

Incidence of stalk rots in corn hybrids influenced by sowing time and nitrogen rates

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ABSTRACT: Stalk rots compromise the translocation of water and nutrients, affecting grain filling. This study aimed to quantify the incidence of stalk rots in corn hybrids depending on sowing time and nitrogen topdressing rate, and to assess any correlation with grain yield. The experiment was conducted under field conditions in Atalanta, Santa Catarina state, Brazil, using a split-split-plot randomized block design. The hybrids AG 9025 PRO3 (super-early) and 30F53 VYH (early) were sown in the preferential (September 20) and late (December 5) sowing seasons with four nitrogen rates (0, 150, 300, and 450 kg·ha⁻¹). The population density was 75,000 plants·ha⁻¹ and the nitrogen topdressing was carried out at the V4, V8, and V12 stages.

The fungus *Colletotrichum graminicola* predominated in both hybrids and its presence was negatively correlated with stalk diameter and yield. The hybrid AG9025 PRO3 was more susceptible to stalk rot (35.1%) than the hybrid 30F53 VYH (8.8%). A higher incidence of stalk rot was observed in the late sowing season (31.1%) than in the preferential sowing season (11.2%). A decrease in the incidence of stalk rot was observed in the hybrid AG9025 PRO3 as nitrogen rates increased, but this behavior was not clearly observed in the hybrid 30F53 VYH.

Key words: *Zea mays*, *Colletotrichum graminicola*, nitrogen fertilization, stalk diseases.

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INTRODUCTION

Corn stalk rots (SRs), characterized by the presence of symptoms or pathogens signs, cause changes in tissue structure that compromise the translocation of water and nutrients (Denti and Reis 2001) and commonly cause lodging, breakage or premature death of plants toward the end of the crop cycle, compromising grain filling.

The fungi frequently associated with SRs are *Colletotrichum graminicola* (Ces.) G.V. Wils. (anthracnose), *Stenocarpella macrospora* Earle and *S. maydis* Berk. (diplodia), *Fusarium graminearum* Schwabe (fusarium head blight) and *F. verticillioides* (Sacc.) Nirenberg (fusariosis) (Ribeiro et al. 2005), and *Macrophomina phaseolina* Tassil (charcoal rot) (Casela et al. 2006).

The general management strategies for SRs include the planting of resistant hybrids (Matiello et al. 2013), rotation and succession of crops with non-host species (Denti and Reis 2001), choosing a suitable sowing density (Blum et al. 2003; Casa et al. 2007), use of healthy seeds and seed treatment with fungicides to avoid pathogen transmission to seedlings (Sartori et al. 2004), and ensuring a balanced nutrition (Casela et al. 2006), mainly in relation to nitrogen (N) and potassium (K) (Carvalho et al. 2013). Crop rotation is a management alternative when the pathogen does not have a wide range of hosts (Zambolim et al. 2000). Aerial chemical control using fungicides is efficient for macrospora leaf strip control, reducing the inoculum for stalk and ear infection (Casa et al. 2006).

Sowing season (SS) may be a management tool to reduce the incidence of SR. In the south of Brazil, when sown during the preferential sowing season (PSS), between mid-September and the end of October, and free of climatic stresses, maize is guaranteed a satisfactory accumulation of photoassimilates (Silva et al. 2010). High availability of radiation during the reproductive stage allows the leaves to maintain photosynthetic efficiency (Forsthofer et al. 2004). Thus, there is less mobilization of sugars from the stalk to fill the grains, delaying senescence. In the late sowing season (LSS), from November onward, the availability of radiation and temperature decreases during the reproductive stage as a function of the reduction of the photoperiod. This reduces photosynthetic efficiency (Forsthofer et al. 2006), resulting in mobilization of carbohydrates from the stalk for grain filling. As consequence the plants become more sensitive, accelerating senescence and increasing the propensity to SRs.

High availability of N has been shown to be a precursor in increasing the intensity of disease since it competes for carbon (during formation of amino acids) in the secondary metabolic routes responsible for the production of plant defense compounds (Dordas 2008). However, N is a component of the chlorophyll molecule and therefore essential for the maintenance of photosynthetic activity.

In this sense, photosynthesis is impaired under N deficiency. To the detriment of grain filling, stalk reserves are rapidly mobilized and may vary in relation to hybrids and SS, making them fragile, senescent, and predisposed to infection. The objective of this study was to quantify the incidence of SR in maize hybrids sown in the different SSs and under varying N rates, and to establish correlations with grain yield.

MATERIAL AND METHODS

The experiment was conducted under field conditions in 2016 and 2017 in Atalanta, Santa Catarina state, Brazil (27°26'03" S, 49°42'06" W; altitude 586 m). According to the Köppen classification, the regional climate is type Cfa, humid mesothermic subtropical with warm summers. The soil of the experimental area is classified as a Dystrudept (Cambissolo Háplico distrófico, Brazilian Soil Classification System) with a silty clay loam texture type 3.

The experimental design was a split-split-plot randomized block design with four replicates. Plots were planted with the hybrids AG9025 PRO3 (single hybrid, super-early, yellow and dent grain, from the company Sementes Agrocere) and 30F53 VYH (simple hybrid, early, semi-flint orange grain, from the company Du Pont do Brasil SA). Subplots consisted of PSS on September 20 and LSS on December 5. Sub-subplots received N at four topdressing rates, 0, 150, 300, and 450 kg N·ha⁻¹ equivalent to 0, 0.5, 1.0, and 1.5 times the additional rate for a yield expectancy of 21,000 kg·ha⁻¹, respectively. Base fertilization was performed in the sowing rows for a yield expectancy of 21,000 kg·ha⁻¹ and consisted of 30 kg N·ha⁻¹, 300 kg P·ha⁻¹, and 200 kg K·ha⁻¹, following crop recommendations.

Sowing was carried out using manual seeders in a no-tillage system under a sequence of crops characterized by monoculture in the winter season (the previous two years with black oat cultivation) and crop rotation in the summer (the previous two years with soybean cultivation). Three to four seeds were distributed linearly and equidistant in each

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hill, with a row spacing of 0.7 m. Thinning was carried out at the V2 (two fully expanded leaves) stage according to the Ritchie scale (Richie et al. 1993) to reach a population of 75,000 plants·ha⁻¹.

The topdressing N rates were applied as urea (45% N). Treatments had their respective rates split into equal portions at stages V4, V8, and V12 (4, 8, and 12 fully expanded leaves, respectively), except for the control (without N). Each experimental unit (sub-subplot) had dimensions of 2.8 × 6 m and contained four rows, the two central rows (64 plants) being considered as the useful area and the two external rows considered as borders.

Seeds were treated with carbendazim (0.45 g a.i.·kg⁻¹) + thiram (1 g a.i.·kg⁻¹) and metalaxyl-M (0.015 g a.i.·kg⁻¹) + fludioxonil (0.038 g a.i.·kg⁻¹). Weed control was performed with atrazine (1.5 kg a.i.·ha⁻¹) + metolachlor (1.7 g a.i.·ha⁻¹) following sowing and tembotrione (100 g a.i.·ha⁻¹) at V3 stage. Fall armyworm (*Spodoptera frugiperda*) was preventively controlled with lambda-cyhalothrin (6 g a.i.·ha⁻¹) + chlorantraniliprole (12 g a.i. ha⁻¹), applied on two occasions as supported by the technologies PRO and Leptra inherent to the hybrids AG9025 PRO3 and 30F53 VYH, respectively, maintaining leaf area intact. No fungicide application was carried out.

At VT (tasseling) stage, stalk base diameter was measured using a digital caliper. At harvest time, when grains contained 18% to 22% moisture, 15 days after the R6 stage (physiological maturation), an assessment of stalk health was carried out. Stalks of all plants from the useful area of the plot were assessed for disease after removing the leaves from their bases. The stalks that presented discoloration of nodes and internodes and/or those exhibiting less pressure resistance when squeezed between the thumb and forefinger were considered symptomatic (Denti and Reis 2003; Casa et al. 2007). The incidence of SR was calculated based on the relationship between diseased and the total assessed stalks.

In the laboratory, fragments (1 × 5 mm) of medulla were detached to identify the fungi associated with symptomatic stalks. These fragments were disinfected in sodium hypochlorite solution (1%) for two minutes. Excess solution was removed by washing with sterile distilled water followed by drying on filter paper. Then, in a flow chamber, the fragments were arranged in gerbox acrylic boxes (11 × 11 × 3.5 cm) previously disinfected with alcohol (70%) and containing the culture medium PDA+A (potato-dextrose-agar + streptomycin sulfate antibiotic at 200 mg·L⁻¹).

The boxes were maintained in a growth chamber at 23 ± 2 °C under a 12-h photoperiod for 7 days. We considered as infected the fragments on which colony or structures of the fungi were identified under a stereoscopic microscope at 40× magnification. The presence of fungi was confirmed under a light microscope by analyzing fungal structures and comparing them with those described in the literature (Barnett and Hunter 1998).

Ears from plants with SR (symptomatic) and without SR (asymptomatic) were harvested separately. After threshing, the thousand-grain weight (TGW) was determined at 13% moisture for each sample, as well as the total mass of samples, with yield results being expressed in kg·ha⁻¹.

The data were submitted to analysis of variance in a factorial scheme using the *F*-test at 5% error probability. The SR incidence and specific incidence of fungi data were arcsine [$\sqrt{(x/100)}$] transformed. When significant, the Tukey's test at 5% probability error was used. The correlation between stalk diameter, incidence of SR, and yield was determined using the Pearson correlation coefficient. Statistical analyses were performed using the statistical software SISVAR 5.6 (Ferreira 2011) and SAS 9.1 (SAS Institute, Cary, NC, USA).

Climatic data from the automatic meteorological station of the Brazilian National Institute of Meteorology, located approximately 10 km from the experimental location in Ituporanga, Santa Catarina state, were used to monitor temperature and precipitation variations during the crop cycle in both sowing seasons (Fig. 1).

RESULTS AND DISCUSSION

Triple interactions were observed between topdressing N rates, hybrids, and SS for the variable incidence of SR (Fig. 2a), as well as for the specific incidence of anthracnose rot caused by *C. graminicola* (Fig. 2b). The results show that the incidence of SR (Fig. 2a) was higher than the incidence of *C. graminicola* (Fig. 2b). The difference is explained by SR caused by (albeit less significant) infection with *F. graminearum* and *Stenocarpella*. Because no significant interaction was observed between N rates and the pathogens *F. graminearum* and *S. macrospora*, the interaction observed for incidence of SR was attributed to the fungus *C. graminicola*. The incidence of SR (as well as the incidence of *C. graminicola*) decreased as topdressing N rates increased. The decrease in the incidence of SR can be attributed to the increase in photosynthetic

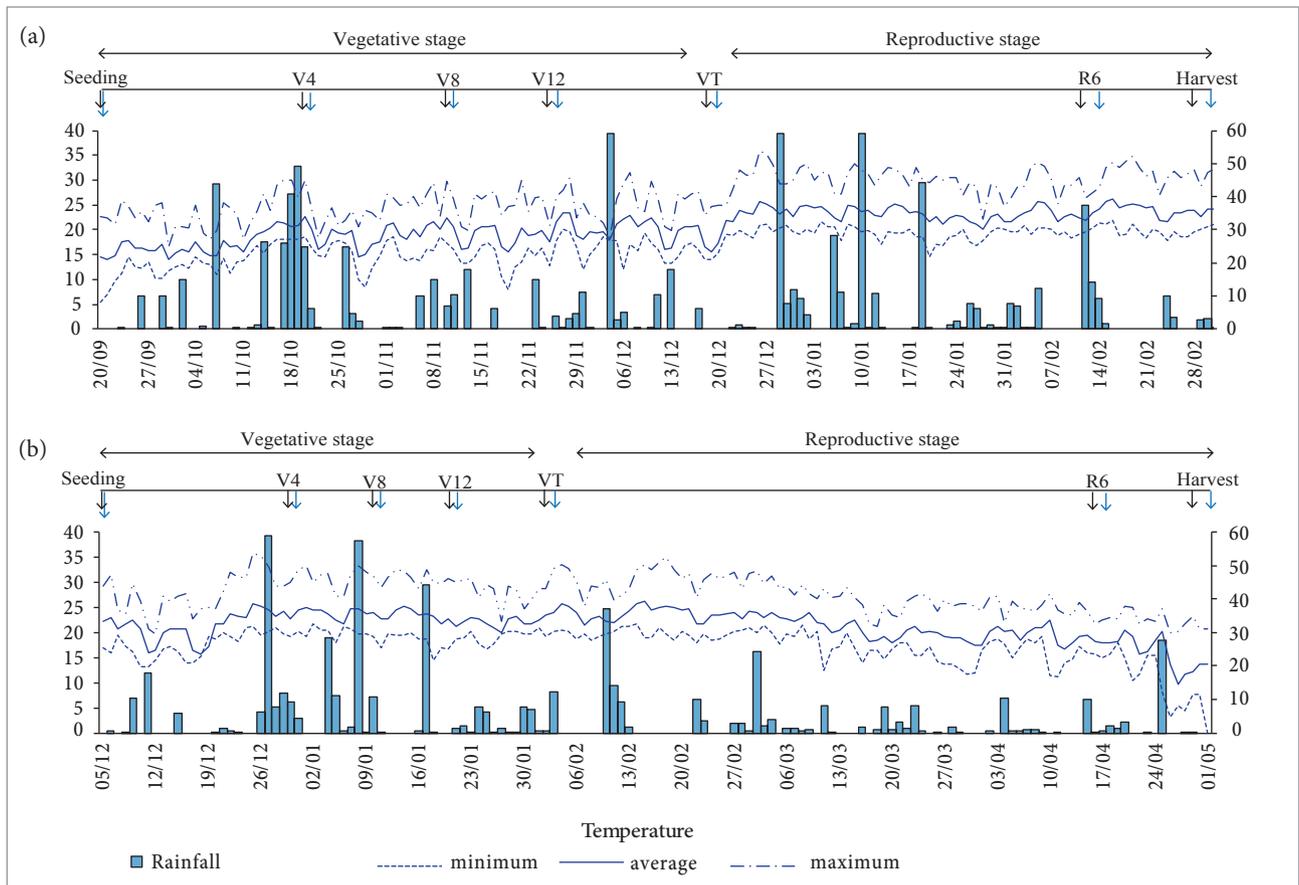


Figure 1. Daily temperature data in °C (rows) in the lower session, and precipitation in mm (bars) in the upper session, during maize crop cycles at (a) preferential and (b) late sowing seasons. Black arrows indicate the hybrid AG9025 PRO3 and blue the 30F53 VYH. Atalanta, Santa Catarina state, data from 2016 and 2017.

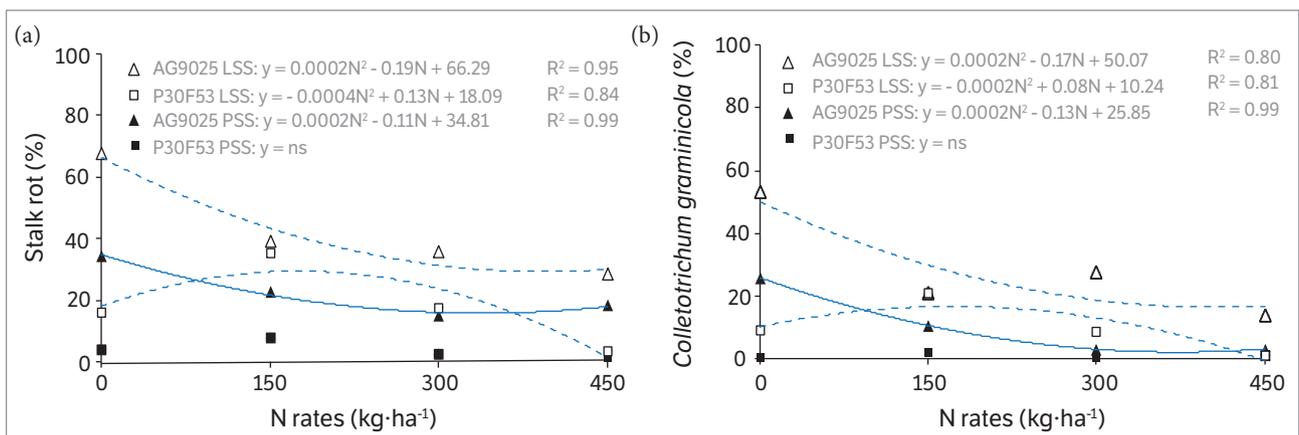


Figure 2. Regression of the incidence of a) stalk rot, and b) *Coletotrichum graminicola* for the hybrid factors and sowing season as a function of the N rate in the cover. The square markers represent the 30F53 VYH hybrid and the triangles the hybrid AG9025 PRO3. Both when filled represent the preferential sowing season (PSS) and when empty the late sowing season (LSS). ns = not significant. Atalanta, Santa Catarina state.

efficiency due to the addition of N, which in the period of grain filling delays the use of photoassimilates of the stalks. At super optimal doses the plant can restrict the absorption of the nutrient, formation of chlorophyll molecules reaches

a plateau to the point that the response of the nutrient in the photosynthetic rate becomes less (Sangoi et al. 2016) and the accumulation of photoassimilates in the stalk becomes similar, as in the doses of 300 and 450 kg·ha⁻¹. Likewise,

similarity is observed in grain yield when N is surplus, so the best results (maximum technical efficiency) will not always be tied to the highest dose (Caires and Milla 2016). With respect to mean N rates and ES, AG9025 PRO3 exhibited a higher incidence of SR (35.1%) compared to 30F53 VYH (8.8%). Regarding mean of the hybrids and N rates, the incidence of SR predominated in EST (31.1%) compared to ESP (11.2%).

The incidence of *C. graminicola* at the average N topdressing rate was higher in AG9025 PRO3 (17.7%) than in 30F53 VYH (3%). A lower incidence of fungus was observed in PSS (3.3%) than in LSS (16.9%). The regression of N rates for incidence of SR and *C. graminicola* was not significant for 30F53 VYH in PSS but presented a decreasing trend for LSS, although different behavior was observed for the control (zero N rate), in which SR incidence was maintained at N rates below 150 kg·ha⁻¹. The points related to the control show an incidence gap between the hybrids. The AG9025 PRO3 super-early hybrid exhibited a higher incidence of both *C. graminicola* and SR, reducing sharply with increasing rates of N, while the 30F53 VYH early hybrid showed lower incidence and less oscillation as N rates increased.

Reduction of the N/K ratio is a way to attenuate the intensity of foliar lesions of *C. graminicola* (Carvalho et al. 2013), indicating that K is an essential component of defense. We maintained K at a level suitable for productivity, only increasing levels of N. This resulted in an increase in the N/K ratio, which contributed to a reduction in SR susceptibility caused by *C. graminicola*. Despite its incidence on the corn stalks, *C. graminicola* was not found to be causing leaf spots, perhaps because of the greater distance between the source of inoculum and stalks, as well as dispersion favored by water splash. The greatest amounts of solar radiation are intercepted by leaves. This may cause dehydration of fungus propagules, reducing its effectiveness to cause infection in stalks.

Fungi of the genera *Stenocarpella* and *Fusarium* were not submitted separately to statistical analysis since their incidence was < 5%. No pathogens were detected in 14.5% of deteriorated stalks. Therefore the deterioration was attributed to early senescence due to reasons other than pathogens. The highest prevalence among infected stalks was of the fungus *C. graminicola* regardless of hybrid or SS. Values ranged from 56% to 73%, attributable to the monoculture of oats (a host for this pathogen) in the experimental area during the four preceding winters (Chester 1947; Tarr 1962; Mordue

1967; White 1999). The presence of infected crop residues ensures pathogen survival in the crops. The prevalence of *C. graminicola* in relation to the fungi of the genera *Fusarium* and *Stenocarpella* was observed by Denti and Reis (2003) in Rio Grande do Sul state, Brazil.

The greater the precocity of a hybrid, the lower is its leaf area (Fancelli 2000). This reduction in leaf area tends to increase the contribution of the stalk in the supply of photoassimilates to grain filling, resulting in a higher carbohydrate mobilization in the stalk of precocious hybrids compared to late hybrids (Blum et al. 2003). The hybrid AG9025 PRO3 has a shorter cycle (super-early) when compared to the hybrid 30F53 VYH (early). The latter maintains a high source-sink relationship, supports grain filling through photosynthesis for a longer period, and develops less fragile stalks, reducing susceptibility to pathogens. Hybrid characteristics such as a low source-sink relationship are also associated with higher stalk rot severity (Blum et al. 2003).

In PSS, the temperatures during crop vegetative stage are lower when compared to those observed in LSS, where the sub-period emergence-silking is submitted to a higher accumulation of thermal units. Temperature also influences growth and development; the stalks of corn plants from LSS tend to have longer internodes making them more fragile and prone to infection. Intraspecific competition for light reduces the photosynthetic activity of plants (Sangoi 2001) due to an excess of leaf area, leading to a lower carbohydrate accumulation in the stalk.

In LSS, in addition to a higher availability of inoculum from anticipated sowing corn and PSS (Fig. 1), the crop reproductive stage coincides with a reduction in both temperature and solar radiation availability during the months of March-April. An increase in *C. graminicola* intensity has been observed under low luminosity conditions (Schall et al. 1980). Under these conditions, photosynthetic capacity is compromised, leading to carbohydrate mobilization from stalks for grain filling. A reduction in degree-days lengthens the crop reproductive cycle, increasing the predisposition period of plants to infections and tissue colonization by pathogens.

Regarding susceptibility, AG9025 PRO3 was classified as tolerant and 30F53 VYH was classified as resistant to stalk rot (Pereira Filho and Borghi 2016). As observed in this study, distinct behaviors occurred between pathogens. Hence they should be studied separately for the attribution of degrees of susceptibility.

A significant correlation was observed only for the incidence of *C. graminicola*, which was negatively correlated with stalk diameter and yield, with values of -39% and -46%, respectively (Table 1). This indicates that the larger the stalk diameter, the lower the incidence of *C. graminicola* and the higher the yield. Infection with *F. graminearum* or *S. macrospora* did not influence stalk diameter or significantly alter yield.

An increase in applied N leads to a significant increment in stalk diameter (Fig. 3a). Although this increment is low, with a maximum difference of 3 mm between the averages of the tested rates, it has an effect on the content of stored carbohydrates, which may delay plant senescence, altering *C. graminicola* incidence and yield. This fungus can start its infection even in the vegetative stage of the plant. During infection, hyphal production and elongation occur within cellular fibers associated with vascular bundles and bark (Venard and Vaillancourt 2007), in detriment to the plant. Senescence accelerates via remobilization of reserves for grain filling, and defragmentation of vessels and tissue death occur. Increased necrotrophic fungal colonization makes stalks more flaccid, which is easily

perceptible when squeezed between thumb and forefinger. Stalks of larger diameter can withstand the export flow of carbohydrates, delaying their senescence. In a study with *C. graminicola* inoculation on stalks of susceptible and resistant hybrids at V8, V12, and VT, Matiello et al. (2013) did not find differences between treatments regarding the size of internal lesions in the stalk, and yield reduction was observed only in susceptible hybrids inoculated at V8 due to more plants dying prematurely. The authors suggested that early infection could interfere with the process of sugar accumulation in the stalk.

Significant interactions were observed between N topdressing rates and SS for TGW from plants with symptomatic and asymptomatic stalks. However, no interaction was observed between N topdressing rates and hybrids in both cases (Fig. 3b). Regardless of grain origin (from symptomatic or asymptomatic stalks), a significant reduction was also observed in TGW in LSS compared to PSS (Fig. 3b). This reduction is related to physiological aspects of the plant in relation to climatic differences between SS, mainly in the availability of differentiated radiation between the vegetative and reproductive stages,

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Table 1. Coefficient of correlation between stalk diameter and yield grains with incidence of colonized stalk by *Colletotrichum graminicola*, *Fusarium graminearum* and *Stenocarpella*. Atalanta, Santa Catarina state.

Variables	<i>C. graminicola</i>	<i>F. graminearum</i>	<i>Stenocarpella</i> ¹	Yield
Stalk diameter	-0.39 **	0.12 ns	-0.14 ns	0.27*
Yield	-0.46 **	0.02 ns	-0.07 ns	

¹Sum of the incidence of *Stenocarpella maydis* and *S. macrospora*; * and ** = significant at p < 0.05 and < 0.01 probability of error, respectively; ns = not significant.

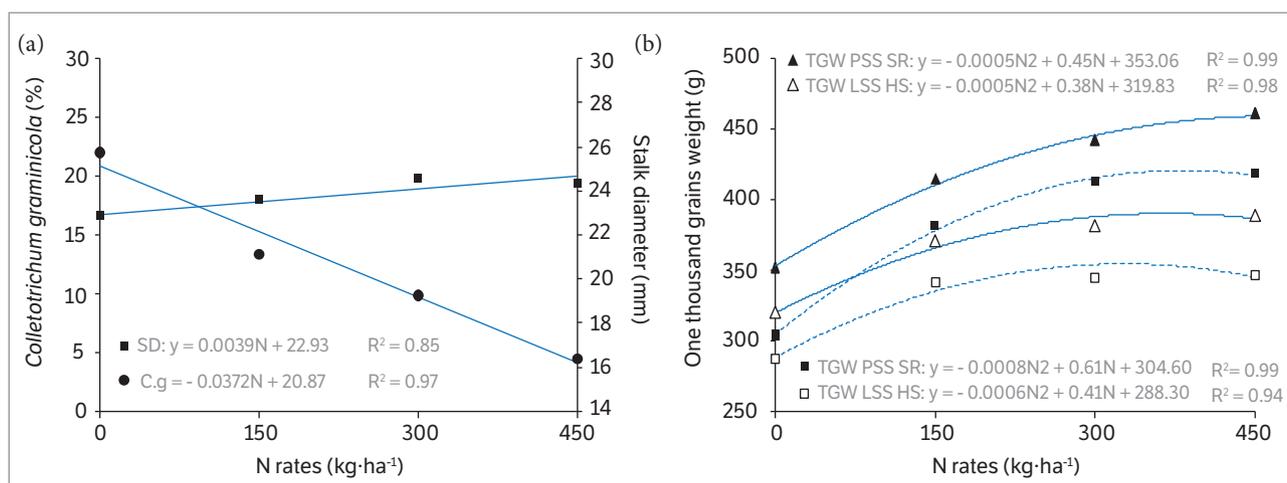


Figure 3. Regression of the incidence of (a) *Colletotrichum graminicola* and stalk diameter, and (b) one-thousand-grains weight of symptomatic and asymptomatic stalks of rot as a function of the rates of N under cover. In (b) the square markers represent the weight of a thousand grains with stalk rot (SR) and the triangles represent healthy stalk (HS). Both when filled represent the preferred sowing season (PSS) and when empty the late sowing season (LSS). Atalanta, Santa Catarina state.

which result in changes in photosynthetic efficiency and source-sink relationships. Grain from symptomatic plants presented a TGW 9.3% lower (354.6 g) when considering the average rates, hybrids, and SS compared to asymptomatic plants (390.9 g). This occurs because stalk rot interrupts the flow of water and nutrients in the plant, especially during grain filling, when stalks become more fragile and prone to colonization due to carbohydrate remobilization.

An increase in N topdressing rate generated an increase in TGW, with a tendency to stabilize above 300 kg·ha⁻¹ regardless of SS or presence or absence of stalk rot. In addition, the higher the N rate, the greater is the difference between TGW values for PSS and LSS, from both symptomatic and asymptomatic plants. This behavior suggests higher N use efficiency in PSS. The maximum TGW value (460 g) was observed in asymptomatic plants grown in PSS with N at a rate of 450 kg·ha⁻¹, while the minimum TGW value (285 g) was observed in symptomatic plants grown in LSS with N at a rate of 0 kg·ha⁻¹.

CONCLUSION

The super-early hybrid AG9025 PRO3 is more susceptible to SRs when compared to the early hybrid 30F53 VYH.

The LSS presents higher incidence of SRs when compared to the PSS.

The incidence of stalk rot caused by the fungus *Colletotrichum graminicola* is significantly reduced with increasing N rates.

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AUTHOR'S CONTRIBUTION

Conceptualization, Berghetti J., Casa RT. and Sangoi L.; Methodology, Ferreira EZ., Zanella EJ., Scheidt BT and Casa RT.; Investigation, Berghetti J., Zanella EJ. and Scheidt BT.; Writing – Original Draft, Berghetti J.; Writing – Review and Editing, Berghetti J., Casa RT. and Sangoi L.; Funding Acquisition, Casa, RT.; Resources, Casa RT.; Supervision, Casa RT. and Sangoi L.

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