Christ D, Kreibich HH, Valmorbida R, Savi GD, Silva J, Soares C, Scussel VM (2016) Gaseous ozone fungi growth inhibition during controlled storage of naturally contaminated maize (*Zea mays*) Grains. Pp. 32–36. In: Navarro S, Jayas DS, Alagusundaram K, (Eds.) Proceedings of the 10th International Conference on Controlled Atmosphere and Fumigation in Stored Products (CAF2016), CAF Permanent Committee Secretariat, Winnipeg, Canada.

Gaseous ozone fungi growth inhibition during controlled storage of naturally contaminated maize (*Zea mays*) grains

D CHRIST[1], H H KREIBICH[2], R VALMORBIDA[1], G D SAVI[1], J SILVA[1], C SOARES[1], V M SCUSSEL[*]

Federal University of Santa Catarina, P.O. Box 476, Florianopolis, SC, Brazil

ABSTRACT

Antifungal properties of ozone (O_3) gas were evaluated using 2^2 factorial design in stored naturally contaminated dry (*Zea mays* L.) maize grains. Three concentrations (20, 40, 60 µmol/mol) and different exposure times (30,105 and180 min) were used for Groups I to III, respectively. The antifungal efficiency of O_3 gas was evaluated at day zero and after 30 days of storage, at the lower and upper layers of each experimental silo. The results showed that with the increase of O_3 concentration (from 20 to 60 µmol/mol), a decrease of 2.5×10 (16.2%) and 0.5×10 (3.2%) CFU (colony forming units)/g was noticed in the silo upper and lower layers, respectively. On the other hand, when the ozonation time was increased (from 30 to 180 min) on contaminant mycoflora (*Aspergillus* sp. and *Fusarium* sp.), there was decrease of 1.0×10 (6.5%) and 0.5×10 (3.2%) CFU/g in total fungi load in the upper and lower layers, respectively. The surface response exhibited the maximum of 94.5% of spore inhibition. After 30 days of storage, both treatments were effective. The O_3 treated, at day 30 had in the upper layer (1.3×10 CFU/g) and in lower layer (0.4×10 CFU/g), representing spore inactivation of 93.8% (max 97.7%) and 98.1% (max 100%, i.e., NG= no growth) in the upper layer and lower layer, respectively. In this study, spores could be efficiently destroyed by the O_3 gas, with 88 and 100% (NG) spore inhibition immediately after application and at day 30 of storage, respectively, under the conditions of 60 µmol/mol and 180 min.

Key words: Fungi, Maize, Naturally contaminated, Ozonation, Quality

Maize (*Zea mays* L.) grain production has a high economic importance in Brazil, the third largest producer with 70,000,000 tonnes during 2015-16 harvest. It is commercialized for different applications, ranging from its consumption in natura to high-technology food industry processes to obtain diverse maize product (for human consumption), as well as the main ingredient for animal feeds.

The purpose of the storage is the preservation of the characteristics and quality of grain over time to meet the market demands. In this scenario, the storage conditions are determinant for the final quality of the product, as the mass of stored maize consists of a living system with mutual influences of physical, chemical and biological internal and external sources (Faroni 1998). According to Travaglia (2011), if the storage conditions are not suitable for these grains, they will be susceptible to decay and exposed to fungal contamination, which is one of the most worrying factors today. Zummo and Scott (1992) suggest that maize is one of the most vulnerable cereals to the development of toxigenic fungi and therefore is a major product contaminated with mycotoxins. It is necessary to evaluate and control storage conditions to enable food safety maintenance during long-term storage (Kawashima and Soares, 2006).

Any detoxification strategies that aim to remove those contaminant (fungi and toxins), either when grains are still in the plant or during storages/processing, without compromising the food nutritional quality, are important and necessary (Leung et al., 2006), Ozone (O_3) gas has an oxidizing power and has been studied to reduce contamination with advantages over traditional methods.

The effective use of O_3 has been reported to control fungi growth, degrading mycotoxins and pesticide residues in a broad variety of raw and processed

[1]Laboratory of Mycotoxicology and Food Contaminants, Food Science and Technology Department, Center of Agricultural Sciences;

[2]Storage Laboratory and Prototype Drying Facilities, Western Parana State University, Cascavel, PR, Brazil

[*]Corresponding author e-mail: *vildescussel_2000@yahoo.co.uk*

foods, either at postharvest or in the industry (without reducing the nutritional value) (Mendez et al., 2003; Tiwari et al., 2010; Graham et al., 2011; Mcdonough et al., 2011; Alencar et al., 2011, 2012; Scussel et al., 2011; El-Desouky et al., 2012; White et al., 2011, 2013; Beber-Rodrigues et al., 2014; Savi and Scussel 2014; Savi et al., 2015; Kreibich et al., 2016).

Therefore, the antifungal properties of O_3 atmosphere at different concentrations and time of exposure, under controlled storage conditions, was investigated to determine its efficacy on maize grains with possible effects on quality.

MATERIALS AND METHODS

Maize grains (50 kg), naturally contaminated with fungi (15.5×10 CFU/g) from 2014-15 harvest (m.c. 11.5%) were used. The maize kernels (6.0 kg) were loaded into silos (Groups: I, II, III - O_3 treated/C - not treated - Fig. 1) for gas application.The 2^2 factorial design was applied to evaluate the effect of O_3 treatment at different concentrations (20, 40 and 60 µmol/mol) and exposure times (30, 105 and 180 min) as reported by Kreibich et al. (2016). The study was carried out at 23 ± 2°C temperature. The efficacy of O_3 in fungi inactivation was checked immediately (day zero) and after 30 days of storage. Equation 1 below show the model utilized.

The maize samples were analysed to measure the effect of O_3 on fungal population, i.e. *(a) total fungi load* - remaining. The number of CFUs was counted prior and after O_3 treatment and so after 30 days (Silva et al., 2010), and *(b)* humidity–m.c. was determined by the AOAC gravimetric method (AOAC 2005). The statistical analysis was carried out for the main effects and the variables interactions on responses. Thus to determine, which the significant factors ($P < 0.1$) were, and to adjust a model (Eq.1) to correlate variables and the significant coefficients, analysis of variance (ANOVA) was used.

$$\hat{y} = b_0 + b_i X_i + b_j X_j + b_j X_i X_j ... \quad [Eq.1]$$

where b_0, mean/intercept; b_i, b_j, b_{ij}, the model of regression coefficients; X_i (O_3 concentration) and X_j (exposure time): independent factors evaluated in coded values.

RESULTS AND DISCUSSION

The effect of O_3 on fungal spores of naturally contaminated maize grain (Groups I, II and III), was analysed at day zero and after 30 days of storage. The analysis included the effects at the bottom layer (zero to 100 mm) and at the top layer (400 to 500 mm), under the factorial design (2^2). Results shown in Table 1 and Fig 2 a,b and Fig 3 a,b were compared with the control group (maize without O_3 treatment).

Effect of O_3 treatment on fungi counts

Day zero, upper layer (400 – 500 mm): The effect of O_3 concentration and exposure time at day zero in the fungi spore inactivation (CFU/g) was -2.5 (concentration O_3), -1.0 (time) and -0.5 (concentration O_3 × time). Increasing the concentration of O_3 from 20 to 60 µmol/mol caused destruction of 2.5×10 CFU/g, i.e. from 15.5×10 to 4.25×10 CFU/g (low concentration) and 15.5×10 to 1.75×10 CFU/g (high concentration). With the increase in ozonation time (from 30 to 180 min), there was a reduction in fungi population that resulted from 15.5×10 to 3.5×10 CFU/g (shorter time) and 15.5×10 to 2.5×10 CFU/g (longer time). The mathematical model (Eq. 1) used to estimate the total count in CFU/g depending on the O_3 concentration (b_i=-1.25), the exposure time (b_j=-0.5) and mean (b_0=2.64) was found to be predictive (based on F-test).The correlation coefficient (r^2) of 0.89 and the surface response (Fig. 2a) represents the

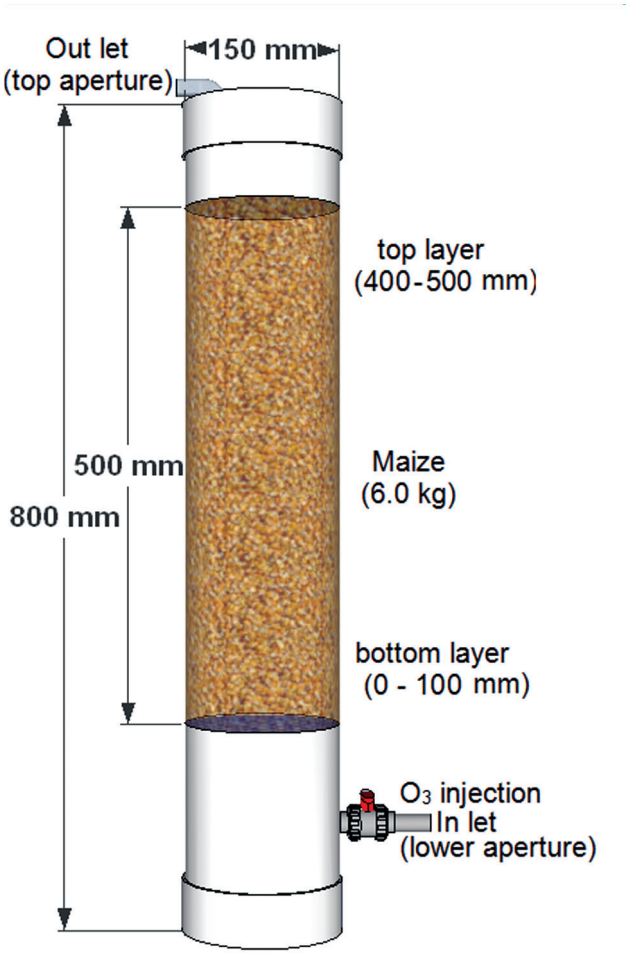


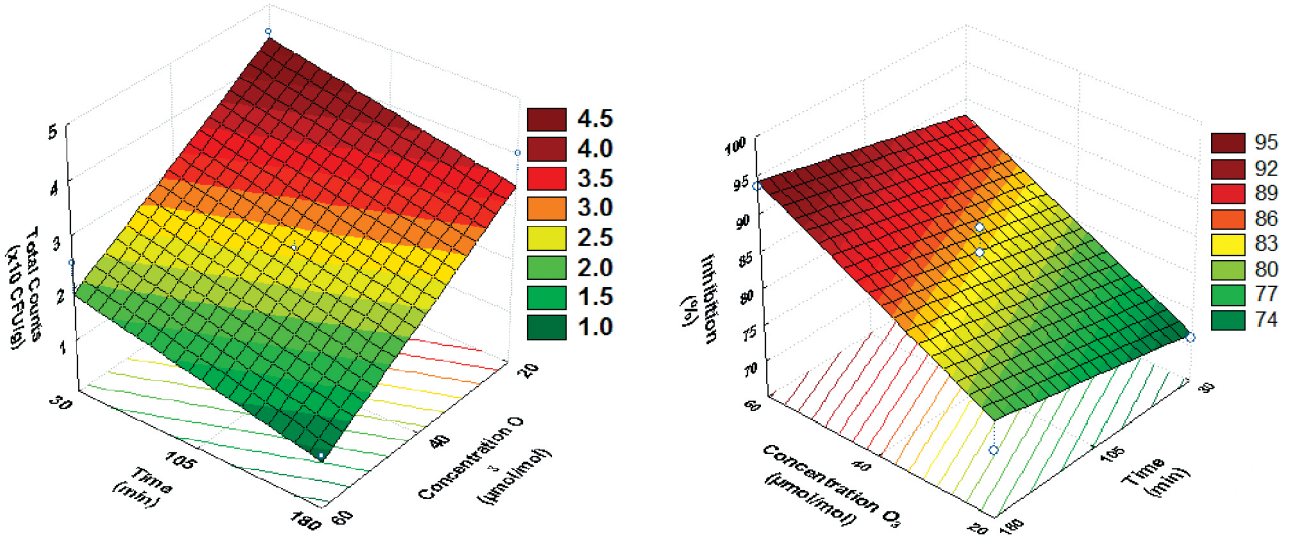
Fig. 1. Silo system for maize ozone treatment details and dimensions in mm

Table 1 Levels of spore decontamination by ozone in dry maize kernels at different gas concentration, ozonation time, layer distribution and days of storage (at 23±2°C)

O_3[a] treatment[b]				Total load						Humidity	
				Count[c] ('x'10 CFU/g)				Reduction[d] (%)			
Concetration (µmol/mol)		Time (min)	Layer (mm)	Control at day		O_3 treatment at day		Storage at Day		m.c.(%) at day	
				Zero	30	Zero	30	Zero	30	Zero	30
LOWER Layer	20[e]	30[e]				2.0	1.0	87.1	95. 5	11.6	11.4
	60[g]	30[g]				1.0	NG	93.6	100.0	11.8	11.2
	20[e]	180[e]				1.0	NG	93.6	100.0	11.5	11.3
	60[g]	180[g]	zero-10	15.5	22.0	1.0	NG	93.6	100.0	11.6	11.0
	40[f]	105[f]				2.0	0.5	87.1	97.7	11.3	10.9
	40[f]	105[f]				1.0	1.0	93.6	95.5	11.3	11.1
	40[f]	105[f]				1.5	0.5	90.3	97.7	11.2	11.0
UPPER Layer	20[e]	30[e]				4.5	1.5	71.0	93.2	11.5	11.3
	60[g]	30[g]				2.5	2.0	83.9	90.9	11.7	11.5
	20[e]	180[e]				4.0	1.0	74.2	95.5	11.5	11.2
	60[g]	180[g]	40-50	15.5	22.0	1.0	0.5	93.6	97.7	11.5	11.2
	40[f]	105[f]				2.0	0.5	87.1	97.7	11.5	11.2
	40 [f]	105[f]				2.0	1.0	87.1	95.5	11.6	11.3
	40 [f]	105[f]				2.5	3.0	83.9	86.3	11.4	11.2

model. The efficacy of O_3 concentration and exposure time on inactivating fungi spores (%) was 16.1% (O_3concentration), 6.5 (time) and 3.2 (concentration O_3 × time). Increasing from 20 to 60 µmol/mol, the gas concentration promoted an increase of 16.1% and with the increase ozonation time (from 30 to 180 min), there was an increase by 6.5%, i.e. 93.5% compared with untreated (Control group). The mathematical model (Eq.1) for estimating the efficacy depending on the concentration of O_3 (b_i=8.0) and the exposure time (b_j=3.2) and mean (b_0=83.0) using "F" test was found to be predictive, with a r^2 of 0.89. The surface response (Fig. 3b) represented the model.

Day Zero, lower layer (zero – 100 mm): The inactivation of fungi spores from the initial count (CFU/g) in the bottom layer (zero–100 mm) after the application O_3 was 1.35 × 10 CFU/g, which represents 91.2% reduction of viable spores regarding the initial



(a) (b)

Fig. 2. Surface response of O_3 concentration (20, 40 and 60 µmol/mol) and time of exposure (30, 105 and 180 min) on maize on (*a*) total load (*CFU*) inhibition (*b*) total reduction (%) – day zero, upper layer (40 – 50 cm) at 23 ± 2°C

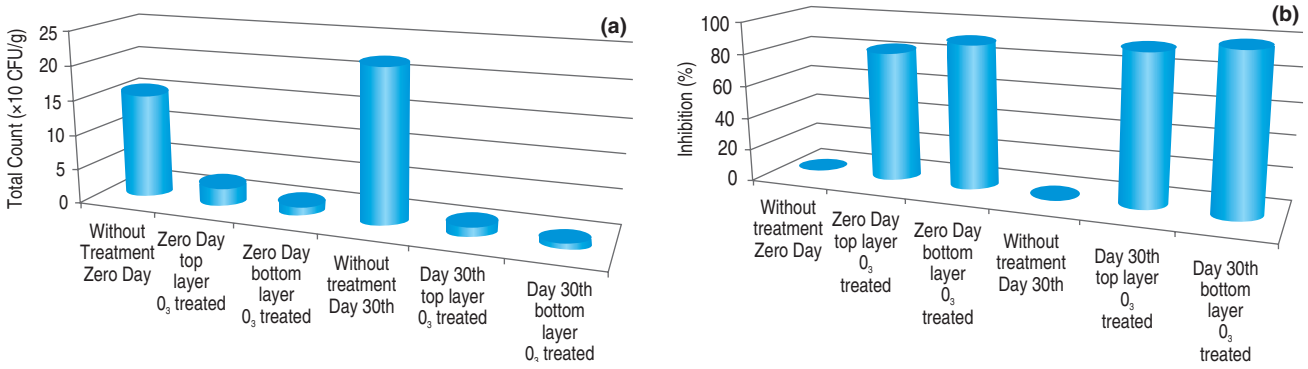


Fig. 3. Latent effect (day 30) of O_3 treatment (20, 40 and 60 µmol/mol) and time of exposure (30, 105 and 180 min) on maize on (*a*) total count (CFU/g), (*b*) inhibition efficiency (%) of fungal sporesat 23 ± 2°C

count. The concentration of O_3 of 60 µmol/mol and time of 180 min (high concentration and longer time) caused fungi spores destruction of 14.5×10 CFU/g from 15.5×10, i.e. 93.5% of reduction in total counts of fungi spores.

Day 30, lower and upper layers – latent effect: The latent effect of O_3 application in maize is shown by the total fungi count after 30 days of storage (Fig 3). Both treatments were effective compared to the control group, which at day 30 had 22×10 CFU/g. On the other hand, in the treated maize, it was 1.3×10 CFU/g in the upper layer and 0.4×10 CFU/g in the lower layer which represents 93.8% (max 97.7%) and 98.1% (max 100%) of spores inactivation in the top and bottom layers, respectively. Studies have demonstrated the economic viability of O_3 application to fumigate stored grain as a viable alternative for both environmental and economic perspectives (Tiwari et al., 2010). Reductions of as high as 10^3 CFU/g of microorganisms associated with stored grains were achieved with the O_3 treatment, as well as significant reductions in the levels of mycotoxin (Christ et al., 2016). Moreover, investigations of grain treated with O_3 indicate that it has no impact on the intrinsic quality of the grain and the nuts (Mendez et al., 2003; Scussel et al., 2011; Giordano et al., 2012; Savi et al., 2014a, b; 2015). Savi et al. (2014a) showed that O_3 gas did not change the physico-chemical characteristics at effective concentrations and exposure times applied against contaminants (fungi and mycotoxins) in wheat grains. Kreibich et al. (2016) showed around 90% spore inhibition of *A. flavus* immediately after the maximum gas concentration and time of exposure (60 µmol/mol and 180 min) reached cocoa beans, followed by total inhibition (NG: no growth) as the time of storage increased.

Effect of O_3 treatment on grain moisture content

Grain moisture content after treatment with O_3 reached a mean of 11.5% and there were no significant differences in moisture content between the treatments (P<0.1). After 30 days of storage, maize had a slight decrease in moisture content to a mean of 11.2%, but was similar for all treatments (P<0.1). This slight reduction was probably due to the temperature and the relative humidity during that period, which established new moisture content equilibrium (external environment × inner silos stored grains). The fact that there was no statistical difference among the moisture content at different O_3 treatments corroborates that moisture content did not influence the treatments too.

CONCLUSION

Fungal spores were effectively destroyed by O_3 gas under the conditions of 60 µmol/mol and 180 min exposure time (93.5% of spores did not germinate). The effect of $O_3$with the increase in concentration was more pronounced than the increase in exposure time for the destruction of fungi spores on maize. There was a latent effect of O_3 on fungi spores, which resulted in 100% inhibition (NG) after 30 days exposure.

REFERENCES

Alencar E R, Faroni L R D, Soares N F F, Carvalho M C S, Pereira K F (2011) Effect of the ozonization press on the quality of peanuts and crude oil. Ver Bras Eng Agríc Ambiental **15**: 154–160.

Alencar E R, Faroni L R D, Ferreira S N F, Da silva W A, Carvalho M C (2012) Efficacy of ozone as a fungicidal and detoxifying agent of aflatoxins in peanuts. Journal of Science Food and Agriculture **4**: 899–905.

AOAC - Association Official Method of Analysis of AOAC Internacional (2005) (In) Thiex NJW (ed.) Official Methods of Analysis. 18ed Maryland.

Beber-Rodrigues M, Savi G D, Scussel V M (2014) Ozone effect on fungi proliferation and genera susceptibility of treated stored dry paddy rice (*Oryza sativa* L.). Journal of Food Safety. doi: 10.1111/jfs.12144.

Christ D, Savi G D, Scussel V M (2016) Effectiveness of ozone gas in raw and processed food for fungi and mycotoxin decontamination— A Review. Journal of Chemistry Biology and Physical Science **6**: 326–348.

El-Desouky T A, Sharoba A L, El-Mansy H A, Naguib K (2012) Effect of ozone gas on degradation of aflatoxin B1 and *A. flavus* fungal. Journal of Environment and Toxicology **2**: 128–133.

Giordano B N E, Nones J, Scussel V M (2012) Susceptibility of the in-shell Brazil nut mycoflora and aflatoxin contamination to ozone gas treatment during storage. Journal of Agriculture Science **8**: 1–10.

Faroni L R D (1998) Fatores que influenciam a qualidade dos grãos armazenados. Revista Iberoamericana de Tecnologia Postcosecha **5**: 34–41.

Graham T, Zhang P, Woyzbun E, Dixon M (2011) Response of hydroponic tomato to daily applications of aqueous ozone via drip irrigation. Science Horticulture **129**: 464–471.

Kawashima L M, Soares L M S (2006) Incidência de fumonisina B_1, aflatoxinas B_1, B_2, G_1 e G_2, ocratoxina A e zearalenona em produtos de milho. Ciência e Tecnologia de Alimentos, Campinas **26**: 516–521.

Kreibich H H, Christ D, Maria G S, Silva J R, Savi G D, Scussel V M (2016) Decontamination of cocoa beans (*Theobroma cacao* L.) inoculated with *Aspergillus flavus* by ozone gas. Journal of Chemistry Biology and Physical Science **6**: 560–70.

Leung M C K, Diaz-Llano G, Smith T K (2006) Mycotoxins in pet food: a review on worldwide prevalence and preventative strategies. Journal of Agriculture Food and Chemistry **54**: 9.623–9.635.

Mcdonough M X, Campabadal C A, Mason L J, Maier D E, Denvir A, Woloshuk C (2011) Ozone application in a modified screw conveyor to treat grain for insect pests, fungal contaminants, and mycotoxins. Journal of Stored Products Research **3**: 249–254.

Mendez F, Maier D E, Mason L J, Woloshuk C P (2003) Penetration of ozone into columns of stored grains and effects on chemical composition and processing performance. Journal of Stored Products Research **1**: 33–44.

Savi G D, Scussel V M (2014) Effects of ozone gas exposure on toxigenic fungi species from *Fusarium*, *Aspergillus*, and *Penicillium* genera. Ozone-Sci Eng **2**: 144–152.

Savi G D, Piacentini K C, Bittencourt K O, Scussel V M (2014a) Ozone treatment efficiency on *Fusarium graminearum* and deoxynivalenol degradation and its effects on whole wheat grains (*Triticum aestivum* L.) quality and germination. Journal of Stored Products Research **59**: 245–253.

Savi G D, Piacentini K C, Scussel V M (2014b) Ozone treatment efficiency in *Aspergillus* and *Penicillium* growth inhibition and mycotoxin degradation of stored wheat grains (*Triticum aestivum* L.). Journal of Food Processing and Preserventions **3**: 1–9.

Savi G D, Piacentini K C, Scussel V M (2015) Reduction in residues of deltamethrin and fenitrothion on stored wheat grains by ozone gas. Journal of Stored Products Research **61**: 65–69.

Scussel V M, Giordano B N, Simao V, Manfio D, Galvão S, Rodrigues M N F (2011) Effect of oxygen-reducing atmospheres on the safety of packaged shelled brazil nuts during storage. International Journal of Analytical Chemistry. doi:org/10.1155/2011/813591.

Silva N, Junqueira V C A, Silveira N F A, Taniwaki M H, Santos R F S, Gomes R A R (2010) Manual de métodos de análise microbiológica de alimentos e água. 4th ed. São Paulo: Varela.

Tiwari B K, Brennan C S, Curran T, Gallagher E, Cullen P J, O'Donnel C P (2010) Application of ozone in grain processing. Journal of Cereal Science **3**: 248–255.

Travaglia D P (2011) Crescimento de *Aspergillus flavus* e produção de aflatoxina em grãos de milho armazenados sob diferentes temperaturas. Dissertation,Universidade Federal de Viçosa.

White S D, Murphy P T, Bern C J, Leeuwen J Van (2010) Controlling deterioration of high-moisture maize with ozone treatment. Journal of Stored Products Research **46**: 7–12.

White S D, Murphy P T, Leandro L F, Bern C J, Beattie S E, Van Leeuwen J (2013) Mycoflora of high-moisture maize treated with ozone. Journal of Stored Products Research **55**: 84–89.

Zummo N, Scott G E (1992) Interaction of *Fusarium verticilioides* and *Aspergillus flavus* on kernel infection and aflatoxin contamination in maize ears. Plant Disease **76**: 771–773.