International Journal of Food Microbiology 136 (2010) 304–309

Contents lists available at ScienceDirect

International Journal of Food Microbiology

journal homepage: www.elsevier.com/locate/ijfoodmicro

Activity of natural compounds on *Fusarium verticillioides* and fumonisin production in stored maize kernels

A.M. Menniti *, R. Gregori, F. Neri

Department of Protection and Improvement of Agricultural Food Products, Alma Mater Studiorum, University of Bologna, Viale Fanin 46, Bologna 40127, Italy

ARTICLE INFO

Article history:
Received 17 April 2009
Received in revised form 18 September 2009
Accepted 9 October 2009

Keywords:
Fusarium
Trans-2-hexenal
Carvacrol
Eugenol
Fumonisins
Maize

ABSTRACT

The ability of *trans*-2-hexenal, carvacrol and eugenol to control *F. verticillioides* was explored *in vitro* and in artificially infected kernels. The effect of the *trans*-2-hexenal fumigation on *F. verticillioides* control, fumonisin production and kernel germination was also investigated in naturally infected kernels. *Trans*-2-hexenal, carvacrol and eugenol vapour showed fungicidal activity against *F. verticillioides*, in *in vitro* trials. *Trans*-2-hexenal was the best pathogen inhibitor, followed by carvacrol and eugenol. In maize kernels, fumigations with *trans*-2-hexenal provided a high inhibitory effect on *F. verticillioides* growth and its efficacy depended on concentration and time of incubation. The most effective dose of *trans*-2-hexenal was 369 μL/L, but this concentration induced off-odour in maize. The exposure to 246 μL/L *trans*-2-hexenal provided the best control of *F. verticillioides* and no phytotoxic symptoms or off-odour in kernels was observed. In contrast *trans*-2-hexenal fumigations were ineffective in the reduction of fumonisin concentration and high concentration (369 μL/L) stimulated fumonisin levels. Reduction or delay in the germinability of the kernel was observed after *trans*-2-hexenal exposure. The results showed that *trans*-2-hexenal postharvest fumigation is effective in *F. verticillioides* control also in asymptomatic maize kernels, but cannot reduce fumonisin production.

1. Introduction

Fusarium verticillioides (Sacc.) Nirenberg (synonym: *F. moniliforme* J. Sheld.; teleomorph: *Gibberella moniliformis* Wineland) is one of the most common fungal pathogens in maize (*Zea mays* L.) worldwide. Under favourable conditions, the pathogen causes root, stalk, ear, kernel and seedling rot, resulting in serious production losses in maize. In addition, *F. verticillioides* is considered to be an important producing species of the toxin fumonisin. The pathogen generally produces a whitish-coloured mould growth that tends to be scattered on the ear; however *F. verticillioides* is recognized also as an endophyte of maize. Many reports indicate that not all infected kernels are symptomatic and fumonisins have also been detected in symptomless infected kernels (Bacon et al., 1992; Munkvold et al., 1997; Desjardins et al., 1998). The main forms of fumonisins present in naturally contaminated maize are fumonisin B_1 (FB_1), B_2 (FB_2) and B_3 (FB_3) (Ross et al., 1990; Scott 1993). Since their discovery in 1988 (Gelderblom et al., 1988), FB occurrence has been reported in maize, maize-based foods and feeds in many areas of the world (Visconti and Doko, 1994; Shephard et al., 1996; Kedera et al., 1999; Hennigen et al., 2000; Shephard et al., 2000; Logrieco et al., 2002; Lombaert et al., 2003; Cirillo et al., 2003; Silva et al., 2007). Contamination of maize by toxigenic fungi and mycotoxins can occur in the field during harvest and storage and at any time until consumption. Environmental conditions play an important role in the growth of *F. verticillioides* and in fumonisin accumulation before and after harvest (Miller, 2001; Ono et al., 2002).

The implications of fumonisin toxicity in foods and feeds are serious (Shephard, 2008; Miller, 2008). FB_1, the most abundant of the fumonisin analogues, has been classified by the International Agency for Research on Cancer (IARC) in Group 2B as a possible carcinogen to humans (IARC, 2002). It has been associated with human oesophageal cancer (Rheeder et al., 1992; Chu and Li, 1994; Yoshizawa et al., 1994; Marasas, 2001). FB_1 can cause leukoencephalomalacia in horses, pulmonary oedema and hepatic syndrome in swine, and liver damage and cancer in laboratory animals (Gelderblom et al., 1988; Ross et al., 1990; Bacon et al., 1992; Caramelli et al., 1993). The United States Food and Drug Administration (FDA) has established recommended guidelines for fumonisin content in human and animal food. The recommended maximum level of fumonisins in human foods is 2–4 mg/kg according to the particular corn-based product, while in animal feeds it is from 5–100 mg/kg depending on the animals that the feed is intended for (FDA, 2001). The European Union also recently regulated fumonisins ($B_1 + B_2$): the maximum levels are 1 mg/kg for maize-based foods and 4 mg/kg for unprocessed maize (European Commission, 2007). FB occurrence in foods and feeds has wide economic implications (Miller, 2008), and the development of management strategies for controlling *Fusarium* infection and fumonisin contamination is needed in order to limit

* Corresponding author. Tel.: +39 051 766563.
E-mail address: annamaria.menniti@unibo.it (A.M. Menniti).

0168-1605/$ – see front matter © 2009 Elsevier B.V. All rights reserved.
doi:10.1016/j.ijfoodmicro.2009.10.008

their harmful effects on human and animal health (Munkvold and Desjardins, 1997). Biocontrol agents and a combination of strategies using biocompetitive fungi and enhancement of host-plant resistance may adequately prevent mycotoxin contamination in the field (Pereira et al., 2007; Cleveland et al., 2003). The other methods to reduce the risk of mycotoxin contamination are models for predicting *Fusarium* kernel rot and fumonisin content, early harvest, and processing to remove infected kernels and prevent continued fungal development after harvest (Stewart et al., 2002; Bush et al., 2004).

In recent years there has been increasing interest in the scientific community for natural plant extracts as alternatives to chemicals to control several pathogens in food (Fu et al., 2007; Rota et al., 2008). Studies on cinnamon, clove, lemon grass, palmarosa and oregano essential oil activity to control the growth of *F. verticillioides*, *F. proliferatum* and *F. graminearum* have recently been published (Juglal et al., 2002; Velluti et al., 2003; Velluti et al., 2004).

Less studied is the activity against *F. verticillioides* of the single constituents of the essential oils or other aroma compounds of plants. In our previous studies, *trans*-2-hexenal, an aroma constituent of many fruits and vegetables, carvacrol, the main constituent of oregano and thyme essential oils and eugenol, a constituent of cinnamon and clove essential oils, proved to control *Penicillium expansum* and *Monilinia laxa* in *in vitro* and *in vivo* experiments (Neri et al., 2006a,b, 2007).

The objective of our study was to evaluate: *i*) the antifungal activity of *trans*-2-hexenal, carvacrol and eugenol vapour against *F. verticillioides in vitro* and in artificially infected kernels; *ii*) the effect of *trans*-2-hexenal fumigation on *F. verticillioides* control and kernel germination in naturally infected kernels; iii) the effect of *trans*-2-hexenal on fumonisin production in artificially and naturally infected kernels. This is the first time that *trans*-2-hexenal was studied for controlling *Fusarium* kernel rot and fumonisin production.

2. Materials and methods

2.1. Maize kernel

Ears of Maize hybrid 4XLO1016 were harvested in the second week of September in an experimental field (Bologna, Italy). Four hours after harvesting, the material was dried with forced air at 40 °C for 48 h to reach approximately 14% (0.70 a_w) kernel moisture, condition generally recommended during storage period. No visible disease was observed on maize ears. Kernels were shelled from 400 ears and a 20 kg sample was taken randomly, divided in 40 polypropylene vacuum sealed bags of 500 g each and stored for a week at room temperature until testing. Before each trial, the lots of kernel were surface disinfected for 2 min in a 2% sodium hypochlorite solution at 20 °C and subsequently rinsed twice (2 × 2 min) in sterile water to eliminate any hypochlorite residues. The kernels were then left to dry for 1 h over a sterile filter paper in a sterile cabinet before treatments.

2.2. Pathogen and inoculation

The pathogen had been isolated from maize meal, identified as *F. verticillioides* by PCR and kept in our collection (Iotti and Zambonelli, 2006). The culture was grown on potato dextrose agar (PDA, Oxoid) with the addition of a 10% lactic acid solution (pH 2.8) for 9 days at 25 °C and stored at 4 °C in the dark. The lactic acid solution was added to avoid the growth of yeast and bacteria. Maize kernels were inoculated by dipping for 1 min in a conidial suspension (10^4 conidia/ml) of *F. verticillioides*. The conidial suspension was prepared by washing a 9 day old, colony grown on acidified PDA with sterile distilled water containing 0.05% (v/v) Tween 80. The conidial concentration was adjusted by dilution after observation with a haemocytometer.

2.3. Compounds

The aldehyde (*trans*-2-hexenal) and the phenols (carvacrol and eugenol) were purchased from Sigma-Aldrich (Milan, Italy). The reported concentrations are expressed as liquid volume of compound on filter paper per dish volume.

2.4. In in vitro trials

The effect of *trans*-2-hexenal, carvacrol and eugenol on conidia germination and mycelial growth of *F. verticillioides* was tested. For conidia germination, an aliquot of 100 μL of *F. verticillioides* conidia suspension (10^4 conidia/ml) was spread onto dishes containing 20 ml of acidified PDA. For mycelial growth, a plug (6 mm diameter) taken from the margin of a 9 day old colony grown on acidified PDA at 25 °C was placed at the centre of each plate.

Liquid volumes of compounds were added by means of a micro-syringe to 90 mm paper filter (ALBET® 400) placed inside the dish cover. Concentrations of compounds varied from 6.2 to 147.6 μL/L and from 3.1 to 49.2 μL/L for conidia germination and mycelial growth respectively. The dishes were quickly wrapped in Parafilm and incubated at 25 °C in the dark, for 3 and 9 days for conidia germination and mycelia growth respectively (Neri et al., 2007). The number of germinated conidia and colony diameter were recorded after incubation. Germinating conidia were counted by observing the conidia directly in Petri dishes. Data were expressed as percentages of conidia germination or mycelial growth compared to the control. The lowest concentration of the compounds at which no conidia germination or mycelial growth was observed was considered as the minimum inhibitory concentration (MIC). After removing the paper filter, the plates were incubated for a further 5 days at 25 °C to evaluate whether the activity of the compounds was fungistatic or fungicidal. Plates inoculated with the pathogen and treated with distilled water were used as a control. Eight replications were used for each concentration and compound. All experiments were performed twice.

2.5. Trials with artificially inoculated kernels

To evaluate the activity of *trans*-2-hexenal, carvacrol and eugenol, in *in vivo*, kernels were surface disinfected and inoculated as described above were divided into samples of 25 g and placed in sterile Petri dishes. Aliquots of 24.6, 43.1 and 147.6 μL/L for *trans*-2-hexenal, carvacrol and eugenol, respectively, were added to 90 mm paper filters placed inside the cover of the dish. For each trial and treatment, 10 Petri dishes were used. The dishes were quickly closed, sealed with Parafilm and incubated at 25 °C in the dark. After 24 h of treatment, the paper filter was removed and the dishes were incubated for 7 days. To determine the incidence of kernel infection when no symptoms of infection were observed, 50 kernels for each treatment were transferred on acidified PDA in 10 Petri dishes (5 kernels per plate) at 25 °C for a further 4, 7 and 12 days of incubation to promote the pathogen growth. After incubation one colony per kernel was transferred to PDA for identification based on morphology according to the classification system of Nelson et al. (1983). The control was represented by untreated kernels.

The subsequent trials were performed with the most effective compound, *trans*-2-hexenal. After surface sterilization and inoculation, a 500 g sample of kernels was placed in 20 sterile Petri dishes (approximately 25 g per plate) and treated with concentrations of *trans*-2-hexenal ranging from 92.3 to 369 μL/L. Treatments were performed using the same method described above. At the end of the incubation period, the remaining kernels (400 g for each treatment) were frozen at −20 °C for FB analysis. In all trials, the incidence of infected kernels was recorded after 11, 14 and 19 days of incubation. All experiments were performed twice.

2.6. Trials with naturally infected kernels

Another set of experiment was performed on asymptomatic naturally infected kernels. The presence of internal infection in kernels was previously determined by preliminary trials. For each treatment, a 500 g sample of kernels was surface disinfected and treated with *trans*-2-hexenal at 92.3, 184.5 and 369 μL/L. To determine the most effective concentration for controlling the pathogen without producing off-odour in maize, further concentrations of 123 and 246 μL/L *trans*-2-hexenal were tested using the method described above. After 11, 14 and 19 days of incubation the percentage of infected kernels was recorded. In addition the percentage of kernel germination was determined by counting the kernels sprouted.

2.7. Semi-commercial trials

Naturally infected kernels were fumigated inside plastic jars with a capacity of 10 L. The jar was connected by silicon pipes to an air pump with a continuous flow of 11.5 L/min and to a flask in closed circuit. A 500 g sample of naturally internal infected kernels was surface disinfected and placed in mesh bags suspended by a hook fixed on the jar cap. An aliquot of 246 μL/L of *trans*-2-hexenal was injected inside the flask and the kernels were treated by its vapour for 24 h at 25 °C. After treatment, the kernels were removed from the jar and incubated for 7 days at 25 °C. Fifty kernels were then transferred on acidified PDA in 10 Petri dishes (5 kernels for plate) at 25 °C. After the incubation period the remaining kernels (400 g for each treatment) were frozen at −20 °C for FB analysis. The incidence of infected kernels was recorded after 11, 14 and 19 days of incubation. All experiments were performed twice.

2.8. Fumonisin analysis

Fumonisins were determined on a 200 g subsample of kernels from each treatment and repetition. The protocol used for analysis was based on AOAC official method (2002) with some modifications. FB_1 and FB_2 were extracted by adding 100 mL of acidified water (0.4% acetic acid), 50 mL of methanol and 50 mL of acetonitrile to a 10 g of finely ground sample. The sample was mixed and shaken in a mechanical shaker for 60 min and then ultra-sonicated for 5 min. A portion of extract was filtered and analysed by reversed-phase liquid chromatography with mass spectometric detection LC-MS/MS (Triple Stage Quadrupole). A Waters (Acquity UPLC) LC system was used. The LC column was an ACQUITY UPLC BEH C_{18} (1.7 μm, 2.1 μm × 50 mm). The mobile phase A was water acetronitrile solution (95:5) added to 0.1% of formic acid; the mobile phase B was acetonitrile added to 0.1% of formic acid. The MS/MS system was an API 4000™. Mass spectra were obtained by scanning from *m/z* 300 to 800. For FB_1 the positive ion *m/z* was 722.3; for FB_2 the positive ion *m/z* was 706.3. The detection limit of the methods was 50 μg/kg for both FB_1 and FB_2.

2.9. Statistical analysis

For each compound, the effective dose (ED) for 50 and 95% inhibition (ED_{50} and ED_{95}, respectively) was calculated using probit-analysis applied to the percentages of conidial germination and mycelial growth obtained from in *in vitro* experiments. Regression lines between the logarithm of the compound concentrations and the effectiveness indices transformed in probit were calculated. Infected kernel percentages were arcsin-transformed (Snedecor and Cochran, 1980) before analysis of variance and the data are presented as untransformed. Statistical analysis was performed using the STATISTICA 5.1 software package. The data were processed with the variance analysis (ANOVA) according to LSD test at $P<0.05$.

3. Results

3.1. In in vitro trials

Trans-2-hexenal, carvacrol and eugenol inhibited *F. verticillioides* conidial germination and mycelial growth with fungicidal activity (Table 1). *Trans*-2-hexenal was the best pathogen inhibitor for conidia germination (ED_{50} 7.5 μL/L; ED_{95} 12.7 μL/L; MIC 24.6 μL/L). Carvacrol showed the best ED_{50} and ED_{95} (7.5 and 15.5 μL/L, respectively) against mycelia growth, but the same MIC of *trans*-2-hexenal (MIC 24.6 μL/L). Higher concentrations were required to inhibit conidial germination and mycelial growth for eugenol (MIC 147.6 and 49.2 μL/L, respectively).

3.2. Trials with artificially inoculated kernels

In preliminary trials performed on artificially infected kernels, treatments with *trans*-2-hexenal, carvacrol and eugenol, at MIC_s on conidia germination (24.6, 43.1 and 147.6 μL/L, respectively), failed to control the pathogen. No phytotoxic symptoms were observed in kernels after treatments with volatile compounds, while the exposure to carvacrol and eugenol induced off-odours, described by subjective evaluation as characteristic odour of oregano and clove, respectively. As the absence of negative effects on kernels, only *trans*-2-hexenal was tested at higher concentrations in subsequent experiments.

The results reported in Fig. 1 clearly show that treatments at 92.3, 184.5 and 369 μL/L *trans*-2-hexenal were effective in controlling *F. verticillioides* growth and that the efficacy varied with concentration and time of incubation. The efficacy of *trans*-2-hexenal at 369 μL/L (100, 97 and 97% of efficacy, following 11, 14 and 19 days of incubation, respectively) was higher than at 92.3 μL/L (83, 40 and 37% of efficacy, following 11, 14 and 19 days of incubation, respectively). However, this high concentration induced an off-odour in maize, like “green” aroma. No visible injury was observed.

3.3. Trials with naturally infected kernels

The incidence of kernels naturally infected by *F. verticillioides* was very high (86.7, 93.3 and 96.7% following 11, 14 and 19 days of incubation) in the control. Exposure to 184.5 and 369 μL/L *trans*-2-hexenal achieved a good control of *F. verticillioides* (Fig. 2a). Only kernels treated with 369 μL/L had an anomalous odour, like “green” aroma. Concentrations of 123 and 246 μL/L also strongly reduced the percentage of infected kernels (Fig. 2b). In addition, the application of 246 μL/L showed the best control of the pathogen also after 19 days of incubation (efficacy of 86.7%). No phytotoxic symptoms were observed.

Trans-2-hexenal caused a reduction or delay in the germinability of maize kernels. In particular, exposure to 369 μL/L *trans*-2-hexenal caused reductions of 63.3, 26.7 and 23.3% in kernel germination following 11, 14 and 19 days of incubation. At lower concentrations (123, 184.5 and 246 μL/L), germination was only delayed and at 92.3 μL/L concentration, no germination reduction was noted.

Table 1
In vitro activity of *trans*-2-hexenal, carvacrol and eugenol on conidia germination and mycelial growth of *F. verticillioides* following 3 and 9 days of incubation at 25 °C, respectively.

Compounds	Conidia germination			Mycelial growth		
	ED_{50}	ED_{95}	MIC	ED_{50}	ED_{95}	MIC
Trans-2-hexenal	7.5	12.7	24.6	13.4	19.6	24.6
Carvacrol	15.0	26.3	43.1	7.5	15.5	24.6
Eugenol	37.0	102.2	147.6	21.2	35.2	49.2

ED_{50} ED_{95} MIC values μL/L.

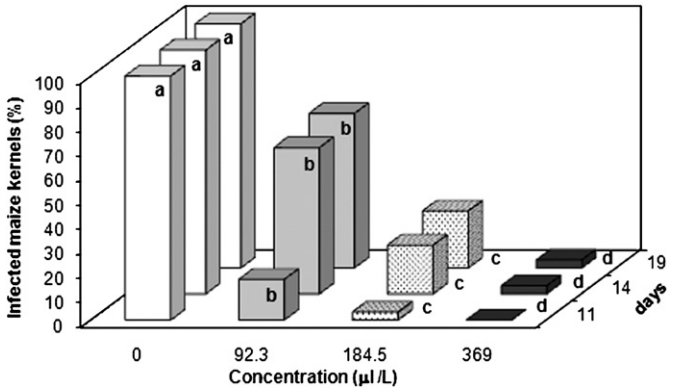

Fig. 1. Effect of *trans*-2-hexenal concentration on *F. verticillioides* growth, in artificially infected kernels, after 11, 14 and 19 days of incubation at 25 °C. Between several concentrations, bars having different letters are significantly different by LSD test at $P<0.05$.

3.4. Semi-commercial trials

Trans-2-hexenal at 246 µL/L confirmed the effectiveness in reducing infection (efficacy of 100, 96 and 82% following 11, 14 and 19 days of incubation, respectively) (Fig. 3).

3.5. Effect of trans-2-hexenal on fumonisin levels

In all trials *trans*-2-hexenal fumigation failed to reduce the concentration of fumonisins (FB_1+FB_2). In trials with artificially infected kernels, exposure to 92.3 µL/L *trans*-2-hexenal did not affect fumonisin production while high *trans*-2-hexenal applications (184.5 and 369 µL/L) increased fumonisin concentrations to 1640 and 1510 µg/kg, respectively, comparing to control (660 µg/kg). In trials with naturally infected kernels, fumigation with 92.3 and 184.5 µL/L did not reduce fumonisin concentrations. High *trans*-2-hexenal applications (369 µL/L) stimulated fumonisin concentration to 486 µg/kg comparing to 373 µg/kg in the control. Also in the semi-commercial trial, *trans*-2-hexenal at 246 µL/L did not lead to any significant reduction of the mycotoxins (Table 2).

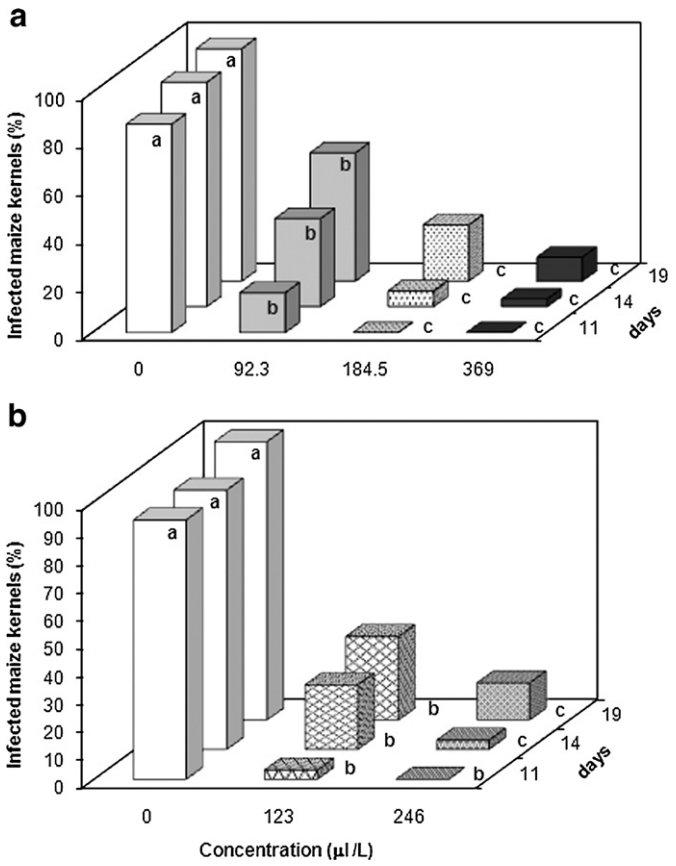

Fig. 2. Effect of *trans*-2-hexenal on *F. verticillioides* growth, in naturally infected kernels, after 11, 14 and 19 days of incubation at 25 °C, at concentrations of 92.3, 184.5, 369 µL/L (a) and 123, 246 µL/L (b). Between several concentrations, bars having different letters are significantly different by LSD test at $P<0.05$.

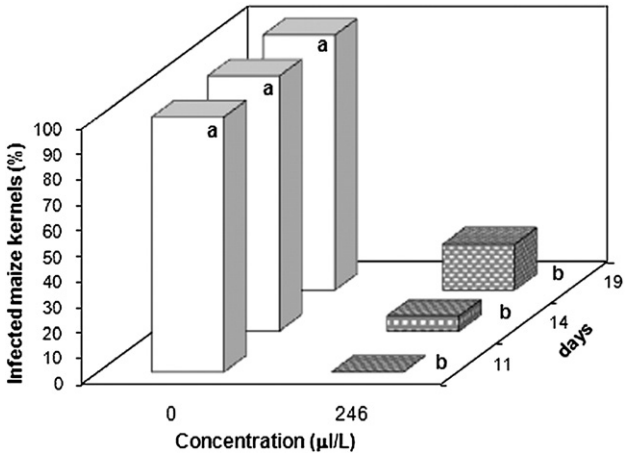

Fig. 3. Effect of *trans*-2-hexenal on *F. verticillioides* growth, in semi-commercial trials, after 11, 14 and 19 days of incubation at 25 °C, at 246 µL/L concentration. Bars having different letters are significantly different by LSD test at $P<0.05$.

4. Discussion

In recent years, the interest in natural compounds for plant disease control has been considerable.

Several studies, concerning the antifungal activity of natural plant extracts, showed that cinnamon, clove, lemon grass, palmarose and oregano essential oils had the best activity on growth of *F. verticillioides*, *F. proliferatum* and *F. graminearum* (Juglal et al., 2002; Velluti et al., 2003; Velluti et al., 2004). The other natural compounds like limonene and thymol showed the highest inhibitory activity on *F. verticillioides* development and fumonisin biosynthesis (Dambolena et al., 2008).

In our present study the ability of some natural compounds to control *F. verticillioides* in stored maize kernels was explored, and the possibility of *trans*-2-hexenal being used as a postharvest biofumigant to reduce fumonisin production was investigated. The aldehyde (*trans*-2-hexenal) and the phenols (carvacrol and eugenol) had a fungicidal activity against *F. verticillioides* in *in vitro* trials. *Trans*-2-hexenal was the best inhibitor of the fungus, according to our previous studies on *P. expansum* (Neri et al., 2006a,b) and *M. laxa* (Neri et al., 2007). In maize

Table 2
Effect of *trans*-2-hexenal on fumonisins in artificially and naturally infected kernels and semi-commercial trial.

Trans-2-hexenal (µL/L)	Artificially infected kernels	Naturally infected kernels	Semi-commercial trial
	FB_1+FB_2 (µg/kg)[a]	FB_1+FB_2 (µg/kg)	FB_1+FB_2 (µg/kg)
0	660[b]	373[b]	458[a]
92.3	587[b]	330[b]	nd
184.5	1640[a]	390[b]	nd
246	nd[b]	nd	478[a]
369	1510[a]	486[a]	nd

Results are means of two replicates per treatment. Values in the same column followed by the same letter indicate no significant difference by LSD test ($p<0.05$). The letters indicate the results of the statistic analysis.

[a] Detection limit of method: 50 µg/kg.

[b] nd = not determined.

kernels, *trans*-2-hexenal was very effective in controlling *F. verticillioides* growth at higher concentrations than found in previous work on fruit (Neri et al., 2006a,b, 2007). Probably, the adsorption of the volatile compound is easier in fruit than in dried kernels.

In our results the exposure to 2.46 μL/L *trans*-2-hexenal provided the best control of *F. verticillioides* and no phytotoxic symptoms or off-odour in kernels was observed. These results showed that *trans*-2-hexenal was effective in controlling the internal fungus located in asymptomatic maize kernels. *Trans*-2-hexenal, applied as a fumigant, was able to penetrate into the internal part of kernels and the antifungal activity may be attributed to its volatility. The most effective dose was 369 μL/L, but this concentration induced off-odour, like "green" aroma in maize. In accordance with the results on pome and stone fruit, *trans*-2-hexenal treatment can also induce off-odour in kernels (Neri et al., 2006a,b, 2007).

Trans-2-hexenal caused reduction or delay in the germinability of the kernel, therefore it is clear that treated kernels cannot be used for seed, but only for human or animal consumption. Paster et al. (1995) also reported a germination reduction in wheat grain following exposure to oregano, carvacrol and thymol.

In our study, fumonisins remained after harvest in kernels that were surface disinfected because the mycotoxin was produced in the field. In symptomless kernels mean levels ranged from 330 to 478 μg/kg, in agreement with the observations of other researchers (Bacon et al., 1992; Munkvold et al., 1997) who detected fumonisin occurrence in apparently healthy maize kernels. In addition, we observed that *trans*-2-hexenal was ineffective in reducing fumonisin ($FB_1 + FB_2$) concentrations and at high concentration (369 μL/L) stimulated fumonisin levels. A mycotoxin increase was also noted in field trials by other authors (Marín et al., 1998), reporting that fumonisin production was stimulated by some fungal species, e.g., *Aspergillus niger*. However, in our trials fumonisin levels ranged from 330 to 1640 μg/kg, values lower than 4000 μg/kg, which is the maximum level of contamination allowed in the EU (European Commission, 2007).

Trans-2-hexenal was effective in pathogen control, but not in reducing fumonisins. According to results by Marín et al. (2001) there was no correlation between inhibition of pathogen growth and FB_1 reduction. On the other hand, also toxin production may sometimes be inhibited without fungal growth being affected. Some volatile aldehydes (n-decyl aldehyde, hexenal, octanal) have been shown to inhibit the growth of *A. parasiticus*, but only n-decyl aldehyde reduced the aflatoxin produced by the fungus, whereas octanal stimulated aflatoxin biosynthesis (Wright et al., 2000). Other studies (Marín et al., 2001) have examined the effects of interaction between fumonisin and aflatoxin-producing species under different environmental conditions. In general, *A. parasiticus* reduced *F. verticillioides* and *F. proliferatum* infection but did not affect FB_1 production by these species. While the *Fusarium* species were not able to reduce *A. parasiticus* populations, they did reduce aflatoxin B_1 concentration.

Environmental conditions play an important role in the contamination of maize kernels before and after harvest. Recent studies suggest that fumonisin production and reduction were therefore very dependent on the moisture of the kernels. Some authors (Velluti et al., 2004) found that the essential oils tested (cinnamon, clove, lemon grass, palmarose and oregano) inhibited growth of *F. verticillioides,* however FB_1 production was only reduced at 0.99 a_w, while at 0.95 a_w none of the essential oils tested had any significant effect on fumonisin. Torres et al. (2003) reported that the activity of antioxidants (butylated hydroxyanisole and propyl paraben) for controlling of *F. verticillioides* and *F. proliferatum* growth and fumonisin production was dependent on the dose used and the a_w treatment. Antioxidants significantly reduced the production of fumonisin especially at 0.98 and 0.95 a_w conditions in maize kernels. Our results can be affected by the low moisture present in kernels (14% = 0.70 a_w).

In conclusion, the results of our study showed that *trans*-2-hexenal postharvest fumigation was effective in *F. verticillioides* control also in asymptomatic maize kernels, but was not active in reducing fumonisins. From the survey carried out, it has emerged that pathogen development and mycotoxin production are not strictly related. Further studies should be performed to evaluate the molecular level the mechanisms of action of primary and secondary metabolism of pathogen. In addition the role of the moisture of kernels on the activity of *trans*-2-hexenal in reducing the fumonisin production should be investigated. Finally, it is necessary to assess the activity of other compounds in the reduction of fumonisins.

Acknowledgements

The research was funded by the Ricerca Fondamentale Orientata (RFO) of *Alma Mater Studiorum*-University of Bologna.

References

AOAC Official Method 2001.04, 2002. Fumonisins B_1 and B_2 in corn and corn flakes. AOAC International.
Bacon, C.W., Bennett, R.M., Hinton, D.M., Voss, K.A., 1992. Scanning electron microscopy of *Fusarium moniliforme* within asymptomatic corn kernels and kernels associated with equine leukoencephalomalacia. Plant Dis. 76, 144–148.
Bush, B.J., Carson, M.L., Cubeta, M.A., Hagler, W.M., Payne, G.A., 2004. Infection and fumonisin production by *Fusarium verticillioides* in developing maize kernels. Phytopathology 94, 88–93.
Caramelli, M., Dondo, A., Cantini Cortellazzi, G., Visconti, A., Minervini, F., Doko, M.B., Guarda, F., 1993. Leucoencefalomalacia nell'equino da fumonisine: prima segnalazione in Italia. Ippologia 4, 49–53.
Chu, F.S., Li, G.Y., 1994. Simultaneous occurrence of fumonisin B_1 and other mycotoxins in moldy corn collected from the People's Republic of China in regions with high incidences of esophageal cancer. Appl. Environ. Microbiol. 60, 847–852.
Cirillo, T., Ritieni, A., Galvano, F., Amodio Cocchieri, R., 2003. Natural co-occurrence of deoxynivalenol and fumonisins B_1 and B_2 in Italian marketed foodstuffs. Food Addit. Contam. 20, 566–571.
Cleveland, T.E., Dowd, P.F., Desjardins, A.E., Bhatnagar, D., Cotty, P.J., 2003. United States Department of Agriculture—Agricultural Research Service research on pre-harvest prevention of mycotoxins and mycotoxigenic fungi in US crops. Pest Manag. Sci. 59, 629–642.
Dambolena, J.S., Lopez, A.G., Canepa, M.C., Theumer, M.G., Zygadlo, J.A., Rubinstein, H.R., 2008. Inhibitory effect of cyclic terpenes (limonene, menthol, menthone and thymol) on *Fusarium verticillioides* MRC 826 growth and fumonisin B1 biosynthesis. Toxicon 51, 37–44.
Desjardins, A.E., Plattner, R.D., Lu, M., Claflin, L.E., 1998. Distribution of fumonisins in maize ears infected with strains of *Fusarium moniliforme* that differ in fumonisin production. Plant Dis. 82, 953–958.
European Commission. 2007. Commission Regulation (EC) 1126/2007 of 28 September 2007 amending Regulation (EC) 1881/2006 setting maximum levels for certain contaminants in foodstuffs as regards *Fusarium* toxins in maize and maize products. Off J Eur Union L. 255, 14–17.
FDA (Food and Drug Administration). 2001. Fumonisin levels in human foods and animal feeds. Available from http://www.cfsan.fda.gov/~dms/fumongu2.htm.
Fu, Y., Zu, Y., Chen, L., Shi, X., Wang, Z., Sun, S., Efferth, T., 2007. Antimicrobial activity of clove and rosemary essential oils alone and in combination. Phytother. Res. 21, 989–994.
Gelderblom, W.C., Jaskiewicz, K., Marasas, W.F., Thiel, P.G., Horak, R.M., Vleggaar, R., Kriek, N.P., 1988. Fumonisins—novel mycotoxins with cancer-promoting activity produced by *Fusarium moniliforme*. Appl. Environ. Microbiol. 54, 1806–1811.
Hennigen, M.R., Sanchez, S., Di Benedetto, N.M., Longhi, A., Torroba, J.E., Valente Soares, L.M., 2000. Fumonisin levels in commercial corn products in Buenos Aires, Argentina. Food Addit. Contam. 17, 55–58.
IARC (International Agency for Research on Cancer) 2002. Fumonisin B1 Summaries & Evaluations. 82, 301.
Iotti, M., Zambonelli, A., 2006. A quick and precise technique for identifying ectomycorrhizas by PCR. Mycol. Res. 110, 60–65.
Juglal, S., Govinden, R., Odhav, B., 2002. Spice oils for the control of co-occurring mycotoxin-producing fungi. J Food Prot. 65, 683–687.
Kedera, C.J., Plattner, R.D., Desjardins, A.E., 1999. Incidence of *Fusarium* spp. and levels of fumonisin B_1 in maize in Western Kenya. Appl. Environ. Microbiol. 65, 41–44.
Logrieco, A., Mulè, G., Moretti, A., Bottalico, A., 2002. Toxigenic *Fusarium* species and mycotoxins associated with maize ear rot in Europe. Eur. J. Plant Pathol. 108, 597–609.
Lombaert, G.A., Pellaers, P., Roscoe, V., Mankotia, M., Neil, R., Scott, P.M., 2003. Mycotoxins in infant cereal foods from the Canadian retail market. Food Addit. Contam. 20, 494–504.
Marasas, W.F.O., 2001. Discovery and occurrence of the fumonisins: a historical perspective. Environ. Health Perspect. 109, 239–243.
Marín, S., Albareda, X., Ramos, J.A., Sanchis, V., 2001. Impact of environment and interactions of *Fusarium verticillioides* and *Fusarium proliferatum* with *Aspergillus parasiticus* on fumonisin B_1 and aflatoxins on maize grain. J. Sci. Food Agric. 81, 1060–1068.
Marín, S., Sanchis, V., Rull, F., Ramos, A., Magan, N., 1998. Colonization of maize grain by *Fusarium moniliforme* and *Fusarium proliferatum* in the presence of competing fungi and their impact on fumonisin production. J. Food Prot. 61, 1489–1496.

Miller, J.D., 2001. Factors that affect the occurrence of fumonisin. Sci. Total Environ. 109, 321–324.
Miller, J.D., 2008. Mycotoxins in small grains and maize: old problems, new challenges. Food Addit. Contam. 25, 219–230.
Munkvold, G.P., Desjardins, A.E., 1997. Fumonisins in maize: can we reduce their occurrence? Plant Dis. 81, 556–565.
Munkvold, G.P., Hellmich, R.L., Showers, W.B., 1997. Reduced *Fusarium* ear rot and symptomless infection in kernels of maize genetically engineered for European corn borer resistance. Phytopathology 87, 1071–1077.
Nelson, P.E., Toussoun, T.A., Marasas, W.F.O., 1983. *Fusarium* species. The Pennsylvania State University Press, An illustrated manual for identification.
Neri, F., Mari, M., Menniti, A.M., Brigati, S., 2006a. Activity of trans-2-hexenal against *Penicillium expansum* in 'Conference' pears. J. Appl. Microbiol. 100, 1186–1193.
Neri, F., Mari, M., Menniti, A.M., Brigati, S., Bertolini, P., 2006b. Control of *Penicillium expansum* in pears and apples by trans-2-hexenal vapours. Postharvest Biol. Technol. 41, 101–108.
Neri, F., Mari, M., Brigati, S., Bertolini, P., 2007. Fungicidal activity of plant volatile compounds for controlling *Monilinia laxa* in stone fruit. Plant Dis. 91, 30–35.
Ono, E.Y.S., Sasaki, E.Y., Hashimoto, E.H., Hara, L.N., Corra, B., Itano, E.N., Sugiura, T., Ueno, Y., Hirooka, E.Y., 2002. Post-harvest storage of corn: effect of beginning moisture content on mycoflora and fumonisin contamination. Food Addit. Contam. 19, 1081–1090.
Paster, N., Menasherov, M., Ravid, U., Juven, B., 1995. Antifungal activity of oregano and thyme essential oils applied as fumigants against fungi attacking stored grain. J. Food Prot. 58, 81–85.
Pereira, P., Nesci, A., Etcheverry, M., 2007. Effects of biocontrol agents on *Fusarium verticillioides* count and fumonisin content in the maize agroecosystem: imact on rhizospheric bacterial and fungal groups. Biol. Control 42, 281–287.
Rheeder, J.P., Marasas, W.F.O., Thiel, P.G., Sydenham, E.W., Shephard, G.S., van Schalkwyk, D.J., 1992. *Fusarium moniliforme* and fumonisins in corn in relation to human esophageal cancer in Transkei. Phytopathology 82, 353–357.
Ross, P.F., Nelson, P.E., Richard, J.L., Osweiler, G.D., Rice, L.G., Plattner, R.D., Wilson, T.M., 1990. Production of fumonisins by *Fusarium moniliforme* and *Fusarium proliferatum* isolates associated with equine leukoencephalomalacia and a pulmonary edema syndrome in swine. Appl. Environ. Microbiol. 56, 3225–3226.
Rota, M.C., Herrera, A., Martínez, R.M., Sotomayor, J.A., Jordán, M.J., 2008. Antimicrobial activity and chemical composition of *Thymus vulgaris*, *Thymus zygis* and *Thymus hyemalis* essential oils. Food Control 19, 681–687.
Scott, P.M., 1993. Fumonisins. Int. J. Food Microbiol. 18, 257–270.
Shephard, G.S., Marasas, W.F.O., Leggott, N.L., Yazdanpanah, H., Rahimian, H., Safavi, N., 2000. Natural occurrence of fumonisins in corn from Iran. J. Agric. Food Chem. 48, 1860–1864.
Shephard, G.S., Thiel, P.G., Stockenstrom, S., Sydenham, E.W., 1996. Worldwide survey of fumonisin contamination of corn and corn-based products. J AOAC Int. 79, 671–687.
Shephard, G.S., 2008. Impact of mycotoxins on human health in developing countries. Food Addit. Contam. 25, 146–151.
Silva, L.J.G., Lino, C.M., Pena, A., Molt, J.C., 2007. Occurrence of fumonisins B_1 and B_2 in Portuguese maize and maize-based foods intended for human consumption. Food Addit. Contam. 24, 381–390.
Snedecor, G.W., Cochran, W.G., 1980. Statistical Methods. Iowa State University Press, Ames, USA.
Stewart, D.W., Reid, L.M., Nicol, R.W., Schaafsma, A.W., 2002. A mathematical simulation of growth of *Fusarium* in maize ears after artificial inoculation. Phytopathology 92, 534–541.
Torres, A.M., Ramirez, M.L., Arroyo, M., Chulze, S.N., Magan, N., 2003. Potential use of antioxidants for control of growth and fumonisin production by *Fusarium verticillioides* and *Fusarium proliferatum* on whole maize grain. Int. J. Food Microbiol. 83, 319–324.
Velluti, A., Sanchis, V., Ramos, A.J., Egido, J., Marín, S., 2003. Inhibitory effect of cinnamon, clove, lemongrass, oregano and palmarose essential oils on growth and fumonisin B_1 production by *Fusarium proliferatum* in maize grain. Int. J. Food Microbiol. 89, 145–154.
Velluti, A., Sanchis, V., Ramos, A.J., Marín, S., 2004. Effect of essential oils of cinnamon, clove, lemon grass, oregano and palmarosa on growth of and fumonisin B_1 production by *Fusarium verticillioides* in maize. J. Sci. Food Agric. 84, 1141–1146.
Visconti, A., Doko, M.B., 1994. Survey ao fumonisin production by *Fusarium* isolated from cereals in Europe. J. AOAC Int. 77, 546–550.
Wright, M.S., Greene-McDowelle, D.M., Zerngue Jr., H.J., Bhatnagar, D., Cleveland, T.E., 2000. Effects of volatile aldehydes from *Aspergillus*-resistant varieties of corn on *Aspergillus parasiticus* growth and aflatoxin biosynthesis. Toxicon 38, 1215–1223.
Yoshizawa, T., Yamashita, A., Luo, Y., 1994. Fumonisin occurrence in corn from high- and low-risk areas for human esophageal cancer in China. Appl. Environ. Microbiol. 60, 1626–1629.