

Detection and Transmission of *Drechslera avenae* from Oat Seed

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ABSTRACT

Detection, symptoms and symptomless transmission of *Drechslera avenae* (teleomorph *Pyrenophora avenae*) from seed were investigated. The present study reported that the *D. avenae* is frequently found in oat (*Avena sativa*) seed in Argentina. The prevalence of the seed lot infected was 54%. The incidence of seeds infected by *D. avenae* in the various seed lots from different regions ranged from 0 to 52% (overall mean of 7%). Besides conidia on conidiophore and immature pseudothecia,

D. avenae produced small, spherical to pear-shape picnidia containing tiny conidia, on the seed surface. The pathogen was efficiently transmitted at a rate of 55% from seed to coleoptile tips in symptomless seedlings and at 12.5% to plumules. The importance of the infected seed and its epidemiological role are discussed.

Additional keywords: *Pyrenophora avenae*, *Avena sativa*, seed-borne pathogen, transmission.

RESUMO

Detección y transmisión de *Drechslera avenae* en semillas de avena

El presente trabajo tuvo como objetivos detectar y cuantificar a *Drechslera avenae* en muestras de semillas y evaluar su transmisión hacia los coleoptiles y plúmulas de la avena (*Avena sativa*). Un total de trece muestras resultaron infectadas por *D. avenae*, representando un 54% de prevalencia. Los valores máximos y mínimos de infección obtenidos fueron 0 y 52% con un promedio de 7%. Sobre las semillas,

D. avenae produjo además de conidióforos, conidios y pseudotecios inmaduros, picnidios pequeños, esféricos y con forma de pera conteniendo conidios diminutos. El patógeno fue detectado en los coleoptiles, sin síntomas en las plúmulas con una eficiencia de transmisión del 55%. La tasa de transmisión sintomática fue del 12,5%. Se discuten la importancia de la semilla infectada y su papel epidemiológico.

The leaf spot of oats (*Avena sativa* L.) caused by the necrotrophic pathogen *Drechslera avenae* (Eida) Sharf (Teleomorph *Pyrenophora avenae* Ito & Krib), has been recently observed throughout the Argentinean pampas region (Carmona, unpublished). In some countries, it is a very important leaf spotting disease (Fernandez Valiela, 1978). In Brazil it is the fastest spreading oat disease, mainly due to the trend towards conservation tillage and monoculture, which favours development of the disease (Blum *et al.*, 1999). Although *D. avenae* has been detected on oat seed, there is little published information on detection, transmission and its epidemiological role. In Argentina, even though the pathogen was reported (Fernandez Valiela, 1978), there is no published research the sanitary status of the seed nor the potential role of seed borne *D. avenae*. The objectives of the present work were to detect *D. avenae* in seed samples and to evaluate the potential role of seed borne *D. avenae*, as primary inoculum for oat leaf spot epidemics.

Twenty-four seed lots of white oat were obtained from fields the provinces of Buenos Aires, Entre Ríos and La Pampa in January 2001. Samples of 400 seeds from each lot were evaluated for *D. avenae* by means of Jorgensen's (1983) deep-

freezing blotter test. From each seed lot, ten conidia and ten conidiophores were measured. Minimum, maximum and average measurements were registered.

To test for pathogenicity, isolation was made on potato dextrose agar (PDA) from the seed lot with the highest infection (cv. Millauquén with a *D. avenae* infection incidence of 52%).

Studies of seed transmission were carried out using the same seed lot. The technique applied was the one reported in a previous work (Carmona *et al.*, 1999) but without use of the selective agar medium developed for *Cochliobolus sativus* (Ito & Kuribay) Drech. ex Dastur (Reis, 1983).

One thousand seeds were sown in multipot trays containing soil mix (soil: sand: vermiculate 1:1:1), one seed per pot, and maintained in a greenhouse at 10-30 °C (mean 15.5 °C). The moisture content of the soil was maintained below field capacity. At 30 days after sowing, coleoptiles of 200 symptomless seedlings were cut separately and aseptically at 1.0 to 1.5 cm above the soil, placed without surface disinfestation on three layers of moist filter paper in a 9-cm-diameter petri dish (ten coleoptiles/dish), and incubated for ten days as described earlier.

Transmission efficiency (TE) of *D.avenae* from seed to

coleoptile was estimated from the incidence of coleoptiles colonized by *D. avenae* (C) and the incidence of seeds infected by the pathogen (S): $TE = C(\%) / S(\%) \times 100$. The incidence of plumules with oat leaf spot (PS) was estimated based on observations of 200 seedlings taken at random at 30 days after sowing. Plumules with symptoms were kept in high humidity for two-three days and observed for sporulation of *D. avenae*. Efficiency of symptomatic transmission of the pathogen (STE) was determined as $STE = PS(\%) / S(\%) \times 100$.

The results from seed infection assays showed that a total of 13 samples were infected by *D. avenae*. The prevalence of the seed lot infected was 54%. Incidence of seeds infected by *D. avenae* in the various seed lots ranged from 0 to 52 (overall mean of 7%) (Table 1). The high seed infection and the wide prevalence of *D. avenae* from some regions of Argentina indicate that effective control measures should be investigated and applied before planting. This is the first report about the sanitary status of oat seed in Argentina indicating that considerable attention has to be paid to production and management of oat seed. No tillage farming production without rotation, and the susceptibility of the cultivar, probably would favor seed infection.

The anamorph was encountered in all assays tested. Conidiophores arising singly or in a two-four group straight or flexuous, frequently geniculate, dark, septate, 120 - 364 μm ($X = 234$) \times 8 - 12 μm ($X = 8,5$). The conidia were subhyaline or pale yellow, one-six septate (average four); and 32 - 84 μm (59) \times 10 - 16 μm (11,5). The conidia were cylindrical with a rounded apex, and an inconspicuous scar similar to those described by Ellis (1971). Sometimes, the fungus produced black immature pseudothecia on the seeds. The pseudothecia were black, erumpent with dark spines surrounding the short beak. Although the perfect stage of *D. avenae* has been detected on seed in some countries (Kenneth, 1962; Blum *et al.*, 1999), the mature perfect stage has not been found yet in Argentina (Fernandez Valiela, 1978). Besides conidia and immature pseudothecia, *D. avenae* also produced small, spherical to pear-shape picnidia containing tiny conidia on the seed. This observation confirms the results recorded by other researchers (Dennis, 1933; Kenneth, 1962; Sivanesan, 1987; Mehta, 1999; Blum *et al.*, 1999). This is the first report about the picnidial stage of *D. avenae* in Argentina.

The morphology of conidia, conidiophores, typical large white "tuffs" and characteristics of the culture of *D. avenae*, agree with the descriptions of this species by Drechsler (1923) and Ellis (1971).

At ten days, all seedlings inoculated showed typical symptoms of oat leaf spot. The spots first appear as a small oval brown lesion surrounded by a red or anthocyanin margin. Later the lesion increased in size and produced necrotic foliar tissue. Reisolation confirmed the causal agent as *D. avenae*.

The seed transmission studies showed that at 30 days after sowing, *D. avenae* was detected in symptomless seedling coleoptile tips with a TE from seed to coleoptile of 55%. At the same time, 6.5% of primary leaves from 200 examined, showed typical oat leaf spot symptoms. Symptoms usually appeared as

minute brown spots that become larger with red or purple margins. After incubation, abundant sporulation of the fungus was registered on spots. The rate of this STE, from seed to plumule, was 12.5%. Although these rates appeared low when compared to high seed infection the coleoptile infection, and abundant fructification on the leaf spots would have a considerable role under optimum environmental field conditions. There are many environmental factors that affect the transmission process of seed borne pathogens. Environmental factors such as soil moisture and temperature, rain, light are important because they favour an efficient transmission, and can incite a rapid spread and increase of the disease (Leach, 1979). Once the pathogen is established by seed transmission, it will multiply and spread repeatedly. In this case, wind blown rain is essential for the rapid spread of *D. avenae* in the field. Later, the disease will progress upward in the crop, ending by infecting the seed. Thus, the significance of seed transmission under field conditions may be higher than the one studied in the greenhouse. Similar or higher rates of transmission were reported in other species of *Drechslera* such as *D. teres* (Sacc.) Shoem. (Hamptom, 1980; Carmona *et al.*, 1999) and *D. tritici repentis* (Died.) Shoem. (Schilder & Bergstron, 1995; Carmona *et al.*, 1998). The importance of the infected seed and its epidemiological role were confirmed in this work.

TABLE 1 - Incidence of seed borne *Drechslera avenae* in oat (*Avena sativa*) seed using the deep-freezing method (Jorgensen, 1983)

Sample number	Cultivar	Location	% of Seed Infection
1	Cristal INTA	Bragado, Buenos Aires	13.5%
2	Millauquén	Catriló, La Pampa	52%
3	Cristal INTA	Gualeduaychú Entre Ríos	0%
4	Cristal INTA	Gualeduaychú	2.5%
5	Millauquén	Gualeduaychú Entre Ríos	1%
6	Cristal INTA	Míramar, Buenos Aires	0%
7	Cristal INTA	Castex, La Pampa	0%
8	Sure Grain	Macia, Entre Ríos	8%
9	Cristal INTA	Balcarce, Buenos Aires	0%
10	Cristal INTA	Castex, La Pampa	45.5%
11	Tucana	Macia, Entre Ríos	7%
12	Bonaerense Poyé	Balcarce, Buenos Aires	0%
13	Cristal INTA	Necochea, Buenos Aires	0%
14	Cristal INTA	Míramar, Buenos Aires	0%
15	Cristal INTA	Coronel Suarez, Buenos Aires	2%
16	Millauquén	Coronel Suarez, Buenos Aires	0%
17	Pilar	Coronel Suarez, Buenos Aires	0%
18	Cristal INTA	Gualeduaychú Entre Ríos	16%
19	Cristal INTA	Gualeduaychú Entre Ríos	10%
20	Polaris	Gualeduaychú Entre Ríos	0.5%
21	Pionera	Macia, Entre Ríos	7.5%
22	Polaris	Macia, Entre Ríos	0%
23	Cristal INTA	Gualeduaychú Entre Ríos	4.5%
24	Cristal INTA	Gualeduaychú Entre Ríos	0%
Prevalence			54%
Mean			7%

Data are means of 400 seeds per sample

Seed transmission studies showed that infected seed is an important means by which *D. avenae* survives, and initiates oat leaf spot epidemics in Argentina. The infected seed is also considered to be the potential source of long distance dissemination. Since *D. avenae* was able to infect seedlings and to colonize the coleoptile, with or without symptom, early on, special attention should be paid to efficient seed control measures. Crop rotation plus an effective seed treatment can guarantee a healthy crop that avoids the introduction of the fungus. The results indicate that the *D. avenae* is frequently found in oat seed in Argentina and may be easily detected by a deep freezing blotter method. The fungus is transmitted from seeds to plumules and coleoptiles with or without symptoms expression respectively. Therefore, further field studies are needed to research, the relationship between seed infection and primary inoculum, the influence of biotic and abiotic factors in the seed transmission process, and the effects of seed treatment and level of infection tolerance.

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