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ORIGINAL ARTICLE

Physiological and biophysical alterations in maize plants caused by *Colletotrichum graminicola* infection verified by OJIP study

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Abstract

Anthracnose caused by *Colletotrichum graminicola* (Ces.) Wils is among the most aggressive maize diseases worldwide, considerably hindering crop productivity. To investigate the responses underlying the host–pathogen interaction involved in anthracnose infection, we conducted two field experiments in some of the main grain producing areas of the State of Tocantins, in Brazil. Four maize hybrids (with differential tolerance to *C. graminicola*) were sown in February and March, 2017. Chlorophyll fluorescence measures were performed according to the OJIP protocol; additionally, SPAD readings were conducted and productivity components were assessed. Inoculation with the fungus spore suspension affected the response curve of the chlorophyll fluorescence transient, SPAD index, and productivity components. Anthracnose stalk rot caused 20–25% reduction in grain weight, 6–8% reduction in maize ear size, and 20–23% reduction in ear weight. Furthermore, plants experiencing *C. graminicola* rot showed reduced SPAD index and a 24–34% reduction in the activity indicator of PSII (PI_{Total}). These changes in the photosynthetic apparatus were successfully observed via chlorophyll fluorescence measurements.

Keywords Anthracnose · Chlorophyll fluorescence · Corn · Photosystem II · Productivity

Introduction

Anthracnose, *Colletotrichum graminicola* (Ces.) Wils, is one of the most important and aggressive corn diseases worldwide (Bergstrom and Nicholson, 1999; Jirak-Peterson and Esker, 2011). Although *C. graminicola* can affect all plant parts, stem infections are more aggressive and result in greater losses in productivity (Bergstrom and Nicholson, 1999; Cota et al., 2012). Infection of the stalk can occur directly by conidia produced on leaf lesions or crop residues, or by systemic infection via the root system (Bergstrom and Nicholson, 1999; Venard and Vaillancourt, 2007; Sukno et al, 2008).

A synergistic relationship has been established between stalk injuries caused by insects and stalk anthracnose incidence and stalk rot caused by other pathogens (Keller et al., 1986). The use of Bt transgenic corn hybrids, which reduce the injuries caused by insects to the stalk, also reduces the incidence of stalk anthracnose (Gatch and Munkvold, 2002). According to Cota et al. (2012), maize productivity is strongly affected by stalk anthracnose, with a marked reduction in the main productivity components, such as grain weight (28%), ear weight (29%), and 1000-grains weight (18%). Significant losses due to stalk anthracnose in maize have been previously reported (Mueller, et al., 2020).

Photosynthesis is a major driver of crop productivity and the main physiological process affected by phytopathogenic infections (Bastiaans, 1991; Rios et al., 2017). Indeed, photosynthesis impairment may lead to reduced productivity if the plant cannot maintain a high photosynthesis rate under stress (O'Neill et al., 2006). Assessment of photosynthetic

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efficiency in infected plants can reveal useful information about the physiological processes affected by a pathogen, in support of our permanent quest for new, effective strategies for disease control (Rolfe and Scholes, 2010). As for anthracnose in maize stalks, it is likely to hinder water flow from the roots to the aboveground plant parts, as it affects the conductive tissues. This situation could make changes in the physiological and photosynthetic processes more intensive under conditions of drought stress (Martínez-Ferri et al., 2016).

Some studies have evaluated the efficiency of the plant photosynthetic apparatus upon infection by leaf pathogens via Chl*a* fluorescence measurements (Aucique-Pérez et al., 2014; Kuckenberg et al., 2009; Warzecha et al., 2019). Bermúdez-Cardona et al. (2015) showed that maize leaf photosynthesis was consistently severely affected by *Stenocarpella macrospora* infection. Other than this, there are few other studies that have focused the effect of damage caused by stalk rot disease on the photosynthetic apparatus and photosynthetic efficiency (via chlorophyll fluorescence) of agricultural cultivars, specifically in maize. The study of chlorophyll fluorescence kinetics in a dark-adapted leaf can be defined as the OJIP method (where "O" stands for minimum fluorescence, "J" corresponds to the reduction of plastoquinone A-Q_A molecules, "I" corresponds to the reduction of plastoquinone B-Q_B and "P" represents the peak of the fluorescence quantum yield), whereby a dark-adapted leaf is subjected to a saturating pulse of actinic light. The fluorescence curve, or OJIP curve, in response to this light pulse is a useful tool to verify kinetic changes in Chl*a* fluorescence caused by biotic and abiotic stress factors, thus providing detailed information on the structure and function of the photosynthetic apparatus, specifically that of photosystem II (Lazár, 2006).

A clear understanding of the influence of stalk rot fungi on plant physiological processes is essential for understanding pathogen-host interactions and for developing new control strategies for these phytopathogens. Therefore, this work aimed to evaluate the biophysical and physiological changes that occur in the leaf due to infection by *C. graminicola*, which might allow a quick monitoring and phenotyping of maize plants in response to fungal colonization of the stem.

Materials and methods

Two field experiments were conducted in grain producing areas of the State of Tocantins (TO), in Porto Nacional (PN) and Aparecida do Rio Negro (ARN) Counties. Four maize commercial hybrids including, MG600 PW, P30S31 VYH, DKB290 PRO3, and Formula VIP, were sown on February 24, 2017 in PN (10° 29′ 23″ S, 48° 19′ 55″ W; elevation: 245 m) and on March 14, 2017 in ARN (10° 00′ 46″ S, 48° 09′ 18″ W; elevation: 553 m).

The hybrids were chosen to represent a range of tolerance levels to the fungus in field. MG600 PW was the most resistant while Formula VIP was the least resistant, and considered as a positive control treatment.

Fertilization consisting in 130 kg P_2O_5 ha^{-1} and 27 kg N ha^{-1} was applied at seeding, and 90 kg N ha^{-1} in the form of urea was applied when plants had 4–6 leaves. Seeds (60,000) were treated with tiametoxan at 0.08 L $seeds^{-1}$.

Production and preparation of the inoculum

The cg15 isolate of *C. graminicola* from a collection of phytopathogenic fungi held by Embrapa Maize and Sorghum was used in this study. The isolate was replicated in Petri dishes containing an oat flour-agar (OFA) culture medium (60 g oat flour, 15 g agar, 1000 mL deionized water). The dishes were kept in a growth chamber at 28 ± 2 °C under continuous fluorescent light. After 5 days of growth, mycelia were scraped to induce sporulation. For inoculum production, the isolate was again replicated in the OFA medium petri dishes, and the same procedures were followed to obtain abundant sporulation. The inoculum was prepared by the addition of deionized water with Tween (0.02%) in the Petri dishes prior to scraping for conidia release. A hemocytometer was used to adjust the inoculum concentration to 10^6 conidia mL^{-1}.

The inoculation process

Stalks were inoculated at pre-tasseling by using a toothpick immersed in the spore suspension according to Cota et al. (2012). The toothpicks were boiled in water for 30 min to eliminate any toxic substances and then autoclaved for 30 min at 121 °C. Before inoculation, the leaves and internode sheaths on the stalks targeted for inoculation were removed, and the stalks were immediately disinfected with 70% alcohol. Immediately after superficial disinfection, the second internode at the base of the stalk was punctured using a perforator with a diameter similar to that of the toothpick. After the removal of the perforator, toothpicks soaked in a conidia suspension of *C. graminicola* were inserted into the holes drilled to a depth of approximately 1/3 of the length of the toothpicks. The control treatment consisted in the insertion of sterilized toothpicks without inoculum, following the same procedure used for inoculated stalks.

Anthracnose severity was assessed 30 days after inoculation (Fig. 1). All the leaves and the stalk section between the first internode and the ear insertion internode were removed. Once in the laboratory, stalks were cut longitudinally, and the extent (severity) of the lesions was assessed using the scale reported by Nicoli et al. (2015). This scale comprises

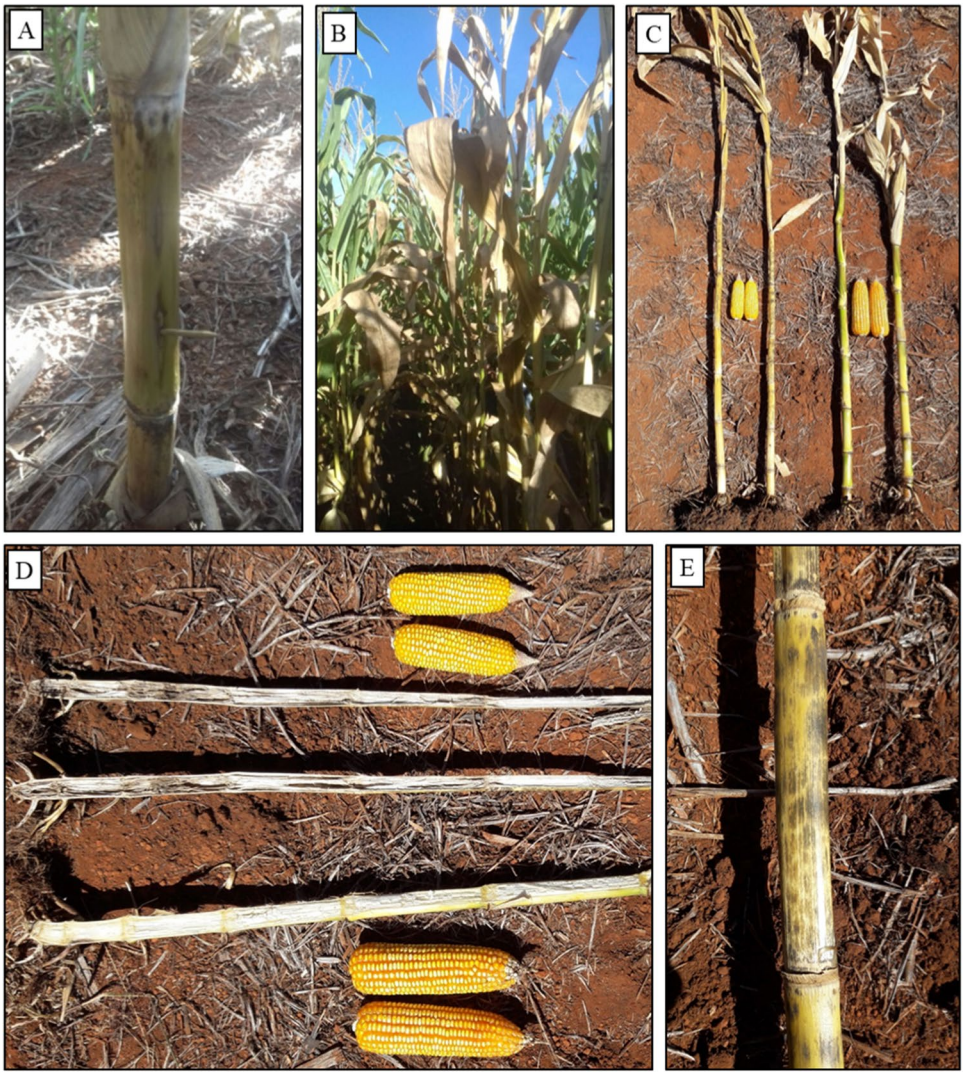

Fig. 1 Panels **A** and **B** are symptoms of *Colletotrichum* in maize stalks and leaves, respectively, in hybrid Fórmula Vip; **C** differences in ear size of infected (left) and healthy (right) maize plants; **D** detailed view of maize ear size and stalk color of infected (above) and healthy (below) maize plants; **E** detailed view of symptoms of *Colletotrichum* sp. infection on the stem surface of a maize stalk

eight severity scores, varying from 1 (6.2% of the internode is necrotic tissue) to 8 (93.8% of the internode is necrotic tissue). All experimental plants were assessed and mean severity grades were obtained for each treatment.

Photosystem II monitoring

The systemic action of the fungus in the stalk is likely to alter the bidirectional flow of water and other substances through the vascular tissues between the root system and the aerial plant organs. A chlorophyll fluorescence study was conducted in order to detect possible photosynthetic changes caused by fungal action on the maize stalk. Chlorophyll fluorescence was evaluated by using a portable fluorometer (FluorPen FP 100, Photon Systems Instruments—PSI, Czech Republic) that measured OJIP (transient fluorescence) according to the protocol described previously (Strasser and Strasser, 1995; Strasser et al. 2000; Kalaji et al. 2016). The protocol is based on the rapid increase in chlorophyll fluorescence quantum yield *in vivo*, after the application of a series of pulses with saturating light (~ 3,000 µmol m^{-2} s^{-1} at 50 µs—"O" point, at 2 ms—"J" point, at 30 ms—"I" point and "P" point with maximum F_M fluorescence, when all reaction centers are closed) to obtain an OJIP transient of the chlorophyll fluorescence. This protocol was applied to fully developed leaves that had been adapted to darkness for at least 20 min. Leaves immediately above the ear of the five inoculated and the five uninoculated plants from each plot were measured. Measurements were performed on the adaxial surface of the leaves at a distance of 15 cm from the stalk. For both groups, three assessments were conducted over the growing season. The first assessment was conducted before inoculation (EP1) and the others two at weekly intervals post-inoculation (EP2 and EP3 at 7 and 21 days after inoculation, respectively). Specific energy flows through reaction centers may be compared and analyzed through biophysical variables reflected through the

induction of fluorescence; thus, various relevant parameters were calculated according to detailed formulas shown in Table 1.

SPAD index

A SPAD-502 chlorophyll meter (Minolta, Japan) which calculates SPAD (Soil Plant Analysis Development) index values was used to determine the leaf green intensity that could be related to chlorophyll content. Three SPAD readings were conducted on the flag leaf for each plant. The first reading was performed at pre-inoculation (EP1), and the other two at 7 and 21 days post-inoculation (EP2 and EP3 at 7 and 21 days after inoculation, respectively).

Yield components

Maize ears from the five inoculated and the five uninoculated plants were harvested from each plot at maturity. The ears were individually harvested, trimmed, and weighed. Ear size (ES) (cm) and weight (WE) (g), number of rows per ear (NRE), number of grains per row (NGE), and grain weight per ear (WG) (g) were measured.

Statistical analysis

The experiment was laid out in a randomized block design, with treatments arranged in subdivided plots with three replicates. Cultivars were designated as plots, and inoculation treatments, either applied or not applied, were used as subplots. The experimental plots consisted of four 5-m rows, with spacing of 0.5 m between rows and 0.3 m between plants for a planting density of 65,000 plants ha^{-1}. Only plants in the two central rows in each experimental plot were used for sampling and measurements, while the two outside rows were considered borders. Five plants randomly selected in the central rows in each plot were marked for inoculation with *C. graminicola*, and five more were marked for use as uninoculated control plants.

Data on yield components, SPAD index values, and biophysical variables were submitted to analysis of variance (ANOVA) to detect treatment effects, and the means, when needed, were compared via the Scott Knott test at 5%

Table 1 Biophysical parameters used in fluorescence studies; formulas and definitions (adapted from Strasser et al., 2000)

OJIP transient fluorescence		Definition
F_0		Minimum fluorescence of chlorophyll adapted to the dark, measured after 50 µs (step O)
Fi		Fluorescence intensity after 2 ms (step J)
Fj		Fluorescence intensity after 60 ms (step J)
F_M		Maximum fluorescence of chlorophyll when all of the PSII reaction centers are closed (step P)
F_V	$=F_M-F_0$	Maximum variable fluorescence
Vj	$Vj=(Fj-F_0)/(F_M-F_0)$	Ratio of reaction center closed after 2 ms, expressed as a proportion of reaction centers that can be closed
Vi	$\mathrm{Vi}=(Fi-F_0)/(F_M-F_0)$	Ratio of reaction center closed after 60 ms, expressed as a proportion of reaction centers that can be closed
F_V/F_0		Transference efficiency of the PSII reaction center to Q_A
F_V/F_M		Maximum photochemical efficiency of PSII
Mo	$= \mathrm{TR_0/RC} - \mathrm{ET_0/RC}$	Net rate of reaction center closure
Area		Area between the fluorescence curve and F_M (without background)
Ss		Necessary energy to reduce a Q_A (single turnover) molecule
φ_{Po}	$= 1 - F_0/F_M$	Maximum quantic yield of primary photosynthesis
φ_{Eo}	$= \left(1 - \left(F_0/F_M\right)\right) * \psi 0$	Quantic yield (at $t=0$) for electron transport
φ_{Do}	$= 1 - \varphi\mathrm{Po} - \left(F_0/F_M\right)$	Quantic yield for energy dissipation
φ_{Pav}	$= \varphi\mathrm{Po}\left(S_M/t_{FM}\right)$	Quantic yield index of primary photochemistry
ψ_0	$= \left(1 - \left(F_0/F_M\right) *\right.$	Probability (at $t=0$) of an exciton to move an electron for CTE after Q_A^-
$\mathrm{PI_{Abs}}$	$= (RC/ABS).\left[\varphi\mathrm{Po}/(1-\varphi\mathrm{Po})\right].\left[\psi_0/(1-\psi 0)\right]$	PSII activity indicator
$\mathrm{PI_{Total}}$	$= PI_{Abs} * \{(1-Vi)/(1-Vj)\}/\left[1-\{(1-Vi)/(1-Vj)\}\right]$	Total performance indicator of PSII
ABS/RC	$= Mo.(1/Vj).(1/\varphi\mathrm{Po})$	Absorbed energy by a PSII reaction center
TRo/RC	$= Mo.(1/Vj)$	Stored energy per PSII reaction center
ETo/RC	$= Mo.(1/Vj)\psi 0$	Electron transport flow from Q_A to Q_B by PSII reaction center
DIo/RC	$= (\mathrm{ABS/RC}) - \left(\mathrm{TR_o/RC}\right)$	Dissipated energy per PSII reaction center

t_{FM}, time to reach F_M (in ms)

probability. Statistical analyses were performed by using the R statistical software (R Core Team. 2020).

Results

Inoculation of corn stalks with *C. graminicola* promoted the development of stalk rot in all four experimental hybrids planted at the two locations, with mean severity scores ranging from 6 to 8 (i.e., lesion extent ranged from 56.3 to 93.8%); however, the severity of the infection differed with hybrid. The mean anthracnose severity in plants of hybrid Formula VIP was significantly higher than the severity recorded for plants of the other hybrids at both locations, while the severity was lowest for plants of the hybrid MG 600 PW (Tables 1 and 2). However, high average severity rates (above 55%, Table 2) were observed across hybrids mean severity rates in inoculated hybrids (68.2% in ARN and 71% in PN) confirming the effectiveness of the inoculation in reproducing anthracnose symptoms. The maximum difference in average disease severity among hybrids was 37.5% in ARN and 34.6% in PN (Table 2), while the maximum difference between inoculated and uninoculated plants across hybrids was 61.7% in ARN and 65% in PN (Table 2).

The hybrid $_{\times}$ inoculation treatment interaction was not significant. Hybrids differed regarding yield components, with the exception of ES in PN and NGE in ARN. Hybrid Formula VIP showed lower WG, ES, and WE and larger NRE than the other hybrids in ARN (Table 2), while hybrid DKB290 showed higher WG and WE values than any other hybrid in PN and, along with Formula VIP, both showed higher NRE values (Table 3). WG, ES, and WE were lower in inoculated plants across hybrids and at both locations. NRE and NGE were unaffected by the fungus (Table 2).

There was no difference among SPAD index values between inoculated and uninoculated plants in the first assessment (Ep 1), conducted before inoculation, at either

Table 2 Mean disease severity (DS), grain weight (WG), ear weight (WE), ear size (ES), number of rows per ear (NRE), and number of grains per row (NGE) for *Colletotrichum graminicola*-inoculated (inoc.) and uninoculated (uninoc.) maize plants

	Hybrids	Variables					
		DS	WG	ES	WE	NRE	NGE
ARN	Formula VIP	93.8 a	88.9 b	13.8 c	107.1 b	18.9 a	589.2 a
	MG600 PW	56.3 b	127.2 a	15.2 b	147.2 a	15.1 d	530.8 a
	DKB290 PRO3	62.5 b	131.2 a	15.4 b	155.1 a	17.5 b	557.6 a
	P30S31 VYH	60.4 b	142.6 a	16.4 a	166.7 a	16.7 c	525.6 a
	Uninoc (−)	6.5 b	136.5 a	15.7 a	160.2 a	17.3 a	581.2 a
	Inoc. (+)	68.2 a	108.5 b	14.7 b	127.9 b	16.8 a	520.5 a
PN	Formula VIP	93.8 a	122.9 b	15.7 a	142.6 b	17.5 a	583.4 a
	MG600 PW	59.2 b	138.7 b	16.4 a	158.1 b	15.3 b	520.8 a
	DKB290 PRO3	68.8 b	172.3 a	16.1 a	193.9 a	17.6 a	538.7 a
	P30S31 VYH	62.5 b	142.1 b	16.5 a	162.4 b	15.4 b	446.9 b
	Uninoc (−)	6.0 b	165.1 a	16.9 a	185.8 a	16.5 a	530.7 a
	Inoc. (+)	71.0 a	122.9 b	15.5 b	142.5 b	16.4 a	514.2 a

Means within columns followed by the same lowercase letter do not significantly differ according to the Scott Knott test ($p \leq 0.05\%$)

Table 3 Mean SPAD index values obtained in maize hybrids (P30S31 VYH, DKB290 PRO3, Formula VIP, and MG 600 PW) inoculated (Inoc.) or uninoculated (N. inoc.) with *Colletotrichum graminicola* (+ or −) in Porto Nacional (PN) and Aparecida do Rio Negro (ARN) at three times: before inoculation (Ep 1), 7 days after inoculation (Ep 2), and 21 days after inoculation (Ep 3)

Hybrids	Porto Nacional			Aparecida do R. Negro		
	Ep 1	Ep 2	Ep 3	Ep 1	Ep 2	Ep 3
P30S31 VYH	52.7 a	53.6 b	51.5 b	53.2 a	58.0 a	50.2 a
DKB290 PRO3	50.9 a	56.3 a	49.0 b	55.3 a	55.2 b	49.9 a
Formula VIP	51.7 a	53.3 b	52.5 b	47.6 b	54.1 b	24.9 b
MG600 PW	53.4 a	56.8 a	58.3 a	54.9 a	60.0 a	52.0 a
Inoc. (+)	52.0 a	53.1 b	51.5 b	52.3 a	55.1 b	37.7 b
N. inoc (−)	51.0 a	56.9 a	54.2 a	53.2 a	58.5 a	50.8 a

Means within columns followed by the same lowercase letter do not significantly differ according to the Scott Knott test ($p \leq 0.05\%$)

location (Table 3). However, inoculation with *C. graminicola* reduced SPAD index in assessments 2 (Ep 2) and 3 (Ep 3), as reflected by lower SPAD index values, at both locations. The hybrid × inoculation treatment interaction for variations in SPAD index values was not significant. Plants of hybrid MG600 PW showed the highest SPAD index values in all assessments at both locations. Conversely, plants of hybrid Formula VIP showed the lowest SPAD index values at both sites, with an extremely low value in assessment 3 (Ep 3) in ARN (Table 3).

Inoculation with *C. graminicola* affected the response curve of chlorophyll transient fluorescence, SPAD index, and yield components. An analysis of the biophysical parameters obtained via the OJIP method indicated a significant difference between inoculated and uninoculated plants for F_0, *Fj*, *Fi*, F_M, *Fv*, *Vj*, *Vi*, *Mo*, *Sm*, Ss, ψ_0, φEo, φPav, TRo/RC, ETo/RC, and PI_{Total} in at least one of the assessments, at one location. No significant difference was observed among inoculated hybrids regarding the biophysical parameters of fluorescence.

F_0 values significantly increased in inoculated plants in all assessments at both sites. φPav was also elevated in the inoculated plants, except for Ep 2 at PN (Table 4). The mean values of parameters *Fj*, *Fi*, F_M, *Fv*, and *Vi* showed differences only in Ep 3 at both sites. For all the aforementioned parameters, inoculated plants showed higher mean values than those shown by control plants (Table 4).

The biophysical parameters *Vj*, *Mo*, φEo, and ETo/RC were different between inoculated and control plants only in Ep 2, in ARN (except ETo/RC, which also showed differences in Ep3), while *Sm*, *Ss*, and TRo/RC only showed changes in Ep 3, in ARN (data not shown). When they varied, *Vj* and *Mo* values were higher in inoculated plants, while φEo, ETo/RC, *Sm*, and *Ss* values were lower in inoculated plants.

Discussion

Inoculation with *C. graminicola* using the method of Parreira et al. (2016) successfully induced infection and disease in maize stalks. In control plants, the insertion of sterile toothpicks without inoculum did not cause lesions in the stalks or vascular tissues, thus, allowing plants to grow and develop normally throughout their life cycle.

Yield components including, WG, ES, and WE were significantly reduced in plants inoculated with *C. graminicola*. Stalk rot induced a 20% loss in grain production per ear, in ARN, along with a 6% reduction in ear size and a 20% reduction in ear weight. Similarly, the disease resulted in a 25% reduction in WG, and 8% reduction in ES, and a 23% reduction in WE in PN. There is no interaction between genotypes × inoculation.

Similar results have been previously reported (Callaway et al., 1992; Cota et al. 2012). Yield losses caused by stalk rot are related to the colonization and degradation of vascular bundles, whereby translocation of water and nutrients to the aerial plant parts becomes severely restricted (Bandara

Table 4 Mean biophysical parameter values of chlorophyll A transient fluorescence obtained in maize hybrids inoculated (Inoc.) or uninoculated (N. inoc.) with *Colletotrichum graminicola* in Porto Nacional (PN) and Aparecida do Rio Negro (ARN) at two times: 7 days after inoculation (EP2) and 21 days after inoculation (EP3). All parameters are described in Table 1

			F_0	*Fj*	*Fi*	F_M	*Fv*	*Vj*	*Vi*	*Mo*
PN	EP2	N. inoc. (−)	6118 b	15,542 a	22,099 a	29,156 a	23,038 a	0.412 a	0.689 a	0.735 a
		Inoc. (+)	6488 a	16,083 a	22,897 a	30,257 a	23,825 a	0.411 a	0.684 a	0.742 a
	EP3	N. inoc. (−)	5476 b	13,358 b	18,254 b	26,190 b	20,714 b	0.379 a	0.599 b	0.690 a
		Inoc. (+)	5896 a	14,959 a	21,962 a	29,951 a	24,055 a	0.381 a	0.662 a	0.674 a
ARN	EP2	N. inoc. (−)	5886 b	12,923 b	15,055 a	19,155 a	13,376 a	0.664 b	0.747 a	1.165 b
		Inoc. (+)	6871 a	15,831 a	16,557 a	19,809 a	12,841 a	0.771 a	0.805 a	1.397 a
	EP3	N. inoc. (−)	5916 b	14,769 b	21,896 b	27,838 b	21,922 b	0.401 a	0.723 b	0.750 a
		Inoc. (+)	6611 a	16,337 a	25,267 a	31,125 a	24,514 a	0.410 a	0.756 a	0.759 a
			Sm	*Ss*	*ψ0*	*φ*Eo	*φ*Pav	TRo/RC	ETo/RC	PItotal
PN	EP2	N. inoc. (−)	293.378 a	0.565 a	0.588 a	0.465 a	928.723 a	1.774 a	1.040 a	2.929 a
		Inoc. (+)	294.712 a	0.560 a	0.589 a	0.464 a	929.042 a	1.794 a	1.052 a	3.071 a
	EP3	N. inoc. (−)	360.710 a	0.565 a	0.621 a	0.487 a	914.861 b	1.831 a	1.141 a	6.130 a
		Inoc. (+)	328.469 a	0.567 a	0.619 a	0.498 a	929.766 a	1.771 a	1.097 a	4.035 b
ARN	EP2	N. inoc. (−)	313.280 a	0.565 a	0.384 a	0.236 a	948.633 b	1.786 a	0.699 a	3.286 a
		Inoc. (+)	322.724 a	0.561 a	0.233 b	0.165 b	960.644 a	1.797 a	0.427 b	5.998 a
	EP3	N. inoc. (−)	299.697 a	0.558 a	0.590 a	0.465 a	932.584 b	1.815 b	1.065 b	2.323 a
		Inoc. (+)	274.670 b	0.532 b	0.599 a	0.472 a	935.477 a	1.887 a	1.127 a	1.751 b

Means within columns followed by the same lowercase letter do not significantly differ according to the Scott Knott test ($p \leq 0.05\%$)

et al. 2019), leading to reduced growth and even premature plant death and stalk lodging and breaking, thereby resulting in considerable loss of crop yield (Costa et al. 2019).

No effect of *C. graminicola* inoculation was observed on either NRE or NGE. According to Szareski et al. (2018), these variables are determined genetically before flowering, between stages V9 (i.e., ninth leaf) and fertilization of the ovule. Hence, as plant inoculation was performed at pre-tasseling, these two variables had already been defined.

A significant difference was observed among hybrids regarding yield components, suggesting genetic variability for disease susceptibility, in terms of productivity parameters evaluated at both study sites. In terms of the yield components evaluated at both sites, Formula VIP and DKB 290 PRO3 were the most and the least affected hybrids, respectively.

Chlorophyll (Chl*a*) fluorescence is associated with photosystem function and has been used as an indicator of the performance and integrity of the plant photosynthetic apparatus (Maxwell and Johnson. 2000; Murchie and Lawson. 2013). Chl*a* fluorescence monitoring is a non-destructive technique that reveals changes in photosystem II during an infectious process (Rios et al. 2017; Rolfe and Scholes, 2010). Changes in Chl*a* fluorescence parameters and their ratios have been previously reported for several foliar pathogens, including *Pyricularia oryzae*, *Puccinia triticina*, and *Blumeria graminis* f. sp. *tritici* in wheat (Aucique-Pérez et al. 2014; Kuckenberg et al. 2009), but only for one stalk rot pathogen *Stenocarpella macrospora* in maize (Bermúdez-Cardona et al. 2015). All of these pathogens resulted in reduced photosynthetic efficiency.

C. graminicola affected the growth and development of maize plants by interfering with plant physiological processes. The reduced amounts of green leaf revealed by post-inoculation SPAD index readings suggest that the pathogen caused physiological changes impacting pigment production. Similar results were obtained by Baghbani et al. (2019), in other pathosystems. The differences in SPAD index observed between ARN and PN may be attributed to a greater disease pressure due to a more severe water restriction after flowering in ARN, where sowing was performed at a later date, whereby the end of the rainy season coincided with the reproductive stage, resulting in a greater degree of plant water stress at this location.

The colonization of root and stalk tissues by phytopathogens may hinder water absorption and flux, leading to water stress, which is counterbalanced by stomatal closure and the generation of oxygen reactive species, thereby affecting photosynthesis activity (Martínez-Ferri et al. 2016).

As shown by Bandara et al. (2016; 2019), a chlorophyll reduction in the leaves of sorghum in association with translocation of water potential, degradation of vascular tissues imposed by stalk rot can leads to chlorophyll degradation. Consistently, Silva et al. (2014) showed that SPAD index valued were reduced by water stress in sugarcane, suggesting that SPAD index provides important information regarding the integrity of the photosynthetic system.

The kinetics of induction of chlorophyll fluorescence—OJIP and the efficiency of electron transfer via the *Z* scheme until the final acceptor of electrons in PSI—support the analysis of biophysical parameters derived from this study (Table 1; Strasser and Strasser. 1995). All the obtained curves suggested typical OJIP values, indicating that the samples were photosynthetically active, and allowing a detailed analysis of biophysical parameters, whose values obtained based on OJIP fluorescence (Table 4), were similar to the values obtained by Thwe and Kasemsap (2014) and by Srinivasarao et al. (2016).

Fungal infection led to high minimum fluorescence (F_0) values in Ep 2 and EP 3 at both sites (Table 4). The F_0 value is obtained when all PSII reaction centers are open, and increased F_0 values may imply irreversible damage to PSII (Krause. 1988; Krause and Weis, 1991) and photoinhibition (Pan et al. 2010), indicating that heat dissipation is out of control (Thwe and Kasemsap, 2014). The higher F_0 observed in inoculated plants (Table 4) suggests a possible loss of photosynthetic efficiency, which is likely, partly responsible for the observed reduction in grain weight (Table 3).

Previous studies showed that *Fusarium* spp. damaged PSII in various plant species (Bauriegel et al. 2011). Similarly, Pan et al. (2010) observed increased F_0 values, along with reduced PSII activity in monitored leaves of maize plants growing in antimony-contaminated water. However, no significant changes in F_0 were observed in tomato plants treated with ozone for 20 min or 20 h (Thwe and Kasemsap. 2014). Martinazzo et al. (2012) recorded higher F_0 values in association with increased temperature in peach leaves. In the present study, a relationship was identified between higher F_0 values and reduced chlorophyll content (as per SPAD index values), consistently with the well-known fact that chlorophyll serves to protect PSII.

According to Lichtenthaler (1988), reduced chlorophyll content is related to changes in other parameters of chlorophyll fluorescence, such as the F_0 and F_M values. In a study in which the effects of maize inoculation with *Fusarium verticillioides* were evaluated, no changes in F_0 were caused by inoculation (Baghbani et al. 2019). However, the authors conducted measurements only 5 days after inoculation, while measurements were performed seven and 21 days after inoculation (EP 2 and EP 3) in the study reported herein (Table 4).

Similar to F_0, parameters *Fj*, *Fi*, F_M, *Fv*, *Vi*, and PI_{Total} were higher in inoculated plants, but only in EP 3 (except *Fj* in ARN Ep 2). The increase in the fluorescence curve in the corresponding *O–J* section is related to the closure of reaction centers in response to the plastoquinone A reduction

(Q_A; first PSII plastoquinone acceptor) due to reoxidation of Q_A by Q_B (second PSII plastoquinone acceptor) via electron transfer through the rest of the chain. The *J–I* section of the curve corresponds to Q_B reduction of plastoquinone (PQ), cytochrome (Cyt b_6/f), and plastocyanin (PC) (Strasser et al., 2000; 2004). The higher *Fj* and *Vj* values suggested an increase in reaction center closures due to infection.

Increased *Fi* values are related to the reduction of electron transport by Q_B, PQ, Cyt b_6/f, and PC, as described above. Despite the higher F_0, *Fv*, and F_M values in EP 3, the area below the fluorescence curve did not vary with study site or assessment time.

C. graminicola-induced stalk rot may cause the degradation of stem vascular bundle tissues, consequently reducing upward water flow and leaf hydration, which in turn would reduce photosynthesis. However, PSII reportedly tolerates water deficit well, compared to PSI; thus, PSII experiences negative impacts only under extreme conditions (Kalaji et al. 2016). PI_{Total} is the parameter most widely used in the analysis of OJIP transient fluorescence, as it reflects the overall status of plant health and vitality, and is often used to assess plant tolerance to water stress (Kalaji et al. 2016; Zivcak et al. 2008). In this study, a 34% reduction in PI_{Total} in PN and a 24% reduction in ARN were observed in plants inoculated with *C. graminicola* at 14 days after inoculation (Ep 3, Table 4); this finding may be attributed to a greater severity of water stress associated with the effects of fungal infection.

The quantic yield of PSII (F_V/F_M), the net rate of PSII reaction center closures (*Mo*), and the PSII performance index (PI_{Abs}) may also be used to verify the sensitivity of plants to abiotic stress (such as water deficit). Nevertheless, these parameters did not differ between inoculated and uninoculated plants in EP 3 at either site (Table 4). These findings are similar to those of Zivcak et al. (2008), who showed that F_V/F_M was not always sensitive to low-intensity water stress in wheat.

Our experiments showed differences in the plant physiological responses to stalk anthracnose. However, no significant differences were observed regarding the biophysical parameters of fluorescence among hybrids. Furthermore, despite the significant differences among hybrids regarding disease severity, the median level of anthracnose stalk rot severity was very high (above 56%) for all hybrids, which might explain the lack of variation among hybrids regarding the investigated biophysical parameters. Hence, hybrids experiencing less severe anthracnose may show more varied biophysical responses, thus allowing differentiation among hybrids. Tůmová et al. (2018) found differences in fluorescence parameters in maize plants in response to drought but not between water stress susceptible and tolerant cultivars. Several studies have found differential cultivar responses to various forms of biotic and abiotic stress using the OJIP method under controlled conditions (Sarkar and Panda, 2009; Simko et al., 2016). However, field plants experience frequent cycles of stress and recovery. Some studies have suggested that plants recovered photosynthetic capabilities after periods of stress, as indicated by improvements in their fluorescence curve (Jedmowski et al. 2013; Stefanov et al. 2011). Significant variations in temperature, soil water content, and air relative humidity are common in field experiments; such conditions likely influence stress and recovery cycles, as well as plant biophysical parameters.

The results presented in this work demonstrate a reduction in grain weight, ear size, chlorophyll content, and changes in FSII caused by inoculation with *C. graminicola*. The chlorophyll fluorescence parameters can show alterations in the FSII functioning, and these could be used to monitor diseases in corn. However, not all parameters were efficient in distinguishing these changes.

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Data availability Raw data were generated at Embrapa. Derived data supporting the findings of this study are available from the corresponding author Costa, R.V. on request.

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