

DISEASE NOTES OR NEW RECORDS

Occurrence of *Ascochyta hordei* var. *europaea* on wheat (*Triticum aestivum*) leaves in Argentina

A. E. Perelló^{A,B,C} and M. V. Moreno^{A,B}

^ACentro de Investigaciones de Fitopatología, Facultad de Ciencias Agrarias y Forestales de la Universidad Nacional de La Plata (CIDEFI). Calle 60 y 119 (1900) La Plata, Provincia de Buenos Aires, Argentina. CC 31.

^BCONICET.

^CCorresponding author; email: fitopato@ceres.agro.unlp.edu.ar

Abstract. During the course of a 2002 survey in the wheat cropping area of Buenos Aires Province, Argentina, an unusual disease was found on wheat leaves. From the symptomatic tissues, a fungus was isolated and identified as *Ascochyta hordei* var. *europaea*. To test its pathogenicity and fulfill Koch's postulates, inoculations under greenhouse conditions were carried out. The symptoms of the disease and the causal agent are described.

Ascochyta leaf spot is often overlooked in association with other leaf spot diseases (Wiese 1977; Zillinsky 1984). It is reported on wheat in Japan, Europe and North America as a pathogen of minor economic importance but there are some reports pointing out that high humidity conditions could favour the occurrence of outbreaks of the disease (Scharen and Krupinsky 1971). Its distribution and frequency may be greater than realised, because most cereals and grasses throughout the world are susceptible to *Ascochyta* species and isolates from wheat have a broad host range among the Gramineae (Wiese 1977).

During September and October 2002, leaf spot symptoms on wheat cv. Baguette 10, growing in farmers' fields of Tandil, an eastern area of Buenos Aires Province, were commonly observed. In most of the plants, leaves showed symptoms similar to those described for other necrotrophic foliar pathogens (*Drechslera tritici-repentis* and *Stagonospora nodorum*) suggesting that any of these might have been involved. *Ascochyta* was commonly isolated from affected tissues of the samples collected. *Ascochyta tritici* is generally accepted as the cause of *Ascochyta* leaf spot, but *A. graminicola* is cited in some literature (Zillinsky 1984). The status of *A. tritici* is uncertain but it may be a synonym of *A. hordei* (Punithalingam in Farr *et al.* 1989). Sprague and Johnson (1950) also stated that *A. tritici* is close to *A. hordei*, differing mainly in the symptoms on barley. The identity of the culture of *Ascochyta* isolate A1102 of this study, was determined as *A. hordei* var. *europaea* by experts of the Centraalbureau voor Schimmelcultures (CBS), The

Netherlands, and deposited in the CBS culture collection under the number 112525.

To our knowledge, there are no previous reports of *A. hordei* var. *europaea* on wheat and other grasses in Argentina. In this sense, the first occurrence of this fungus as a member of the leaf spotting complex on wheat plants in the Argentinian cropping area is significant.

Diseased leaves were collected, stored in paper bags and transported to the laboratory. The pathogen was isolated from typical necrotic symptoms. Small sections of diseased leaves were disinfested in 70% ethanol and 0.1% mercuric chloride for 1 min, then, rinsed twice in sterile distilled water and placed on 2% potato-dextrose agar (PDA). Petri dishes were maintained at 22°C. Morphobiometrical and cultural studies of the fungus were conducted on single-spore colonies grown on PDA, cultured at 20 ± 2°C under cool-white fluorescent light supplemented with near UV with a 12 h photoperiod.

Inoculation experiments to confirm pathogenicity were performed in the greenhouse at 15–25°C and 80% relative humidity on 16 wheat cultivars: Buck Arriero, Buck Yatasto, Buck Poncho, Buck Charrúa, Buck Halcón, ProInta Granar, ProInta Cinco Cerros, Desimoni Caudillo, ProInta Imperial, ProInta Puntal, ProInta Guazú, ProInta Colibri, ProInta Elitte, Klein Estrella, Klein Cacique and Klein Dragón. Plants were grown in plastic pots (four seeds/pot in all samples) with a standard potting mix. Plants were inoculated when they had reached the third expanded leaf stage. Inoculum was prepared from 10-day-old cultures of

A. hordei var. *europaea* (isolate A1102) growing on PDA and was obtained by flooding each sporulating plate with sterile distilled water and gently scraping the fungal colony with a flame-sterilised scalpel to dislodge conidia. The conidial suspension was filtered once through a single layer of cheesecloth and spore concentration was determined with a haemocytometer. The inoculum consisted of 1.2×10^7 conidia/mL. Twenty seedlings of each cultivar were used for the inoculation. Control plants were sprayed with sterile distilled water only. Leaves were sprayed to run-off with a manually operated sprayer. The inoculated plants and controls were kept in a moist chamber for 48 h.

The first symptoms appeared 92 h after inoculation. Between 40 and 60% of inoculated leaves were necrotic 18 days after inoculation. Re-isolation from leaves with lesions was performed and the isolates were compared morphologically with those used for inoculation to fulfill Koch's postulates.

In natural field infection, spots were observed that affected especially the basal leaves rather severely. Lesions at first are distinct, chlorotic, ellipsoidal or round and 1–5 mm across. Later they become diffuse and grey-brown internally. Pycnidia sometimes form and appear as black dots within necrotic lesions. They are submerged in host tissues except for a papillate projection. In culture on PDA, pycnidia measured $142.5\text{--}225 \times 93.75\text{--}206.25$ μm . Conidia (pycnidiospores) are straight, hyaline and oblong, $3.75\text{--}5.60 \times 5\text{--}18.70$ μm typically with one median septum.

All wheat plants inoculated with *A. hordei* var. *europaea* in the greenhouse developed symptoms identical to those observed on naturally infected plants in the field. Differences in degree of infection were noted among the 16 cultivars tested. The amount of damage to seedlings was measured as percent necrotic leaf area from the first leaf of 15 plants of each inoculated cultivar in comparison with controls. Nine days after inoculation, cultivars Buck Arriero and Buck Poncho showed the most conspicuous symptoms, with a disease severity rating ranging from 8 to 35% of necrotic leaf area. The remaining cultivars showed no symptoms or had low disease severity with few, scattered

lesions (1–2% of necrotic leaf area) and a slight yellowing of leaf tips. Twenty days after inoculation Buck Arriero, Buck Charrúa, Buck Halcón, Buck Poncho, Buck Yatasto and Klein Dragón showed the most severe symptoms (between 12 and 45% necrotic leaf area). The rest of the cultivars showed little evidence of infection with up to 10% of the foliar area necrotic, except ProInta Imperial and ProInta Puntal that showed no evidence of infection. These observations are consistent with other reports (Wiese 1977) which indicate that the fungus is apparently of little economic consequence as a foliar pathogen of wheat. No symptoms were observed in non-inoculated plants. Isolations from symptomatic tissue from the inoculated cultivars have consistently yielded cultures of *A. hordei* var. *europaea*. The fungus produced pycnidia on the diseased tissue in Petri dishes.

Inoculation studies proved that *A. hordei* var. *europaea* was the cause of this outbreak on wheat in Argentina. The relationship of this disease to new cultural practices (reduced tillage, nitrogen fertilisation, irrigation), the use of new germplasm and favourable environmental conditions need to be studied.

The fact that other wheat cultivars apart from those checked might also be susceptible to isolates of the pathogen shows the importance of conducting thorough research to determine the reactions of other cultivars currently used in the Argentinian cropping area.

References

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