TAN SPOT OF WHEAT CAUSED BY *Drechslera tritici-repentis*: DETECTION, TRANSMISSION, AND CONTROL IN WHEAT SEED

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Summary

Tan spot of wheat caused by *Drechslera tritici-repentis* is a major leaf spot disease. The objectives of this work were to estimate the incidence of *D. tritici-repentis* in wheat seed using different techniques, determine the rate of seed transmission under greenhouse and field conditions, and to evaluate fungicide seed treatments for disease control. The incidence of seed infection was 22.5%; 17.5% and 11.25% on a selective medium for *Cochliobolus sativus*, lima bean agar and in the deep-freezing method respectively. On agar, the fungus produced black immature pseudothecia on the seeds and sometimes on the roots; the pseudothecia maturated after 30 days in darkness at 5°C. The pathogen was transmitted from seed to coleoptiles of symptomless plumules seedlings at a rate of 15.5% under greenhouse conditions, and to the plumule with tan spot symptom at a rate of 31% under field conditions. The seed treatment study indicated that a mixture of iprodione + triticonazole was the most effective treatment, reducing the rate of transmission from 31% to 8.8%. This is the first report of detection and transmission of *D. tritici repentis* in wheat seed in Argentina. The importance of infected seed and its epidemiological role are discussed.

Key Words: *Pyrenophora tritici-repentis*, seed health tests, seed transmission, seed treatments, fungicides.

Introduction

Tan spot, caused by *Drechslera tritici-repentis* (Died.) Drechs., is a major disease of wheat worldwide (Wiese, 1987). Its prevalence and severity started increasing in Latin America in 1982 (Kohli *et al.*, 1992). In Argentina there has been a marked increase in its intensity mainly due to the trend towards conservation tillage and monoculture which favor disease development. Wheat is the most important cereal crop in Argentina. During 2004/05, 16 million tons were harvested from 6.2 million hectares. The higher production is located in the Pampeana Region representing 92.2 % of the total production of the country (Yalungo, 2006). During 2000, out of 19 wheat cultivars, 14 were susceptible to tan spot in natural epidemics. In 2003, 67% (on 12 cultivars) were still recorded as susceptible. This situation changed in 2004, where only 3 cultivars from 27 evaluated, were susceptible (Annone and Garcia, 2005).

One of the most important source of inoculum is infected wheat residues, where the pathogen remains viable, in saprophytic stage, between crop cycles. Although the disease is well known, a few reports are available on the impact of wheat seed-borne infection and seed

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transmission. The introduction of the pathogen into new wheat areas or areas under rotation has likely been by seed. Although *D. tritici-repentis* has been isolated from wheat seed and seed transmission demonstrated (Schilder and Bergstrom, 1992; 1993; 1995; Fernandez *et al.* 1996), the detection and control of the fungus in the seed before sowing are not frequent practices in South America. In Argentina, in spite of the importance of the disease, seed is mainly treated against *Ustilago nuda* (Jens.) Rostr. and *Gibberella zeae* Schwein (Petch), causal agents of the loose smut and seedling blight diseases, respectively. Since some field are still free of tan spot, information on the seed transmission under local conditions, is needed to avoid the introduction of inoculum into new fields or under crop rotation, and to choose effective fungicides for control.

The objectives of the present study were to compare methods for detection of *D. tritici-repentis* in wheat seed, determine the rate of seed transmission in the greenhouse and the field, and to evaluate fungicide seed treatments for disease control. Some preliminary results from this work have already been reported (Carmona *et al.* 1998).

Materials and methods

Seed health testing. A seed lot of a very susceptible wheat cultivar INTA Super was obtained from a severely tan spot-infected field in Buenos Aires Province in 1999. A sub-sample of seed was surface-disinfected by soaking in 95% ethanol for 30 sec and in 1% sodium hypochlorite for 30 sec, followed by rinsing in sterile distilled water (Schilder and Bergstrom, 1993). Detection of the pathogen in the seed was carried out using the deep-freezing blotter test (Jorgensen, 1983), a selective agar medium for *Cochliobolus sativus* (Ito & Kurib.) Drechsler ex Dastur (Reis, 1983) and lima bean agar (Dhingra and Sinclair, 1986). A total of 400 seeds (4 replicates of 100 seed) were used for each method. In the deep freezing method, 25 seeds were placed on 3 layers of moist filter paper per plate (4 plates per replicate). The plates were incubated at 20-25°C for 1 day, at -20°C on the second day, and at 20-25°C under alternating periods of 12-h near-ultraviolet light (Philips Black-light lamps, TL 40w/08) and 12-h darkness for the following six days. In the agar plate methods, ten seeds were plated on either selective medium or lima bean agar in each plate (10 plates per replicate). Selective medium was prepared with potato, sucrose, amended with fresh solution of captan, benomyl, dicloran, streptomycin sulfate, and neomycin sulfate (Reis, 1983). Fungicides and antibiotics were added to the medium after cooled down to 55°C. After 10 days of incubation, seeds were observed by a low power stereo-microscope with up to 40x magnification. Identification of the fungus was based on the presence of conidiophores and conidia of the pathogen. In the case of pseudothecia, seeds were incubated for a longer period to allow their maturation. Infection incidence (%) values in the three assays were transformed to $\sqrt{(x + 0.5)}$ to reduce skewness and stabilize distributional variance. Values were subjected to analysis of variance (ANOVA), followed by mean separation using Tuckey's test.

Greenhouse seed transmission. This study was conducted using the same seed lot as indicated above. Five hundred seeds were sown in cells of plant flats containing soil mix, one seed per cell, and maintained in a greenhouse at 10-25°C (17.5°C average) and watered from below. After 30 days, at Zadoks growth stage (ZGS) 1.2 (Zadoks *et al.* 1974), 200 coleoptiles from symptomless plumules seedlings were cut separately and aseptically at 1.0 to 1.5 cm

above the ground, aseptically placed without disinfection on selective medium (Reis, 1983) at the rate of 10 coleoptiles per 9-cm culture plate, and incubated for 10 days under alternating periods of 12-hr NUV light on/off cycle. Between each extraction, pincers and scissors were soaked in alcohol 95% and flame-sterilized. Coleoptiles were examined for the presence of the fungus conidiophores and conidia and incidence was recorded.

The transmission efficiency (TE) of *D. tritici repentis* from seed to coleoptile was estimated from the incidence of coleoptiles colonized by the fungus (C) and the incidence of seeds infected by the pathogen (S) as follows: TE = C (%) / S (%) × 100.

Field seed transmission and efficacy of fungicide seed treatments. This assay was conducted using the same seed lot of INTA Super cultivar with a *D. tritici-repentis* infection incidence of 22.5% based on the selective seed health testing assay. A commercial sandy loam field at Alberti, Buenos Aires province, was used for the field trial. To avoid inoculum from crop residues, this plot had never been sown to wheat before. The plot was seeded in August 4, 2000.

Untreated and fungicide treated seeds were sown in plots of 6 m long and 3 m wide, and arranged in a randomized complete block design with four replications. During the 30 days period of this trial, the temperatures were cool (4-18°C), with 22 mm as total precipitation. Three fungicides were applied as slurries: tebuconazole (Raxil 2%) at 42 cc of product per 100 kg of seed, iprodione + triticonazole (Rovral 50% FS + Premis 2.5% FS) at 100 cc + 100 cc per 100 kg of seed, and difenoconazole (Dividend 3%) at 250 cc per 100 kg of seed. Each treatment was applied to 500 g of seed in a 500-ml Erlenmeyer flask plus 2% water (vol/vol). Untreated seed, processed using sterile distilled water, was used to evaluate the maximum degree of transmission from seed to seedling. The rate of symptomatic seed transmission for each treatment was estimated on 50 seedlings at ZGS 1.2 taken at random from each plot 30 days after sowing. Plumules with tan spot symptoms were kept in a humid chamber at 20-25°C and alternating periods of 12-hr NUV on/off cycle for 3-4 days in order to induce sporulation. Plumules were examined after 3-4 days for presence of conidiophores and conidia.

The seed transmission efficiency (STE) for each treatment was determined using the following formula (Carmona *et al.* 1999; 2004): STE = PS (%) / S (%) × 100, where PS denotes incidence of tan spot on plumules and S denotes incidence of seed infection by *D. tritici-repentis*. The effectiveness of fungicide seed treatments in controlling the pathogen also was recorded. Data were transformed to $\sqrt{(x + 0.5)}$, and subjected to analysis of variance (ANOVA), followed by mean separation using Tukey's test.

Results

Seed health testing. Results of the three types of assays used to detect *D. tritici repentis* are shown in Table 1. The agar methods were more sensitive in detecting the fungus than the deep-freezing method. Similarly, visual sporulation of the fungus on the seed was generally heavier in the agar methods than in the deep-freezing method. However, the selective medium was the most effective and revealed a higher incidence of seed infection than the other methods. This assay was also easy and quick for recording the presence of *D. tritici-repentis* on the seed.

Table 1. Incidence of *Drechslera tritici-repentis* in seed health assays of wheat cultivar INTA Super harvested in Buenos Aires Province, Argentina in 1999.

Seed health testing method	% Seed infection
Deep-freezing	11.25a*
Lima bean agar	17. 5 ab
Selective medium for *C. sativus*	22.5 b

*Average values of 400 seeds per method (4 replicates of 100 seeds). Values followed by the same letter are not statistically different according to Tukey's test at $P = 0.05$.

Observations on the anamorphic and teleomorphic stages. The anamorph was encountered in all assays tested. The conidia were sub-hyaline or pale yellow, 4-10 (6) septate; and 138-229 (180) μ × 9.25-14.8 (13.8) μ in size. The conidia were cylindrical with a rounded apex and a characteristic conical basal cell, suggestive of a snake's head, with an inconspicuous scar as described by Ellis (1971) and Drechsler (1923). In the agar tests, the fungus produced black immature pseudothecia in the seeds and sometimes in the roots. These pseudothecia matured after 30 days in darkness at 5°C. The pseudothecia were black, erumpent with dark spines surrounding the short beak. The mature ascospores were oval, yellowish-brown with 3 transverse and sometimes 1-2 longitudinal septa. They measured 37-48.1 (41.6) μ × 14.8-22 (19.1) μ.

Seed transmission in the greenhouse and in the field and efficacy of fungicide seed treatments. The seed transmission studies under greenhouse conditions showed that 30 days after sowing, *D. tritici-repentis* was detected in coleoptiles from symptomless plumules seedlings with a transmission efficiency (TE) from seed to coleoptile of 15.5%. In the field trial, tan spot was the only disease observed on the seedlings 30 days after sowing. The maximum rate of symptomatic transmission from untreated seed to the plumule was 31%. In comparing fungicide seed treatments, the most effective fungicide was the mixture of iprodione + triticonazole, which provided 71% control (Table 2).

Table 2. Effect of seed treatment fungicides on seed transmission of *Drechslera tritici-repentis* in wheat cultivar INTA Super (22.5% seed infection) under field conditions in Alberti, Argentina, in 2000.

Treatment	Product dosage per 100 kg seed (cc)	Tan spot Incidence on the plumule (%)	Seed transmission efficiency (%)	Control (%)
Untreated	--	7.0 a*	31.0	--
Tebuconazole 2%	42	6.0 a	26.6	14
Difenoconazole 3%	250	4.5 a	20.0	36
Iprodione 50% + Triticonazole 2.5%	100 + 100	2.0 b	8.8	71

*Average values of 50 seedlings from each plot of 4 replications. Values followed by the same letter are not statistically different at according to Tukey's test at $P = 0.05$.

Discussion

The importance of *D. tritici-repentis*-infected seed and its epidemiological role was confirmed in this study. Detection of cereals pathogens on seed is commonly carried out by using the routine deep- freezing blotter test (Jorgensen,1983). However, this study showed that the selective medium developed for *Cochliobolus sativus* (Reis, 1983) was the most sensitive. The incorporation of some fungicides (captan, benomyl, and dicloran) and antibiotics into the medium not only did not inhibit the growth of *D. tritici- repentis*, but also suppressed the growth of other fungi (*Aspergillus, Penicillium,* and *Fusarium*) and some bacteria, as *Bacillus*, observed frequently in the deep-freezing method, that could mask the sporulation of *D. tritici-repentis* on seed. This fact facilitated the detection of the target fungus and gave a higher record of the incidence. A previous report showed that this selective medium was consistently more sensitive than routine tests in detecting *Drechslera teres* (Sacc.) Shoem, causal agent of net blotch, in barley seeds (Carmona *et al.* 1999).

The fungus was usually observed as *Drechslera* anamorph. However, the uncommonly teleomorphic stage has had also observed as inmature pseudothecia in the seeds and sometimes in the roots in the agar tests. Schilder and Bergstrom (1993) and Fernandez *et al.* (1996) also reported immature pseudothecia on seed pericarp and roots.

The transmission studies in the greenhouse showed that the pathogen could colonize the coleoptile with a transmission efficacy of 15.5% and therefore may provide another source of dissemination apart from the plumule. Similar observations were reported in the transmission of *D. teres* from barley seed (Carmona *et al.* 1999).

Since field transmission was carried out in a non-wheat-plot, infections of seedlings came from infected seed and reached a seed transmission efficacy of 31% from seed to plumule. In the field trial the air temperature (4-18°C) was lower than at the greenhouse (10-25°C), and field precipitation occurred (22 mm) indicating that different environmental conditions may affect seed transmission.

The presence of *D. tritici-repentis* in wheat seed and its transmission to coleoptile and plumule were confirmed in the present investigation, and could explain the reasons for the sudden outbreak of tan spot in Argentinian fields which had never been seeded to wheat or in fields that were previously under rotation. Although seed transmission of *D. tritici-repentis* was shown previously (Schilder and Bergstrom, 1993), our results confirm the importance of its contribution to tan spot introduction, indicating that the fungus can be transmitted from seed to coleoptiles of symptomless plumules seedlings as well as to plumules causing typical necrotic lesions.

The transmission of *D. tritici repentis* to the coleoptiles as well as plumules agreed with studies made by Schilder and Bergstrom (1993) but not with the observations made by Fernandez *et al.* (1996) who only found seed transmission to coleoptiles. Several researchers studied the transmission of *D. tritici-repentis* from seed. Schilder and Bergstrom (1995) reported seed transmission efficiency as high as 92% *in vitro,* and 60% in potting soil outdoors. Obst (1989) reported a transmission rate to seedlings of 15% and Hyltén-Cavallius (1984) recorded a 59% seed-to-seedling transmission rate. These results should be analyzed taking into account that the seed transmission process can be affected by many factors. The susceptibility of cultivar, percentage of seed infection, method of detection, agressiveness of the isolate, soil moisture, temperature, cultural practices, sowing depth, rain and light can

potentially affect seed transmission rates. Since *D. tritici-repentis* was able to infect seedlings and colonize the coleoptile early, special attention should be paid to efficient seed transmission control measures. The seed incidence of *D. tritici-repentis* and the efficiency of its transmission showed that a specific test for the detection of this fungus should be applied before sowing. The selective agar medium used for *C. sativus* would be most suitable for this purpose.

Seed fungicide treatment, as an attempt to remove seed–borne inoculum, is usually not completely successful. In this study an important control of tan spot infection under field conditions was obtained with the mixture of iprodione + triticonazole which reduced the rate of seed transmission of. *D. tritici-repentis* from 31% to 8.8%. In vitro assays on the related fungus *D. teres* on barley seed indicated that this fungicide mixture reduced the incidence of this fungus from 78.5% (untreated control) to 2.25% (Carmona *et al.* 1999).

To summarize, this study indicates that *D. tritici-repentis* may be detected in wheat seeds by a selective medium for *C. sativus* (Reis, 1983) and is transmitted from seeds to plumules and coleoptiles. In this way, inoculum in the seed could introduce the pathogen or its strains in new fields and provide a source of inoculum for epidemics. Conidia produced on the infected coleoptile or on necrotic lesions on the plumule will be carried by the wind to the leaves where secondary cycles will develop. Seed treatments with an effective fungicide plus crop rotation would help in obtaining a healthy crop. More research is needed to investigate the relationship between seed infection and tan spot epidemics in fields, the influence of environmental and biotic factors on the seed transmission process, the effect of particular levels of seed infection on yield losses, the effects of fungicide seed treatments and the determination of seed infection thresholds for seed treatment applications.

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