RESEARCH

# Fungicide Applications and Grain Dry Milling Quality in Late-Sown Maize

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## ABSTRACT

Argentinean maize (*Zea mays* L.) is known for its grain hardness, and exporting grain lots are subject to strict regulations regarding physical characteristics and mycotoxin concentrations. Our production system is changing to later sowings, and the use of foliar fungicides is becoming more common due to positive yield responses. In this study, we evaluated the effects of fungicide treatments at different application timing on specific physical grain quality traits and fumonisins in late-sown maize. For this we used four commercial genotypes differing in grain hardness. Measured traits were yield, individual grain weight, dry milling grain quality attributes (test weight, floaters, vitreousness, and 8-mm screen retention), grain composition (oil, protein, and starch), and total fumonisin concentrations. Yield was affected by genotype and fungicide treatments ($p < 0.05$). Genotypes differed ($p < 0.001$) in all grain quality attributes and fumonisin concentrations. Particular genotypes had fumonisin values higher than maximum levels accepted for human consumption (4 μg $g^{-1}$). Fumonisin concentration differences among genotypes were negatively related to their grain hardness. Fungicide treatments, regardless of type and application timing, increased yield in all genotypes by ~350 kg $ha^{-1}$. However, fungicides had no significant positive effect on any grain physical quality trait, or on fumonisin concentrations. These results emphasize the importance of genotype selection as a critical crop management option to manage grain physical quality traits and fumonisin contaminations. Fungicides can positively affect yield, but positive effects on dry milling grain quality should not always be expected.


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**Abbreviations:** ELISA, enzyme-linked immunosorbent assay; GMO, genetically modified organism.

Argentinean maize (*Zea mays* L.) is internationally known for its grain hardness and is the main supplier of hard endosperm maize grain to the European Union. This maize is commonly used for dry milling and human consumption, and grain lots are subject to strict regulations regarding physical characteristics and mycotoxin concentrations.

Grain physical characteristics are of great importance in terms of feedstock quality for dry milling processing (Kirleis and Stroshine, 1990; Cirilo et al., 2011; Blandino et al., 2012b). Argentina and the European Union have set minimum quality standards to ensure high grain hardness quality (MAGyP, 2015). At present, the hardness traits that a grain lot needs to meet are: minimum test weight (76 kg $hL^{-1}$), a maximum number of floaters at a standardized solution (25%), and a minimum number of grains with 50% or more of vitreous endosperm (92%). Although screen retention is not contemplated in the specific standards to attain high-quality maize, the dry milling industry demands that most grains be retained in an 8-mm sieve (ideally >50%) to achieve optimum milling quality. In terms of mycotoxins, the European Commission establishes 4 μg $g^{-1}$ as the maximum level of total fumonisin concentration for unprocessed maize (EFSA, 2014).

The Argentinean maize production system has changed drastically in recent years, especially in relation to modifications in sowing date. The sowing date for the central region has moved to later in the growing season, from September to December. A

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longer fallow period allows for more accumulation of water and N at sowing and locates the flowering period under a lower drought stress probability. Later sowings have lower yield potential than earlier ones, but farmers are obtaining acceptable yields with higher yield stability. At present, 60% of the total maize produced in Argentina is considered late sowing (Bolsa de Cereales, 2015), and specific crop management options for adequate management under these later sowings are becoming available (Gambín et al., 2016). Under these environments, optimum grain physical quality can be obtained (Abdala et al., 2018), but disease pressure is higher, making fungicide applications a common management recommendation for yield improvements (Bradley et al., 2010). Recent evidence has shown that fungicide applications can also affect grain hardness (Testa et al., 2015).

Mycotoxins are secondary metabolites produced by mold-infected crops. The presence of mycotoxins in foodstuffs is known as a global problem (Hussein and Brasel, 2001), and worldwide regulations concerning food mycotoxin contamination are followed in many countries (van Egmond and Dekker, 1995). In maize, the most important mycotoxins are fumonisins, aflatoxin, deoxynivalenol, ochratoxin A, and zearalenone. Fumonisins are produced primarily by *Fusarium verticillioides* (Saccardo.) Nirenberg and are the most common mycotoxins coming from initial field infection. This toxin includes many analogues, FB1 being the most common (Marasas, 1995; Shephard et al., 1996). Daily consumption of maize contaminated with fumonisins has been epidemiologically associated with esophageal cancer in humans (Rheeder et al., 1992) and is the causative agent of leukoencephalomalacia in horses (Marasas et al., 1988), pulmonary edema in swine (Osweiler et al., 1992), and liver cancer in rats (Gelderblom et al., 1991). Although grain contamination with fumonisins can occur at any step in the food chain, reducing initial field infection is considered critical (Chulze et al., 1996). There are many studies reporting the effect of field management practices to reduce mycotoxin contamination (Cleveland et al., 2003; Munkvold, 2003), and genotype selection is considered a critical crop management option (Hammond et al., 2004; Barros et al., 2009). In particular, Munkvold and Hellmich (1999) found that genetically modified organism (GMO) Bt maize had lower fumonisin concentrations than its non-Bt counterpart. However, the effects of field fungicide applications in late-sown maize have never been described.

We are particularly interested in further understanding fungicide effects on maize grain quality for dry milling and total fumonisin concentrations. Our working hypothesis is that fungicides would not only increase yield but also grain hardness. A healthier canopy during grain filling should affect the amount of available assimilates per growing grain, increasing grain protein concentration (Borrás et al., 2002). Grain protein is key for grain hardness (Gerde et al., 2016). Blandino et al. (2012a) found increases in grain yield and test weight after fungicide treatments in both vegetative and reproductive stages, supporting our initial hypothesis.

In the present manuscript, we evaluated the effect of genotype and fungicide treatments on yield, grain weight, physical grain quality traits related to dry milling, and total fumonisin concentrations. Four commercial maize genotypes currently grown in Argentina differing in grain hardness were tested. We specifically focused on fumonisin concentrations and specific traits used for exporting hard endosperm maize from Argentina to the European Union. A treatment with silk channel inoculation with *F. verticillioides* was added for testing maize genotype susceptibility and fungicide performance under high infestation levels.

## MATERIALS AND METHODS

### Crop Management

Field experiments were conducted at the Campo Experimental Villarino, Facultad de Ciencias Agrarias, Universidad Nacional de Rosario at Zavalla, Santa Fe, Argentina (33°1′ S, 60°53′ W), during two growing seasons (2014–2015 and 2015–2016, Years 1 and 2, respectively). The soil was a silty clay loam Vertic Argiudoll, Roldán series. Planting dates were 18 Dec. 2014 and 19 Dec. 2015. Both experiments were arranged in a completely randomized design with four replicates. Four commercial genotypes and four fungicide treatments were evaluated in conditions of natural infection and after inoculation with *F. verticillioides*, totaling 32 treatment combinations randomly assigned in the field. We tested one commercial hard endosperm non-GMO genotype (ACA2002, developed by the Asociación de Cooperativas Argentinas), and three regular GMO softer endosperm genotypes from different seed companies (DK7210 VT3Pro, AX7822 HCLMG, and NK900 Vip3, developed by Monsanto, Nidera, and Syngenta, respectively). Genotypes represent common commercial genotypes used by farmers in the temperate central Argentinean region. The three regular dented genotypes were also known to differ in their grain hardness (Abdala et al., 2018). Hybrids differed in phenology, but differences were minor. Genotype maturity groups were 117, 122, 126, and 128 for AX7822, DK7210, NK900, and ACA2002, respectively, and average days from sowing to silking across both years were 57, 59, 59, and 57 d for AX7822, DK7210, NK900, and ACA2002, respectively. Fungicide applications had no effect on time to silking.

Plots were four rows 6 m long, with 0.52 m of inter-row spacing. A uniform stand density of 8 plants $m^{-2}$ was used across treatments. Plots were oversown, hand thinned at V3 (Ritchie and Hanway, 1982), and managed following common agronomic practices for the region for weeds and diseases. Weeds were also periodically removed by hand whenever necessary. Insect pressure of *Diatraea saccharalis* (Fabricius) and *Spodoptera frugiperda* (J.E. Smith) were specifically monitored and controlled with recommended products only during vegetative stages. No insecticide was applied after reproductive fungicide was sprayed over plots. Total rainfall was 383 and 691 mm for

Years 1 and 2, respectively. Average temperatures were also different (21.8 and 22.2°C for Years 1 and 2, respectively), making Year 1 a warmer and dryer growing season.

At sowing, monoammonium phosphate (10–50–0, N–P–K) was applied at a rate of 120 kg $ha^{-1}$ to all plots. The crops were all fertilized with 60 kg N $ha^{-1}$ as urea (46–0-0 N-P-K) between V5 and V6. The urea was broadcasted manually over the plots.

Four fungicide treatments were established: (i) a control, where no fungicide was applied; (ii) a vegetative fungicide application, with commercially available fungicide Opera (Basf Argentina) applied between V10 and V14 (Ritchie and Hanway, 1982); (iii) a reproductive fungicide application, with commercially available fungicide Duet Plus (Basf Argentina) applied 1 d after each plot reached 50% silking (R1 stage; Ritchie and Hanway, 1982); and (iv) a double vegetative and reproductive fungicide application, using Opera in V10 through V14 and Duet Plus 1 d after R1. Opera fungicide is a mixture of pyraclostrobin and epoxiconazole in a suspoemulsion formulation, whereas Duet Plus is a suspension concentrate containing metconazole and epoxiconazole. Fungicides were applied in each plot using a hand sprayer. Fungicide rates (0.75 and 1.5 L $ha^{-1}$ for fungicides Opera and Duet Plus, respectively) and water volume (65 L $ha^{-1}$) followed commercial recommendations. During flowering time, visual observations were made on each plot to determine when 50% of plants reached silking, as in Borrás et al. (2009).

Ears were inoculated with a 2-mL sample containing a conidial suspension of *F. verticillioides* ($1 \times 10^6$ conidia $mL^{-1}$). The inoculation treatments were done 2 d after each plot had 50% of plants with visible silks (R1). The isolate used for the mentioned treatments was P364, following Presello et al. (2007). This isolate was obtained from naturally infected maize growing in Pergamino (Presello et al., 2008). Isolate P364 is a high fumonisin producer belonging to *F. verticillioides* (Iglesias et al., 2010).

## Phenotypic Measurements

At maturity, the central two rows from each plot were manually harvested and used for determining grain yield, average individual grain weight, and all other phenotypic traits (described below). Yield is described on a 14.5% moisture basis. Individual grain weight was determined by weighing two sets of 100 grains per plot.

Fumonisin concentration was determined using an enzyme-linked immunosorbent assay (ELISA) kit test RIDASCREENFAST Fumonisin (R-Biopharm). It is a competitive enzyme immunoassay for the quantitative analysis of fumonisins in cereals and feed. The detection limit of the RIDASCREENFAST Fumonisin method is 0.22 μg $g^{-1}$.

Fumonisin concentration was immediately analyzed after harvest. Each grain sample coming from individual field plots weighed ~3 kg. After grain sample homogenization, subsamples of 250 g were separated, and grounded in a laboratory grinder (Tecnodalvo). Milled subsamples were thoroughly mixed, and 5 g was separated and mixed with 25 mL of 70% methanol. This mix was shaken for 3 min by hand. The mix was later centrifuged at 14,000*g* and 20°C for 5 min, and an aliquot of the supernatant was diluted to 1:14 with sterile distilled water. Diluted extracts and five standards, with different fumonisin concentrations (0.000, 0.222, 0.667, 2.000, and 6.000 μg $g^{-1}$) were subjected to ELISA for fumonisin determination.

Test weight was determined after grain sample homogenization (MAGyP, 2015) using a Schopper chondrometer (Cuenca). Results were expressed as kilograms per hectoliter.

Floaters percentage (%) was measured by introducing a 100-grain aliquot in a $NaNO_3$ solution (density = 1.25 g $cm^{-3}$) at 35°C and thoroughly shaking every 30 s for 5 min to eliminate any bubbles. At the end of this time period, floating grains were counted and reported as a percentage. The test was done two times per field replicate, following Gerde et al. (2016).

To determine vitreousness (%), 200 grains per plot were longitudinally dissected and visually inspected. The grains that were not indented in the crown, that had central starchy endosperm completely surrounded by horny endosperm, and horny endosperm representing 50% or more of the endosperm were considered vitreous grains and reported as a percentage of the total number of inspected grains. For a particular maize lot to fulfill the EU requirements for hard endosperm maize (flint maize), percentage grain vitreousness needs to be >95%. However, there is a 3% tolerance that sets the limit value at 92% (MAGyP, 2015).

The proportion of grains sized >8 mm was measured by using a Ro-Tap-like sieve shaker (Zonytest, Rey & Ronzoni). A 100-g grain aliquot was loaded on top of an 8-mm roundhole stackable standard sieve. The weight of the aliquots retained before and after the 8-mm sieve was determined after 2 min of shaking. This test was done twice per sample. The percentage of grain retained by the 8-mm sieve over the total sample was reported (Tamagno et al., 2016).

Grain starch, protein, and oil percentages were determined by near-infrared spectroscopy with an Infratec 1241 instrument (Foss), as in Borrás et al. (2002), using 400 g of grain per plot. Values were reported on a dry-weight basis.

## Statistical Analysis

Data were analyzed using linear mixed-effects models (nlme package; Pinheiro et al., 2016) in R (R Core Team, 2016; version 3.3.0). For each trait, the model considered genotype, fungicide, inoculation treatments, and all their interactions (genotype × fungicide, genotype × inoculation, fungicide × inoculation, and genotype × fungicide × inoculation) as fixed effects and year as random effect. Models were fitted using the restricted maximum likelihood method (Zuur et al., 2009).

We checked the Gaussian and homoscedasticity assumptions for the standardized residuals of the models with graphical analysis (Zuur et al., 2009). Depending on the trait, variance heterogeneity was found across genotypes, fungicides, or inoculation treatments. Heterogeneity was incorporated into the models using the varIdent variance structure (Zuur et al., 2009). Thus, each genotype, fungicide, or inoculation treatment was allowed to have a different variance. Models with and without variance structure were compared using the log-likelihood ratio test. Multiple comparisons between means were performed using the predictmeans function in R (Luo et al., 2014).

# RESULTS

## Crop Yields

Significant yield differences were observed among the four genotypes tested ($p < 0.001$, Table 1). AX7822 and DK7210 were the highest yielding genotypes, whereas

Table 1. Yield, grain weight, fumonisins, test weight, vitreousness, flotation index, 8-mm screen retention, oil, protein, and starch for four genotypes (AX7822, DK7210, NK900 and ACA2002), fungicide treatments (control, vegetative fungicide [Fung. veg.], reproductive fungicide [Fung. rep.], and vegetative plus reproductive fungicide [Fung. veg. & rep.]), and pathogen inoculation. Only main effects are described here. Significant interactions are described in Fig. 1. Data are the mean of 2 yr.

| Treatment | *n* | Yield | Grain weight | Test weight | Vitreousness | Flotation index | 8-mm screen retention | Oil | Protein | Starch | Fumonisins |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | kg ha$^{-1}$ | mg grain$^{-1}$ | kg hL$^{-1}$ | % | | | g 100 g$^{-1}$ | | | μg g$^{-1}$ |
| AX7822 | 64 | 13,434 | 308 | 77.5 | 1 | 39 | 49 | 4.5 | 8.2 | 75.9 | 9.9 |
| DK7210 | 64 | 13,293 | 295 | 79.3 | 1 | 23 | 49 | 4.6 | 8.3 | 75.9 | 3.0 |
| NK900 | 64 | 11,188 | 243 | 80.8 | 67 | 4 | 10 | 5.1 | 9.2 | 74.7 | 4.3 |
| ACA2002 | 32 | 9,035 | 231 | 78.8 | 87 | 11 | 25 | 5.0 | 9.5 | 72.6 | 2.9 |
| Control | 56 | 11,867 | 273 | 79.2 | 33 | 20 | 35 | 4.8 | 8.7 | 75.2 | 5.0 |
| Fung. veg. | 56 | 12,221 | 275 | 78.9 | 32 | 22 | 33 | 4.7 | 8.7 | 75.0 | 5.6 |
| Fung. rep. | 56 | 12,115 | 280 | 79.2 | 32 | 20 | 35 | 4.8 | 8.7 | 75.0 | 5.6 |
| Fung. veg. & rep. | 56 | 12,291 | 277 | 79.2 | 32 | 21 | 34 | 4.8 | 8.7 | 75.1 | 5.1 |
| Control | 112 | 12,179 | 278 | 79.2 | 32 | 19 | 34 | 4.8 | 8.7 | 75.0 | 4.5 |
| Inoculation | 112 | 12,068 | 274 | 79.1 | 32 | 23 | 34 | 4.8 | 8.7 | 75.1 | 6.2 |
| Genotype (G) | | *** (236)† | *** | *** (0.3) | *** (5) | *** | *** (1) | *** (0.1) | *** (0.3) | *** (0.3) | *** (2.5) |
| Fungicide (F) | | * (236) | *** | ns‡ | ns | ns | ns | ns | ns | ns | ns |
| Inoculation (I) | | ns | *** | ns | ns | ns | ns | ns | ns | ns | ns |
| G × F | | ns | * (8) | ns | ns | ns | ns | ns | ns | ns | ns |
| G × I | | ns | * (5) | ns | ns | * (1) | ns | ns | ns | ns | ns |
| F × I | | ns | ns | ns | ns | ns | ns | ns | ns | ns | ns |
| G × F × I | | ns | ns | ns | ns | ns | ns | ns | ns | ns | ns |

*, *** Significant at the 0.05 and 0.001 probability levels, respectively.

† Parenthetical values indicate LSD ($P < 0.05$).

‡ ns, not significant.

ACA2002 was the lowest one. Yield was significantly affected by fungicide applications ($p < 0.001$, Table 1), where all fungicide treatments, regardless of the type and moment of application, increased yield for all genotypes similarly (no fungicide × genotype interaction, $p > 0.05$). Fungicide applications increased yield by 248 to 424 kg ha$^{-1}$, and there were no differences among the application at V14 targeting the foliar system or at R1 focusing on the middle canopy and extruded silks.

*Fusarium* inoculations at R1 did not reduce yield in any genotype ($p > 0.05$, Table 1) and showed no interactions with fungicide treatments for yield.

Individual grain weight differed among genotypes, fungicides, and inoculation treatments ($p < 0.001$). Genotype differences in grain weight followed yield differences: AX7822 and DK7210 showing the highest grain weight and ACA2002 the lowest one (Table 1). Grain weight increased when fungicides were applied, irrespective of the application timing, and was lower under the inoculation treatment (Table 1). There were significant genotype × fungicide and genotype × inoculation interactions ($p < 0.05$; Table 1, Fig. 1). Genotypes DK7210 and ACA2002 increased their individual grain weight when fungicide was applied during both vegetative and reproductive or reproductive stages, whereas no differences were evident for AX7822 and NK900. Genotype DK7210 reduced its individual grain weight after inoculation, whereas no differences were evident for the rest of the genotypes (Fig. 1).

## Grain Hardness and Composition

Physical grain quality for dry milling was tested using four different traits: test weight, floaters, grain vitreousness, and screen retention. Grain composition was evaluated by measuring grain oil, protein, and starch concentration.

Test weight was only affected by genotype ($p < 0.001$, Table 1), where NK900 and AX7822 showed the highest and lowest values, respectively. No fungicide or inoculation significant effects were evident on test weight.

Grain vitreousness was only affected by genotype ($p < 0.001$, Table 1). ACA2002 had the highest values. No fungicide treatment effect was evident on grain vitreousness ($p <$

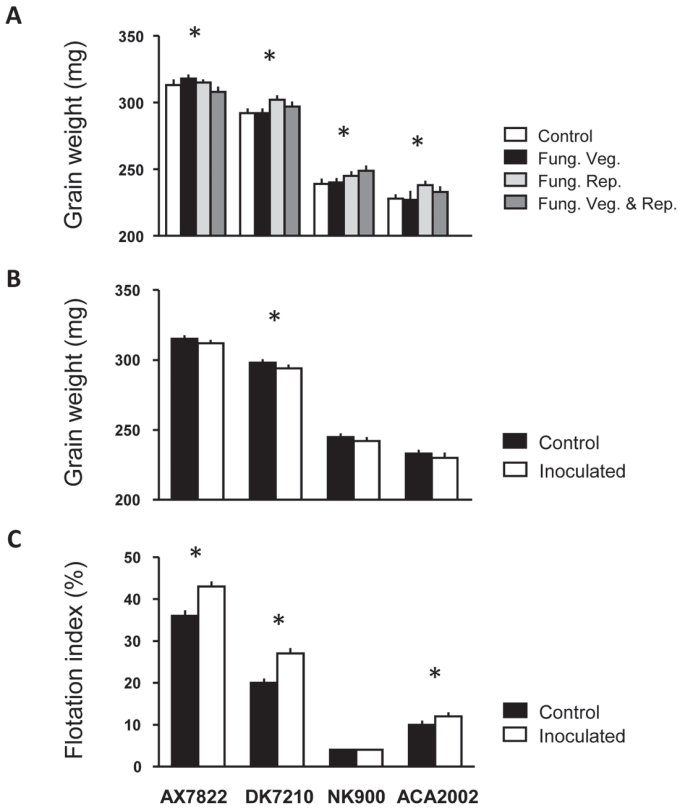


Fig. 1. Description of (A) fungicide treatments × genotype interaction differences for grain weight, (B) *Fusarium* inoculation × genotype interaction differences for grain weight, (C) *Fusarium* inoculation × genotype interaction differences for flotation index. Asterisk presence describes significant differences ($p < 0.05$) among fungicide treatments for (A), and significant differences ($p < 0.05$) between control and inoculation treatments for Fig. (B and C). Fung., fungicide; Veg., vegetative; Rep., reproductive.

0.05, Table 1), and *Fusarium* inoculations had no effect on grain vitreousness either ($p > 0.05$, Table 1).

Flotation index showed significant genotype differences ($p < 0.001$, Table 1), where the lowest values were evident for NK900 and ACA2002. Fungicide treatments had no effect ($p > 0.05$) on flotation index. However, flotation index showed a significant genotype × inoculation treatment interaction ($p < 0.05$, Table 1, Fig. 1). Genotypes AX7822 and DK7210 increased their floater percentages under the *Fusarium* inoculation treatment (Fig. 1).

Screen retention was only affected by genotype ($p < 0.001$, Table 1). No differences were evident in screen retention among fungicide applications or *Fusarium* inoculation treatments ($p > 0.05$, Table 1).

Genotypes differed in grain oil concentration ($p < 0.001$, Table 1). Oil concentration was higher in ACA2002 and NK900, and lower in AX7822 and DK7210. Oil concentration was not affected by fungicide or inoculation treatments (Table 1).

Grain protein concentration showed significant genotype differences ($p < 0.001$), where ACA2002 had higher values than AX7822 or DK7210 (Table 1). Fungicide applications and *Fusarium* inoculation treatments had no effects over grain protein concentration.

Starch concentration showed genotype differences ($p < 0.001$, Table 1). Neither fungicide applications nor *Fusarium* inoculation treatments had any significant effect over this trait.

In brief, test weight, grain vitreousness, flotation index, screen retention, oil, protein, and starch concentration were only affected by genotype ($p < 0.001$, Table 1). No fungicide treatment effect was evident on physical grain quality attributes, even though fungicides consistently affected crop yield. The *Fusarium* inoculation treatment showed no detectable effects on physical grain quality traits. Neither fungicides nor the *Fusarium* inoculation treatment modified final grain composition.

## Fumonisin Concentrations

Genotypes differed in grain fumonisin concentrations ($p < 0.001$, Table 1). The lowest values were observed in ACA2002 and DK7210 (non-GMO and GMO, respectively). Only these two genotypes reached acceptable levels of total fumonisin concentrations for human consumption (<4.0 $\mu g\ g^{-1}$) according to the EU approved levels (EFSA, 2014). Fungicides had no evident effects on total fumonisin concentrations ($p > 0.05$, Table 1), and total fumonisin concentrations were not different between the control and inoculated treatments at $p < 0.05$ (Table 1). *Fusarium* inoculation, however, increased fumonisin levels at $p < 0.10$, with no evident genotype or fungicide interactions.

In summary, fumonisin concentrations decreased with increasing grain hardness, especially with floater percentage (Fig. 2).

## DISCUSSION

Field treatments allowed testing yield, grain physical quality, and fumonisin concentration responses to fungicide applications. In our experiments, fungicide applications increased yield in all genotypes by ~350 kg $ha^{-1}$, but fungicide applications had no effect on any physical grain quality trait, despite the large differences in grain hardness traits among genotypes. Maize genotypes were sown at a late date with no major nutrient limitations (i.e., nitrogen, sulfur, or phosphorous), and fungicide applications evidently play a minor role when targeting maize grain hardness in our growing environments. Nitrogen fertilizer rates (Tamagno et al., 2016) or genotype selection (Abdala et al., 2018) are management practices playing a significantly more important role than fungicide applications for attaining high-quality maize in our region. We hypothesized that fungicides would not only increase yield but also grain hardness.

No differences were observed in the concentration of fumonisins as a result of fungicide treatments. Our results have shown that the two highest yielding genotypes

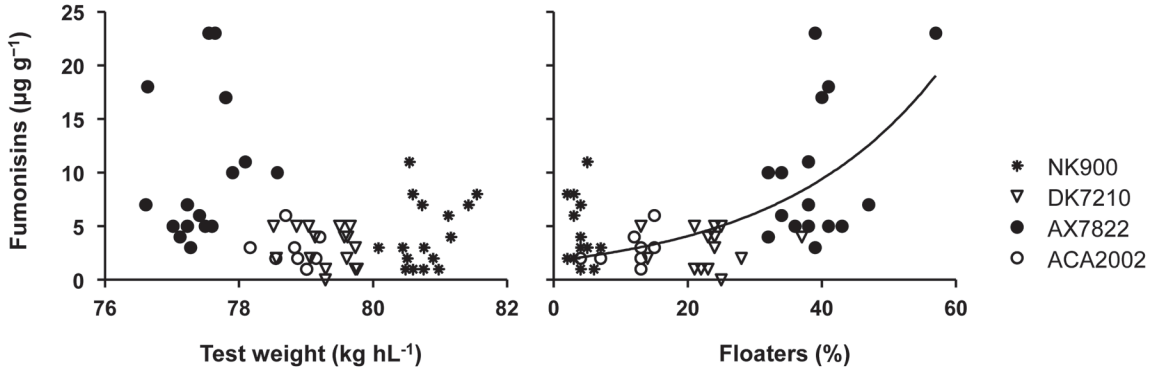


Fig. 2. Relationship between grain hardness attributes (test weight and floater percentage) and fumonisin concentrations in four genotypes (AX7822, DK7210, NK900, and ACA2002). A significant exponential increase curve was fitted between grain fumonisin concentrations and floater percentage ($Y = 1.78e^{0.042X}$, $r^2 = 0.36$, $p < 0.05$).

(AX7822 and DK7210, both Bt-genotypes) presented significant differences in total fumonisin concentrations between both genotypes, demonstrating the possible presence of fumonisins regardless of genotypes yield performance. These results reinforce the importance of genotype selection as a critical crop management option for minimizing fumonisin accumulation in maize grain, in general agreement with some earlier reports (Munkvold and Hellmich, 1999; Hammond et al., 2004; Bowers et al., 2014).

Munkvold et al. (1997) showed that *Fusarium* sp. infections can adversely affect yield and grain quality, and Presello et al. (2008) reported that inoculation treatments might affect yield only in susceptible genotypes. We found no significant yield losses due to *F. verticillioides* inoculation, but detrimental effects on grain physical quality were detected for some genotypes. Flotation index increased under inoculation, in agreement with increased fumonisin levels. There was a general positive correlation between fumonisin levels and flotation index (Fig. 2), suggesting that fumonisin levels are correlated with this quality trait. Huff (1980) reported grain density differences between contaminated with mycotoxins and noncontaminated grains. The concept of Shetty and Bhat (1999), sorting grains through a flotation device for discriminating grains with different fumonisin concentrations, seems reasonable based on our results.

Presello et al. (2007) showed that the hard grain endosperm genotype ACA2002 accumulated fewer mycotoxins than other softer endosperm commercial germplasm. Our results agree with this. We also showed that the genotype with the highest flotation index (AX7822) had the highest values of fumonisin concentrations (9.9 µg $g^{-1}$). However, it is evident that there are other genotypic traits not explored here that affect susceptibility to fumonisins. DK7210 showed comparable fumonisin levels with ACA2002, one being a Bt-genotype and one being a non-GMO genotype. Only these two genotypes presented fumonisin concentration levels lower than the ones considered safe for unprocessed maize (EFSA, 2014). Total fumonisin concentrations of NK900 were barely higher (4.3 µg $g^{-1}$) than the maximum allowable levels (4 µg $g^{-1}$).

Disease susceptibility is commonly used in breeding programs to discard susceptible hybrids (Hallauer et al., 1988; Munkvold and Desjardins, 1997), and many have reported associations between disease severity and mycotoxin concentrations (Munkvold and Hellmich, 1999; Presello et al., 2007; Bowers et al., 2014). With no disease severity data, it is difficult to judge the effectiveness of our fungicide and inoculation treatments. However, fungicide applications increased grain yield, indicating significant disease presence.

## CONCLUSIONS

Significant differences were observed for yield, physical grain quality traits, and fumonisin levels among the evaluated maize genotypes under late sowing. Fungicide applications increased yield but had no impact on grain hardness or composition. Fungicides had no effect on grain fumonisin concentrations either, making genotype selection a critical crop management option to affect initial field fumonisin contamination in later sowings. Several genotypes showed fumonisin concentration values higher than the maximum permitted for human consumption. In general, lower fumonisin concentrations were observed in harder endosperm genotypes.

### Conflict of Interest

The authors declare that there is no conflict of interest.


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