Drechslera avenae* (Carmona et al. 1999; Carmona et al. 2004) and *D. tritici-repentis* (Carmona et al. 2006; Schilder and Bergstrom 1992). Several factors are known to influence seed to crop infection, such as the number of infected seeds brought into the soil, the seeding rate, and the edaphic and climatic conditions. Among the major environmental factors, the dew hours, the amount of rain and the intensity of wind-blown rain determine the length of the latent period for production of new spores and the spread of conidia from the infection court (Leach 1979). These factors can also explain the different seed-transmission rates data between the greenhouse and the field experiments. The yield losses and the rate of re-establishment of seed-borne inoculum in the harvested seed are also crucial factors in setting inoculum threshold in seed.
Crop losses, determined from both untreated and treated seed samples planted into the field, indicated a significant difference between yield components. At harvest, an increase in both seed weight and 1000-grain weight in the treated plot (22.4% and 11.0%, respectively) was significantly different from the control plot. The increased yield in treated seeds was probably due to reduced intensity of the disease and the contribution of the green leaves, including the flag leaf. Similar losses have been reported by Jordan (1981), Sutton and Steele (1983) and Smedegard-Petersen (1974). The re-infection of the pathogen in the harvested seed was significantly higher in the non-treated plot (26%) than in the treated one, where the infection reached only 3% in the harvested seed. According to the results of re-establishment, seed treatment is not recommended for seeds harvested from the non-treated plot, since it did not reach the infection level of 5%, indicated as the tolerance level for sowing seeds under Denmark’s conditions (Jorgensen 1983).

In the present study an approach was made to establish the effect of planting infected seeds on the extent of net blotch from seed to crop and the advantage of planting seeds with efficient fungicides before sowing. The seed-borne inoculum resulted in a risk to barley crop and economic yield loss. Control of seed-borne inoculum of this pathogen plus crop rotation is needed to protect plants from an epidemic, and consequently to avoid the expensive application of a foliar program with fungicides.

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