Effects of calcium silicate on the protection of *Brachiaria* seeds against *Sclerotium rolfsii*

Efectos del silicato de calcio en la protección de las semillas de *Brachiaria* contra *Sclerotium rolfsii*

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Originales: Recepción: 13/11/2019 - Aceptación: 28/04/2020

Abstract

Sclerotium rolfsii, a widespread fungus in tropical and subtropical regions, is a phytopathogen that affects a large number of cultivated and wild plants-*e.g. Brachiaria* species-, causing plant damping-off events. Current disease control strategies include chemical treatment of seeds, genetic resistance and crop rotation. Compared to synthetic agrochemicals, silicon may induce plant resistance. This study examined the efficiency of calcium silicate in the coating of *Brachiaria* seeds on the incidence of the disease during plant germination and establishment. The experiment consisted of six *Brachiaria brizantha* seed-coating treatments involving different calcium silicate and sand proportions and two controls. The fungus was inoculated at three different times in relation to seed sowing: inoculation at sowing (IN0); inoculation 10 days after sowing (IN10); inoculation 20 days after sowing (IN20); and no inoculation (control). Radicle emergence, germination, abnormal seedlings, damped-off seedlings and dead seeds were evaluated. Increase in the germination rate of *B. brizantha* cv. MG5 seeds coated with calcium silicate was observed, which showed protective effect against the incidence of *S. rolfsii*, directly reflecting in lower damping-off percentages. The application of calcium silicate as coating provided protection to MG5 seeds and plants inoculated with *S. rolfsii*.

Keywords
Brachiaria brizantha • *Sclerotium rolfsii* • damping-off • seed coating • silicon

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Resumen

El hongo *Sclerotium rolfsii* es un fitopatógeno distribuido en regiones tropicales y subtropicales que infecta una gran cantidad de plantas cultivadas y silvestres, entre ellas la brachiaria, causando el vuelco. Las estrategias actuales de control de enfermedades implican el tratamiento químico de semillas, la resistencia genética y la rotación de cultivos. En este trabajo se evaluó la eficiencia del silicato de calcio en el recubrimiento de semillas de brachiaria bajo la incidencia de la enfermedad durante la germinación y el establecimiento de la planta. El experimento consistió en seis tratamientos de recubrimiento de semillas de *Brachiaria brizantha* con diferentes proporciones de silicato de calcio/arena y dos controles. La inoculación del hongo ocurrió en tres momentos diferentes en relación con la siembra de semillas: inoculación junto con siembra (IN0); inoculación 10 días después de la siembra (IN10), inoculación 20 días después de la siembra (IN20) y control no inoculado. Se evaluó emisión de radícula, germinación, plántulas anormales, plántulas caídas y semillas muertas. Germinación de *B. brizantha* cv. MG5 recubierto con silicato cálcico, que tuvo un efecto protector sobre la incidencia de *S. rolfsii*, reflejando directamente el menor porcentaje de inclinación de la planta.

Palabras clave
Brachiaria brizantha • *Sclerotium rolfsii* • damping-off • recubrimiento de semillas • silicio

Introduction

According to Land Coverage and Use Monitoring data from the Brazilian Institute of Geography and Statistics (IBGE), the total managed pasture area in Brazil in 2016 was 1,118,893 km^2 (8). In addition, Brazil stands out as the largest producer, consumer and exporter of forage-grass seeds worldwide (3, 9). However, despite the economic importance of the sector, the grass-seed production chain has faced obstacles such as low seedling survival rates after sowing due to pests and diseases; low soil fertility and compaction; and adverse climatic conditions in producing regions (7, 22).

Sclerotium rolfsii Sacc. is a pathogenic fungus from tropical and subtropical regions, mainly from temperate climate regions (16). This soilborne fungus has a wide variety of hosts, including horticultural, ornamental, leguminous and forage crops such as tomato, bean, almond, potato, cotton, wheat, corn (6, 10, 17) and *Brachiaria* species (1). *S. rolfsii* is responsible for rotting of the root system and root base, and for plant damping-off, generating significant economic losses in agriculture (5). Moreover, studies have reported difficulty in controlling this pathogen due to the formation of sclerotia that can persist in the soil for years (21, 27).

Silicon (Si) has been shown to influence pathogen resistance in plants. This nutrient induces changes in the plant anatomy that interferes with the plant × pathogen × environment interaction (26). Thus, plants originating from silicate-coated seeds would have the nutrient readily available for absorption during seed germination, growth and initial development of seedling. This would allow the beneficial effects of Si, preventing plants stress in response to edaphoclimatic conditions (26).

Additional known benefits of the action of Si are increased thickening and lignification of epidermal cells. Grasses of the genera *Brachiaria* and *Panicum* are considered silicon accumulators, which means that the benefits attributed to silicon can be obtained (20). Furthermore, Si improves plant defense mechanisms through the synthesis of secondary compounds such as polyphenol, lignin and peroxidase (14); production of phytoalexins, glucanases and peroxidases; and contribution to the regulation of pathogenicity or stress-related gene expression, limiting pathogen invasion and colonization (26).

In view of the search for environmentally sustainable-*i.e.*, more efficient and less environmentally toxic-alternatives for the control of *S. rolfsii*, the present study examined the effect of calcium-silicate coating for *Brachiaria brizantha* seeds on seedling damping-off caused by *S. rolfsii*.

Material and methods

Seed purchase and processing

Commercial *Brachiaria brizantha* cv. MG5 seeds (export class) were used in the experiment. Seeds were previously seived with De Leo® blower, using mesh size determined in a pre-test to eliminate empty seeds and minor impurities. Subsequently, all remaining impurities were removed by visual separation so that only pure seeds would remain. These were then immersed in 98% sulfuric acid for 10 min, washed in abundant running water and left to dry at room temperature for 24 h.

Seeds were coated with sand sieved through 100- and 500-mesh size, generating material with particle size of approximately 0.25 mm. Cascorez® Extra, a polyvinyl acetate-based glue, was used as cementing material after being diluted in water warmed to 70°C at 1:1 ratio (v/v), in accordance with methodology described by Mendonça *et al*. (2007).

Six coating treatments were prepared using different calcium silicate and sand proportions, in addition to two controls, namely, treatment 1 (T1) = 200 g/200 g; treatment 2 (T2) = 250 g/150 g; treatment 3 (T3) = 300 g/100 g; treatment 4 (T4) = 350 g/50 g; treatment 5 (T5) = 400 g/0 g; treatment 6 (T6) = 0 g/400 g; treatment 7 (T7) = Control (uncoated and scarified seeds); and treatment 8 (T8) = Control without scarification.

The proportion between filler material (*i.e.*, sand/glue) and seed was 4:1 (p/p), and the material was applied as two 12.5-g layers using benchtop seed-coating machine (N10, Newpack®) equipped with stainless-steel pan, a spraying device to apply the adhesive material and hot-air blower for drying. The equipment was regulated at 90 rpm; the cementing solution was sprayed for 1 s; and the air blower temperature was set at 40°C, according to methodology of Xavier *et al*. (2015).

Seeds to be coated were placed in the coating pan together with a portion of filler material (12.5 g). The cementing solution spray was activated three consecutive times, followed by another portion of filler material (12.5 g) on the seed mass and, again, another application of cementing solution. After this process, the air blower (40°C) was activated for 1 min for drying. This procedure corresponded to one coating layer and was repeated until 16 layers were applied. After the entire coating process, seeds were packed in multipurpose paper bags until use.

Production of *Sclerotium rolfsii* Sacc. inoculums and physiological seed quality evaluation

S. Rolfsii inoculum was produced from fungi sclerotia that had been isolated from *B. brizantha* seeds. These were sown in Petri dishes containing potato-dextrose-agar (PDA) culture and incubated in germination chambers at 27°C for seven days, after which, seeds were used in the experiment.

Pathogenicity assays were assembled in plastic germination boxes disinfected with 0.3% sodium hypochlorite. Each germination box received two sterilized leaves (moistened with sterilized water) on germination paper, in the proportion of 2.5 times their dry weight. Four replicates of 50 seeds were sown per germination box per treatment.

Sclerotium rolfsii was inoculated at three different times in relation to seed sowing, using five 0.5-cm-diameter fungus colony discs per germination box as follows: incubation at sowing (IN0); inoculation 10 days after sowing (IN10); and inoculation 20 days after sowing (IN20); plus non-inoculated control. Non-inoculated seeds were termed INot. Germination boxes containing seeds inoculated with *S. rolfsii* were placed in a growth room at average temperature of 27°C, under 12-h photoperiod.

As the *B. brizantha* germination test lasted 21 days, the duration of assays varied in each treatment, since evaluations were performed based on the pathogen inoculation. However, the time of contact between pathogen and seeds was fixed at 21 days for all treatments.

Evaluations were carried out by calculating the percentages of seeds that produced radicle; germination; abnormal seedlings; damped-off seedlings; normal damped-off seedlings; abnormal damped-off seedlings; and dead seeds, by adopting criteria established by Brasil (2009). To evaluate disease incidence, the presence of rotting, wilting, discoloration or seedling damping-off symptoms was considered. The percentage of seeds that produced radicle was the parameter adopted to indicate the number of seeds that started the germination process, regardless of whether or not they became normal seedlings, since radicle

emergence might not occur due to infection by *S. rolfsii*. This parameter also differs from the germination percentage evaluation, which reflects the number of normal seedlings formed.

Statistical analysis

The experiment was set up as a completely randomized design in an 8 × 4 factorial arrangement consisting of six seed-coating treatments with different calcium silicate proportions and two controls (scarified and non-scarified seeds); and three inoculation times of fungus *S. rolfsii* after sowing plus control (no inoculation), with four replicates of 50 seeds. Data were submitted to analysis of variance and means were compared by the Duncan test ($p<0.05$).

Results and discussion

Germination and development of *B. brizantha* seedlings were significantly affected by the interaction between different calcium silicate and sand proportions in the seed coating and different inoculation times. Radicle emergence percentage differed for the different inoculation times (0, 10, or 20 days after sowing) and in non-inoculated seeds (figure 1).

CS*= calcium silicate
CSW*** = Control, non-scarified seeds.

CS * = silicato de calcio
CSW *** = Control, semillas sin carificar.

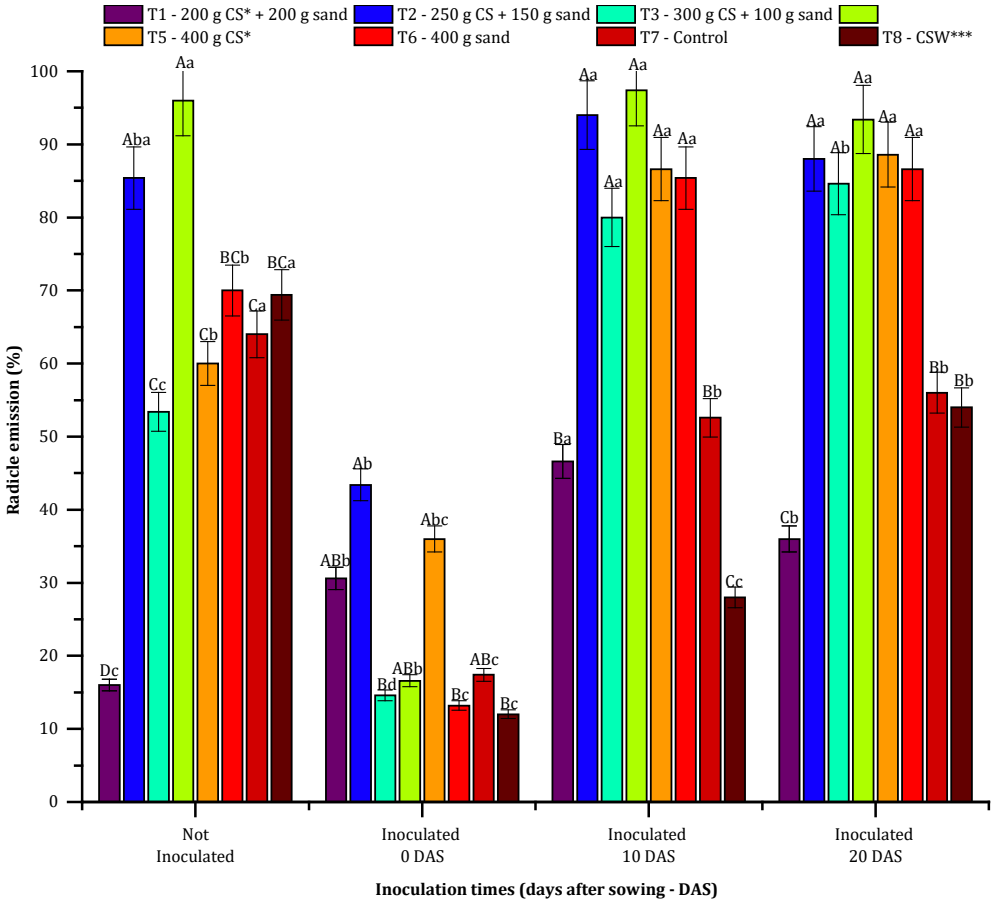

Figure 1. Radicle emergence in *B.brizantha* cv. MG5 as a function of seed coating with different calcium silicate + sand proportions and different inoculation times of fungus *S.rolfsii*. Uppercase letters compare treatments and lowercase letters compare inoculation times by the Duncan test ($p<0.05$).

Figura 1. Emergencia de radículas en *B. brizantha* cv. MG5 en función del recubrimiento de semillas con diferentes proporciones de silicato de calcio + arena y diferentes tiempos de inoculación del hongo *S. rolfsii*. Las letras mayúsculas comparan los tratamientos y las letras minúsculas comparan los tiempos de inoculación de hongos, mediante la prueba de Duncan ($p <0,05$).

Seeds not inoculated with the fungus (INot) exhibited good viability for most coatings, especially in T2 and T4 treatments (250 and 350 g of calcium silicate, respectively). This group presented radicle emergence percentages greater than 85% (figure 1, page 292). Additionally, the results for radicle emergence in control treatment (T7), which exceeded that of the seeds not inoculated with the fungus by 60% (figure 1, page 292), indicated good seed health, since pathogens, carried by seeds or otherwise, tend to reduce their vigor (4).

When *S. rolfsii* was inoculated at sowing (IN0), reduction in radicle emergence was observed, suggesting a pathogenic effect of the fungus on *B. brizantha* (19). However, radicle emergence percentage was higher in T2 and T5 compared to the other treatments, reaching values between 35 and 44% (figure 1, page 292).

In IN10 and IN20 inoculation times, radicle emergence percentage reached values between 80 and 97.4% in T2, T3, T4, T5 and T6 treatments (figure 1, page 292). Lower means were observed in T1, T7 and T8 treatments, where results ranged from 36 to 56% (figure 1, page 292).

Seed germination test results are described in figure 2. Germination percentage was significantly lower in IN0 compared to INot, IN10 and IN20 (figure 2). Additionally, regardless of inoculation time (IN10 or IN20), in T2, T3, T4, T5 and T6 treatments, the germination percentages of *B. brizantha* seeds were higher than those obtained in T1, T7 and T8 treatments (figure 2).

CS*= calcium silicate
CSW*** = Control, non-scarified seeds.

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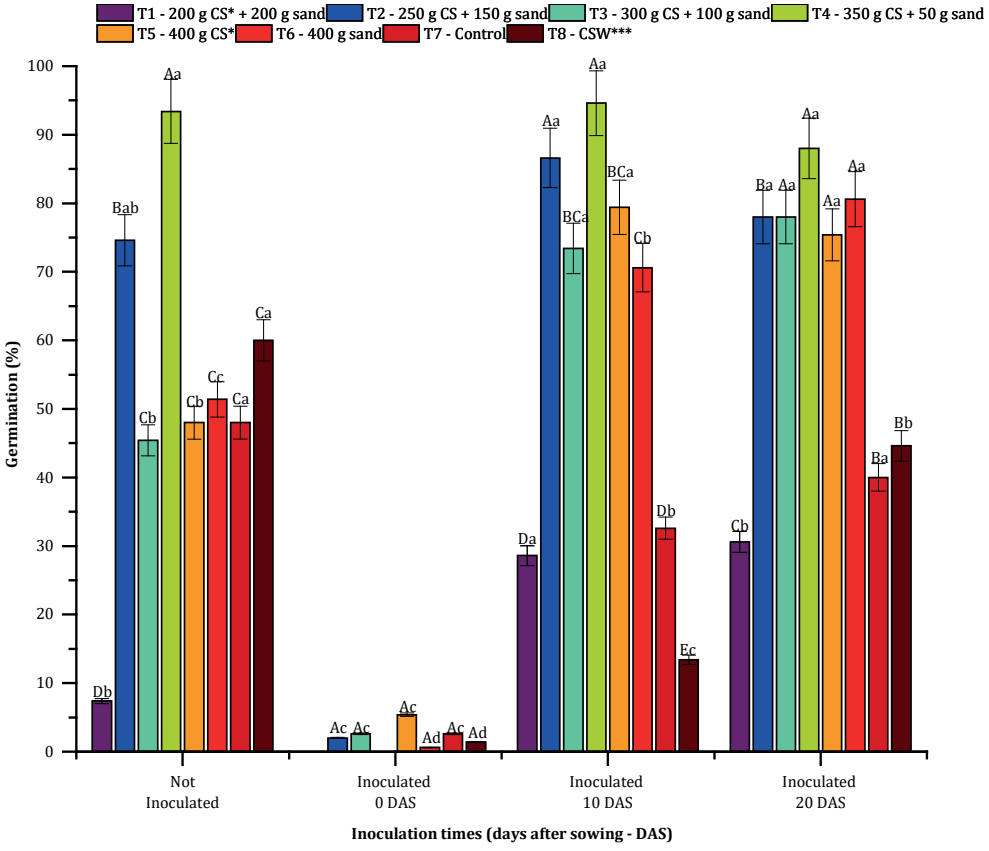

Figure 2. Germination of *B. brizantha* cv. MG5 seeds as a function of seed coating with different calcium silicate + sand proportions and different inoculation times of the fungus *S.rolfsii*. Uppercase letters compare treatments and lowercase letters compare inoculation times by the Duncan test ($p<0.05$).

Figura 2. Germinación de *B. brizantha* cv. Semillas MG5 en función del recubrimiento de semillas con diferentes proporciones de silicato de calcio + arena y diferentes tiempos de inoculación del hongo *S.rolfsii*. Las letras mayúsculas comparan los tratamientos y las letras minúsculas comparan los tiempos de inoculación de hongos, mediante la prueba de Duncan ($p <0,05$).

Seed germination was negatively affected by the pathogen in all treatments when fungus was inoculated at sowing (figure 2, page 293). Moreover, in T6 treatment and IN20 inoculation time, in which only sand was used in seed coating, the average germination percentage was statistically equal to those obtained in T2, T3, T4 and T5 treatments, in which calcium silicate was also used (figure 2, page 293). This increase may be explained by the fact that germination began before inoculation, allowing the absorption of silicate by the seedling, consequently providing physical protection against the pathogen; or by the action of silicon as anti-stress element (26). Since seeds were coated only a few days before use, calcium silicate absorption was not likely of providing protection against *S. rolfsii*, which leads to increase in the number of abnormal seedlings and restricted germination (figures 2, page 293 and figure 3).

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CSW *** = Control, semillas sin carificar.

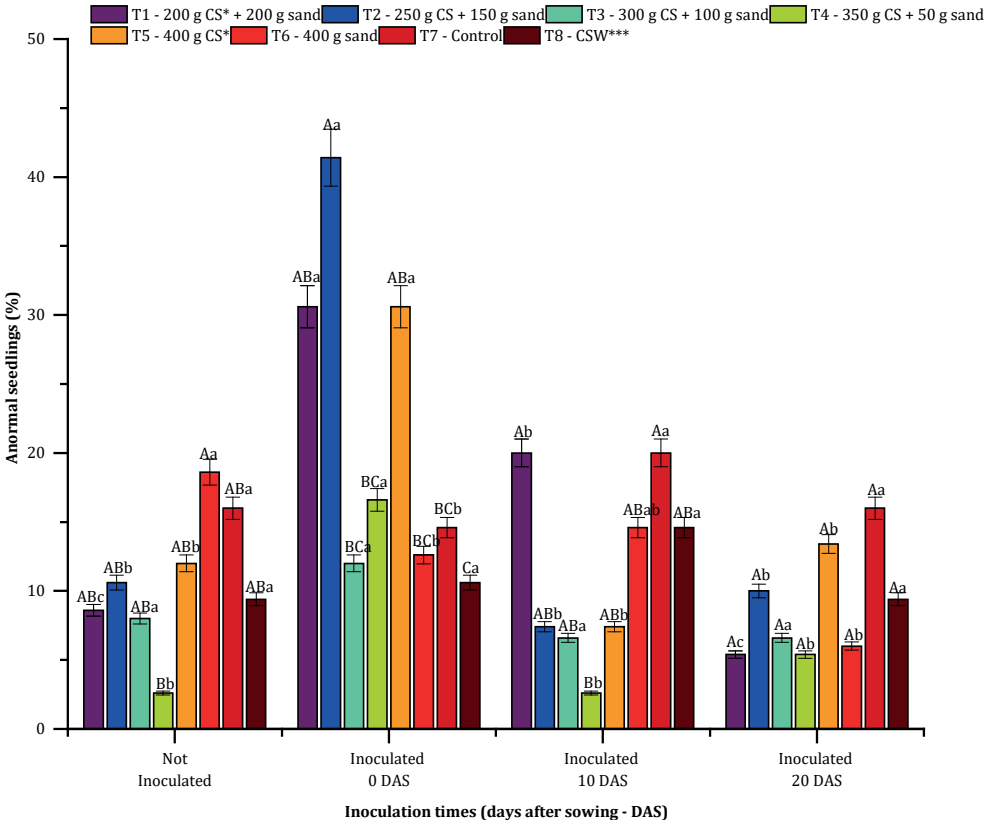

Figure 3. Percentages of abnormal *B. brizantha* cv. MG5 seedlings as a function of seed coating with different calcium silicate + sand proportions and different inoculation times of *S. rolfsii*. Uppercase letters compare treatments and lowercase letters compare inoculation times by the Duncan test ($p<0.05$).

Figura 3. Porcentajes de plántulas anormales de *B. brizantha* cv. MG5 en función del recubrimiento de semillas con diferentes proporciones de silicato de calcio + arena y diferentes tiempos de inoculación del hongo *S. rolfsii*. Las letras mayúsculas comparan los tratamientos y las letras minúsculas comparan los tiempos de inoculación de hongos, mediante la prueba de Duncan ($p <0,05$).

The percentage of abnormal seedlings formed was lower when they originated from seeds coated with calcium silicate and when inoculation was performed at 10 (IN10) and 20 (IN20) days after sowing (figure 3). The number of abnormal seedlings was not affected by the pathogen in T3 treatment (figure 3); *i.e.*, treatment containing 300 g of calcium silicate and 150 g of sand. In T1, T2, T4 and T5 treatments, decrease in the percentage of abnormal *Brachiaria* seedlings was observed when fungus was inoculated at 0, 10 and 20 days after sowing. This variable decreased from 30.6% (IN0) to 5.4% (IN20) in T1; from 41.4% (IN0) to 7.4% (IN10) in T2; from 16.6% (IN0) to 2.6% (IN10) in T4; and from 30.6% (IN0) to 7.4% (IN10) in T5 (figure 3).

Although *S. rolfsii* developed in all inoculated plots, producing a pathogen resistance structure (sclerotia), infection and colonization were not completed, particularly in calcium-silicate coated seeds. Promising results regarding the beneficial effects of silicon on seed germination were also observed by Vieira *et al.* (2011) following application of calcium silicate in soil 30 days before rice was planted. In this case, there was an upward trend in seed germination up to the dose of 1,600 kg ha^{-1}.

Since infestation by microorganisms is the most influential biotic factor affecting seed vigor loss, protection through seed coating can be a promising alternative to maintain the viability of *Brachiaria* seeds against *S. rolfsii* over time, coupled with previous effective seed treatments with fungicides for the control of seedling damping-off. It could be inferred that, in stressed plants, the beneficial effect of calcium silicate tends to be more pronounced as the contact time increases, which also increases its absorption rate by seedling (26).

Considering the results obtained, the use of silicon in the control of seedling damping-off caused by *S. rolfsii* may be promising, as reported for foliar diseases; *e.g.* reduced incidence and severity of Asian soybean rust in plants supplied with silicon. In this case, disease intensity decreased in response to increased silicon doses in the nutrient solution (12). In the study above, the authors also reported that silicon addition contributed to increase phosphorus, calcium, sulfur and zinc contents as well as chlorophyll B, carotenoid and lignin contents in soybean plant shoots.

Although in this study, silicon content was not quantified in *B. brizantha* seedlings, it was possible to measure its protective effect on the incidence of damping-off caused by *S. rolfsii* on seedlings (figure 4).

CS*= calcium silicate
CSW*** = Control, non-scarified seeds.

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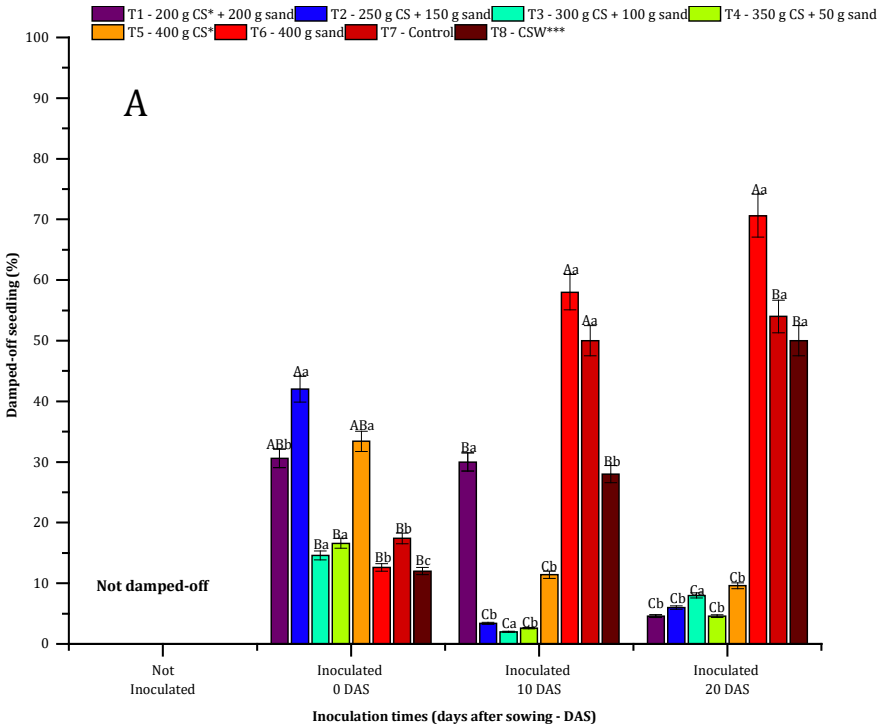

Figure 4. Percentages of damped-off (A), damped-off normal (B) and damped-off abnormal (C) *B. brizantha* cv. MG5 seedlings as a function of seed coating with different calcium silicate + sand proportions and different inoculation times of *S. rolfsii*. Uppercase letters compare treatments and lowercase letters compare inoculation times by the Duncan test ($p<0.05$).

Figura 4. Porcentajes de plántulas amortiguadas (A), plántulas normales amortiguadas (B) y plántulas anormales amortiguadas (C) de *B. brizantha* cv. MG5 en función del recubrimiento de semillas con diferentes proporciones de silicato de calcio + arena y diferentes tiempos de inoculación del hongo *S. rolfsii*. Las letras mayúsculas comparan los tratamientos y las letras minúsculas comparan los tiempos de inoculación de hongos, mediante la prueba de Duncan ($p <0,05$).

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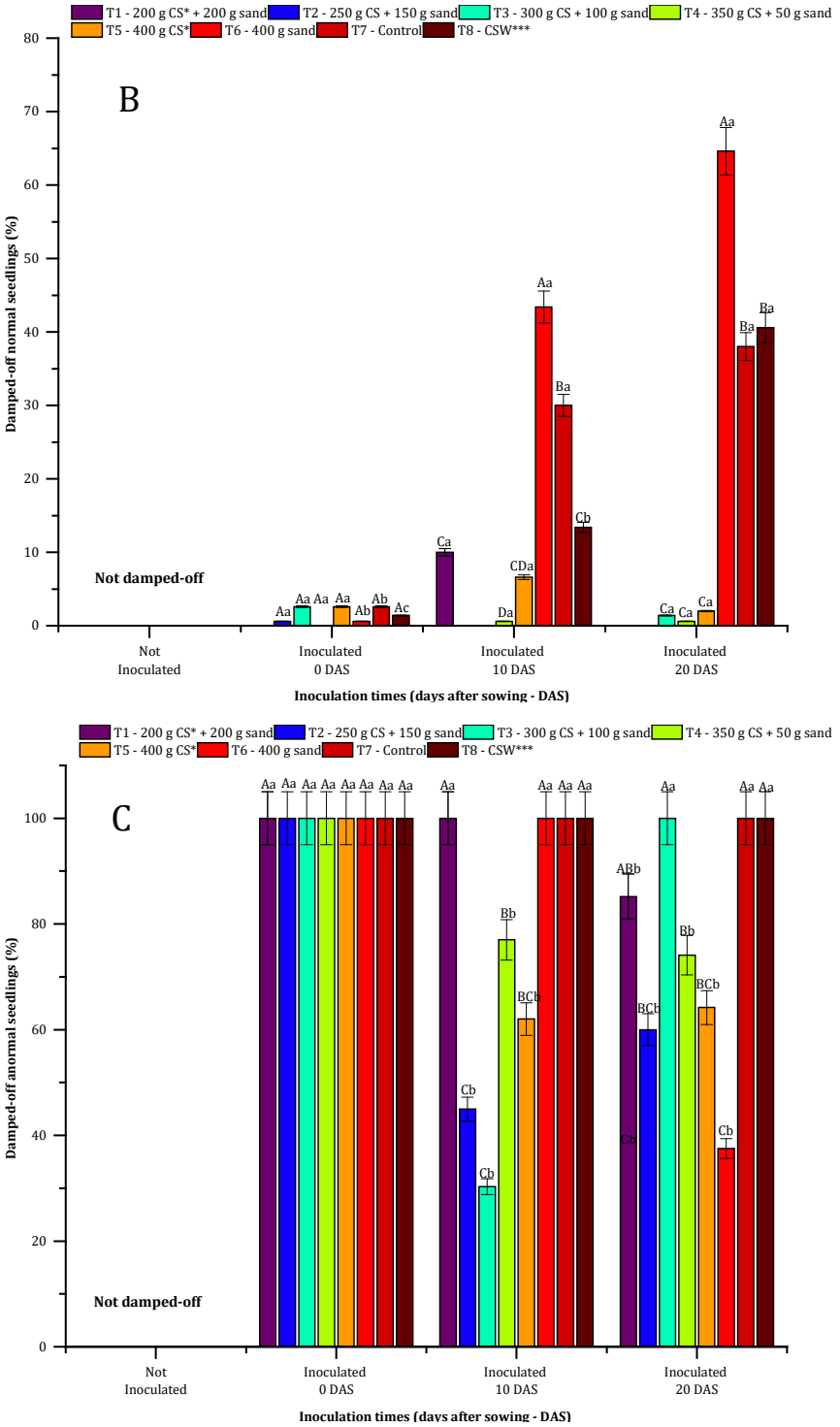

Figure 4 (cont). Percentages of damped-off (A), damped-off normal (B) and damped-off abnormal (C) *B. brizantha* cv. MG5 seedlings as a function of seed coating with different calcium silicate + sand proportions and different inoculation times of *S. rolfsii*. Uppercase letters compare treatments and lowercase letters compare inoculation times by the Duncan test ($p<0.05$).

Figura 4 (cont). Porcentajes de plántulas amortiguadas (A), plántulas normales amortiguadas (B) y plántulas anormales amortiguadas (C) de *B. brizantha* cv. MG5 en función del recubrimiento de semillas con diferentes proporciones de silicato de calcio + arena y diferentes tiempos de inoculación del hongo *S. rolfsii*. Las letras mayúsculas comparan los tratamientos y las letras minúsculas comparan los tiempos de inoculación de hongos, mediante la prueba de Duncan ($p <0,05$).

The pathogenicity of the fungus *S. rolfsii* on the percentage of seedling damping-off was proved, since this phenomenon was not observed in control treatment. The increasing contact time between seedlings and calcium silicate contributed to lower percentages of seedling damping-off in T2, T3, T4 and T5 treatments at IN10 and IN20 inoculation times (figure 4A, page 295). In contrast, in control, T7 and T8 treatments and treatments without the presence of calcium silicate in seed coating (T6), the percentage of seedling damping-off was higher, reaching values between 28 and 70.6% (figure 4A, page 295). This suggests that lack of calcium silicate in coating makes seeds more susceptible to the attack of the pathogen, because when silicate was used in coating, seeds and seedlings were protected.

Similar results were obtained for the damping-off of normal seedlings (figure 4B, page 296), whereas the presence of silicate significantly reduced the occurrence of this phenomenon in abnormal seedlings. In contrast, the damping-off of abnormal seedlings (figure 4C, page 296) reached 100% in IN0 inoculation time for all treatments, with and without silicate in seed coating. However, in IN10 and IN20 inoculation times, treatments in which calcium silicate was used for seed coating (T2, T3, T4 and T5) resulted in the lowest damping-off percentages of abnormal seedlings (figure 4C, page 296), indicating that silicate protected seedlings against infection by *S. rolfsii*. This protection may be due to the formation of a barrier on the internal membrane of cells, preventing the penetration of the pathogen. According to Wang *et al*. (2017), silicon acts by strengthening the cell wall structures and increasing lignification, thus being a passive resistance. Additionally, silicon may enhance specific defense mechanisms such as phytoalexin production and increase the activity of pathogenesis-related enzymes.

Considering that seeds and seedlings had the same contact time with the pathogen regardless of inoculation time, the positive effect of coating with calcium silicate could be observed. The treatments with the silicate efficiently protected the *Brachiaria* seedlings from damping-off; overall, the same percentage of normal plants detected initially was maintained, which directly reflected in a lower percentage of damping-off.

These results corroborate those published by Tunes *et al*. (2014), who evaluated the effect of coating rice seeds with different silicon sources on pathogen susceptibility. The authors found reductions of 89 and 87% in the incidence of *Alternaria* sp. and *Fusarium* sp., respectively, after using carbonized rice hulls, and practically 100% and 94% when the source used was kaolin. Moreover, silicon has proven to mediate physical (wax, cuticle, cell wall), biochemical interactions (activation of signaling enzymes) and molecular mechanisms (expression of genes involved in defense response) between plant and pathogen (26).

In IN0, regardless of coating treatment applied, the percentage of dead seeds ranged from 56.6% (in T2) to 88% (in T8), indicating that pathogen incidence and colonization were efficient (figure 5, page 298).

Increase in the percentage of dead seeds was observed at later inoculation times. In IN10 and IN20 inoculation times, some treatments showed lower seed mortality or the same results found for non-inoculated seeds (INot). Seed mortality in T7 and T8 treatments differed statistically without inoculation and when inoculation occurred at 10 days after sowing. The relevance of this information lies in the fact that, in the presence of *S. rolfsii*, there is already an indication of an increase in the number of dead seeds. However, when the contact between seed and calcium silicate was longer-10 and 20 days before the pathogen was inoculated (IN10 and IN20)- seeds were found to gain protection, which prevented colonization and more advanced deterioration of tissues as observed when inoculation was performed at sowing (IN0).

Many studies have reported the importance of silicon to plant development, seed production and even in cases of salt, water, or disease-related stress (11, 12, 15, 18, 26). However, there is little research relating seeds to pathogenic microorganisms, especially when silicate is used in seed coating. Therefore, further studies should be carried out to expand this type of investigation and elucidate the silicon-regulated plant × pathogen interactions. Thus, silicon can contribute to increase crop yield and disease resistance, given the benefits demonstrated in the current experiment.

CS*= calcium silicate
CSW*** = Control, non-scarified seeds.

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CSW *** = Control, semillas sin carificar.

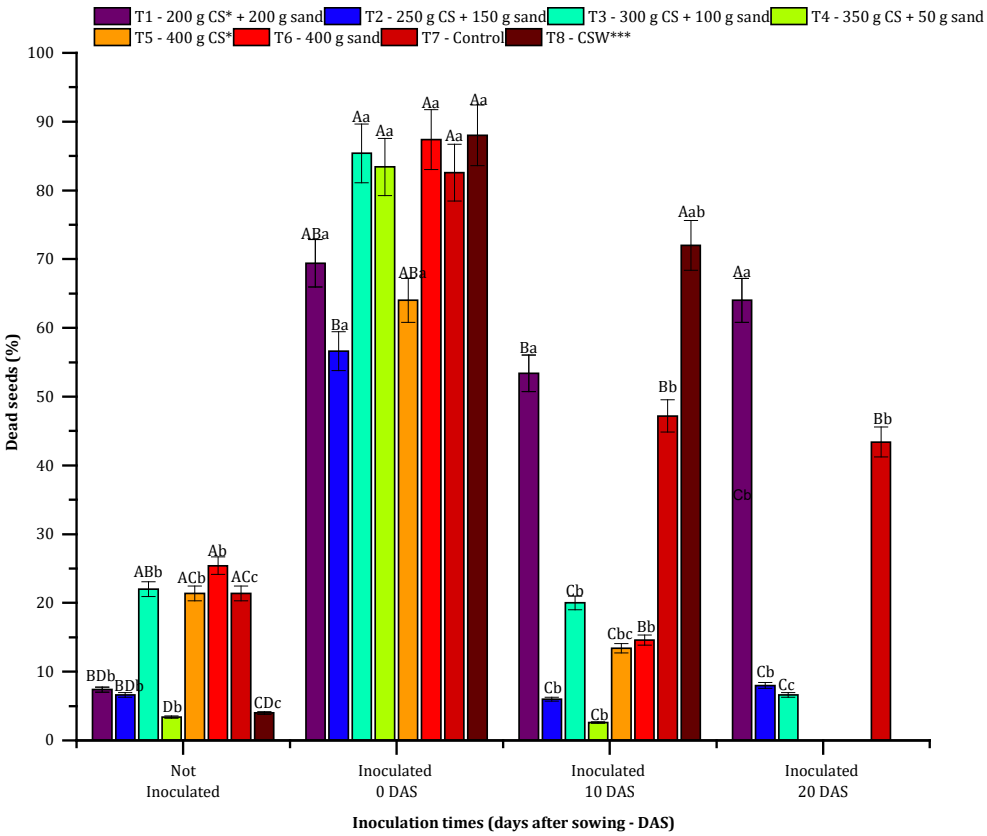

Figure 5. Percentage of dead *B. brizantha* cv. MG5 seeds as a function of seed coating with different calcium silicate + sand proportions and different inoculation times of *S. rolfsii*. Uppercase letters compare treatments and lowercase letters compare inoculation times by the Duncan test ($p<0.05$).

Figura 5. Porcentaje de semillas muertas de *B. brizantha* cv. MG5 en función del recubrimiento de semillas con diferentes proporciones de silicato de calcio + arena y diferentes tiempos de inoculación del hongo *S. rolfsii*. Las letras mayúsculas comparan los tratamientos y las letras minúsculas comparan los tiempos de inoculación de hongos, mediante la prueba de Duncan ($p <0,05$).

Conclusions

The germination rate of *B. brizantha* cv. MG5 seeds coated with calcium silicate increases in seeds inoculated with *S. rolfsii* at 10 and 20 days after sowing.

Seed coating with calcium silicate reduces damping-off in *B. brizantha* cv. MG5 plants.

The application of calcium silicate in coating provides protection to *B. brizantha* cv. MG5 seeds and plants inoculated with *S. rolfsii*.

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