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Note

Influence of harvest time on fumonisin contamination of forage maize for whole-crop silage

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ABSTRACT

Forage maize growing in agricultural fields is often contaminated with fumonisins, a group of mycotoxins produced by *Fusarium* species. We investigated fumonisin accumulation in maize plants from 2 to 10 wk after silking. Our aim was to determine whether harvesting forage maize earlier could be a practical control measure to reduce the fumonisin contamination in whole-crop silage. In a year with high fumonisin levels, the total fumonisin content (consisting of fumonisins B1, B2, and B3) of the maize plants increased 4–8 wk after silking, indicating that the risk of contamination increases with later harvest times.

Fumonisins are mycotoxins produced by members of the *Gibberella fujikuroi* species complex, a group of plant pathogens that cause Fusarium ear or stalk rot in maize (*Zea mays* L.) (Payne 1999; White 1999; Desjardins 2006). *Fusarium verticillioides* (Saccardo) Nirenberg (= *G. fujikuroi* mating population A),*F. proliferatum* (Matsushima) Nirenberg (= *G. fujikuroi* mating population D), and *F. fujikuroi* Nirenberg (= *G. fujikuroi* mating population C) are common members of this species complex in Japan (Tsukiboshi et al. 2011). Although these species are known to be plant pathogens, they can infect maize plants without showing obvious symptoms (Yates et al. 1997).

Fumonisin contamination is a major concern for feed safety in livestock farming (European Food Safety Authority 2005). Corn silage produced in Japan is frequently contaminated with fumonisins (Hiraoka 2007). Although the total fumonisin content (composed of fumonisins B1, B2, and B3) found by Hiraoka (2007) was low (a maximum of 782 μg/kg dry matter), these mycotoxins were found in 6 of 18 corn silage samples (Hiraoka 2007).

Since maize plants are contaminated with fumonisins during the normal course of development under field conditions (Driehuis et al. 2010), good agricultural practices (GAPs) during the pre-harvest stages should be considered in risk-

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control strategies to combat fumonisin contamination (Deguchi 2008). The GAPs for forage maize should be different from those for maize grown for grain production, because fungicide/pesticide application in forage crop fields is not cost-effective. Among the various control methods, we focused our attention on maize harvest time because kernel fumonisin content increases rapidly as the infected ear matures (Bush et al. 2004).

Early harvest could be an effective control strategy to reduce fumonisin contamination (Bush et al. 2004). This practice has not been widely implemented because maize grown for grain production must be harvested at full maturity. However, we reasoned that early harvest might be a practical control measure for forage maize, which is harvested at the dent stage, a few weeks before physiological maturity, to improve the digestibility of silage. This agricultural practice requires careful study because farmers have recently come to prefer a delayed harvest time due to technological innovations. For example, the introduction of kernel processors on forage harvesters enables the production of digestible silage from maize plants at a more advanced stage (Johnson et al. 1999). Although harvesting fully mature maize for silage increases the dry matter yield, this practice may also extend the fumonisin accumulation period.

To determine the latest recommended time for harvest, we quantified the total fumonisin content (fumonisins B1, B2, and B3) in maize plants during the maturation process in 2008 and 2009. We sampled a mixture of tissues derived from maize ears, stalks and leaves because *Fusarium* spp. infect maize stalks as well as kernels and because silage is made from the whole maize plant. Fumonisin-producing fungi in the plant samples were detected using PCR amplification of a fumonisin biosynthesis gene, *FUM1*. We also examined the effect of seeding date because the timing of maize seeding may influence the course of fungal infection and mycotoxin contamination (Blandino et al. 2009). A part of this work (fumonisin content in maize plants in 2008) has been presented previously (Okabe et al. 2009; Okabe 2010).

Maize samples

Field experiments were conducted in 2008 and 2009. Maize plants were grown in a naturally infested field at the Nasu Research Station, National Institute of Livestock and Grassland Science (NILGS), Nasushiobara, Tochigi, Japan. Maize (May to September) and rye (*Secale cereale* L.; October to April) had been planted at this site according to the conventional culture system since 2006, and fumonisin-producing *Fusarium* spp. (*F. verticillioides* and *F. fujikuroi*) were isolated from maize kernels in 2006 and 2007 (Okabe et al. 2008). The maize hybrids used in this study were 32K61 (relative maturity [RM] 122; Pioneer Hi-Bred Japan Co. Ltd., Tokyo, Japan) and SH3815 (RM125; Snow Brand Seed Co., Ltd., Sapporo, Japan); these hybrids had previously been classified as susceptible and resistant to Fusarium ear rot, respectively (Miki et al. 2008). A two-replicate split-plot design was used with seeding date as the main-plot treatment and hybrid as the subplot treatment. In each year, the two replicates of early-sown plants were designated as '-1' and '-2' and the two late-sown replicates were designated as '-3' and '-4'. The subplots consisted of two rows 30 m in length and spaced 0.75 m apart. The temperature and precipitation data for each growing season were provided by the weather station at the Nasu Research Station, NILGS (Supplementary Fig. S1).

In 2008, the maize hybrids were seeded on 1 May (early-sown group) and 28 May (late-sown group). The silking dates (defined as the date when approximately 50% of the plants had silks) were in late July for the early-sown group and in early August for the late-sown group (Supplementary Table S1). Five plants in each subplot were harvested every week from 2 to 10 wk after silking by cutting the stalks about 7 cm above the soil surface. Plant material was chopped into fragments approximately 15 mm long, weighed, dried in a forced-air oven at 70 °C for 3 d to achieve 6–9% moisture content, and reweighed to determine the percentage of dry matter at harvest. The dry matter content reached 28% suggesting that 6–7 wk after silking was the optimal harvest time, i.e., middle to late dent stage (Oshita 2006) (Supplementary Fig. S2). Plant material was then ground in a Wiley mill, passed through a 1-mm screen and stored at room temperature until further use. Another 10 ears in each subplot were sampled 10 wk after silking and the percentage of kernels with symptoms was rated for each ear. The percentage of diseased kernels for each treatment (average of 20 ears) was 2.9% for early-sown 32K61, 0.7% for early-sown SH3815, 2.4% for late-sown 32K61 and 1.4% for late-sown SH3815.

In 2009, the seeding dates for the early- and late-sown groups were 1 May and 27 May, respectively. Plants were harvested every week from 2 to 12 wk after silking and the position of the kernel milkline was rated to monitor the grain maturity, because this visual indicator is more popular with farmers than the dry matter percentage (Wiersma et al. 1993; Oshita 2006) (Supplementary Table S1). The kernel milkline also indicated that 6–7 wk after silking was the middle to late dent stage (half to 3/4-milkline stage) for 32K61, but half-milkline stage for SH3815 was 8 wk after silking.

Detection of *F. verticillioides* and *F. fujikuroi* by PCR

A PCR assay targeting fumonisin biosynthesis gene, *FUM1* (=*FUM5*), has been used to detect the fumonisin-producing strains *F. verticillioides* in plant tissues (Bluhm et al. 2002; Sanchez-Rangel et al. 2005). We combined the methods of Bluhm et al. (2002) and Sanchez-Rangel et al. (2005) to develop a semi-nested PCR. The primers used in our semi-nested PCR strategy were FUM53F (5′-CTTGAACGCGGAGCTAGATTAT; Sanchez-Rangel et al. 2005), the forward primer in the first amplification; Fum5F (5′-GTCGAGTTGTTGACCACTGCG; Bluhm et al. 2002), the forward primer in the second amplification; and Fum5R (5′-CGTATCGTCAGCATGATGTAGC; Bluhm et al. 2002), the reverse primer in both amplifications. Those primers successfully amplified the *FUM1* of *F. verticillioides* strain MAFF 511481 isolated from a maize kernel from the experiment site in 2006. However, they failed to amplify the *FUM1* of *Fusarium* sp. strains MAFF 511484 and 511485, which were also isolated from the same field in 2007 (data not shown). Since partial sequences of the EF-1α genes from these