

Physicochemical characteristics and nutritional quality of corn grains related to a foliar disease

¹Henao López, M.J., ¹Montoya-Estrada, C.N., ²Hernández Jorge, F.E. and ^{1,*}Castro-Ríos, K.

¹Microbiology and Agroindustrial Biotechnology Research Institute, Faculty of Health Sciences,
Universidad Católica de Manizales

²Research and Projection Group in Agricultural Production (GIPPA), Faculty of Agricultural Sciences,
Universidad de Caldas, Manizales, Colombia

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Abstract

Corn (*Zea mays*) is the most produced cereal globally, with a high economic and nutritional impact. The crop is affected by a foliar disease known as the tar spot complex disease (TSC), induced by three fungi, generating losses of more than 50% of yield and impacting the grain's quality. This research aimed to assess potential physicochemical and nutritional quality alterations in corn grain intended for human consumption caused by the TSC. A field crop of yellow corn hybrid ATL200 was established with two homogeneous lots, one with fungicide application for fungus control and another without fungicide application. The corn ears were harvested when the corn grains had less than 16.4% moisture content. Physical, chemical, and nutritional variables were measured, and a correlation was made between these parameters and disease severity. The TSC had an impact on grain quality, corn ear, corn grain, and grain weight, showing a negative correlation with these measures, as well as an impact on pH, water activity (a_w), moisture, fat, carbohydrates, protein, and micro-nutrients such as iron, phosphorus, and calcium. The results show that the presence of the TSC disease impacts grain quality.

1. Introduction

With 1124 million tons in the 2019–2020 cycle, corn (*Zea mays*) is the most produced cereal in the world; the leading exporters are the United States, Brazil, and Argentina, which produce 369, 113 and 57 million tons/year, respectively (International Grains Council (IGC), 2021). It is estimated that by 2025, global corn production will increase by 13% due to the demand for food use (Urango, 2018; FAO, 2020). Corn is generally used for the production of biofuels, animal feed, and foodstuffs for human consumption, such as starches, oils, and sweeteners; and in Latin American countries, it is the basis for the production of various ethnic products due to its nutritional characteristics, specifically for its carbohydrate, protein and micronutrient content (Urango, 2018; Hernández *et al.*, 2019; IGC, 2021).

The corn crop has been historically affected by biotic and abiotic factors; however, diseases are the main limiting factors in corn grain productivity and quality (Urango, 2018; Silva Valle, 2019). Among the diseases that affect corn with greater severity is the tar spot complex (TSC), which is one of the main foliar diseases of corn in several Latin American countries, and was

recently detected in the United States (Mottaleb *et al.*, 2019; Valle-Torres *et al.*, 2020). TSC is caused by an obligated pathogen *Phyllachora maydis*, but in Latin America it is also related to the interaction with other two fungi *Monographella maydis* and *Coniothyrium phyllachorae* (Valle-Torres *et al.*, 2020). The disease is characterized by slightly elevated black spots distributed throughout the leaf blade, which after several days, become lesions that coalesce to form necrotic areas, with a final economic impact on grain yield (Mottaleb *et al.*, 2019). Grain yield losses associated with TSC reach up to 51%, depending on the degree of susceptibility of corn phenotype and the presence of specific environmental factors that favor disease development (Hock *et al.*, 1989; Hock *et al.*, 1995). Recently was demonstrated that TSC could cause up to 58% yield losses in the most susceptible corn phenotype (Loladze *et al.*, 2019).

The tar spot complex also generates quality defects like poor filling of the corn ear with the presence of wrinkled grains, premature germination, and lower weight when it appears at the beginning of the production cycle; causing a greater susceptibility of corn to contamination in storage by other fungi, especially

*Corresponding author.

Email: katherin.castro@protonmail.ch

mycotoxin-forming fungi (Mahuku *et al.*, 2013; Mohapatra *et al.*, 2017). Fungal contamination in the corn plant can generate competition for nutrients between the plant and the microorganism, possibly impacting the nutritional quality of the grain (Kalebich and Cardoso, 2017). This has been observed in other cereals like sorghum and wheat, where foliar diseases can affect grain yield, macronutrients, and micronutrients (Dimmock and Gooding, 2002; Santa-Rosa *et al.*, 2016). For example, Bandara *et al.* (2017) established the impact of fungal stalk rot in sorghum, with a significant reduction of micro and macronutrients with increasing severity; generating significant effects of pathogens on the levels of phosphorus, potassium, calcium, copper, manganese, zinc, and on proteins, fats and starches when compared to control grains.

Nonetheless, no studies have been evidenced that relate the impact of grain quality with foliar diseases in corn, especially the tar spot complex, although an impact on physical and nutritional quality is expected, as it has occurred with other fungi in sorghum and wheat crops previously mentioned. So far, have focused on the quality characteristics of the grain during harvest or the yield losses caused by diseases (Mottaleb *et al.*, 2019; Mueller *et al.*, 2020; Valle-Torres *et al.*, 2020). Therefore, the objective of the present work was to evaluate if the physicochemical characteristics and nutritional quality of corn can be affected by the foliar disease tar spot complex.

2. Materials and methods

2.1 Location, treatments and harvesting

This study was conducted from August to December 2019 at Montelindo farm of Caldas University, located at 5°05'10.2" N and 75°41'20.0" W in Santagueda, Palestina, Colombia (Figure 1). With an average



Figure 1. Study Localization (Adapted from: <https://commons.wikimedia.org/w/index.php?curid=2010597> and <https://earth.google.com/>).

temperature of 25.8°C, an altitude of 1010 m.a.s.l., average annual rainfall of 2100 mm and relative humidity of 76%, annual solar brilliance of 2010 h, and sandy loam soils with a slope lower than 3%. The corn plants used belong to the yellow corn hybrid ATL 200.

Two homogeneous lots of approximately 1 ha each were used; one of them had integrated management that included the application of the fungicide fenbuconazole at a concentration of 500 mL/ha (Lot 1, treated with fungicide) and while the other lot did not receive any fungicide treatment (Lot 2, untreated with fungicide). The corn plants were sown at a distance of 80 cm between rows, 7 seeds/m². A completely randomized block design was established, the lot was divided into three blocks, and the experimental unit was composed of 5 plants (Figure 2).

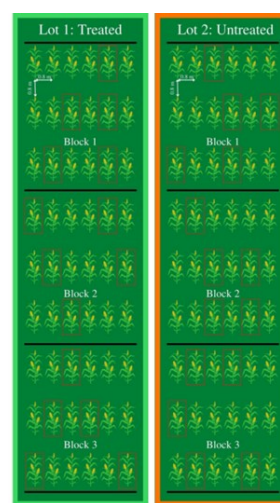


Figure 2. Randomization scheme of the trial.

Harvest was done using a grain harvester (Jumil JM 390) upon reaching grain moisture of 16.4%, which was determined with a moisture meter (Wile 200). Subsequently, the grains were weighed, and the harvest yield was established by taking a small plot, determining the m² harvested, and then extrapolating this value to a hectare. Samples were randomly collected, placed in paper bags, and subsequently taken and analyzed at the Food Microbiology Laboratory of the Catholic University of Manizales (Manizales, Colombia).

2.2 Morphological characterization of the pathogen tar spot complex and assessment of foliar disease severity determination

For the morphological characterization, the samples were collected weekly from August to October 2019. These were packed in paper bags and taken to the Food Microbiology Laboratory of the Catholic University of Manizales, where they were photographed and placed in a humid chamber for 24 h; subsequently, they were analyzed for evidence of the presence of sporulation.

The diseased material was carefully examined with a Leica stereoscope microscope (4×), and longitudinal cuts were made to the stroma structures; the fungal staining was made in slides with lactophenol cotton blue and observed with an optical microscope in 40× objective to make the identification according to taxonomic keys according to Castaño-Zapata and Salazar (1998). For the severity evaluation of the tar spot complex disease, the diagrammatic scale of Hernández Ramos and Sandoval Islas (2015) was used, analyzing the affected area by the pathogens in leaves by visual comparison with the scale to determine the percentage of severity.

2.3 Physicochemical analysis

The weight of the cob, the corn ear, and the corn grains were determined with a semi-analytical scale Kern brand of 500 g. For pH and soluble solids, the samples were ground for 10 s at maximum speed in a commercial mill XPERT series. The potentiometric technique was used to determine the pH of the samples with the SIAanalytics Lab 855 pH equipment, the soluble solids with a refractometer (Bellingham Stanley 60/70 Abbe), and the water activity (a_w) of the whole grains was quantified utilizing a_w Schaller 3115 (mod RH2 + a_w -Wert).

2.4 Nutritional analysis

A sample of 300 g of corn was used for nutritional analysis. Moisture content was determined by oven drying at 100°C (AOAC Official Method 925.45-1925), ash content by gravimetric technique (ICONTEC, 2002b), protein by Kjeldahl method 6.26 (ICONTEC, 1999; HACH Company, 2019), crude fat by Soxhlet extraction (ICONTEC, 2002a), total carbohydrate content (United States Department of Agriculture (USDA), 1973), iron and calcium (American Public Health Association (APHA), 1999), phosphorus by colorimetric method (AOAC Official Method 981.14-1981) and sodium (AOAC Official Method 976.25-1977).

2.5 Statistical analysis and correlation with disease severity

Normality and homogeneity of variances tests were performed, and then the data were analyzed by ANOVA; also, a Pearson correlation analysis was performed to identify the correlation between disease severity and physicochemical parameters using the software package Jamovi version 1.2 (Jamovi, 2020). Significant differences were determined by Tukey's test with a significance level of 5%. Experimental results were expressed as mean $\pm$ standard deviation (SD), and the experimental unit for data analysis was the sample per lot.

3. Results and discussion

3.1 Morphological characterization of the pathogen complex in tar spot complex in corn plants and crop yield

During the experiment, 15 samplings were performed from August to October 2019; 225 samples were collected, of which 152 showed symptoms of the foliar disease known as tar spot complex (TSC), and structures corresponding to the fungus *Phyllachora maydis* were observed (Figure 3). The severity was classified and evaluated as follows: Class I. Severity 1-6%; Class II. Severity 7-22%; Class III. 23-55%; Class IV. 56-84%; Class V. 85-95%; Class VI. 96-100% (Hernández Ramos and Sandoval Islas, 2015). Taking into account this classification, the disease severity results were as follows: in Lot 1, the severities ranged from 0 to 0.48%, with an average of 0.14%; in Lot 2, the severities ranged between 1% and 48%, with an average during the whole experiment of 12%.



Figure 3. Samples with different severities of the foliar disease tar spot complex (TSC). Class I. Severity 1-6%; Class II. Severity 7-22%; Class III. 23-55%; Class IV. 56-84%; Class V. 85-95%; Class VI. 96-100% Adapted (Hernández Ramos and Sandoval Islas, 2015).

Approximately four months after sowing, harvesting began for the two lots. The treatment with fungicide obtained the highest yield with values of 4.75 ton/ha, and for the lot treated without fungicide, the production values were lower at 1.86 ton/ha. Depending on the degree of susceptibility of the corn genotype and the existence of particular environmental variables that favor disease development, TSC can result in grain yield losses of up to 58% (Hock *et al.*, 1995; Loladze *et al.*, 2019). Other cereal crops, such as sorghum and wheat, have also shown crop yield loss related to grain weight loss linked to foliar disease (Santa-Rosa *et al.*, 2016; Bandara *et al.*, 2017).

3.2 Physical analysis: weight loss of the cob, corn ear, and grains

When moisture and corn grain growth were adequate, samples (corn ear) were harvested for physicochemical analysis. As shown in Figure 4, the tar spot complex disease (TSC) affected the weight of the corn ear, with an average weight of 85.66 ± 31.92 g (Lot

2, untreated, with disease severity of 1-48%). In contrast, the lot with less severity of the disease (Lot 1, treated, with severity between 0-0.48%) had an average weight of 235.28 ± 60.71 g, representing a difference of 192.57 g, corresponding to a loss of 75% of the weight generated by the TSC.

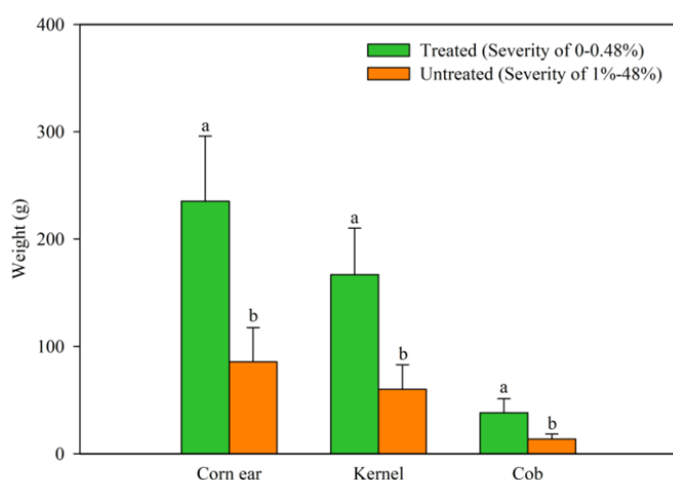


Figure 4. Corn ear, grain, and cob weight affected by tar spot complex disease. Bars with different notations are statistically significantly different ($p \leq 0.05$) between lots; the error bars in the columns represent the standard deviation of the values.

Regarding grain weight, the average for Lot 2 was 60.07 ± 22.81 g, while for Lot 1 with fungicide treatment was 166.89 ± 43.25 g; this same weight behavior was observed in the corn ear with an average of 13.62 ± 4.83 g for Lot 2 and 38.17 ± 13.12 g for Lot 1 with low TSC severity. The analysis of variance showed a significant statistical difference ($p < 0.05$) between the values of corn ear, corn grain, and ear weight between Lot 2 without treatment for the control of TSC and Lot 1 treated with a fungicide that presented a low severity of the disease, which was verified by Tukey's test.

The loss of weight in grains related to the presence of the disease in the flowering stage causes a reduction of photosynthates which are generally sugars, impacting the availability of starches that will be deposited in the tissues of the plant; therefore, reducing the weight of corn, and affecting the grains which shrink and have a lower weight (Guzmán de Serrano *et al.*, 2014).

Among the physical characteristics of the corn ear (Figure 5), it was found that the samples affected by the tar spot complex, have an inadequate filling of the grains and a small size, in addition to wrinkled grains, and a cob also small and deformed; while the corn from the lot with low severity (Figure 5) of the tar spot complex had a larger corn ear, with adequate filling, the grains were large and with characteristic golden color, the cob was large and without deformations. Mahuku *et al.* (2013) evidenced that the affected corn ears with the foliar disease TSC at the beginning of the cycle shrivel, do not

have a good filling, and have little weight, impacting crop yield, with reported losses up to 58%, but this also depends on the degree of susceptibility of corn phenotype and the presence of specific environmental factors that favor disease development (Hock *et al.*, 1995; Loladze *et al.*, 2019). Grain weight loss and its impact on yield, associated with foliar disease, has also been observed in other cereals like sorghum and wheat (Santa-Rosa *et al.*, 2016; Bandara *et al.*, 2017).

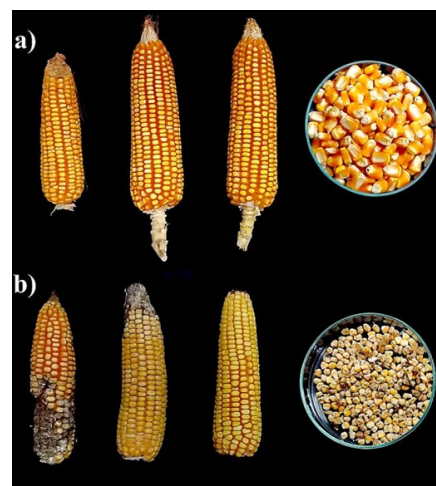


Figure 5. Physical quality of corn ears and grains affected by the foliar disease tar spot complex (TSC). a. Samples from Lot 1 (treated, severities between 0-0.48%) b. Sample from Lot 2 (untreated, severities between 1-48%).

3.3 Physicochemical analysis: pH, a_w , °Brix

Figure 6 shows the results of pH, a_w , and °Brix of the grains from the samples of corn ears from Lot 1 and Lot 2, respectively. The average pH of the grains in Lot 2 affected with TSC was 6.47 ± 0.20 , while Lot 1 with control for the disease was 6.08 ± 0.24 , with a difference in pH of 0.39. This was verified by statistical analysis, in which a significant statistical difference ($p < 0.05$) was obtained between the pH of the samples from Lots 1 and 2.

Regarding a_w of corn grains in the lot without fungicide was 0.70 ± 0.03 , and the lot with fungicide application was 0.83 ± 0.03 with a difference of 0.13 between both lots; through the statistical analysis was obtained, a significant statistical difference ($p < 0.05$) for the evaluated lots. The average °Brix of Lot 2 corn grains was 0.82 ± 0.17 , and for Lot 1 was 0.83 ± 0.34 , with a difference of 0.01 between the lots. However, there is no significant statistical difference ($p > 0.05$) between the °Brix values from the samples of both lots.

In general, the presence of a fungal disease causes nutrient competition with the plant; this generates changes reflected in the chemical composition and therefore affects physicochemical parameters, as evidenced in the results of this work (Venancio *et al.*, 2003; Kalebich and Cardoso, 2017). According to

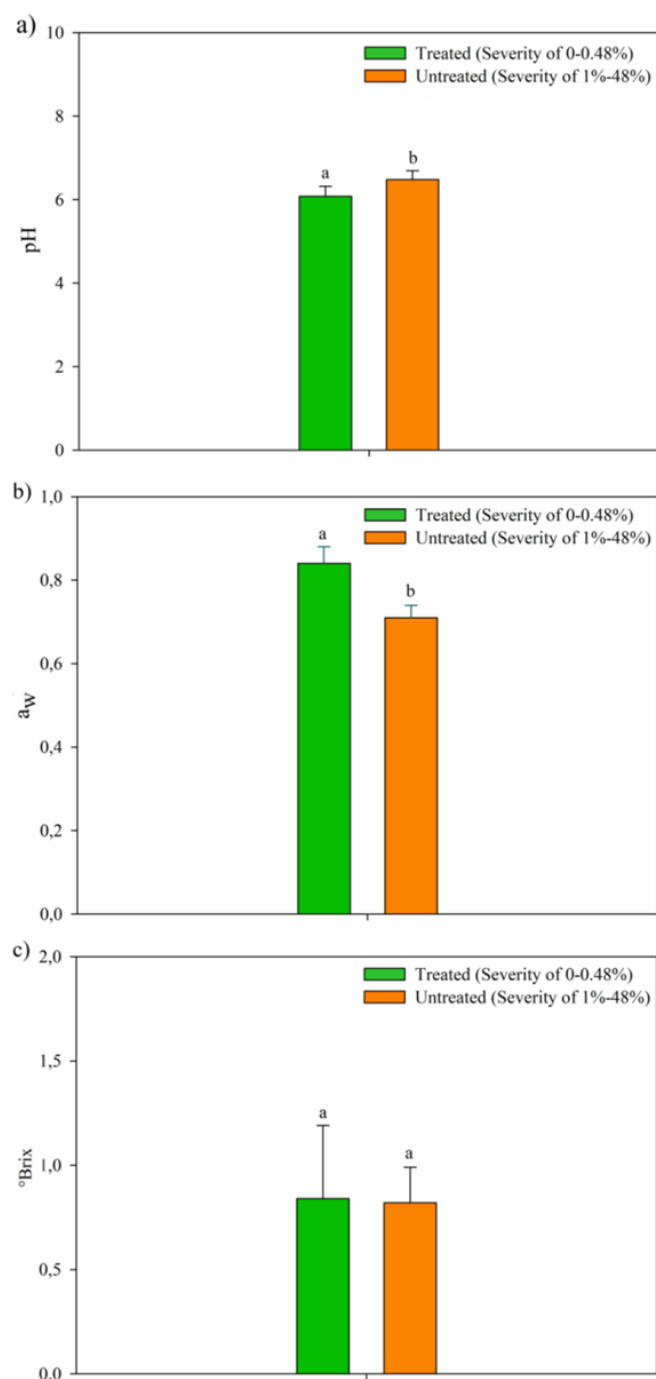


Figure 6. Physicochemical parameters of grains affected by tar spot complex disease. a. pH, b. a_w , and c. °Brix. Bars with different notations are statistically significantly different ($p \leq 0.05$) between lots; the error bars in the columns represent the standard deviation of the values.

Bandara *et al.* (2017), the presence of foliar disease in sorghum affects the macro and micronutrients in the grain and reduces the absorption of water and nutrients in the plant; this could explain the effect on both the pH and the a_w of the grains.

3.4 Correlation between disease severity and physicochemical parameters

Pearson's correlation analysis was applied to the different physicochemical parameters and TSC disease

severity. As shown in Table 1, a negative correlation was found between severity and corn ear and cob weight. Corn ear weight was positively correlated with grain weight, cob weight, pH, and a_w ; and negatively correlated with pH. Grain weight is positively correlated with cob weight and a_w , and negatively correlated with pH. Furthermore, finally, a_w is negatively correlated with pH. The results indicate that the severity of the foliar disease TSC has a relationship with the weight of the corn ear, impacting negatively when the disease is present, directly affecting crop yield and grain quality. The impact of TSC has also been described by Guzmán de Serrano *et al.* (2014), which indicated that the disease generates weight loss in the corn ears. This is caused by an incorrect filling of the corn ears generated by premature germination of the grain (Mahuku *et al.*, 2013).

3.5 Nutritional analysis

Table 2 shows the macro and micronutrient content of corn grains from samples of corn ears from Lot 1 with the application of fenbuconazole and Lot 2 without fungicide. A decrease in moisture, crude fat, iron, phosphorus, high protein content, total carbohydrates, and calcium was observed for the lot affected by TSC compared to the lot with fungicide application for disease control. Similar results were found by Bandara *et al.* (2017) in a study conducted in sorghum on the impact of fungal stalk rot pathogens on grain nutritional properties. They found that the presence of the disease affected protein, fat and starch content, and minerals N, P, K, Ca, Mg, Cu, Fe, Mn, and Zn; attributing this to a reduction of water and nutrient uptake in the plant, having a direct impact on the grain. Finding that the reduction of macronutrients and minerals is directly associated with the sorghum hybrid evaluated and environmental conditions, the hybrid 84G62 the one that presented higher resistance to this fungal complex and consequently lower impact on macronutrient and mineral reduction compared to the hybrid DKS37-07.

Regarding the increase of protein in grains, something similar was observed by Fleitas *et al.* (2017) in a wheat crop, where protein increased in grains from lots affected by the fungus *Mycosphaerella graminicola*; while protein decreased in lots with fungicide application (triazole, triazole-strobilurin). The authors indicated that disease progression was lower in crops with more nitrogen, impacting grain protein levels. Martínez *et al.* (2019) also observed an increase in wheat grain protein content with increasing severity of wheat blast disease caused by isolates of *Pyricularia oryzae*.

Regarding the changes in the mechanisms, Lucas (2009) reported that rust-causing pathogens generate a

Table 1. Pearson correlation matrix between physicochemical analysis, and tar spot complex disease severity.

	Severity	Corn ear weight	Grain weight	Cob weight	a _w	pH	°Brix
Severity	-						
Corn ear weight	-0.386*	-					
Grain weight	-0.363	0.993***	-				
Cob weight	-0.386*	0.952***	0.917***	-			
a _w	-0.366	0.863***	0.853***	0.853***	-		
pH	0.279	-0.576**	-0.584***	-0.507**	-0.546**	-	
°Brix	-0.102	0.084	0.077	0.058	0.069	0.28	-

Table 2. Comparison of the effect of tar spot complex disease on grain nutritional parameters.

Analysis	Lot 1 with disease severity between 0-0.48%	Lot 2 with disease severity of 1%-48%
Humidity	14.39 g/100 g	12.54 g/100 g
Ash	1.17 g/100 g	1.17 g/100 g
Protein	7.63 g/100 g	8.48 g/100 g
Crude fat	3.03 g/100 g	2.18 g/100 g
Total carbohydrates	73.78 g/100 g	75.63 g/100 g
Iron	4.6 mg Fe/100 g	2.5 mg Fe/100 g
Calcium	71.9 mg Ca/100 g	79.8 mg Ca/100 g
Phosphorus	113 mg P/100 g	108 mg P/100 g
Sodium	< 3 mg Na/100 g	< 3 mg Na/100 g

nutritional imbalance in plants due to physical damage to leaves that directly affect photosynthetic processes, decreasing nutrient translocation. In addition, transpiration increases due to cuticle damage and causes the leaves to age prematurely, which increases the retention of sugars and amino acids in diseased leaves and restricts mobilization to the developing grains. Likewise, Hartill (1961) reported that wheat plants in normal physiological development increase their nitrogen concentration in the last stages of grain filling; however, if they are infected by *Puccinia striiformis*, grain maturation is affected, and a reduction in protein concentration is evidenced.

4. Conclusion

Regarding the physical parameters, the lot that presented lower severities of tar spot complex between 0 and 0.48% had better-looking corn ears and larger size, the filling was homogeneous, and had larger grain size with yellow and uniform coloration. The lot that presented severity of the disease was between 1%, and 48% presented smaller corn ears, with dehydrated appearance, incorrect filling of grains, and variable size. In addition, nutritional parameters showed a decrease in moisture, crude fat, iron, and phosphorus, as well as an increase in protein content, total carbohydrates, and calcium in the grains. Additionally, a negative correlation between severity and corn ear weight, a_w, and °Brix was evidenced, demonstrating the negative impact

of the tar spot complex foliar disease on the physicochemical and nutritional parameters of corn.

Conflict of interest

The authors declare no conflict of interest.

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