

## Smudge and other diseases of onion caused by *Colletotrichum circinans*, in southern Argentina

### Tizne y otras enfermedades de cebolla causadas por *Colletotrichum circinans*, en el sur de Argentina

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**Abstract.** While studying the etiology of onion diseases in southern Argentina, a coelomycetous fungus was found associated with diverse disease expressions. They included the typical smudge symptoms in white onion bulbs in local markets as well as field infections on yellow- and red-coloured cultivars, with severe infections on transplants in the seed-bed. The fungus was isolated and identified as *Colletotrichum circinans* on the basis of its morphological, morphometric, biological and pathological characteristics. Two isolates, obtained from white bulbs and from transplants, were inoculated on bulbs of white and yellow-coloured cultivars and produced the typical smudge symptoms on the outer scales. Koch's postulates were fulfilled by re-isolating the inoculated fungus. This is the first record of *C. circinans* in southern Argentina and the first observation of the disease in the field in this country.

**Keywords:** *Colletotrichum circinans*; Onion; Smudge; Southern Argentina.

**Resumen.** Durante estudios etiológicos de enfermedades de cebolla en la región cebollera sur argentina, un hongo celomiceto ha sido encontrado asociado con diversas patologías. Éstas incluyeron los típicos síntomas de tizne en bulbos de catáfilas blancas en el mercado minorista, infecciones a campo en plantas y bulbos de catáfilas amarillas y coloradas así como en plantines en almácigo. El hongo ha sido aislado e identificado como *Colletotrichum circinans*, sobre la base de sus características morfológicas, morfométricas, biológicas y patológicas. Con los aislamientos obtenidos de bulbos blancos con tizne y de plantines afectados, se realizaron pruebas de inoculación en bulbos de cultivares blancas y coloreadas, en los cuales se reprodujeron los típicos síntomas de tizne. Se logró reaislar al hongo inoculado dando cumplimiento a los postulados de Koch. Éste es el primer registro de *C. circinans* en condiciones de campo, en la Argentina, y el primero de la enfermedad en la región cebollera sur de este país.

**Palabras clave:** *Colletotrichum circinans*; Cebolla; Tizne; Sur argentino.

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## INTRODUCTION

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Onion is the principal horticultural crop in the Lower Río Colorado Valley (LRCV) and other areas of southern Argentina; reciprocally, the LRCV is by far the most important onion producing area of Argentina. More than a dozen diseases, caused by fungi, bacteria and viruses, are the principal constraint for onion production and marketing (Kiehr et al., 1996; Kiehr & Delhey, 2007). Here we report on smudge (called “tizne” in Spanish) and related diseases of onion, caused by the coelomycete *Colletotrichum circinans* (Berk.) Voglino. This fungus has been described for the first time in the United Kingdom and is now widespread, especially in the Northern Hemisphere. Smudge is normally considered one of the minor diseases of the onion crop (Entwistle, 1990; Sumner, 1995).

In Argentina, smudge-infected onions have been detected for the first time in 1928 by J.B. Marchionatto, in Bell Ville, province of Córdoba (*vide* Gatica et al., 2010). Later, it was recorded on white onion in Colonia Tirolesa, Córdoba (Docampo & Nome, 1970); on bulbs of the white cv. Chata Blanca, at the Central Market of Buenos Aires (Vigliola & Calot, 1982) and at local markets of the province of Jujuy (Alcoba et al., 1986). We are not aware of any field record of *C. circinans* in Argentina, and so far smudge disease has not been detected in the LRCV or other southern production areas. To our knowledge, there is no detailed description of the fungus in Argentina and no inoculation experiments have been reported.

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## MATERIALS AND METHODS

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*Colletotrichum circinans* was isolated from the outer scales of onion bulbs and from lesions on the leaf sheaths of onion transplants. The affected tissue was superficially disinfected with NaOCl (0.8% of active Cl) during 0.5 min, dried with sterile paper and small pieces were cut and deposited on potato dextrose agar (PDA) and water agar in Petri dishes.

Inoculation experiments were carried out with two isolates, one obtained from white-scaled onion bulbs and the other from transplants infected in the seed-bed. Medium-sized bulbs of an unidentified white onion cultivar and of the yellow-scaled cv. Valcatorce were cleaned from adhering soil, roots and loose outer scales, submerged in ethanol 96% and flamed shortly at their basal portion. Then they were planted in pots with twice autoclaved soil amended with about 15 day-old actively growing mycelial colonies (one PDA-plate of 9 cm/600 ml of soil). The pots were placed at ambient temperature in the laboratory. After three to four weeks the plants were uprooted and observed for any symptoms on outer scales and roots. Non-inoculated check plants were grown in non-infested soil.

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## RESULTS

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**Geographic distribution and symptoms.** Diseases caused by *C. circinans* were found four times in the field and twice at local markets of the city of Bahía Blanca. In the latter cases they were white-scaled onions of unknown cultivar and origin, showing typical symptoms of smudge: dark spots frequently organized in concentric rings, forming patches of up to 1 cm of diameter (Fig. 1, A). These spots are made up of stromatic masses (Fig. 2, A) and small acervuli with stiff dark setae (Fig. 2, B) which can be seen with a hand lens.

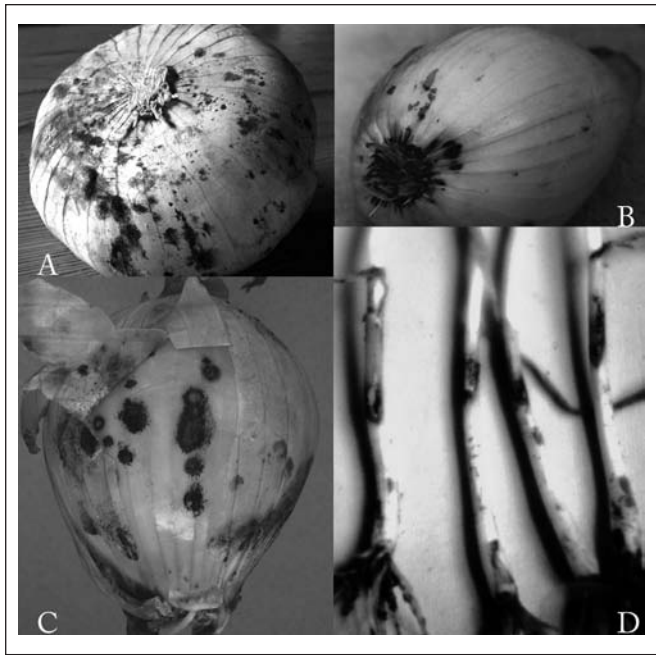
Under field conditions, we observed wilting and death of transplants of cv. Valcatorce in a very dense seed-bed in the locality of Pedro Luro, LRCV. Expanding lesions with a dark brown border and a white centre appeared on the leaf sheaths; later, this central part became necrotic and fell off (Fig. 1, D). Acervuli with dark setae were observed on such lesions.

In a field of green salad onion of the Green Belt of Bahía Blanca (G BBB), we observed a beginning of wilting in plants of an unidentified red-scaled cultivar. Dark olivaceous-brown lesions with typical acervuli were seen on the basal part of the external bulb scales. In another field of the same region grown with cv. Valcatorce for bulb production, close to harvest we observed dark olivaceous spots in the still uncoloured basal part of the outer scales (Fig. 1, B), with typical stromata and numerous acervuli.

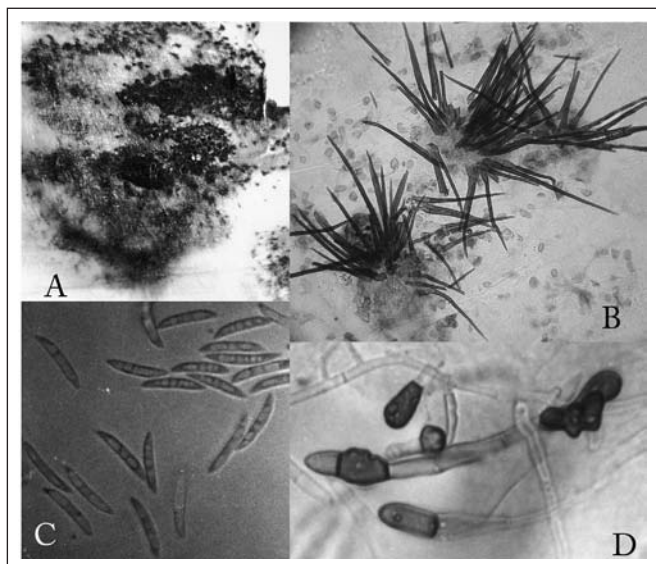
Finally, in an experimental lot of the G BBB planted with cvs. Torrentina and Valcatorce which partially remained unharvested, at the end of autumn on many of the remaining bulbs of cv. Torrentina large patches of smudge appeared, frequently in concentric rings (Fig. 1, C). There were far fewer smudge patches on the Valcatorce bulbs.

**Fungal morphology.** The isolates obtained were grown on PDA. Acervuli with dark, almost black, acicular setae having a bulbous base and 1 to 3 septae, measured up to 290 by 8  $\mu$ m (Fig. 2, B). Conidia were hyaline, unicellular, 16–25 x 3–5  $\mu$ m, slightly falcate, guttulated (Fig. 2, C). Apressoria, formed in the borders of Petri dishes, were subglobose, ellipsoid, clavate or irregular, 1- or 2-celled, measuring 6.5–13.5 x 5.5–8  $\mu$ m (Fig. 2, D). In several month-old cultures, secondary, tertiary and quaternary appressoria may be formed by successive processes of germination.

**Pathogenicity test.** Both isolates produced similar symptoms, consisting of typical patches of smudge on the white-scaled onion bulbs, with stromatic masses and the formation of acervuli. On the yellow-scaled Valcatorce bulbs only few lesions developed and only on still uncoloured scales. *C. circinans* was recovered from the inoculated bulbs of both cultivars. No symptoms were observed on the un-inoculated controls.



**Fig. 1.** Disease symptoms on: (A) an unidentified white-scaled cultivar; (B) cv. Valcatorce; (C) cv. Torrentina; (D) transplants of onion.  
**Fig. 1.** Síntomas de enfermedades en: (A) un cultivar de catáfilas blancas no identificado; (B) cv. Valcatorce; (C) cv. Torrentina; (D) transplantes de cebolla.



**Fig. 2.** *Colletotrichum circinas*: (A) stromatic masses; (B) acervuli with setae; (C) conidia; (D) appressoria.  
**Fig. 2.** *Colletotrichum circinas*: (A) masas estromáticas; (B) acérvulos con setas; (C) conidios; (D) apresorios.

## DISCUSSION

Several *Colletotrichum* spp. have been reported as disease agents of onion worldwide (Farr & Rossman, 2012). *C. gloeosporioides* and *C. coccodes* have straight conidia and infect mainly the leaves and necks of onion plants (Hill, 1995; Rodríguez-Salamanca et al., 2012), while *C. circinans* causes primarily, but not exclusively, the typical smudge symptoms on the bulbs, and the conidia are falcate (Sumner, 1995). This latter species is closely related to *C. spinaciae* on Amaranthaceae (Damm et al., 2009). On the basis of its morphological, morphometric, biological and pathological traits, the fungus studied by us is identified as *C. circinans*.

These are the first records of *C. circinans* in southern Argentina. This is also the first time that field infections are described in Argentina. During our studies, infections in very diverse situations have been observed indicating that *C. circinans* is a rather versatile pathogen. However, it is normally associated with the typical smudge symptoms on white onion bulbs during postharvest. This latter aspect has been recorded only in supermarkets of the city of Bahía Blanca, but never in the local production of the LRCV where at present smudge is not economically important. White onions are mostly grown for the European market, and growers practice an adequate rotation scheme which may explain the absence of smudge. This situation might change if the acreage of white onions in the area should increase.

It is quite uncommon that *C. circinans* affects coloured onion bulbs. If this were the case the symptoms tend to appear in the neck of the bulb (Walker, 1921). We found symptoms in the yellow-scaled cvs. Valcatorce and Torrentina and in an unidentified red-coloured cultivar. In cv. Valcatorce it was the basal part of the scales that was normally affected; and this occurred mainly in scales still not completely coloured. It is known that phenolic substances in the outer scales of coloured onions inhibit the growth of *C. circinans* (Walker, 1923; Walker & Stahmann, 1955). We also found a serious attack of *C. circinans* on onion transplants which is observed very infrequently, although in occasions this fungus may kill entire seed-beds (Heinze, 1974).

Both isolates of *C. circinans* caused identical symptoms when inoculated on white and yellow-coloured plants. Koch's Postulates were fulfilled by recovering the fungus from the inoculated bulbs.

We conclude that *C. circinans* is at present a minor pathogen of onion in southern Argentina. Nevertheless, producers should take cautionary measures in order not to introduce the fungus into new production areas or fields. Where it is already present, the build-up of large populations of the fungus should be prevented by a proper rotation scheme, and by avoiding that unharvested bulbs and other residues remain for too long in the field.

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