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Occurrence of the complete cycle of *Puccinia sorghi* Schw. in Argentina and implications on the common corn rust epidemiology

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Abstract The life-cycle of *Puccinia sorghi*, a heteroecious fungus, consists of five well-defined spore stages. The uredinial and telial stages are completed on the primary host (maize) whereas spermagonial and aecial stages occur on *Oxalis* spp., a perennial and widespread weed. Portions of corn leaves with telia were surface sterilized and placed in Petri dishes with 2% water agar and maintained in a growth chamber at 25 ± 1 °C and photoperiod of 16 h light and 8 h dark for 48 h to induce the formation of basidia and basidiospores. *Oxalis conorrhiza* plants were inoculated with those basidiospores, to confirm the generation of spermagonia with spermatia, and subsequently aecia with aeciospores. Corn plants were then inoculated with aeciospores to confirm the formation of urediospores and teliospores. The aecial phase of common corn rust was confirmed to occur on *O. conhorriza* and the descriptions of spore stages in Argentina are now reported in this work, confirming a potential sexual source of variability of *P. sorghi*. The natural occurrence of aecial infections on *O. conhorriza* in Córdoba may play an important role in generating new variants of *P. sorghi* in Argentina, allowing a constant adaptation of the pathogen to the environment of the different corn production zones.

Keywords Common rust · Maize · Corn · *Oxalis* · *Zea mays* L.

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Introduction

Common corn rust, caused by *Puccinia sorghi* Schw. (*P. sorghi*), is one of the most important leaf diseases in the corn cropping area of Argentina (INTA 1980; Gonzalez 2005). It is a very common disease, appearing every year with different levels of incidence and severity in the corn producing areas of Argentina (Gonzalez 2005). The causal agent is *P. sorghi*, a biotrophic, heterocius and macrocyclic fungus, belonging to the Basidiomycota group. The life cycle involves corn (*Zea mays* L.) as the telial host and several *Oxalis* species as aecial hosts. The complete cycle of *P. sorghi* includes the generation of five spores stages: teliospores, basidiospores, spermatia, aeciospores and urediospores (Biswanath 2016).

Teliospores, basidia and basidiospores are the main phases in rust life cycles for overwintering and they are responsible for the formation of new physiologic races (Anikster and Wahl 1979). Some teliospores are constitutively dormant, which is often a mechanism to protect the fungus during unfavourable environmental period (Anikster 1986) and require processes for germination

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of the teliospore. These processes can be activated by: storage outside, different humid and temperature range conditions, alternation of wet/dry conditions of the spore, exposure to different light regimes, and/or internal factors (Mendgen 1984).

In some areas of the world, *P. sorghi* teliospores germinate (usually in the spring) to generate basidia and basidiospores. The latter germinate and penetrate only into *Oxalis* spp. leaves (Pataky 2016). In most temperate areas of the world, *P. sorghi* does not infect *Oxalis* spp. after surviving the winter; hence, aeciopores are not the primary inoculum of corn rust. Initial infection of corn coincides with the arrival of air-borne urediospores from nearby tropical and subtropical regions, where the aecial stage appear on *Oxalis* spp. In other areas, the pathogen survives on volunteer maize plants and urediospores are dispersed by wind until they reach susceptible corn plants, infecting them and developing the uredinal stage continuously (Reis et al. 2004).

Ten *Oxalis* species have been reported as hosts of *P. sorghi* over the world (Farr and Rossman 2016). In Argentina Godoy and Bruni (1952) reported *P. sorghi* on *O. corniculata* in the Buenos Aires province at an urban environment, but not in rural fields or near corn crops fields. In Córdoba province, Guerra et al. (2016) reported natural infections of *P. sorghi* on *O. conorrhiza* in different rural locations near corn crops. Among the alternate hosts of *P. sorghi*, *O. conorrhiza* is a South American native perennial specie distributed throughout Argentina, Brazil, Paraguay and Uruguay (Belgrano et al. 2008). Its geographical distribution coincides with the greatest corn production areas of Argentina (MAGyP 2016). Although the aecial stage of *P. sorghi* has been previously reported in Argentina (Godoy and Bruni 1952; Guerra et al. 2016), the generation in nature of all five spores stages, including basidiospores and determination of the environmental conditions required for the development of the complete cycle, have not been documented. Here we present evidence that all five stages of *P. sorghi* occur in the field in Argentina.

Materials and methods

Collection and conditioning of samples and germination of basidia

At the end of the 2015–16 corn crop campaign on a field located in Córdoba province (31°10′04.1“ S 64°09’33.1” W), telia from corn leaves were collected and stored in a herbarium. After 5 months, portions of those leaves with telia were surface sterilized with 2% sodium hypochlorite for three minutes, rinsed three times with sterile water for two minutes and placed in Petri dishes with 2% water agar. Plates were put into a growth chamber at 25 ± 1 °C and photoperiod of 16 h light and 8 h dark, and the germination was monitored every 12 h for production of basidia with basidiospores.

Inoculation of *O. conorrhiza* plants

Seeds of *O. conorrhiza* were sowed in 850 ml plastic pots containing a substrate composed of 70% fertile soil and 30% vermiculite. Plants were inoculated at the 20 leaflet stage and after being kept for 24 h. in a saturated humidity environment.

Portions of corn leaves with telia were disinfected and placed in Petri dishes with 2% water agar for 24 h with the aforementioned methodology.

Two inoculation methodologies were applied; one (treatment A) simulating infected stubble on the soil, and the other (treatment B) simulating inoculum from unharvested corn stalks. In treatment A, leaf portions with basidiospores were removed from the plates and placed on the soil surface of a pots where *Oxalis* plants were growing and covered with transparent plastic bags to maintain a saturated environment for 48 h. In treatment B, the methodology described by Morin et al. (1993) was applied as follows: a transparent plastic tube was placed over the pots where *Oxalis* plants were growing and an inverted plate containing leaves with basidiospores was placed on top, and was kept closed for 48 h. The control treatment consisted of the same procedures but without inoculum. The procedures were repeated four times each. Inoculated *Oxalis* pots were kept in a plant growth chamber at 25 ± 1 °C, with 70 ± 10% relative humidity and a photoperiod of 16 h of light and 8 h of darkness.

Inoculation of corn plants

Seeds of a *P. sorghi* susceptible hybrid corn (experimental hybrid, KWS Argentina S.A.) were sowed in 850 ml. plastic pots, containing a substrate composed of 70% fertile soil and 30% vermiculite. When plants reached the three fully expanded leaf stage, they were placed for 24 h in a saturated humidity environment. Aeciospores were manually extracted from mature aecia on

O. conorrhiza infected leaves and placed in a 1.5 ml eppendorf tube with 1 ml of sterile water. After shaking, the suspension of aeciospores was sprinkled on the seedlings. Inoculated corn were kept in a humid saturated environment for another 24 h and then, the pots were placed in a plant growth chamber at 23 ± 1 °C, with 70 ± 10% relative humidity and a photoperiod of 16 h light and 8 h dark.

Microscopy, morphometry and image capturing

For morphometric evaluation and image capturing, the following methodologies were carried out: i) extraction of produced spores (urediospores, teliospores, aeciopores and basidiospores); and ii) thin sections of the fruiting bodies (aecia and spermagonia). Urediospores, teliospores and aeciospores, collected from portions of symptomatic tissue from leaves of each host, were placed in a 1.5 ml tube with 0.5 ml of sterile water and shaken for 60 s. Subsequently, 10 μl of the suspension were placed on a microscope slide and observed under the microscope. Alternatively using a stereoscopic microscope and a histological needle, structures developed within the telia were collected and placed on a slide. A Zeiss Primo Star optical microscope was utilized, and photographs were taken using a Canon Power Shot SX 30 IS camera. The morphometric parameters for the pathogen characterization were determined and processed with the AxioVision LE V4.6.3.0 program and compared with those described in the literature for urediospores, teliospores, spermagonia, aeciopores and aecia.

Results and discussion

Collection of samples, conditioning and germination of basidia

The generation of basidia and basidiospores from teliospores were first observed at 48 h, and in greater quantity at 72 h, after placing the leaf portions on water agar plates (Figs. 1 and 2). Under stereoscopic microscopy, hyaline structures were observed on some teliospores. Those structures were pickedup with histological needles, placed on a microscope slide, and confirmed as basidia and basidiospores after observation under a microscope. Changes in the appearance of telia were observed when basidiospores began to germinate. The

Fig. 1 Teliosporas of *P. sorghi* with probasidia

telia initially presented as flat, opaque, compact, and dry-looking, and at the time of germination they looked bright, moist, voluminous and lax.

Mendgen (1984) reported that teliospores of *P. sorghi* present an intermediate maturity, and thus dormancy length is variable allowing them to germinate immediately or only after a few months after apearing. Pavgi (1975) stated that termination of the dikaryotic condition is essential for germination. Several authors observed germination of spores from 48 h at 17–21 °C with high humidity (Pavgi 1975; Mendgen 1984; Dunhin et al.2004). Although some authors described

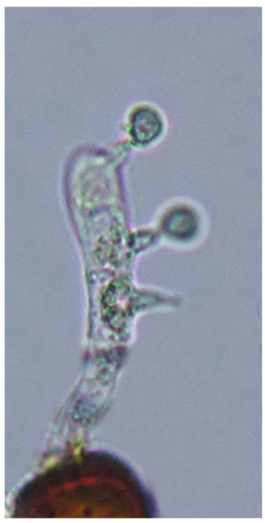

Fig. 2 Basidiospores of *P. sorghi* on probasidia

the need of exposing portions of infected leaves to unfavourable or outdoor environmental conditions to break dormancy (Pavgi 1975; Pavgi et al. 1960; Mendgen 1984), in this work and that of other authors (Dunhin et al. 2004; Godoy and Bruni 1952) germinations were achieved without fulfilling those condition. Pavgi (1975) concluded that although moisture is essential for the normal germination of teliospores of *P. sorghi*, excessive humidity results in delayed development, inhibition, or aberrations in the germination of the basidiospores. We determined that changes in humidity and temperature of the telia are particularly important during the process of germination. Humidity is the most sensitive and indispensable factor, which together with the temperature initiates the germination of basidia and the formation of basidiospores.

Inoculation of *O. conorrhiza* plants

Aecia and aeciospore development were observed seven days post inoculation (dpi) on inoculated *O. conorrhiza* under treatment A, while no symptoms were observed on the control plants. Similar results were observed by Dunhin et al. (2004). Unfortunately, attempts to inoculate *O. conorrhiza* by treatment B were not successful. Under the hypothesis that 48 h of incubation would had not been enough time for the basidiospores to become effective for penetration, the methodology was repeated but with an extended inoculation period from 48 to 72 h of incubation. This modification in the methodology did not improve successful infection, and we also found that *O. conorrhiza* plants did not tolerate a long-time saturation humidity by presenting symptoms of senescence that did not reverse after returning them to a normal environment.

Based on our own observations during the developing of this work and coincident with previous reports (Pavgi et al. 1960; Dunhin et al. 2004; Pataky 2016; Guerra et al. 2016), natural infections and teliospores germination are observed during the beginning of the spring season, when the time of foliar humidity increases, and temperatures and relative humidity are closer to the mentioned optimal ranges.

Inoculation of corn plants

Corn plants inoculated with aeciospores showed small chlorotic spots at 5 dpi. Typical pustules with urediospores were observed at 7 dpi, coinciding with the times described by Pataky (2016). Teliospores were produced on the pustules at 21 dpi on senescent infected leaves.

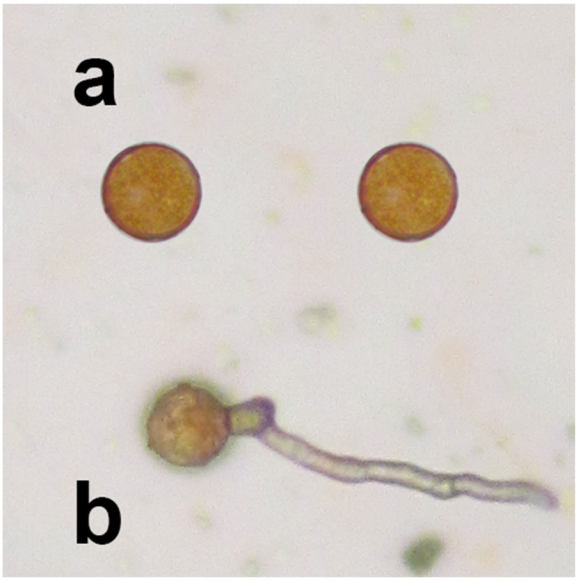

Fig. 3 Urediospores and urediospores with germinative tube of *P. sorghi*

Microscopy, morphometry and image capturing

Urediospores averaged 26.1 μm × 28 μm (Fig. 3) and teliospores 20.1 μm wide × 33.6 μm long (Fig. 4), which coincide with that described by Cummins (1971) and Lindquist (1982). The aeciospore average size was 19.5 μm wide × 21 μm long (Fig. 5a). These values are very close to those reported by Cummins (1971) and Lindquist (1982). Although one of the values exceeds the ranges proposed in the literature,

Fig. 4 Teliospores of *P. sorghi*

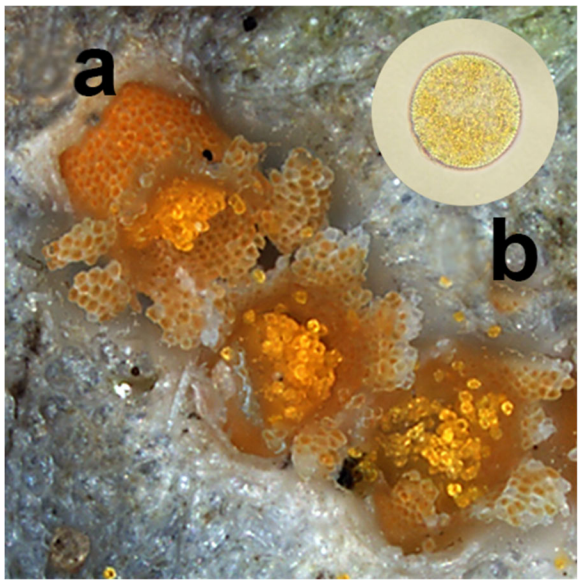

Fig. 5 **a** Aecia of *P. sorghi* on *O. conorrhiza*. **b** Aeciospores of *P. sorghi*

Guerra et al. (2016), in complementary work, performed the molecular characterization of this group of aeciospores confirming *P. sorghi* as the pathogenic agent. The aecia diameter was in average 279.1 μm (Fig. 5b), coinciding with Cummins (1971) and Lindquist (1982). The diameter of spermagonia was 120 μm (Fig. 6a and b). There are no recorded measurements of this structure in the literature consulted.

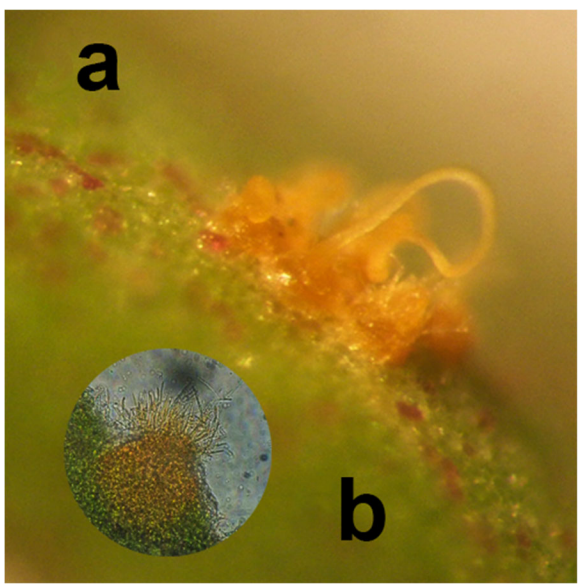

Fig. 6 **a** Spermagonia of *P. sorghi* with spermatia. **b** Spermagonia of *P. sorghi*

Fig. 7 Corn crop with presence of *Oxalis conorrhiza* in Córdoba, Argentina

In conclusion, this study describes the occurrence of the complete cycle of *P. sorghi* in Argentina. All spore stages were recorded in nature and their development was repeated under controlled conditions (Figs. 7 and 8). The infections generated on *O. conorrhiza* by basidiospores produced from teliospores of infected maize leaves of the previous crop cycle, and the subsequent infections on corn by aeciospores generated on *O. conorrhiza*, complete the cycle of the pathogen. Therefore, in Argentina, the

Fig. 8 *Oxalis conorrhiza* with spermagonia and aecia

epiphytic events of corn common rust that are normally observed with high prevalence (Gonzalez 2005; Formento 2010; Parisi et al. 2013; De Rossi et al. 2016) can be initiated from urediospores that migrate from other regions, or from aeciospores generated in situ, if any of the susceptible *Oxalis* species were present, as our work confirms. This stage, important and critical for the natural evolution of the pathogen, develops under environmental conditions that are normal in the spring of Argentina and with a wide distribution of alternative hosts. A potential sexual source of generation of physiological races of *P. sorghi* is confirmed, giving an explanation of the phenomenon observed by Gonzalez et al. (2011) and Darino (2015), who described the presence of a wide population variability of this pathogen in Argentina, although they did not determine its source with certainty. A constant adaptation of the pathogen to the environment of the different productive zones of Argentina can be explained as a consequence of the determination of the occurrence of the complete cycle of *P. sorghi*.

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Compliance with ethical standards

Conflict of interest Authors declare that there are no conflicts of interest in the develop of this research work.

Human and animal rights and informed consent There are no humans and or animals participating in the research and all the authors have been informed and consent to publish this work.

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