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Research Note
Australasian Plant Pathology, 2008, **37**, 589–591

Formation of *Lewia infectoria*, the teleomorph of *Alternaria infectoria*, on wheat in Argentina

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Abstract. The trapping of pseudothecia carrying asci with mature ascospores of *Lewia infectoria* (teleomorph of *Alternaria infectoria*) from wheat stubble under natural field conditions in Buenos Aires Province, Argentina, is reported for the first time. The production of mature pseudothecia in culture is also reported. Monosporic isolates of *A. infectoria*, obtained from infected wheat plants in Argentina, produced conidia within a week and ascomata with fully mature ascospores within 7 months when stored on slants of PCA at 4°C in darkness. The anamorph exhibited the sporulation pattern of *Alternaria infectoria* species-group, and was identified by its axenic colony morphology and the prominence of its secondary conidiophore structure. Critical examination of the teleomorph revealed it to be *Lewia infectoria.* The presence of the teleomorph has implications in the long-distance dispersal of *A. infectoria* and on resistance breeding programs. This is the first confirmed report of the sexual stage of *A. infectoria* in Argentina.

Regional surveys are being conducted to investigate the presence of wheat (*Triticum aestivum*) pathogens on leaves and seeds across the Argentinian cropping area. The dominant genus amongst the field fungi found in cereals is *Alternaria*, with the majority of taxa within this genus coming from the *A. infectoria* species-group (Andersen *et al*. 2002). The *Alternaria infectoria* species-group complex is used here to encompass relatively small-spored *Alternaria* taxa that are distinguished as much by the prominence of their secondary conidiophore structure as they are by the morphology of their conidia. To date, the *A. infectoria* species-group comprises the following known species: *Alternaria arbusti*, *Alternaria conjuncta*, *A. infectoria*, *Alternaria oregonensis*, *Alternaria triticimaculans*, *Alternaria metachromatica*, *Alternaria* v*iburni*, *Alternaria intercepta*, and *Alternaria novae-zelandiae*, as well as an unknown number of distinct taxa yet to be described (Christensen *et al*. 2005). In Argentina, this genus has been studied over the last decade (Perelló *et al*. 1992; Perelló and Sisterna 2006). Although not a major issue for many years, the *A. infectoria* species-group, causing leaf blight and black point of wheat, has becomes a new problem in Argentina (Perelló and Sisterna 2006; Perelló *et al*. 2007). Primary inocula (ascospores and conidia) commonly originate from infected stubble, with secondary spread achieved via conidia produced from infected leaves. The occurrence of the teleomorph has not been investigated nor reported in Argentina.

During the 2005 spring growing season, typical symptoms of tan to dark brown leaf spot were observed on wheat samples collected from several cultivars in the cropping area of Buenos Aires Province. Different isolates of a fungus with characteristics of *Alternaria* were obtained from this material. Cultures of this fungus were grown on Potato Carrot Agar (PCA) under laboratory conditions and produced conidia within a week. These isolates were stored to maintain a fungal collection. Within 7 months, ascomata with fully mature ascospores were observed to have grown from these isolates. Furthermore, during the summer season (December to March), wheat stubble from natural fields in different localities was collected and examined under laboratory conditions. Based on morphological characters, the teleomorph proved to be a *Lewia infectoria* with its *Alternaria* anamorph. The objective of this study was to describe, illustrate and discuss the occurrence of this new record in culture.

Wheat leaves exhibiting necrotic symptoms were collected during September and October 2005 from different cultivars of farmer's fields and research stations in eight localities of north, central and south of Buenos Aires Province. Lesions on leaves were viewed through a stereomicroscope at ×12 magnification, with specific morphological characteristics or signs of sporulating pathogens used for identification. To identify the fungi responsible for leaf spots, small sections of diseased leaves were disinfected in 70% ethanol and 0.06% sodium hypochlorite 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. Screening identified 180 cultures belonging to the genus *Alternaria.* Single conidial isolated were derived by removing conidia from the initial isolation dishes, placing them on the surface of water agar, and later transferring single germinating conidia to PCA. Morphobiometrical and cultural studies of the fungus were conducted on single spore colonies grown in Petri dishes

10.1071/AP08060 0815-3191/08/060589

containing PCA, cultured at 20 ± 2°C under cool white fluorescent light (near UV supplemented) with a 12-h photoperiod. Based on morphological characters, like conidial sporulation pattern and the prominence of their secondary conidiophore structure, most of the strains were identified as members of the *A. infectoria* species-group. The cultures were stored on slants of PCA at 4°C in darkness. Numerous conidia appeared on the surface of the medium within a week and groups of fruiting bodies within 7 months. To determine their stage of development, fungal fruiting bodies were placed on a microscope slide, stained with 0.25% Trypan blue in lactic acid : glycerol : water (1 : 1 : 1), and examined with a light microscope (×400 magnification).

Naturally infested wheat stubble (10 months old) showing pseudothecia was collected from previously infected wheat crops in eight locations of Buenos Aires Province. In experiments, Petri dishes with small stubble sections, each ~2 cm long, were placed on top of water agar (20 g/L) contained in a 100-mm-diameter Petri plate. The Petri plates were sealed with parafilm (American National Can Co., Greenwich, CT). In other experiments, plates were incubated with a 12 h light : 12 h dark photoperiod, or in darkness for 10 days followed by an incubation with 12 h light : 12 h dark photoperiod. Cool white fluorescent lights were used in all light cycles. Plates held in darkness were covered with aluminium foil and exposed to light only when samples were taken. Cultures incubated in continuous light for 1 month failed to produce ascospores in preliminary trials, so this treatment was not used. Observations were made daily on all treatments until ascospores discharged from pseudothecia were detected on the lids of the Petri plates. At this point, pseudothecia were crushed and evaluated for the presence or absence of mature ascospores. Several erumpent pseudothecia were removed from the stubble, soaked in water for 1 hour and crushed on a cavity slide containing a few drops of water. Crushed pseudothecia were viewed at ×200 magnification using a light microscope. In other experiments, pseudothecia were placed on a microscope slide in a drop of 15% glycerol and crushed under a coverslip.

From wheat stubble, the *A. infectoria* teleomorph developed on all treatments tested but there were detectable differences in the level of ascospore maturity. Better development occurred on stubble incubated in a 12 h light : 12 h dark photoperiod, with ascospore discharge evident by days 14–24. PDA cultures of isolates from wheat cv. Klein Estrella, obtained from Balcarce, Buenos Aires Province, developed groups of fertile ascomata (pseudothecia) with septate, hyaline and mature ascopores after 7 months (Fig. 1). There were detectable differences between isolates with regard to pseudothecia density and speed of ascospore maturity. In some cases, the production of immature asci was observed.

The morphobiometrical and cultural features of these ascomata, both on PCA and from stubble, allowed the teleomorph of *A. infectoria*, *Lewia infectoria*, to be identified. It appears as follows: ascomata, 400–500 × 150 µm in size, ellipsoid with a short papillate beak, dark and thin-walled at maturity; asci, 105–125 × 13–16 µm in size, subcylindrical, straight or somewhat curved, usually eight spores; ascospores, 18–22 × 7–8 µm in size at full development, broadly elliptic, muriform, becoming 5-septate (three primary septa), only end cells not longitudinally septate, constricted, yellow-brown

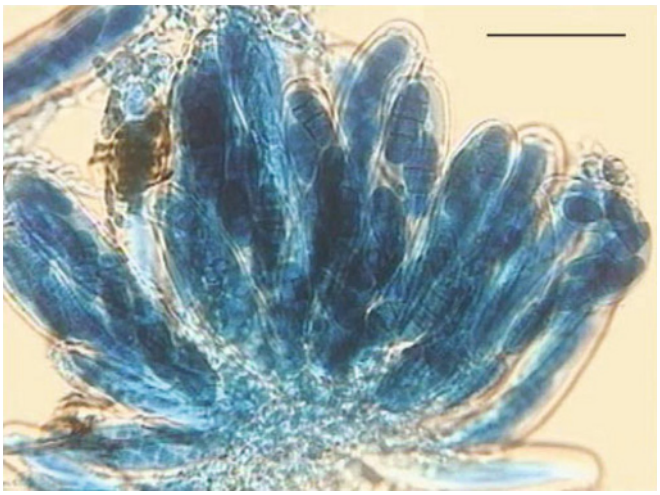

Fig. 1. Mature pseudothecia of *Lewia infectoria* on 2% PDA. Scale bar = 50 µm.

(Fig. 2). Asci were transferred to PDA and incubated at 20°C under near ultraviolet light with a 12-h photoperiod. After 1 week, typical colonies of *A. infectoria* were observed. The strain was deposited in the culture collection of the Instituto de Micología Spegazzini (LPSC) (La Plata, Buenos Aires, Argentina), No. 1022.

To further confirm the identity of the ascospores isolated, 3-week-old wheat cv. Klein Estrella seedlings were inoculated using mycelium from ascospores. After inoculation, the seedlings were placed in a moist polythene chamber for 48 h. After 10 days, typical spot symptoms were observed. The present study indicates that the pathogen associated with foliar blight produces asci with matured ascospores, either on stubble or in culture under our conditions.

Although most *Alternaria* species do not have teleomorphic affinities, several anamorphic taxa within the Pleosporaceae have recognised teleomorphs and most are not commonly encountered (Simmons 1986, 2002). These teleomorphs are representative of

Fig. 2. Asci and ascospores of *Lewia infectoria* on 2% PDA. Scale bar = 20 µm.

nearly all major lineages within the Pleosporaceae. An evaluation of the teleomorphic characters of well known *Pleospora* spp. with anamorphs of *Alternaria*, namely *P. infectoria* and *P. scrophularieae*, revealed that *Pleospora* spp. with *Stemphylium* anamorphs were morphologically distinct from *Pleospora* spp. with *Alternaria* anamorphs, particularly in the size of the ascomata and ascospores. This resulted in the designation of the genus *Lewia* for *Pleospora*-like fungi with *Alternaria* teleomorphs (Simmons 1986). Evidence of obtaining *Alternaria*-related teleomorphs in axenic culture was previously reported in *L. infectoria* (Bilgrami 1994), *L. photistica* (Simmons 1986), *L. avenicola* (Kwasna and Kosiak 2003) and *L. hordeicola* (Kwasna *et al*. 2006). Others described *Lewia* species, such as *L. chlamidosporiformans*, *L. ethzedia*, *L. intercepta*, *L. sauropi*, *L. viburni and L. eureka*, that usually produced ascomata on tissues of infected plants (Simmons 1986; Vieira and Barreto 2005). In our findings, ascomata of *L. infectoria* were produced *in vitro* in axenic culture on PCA slants, in connection with the anamorph (Fig. 3). However, the fungus does not often produce both anamorph and teleomorph forms on the same slant. Crossing between isolates is evidently not necessary for production of the teleomorph, as single-ascospore axenic cultures continued to produced ascomata on PCA. These results agreed with observations of Kwasna and Kosiak (2003) for *L. avenicola.* In addition, its finding in Argentina has provided an important framework for hypothesis-testing in advanced studies on *Alternaria*/*Lewia* epidemiology and pathogenicity variability on wheat plants.

L. infectoria forms pseudothecia on wheat straw in the field under determined weather conditions. This could be an important source of inoculum in *Alternaria*/*Lewia* disease, with the dispersal of air-borne ascopores able to infect wheat and wild grasses. The discovery of the sexual stage in nature indicates that new combinations of virulence genes may arise that could have a large influence on localised development of the diseases in different regions of the country. Further work is required to verify the role of ascospores as primary inoculum, as a potential source of genetic variation under field conditions, and its relative contribution to disease development during the wheat growing season. Furthermore, the need for appropriate disease management strategies to specifically counteract this phase of the disease requires further investigation.

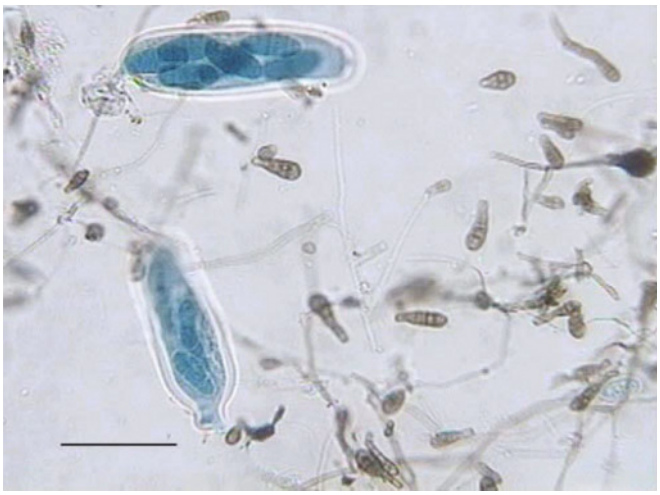

Fig. 3. Connection *Lewia*/*Alternaria* on 2% PDA. Scale bar = 50 µm.

Acknowledgements

This work was funded by the University National of La Plata, Facultad de Ciencias Agrarias y Forestales, Teaching and Research Project A11/142.

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Manuscript received 7 March 2008, accepted 21 July 2008