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ORIGINAL ARTICLE

Effects of loose kernel smut caused by *Sporisorium cruentum* onrhizomes of *Sorghum halepense*

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Abstract

The effect of loose kernel smut fungus *Sporisorium cruentum* on *Sorghum halepense* (Johnson grass) was investigated *in vitro* and in greenhouse experiments. Smut infection induced a decrease in the dry matter of rhizomes and aerial vegetative parts of the plants evaluated. Moreover, the diseased plants showed a lower height than controls. The infection resulted in multiple smutted buds that caused small panicles infected with the fungus. In addition, changes were observed in the structural morphology of the host. Leaf tissue sections showed hyphae degrading chloroplasts and vascular bundles colonized by the fungus. Subsequently, cells collapsed and widespread necrosis was observed as a symptom of the disease. The pathogen did not colonize the gynoecium of Sorghum plants until the tassel was fully developed. The sporulation process of the fungus led to a total disintegration of anthers and tissues. When panicles were inspected before emergence, fungal hyphae were observed on floral primord. Histological sections of panicles showed fungal hyphae located in the parenchyma tissue and the nodal area. Infection occurred in the floral primordium before the tassel had fully developed and emerged from the flag leaf. Grains were replaced by sori surrounded by a thin membrane that usually was broken before or after the emergence of the panicle. The results, together with the significant decrease of the dry matter of rhizomes and seeds of *S. halepense,* suggest that *S. cruentum* could be considered as a potential biocontrol agent in the integrated management of this weed.

Key words: biocontrol, host-pathogen interactions, Johnson grass, Kernel smut, smut, systemic diseases

Introduction

Loose kernel smut, caused by the fungus *Sporisorium cruentum* (*Sphacelotheca cruenta*), attacks all groups of sorghum, including Johnson grass (*Sorghum halepense*), although certain varieties in some groups are immune or highly resistant. *Sporisorium cruentum* is the smut with the least incidence as a disease in the genus Sorghum (Fischer and Holton 1953; Vánky 1985; Hirschhorn 1986; Duran 1987). Unlike plants covered with kernel smut, plants affected by loose kernel smut are stunted, have thin stalks, and heads emerging earlier than those of healthy plants. Abundant side branches (tillers) may also develop. Occasionally, the tillers are smutted, while the primary head is not. In addition, secondary infection may occur when spores from a smutted head infect late developing heads of healthy plants, causing them to become smutted. *Sorghum halepense* is one of the ten most important perennial weed species in the world (Holm *et al.* 1977). In Argentina, it is spread over more than 7,000,000 Ha (Mitidieri 1984). The perpetuation capacity of alepo sorghum is through the seeds and sprouting of rhizomes. After 40–50 days of emergence, it is very difficult

to establish whether the aerial structure has been generated by rhizomes or by seeds. From biological, population and management points of view, there are no differences between adult plants generated by a seed or a rhizome. In the case of *S. halepense* seeds, this gives the species the possibility of being the main source of dispersion at a distance. Also they provide genetic variability that allows the adaptation of the population to different environments that affect the activity (Piper 1928; Ghersa and Satorre 1981; Satorre *et al.* 1981; Méndez Fernández *et al.* 1983; Leguizamón 2012). In Argentina, *S. halepense* can be infected by various pathogens such as *S. cruentum, Alternaria* spp., *Drechslera* spp., *Curvularia* spp., *Phyllostictas*pp, *Phoma*spp, and *Bipolaris sorghicola* (Hirschorn 1986; Verdejo *et al.* 1995; Acciaresi and Mónaco 1999). These pathogens affect the vegetative development of the plant but have little importance in the reproductive stage. *Sporisorium cruentum* prevents seed development, and black, small smut galls (sori) surrounded by a gray thin membrane replace normal kernels. The powdery black spores (teliospores) are spread by the wind and adhere to the surface of healthy kernels ofneigh boring plants in the same field. Postharvest the disease may persist in the soil, and infected seeds or rhizomes. Previous reports indicated that *S. cruentum* also reduces vegetative and reproductive biomass of infected plants (Millhollon 2000, Astiz Gassó *et al.* 2001). The objective of this work was to analyze the effect of *S. cruentum* on the vegetative development of rhizomes of *S. halepense*.

Materials and Methods

Our methodology for the study of the smut fungi was done, with some modification, according to Fernández *et al.* (1978), Matyac (1985), Craig and Frederiksen (1992), Kosiada (2011).

Plant material

Rhizomes of *S. halepense* plants naturally infected with loose kernel smut were collected from the Experimental Field of the Instituto Fitotécnico de Santa Catalina, Llavallol, Province of Buenos Aires, Argentina, during the summer of 2014.

The rhizomes were sectioned into small pieces, washed in water for 2 h and surface-disinfected with 30% sodium hypochlorite for 20 min. Finally, they were rinsed in sterile distilled water.

Teliospore germination

For inoculum preparation, teliospores from heads infected with loose kernels were collected and allowed to dry at room temperature for a week under laboratory conditions at 20°C (±2°C). The teliospores were passed through a fine metal sieve to separate them from plant debris. Teliospores were sown on Petri dishes with Potato Dextrose Agar 2% (PDA) at 20°C (±2°C) in darkness. After 48 h, direct microscopic observations were made, and the number of germinated teliospores per microscopic field at x 100 magnification were counted. Teliospore germination was defined by the production of either promycelia or elongated hyphae. Morpho--cultural studies of the colonies which developed on PDA were made.

Fungal inoculum

The harvested teliospores were washed in 3% sodium hypochlorite for 3 min, suspended in sterile distilled water, plated on PDA and incubated in the dark at 25°C (±2°C) for 48–72 h. Isolated sporidial colonies were transferred to new PDA plates and incubated for an additional 48 h. A few colonies were transferred to flasks containing 2% Potato Broth (PB) and allowed to grow on a rotary shaker (CAT-S20) at 200 rpm for approximately 4–5 days (Astiz Gassó *et al.* 2001). Liquid medium containing sporidia (10^6–10^8 sporidia/ml) was used for inoculations following two techniques: T1) hypodermic injection and T2) vacuum technique.

In T1, inoculation of sorghum seedlings by means of hypodermic injection was performed. Two disinfected rhizomes per pot were sown in fifteen 10 × 13 × × 15 cm plastic pots filled with sterile sand and inoculated at the 3–4 leaf stage according to Edmunds technique (Edmunds 1963), maintained in a greenhouse at 20°C (±5°C) with 80–90% relative humidity andcycles of 16–8 h light-darkness. As a control, an equal number of plants was inoculated with the broth only. Three days later, five plants per stage were removed until the heading stage or until visible symptoms were observed. Samples were fixed in formaldehyde/acetic acid and ethyl alcohol (FAA) and storedin the laboratory. The presence of mycelium in apical buds, leaves, stalks and heads was assessed.

In T2, inoculation of rhizomes with teliospores and sporidial cultures by the vacuum technique was done. Two assays were conducted using two inoculum types: **T2i**. Immersion of rhizomes in a suspension of potato broth + teliospores (10^6spores/ml) with vacuum (0.1 MPa for 5 min) and **T2ii**. Immersion of rhizomes in potato broth + sporidia (10^6sporidia/ml) with vacuum (0.1 Mpafor 5 min). As controls, rhizomes were immersed in broth only. The inoculated material was sown in plastic trays (10 × 13 × 15 cm) containing sterile sand watered with distilled water and maintained in achamber "BINDER KBF LQC" with favorable environmental conditions for plant growth and pathogen (temperature 20°C (±5°C), relative humidity 40–60%

and cycles of 16 h light + 8 h darkness). When seedlings reached the 2–3 leaf stage, 2 plants per pot were transplanted into 4 liter pots containing sterile black substrate, to avoid contamination with other microorganisms. Plants were then transferred to the greenhouse to complete their growing cycle.

A completely randomized design with three replications, and 2 seedlings/pot in 20 pots/treatment and controls were used. At the end of the growing cycle of the weed, the aerial dry matter (ADM g.pl-1) and rhizome dry matter (RDM g.pot-1.) was determined according to Ward *et al.* (1978). Data were analyzed and means were compared using the method of least significant differences (LSD) ($p < 0.05\%$) using SAS 6.03 (SAS, 1989).

Histological techniques

Samples of leaves with symptoms of the disease and vegetative and floral buds were fixed in 4% glutaraldehyde, then sectioned in 1 × 1 mm pieces with a microtome Sorvall MT 2–13 equipped with a glass blade to obtain 1-µm sections, and stained in Toluidine Blue before light microscope observations (Optical Olympus CX 21) to detect the presence of pathogen hyphae.

Samples of infected stalk nodes with apical buds, and panicles and controls were fixed in FAA, embedded in paraffin wax and then sectioned longitudinally and transversely (10–12 µm) using a Senior Rotary microtome "Model RMT-30". Fast-green staining was selected because this method gives good differentiation between the host and fungal components of the sorus. Sections were observed with a light microscope (Bracegirdle and Miles 1975; D´Ambrogio Argueso 1986).

The mature teliospores were mounted on a metal plate metallized coated gold-palladium and then observed and photographed. The observations were performed with a Philips XL 30 scanning electron microscope (SEM).

For transmission electron microscopy (TEM) studies, the samples were fixed in 2.5% glutaraldehyde in 0.1 M sodium phosphate buffer, pH = 7.2 at 4°C overnight. The materials were post-fixed in 1% OsO_4 in the same buffer for 4 h at room temperature and then rinsed in three 20-min changes of buffer. For dehydration an acetone series was used, and Spurr's resin was used for embedding (Spurr 1969). Sectioning was done with an ultramicrotome and thin sections were stained with uranyl acetate and lead citrate (Reynolds 1963; O'Brien and McCully 1981).

Inoculations by the vacuum technique

Samples of different organs such as leaves, apical meristems, and inflorescences were observed under a light microscope in order to detect the presence of the pathogen. Trypan Blue 2% in lactophenole was used as amounting medium.

To evaluate the development of *S. cruentum* and the host-pathogen relationship with *S. halepense*, samples were taken before apical meristem stem elongation, before inflorescence emergence, and at smutted panicles at the end of the vegetative cycle.

Results

Taxonomic identification of *S. cruentum*

Macroscopic observations confirmed that the sori, which showed a curved columella with fragile peridium, were typical of *S. cruentum* (Fig. 1a). Teliospores, which were found in preparations using scanning electron microscopy (SEM) and optical microscopy (Fig. 1b, c) were rounded to subglobose, with an echinulate episporium, 6–8 µm in diameter and dark brown. Teliospores germinated *in vitro* and produced metabasidia (= promycelia) with 4 cells and lateral basidiospores (= sporidia). Multiplication by budding generated an abundance of new sporidia (Fig. 1d). The sporidia quickly formed light-brown to ocher, yeast-like colonies with smooth edgeson PDA (Fig. 1e).

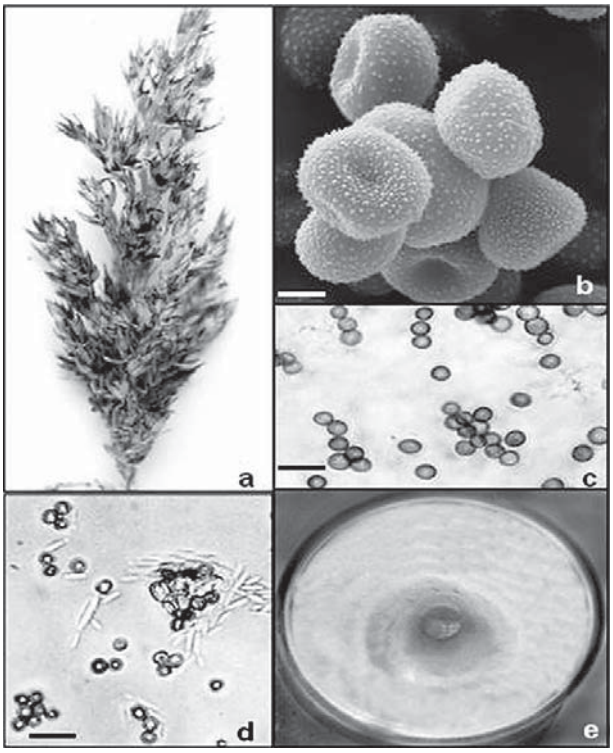

Fig. 1. Macroscopic and microscopic observations of *S. cruentum*. a – smutted panicle; b – photomicrograph of teliospores with echinulate episporium observed under electron microscope (SEM). Scale bar = 22 µ; c – photomicrograph of teliospores observed in optical microscope (OM). Scale bar = 14 µ; d – *in vitro* germination of sporidial type of teliospores on PDA. Scale bar = 14 µ; e – colony developedon PDA

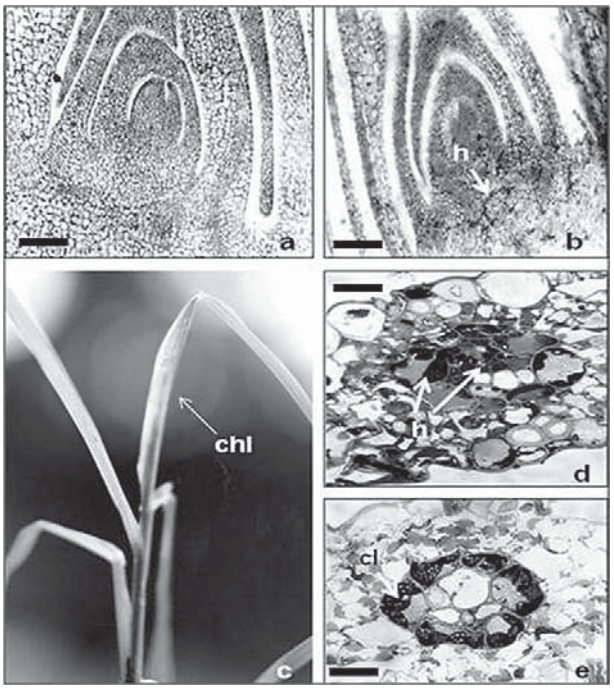

Fig. 2. Longitudinal sections of meristems of Johnson grass rhizomes. a – apical meristem and leaf primordium from uninfected control. Scale bar = 169 µ; b – apical meristem with fungal hyphae (h) from rhizomes inoculated with *S. cruentum*. Scale bar = 169 µ; c – leaves of inoculated plants showing chlorotic spots (chl); d–e – cross sections of leaves: d. Leaf of inoculated plants showing absence of chloroplasts and/or degraded by the hyphae of the fungus that are located in the vascular bundles (h). Scale bar = 16 µ; e – uninoculated with normal chloroplasts (cl) in the parenchyma of vascular bundles. Scale bar = 20 µ

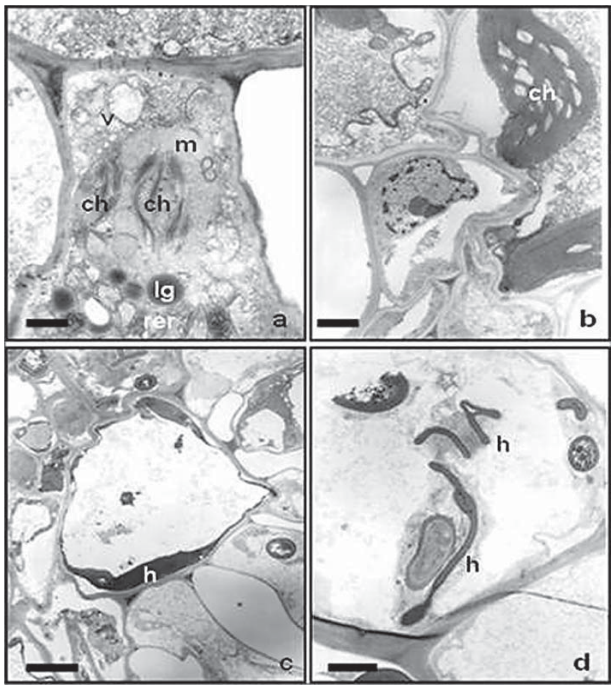

Fig. 3. Photomicrographs of Transmission Electron Microscopy (TEM) in *S. halepense*. a–b – ultrastructure of the mesophyll cells of the healthy leaves. a – healthy chlorenchyma cells containing chloroplasts (ch), lipid globules (lg), mitochondria (m), rough endoplasmic reticulum (ret) and vesicles (v) in the cytoplasm. Scale bar = 0.5 µ. b – parenchymal cells of vascular bundles with normal chloroplasts. Scale bar = 1 µ. c–d – parenchymal sheath cells infected with the hyphae (h) of *S. cruentum*. Scale bars = 2 µ

Inoculation with sporidia by the hypodermic syringe technique

Plants from rhizomes at the 4 to 6 leaf stage were inoculated with sporidia. Three days after inoculation, samples from apical meristems and leaves of treated plants and controls were taken for the longitudinal section. Samples from healthy plants showed no pathogen infection (Fig. 2a), whereas those from infected plants showed inter- and intracellular hyphae (Fig. 2b). Between 10 to 15 days after inoculation 4 plants were recorded and hypoplastic symptoms with mild to marked chlorosis (Fig. 2c). Metaplastic symptoms with the production of anthocyanin pigments were observed. There was also a loss of leaves by vascular necrosis. Cross sections of the sheath from the vascular parenchyma showed the absence of or degraded chloroplasts (Fig. 2d). Controls showed a normal location of the chloroplasts (Fig. 2e). In plants where chlorosis was marked, there was a minor effect on growth without death. These observations were confirmed at the ultrastructural level with TEM. Healthy leaves showed normal chloroplasts in the chlorenchyma of the mesophyll and parenchyma sheath of the vascular bundles and normal development of structures in the cytoplasm, such as lipid globules, rough endoplasmic reticulum, mitochondria, and vesicles (Fig. 3a, b). In contrast, infected leaves of inoculated plants showed the presence of the pathogen hyphae (Fig. 3c, d). About 18–25 days after inoculation, the reproductive stage induced the production of multiple buds (between 3 and 7 buds) originating at a common node in one of the upper nodes. This type of abnormality is typical for *S. cruentum*. Subsequently, we observed the emergence of small infected panicles or the formation of sterile panicles or phyllodes and small stems (Fig. 4a). These abnormalities were not seen in the controls. When histological sections of small stems were analyzed, bud formation, i.e. vegetative and reproductive buds protected by the formation of leaf primordia, was visible (Fig. 4b). Hyphae were observed with an optical microscope at the base of the meristem of the stem (Fig. 4c, d) and confirmed in detail with TEM (Fig. 6a, b). The longitudinal and transverse sections of stems showed that the pathogen was not present intreated plants. We also determined that the pathogen does not grow until the gynoecium is fully developed within the spikelets. Thus, the invasion of the fungus begins primarily by the gynoecium, which shows that hyphal fragments and spermogenesis occur with the formation of teliospores. The sporulation process continues in the

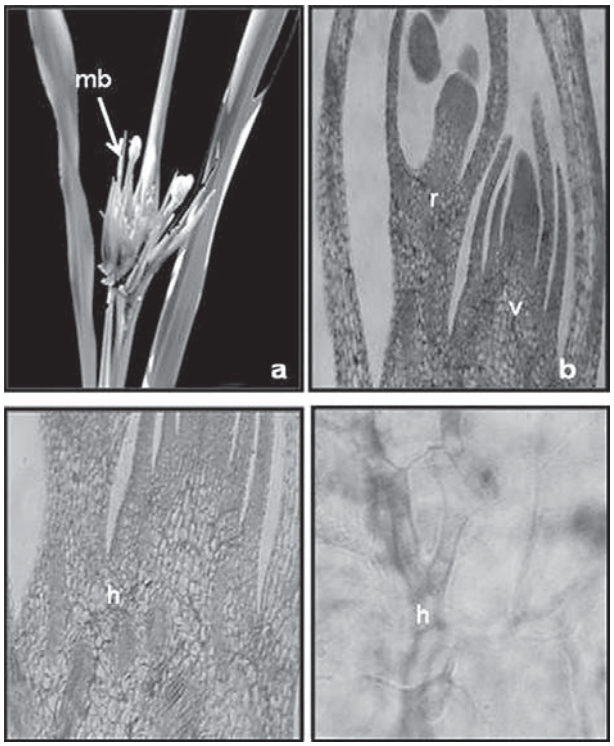

Fig. 4. Morphological changes produced by *S. cruentum*. a – multiple buds that cause small panicles infected with the fungus; b–c – longitudinal section of a multiple bud (mb), b – vegetative (v) and reproductive (r) buds. Scale bar = 169 µ, c – bud bottoms with the presence of fungal hyphae (h). Scale bar = 74 µ; d – detail of intracellular branched hyphae (h). Scale bar = 8.5 µ

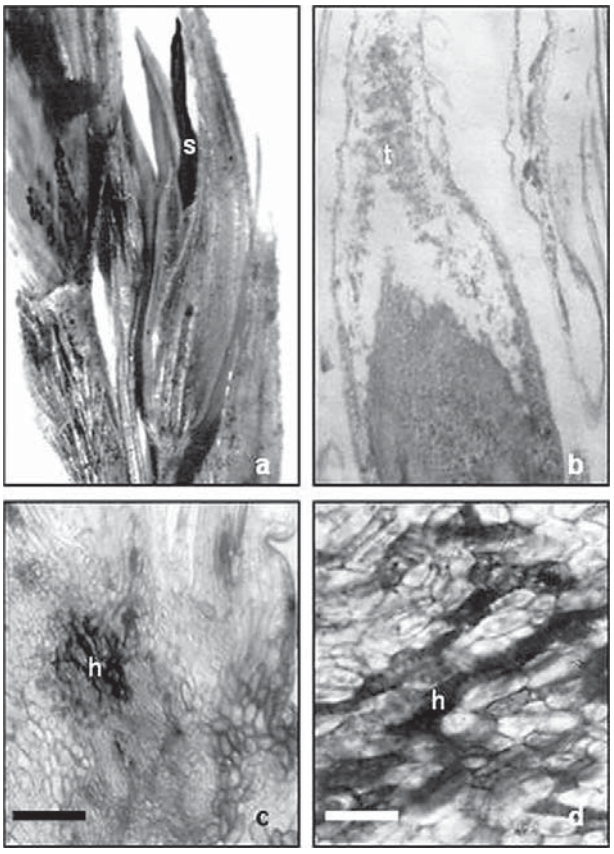

Fig. 5. a – Spikelets infected with smut with a sorum (s) formed by a curved columella, b – longitudinal section of gynoecium with the presence of teliospores (t), c–d – detail of a spikelet infected by the fungus (h). Scale bars = c, 50 µ; d, 8,5 µ.

anthers to produce the total disintegration of the tissues involved. The grains were replaced by 2.5 cm medium-sized sorior surrounded by a thin gray membrane. This membrane usually breaks before or after the emergence of the panicle and brown to black teliospores are detached and a curved structure (columella) (Fig. 5a, b) formed by fungal tissues and the host can be seen with the naked eye (McTaggart *et al.* 2012). Glumes and bracts remain intact to protect the sorus. When spikelets in formation were extracted, the presence of fungal hyphae was observed at the base of the flower primordia in all five specimens analyzed. At a more advanced stage, histological sections of basal spikelets showed that hyphae were located in the conducting tissues and the parenchyma of the floral pedicel (Fig. 5c, d). This was observed in greater detail in the TEM: cells infected by fungal hyphae, cytoplasm of the infected cell containing starch granules and bottoms of spikelet cells infected by *S. cruentum* (Fig. 6a, b, c, d).

Inoculation with teliospores by the vacuum technique

The development of reproductive and vegetative mycelium of *S. cruentum*. We also examined the base of 10 outbreaks in 3–4 weeks old inoculated plants. In outbreaks, only bits of hyphae in meristematic tissue

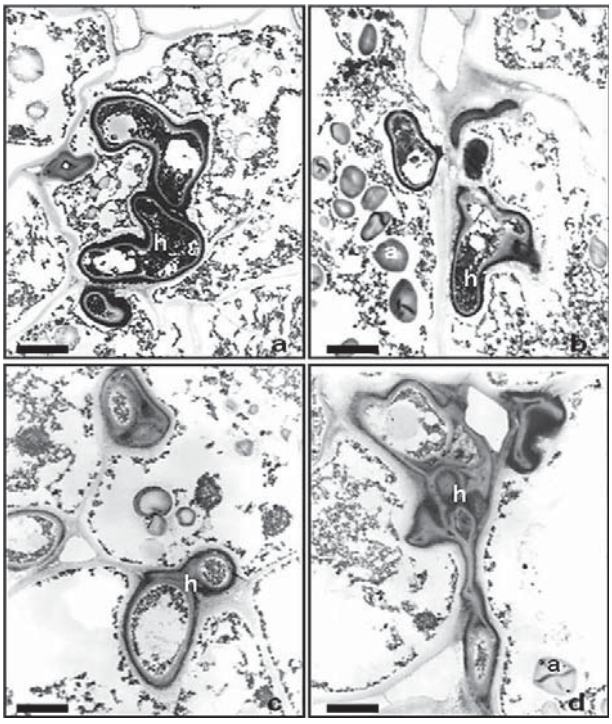

Fig. 6. Photomicrographs of Transmission Electron Microscopy (TEM) in *S. halepense*, a–b. Ultrastructure of vegetative meristem. Scale bars = 2 µ, a – cells infected by fungal hyphae (h); b – cytoplasm of the infected cell containing starch granules (a), c–d – bottoms of spikelet cells infected by *S. cruentum*. Scale bars = 1 µ

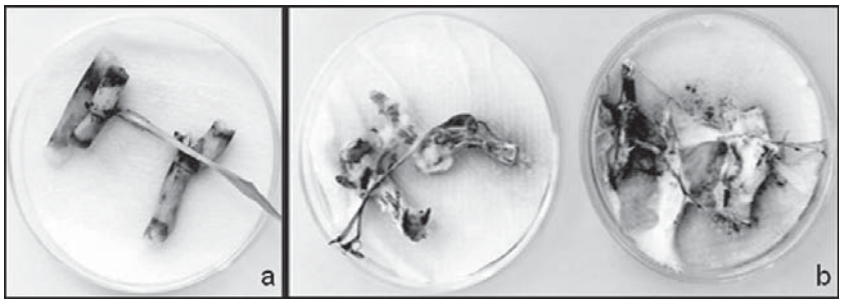

Fig. 7. Shoots of Johnson grass rhizomes in Petri dishes; a – healthy control; b – rhizomes and necrotic shoots colonized by the fungus (left). Detail of the whitish mycelium of *S. cruentum* (right)

were inactive, while some developed shoot apices were fully colonized in relatively early stages of development. In other cases, plants showed symptoms of metaplastic type in leaves but not as marked as in the case of infection with the hypodermic syringe technique. The presence of hyphae was not determined in stems.

Inoculation with sporidia by the vacuum technique

We examined 80 rootstocks inoculated with sporidia of the fungus and found a white mycelium which rapidly colonized the surface and prevented the development of seedlings. Inoculated plants showed inhibition of bud sprouting, dehydration and partial or total necrosis until death of the rhizome, unlike the controls, which grew normally. To check if these changes were caused by insufficient disinfection, contaminants or other causes, rhizomes inoculated with sporidia by the vacuum technique (**T2ii**) were placed in Petri dishes on paper moistened with sterile distilled water to confirm the presence of *S. cruentum* by reisolation of the fungus (Fig. 7a, b). Also, in this case, the intercellular mycelia and pathogen hyphae were found at the base of the apical meristem and the region of buds. Fusion between sporidia was not observed, but the development of intracellular hyphae was seen. Other plants showed symptoms of chlorosis in the leaves, and histological sections confirmed the presence of the hyphae of the fungus. Also, in this case, we observed the presence of aerial shoots at the nodes, multiple panicles and sterile buds induced by the pathogen, unlike the controls, which showed normal development of plants.

Aerial dry matter (ADM)

ADM showed no significant differences between the inoculation treatment with teliospores by the vacuum technique **T2i** (0.16 $g.^{pl-1}$) and withsporidia **T2ii** (0.17 $g.^{pl-1}$), but there were highly significant differences ($p \leq 0.05$) when compared with the controls (0.52 $g.^{pl-1}$) (Fig. 8). The plants showed both a shortening of the internodes and a decrease in height compared to the control. An early flowering in plants that had smut as opposed to controls was also observed.

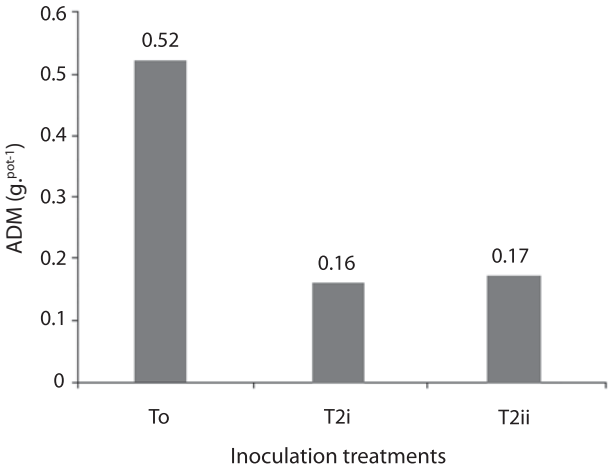

Fig. 8. Dry mass of aerial parts (ADM) of Johnson grass plants at the end of the crop cycle: T0 – control treatment; T2i – rhizome immersion in liquid culture suspension + teliospores; T2ii – rhizome immersion in liquid media + sporidia

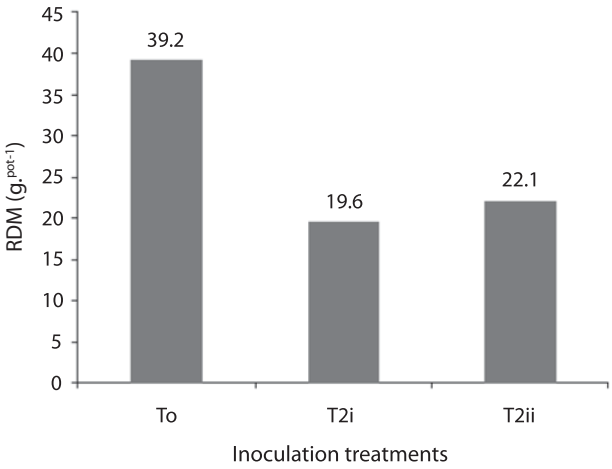

Fig. 9. Dry mass of rhizomes (RDM) of Johnson grass plants at the end of the crop cycle: T0 – control treatment; T2i – rhizome immersion in liquid culture suspension + teliospores; T2ii – rhizome immersion in liquid media + sporidia

Rhizome dry matter (RDM)

RDM showed a behavior similar to that of ADM with **T2i** and **T2ii** treatments. The production of rhizomes obtained was similar to **T2i** (19.60 $g.^{pl-1}$) and **T2ii** (22.10 $g.^{pl-1}$). A significant reduction ($p \leq 0.05$) of the weight of the rhizomes with respect the controls was found (39.2 $g.^{pl-1}$) (Fig. 9).

Discussion

Several factors associated with infection of the rhizomes of Johnson grass (*S. halepense)* seedlings were found during our experiments. Inoculations with the hypodermic syringe method had a more drastic effect than the vacuum technique since we found a higher number of plants with leaves with chlorotic spots and more anthocyanin pigments (Hanna 1929). In infected leaves high amounts of anthocyanins were present as a result of the accumulation of this pigment in the cells of the epidermis. Results showed the presence of mycelium of the fungus in the parenchymal tissue and in the nodal area. The scattered distribution of the mycelium was probably due to the inoculation method used, as the sporidia were forced to penetrate into the plant tissue. The mycelium does not usually invade the apical meristem or vascular tissue of the shoots. Other researchers have reported the presence of chlorotic spots containing branched hyphae that developed and emerged on the leaves of maize and sorghum seedlings inoculated with *S. reilianum*. They also have found a correlation between field and greenhouse experiments which indicated that the hypodermic syringe method of inoculation is useful for the evaluation of different genotypes resistant or susceptible to smut. Here, we determined that the method was efficient in replacing field testing in screening assays (Wilson and Frederiksen 1970; Matyac 1985; Matyac and Kommedaha 1985; Craig and Fredenksen 1992; Snetselaar and Mims 1994). According to our observations of the cross sections of leaves, the hyphae of the fungus degraded chloroplasts and invaded vascular bundles. Subsequently, cells collapsed and widespread necrosis was observed. In several cases, leaves withered, but the plant continued its development to complete its cycle, and showed the characteristic symptoms when the smut panicle emerged. Snetselaar and Mims (1994), Martinez *et al.* (1999) and Martinez *et al.* (2002) observed that after inoculation of *S. reilianum*, young plants were slightly chlorotic and contained scattered hyphae. The latter developed in the epidermal cells and vascular parenchyma, but not in the intercellular spaces.

Our results suggest the aggressiveness of *S. cruentum* because multiple buds which in turn developed into vegetative and reproductive buds enclosed in leaves were frequently observed. Similar morphological alterations such as the formation of convoluted whips emerging from lateral buds from the stems, producing deformation, have been reported in *Ustilago scitaminea* Sydow (sugarcane smut) in stems, leaves and inflorescences (Sharma 1956; Byther and Steiner 1974). Other studies also described the formation of single or multiple inflorescences and the formation of multiple whips emerging from the same point at the apex of the stem due to the smut infection. In addition, a reduction in plant growth and a shortening of internodes were reported (Nasr 1976; Astiz Gassó 1988). One hypothesis to explain these changes could be the excessive production of hormones that stimulate stem elongation, shortening of internodes, formation of phyllodes and induction of changes in vegetative to reproductive primordia anticipating flowering. This stage of the plant is essential for the fungus to infect inflorescences and quickly to produce fragmentation of the mycelium and sporogenesis. While studying the involvement of gibberellins in *S. reilianum*, Matheussen *et al.* (1990) observed that the fungus produced these hormones on the first day of infection, and also when the fungus was cultured in vitro. Other studies on *S. reilianum* infected plants showed that changes in the inflorescence and the branching of maize also led to an increase in the auxin content of the inflorescence as well as an accumulation of reactive oxygen species (Ghareeb *et al.* 2011). In the case of *S. cruentum* further research is needed to confirm if the fungus produces these hormones to induce the abnormalities showed in this assay.

The longitudinal sections of the vegetative apex analyzed in the present work showed, in some cases, the presence of broken hyphae of the fungus, but not the fusion of sporidia. Similar results have been recorded in artificial inoculations of *S. reilianum* in *S. bicolor* (Wilson and Frederiksen 1970; Osorio and Frederiksen 1998) and *S. sorghi* (Moharam *et al.* 2012). Alternatively, intracellular branched hyphae below the dormant vegetative apex was observed previously to the differentiation of floral primord and the beginning of the mycelium colonization to the spikelets.

In our study, intracellular branched hyphae of the fungus below the vegetative apex observed in the dormant stage until the differentiation of floral primordia occurred and the mycelium began to move to the spikelets of the inflorescence. These were infected at ground level prior to stem elongation.

Regarding the histopathological techniques used in this study, it is important to point out that previously they were successfully used in studies of smuts affecting other plant species. In the work of Millhollon (2000), for example, histological nodal segments of seedlings of *S. halepense* were used to confirm the

effectiveness of field inoculations with suspensions of teliospores and sporidia of *S. cruentum*. Likewise, Sinha *et al.* (1982) used histology staining with trypan blue in lactophenol to detect the presence of the mycelia of *U. scitaminea* from nodes, which were confirmed in the basal part of apical meristem.

Our results showed that fungal infection occurs in the floral primordium and not when the tassel is fully differentiated and emerged from the flag leaf. In relation to the infection of spikelets of the panicle, we also noted that the latter must be fully formed, because there is a dependence on nutritional compounds. Such elements are apparently provided by the gynoecium, stamens and flower stigma to induce fragmentation of hyphae followed by sporulation and maturation of teliospores. Since we found no other reports in *S. cruentum*, we consider it important to undertake further studies to elucidate this process.

According to our results, it is important to consider the effect of the pathogen on the rhizomes' dry matter, as shown by the below-ground biomass, which decreased at a time when the weed recorded an increase in the production of rhizomes at the end of the crop cycle. In this sense, Williams and Ingber (1977) demonstrated that in the absence of competition and under non-limiting water and nutritional conditions, *S. halepense* allocated 27% of the dry matter to the rhizomes and only 4% to the production of seeds. These researchers stated that in the presence of intraspecific competition, weeds retard the formation of rhizomes. Smith and Holt (1997) established that the use of additional weed control and biological control are not antagonistic, but rather, that there are synergistic effects (where the combined effects are more than additive) that depend on the reproductive rate of weeds and the density at which they grow. In the present work, we also noted that the ADM was reduced because the diseased plants had lower height than controls. Reports made by Luttrell *et al.* (1964) and Millhollon (2000) on the species mentioned differences in growth of plants treated with smut.

In this work, a high level of fungal infection was obtained using the different inoculation methods tested and under the environmental conditions under which the experiments were performed. In addition, changes in the structural morphology of the host were found similar to those reported in previous research conducted in *S. reilianum* (Craig and Fredenksen 1992; Martinez *et al.* 1999). Similarly, it was confirmed that *S. cruentum* produced structural morphological changes in the host.

As in the rest of Poaceae, the true stem of *S. halepense* is compressed in a basal plate with nodes and internodes compressed that elongate at the reproductive stage (Maddaloni and Ferrari 2001). Due to this form of plant growth, we can say that, according to the observations made, the pathogen located in the base of the buds migrates to the inflorescence producing the disease.

Regarding the management of this weed, the most effective method to control Johnson grassis is to cause a decrease in the production of rhizomes, as rhizomes have an important role in the reproductive dynamics of this weed (Williams and Ingber 1977). As our results showed, a marked reduction of rhizomes (RDM) has a potential role for *S. cruentum* as biocontrol agent.

As part of our research for integrated weed control, we studied the possible use of fungi as potential biocontrol agents of the Ustilaginales group. The use of *Ustilago* spp. to control *Paspalum* on switch grass (Arévalo *et al.* 2000) and *U. bullata* on cheat grass was effective due to the reduction in seed production because it was replaced by teliospores mass demonstrating its potential as a biocontrol agent. Moreover, in these studies the fungal optimum environmental conditions to cause infection in plants were established (Meyer *et al.* 2001; Boguena 2003, Boguena *et al.* 2007). Therefore, the feasibility of biological control using *S. cruentum* could be reliably established within an integrated framework of alternatives.

Moreover, it is necessary to establish the possible interactions between the implementation of biological control with other management alternatives in Johnson grass, such as the use of herbicides at reduced doses and the use of the competitive ability of crops to compete with weeds. Further experiments should be carried out to elucidate the effect of the pathogen in weeds under field conditions, given the high efficiency of *S. cruentum* to produce infections in *S. halepense*. This fungus could be used as a biocontrol agent in areas where the weed grows and limits agricultural production, by improving crop fields and promoting a sustainable agriculture.

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